

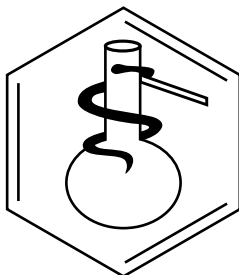
*Facultad de Ciencias
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REVISTA COLOMBIANA DE CIENCIAS QUÍMICO-FARMACÉUTICAS

Universidad Nacional de Colombia
Facultad de Ciencias
Departamento de Farmacia



REVISTA COLOMBIANA DE CIENCIAS QUÍMICO-FARMACÉUTICAS

Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Farmacia.

ISSN 0034-7418, Fax: 3165060, Cra. 30 No. 45-03, Bogotá, D. C., Colombia.

Correo electrónico: rciquifa_fbog@unal.edu.co

<http://www.revistas.unal.edu.co/index.php/rciquifa>

http://www.scielo.org/scielo.php?script=sci_serial&pid=0034-7418&rep=

<https://scholar.google.com/citations?user=TVBT68sAAAAJ&hl=es>

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La *Revista Colombiana de Ciencias Químico-Farmacéuticas* es un órgano de difusión en el cual se publican investigaciones científicas, comunicaciones técnicas y revisiones temáticas originales en las áreas de las ciencias farmacéuticas (véanse Normas para publicación). La revista está destinada principalmente a químicos farmacéuticos, químicos, ingenieros químicos, médicos cirujanos, médicos veterinarios, y a otros profesionales de las ciencias físicas y naturales, de la ingeniería y de las profesiones sanitarias relacionadas con el uso de medicamentos.

VISIÓN

En pro de la difusión de las investigaciones, los contenidos de la *Revista Colombiana de Ciencias Químico-Farmacéuticas* son de acceso libre, con ello se espera llegar a un número mayor de lectores, propiciando la consolidación de comunidades académicas. Además, se proyecta que los contenidos publicados contribuyan al desarrollo e innovación de las ciencias farmacéuticas.

ÉTICA

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Artigo de pesquisa tecnológica / <http://dx.doi.org/10.15446/rcciquifa.v51n3.100051>

Avaliação dos parâmetros de qualidade físico-químicos de creme-géis à base de ácido hialurônico adquiridos em farmácias magistrais do município de Campo Grande-MS

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Recebido: 16 de dezembro de 2021

Revisado: 30 de janeiro de 2022

Aceto: 7 de fevereiro de 2022

RESUMO

Introdução: ao longo dos anos, a busca pela melhora do autocuidado estendeu-se às farmácias com manipulação, onde o cosmético pode ser manipulado de acordo com as preferências de cada indivíduo. Dessa forma, é necessário prover a estabilidade para cada produto manipulado, garantindo que um cosmético seguro e eficaz será dispensado. O ácido hialurônico é um ativo que tem se tornado popular devido à sua capacidade de realizar a manutenção da elasticidade da pele e exercer função hidratante, além de atuar na redução de linhas de expressão. **Objetivo:** analisar a qualidade de dermocosméticos manipulados a base de ácido hialurônico de diferentes farmácias com manipulação no município de Campo Grande-MS, através de análises laboratoriais físico-químicas e de rotulagem. **Material e métodos:** quatro amostras de creme-gel à base de ácido hialurônico 5% foram adquiridas em farmácias magistrais, e realizadas as seguintes análises de controle de qualidade: análise organoléptica, determinação de pH, densidade, teste de centrifugação, espalhabilidade e análise de rotulagem. **Resultados:** em relação às análises, os resultados obtidos foram satisfatórios. A análise organoléptica, o teste de centrifugação e de espalhabilidade se encontraram dentro dos padrões estipulados; porém, destaca-se o ensaio de densidade, em que duas amostras obtiveram valores abaixo do estipulado; e para a análise de rótulos, nenhum produto continha lista de componentes da formulação. **Conclusão:** ao final das análises, pode ser apontado que os produtos apresentaram

desempenho satisfatório em grande parte dos testes propostos e são uma opção segura, de boa qualidade e custo acessível ao público.

Palavras chave: Manipulação, garantia da qualidade, cosmético, estabilidade, autocuidado.

SUMMARY

Evaluation of the physicochemical quality parameters of cream-gels based on hyaluronic acid purchased in compounding pharmacies in the city of Campo Grande-MS

Introduction: Over the years, the search for improving self-care has extended to compound pharmacy, where the cosmetic can be manipulated according to the preferences of each person. Thus, it is necessary to provide stability for each product handled, ensuring that a safe and effective cosmetic will be dispensed. Hyaluronic acid is an active that has become popular due to its ability to maintain skin elasticity and exert a moisturizing function, in addition to acting in the reduction of fine lines.

Aim: To analyze the quality of cosmetics manipulated with the active ingredient of Hyaluronic Acid from different compound pharmacies in the city of Campo Grande-MS, through physical-chemical laboratory analysis and labeling. **Material and methods:** Four samples of 5% hyaluronic acid-based cream-gel were acquired from master pharmacies, and the following quality control analyzes were carried out: organoleptic analysis, pH determination, density, centrifuge test, spreadability and labeling analysis. **Results:** Regarding the analyses, the results obtained were satisfactory. The organoleptic analysis, the centrifugation test and the spreadability were within the stipulated standards; however, the density test stands out, in which two samples obtained results below the stipulated; and for label analysis, no product contained a list of formulation components. **Conclusion:** At the end of the analyses, it can be pointed out that the products presented satisfactory performance in most of the proposed tests and are a safe option, of good quality and accessible to the public.

Keywords: Manipulation, quality warranty, cosmetic, stability, self-care.

RESUMEN

Evaluación de parámetros de calidad fisicoquímica de geles-crema a base de ácido hialurónico adquiridos en farmacias magistrales de la ciudad de Campo Grande-MS

Introducción: a lo largo de los años, la búsqueda por mejorar el autocuidado se ha extendido a las farmacias, donde los cosméticos pueden ser manipulados según las preferencias de cada individuo. Por lo tanto, es necesario proporcionar estabilidad a cada producto manipulado, asegurando que se dispensará un cosmético seguro y eficaz. El ácido hialurónico es un activo que se ha popularizado por su capacidad para mantener la elasticidad de la piel e hidratarla, además de actuar para reducir las líneas de expresión. **Objetivo:** analizar la calidad de los dermocosméticos manipulados a base de ácido hialurónico de diferentes farmacias en Campo Grande-MS, mediante análisis de laboratorio físico-químico y marcaje. **Material y métodos:** se adquirieron en farmacias magistrales cuatro muestras de crema-gel a base de ácido hialurónico al 5%, y se realizaron los siguientes análisis de control de calidad: análisis organoléptico, determinación de pH, densidad, prueba de centrifugación, untabilidad y análisis de marcaje. **Resultados:** en cuanto a los análisis, los resultados obtenidos fueron satisfactorios. Las pruebas de análisis organoléptico, centrifugado y untabilidad estuvieron dentro de los estándares estipulados, sin embargo, se destaca la prueba de densidad, en la que dos muestras obtuvieron valores por debajo del valor estipulado y para el análisis de etiquetas, ningún producto contenía una lista de componentes de la formulación. **Conclusión:** los productos se desempeñaron satisfactoriamente en la mayoría de las pruebas propuestas y son una opción segura, con buena calidad y su costo accesible al público.

Palabras claves: Manipulación, garantía de calidad, cosmética, estabilidad, autocuidado.

INTRODUÇÃO

A indústria farmacêutica possui um amplo espectro de setores de atuação, em que o maior foco é prover saúde à comunidade. Dentre os setores, a farmácia magistral é uma opção favorável para o público, uma vez que os preços são acessíveis para uma grande parcela da população, assim como o processo de manipulação pode ser inserido dentro do conceito de uso racional de medicamentos, por exigir prescrições adequadas, dispensação correta com o consumo nas doses indicadas, havendo uma oferta de serviços a uma faixa de custo alcançável [1].

No entanto, as farmácias magistrais necessitam de um padrão de controle de qualidade alto, desta forma há uma prevenção e redução dos riscos, garantindo a segurança do produto final. Portanto, a Agência Nacional de Vigilância Sanitária (Anvisa) publicou a Resolução da Diretoria Colegiada (RDC) N.º 67/2007, onde dispõe sobre as “boas práticas de manipulação de preparações magistrais e oficinais para uso humano em farmácias” [2].

Ao longo dos anos, o crescimento da indústria mostra-se evidente, voltada aos cuidados estéticos e à higiene pessoal. Com isto, a busca pela melhora do autocuidado estendeu-se às farmácias com manipulação, onde o cosmético pode ser manipulado de acordo com as preferências de cada indivíduo [3].

Dessa forma, é necessário prover a estabilidade para cada produto manipulado, seguindo as exigências listadas pela Anvisa, para que, quando comprovada a estabilidade, seja possível analisar o desempenho e eficácia do cosmético para dispensação [3].

A emulsão pode ser definida como um sistema de duas fases não imiscíveis entre si, uma fase dispersa dentro de uma fase contínua, em que através de um agente emulsionante será formado um sistema homogêneo e estável [4, 5]. A forma farmacêutica creme-gel se trata de uma emulsão com elevado percentual de água e percentual de óleo muito baixo [5].

Essa formulação cosmética se mostra uma opção interessante para o público de pele oleosa ou acneica, por se tratar de um veículo leve, não comedogênico, de rápida absorção e que promove uma sensação de toque seco na pele [6].

Dentre os ativos escolhidos para efetuar a manutenção da elasticidade da pele e exercer função hidratante, o ácido hialurônico (AH) é uma opção popular para ser associado a formulação, uma vez que o composto age na pele sendo responsável pela formação de novos capilares sanguíneos e fibroblastos, além de atuar na redução de linhas de expressão, principalmente aquelas formadas pela perda de água da pele [7].

O AH possui fórmula molecular $(C_{14}H_{21}NO_{11})_n$ e é um polímero natural não-ramificado pertencente ao grupo dos glicosaminoglicanos (GAGs) [8], este grupo possui uma estrutura química composta por unidades de dissacarídeos (N-acetil-D-glucosamina) e ácido glucurônico [7].

Este princípio ativo é uma molécula encontrada no corpo humano e possui um alto grau de ligação com moléculas de água presentes no organismo, podendo reter até 100 vezes o próprio peso molecular, o que resulta na formação de uma estrutura coesa capaz

de preencher finas linhas de expressão e rugas. Outra característica apresentada pelo AH, é o efeito antioxidante que exerce sob a pele, atuando no sequestro de radicais livres e melhorando a proteção da pele contra radiação ultravioleta [9].

Portanto, o presente estudo tem como objetivo analisar a qualidade de creme-géis manipulados a base de AH de diferentes farmácias com manipulação da cidade de Campo Grande-MS, através de análises laboratoriais físico-químicas e de análise de rótulos.

MATERIAL E MÉTODOS

A aquisição da amostra

Para a realização do controle de qualidade, foram adquiridos quatro creme-géis à base de ácido hialurônico 5% em abril de 2021 e a aquisição foi feita a caráter de consumidor. Todos os quatro eram provenientes de farmácias magistrais e foram identificados como A, B, C e D. As identificações foram feitas com o objetivo de não expor os fabricantes. As análises ocorreram durante o prazo de validade estabelecido nos rótulos dos produtos.

Equipamentos

O desenvolvimento do estudo envolveu a utilização dos seguintes equipamentos: balança analítica modelo AG200 Gehaka®; pHmetro de bancada pH Meter TEC-2 Tecnal®, centrífuga modelo Combate Celm®.

Controle de qualidade dos produtos

Os produtos foram analisados quanto a determinação das características organolépticas, pH, densidade, teste de centrífuga, espalhabilidade e análise de rótulos.

As análises foram realizadas conforme preconizado pelo *Guia de controle de qualidade de produtos cosméticos* [3], no entanto, o estudo da espalhabilidade se baseou em uma metodologia proposta por Muenzel *et al.* [10] com adaptações descritas por Ferreira-Silva *et al.* [11], uma vez que este teste não se encontra no guia.

Análise das características organolépticas

Para a realização da análise organoléptica, a amostra foi colocada em um vidro relógio e sua análise foi feita com o auxílio de uma lupa, verificou-se aspectos como aparência, cor e odor.

Determinação do pH

A solução foi preparada com 1g de amostra e 10 mL de água destilada, em seguida a determinação do pH foi feita por potenciometria utilizando o pHmetro de bancada.

Determinação da densidade

A determinação da densidade foi realizada em picnômetro e em quatro passos: primeiramente, o picnômetro vazio foi pesado e o seu peso foi anotado (M_0); em seguida, preencheu-se o picnômetro com água purificada e pesado novamente, o peso foi anotado (M_1); com a vidraria limpa e seca, o picnômetro foi preenchido uma última vez com a amostra, pesou-se e anotou-se o peso; por fim, as anotações foram empregadas no seguinte cálculo:

$$d = (M_2 - M_0) / (M_1 - M_0)$$

Onde:

d: densidade

M_0 : massa do picnômetro vazio, em gramas;

M_1 : massa do picnômetro com água purificada, em gramas;

M_2 : massa do picnômetro com a amostra, em gramas.

Teste de centrifuga

Para o teste de centrifuga, transferiu-se a amostra para um tubo tipo falcon, levou-se para a centrifuga durante 30 minutos a 3300 rpm, a fim de observar a ocorrência de separação de fases.

Teste de espalhabilidade

Empregou-se uma metodologia proposta por Muenzel *et al.* [10] com adaptações descritas por Ferreira-Silva *et al.* [11]. O teste de espalhabilidade foi realizado colocando-se 0,3 g da amostra sobre uma placa de vidro e cobriu-se a amostra com uma folha de acetato A4.

Em seguida, foi adicionado um peso padrão de 50 g sobre o acetato e aguardou-se 3 minutos, após esse tempo mediu-se os diâmetros do círculo com o auxílio de uma régua e calculou-se a sua média; este mesmo processo foi repetido utilizando pesos maiores de 150 g, 250 g e 350 g. Ao final, a espalhabilidade foi calculada pela fórmula:

$$E_i = d_i^2 \cdot \pi / 4$$

Onde:

E_i : espalhabilidade da amostra para o peso i (mm^2);

d_i : diâmetro médio (mm).

Análise de rótulos

A análise foi feita com os rótulos dos produtos provenientes das farmácias com manipulação. Apesar das amostras se tratarem de produtos cosméticos, observou-se que as farmácias magistrais onde as aquisições foram feitas seguiram o padrão de rotulagem para preparações magistrais estabelecido pela RDC N.º 67/2007 [2] e, por isso, optou-se para que as análises fossem executadas seguindo a mesma resolução.

A fim de auxiliar o processo de avaliação, elaborou-se um *check-list* para verificar se os rótulos continham as informações necessárias preconizadas pela RDC N.º 67/2007 citada anteriormente.

Análises estatísticas

Os resultados obtidos durante o estudo foram descritos e dispostos em gráficos e tabelas utilizando os *softwares* Microsoft Office Excel e Word.

RESULTADO E DISCUSSÃO

Todas as amostras possuíam como base da formulação o creme-gel, entretanto, não foi encontrado um produto industrializado nesta mesma base farmacêutica contendo ácido hialurônico a 5%, que preenchesse os requisitos para realizar uma análise de comparação com as amostras magistrais adquiridas.

O controle de qualidade foi iniciado pela avaliação das características organolépticas (figura 1), na qual observou-se que não constavam desvios de qualidade quanto aos aspectos organolépticos. Todas as amostras se apresentaram com aspecto homogêneo, em que não havia presença de grumos e textura consistente, coloração branca e opaca e odor característico da essência.

Em um estudo sobre controle de qualidade de um gel-creme antiacneico feito por Ferreira-Silva *et al.* [11], o resultado obtido foi satisfatório, no qual a amostra não possuía grumos, com uma boa homogeneidade, mantendo-se estável, comprovando a estabilidade dos creme-géis e se assemelhando ao resultado obtido no presente estudo.

A adição de algum tipo de fragrância à formulação é um fator de risco aos pacientes que possuem hipersensibilidade, uma vez que esses tipos de substâncias podem gerar reações alérgicas.

Em diversas áreas industriais o uso de fragrâncias é difundido com o intuito de fornecer um produto mais atrativo ao público, proporcionando uma maior aceitabilidade por conta do fator olfativo mais agradável, e a indústria cosmética não é isenta de utilizar tal prática [12].

Ao longo dos anos nota-se que os diferentes *blends* de essências podem conter ingredientes que acarretam irritações cutâneas ou alergias por contato; em primeiro instante, os indivíduos podem não experimentar tais efeitos colaterais, porém, é um fator que pode vir a ser desenvolvido de acordo com a exposição contínua [12].

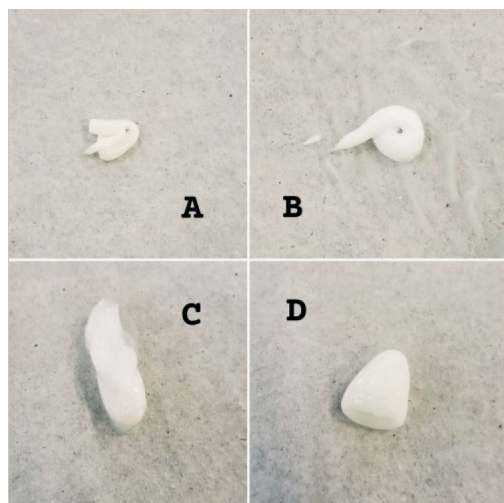


Figura 1. Avaliação das características organolépticas de géis-creme de ácido hialurônico, quanto a seu aspecto, cor e odor.

Fonte: autoria própria.

De acordo com Oliveira de Melo e Maia-Campos [13], a pele possui um caráter levemente ácido por toda estrutura corporal, entre a faixa de 4,5-5,8, variando de acordo com a região, portanto a característica ácida possui uma função preventiva bactericida

e fungicida. Com isto em consideração, o pH em formulações torna-se de suma importância, pois é um fator diretamente relacionado com a estabilidade do produto e com a compatibilidade fisiológica do tecido de aplicação [14].

Em produtos cosméticos, nos quais são utilizados como base creme-gel, deve-se ter como faixa de pH entre 6,0-7,0 [15]. No entanto, pode haver variação em relação aos valores, quando se utiliza um princípio ativo de natureza ácida à composição final, sendo o caso do ácido hialurônico, em que para obter melhor estabilidade é necessário estar entre 5,5-7,5 em cosméticos [16].

Desta forma, as análises de determinação do pH encontraram-se dentro do padrão, entre os valores estabelecidos de 5,5-7,5; e apresenta destaque para o exemplar C, que obteve um resultado bastante próximo ao valor máximo preconizado (tabela 1).

A densidade de emulsões deve ficar dentro do espectro de 0,95-1,05 g/cm³, faixa esta estipulada de acordo com a média encontrada em outros estudos desenvolvidos sobre creme-géis, onde duas das amostras analisadas (B e C) não estavam dentro do padrão estipulado. Para isto, um dos fatores que pode interferir com a densidade do produto final é a incorporação de ar durante a manipulação, resultando na diminuição significativa dos valores da densidade [11].

Tabela 1. Resultados obtidos durante as análises físicas dos creme-géis de ácido hialurônico.

Características físico-químicas	Amostras			
	A	B	C	D
pH	5,7	6,8	7,3	6,8
Densidade (g/cm ³)	0,98	0,92	0,94	0,97
Teste de centrifuga	N	N	N	N

Legenda: N: normal; M: modificada.

Fonte: autoria própria.

O ensaio de centrifugação submete a amostra ao estresse e simula um aumento na força da gravidade, possui a finalidade de antecipar possíveis instabilidades do sistema que podem se apresentar na forma de separação de fases, como cremeação, floculação, coalescência e entre outros [3].

Após a execução do teste de centrifuga, nenhuma amostra deu sinais de instabilidade na formulação, não apresentando separação de fases e, portanto, mantiveram a sua integridade, como mostrado na figura 2.

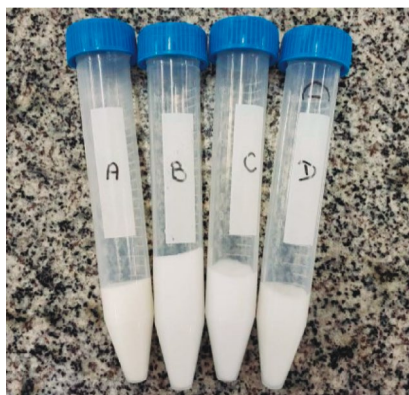


Figura 2. Amostras de creme-géis após o teste de centrifuga.

Fonte: autoria própria.

Em seu trabalho intitulado *Protocolo de para ensaios físico-químicos de estabilidade de fitocosméticos*, Isaac *et al.* [17] realizaram o teste de centrifugação com dois sistemas emulsionados, água em óleo (A/O) e óleo em água (O/A), ao final do processo não houve relato de anormalidades e as amostras também permaneceram estáveis.

De acordo com Moraes [18], a separação de fases é um sinal de instabilidade do sistema e pode ser percebida através de alterações de aparência, de consistência e na performance do produto; a separação de fase ocorre por diversos mecanismos como: cremeação, coalescência, floculação e sedimentação, além disso, podem levar a uma instabilidade química que será refletida na alteração do pH e na hidrólise de tensoativos da formulação, assim também como promover a contaminação microbiana.

Siqueira [19] encontrou instabilidades ao realizar o teste de centrifugação em suas amostras de emulsão cosmética *cold cream*. A autora relatou leve separação de fases em duas amostras, classificando-a como uma separação do tipo coalescência, além disso, outras duas amostras já haviam sido eliminadas anteriormente ao teste por apresentarem instabilidade e separação de fases, tornando-as impróprias para o uso.

A espalhabilidade está relacionada à aceitação do usuário, e se baseia na capacidade de expansão da formulação sobre o local de ação onde será aplicado [20]. Dessa forma, considera-se importante que um produto cosmético possua uma boa característica de espalhamento, uma vez que irá influenciar na percepção do usuário sobre o produto [11].

Conforme mostrado na figura 3, as amostras apresentaram resultado satisfatório no teste da espalhabilidade, uma vez que o valor de espalhabilidade aumentou à medida que pesos maiores eram adicionados. A amostra D teve maior destaque, se mostrando

com maior capacidade de espalhabilidade do que as demais, enquanto a amostra B obteve o menor desempenho neste teste.

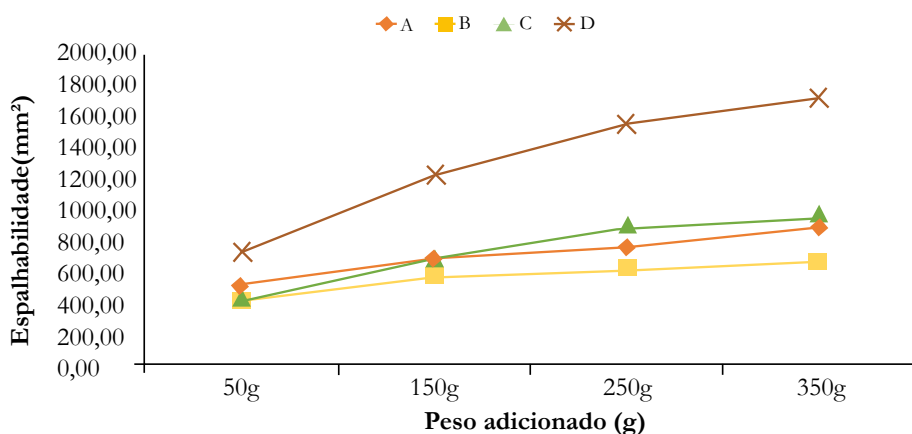


Figura 3. Espalhabilidade das formulações de creme-géis de ácido hialurônico.

Fonte: autoria própria.

No teste de espalhabilidade do creme-gel antiacneico à base do óleo de copaíba desenvolvido por Ferreira-Silva *et al.* [11], os autores obtiveram resultados semelhantes, em que foi observado o aumento da espalhabilidade conforme o peso adicionado, concluindo-se que o creme-gel antiacneico apresentou uma boa espalhabilidade.

De acordo com Favero [21], a espalhabilidade é um parâmetro importante em cosméticos, pois influencia na sua eficácia e na aceitação do produto pelo consumidor. Um cosmético com baixa espalhabilidade pode se espalhar de forma irregular, o que irá afetar a dose aplicada, comprometendo a penetração do princípio ativo na pele; além disso, a percepção do público sobre o produto também poderá ficar comprometida e provocar um baixo índice de aceitação.

As amostras apresentaram rótulos que estavam de acordo, em sua maioria, com as normas impostas da RDC N.º 67/2007, como pode ser visto na tabela 2.

Entretanto, nenhum dos rótulos continha os componentes da fórmula, o que também consta na resolução citada, tendo-se conhecimento somente do princípio ativo, o AH. A presença da lista de componentes de formulação se faz importante uma vez que veículos, conservantes, entre outros excipientes empregados à formulação, na forma de produto acabado podem desencadear uma reação alérgica ou sensibilizante [22].

Tabela 2. Informações previstas na legislação RDC N.º 67/2007 presentes nos rótulos dos produtos.

Informações previstas na legislação	Amostras			
	A	B	C	D
Nome do prescritor	+	+	+	+
Nome do paciente	+	+	+	+
Nº de registro da formulação no livro de receituário	+	+	+	+
Data da manipulação	+	+	+	+
Prazo de validade	+	+	+	+
Componentes da formulação	-	-	-	-
Peso ou volume contido	+	+	+	+
Modo de uso	+	+	+	+
Identificação da farmácia (CNPJ e endereço)	+	+	+	+
Nome do farmacêutico responsável	+	+	+	+
CRF do farmacêutico responsável	+	+	+	+

Legenda: (+) presente; (-) ausente.

Fonte: autoria própria.

Um estudo realizado em Bambuí-MG por Bento e Henriques [23], relacionado à avaliação de rótulos de cremes manipulados contendo ureia 10%, também observou a ausência de itens preconizados pela RDC N.º 67 de 2007 em seus rótulos, como componentes da formulação e suas respectivas quantidades, a posologia e advertências necessárias.

Em seu estudo relacionado a estabilidade de formulações (creme e gel) manipuladas contendo vitamina C a 5%, Santos *et al.* [24] encontraram divergências nos rótulos de suas amostras. Os autores escolheram dois estabelecimentos de Teresina-PI, os denominaram como F1 e F2 e os avaliaram, dentre eles, os rótulos das amostras provenientes da farmácia F1 não continham a advertência de armazenamento em geladeira, assim como não informaram o CNPJ da empresa e o seu endereço completo.

Os estudos citados mostram que farmácias magistrais de outras cidades também constataram a ausência de itens em seus rótulos, demonstrando que os estabelecimentos farmacêuticos com manipulação necessitam de um maior cuidado neste tópico. A rotulagem inadequada pode ter como consequência a ocorrência de efeitos indesejados

decorrentes do uso inadequado do produto, assim também como entendimento equivocado da sua finalidade de uso e das condições de armazenamento.

CONCLUSÃO

O estudo apontou com clareza através de análises laboratoriais que os produtos finais adquiridos em farmácias magistrais da cidade de Campo Grande-MS demonstraram poucos desvios de qualidade.

A partir das análises foi possível identificar um rígido controle de qualidade das farmácias com manipulação em muitos aspectos, entretanto, mesmo assim faz-se necessário uma maior atenção no preparo das formulações e uma maior preocupação com o rótulo dos produtos, para prover o melhor produto possível para o público.

O desenvolvimento deste estudo também mostra que todos os ensaios realizados possuem uma boa aplicabilidade nas farmácias magistrais, uma vez que são testes simples e que requerem a utilização de poucos materiais, estes últimos bastantes presentes na rotina magistral.

Devido à falta de pesquisas que abordem o controle de qualidade de produtos cosméticos a base de ácido hialurônico, sugere-se que mais estudos sejam desenvolvidos acerca deste tema, a fim de enriquecer a busca de dados sobre a aplicação do AH em cosméticos destinados ao uso na face.

AGRADECIMENTOS

Este estudo foi desenvolvido graças à Universidade Católica Dom Bosco, localizada em Campo Grande - MS, que disponibilizou o laboratório de controle físico-químico de qualidade, contendo todos os equipamentos e vidrarias necessários para que as análises fossem realizadas dentro do período de dias desejado.

CONFLITO DE INTERESSES

As autoras declaram que não há conflito de interesses.

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COMO CITAR ESTE ARTIGO

L. Cruz-Rocco, N. Soken-Ikeda, K.d.T.C. Muller, M.R.A. Miranda, Avaliação dos parâmetros de qualidade físico-químicos de creme-géis à base de Ácido Hialurônico adquiridos em Farmácias Magistrais do município de Campo Grande-MS, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1049-1064 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100051>

Premezclados como alternativa para el uso seguro de medicamentos intravenosos en escenarios de atención en salud

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Recibido: 18 de septiembre de 2021

Revisado: 22 de enero de 2022

Aceptado: 1 de febrero de 2022

RESUMEN

Objetivo: presentar el panorama de errores de medicación, los recursos asociados a la preparación de medicamentos intravenosos y el uso de premezclados en la atención en salud. **Metodología:** se realizaron búsquedas en diferentes bases de datos, sin límite de fecha o tipo de estudio. Adicionalmente se realizó un análisis para estimar los costos, validando con expertos los recursos en central de mezclas. **Resultados:** los errores de medicación son un error médico común a nivel global. Los datos disponibles son heterogéneos, pero sugieren que los errores de medicación pueden ser una causa considerable de morbilidad y mortalidad en ciertas poblaciones y contextos, con intervenciones adicionales, estancias hospitalarias prolongadas, mayores costos de atención y reducción en la probabilidad de que el tratamiento sea oportuno y eficaz. En medicinas intravenosas resultan escenarios de mayor gravedad y mayor nivel de costos. Los costos laborales anuales para una central de mezclas en Colombia se estiman entre 281,5 y 422,3 millones de pesos. La estandarización, como parte de los fármacos premezclados proporciona menor riesgo de contaminación, menor posibilidad de error en la preparación, menor incidencia de complicaciones relacionadas con la terapia, disminución del desperdicio, mejora en la oportunidad de dispensación, optimización en el trabajo de los equipos de farmacia y reducción de costos asociados con este proceso. **Conclusiones:** el uso de premezclados, como parte de un programa de reducción de errores de medicación, puede mejorar los indicadores de calidad en administración de medicamentos y garantizar un uso más seguro de la terapia intravenosa.

Palabras clave: Error de medicación, farmacovigilancia, medicamentos premezclados.

SUMMARY

Premixed products, an alternative for safe intravenous medications use in health care settings

Objective: To present an overview of medication preparation and administration errors, the resources implied in the preparation of intravenous medications, and the use of premixed drugs in healthcare settings. **Methodology:** We performed a literature review without limits by date or type of study. Additionally, analysis and validation by pharmaceutical experts were carried out to estimate the use of resources and related costs. **Results:** The available heterogeneous data suggest that medication errors are a significant cause of morbidity and mortality in specific populations and settings. Error medications cause additional interventions, prolonged hospitalization, higher costs of care, and a reduction in the treatment probability of success. Errors involving intravenously administered drugs have more severe consequences and generate higher costs. Annual labor costs for centralized medication mixing in Colombia are estimated between \$281.5 and \$422.3 million. Premixed drugs decrease the risk of contamination, the possibility of error in the preparation, and the incidence of complications related to therapy. Also, its use is related to the reduction of waste, improvement in the timing of dispensing, optimizing the pharmacy team, and reducing costs associated with this process. **Conclusions:** The use of premixes as part of a program to reduce medication errors can improve the quality indicators in drug administration and guarantee a safer use of intravenous therapy.

Keywords: Medication error, pharmacovigilance, premixed medications

RESUMO

As pré-misturas como alternativa para o uso seguro de drogas intravenosas em ambientes de saúde

Objetivo: apresentar o panorama dos erros de medicação, os recursos associados ao preparo de medicamentos intravenosos e o uso de pré-misturas na assistência à saúde. **Metodologia:** as buscas foram realizadas em diferentes bases de dados, sem limite de data ou tipo de estudo. Além disso, foi realizada uma análise para estimar os custos, validando com especialistas os recursos no centro de mistura. **Resultados:** erros de medicação são um erro médico comum globalmente. Os dados disponíveis são heterogêneos, mas sugerem que os erros de medicação podem ser uma causa

significativa de morbidade e mortalidade em certas populações e contextos, com intervenções adicionais, internações hospitalares mais longas, custos mais altos de atendimento e probabilidade reduzida de que o tratamento seja oportuno e eficaz. Nos medicamentos intravenosos, resultam cenários de maior gravidade e maior nível de custos. Os custos anuais de mão de obra para uma usina de mistura na Colômbia são estimados entre 281,5 e 422,3 milhões de pesos. A padronização, como parte dos medicamentos pré-misturados, proporciona menor risco de contaminação, menor possibilidade de erro no preparo, menor incidência de complicações relacionadas à terapêutica, redução de desperdícios, melhoria na oportunidade de dispensação, otimização no trabalho das equipes, da farmácia e redução dos custos associados a este processo. **Conclusões:** o uso de pré-misturas, como parte de um programa de redução de erros de medicação, pode melhorar os indicadores de qualidade na administração de medicamentos e garantir um uso mais seguro da terapia intravenosa.

Palavras-chave: Erro de medicação, farmacovigilância, medicamentos pré-misturados.

INTRODUCCIÓN

La atención sanitaria en el mundo propende cada vez más por un servicio integrado y centrado en los pacientes. Brindar una atención segura y de calidad es un aspecto fundamental pues la minimización de eventos potencialmente perjudiciales para los pacientes puede dar lugar a menos hospitalizaciones evitables e incluso menor discapacidad, secuelas o muerte [1]. En ese sentido, el interés respecto a las causas, consecuencias y posibles soluciones a los eventos que afectan la seguridad del paciente ha derivado en información que amplía el conocimiento sobre el tema, pero su completo entendimiento y control sigue constituyendo un reto para las instituciones y los sistemas de salud.

La vigilancia e intervención oportuna ante los errores potencialmente perjudiciales para las personas son necesarios en los diferentes escenarios de atención sanitaria. Dentro de las cuestiones más frecuentes en un entorno hospitalario está el uso de medicamentos intravenosos, que involucra una serie de procesos desde la evaluación de la necesidad y prescripción hasta la administración y monitoreo del mismo. Cada etapa implica la participación de diferentes actores y procedimientos específicos y sistematizados para minimizar las probabilidades de falla. Sin embargo, los errores relacionados con la preparación y administración de la medicación pueden ocurrir y afectar al paciente, por lo que su medición es importante para comprender la magnitud y la naturaleza del daño en la atención.

Aunque los datos son ampliamente variables en la literatura publicada, los reportes de errores de medicación en diferentes instituciones y territorios a nivel mundial muestran una frecuencia considerable de los errores de administración de medicamentos, específicamente en lo relacionado con la velocidad y frecuencia de administración y la preparación de medicamentos, los cuales aumentan de manera importante el riesgo de afectación al paciente [2]. De hecho, es probable que las tasas de error sean más altas para los medicamentos administrados por vía intravenosa en comparación con otras rutas [3]. El objetivo de esta revisión es exponer el panorama de errores de medicación, los recursos asociados a la preparación de medicamentos intravenosos en una Central de Mezclas y el uso de premezclados como alternativa para reducir los errores de medicación en intravenosos reduciendo el impacto tanto clínico como económico relacionado con el uso de estos.

METODOLOGÍA

Se realizaron diferentes búsquedas de evidencia respecto a seguridad en el uso de medicamentos en bases de datos electrónicas y motores de búsqueda (Medline, Ovid, SciELO, Google scholar) en agosto de 2021. La estrategia de búsqueda se compuso de vocabulario controlado (MeSH, Emtree y DeCS) y lenguaje libre, sinónimos, abreviaturas, variaciones ortográficas y plurales, con base en los términos: “error de medicación”, “farmacovigilancia” y “premezclados”. Esta estrategia se adaptó para las fuentes de información. No se utilizaron filtros de fecha o tipo de estudio y se consideraron aquellos documentos publicados en texto completo, en inglés o español, que reportaran aspectos relacionados con errores de medicación: mediciones y reporte, carga del daño, costos asociados, reducción de errores en preparación de medicamentos intravenosos, premezclados en reducción de errores de medicación. Se extrajo la información relevante priorizando los artículos más recientes (últimos diez años) para completar información de cada eje temático, realizando una síntesis narrativa de la evidencia de la evidencia.

Adicionalmente, por medio de un análisis, para estimar los costos asociados de una central de mezclas intrahospitalaria en Colombia, se identificaron, midieron y valoraron los recursos asociados a la preparación de medicamentos intravenosos en una Central de Mezclas. Los recursos, referidos especialmente a la mano de obra por cada fase del proceso, esta información se validó con cuatro expertos químicos farmacéuticos mediante grupo focal y revisiones asincrónicas.

RESULTADOS Y DISCUSIÓN

Errores de medicación: reporte y medición

Actualmente, no hay una definición unificada de error de medicación, pero se entiende universalmente de acuerdo con el National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP) de los Estados Unidos como: “cualquier evento prevenible que pueda causar o conducir a un uso inadecuado de medicamentos o daños al paciente mientras el medicamento está bajo el control del profesional de la salud, el paciente o el consumidor. Dichos eventos pueden estar relacionados con la práctica profesional, los productos, procedimientos y sistemas de atención sanitaria, incluida la prescripción, la comunicación de la orden, el etiquetado, el empaque y la nomenclatura del producto, la composición, la dispensación, la distribución, la administración, la educación, el monitoreo y el uso” [4].

Como se entiende, cada una de las etapas de la prescripción, preparación y administración intravenosa de medicamentos es susceptible a errores de medicación, cuya evaluación resulta útil para el diseño de intervenciones que permiten mitigar los riesgos y daños asociados. Sin embargo, el conocimiento acerca de los errores de medicación está sujeto a los datos heterogéneos actualmente disponibles acerca de la magnitud del problema. Una variedad de estudios que presentan la información desde diferentes contextos, metodologías y formas para clasificar los errores de medicación [5], limitan la generalización de los hallazgos y dan lugar a una probable subestimación del problema. Además, las prácticas que comprenden la terapia intravenosa y los costos de la morbilidad asociada a la atención médica son diferentes entre las economías y los sistemas de salud, por lo que puede no ser apropiado extrapolar los datos de errores de medicación de un entorno geográfico de atención médica a otro.

Actualmente, la vigilancia de errores de medicación se enfoca principalmente en prevención dirigida a proveedores de atención médica y seguimiento de los reportes voluntarios. Los datos obtenidos de algunos sistemas de seguimiento, análisis y clasificación de errores de medicación, permiten identificar medicamentos de alerta hospitalaria por potencial aumento del riesgo de daño al paciente si se usan por error [6]. Pero esta información es limitada en muchos territorios, entre tanto los diferentes sistemas de salud y escenarios clínicos en los que se utiliza la terapia intravenosa exigen una cultura de reporte fortalecida que permita conocer y limitar en el impacto de los errores de medicación en términos de carga del daño evitable al paciente.

La carga del daño en errores de medicación

Las diferentes publicaciones respecto a errores de medicación muestran que cerca del 90% de estos no generan daños a los pacientes, sin embargo, pueden llegar a ser una causa considerable de morbilidad y mortalidad en ciertas poblaciones y escenarios de atención. Las afectaciones consecuencia de errores de medicación incluyen dolor, flebitis, hipo o hiperglucemia, depresión respiratoria, hipoxia, hipotensión, anafilaxia, alteraciones de la frecuencia cardíaca, convulsiones y paro cardíaco [7], además de una proporción menor de casos que requieren ingreso a unidades de cuidado intensivo (UCI). Aproximadamente una quinta parte de los errores de medicación en UCI son potencialmente mortales y casi la mitad tienen suficiente importancia clínica para justificar tratamientos adicionales de soporte vital [8]. Aunque las consecuencias más evidentes de los errores de medicación son los eventos adversos que resultan en intervenciones adicionales, estancias hospitalarias prolongadas y mayores costos de atención, se conoce además una reducción en la probabilidad de que el tratamiento sea oportuno y eficaz [9].

Para Colombia, un estudio retrospectivo en un sistema multicéntrico de farmacovigilancia determinó 4707 errores de medicación en 45 servicios farmacéuticos hospitalarios entre 2008 y 2013 [10]. De acuerdo con la clasificación taxonómica del NCC MERP, del total de eventos identificados 12,0% afectaron al paciente (categorías C a I) y en 0,36% de los casos causaron daño. El tipo de error, causa del mismo y proceso implicado en los errores de medicación se describen en la tabla 1. Aunque es un estudio a gran escala en el país, no es posible extrapolar los resultados a todos los entornos hospitalarios colombianos. En razón a las limitaciones de reporte de errores de medicamentos, anteriormente descritas, este estudio probablemente registra un menor número de errores de los que ocurren en la práctica habitual.

Tabla 1. Descripción de errores de medicación en estudio de farmacovigilancia en Colombia.

Tipo de errores	Porcentaje
Dosis/cantidad inadecuadas	43,41
Concentración incorrecta	14,65
Medicamentos equivocados	13,69
Forma de dosificación incorrecta	11,15
Causa del error	
Factores humanos	78,84

Tipo de errores	Porcentaje
Mal leído o no leyó la receta	11,09
Confusión en el nombre de los medicamentos	7,37
Embalaje/diseño	1,62
Dispositivos para distribución automática	0,61
Confusión en el nombre del paciente	0,47
Proceso de uso de medicamentos donde se detectó el error	
Alistamiento	28,74
Revisión de recetas médicas	25,55
Dispensación	23,07
Administración o posterior	10,85
Gravedad de los errores de medicación	
A. Circunstancias o eventos que tienen la capacidad de causar errores	48,06
B. Se produjo un error, pero el error no llegó al paciente	45,69
C. Se produjo un error que llegó al paciente, pero no causó daño al paciente	5,95
D. Se produjo un error que llegó al paciente y requirió un seguimiento para confirmar que no resultó en ningún daño al paciente o requirió intervención para evitar daños	0,12
E. Se produjo un error que pudo haber contribuido o dado lugar a daños temporales al paciente	0,14
F. Se produjo un error que pudo haber contribuido o resultado en daños temporales al paciente y requirió hospitalización inicial o prolongada	0,02
G. Se produjo un error que pudo haber contribuido o resultado en daños permanentes al paciente	0,01
H. Se produjo un error que requirió intervención para mantener la vida	0,01

Fuente: elaborado a partir de datos reportados por Machado-Alba *et al.* [10].

Costos asociados a errores de medicación intravenosa

Según la Organización Mundial de la Salud (OMS), los errores de medicación generan daños graves y evitables a nivel mundial, que resultan en escenarios desfavorables y costosos. Por ejemplo, los errores de medicación provocan al menos una muerte diaria

y daños en aproximadamente 1,3 millones de personas al año solo en EE. UU. De igual forma, la OMS estima que para el año 2017, el costo mundial asociado a los errores de medicación fue de US\$ 42 000 millones, representando el 1% del gasto sanitario mundial [11]. Además, estos constituyen la tercera causa de muerte en EE.UU. después de las enfermedades cardiovasculares y los tumores [12].

Los errores de medicación son un error médico común a nivel global, y ocurren principalmente en medicinas intravenosas, lo que es muy grave y conlleva a más costos [13]. Los medicamentos premezclados administrados por vía intravenosa pueden ser una buena alternativa para reducir la tasa de error en medicamentos, pueden ser costo-efectivos y generar ahorros potenciales en el proceso de atención [14]. Adicionalmente, la estandarización (como parte de los medicamentos premezclados) mejora la seguridad del paciente y los indicadores de calidad.

Hay varias revisiones de literatura de los estudios que analizan la prevalencia de errores de medicación y su impacto económico. Por ejemplo, Wash *et al.* [15] realizaron una revisión sistemática de artículos que describen y cuantifican la carga económica de los errores en la medicación en varios países. Entre sus hallazgos fue posible identificar que los errores médicos estaban asociados al incremento en los costos de medicación y los costos de hospitalización. Los costos reportados en la literatura son variables y dependen de cada contexto en particular, estos oscilan entre € 2,58 y € 111 727,08. En Reino Unido, aproximadamente el 10,1% de los casos en que se requiere medicación intravenosa están asociados con algún error de medicación, ya sea por dosis incorrecta, diluyente incorrecto, volumen incorrecto, u omisión de dosis, lo cual evidencia cómo la incidencia de errores asociados a medicamentos intravenosos afecta negativamente al sistema de salud [2].

Los errores médicos son una problemática real en el mundo debido al impacto en los costos asistenciales y económicos y Colombia no es ajena a dicha situación. Sin embargo, para el país es un reto poder contar con reportes por parte de las instituciones de salud, por lo que es necesario reforzar los sistemas de farmacovigilancia y reconocer la naturaleza del fenómeno [10].

Reducción de errores en la preparación de los medicamentos intravenosos

Dentro del ciclo de uso de medicamentos intravenosos, que implica varios pasos antes de la administración, se reconoce como crítica la etapa de preparación de la mezcla y etiquetado (figura 1), por los múltiples procesos involucrados relacionados con los fármacos (dosis, concentración, diluyentes), el etiquetado, y el manejo aséptico necesario (higiene en manipulación y desinfección de material). Los pasos descritos en preparación de medicamentos pueden ocurrir *in situ* en un centro de enfermería o en una

farmacia, y las dosis de medicación intravenosa suelen ser preparadas manualmente por enfermeras o farmacéuticos, en variedad de escenarios con diversos grados de control y automatización. La introducción de tecnología a estos procesos se evidencia en mayor medida en centros de atención más grandes y en territorios más centralizados.

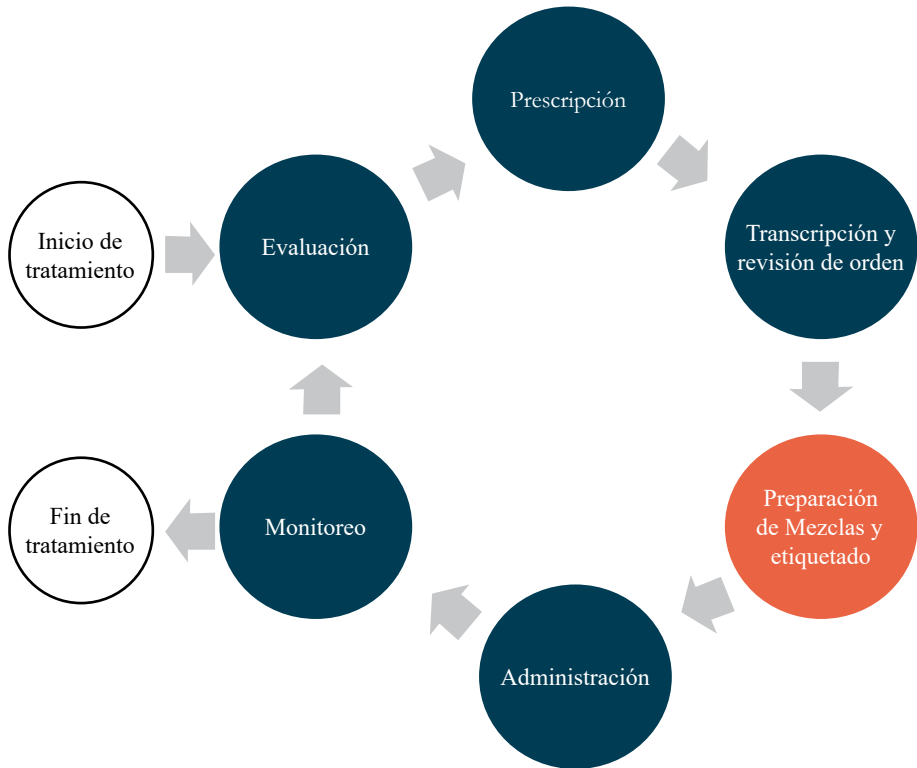


Figura 1. Ciclo de uso de medicaciones intravenosas.

Fuente: adaptado de la referencia [16].

El proceso de mezcla de medicamentos implica una serie de factores cuyo control resulta desafiante en cualquier escenario (figura 2). Los estudios centrados en las prácticas de preparación de medicamentos sugieren que las etapas de mezcla intravenosa y etiquetado plantean una importante posibilidad de errores cuya estimación no se conoce [16], y los reportes disponibles no son específicos en diferenciar los errores asociados a estos procesos de los atribuidos a la administración incorrecta [16]. Los diversos reportes identifican errores en la preparación y administración de medicamentos intravenosos en rangos de 10% [2] hasta más de 95% en instituciones hospitalarias [17]. Un estudio multicéntrico examinó los errores de medicación en los sistemas para la preparación

y administración de terapia intravenosa en hospitales de tres países europeos [18]. La observación directa de 824 dosis preparadas y 798 dosis administradas reveló falta de etiquetado o etiquetado incorrecto en 43%, 99% y 20% de las dosis administradas en los hospitales del Reino Unido, Alemania y Francia, respectivamente; uso de diluyente incorrecto en 1%, 49% y 18% de los casos, respectivamente, y tasa de administración incorrecta en 49%, 21% y 5% de las dosis observadas, respectivamente. Se observó al menos una desviación de la técnica aséptica entre el 100%, 58% y 19% de los casos en los tres países, demostrando riesgos no controlados en procesos estudiados en los tres países.



Figura 2. Proceso para la preparación de medicamentos en central de mezclas.

Costos asociados a centrales de mezcla en hospitales

En un escenario de central de mezclas en Colombia, se ha estimado la distribución del tiempo de demanda de cada una de las profesiones en el proceso de mezclado de medicamentos, descritos en la tabla 2.

Tabla 2. Tiempos y mano de obra en el proceso de preparación de medicamentos intravenosos en una Central de Mezclas*

Personal involucrado	Proceso	Tiempo por proceso (minutos)	Tiempo total (minutos)
Médico tratante	Prescripción	20	20

Personal involucrado	Proceso	Tiempo por proceso (minutos)	Tiempo total (minutos)
Químico farmacéutico de producción	Revisión de fórmulas	20	150
	Elaboración de orden de producción, orden de alistamiento, control de calidad, y etiquetas	20	
	Preparación del ingreso a la central (lavado de manos, cambio de ropa por uniforme de circulación, postura de uniforme estéril y guantes estériles)	25	
	Alistamiento del área de trabajo	10	
	Preparación de dosis unitarias: 100 unidades	60	
	Salida del producto al área de calidad y diligenciamiento de formatos	15	
Químico farmacéutico de control de calidad	Revisión de fórmulas	20	40
	Aprobación de etiquetas	20	
	Preparación del ingreso a la central (lavado de manos, cambio de ropa, traje, y guantes estériles)	25	45
	Control de calidad de 100 unidades	20	
Regente	Alistamiento de insumos y medicamentos	15	90
	Descartonamiento e ingreso a la central	10	
	Preparación del ingreso a la central (lavado de manos, cambio de ropa, traje, y guantes estériles)	15	
	Colocar dentro de bolsas y pegar etiqueta externa a las 100 unidades	30	
	Colocar dentro de neveras de transporte: ubicar pilas o geles refrigerantes y productos en cada nevera según validación de cadena de frío	20	

Personal involucrado	Proceso	Tiempo por proceso (minutos)	Tiempo total (minutos)
Auxiliar	Recepción de insumos y medicamentos	20	125
	Limpieza y desinfección	15	
	Ingreso de insumos y medicamentos al área de preparación	10	
	Preparación del ingreso a la central (lavado de manos, cambio de ropa por uniforme de circulación, postura de uniforme estéril, y guantes estériles)	25	
	Desinfección y ubicación de medicamentos e insumos en el área de preparación (mesas de apoyo)	25	
	Entrega de productos a servicios	30	

*Para un lote de 100 unidades de mezclas estándar.

Considerando los salarios promedio por minuto de cada profesión requerida en el proceso de mezcla y la distribución de la demanda de tiempo en minutos de cada una de estas profesiones en el proceso de mezcla, se determinó que el costo laboral de cada proceso de mezcla es igual a \$97 750. Si se asume que este proceso se realiza entre 8 y 12 veces al día, se cuantifican costos laborales de cada central de mezclas entre \$782 000 y \$1 173 000 por día, entre \$23 460 000 y \$35 190 000 por mes, y entre \$281 520 000 y \$422 280 000 por año. Este análisis constituye una estimación del costo de oportunidad del tiempo empleado que podría haberse usado en actividades de mayor valor por el personal involucrado.

Para determinar la validez de estos hallazgos, se comparó frente al costo mensual de funcionamiento de una central de mezclas para pacientes neonatales en la Clínica Madre Bernarda ubicada en Bogotá-Colombia para el 2012 (valores actualizados a precios de 2021) [19], donde reportaron un valor anual de \$284 655 879 en costos totales de preparación de medicamentos intravenosos (para un lote de 100 unidades de mezclas estándar). Si bien estos costos corresponden a la central de mezclas de una IPS privada restringida a la atención neonatal en Bogotá, hay que considerar que puede existir una variación entre regiones y especialidad o nivel de complejidad. No obstante, la conversión del costo anual de mano de obra para valores del 2021 en este estudio (\$81 881 294) es bastante cercano al estimativo con el análisis propio considerando solamente costos laborales (\$91 748 494).

Medicamentos premezclados, una alternativa

Los medicamentos premezclados han surgido como una alternativa para minimizar la amplia posibilidad de errores relacionados con los procesos de preparación y desde hace décadas se ha documentado su impacto en reemplazo de las preparaciones de medicación intravenosa [20, 21]. Un estudio reciente investigó la probabilidad general de error en la preparación y administración de medicamentos por vía intravenosa, mediante una revisión sistemática y un modelo bayesiano de efectos aleatorios de independencia condicional [22]. La probabilidad general de cometer al menos un error en la terapia intravenosa fue de 0,73 (intervalo de confianza del 95% 0,54-0,90), con una mayor probabilidad en el paso de reconstitución. Introducir la verificación de errores en cada etapa del proceso redujo la tasa general de errores a 0,22 (intervalo de confianza 95% 0,14-0,31) y eliminar el paso de reconstitución mediante el uso de medicamentos premezclados reduciría la tasa de error general a 0,17 (intervalo de confianza 95% 0,09-0,27).

Los estudios sugieren que los premezclados pueden tener ventajas en comparación con los medicamentos que requieren preparación, en razón a menores costos de preparación, menor riesgo de contaminación asociada a la manipulación, menor posibilidad de error y menor incidencia de infecciones y complicaciones relacionadas con la terapia [23]. Del mismo modo, el uso productos premezclados puede reducir el tiempo de la farmacia para procesar, componer y dispensar los pedidos de medicamentos, reducir errores de etiquetado, reducir el desperdicio y ser útiles para establecer estandarización de la dosificación [24].

En un estudio realizado por Witte *et al.* [25], un modelo de decisión para premezclados de administración intravenosa, se demostró que el uso de estos productos puede generar ahorros. Por su parte, Geberbauer *et al.* [26] *IV admixtures*] reportaron que el uso de premezclados permite tener un mejor control del inventario, reducción del desperdicio, disminución de costos en equipos y necesidad de personal experto en la realización de mezclas, logrando garantía de calidad para productos parenterales. Estos resultados, particularmente en reducción del desperdicio, se han mostrado en estudios más recientes con el aumento del uso de premezclados disponibles comercialmente asociado a una reducción anual de miles de dosis intravenosas desperdiciadas, lo que resultaría en un ahorro potencial anual en términos económicos cercanos a \$ 449 208, el cual fue calculado en un estudio específicamente para un departamento de farmacia institucional con base en el costo de desperdicios de material intravenoso, [27].

Un estudio en tres hospitales comunitarios de EE.UU. mostró que el uso de premezclados redujo el tiempo total de mano de obra dedicado a la preparación, almacenamiento y manipulación de materiales en un 64-67% [28]. Del mismo modo, se evidenció para cada institución una reducción promedio de costos anuales de US \$15 000 representa-

dos en materiales, tiempo de trabajo, medicamentos desperdiciados e inventario. Otro estudio europeo de vida real mostró una reducción de 32% en el tiempo de enfermería dedicado a la preparación y administración de mezclas intravenosas, allí los costos totales se redujeron significativamente en 60% con medicamentos listos para usar [29]. Este aspecto resulta importante dado que, en el contexto colombiano, la limitación de capacidad de algunos hospitales hace que la práctica de reconstitución por enfermería sea usual.

Una evaluación económica donde se compararon cuatro sistemas de medicación intravenosa en UCI analizó si las premezclas de medicamentos podrían ser una estrategia útil para disminuir los costos de medicación y los costos de hospitalización. El modelo de decisión se realizó desde la perspectiva del tercer pagador, concluyendo que el uso de medicamentos premezclados puede ser una estrategia de ahorro de costos, que disminuye los errores médicos en la administración de medicamentos en la UCI, particularmente si es parte de un programa integral de reducción de errores [30].

El uso de premezclados tiene beneficios adicionales relacionados con el personal pues no requiere diferencias significativas en educación de administración de medicamentos en comparación con la instrucción requerida para las mezclas específicas. Además de reducir la carga de trabajo de los equipos humanos en la preparación de mezclas, el personal encuentra mayor satisfacción con el uso de las premezclas debido a la mayor facilidad de preparación y el potencial de prevención de errores y riesgos de manipulación, lo que permite prácticas institucionales más conscientes y consistentes. En el caso de farmacias hospitalarias, la farmacia se hace más eficiente y se enfoca en la reconstitución de productos de mayor valor y rentabilidad para la unidad.

Finalmente, las formulaciones premezcladas mantienen los beneficios de las presentaciones estandarizadas y adicionalmente mejoran el flujo y la eficiencia de farmacia. Estas ventajas y la posibilidad de minimizar el riesgo de errores asociado con la concentración de dosificación, llevaron a la Joint commission a apoyar específicamente el uso de formulaciones intravenosas premezcladas [31]. Los organismos relacionados con la atención en salud han dirigido sus esfuerzos en mejorar los procesos de administración de medicamentos y garantizar la seguridad del paciente. El consenso basado en las declaraciones del Institute of Medicine (IOM) [32], el Institute for Safe Medication Practice (ISMP) [33] y la American Society of Health-System Pharmacists' IV Medication Summit [34], es que la terapia intravenosa debe ser considerada como una actividad de alto riesgo y que los proveedores deben instituir procedimientos de manejo del riesgo en cada etapa de la preparación, entrega y administración para minimizar el riesgo de los pacientes y garantizar prácticas cada vez más seguras en los entornos de atención en salud.

CONCLUSIONES

La preparación y administración de medicamentos son procesos complejos, con múltiples etapas altamente sensibles a errores. La terapia intravenosa debe considerarse como una actividad de alto riesgo de alta prioridad para el desarrollo de intervenciones que permiten mitigar los eventos negativos y daños asociados.

Las instituciones de salud alrededor del mundo han tomado la decisión de usar medicamentos premezclados, en lugar de realizar mezclas en la farmacia institucional, para disminuir el desperdicio de medicamentos, mejorar la oportunidad de la dispensación, cumplir con las regulaciones de las autoridades locales, y garantizar un uso más seguro de la terapia intravenosa. La potencial reducción de errores de medicación con el uso de premezclados se acompaña además de los beneficios de asegurar la calidad de los productos administrados y optimizar el trabajo de los equipos de farmacia, reduciendo la carga de trabajo en la preparación de mezclas y los costos asociados con este proceso.

La Organización Mundial de la Salud apoya la reducción del 50% de los errores de medicación que causan daños para 2022; en este sentido, se promueve la verificación de errores en cada etapa de preparación y administración de medicamentos intravenosos, así como la implementación de cambios y procedimientos para generar mejores prácticas en todos los niveles de la atención médica.

CONFLICTOS DE INTERESES

Esta investigación fue realizada con el apoyo de Baxter. Los autores de este manuscrito manifiestan que son independientes con respecto a la institución financiadora, y que durante la ejecución del trabajo o la redacción del manuscrito no hubo influencia de intereses o valores distintos a los de la investigación.

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COMO CITAR ESTE ARTÍCULO

S.P. Martínez-Rojas, L.M. Ibatá-Bernal, A. Taborda-Restrepo, J.G. Ariza, K. Bonilla-Arteaga, Premezclados como alternativa para el uso seguro de medicamentos intravenosos en escenarios de atención en salud, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1065-1082 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100189>

Artigo de pesquisa clínica / <http://dx.doi.org/10.15446/rcciquifa.v51n3.100355>

Instrumento sobre conhecimento, atitudes e práticas da automedicação em estudantes universitários

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Recebido: 11 de janeiro de 2022

Revisado: 25 de janeiro de 2022

Aceto: 1 de fevereiro de 2022

RESUMO

Objetivo: construir e validar instrumento para investigar os conhecimentos, atitudes e práticas de estudantes universitários brasileiros sobre a automedicação. **Método:** estudo metodológico realizado em universidade pública do centro oeste mineiro, Brasil. Um comitê de juízes avaliou clareza, pertinência e abrangência e o pré-teste realizado com público alvo para avaliar compreensão e aceitabilidade. O índice de validade de conteúdo (IVC) foi utilizado para avaliar a proporção de concordância e o coeficiente alfa de Cronbach (α) para mensurar a confiabilidade. **Resultados:** o instrumento foi composto por 38 itens divididos em três seções: caracterização dos participantes (13 itens); conhecimentos e práticas da automedicação (3 itens); e crenças e atitudes (22 itens). A média do IVC de todas as seções do instrumento foi de

0,97 e o coeficiente alfa de *Cronbach* da seção crenças e atitudes >0,70. **Conclusão:** o instrumento foi considerado válido para avaliar conhecimento, prática, crenças e atitudes sobre automedicação em estudantes universitários.

Palavras-chave: Automedicação, estudantes, conhecimentos, atitudes e prática em saúde, estudos de validação.

SUMMARY

Instrument on knowledge, attitudes and practices of self-medication in university students

Objective: To build and validate an instrument to investigate the knowledge, attitudes and practices of Brazilian university students about self-medication. **Method:** Methodological study carried out at a public university in the center of western Minas Gerais, Brazil. A committee of judges assessed clarity, relevance and comprehensiveness and the pre-test carried out with a target audience to assess understanding and acceptability. The content validity index (CVI) was used to assess the proportion of agreement and Cronbach's alpha coefficient (α) to measure reliability. **Results:** The instrument consisted of 38 items divided into three sections: characterization of participants (13 items); knowledge and practices of self-medication (3 items); and beliefs and attitudes (22 items). The mean CVI for all sections of the instrument was 0.97 and the Cronbach's alpha coefficient for the beliefs and attitudes section >0.70. **Conclusion:** The instrument was considered valid to assess knowledge, practice, beliefs and attitudes about self-medication in university students.

Keywords: Self-medication, students, health knowledge, attitudes, practice, validation study.

RESUMEN

Instrumento sobre conocimientos, actitudes y prácticas de automedicación en estudiantes universitarios

Objetivo: construir y validar un instrumento para investigar los conocimientos, actitudes y prácticas de estudiantes universitarios brasileños sobre la automedicación. **Método:** estudio metodológico realizado en una universidad pública del centro del occidente de Minas Gerais, Brasil. Un comité de jueces evaluó la claridad,

la relevancia y la exhaustividad y la prueba previa se llevó a cabo con un público objetivo para evaluar la comprensión y la aceptabilidad. El índice de validez de contenido (IVC) se utilizó para evaluar la proporción de acuerdo y el coeficiente alfa de Cronbach (α) para medir la confiabilidad. **Resultados:** el instrumento constaba de 38 ítems divididos en tres apartados: caracterización de los participantes (13 ítems); conocimientos y prácticas de automedicación (3 ítems); y creencias y actitudes (22 ítems). El CVI medio para todas las secciones del instrumento fue 0,97 y el coeficiente alfa de Cronbach para la sección de creencias y actitudes > 0,70. **Conclusión:** el instrumento se consideró válido para evaluar conocimientos, prácticas, creencias y actitudes sobre la automedicación en estudiantes universitarios.

Palabras clave: Automedicación, estudiantes, conocimientos, actitudes y práctica en salud, estudio de validación.

INTRODUÇÃO

Mundialmente, a automedicação é um comportamento prevalente entre estudantes universitários podendo variar de 49,3 a 94% a depender das diferentes populações, cursos e culturas [1-5]. A automedicação pode ser definida como obtenção e consumo de um ou mais medicamentos sem indicação, prescrição ou vigilância de um tratamento indicado por um médico para uma determinada doença. Inclui também a aquisição de medicamentos sem receitas, reutilização de receitas antigas, compartilhamento de medicamentos entre familiares e pessoas do mesmo convívio social ou utilização de medicamentos armazenados em casa [6].

O comportamento de automedicação é impactado pelo nível educacional e socioeconômico, ou seja, quanto mais conhecimento especializado os estudantes adquirem, somado à influência das mídias sociais, mais intensifica-se a prática da automedicação no cotidiano [6]. Acredita-se que esta prática seja mais comum entre estudantes das áreas de saúde quando comparados à população geral. Tal fato, pode ser explicado pelo conhecimento sobre diferentes doenças e medicamentos ao longo do processo de formação acadêmica [6].

Diversos fatores estão relacionados à prática da automedicação entre estudantes universitários, como acessibilidade informal aos medicamentos, longa evolução clínica da doença, exposição às mídias sociais, falta de tempo, custo da consulta médica (situação econômica), estilo de vida, inacessibilidade dos serviços de saúde, longa espera para consulta, experiência anterior e sensação de ter as informações necessárias para automedicação [2, 3, 7].

A Organização Mundial de Saúde (OMS) [8] reconhece que esta prática pode resultar no uso inadequado ou abuso de medicamentos aumentando os riscos potenciais à saúde. Além disso, pode resultar em reações adversas graves com consequências fatais, interações medicamentosas, surgimento da resistência microbiana, diagnóstico e tratamento incorretos, abuso e dependência [3, 6].

Neste sentido, faz-se necessário desenvolver a conscientização dos estudantes universitários sobre a prática da automedicação durante o processo de formação acadêmica. O contexto universitário oportuniza um ambiente propício à prática de autocuidado, pois disponibiliza recursos humanos e físicos para promover a adoção de mudanças de estilo e autonomia para tomada de decisões em saúde [9].

Diante da importância deste fenômeno, é essencial o desenvolvimento de estudos que identifiquem conhecimento, crenças, atitudes e prática de automedicação entre os estudantes universitários. Para tal finalidade, é necessário a utilização de instrumentos de medida validados que mensurem estes construtos. O questionário KAP (*Knowledge, Attitudes and Practices*) [10] pode mensurar estas variáveis, identificar necessidades e barreiras relacionados a um determinado problema, além de implementar intervenções específicas e efetivas que impactarão na saúde futura, bem como para aqueles que serão profissionais da saúde, melhorar a competência para o cuidado de pessoas no que diz respeito a este assunto. Assim, o objetivo deste estudo foi construir e validar um instrumento para investigar os conhecimentos, atitudes e práticas de estudantes universitários brasileiros sobre a automedicação.

MÉTODO

Estudo metodológico, realizado entre fevereiro de 2020 e maio de 2021, abrangendo três etapas: I. Construção do instrumento do tipo inquérito KAP (conhecimento, atitudes e práticas), II. Validação de conteúdo com comitê de juízes e III. Pré-teste com avaliação da compreensão do instrumento pelo público alvo [11].

Etapas I. Construção do instrumento do tipo inquérito KAP

A construção do instrumento embasou-se em uma revisão de escopo apoiada no método do Joanna Briggs Institute (JBI) [12], com protocolo de pesquisa registrado na *Open Science Framework* (<https://osf.io/2ksny/>). A coleta de dados para esta etapa deu-se entre setembro de 2020 e fevereiro de 2021 nas bases de dados e bibliotecas *Medical Literature Analysis and Retrieval System Online* (Medline-via Pubmed), Literatura Latino-Americana e do Caribe em Ciências da Saúde (Lilacs), Scopus, *Cochrane Central*, *Web of Science*, Portal de Teses e Dissertações da Capes, *DART-Europe E-The-*

ses Portal, Electronic Theses Online Service (EThOS), Repositório Científico de Acesso Aberto de Portugal (RCAAP) e *National ETD Portal*, com a seguinte pergunta norteadora: ¿Quais são as evidências científicas disponíveis para identificar os fatores determinantes da automedicação em estudantes universitários?

Os descritores utilizados foram: (*Students OR “Students, Nursing” OR “Students, Health Occupations” OR “Students, Pharmacy” OR “Students, Medical” OR “Students, Dental” OR “Students, Public Health” OR “Student Health Services”*) AND (*“SelfMedication”*). Foram incluídos estudos publicados nos idiomas português, inglês e espanhol, disponíveis na íntegra de forma gratuita que versassem sobre a temática. Estudos que não contemplaram a pergunta norteadora e que não tinham como objeto de pesquisa a automedicação por estudantes universitários foram excluídos.

Após essa etapa, o instrumento do tipo Inquérito KAP intitulou-se “Conhecimento, atitude e práticas da automedicação em estudantes universitários” sendo composto por três seções, com 38 itens: A. Caracterização sociodemográfica, acadêmica e de saúde (13 itens); B. Conhecimentos e práticas da automedicação (3 itens) e C. Referente às crenças e atitudes (22 itens).

A medida da prática da automedicação pode ser obtida por meio do recordatório do comportamento considerando o período dos últimos 15 dias (item 1, seção B). O conhecimento pode ser considerado satisfatório se o estudante registrar corretamente as informações sobre dose, via de administração e horários dos medicamentos utilizados (item 3, seção B), e não assinalar se tinha alguma dúvida quanto à indicação, contraindicação, à dosagem/administração, à duração máxima de uso, aos efeitos colaterais, às interações medicamentosas, às precauções ou outras dúvidas. Para avaliar esse conhecimento foram consideradas como referência as informações do banco de dados *Drugs.com* (acesso aberto) [13]. A atitude foi mensurada a partir de uma escala tipo *Likert* de cinco pontos que varia de um (discordo totalmente) até cinco (concordo totalmente) e composta por 22 questões (seção C). A soma das respostas dividida pelo número de itens gera uma pontuação que pode variar entre 1 e 5; quanto maior o escore, mais favorável o estudante está para praticar a automedicação.

Etapa II. Comitê de juízes (validação de conteúdo)

Após a construção do instrumento, procedeu-se a etapa de validação de conteúdo com o comitê de juízes, que corresponde o quanto uma amostra de itens é representativa de um universo definido. Para se atingir o tamanho amostral recomendado pelo referencial metodológico proposto por Pasquali (seis 20 juízes) [11], optou-se por convidar um número maior, considerando que alguns poderiam não responder ou recusar o convite. Os critérios de inclusão foram: ser profissional da saúde e possuir, no mínimo, um

ano de experiência (assistencial ou docente/pesquisa) com a temática; e o critério de exclusão foi o preenchimento incompleto do instrumento de coleta de dados utilizado.

A busca pelos juízes ocorreu a partir do currículo *Lattes* com a inserção dos seguintes termos: automedicação; estudantes e comportamento em saúde. Após identificar e verificar se os profissionais atendiam aos critérios de inclusão estabelecidos, um *e-mail* com o convite para participar do estudo foi enviado. No total, 28 juízes foram convidados a participar do estudo; contudo 16 não responderam ao contato realizado, três recusaram-se em participar da pesquisa, dois não retornaram o contato no prazo previsto, o que resultou numa amostra de sete juízes.

Encaminharam-se por *e-mail* a carta convite com orientações sobre a pesquisa e participação, o termo de consentimento livre e esclarecido (TCLE), o instrumento de caracterização sociodemográfica (composto por idade, gênero, cidade onde trabalha, titulação, tempo de formação, ocupação atual e experiência na temática), o inquérito KAP e o guia de avaliação dos itens baseado nos princípios de elaboração de escalas psicológicas [11]. O prazo solicitado para devolutiva da avaliação foram 20 dias, com extensão para 30 dias reforçando o convite.

O comitê de juízes avaliou a clareza, a pertinência e a abrangência de cada item selecionado por meio de uma escala do tipo *Likert*, com pontuação de um a quatro [14]. Quanto à clareza (compreensão da redação dos itens), avaliou-se como: (1) Definitivamente não claro; (2) Não claro; (3) Claro; (4) Definitivamente claro. Em relação à pertinência, ou seja, o quanto os itens são relevantes e adequados para atingir o objetivo proposto, os juízes avaliaram como: (1) Definitivamente não pertinente; (2) Não pertinente; (3) Pertinente; (4) Definitivamente pertinente. Referente à abrangência, se o item compreende o significado do tema abordado, cada item foi julgado como: (1) Definitivamente não abrangente; (2) Não abrangente; (3) Abrangente; (4) Definitivamente abrangente. Além disso, foi solicitado que os juízes indicassem as revisões necessárias e os itens que deveriam ser alterados, incluídos ou excluídos.

Etapas III. Pré-teste (Interrogatório cognitivo)

Após construção da versão final do instrumento, realizou-se o pré-teste ou interrogatório cognitivo (*cognitive debriefing*) [15] para avaliar a compreensão do público alvo. Foram selecionados 30 [16] estudantes universitários, com idade maior ou igual a 18 anos e matriculados nos cursos de bioquímica, enfermagem, farmácia ou medicina de uma universidade pública na região Centro Oeste de Minas Gerais selecionados por amostragem “bola de neve”.

Os estudantes foram convidados a participar do estudo por meio de contato eletrônico e, mediante aceite e leitura/assinatura do TCLE, o inquérito KAP foi autorrespondido;

os estudantes também poderiam opinar sobre dificuldades e dúvidas encontradas ao respondê-lo e sugerir mudanças na versão final do instrumento. O instrumento pode ser acessado na íntegra a partir do seguinte *link*: https://drive.google.com/file/d/1na-1toDEBYvLcXWajQmKhY1IQc_1zXJU/view?usp=share_link

Análise dos dados

Os dados foram analisados pelo *software Statistical Package for the Social Science* (SPSS) versão 21. A partir do índice de validação de conteúdo (IVC), calculou-se o *Item-Level Content Validity Index* (I-CVI), que corresponde à concordância dos juízes para cada item do instrumento, e o IVC geral. O I-CVI foi obtido com base no número de especialistas que atribuíram notas 3 ou 4, divididos pelo número total de especialistas. A proporção mínima de concordância para que o item fosse considerado válido foi de 80% [14].

Em todo o instrumento, a aceitabilidade foi avaliada de acordo com a porcentagem de itens não respondidos e a proporção de estudantes que responderam todos os itens. Na seção C, o coeficiente alfa de *Cronbach* (α) foi utilizado para mensurar a consistência interna dos itens, considerada uma estimativa da confiabilidade. Valores de $\alpha > 0,70$ foram considerados satisfatórios [17].

Aspectos éticos

O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de São João del Rei-CCO (parecer nº 4.752.214).

RESULTADOS

Na primeira etapa, referente a construção do instrumento, realizou-se revisão de escopo, encontrando-se 1315 publicações; destas, 181 foram avaliadas para sustentar a determinação dos itens. Em seguida, na segunda etapa, na validação do conteúdo, o instrumento foi avaliado por 7 juízes com média de idade de 38,4 anos ($\pm 5,9$), sendo quatro (57,1%) enfermeiros e três farmacêuticos (42,9%); o tempo médio de formação foi de 15,7 ($\pm 6,8$) anos. Quanto à titulação, todos (100,0%) eram doutores com publicação envolvendo a temática. Em relação à ocupação atual, seis (85,7%) eram docentes universitários e um (14,3%) atuava na assistência hospitalar.

A avaliação das dimensões do instrumento apresentou IVC total de 0,97, sendo a clareza de 0,96, a pertinência de 0,98 e a abrangência de 0,98. Todos os itens obtiveram IVC superior a 0,8 (tabela 1). Os juízes sugeriram pequenas modificações no instrumento, com o intuito, principalmente, de melhorar a compreensão acerca de cada item.

Tabela 1. Avaliação do comitê de juízes sobre as dimensões do instrumento.

Dimensões		Índice de validade de conteúdo		
		Clareza	Pertinência	Abrangência
Nome do instrumento	-	1,00	1,00	1,00
Texto inicial	-	0,85	1,00	1,00
Sociodemográficas	Item 1 A	1,00	1,00	1,00
	Item 2 A	1,00	1,00	1,00
	Item 3 A	1,00	1,00	1,00
	Item 4 A	1,00	1,00	1,00
	Item 5 A	1,00	1,00	1,00
	Item 6 A	0,85	1,00	1,00
	Item 7 A	0,85	1,00	1,00
	Item 8 A	1,00	1,00	1,00
IES†	Item 9 A	1,00	1,00	1,00
	Item 10 A	1,00	1,00	1,00
	Item 11 A	1,00	1,00	1,00
Saúde‡	Item 12 A	1,00	1,00	1,00
	Item 13 A	1,00	1,00	1,00
Conhecimento e prática da automedicação	Texto inicial-seção B	1,00	1,00	1,00
	Item 1 B	0,85	0,85	0,85
	Item 2 B	0,85	0,85	0,85
	Item 3 B	0,85	0,85	0,85
Crenças e atitudes sobre automedicação	Texto inicial-seção C	1,00	1,00	1,00
	Item 1 C	1,00	1,00	1,00
	Item 2 C	0,85	0,85	0,85
	Item 3 C	1,00	1,00	1,00
	Item 4 C	1,00	1,00	1,00
	Item 5 C	1,00	1,00	1,00
	Item 6 C	1,00	1,00	1,00
	Item 7 C	1,00	1,00	1,00
	Item 8 C	1,00	1,00	1,00
	Item 9 C	1,00	1,00	1,00
	Item 10 C	1,00	1,00	1,00
	Item 11 C	1,00	1,00	1,00
	Item 12 C	1,00	1,00	1,00
	Item 13 C	1,00	1,00	1,00
	Item 14 C	1,00	1,00	1,00
	Item 15 C	0,85	0,85	0,85
	Item 16 C	1,00	1,00	1,00
	Item 17 C	1,00	1,00	1,00
	Item 18 C	1,00	1,00	1,00
	Item 19 C	0,85	1,00	1,00
	Item 20 C	1,00	1,00	1,00
	Item 21 C	1,00	1,00	1,00
	Item 22 C	1,00	1,00	1,00
Avaliação geral	-	0,96	0,98	0,98
total	-	0,97		

†Vinculação à instituição de ensino superior (IES). ‡Acesso e autoavaliação de saúde.

Na terceira etapa, o interrogatório cognitivo foi conduzido com 30 estudantes universitários com média de idade de 23,8 anos ($\pm 2,6$) e na maioria do sexo feminino (70,0%, $n=21$). Em relação aos cursos, 10 estudantes (33,3%) cursavam enfermagem entre o segundo e oitavo período, nove (30,0%) farmácia entre o quarto e sétimo período, sete (23,3%) bioquímica entre o terceiro e sétimo período e quatro (13,3%) medicina entre o primeiro e terceiro período. O total dos participantes responderam a todos os itens; não foram relatadas dificuldades referentes à compreensão dos itens e nem sugeridas alterações. A confiabilidade da seção C foi estimada por meio do coeficiente alfa de *Cronbach* (tabela 2). Todos os itens do instrumento foram satisfatórios com $\alpha > 0,70$.

Tabela 2. Análise descritiva da confiabilidade do instrumento no pré-teste.

	Média (DP)	Correlação item total	Alfa de <i>Cronbach</i> (se item excluído)
Item 1 C	1,93 (1,14)	0,22	0,88
Item 2 C	3,36 (1,24)	0,41	0,87
Item 3 C	3,23 (1,40)	0,25	0,88
Item 4 C	2,43 (1,30)	0,58	0,87
Item 5 C	3,23 (1,35)	0,55	0,87
Item 6 C	2,81 (1,46)	0,42	0,87
Item 7 C	1,66 (0,92)	0,17	0,88
Item 8 C	2,51 (1,30)	0,60	0,86
Item 9 C	1,67 (1,18)	0,41	0,87
Item 10 C	3,00 (1,33)	0,44	0,87
Item 11 C	3,13 (1,13)	0,56	0,86
Item 12 C	3,20 (1,24)	0,48	0,87
Item 13 C	2,64 (1,44)	0,49	0,87
Item 14 C	2,60 (1,52)	0,51	0,87
Item 15 C	2,13 (1,15)	0,64	0,87
Item 16 C	2,23 (1,35)	0,67	0,86
Item 17 C	2,43 (1,40)	0,44	0,87
Item 18 C	2,73 (1,22)	0,19	0,88
Item 19 C	4,05 (1,40)	0,68	0,87
Item 20 C	3,27 (1,01)	0,63	0,87
Item 21 C	2,63 (1,49)	0,45	0,87
Item 22 C	3,74 (1,02)	0,43	0,87
Escore total	2,74 (1,63)	-	0,88

DP: desvio padrão.

DISCUSSÃO DOS RESULTADOS

Este estudo traz inovações com a proposta de um instrumento do tipo Inquérito KAP para a análise da automedicação considerando-se diferentes elementos de interesse da saúde pública sobre esta prática, a saber: a) caracterização socioeconômica, acadêmica e de saúde; b) caracterização completa dos conhecimentos e práticas da automedicação e c) crenças e atitudes associadas a este comportamento. Na seção A do instrumento é disponibilizada a caracterização do respondente; é relevante identificar estas informações porque a literacia em saúde pode ser distinta conforme sexo, idade, condições socioeconômicas e escolaridade [18-20]. Conhecer estas variáveis pode ser fundamental no planejamento de estratégias educativas e comportamentais para o uso racional do medicamento.

Outro aspecto abordado no instrumento, refere-se ao autocuidado, conhecimentos e práticas de automedicação em estudantes universitários [9, 21, 22]. Os principais fatores que podem contribuir para o uso irracional de medicamentos envolvem auto-diagnóstico incorreto, escolha equivocada da terapêutica, falha em reconhecer riscos farmacológicos especiais por outras comorbidades ou características do paciente, risco de ocorrência de efeitos adversos raros (porém graves) e falha em reconhecer ou auto-diagnosticar contraindicações, interações medicamentosas, alertas de segurança ou precauções de uso [23-25].

Estudo internacional [26] conduzido no Irã, reportou que apenas 12,9% dos estudantes foram capazes de identificar corretamente três medicamentos de venda livre ou *over-the-counter* (OTC) sendo que 47,7% conheciam um ou nenhum medicamento deste tipo. O estudo também apresentou que estudantes do curso de farmácia tiveram maior conhecimento sobre o assunto quando comparados a estudantes de medicina ($p < 0,001$); além disso, aqueles alunos que cursavam períodos mais adiantados também tinham um conhecimento significativamente maior do que o primeiro ano de estudo ($p = 0,002$). Neste sentido, o referido instrumento apresenta no item 3B informações sobre o conhecimento do estudante universitário em relação ao medicamento em uso e à condição que proporcionou a automedicação. Isto oportuniza análise quantitativa da correção do emprego da automedicação, bem como da identificação de quais são as principais lacunas para a educação e formação de estudantes que a praticam.

Os comportamentos passados tendem a influenciar as crenças, atitudes e decisões futuras mesmo que a pessoa não consiga identificar tais determinantes [21, 27]. A influência é especialmente relevante quando as experiências pregressas foram bem-sucedidas ou quando a pessoa não tem condições de avaliar adequadamente prós e contras do comportamento naquele momento [27]. Na seção C. Atitude, as questões 2C a 4C,

7C, 8C, 12C e 20C buscaram identificar a influência de situações passadas na decisão de automedicação dos estudantes.

A automedicação entre estudantes universitários é um fenômeno generalizado [21, 22, 28] e uma oportunidade de consolidação de bons padrões de conduta haja visto ambiente propício de formação no meio universitário [9, 28]. Neste sentido, o instrumento inquérito KAP poderá ser utilizado como uma ferramenta de rastreamento da prevalência do comportamento de automedicação nas universidades seguida da avaliação da adequação desta prática (seção B. Conhecimento e prática da automedicação) bem como da investigação das crenças e atitudes (seção C) que podem interferir para esta prática.

Elaborar e validar instrumentos antes de aplicá-los permite ao pesquisador, durante a coleta dos dados, obter resultados mais confiáveis e precisos. Neste estudo, a entrevista cognitiva com o público-alvo apresentou desfecho favorável, uma vez que, ao longo da aplicação do instrumento para validação semântica, não foram identificadas dificuldades no entendimento de termos ou afins. Adicionada à validação do conteúdo por juízes experts, evidencia-se a possibilidade de utilização desta tecnologia na prática clínica, a fim de contribuir para o aprimoramento da realização de intervenções educativas direcionadas para estudantes universitários. Destaca-se também que o período recordatório considerado no instrumento (últimos 15 dias) permitiu uma maior precisão da medida por se tratar de um comportamento complexo que envolve o resgate na memória.

Assim, a utilização de instrumento validado, representa relevante tecnologia em saúde; poderá auxiliar professores, coordenadores de curso e equipes pedagógicas na análise do nível de conhecimento, atitudes e práticas de estudantes acerca da automedicação, identificando precocemente as lacunas e tornando possível a realização de intervenções educativas direcionadas, a fim de intervir, em tempo hábil, e evitar o surgimento e/ou riscos que o uso irracional de medicamentos pode acarretar.

CONCLUSÕES

A versão final do instrumento Inquérito KAP sobre automedicação revelou-se ser uma ferramenta válida e adequada para avaliar o conhecimento, a prática, as crenças e as atitudes sobre a automedicação em estudantes universitários. Vislumbra-se que este instrumento poderá fornecer informações importantes a esta população, de modo a produzir questionamentos acerca deste comportamento complexo, bem como realização da automedicação de modo seguro.

Embora este estudo forneça um instrumento validado, apresenta limitações. Em primeiro lugar, ter incluído apenas estudantes da área da saúde no pré-teste do instrumento; contudo no campus onde o estudo foi desenvolvido há apenas o oferecimento de cursos daquela área. Recomenda-se que durante a aplicação do instrumento outros estudantes sejam incluídos para que seja possível refinar o conteúdo bem como investigar o comportamento de automedicação e seus determinantes em diferentes áreas do conhecimento.

CONFLITOS DE INTERESSE

Os autores não relatam nenhum conflito de interesse.

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COMO CITAR ESTE ARTIGO

G.H. Rabelo-César, H. Nogueira-Guimarães, J.S. Cidade-Santos, A. Carrilho-Menezes, C. Sanches, A.C. de Melo, D. Donizetti-Trevisan, Instrumento sobre conhecimento, atitudes e práticas da automedicação em estudantes universitários, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1083-1097 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100355>

Artigo de pesquisa científica / <http://dx.doi.org/10.15446/rcciquifa.v51n3.106036>

A descrição teórica da determinação eletroquímica do fármaco perilartina, assistida pelo oxihidróxido de cobalto, emparelhado com o seu dióxido

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Recebido: 18 de junho de 2020

Revisado: 30 de janeiro de 2022

Aceto: 7 de fevereiro de 2022

RESUMO

Introdução: perilartina é um adoçante natural potente, considerado 2000 vezes mais doce que o açúcar comum. Ela pode ser alérgica para algumas pessoas. Além disso, ela se usa apenas no Japão, o que pressupõe a possibilidade de adulterar produtos japoneses sem o seu uso. Assim, o desenvolvimento de um método eficiente da sua determinação qualificativa e quantificação é realmente atual. **Metodologia:** um modelo matemático de um processo anódico foi desenvolvido e analisado mediante a teoria de estabilidade linear e análise de bifurcações. O modelo inclui os cenários mais

prováveis do andamento do processo eletroanalítico. **Resultados:** a perilartina é oxidada no meio básico, formando o poliol e o sal do “pseudoácido” correspondente, o que contribui fortemente para a força iônica da dupla camada elétrica. Isto pode ser responsável pela aparição das instabilidades oscilatória e monotônica no processo eletroanalítico. **Conclusão:** malgrado o supracitado, o compósito é um modificador eficiente para a determinação eletroquímica da perilartina no meio básico.

Palavras-chave: Perilartina, sensor eletroquímico, oxihidróxido de cobalto, corantes escurícos, estado estacionário estável.

RESUMEN

Descripción teórica de la determinación electroquímica del fármaco perilartina, asistida por el oxihidróxido de cobalto, pareado con su dióxido

Introducción: perilartina es un edulcorante natural potente, considerado 2000 veces más dulce que el azúcar común. Esta puede causar alergia a algunas personas. Además, ella solo se usa en Japón, lo que presupone la posibilidad de falsificar productos japoneses sin su uso. Así, el desarrollo de un método eficiente de su determinación cuantitativa y cualitativa es realmente actual. **Metodología:** un modelo matemático de un proceso anódico fue desarrollado y analizado mediante la teoría de estabilidad lineal e análisis de bifurcaciones. El modelo incluye los escenarios más probables del curso del proceso electroanalítico. **Resultados:** la perilartinase oxida en el medio básico, formando el poliol y la sal del “pseudoácido” correspondiente, lo que contribuye fuertemente para la fuerza iónica de la doble capa eléctrica. Esto puede ser responsable por la aparición de las inestabilidades oscilatoria y monotónica en el proceso electroanalítico. **Conclusión:** a pesar de lo mencionado, el compuesto es un modificador eficaz para la determinación electroquímica de la perilartina en el medio básico.

Palabras clave: Perilartina, sensor electroquímico, oxihidróxido de cobalto, colorantes escurícos, estado estacionario estable.

SUMMARY

The theoretical description for perillartine electrochemical determination, assisted by cobalt oxyhydroxide in pair with its dioxide

Introduction: Perillartine is a strong natural sweetener, considered 2000 as sweet as a common sugar. It may be allergic to some people. Also, it is used only in Japan, which may presuppose the possibility of falsify Japanese products without its use. Thus the development of an efficient method of its quantitative and qualitative determination is really actual. **Methodology:** A mathematical model of an anodic process has been developed and analyzed by means of linear stability theory and bifurcation analysis. The model includes the most probable scenarios of the course of the electroanalytical process. **Results:** Perillartine is oxidized in basic media, yielding the polyol and the correspondent pseudoacid salt, which strongly contributes to the double electric layer ionic force. This may be responsible for the oscillatory and monotonic instabilities appearance in the electroanalytical process. **Conclusion:** Despite of the above mentioned statements, the composite is an efficient modifier for the electrochemical determination of perillartine in basic media.

Keywords: Perillartine, electrochemical sensor, cobalt oxyhydroxide, squaraine dyes, stable steady-state.

INTRODUÇÃO

Perilartina (figura 1 à esquerda, (S)-4-(prop-1-en-2-ilo)ciclohex-1-enaldoxima, M=165 g/mol, número CAS: 30950-27-7, número PubChem: 5366782), isto é anti-aldoxima de perilaldeído, é um adoçante natural, extraído das folhas da perila japonesa (planta *shiso*) [1-4]. Ela é considerada duas vezes mais doce que a sucralose, 4 vezes mais doce que a sacarina e 2000 vezes mais doce que o açúcar comum.

Além da própria perilartina, o seu derivado etérico (figura 1, à direita) também é usado, apesar de ser muito menos doce. Ele possui o dulçor bastante próximo do de aspártamo.

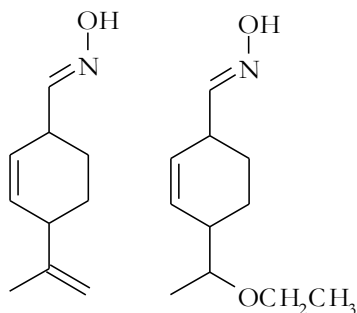


Figura 1. Perilartina e o seu éter.

A oxima é biodegradável e bioacessível, ao contrário dos adoçantes sintéticos como aspártamo, sacarina e sucralose. Outrossim, ela não é considerada tóxica ou perigosa para o ambiente.

No Japão a perilartina é amplamente usada na indústria farmacêutica como corretor de sabor e componente de alimentação especial. Também, ela é componente da receita original do molho de soja (*shoyu*).

Posto que a perilartina se considere segura, ela pode ser alérgica para pessoas alérgicas para produtos da perila japonesa [5, 6]. Outrossim, a hidroxilamina e seus derivados tóxicos podem aparecer durante o seu metabolismo em algumas pessoas. Vale a pena mencionar que a perilartina e derivados muito pouco se usam pela indústria alimentar e farmacêutica fora do Japão, o que pode ser usado para verificar se o produto alegadamente produzido no Japão é realmente japonês. Destarte, o desenvolvimento de um método rápido e eficiente da detecção da perilartina é realmente natural, e os métodos eletroanalíticos podem providenciar-lhe bom serviço [7-15].

Por ora, nenhum método eletroanalítico para a detecção da perilartina há sido desenvolvido. Entretanto, aldoxima pode ser eletroquimicamente ativa, podendo ser tanto reduzida como oxidada. Em ambas as opções, é preferível usar elétrodos quimicamente modificados, que diminuem a sobretensão e aumentam a sensibilidade do elétrodo e a sua afinidade ao analito [16-30].

Tanto processos catódicos como anódicos podem ser aplicados à perilartina. No último caso, é preferível usar oxidantes fortes e específicos, haja vista que o grupo funcional da oxima é um aceitador de elétrons, o que aumenta o potencial da oxidação do analito e sobrevoltagem.

Um desses oxidantes é o dióxido de cobalto. É um oxidante bastante forte, já que o estado de oxidação +4 é bastante instável para cobalto. Assim, vista a instabilidade do dióxido de cobalto, é preferível usar como modificador de eléctrodo um composto bem mais estável, o oxihidróxido de cobalto trivalente, já amplamente usado em sistemas eletrocatalíticos e eletroanalíticos [31-38]. Na etapa electroquímica, o oxihidróxido é oxidado, rendendo o dióxido de cobalto (1):



Depois, o dióxido oxida a perilartina, conforme o exposto abaixo.

Para aumentar o desempenho funcional do oxihidróxido e do dióxido de cobalto, é preferível usá-lo como componente de um compósito híbrido, cuja fase orgânica seria um polímero condutor [39-42] ou composto afim (exemplo, corante esquárico) [43-46]. A fase orgânica desempenha funções de matriz estabilizadora para fase inorgânica, além da função de mediação da transferência de elétron, providenciando a aparição e interpretação do sinal analítico.

No entretanto, o seu uso poderia acarretar uma influência comportamental, nociva à estabilidade do sistema e à sua eficiência eletroanalítica [47-53]. Destarte, o uso prático deste sistema eletroanalítico não pode ser realizado sem uma descrição teórica prévia do comportamento do sistema, o que se faz neste trabalho.

Assim, neste trabalho, pela primeira vez, faz-se uma análise comportamental do sistema eletroanalítico da detecção electroquímica da perilartina sobre o oxihidróxido de cobalto, emparelhado com o dióxido de cobalto (IV), estabilizado pelo corante esquaraínico. A análise comportamental do sistema eletroanalítico faz-se mediante o desenvolvimento e a análise do modelo correspondente. Com isto também se faz a comparação do comportamento do sistema eletroanalítico com o dos semelhantes [54-56].

O SISTEMA E O SEU MODELO

Neste caso, a oxidação da perilartina se realiza no meio ligeira ou moderadamente básico. Nele, o oxihidróxido de cobalto é oxidado conforme a reação (1). Já a perilartina pode ser oxidada por dois mecanismos:

- Os fragmentos alcénicos são oxidados conforme o mecanismo da reação de Wagner, rendendo os polióis correspondentes. O fragmento glicólico, por sua vez, é imediatamente oxidado, rendendo uma dicetona;
- O grupo funcional da oxima é oxidado para render o íon do “pseudoácido”. O “pseudoácido” é uma das formas do nitrocomposto, existente no meio básico (figura 2).

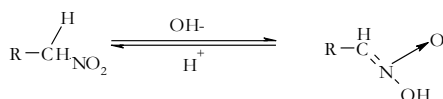


Figura 2. A formação de um “pseudoácido” (memorando).

Como o pseudoácido é ionizado, a formação do seu íon aumenta a condutividade e a força iônica da dupla camada elétrica (DCE), o que pode contribuir para a formação da positiva conexão de retorno no sistema eletroanalítico.

Esquematicamente, o processo eletroanalítico pode ser exposto na figura 3:

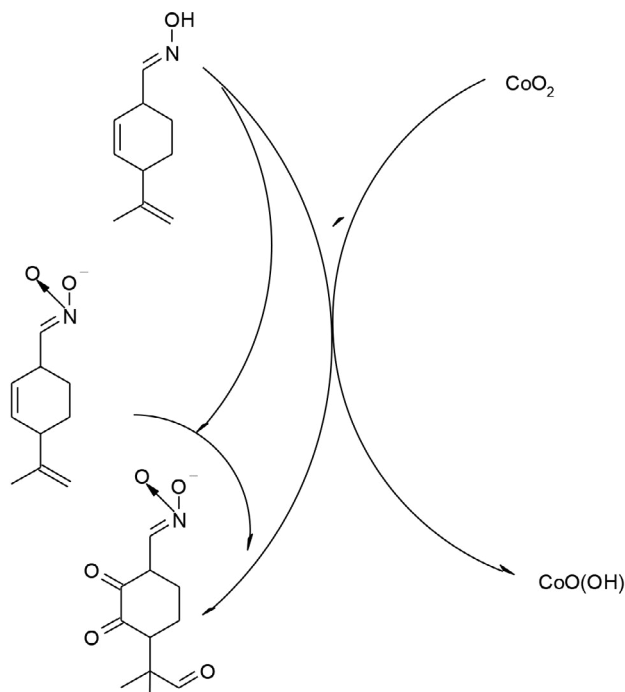


Figura 3. O desempenho do oxihidróxido e do dióxido de cobalto com a perilartina.

Havendo vista o supracitado, para descrever o comportamento do sistema eletroanalítico em questão, usar-se-á o conjunto de três equações diferenciais de balanço com as variáveis:

p : concentração da perilartina na camada pré-superficial;

p^* : concentração do íon do pseudoácido na camada pré-superficial;

c : o grau do recobrimento da superfície pelo oxihidróxido de cobalto.

Para simplificar a modelagem, supomos que o reator esteja sob agitação intensa (destarte, podemos menosprezar o fluxo de convecção), que o eletrólito de suporte esteja em excesso (destarte, podemos menosprezar o fluxo de migração). Outrossim, supomos que o perfil concentracional das substâncias na camada pré-superficial seja lineal, e a sua espessura, constante, igual a δ .

Diante do exposto, o comportamento do sistema será descrito por um conjunto de equações diferenciais (2), conforme:

$$\begin{cases} \frac{dp}{dt} = \frac{2}{\delta} \left(\frac{p}{\delta} (p_0 - p) - r_{21} - r_{22} \right) \\ \frac{dp^*}{dt} = \frac{2}{\delta} (r_{21} - r_{23}) \\ \frac{dc}{dt} = \frac{1}{c} (r_{21} + r_{22} + r_{23} - r_1) \end{cases} \quad (2)$$

Aqui, p é coeficiente de difusão do edulcorante, p_0 é a sua concentração no interior da solução, c é a concentração superficial máxima do oxihidróxido de cobalto, e os parâmetros r são correspondentes velocidades de reações, calculadas conforme:

$$r_1 = k_1 s \exp\left(\frac{F\varphi_0}{RT}\right) \quad (3)$$

$$r_{21} = k_{21} p (1 - s)^2 \exp(-\alpha p) \quad (4)$$

$$r_{23} = k_{23} p^* (1 - s)^5 \exp(-\beta p^*) \quad (5)$$

$$r_{22} = k_{22} p^* (1 - s)^5 \exp(-\beta p^*) \quad (6)$$

Sendo os parâmetros k constantes das velocidades das reações, α e β descrevem as influências da formação dos compostos iônicos na dupla camada eléctrica, F é o número de Faraday, ϕ_0 é salto do potencial, relativo ao potencial da carga zero, R é a constante universal de gases e T é a temperatura absoluta.

Como a formação do íon do pseudoácido contribui para a condutividade e a força iónica da dupla camada eléctrica, isso gera a oposição entre aumento da condutividade e capacitância da capa e diminuição da atividade da forma inicial da perilartina. Isto pode ser responsável pelo comportamento oscilatório. Apesar disto, o compósito $\text{CoO}(\text{OH})$ (corante esquárico) pode ser um modificador eficiente do eléctrodo, conforme exposto abaixo.

RESULTADOS E DISCUSSÃO

Para investigar o comportamento do sistema com a determinação da perilartina, assistida pelo compósito $\text{CoO}(\text{OH})$ (corante esquárico) analisamos o conjunto de equações diferenciais (2) mediante a teoria de estabilidade linear. Os elementos estacionários da matriz Jacobiana podem ser representados como:

$$\begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} \quad (7),$$

em que

$$a_{11} = \frac{2}{\delta} \left(-\frac{p}{\delta} - k_{21}(1-s)^2 \exp(-ap) + \alpha k_{21}p(1-s)^2 \exp(-ap) - k_{22}(1-s)^7 \exp(-ap) + \alpha k_{22}p(1-s)^7 \exp(-ap) \right) \quad (8)$$

$$a_{12} = 0 \quad (9)$$

$$a_{13} = \frac{2}{\delta} (2k_{21}p(1-s) \exp(-ap) + 7k_{22}p(1-s)^6 \exp(-ap)) \quad (10)$$

$$a_{21} = \frac{2}{\delta} (k_{21}(1-s)^2 \exp(-ap) - \alpha k_{21}p(1-s)^2 \exp(-ap)) \quad (11)$$

$$a_{22} = \frac{2}{\delta} (-k_{23}(1-s)^5 \exp(-\beta p *) + \beta k_{23} p * (1-s)^5 \exp(-\beta p *)) \quad (12)$$

$$a_{23} = \frac{2}{\delta} (-2k_{21} p (1-s) \exp(-\alpha p) + 5k_{23} p * (1-s)^4 \exp(-\beta p *)) \quad (13)$$

$$a_{31} = \frac{1}{s} (k_{21}(1-s)^2 \exp(-\alpha p) - \alpha k_{21} p (1-s)^2 \exp(-\alpha p) + k_{22}(1-s)^7 \exp(-\alpha p) - \alpha k_{22} p (1-s)^7 \exp(-\alpha p)) \quad (14)$$

$$a_{32} = \frac{1}{s} (k_{23}(1-s)^5 \exp(-\beta p *) - \beta k_{23} p * (1-s)^5 \exp(-\beta p *) - m k_1 s \exp(\frac{F\varphi_0}{RT})) \quad (15)$$

$$a_{33} = \frac{1}{s} (-2k_{21} p (1-s) \exp(-\alpha p) - 7 k_{22} p (1-s)^6 \exp(-\alpha p) - 5k_{23} p * (1-s)^4 \exp(-\beta p *) - k_1 \exp(\frac{F\varphi_0}{RT}) - j k_1 s \exp(\frac{F\varphi_0}{RT})) \quad (16)$$

Para investigar a estabilidade do estado estacionário, aplicamos ao conjunto de equações diferenciais (2) o critério de Routh-Hurwitz. Evitamos as operações com expressões grandes, introduzindo as novas variáveis e expondo o Jacobiano conforme:

$$\frac{4}{\delta^2 s} \begin{vmatrix} -\sigma - \Sigma - T & 0 & \Phi \\ \Sigma & -P & H - \Phi \\ \Sigma + T & P - \omega & -H - \Phi - \xi \end{vmatrix} \quad (17)$$

Abrindo os parênteses e aplicando o requisito $\text{Det } J < 0$, saliente do critério, nós obtemos a condição de estabilidade do estado estacionário, apresentada como (18):

$$-\sigma(2P\Phi + P\xi + \omega H - \omega\Phi) - \Sigma(2P\Phi + P\xi + \omega H) - T(P\Phi + P\xi + \omega H - \omega\Phi) < 0 \quad (18)$$

Este sistema é bastante parecido com os outros sistemas, envolvendo o cobalto tetravalente e com a determinação da hidrazina sobre o cobre trivalente simultaneamente [56]. Como anteriormente, o processo eletroanalítico é controlado tanto pela difusão como pela cinética do processo. Entretanto, o fator cinético tende a ser mais importante, haja vista a formação do íon do pseudoácido, cujo sal é um eletrólito mais conductor que a própria perilartina.

Este limite é descrito pela *instabilidade monotônica*, descrevendo a margem entre os estados estacionários estáveis e instáveis. A sua realização é condicionada ao requisito de $\text{Det } J=0$, ou:

O comportamento oscilatório neste sistema é passível de observar, já que os elementos da diagonal principal do Jacobiano possuem elementos, que podem ter valores positivos. A sua positividade descreve a já mencionada positiva conexão de retorno.

Neste caso, o comportamento oscilatório pode ser causado pelos dois fatores. Além do elemento $-jk_1 \exp\left(\frac{F\varphi_0}{RT}\right) > 0$ se $j < 0$, descrevendo os câmbios na condutividade da DCE e da superfície durante a reação eletroquímica, os elementos $\alpha K_{21}P(1-S)^2 EX - P(-\alpha P) > 0$ e $\alpha K_{22}P(1-S)^2 \exp(-\alpha P) > 0$ if $\alpha > 0$ e $\beta k_{23}P^*(1-s)^5 \exp(-\beta P^*) > 0$ e $\beta \theta$ esse, também descrevem o comportamento oscilatório. Este comportamento, assim, será causado pelas influências da reação química, cujo produto é o íon do pseudoácido, que, posteriormente, também sofre alterações, na força iônica, estrutura e actividade de cada íon na dupla camada. Isto é responsável pelos câmbios cíclicos na condutividade e capacitância da camada.

As oscilações são semelhantes às já descritas experimentalmente [49-53] e teoricamente [54-56].

O mesmo modelo pode também descrever o processo eletroanalítico análogo, mas que emprega o polímero condutor o material de carbono em vez do corante. Outros oxidantes, capaz de assistir a detecção eletroquímica da perilartina são os compostos de bismuto no estado de oxidação +5, cério tetravalente, os ferratos. O seu comportamento com a perilartina pode ser efetivamente descrito por este modelo.

CONCLUSÕES

A análise teórica do processo eletroanalítico da detecção da perilartina, assistida pelo par $\text{CoO}(\text{OH}) - \text{CoO}_2$, estabilizado por um corante esquaraínico, deixou concluir que:

- Se trata de um processo eletroanalítico eficiente, em que o composto de cobalto funciona como substância ativa, e o corante desempenha o papel de mediador;
- O processo eletroanalítico é controlado pelo tanto pela difusão do analito, como pelos factores cinéticos;
- A realização do comportamento oscilatório é mais provável que no caso mais simples e comum, haja vista os câmbios cíclicos da força iónica da dupla camada elétrica, causados pela formação do íon do “pseudoácido”.

CONFLITOS DE INTERESSE

Os autores não relatam nenhum conflito de interesse.

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COMO CITAR ESTE ARTIGO

V.V. Tkach, M.V. Kushnir, S.C. de Oliveira, Z.O. Kormosh, O.V. Luganska, L. Vaz dos Reis, Y.G. Ivanushko, P.I. Yagodynets', G.M. Pochenchuk, A descrição teórica da determinação eletroquímica do fármaco perilartina, assistida pelo oxihidróxido de cobalto, emparelhado com o seu dióxido, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1098-1113 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.106036>

Bioassay-guided isolation of α -glucosidase inhibitors from *Byrsonima garcibarrigae* Cuatrec.

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Received: July 4, 2021

Corrected: February 2, 2022

Accepted: February 10, 2022

SUMMARY

Introduction: *Byrsonima garcibarrigae* is an endemic tree of Amazonas state, Brazil, with pharmacological and chemical knowledge poorly understood. **Aim:** To investigate the antidiabetic potential of the *B. garcibarrigae* stem bark. **Methods:** The stem bark was sequentially extracted by maceration with hexane (EHBG), ethyl acetate (EABG), and methanol (EMBG). The antioxidant capacity, α -glucosidase inhibitory potentials and anti-glycation capacities were evaluated. A bio-guided fractionation gave compounds that were characterized by MS and NMR. **Results:** 8 compounds were identified by HPLC-MS. EMBG showed the highest α -glucosidase inhibitory activity (1.09 ± 0.32 $\mu\text{g/mL}$), antioxidant activity (9.2 ± 0.23 $\mu\text{g/mL}$) and phenolic compounds content ($61.43 \pm 0.50\%$), thus was fractionated producing hexane (FHX), chloroform (FCL) and hydromethanolic (FHM) fractions. After additional anti- α -glucosidase assays, FHM (1.02 ± 0.49 $\mu\text{g/mL}$) was fractionated giving quercitrin and epicatechin. The anti-glycation assay showed that EMBG, FHM and quercitrin presented higher activities in comparison to the positive control, amino-guanidine. **Conclusions:** *B. garcibarrigae* displayed antidiabetic potential since inhibited α -glucosidase, as well as presented expressive antioxidant and anti-glycation activities were recorded.

Keywords: antidiabetic, oxidative stress, *Byrsonima garcibarrigae*.

RESUMEN

Aislamiento guiado por bioensayo de inhibidores de α -glucosidasa de *Byrsonima garcibarrigae* Cuatrec

Introducción: *Byrsonima garcibarrigae* es un árbol endémico del estado de Amazonas, Brasil, con poco conocimiento farmacológico y químico. **Objetivo:** investigar el potencial antidiabético de la corteza del tallo de *B. garcibarrigae*. **Métodos:** la corteza del tallo se extrajo secuencialmente mediante maceración con hexano (EHBG), acetato de etilo (EABG) y metanol (EMBG). Se evaluó la capacidad antioxidante, los potenciales inhibidores de la α -glucosidasa y las capacidades anti-glicación. Un fraccionamiento bioguiado dio compuestos que se caracterizaron por MS y NMR. **Resultados:** se identificaron 8 compuestos mediante HPLC-MS. EMBG mostró la mayor actividad inhibidora de α -glucosidasa ($1,09 \pm 0,32 \mu\text{g/mL}$), actividad antioxidante ($9,2 \pm 0,23 \mu\text{g/mL}$) y contenido de compuestos fenólicos ($61,43 \pm 0,50\%$), por lo que se fraccionó produciendo hexano (FHX), cloroformo (FCL) e hidrometanólicas (FHM). Después de ensayos adicionales de anti- α -glucosidasa, se fraccionó FHM ($1,02 \pm 0,49 \mu\text{g/mL}$) dando quercitrina y epicatequina. El ensayo antiglicación mostró que EMBG, FHM y quercitrina presentaron actividades más altas en comparación con el control positivo, aminoguanidina. **Conclusiones:** *B. garcibarrigae* mostró potencial antidiabético ya que se registró una inhibición de la α -glucosidasa, así como también presentó actividades expresivas antioxidantes y antiglicación.

Palabras clave: antidiabético, estrés oxidativo, *Byrsonima garcibarrigae*.

RESUMO

Isolamento guiado por bioensaio de inibidores de α -glicosidase de *Byrsonima garcibarrigae* Cuatrec

Introdução: *Byrsonima garcibarrigae* é uma árvore endêmica do estado do Amazonas, Brasil, com pouco conhecimento farmacológico e químico. **Objetivo:** investigar o potencial antidiabético da casca do caule de *B. garcibarrigae*. **Métodos:** a casca do caule foi extraída sequencialmente por maceração com hexano (EHBG), acetato de etila (EABG) e metanol (EMBG). A capacidade antioxidante, potencial inibitório de α -glicosidase e capacidade antiglicação foram avaliadas. Um fracionamento bioguiado isolou compostos que foram caracterizados por MS

e RMN. **Resultados:** 8 compostos foram identificados por HPLC-MS. O EMBG apresentou a maior atividade inibitória de α -glicosidase ($1,09 \pm 0,32 \mu\text{g/mL}$), atividade antioxidante ($9,2 \pm 0,23 \mu\text{g/mL}$) e teor de compostos fenólicos ($61,43 \pm 0,50\%$), por isso foi fracionado produzindo hexano (FHX), clorofórmio (FCL) e hidrometanólico (FHM). Após ensaios anti- α -glicosidase adicionais, FHM ($1,02 \pm 0,49 \mu\text{g/mL}$) foi fracionado, originando a quercitrina e epicatequina. O ensaio de antiglicação mostrou que EMBG, FHM e quercitrina exibiram atividades mais altas em comparação com o controle positivo, aminoguanidina. **Conclusões:** *B. garcibarrigae* apresentou potencial antidiabético, uma vez que foi registrada inibição da α -glicosidase, bem como expressiva atividade antioxidante e antiglicação.

Palavras-chave: antidiabético, estresse oxidativo, *Byrsonima garcibarrigae*.

INTRODUCTION

Diabetes is a metabolic disorder of multiple etiologies characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or in both [1] which can induce non-enzymatic glycosylation of proteins, lipids, and nucleic acids, that lead to the formation of advanced glycation end products (AGEs) and development of chronic clinical complications, such as atherosclerosis, nephropathy, neuropathy and cataracts [2, 3].

Natural products represent an important option for the discovery and development of promising lead candidates with biological activity [4], because of the complexity of their secondary metabolism that results in a large chemical diversity [5, 6]. A promising approach in natural products research is bioassay-guided isolation and identification of lead compounds [7]. As a recent example, performed a bio-guided isolation from the ethanolic extract of *Cordia morelosana* in which allowed to obtain rosmarinic acid and methyl rosmarinate, responsible for activities observed in the extract [8].

Byrsonima is one of the major general of the family Malpighiaceae, including about 150 species with different biological properties reported, such as spasmogenic, immunostimulatory, anti-inflammatory, anti-hemorrhagic, anti-hyperlipidemic, antiulcer and antidiarrheal [9-11].

Byrsonima garcibarrigae Cuatrec., a member of this genus, is an endemic arboreal species in northern Brazil, one of the thousands of species in the Amazon region with pharmacological potential still under investigation. In a project of this research group, five species of *Byrsonima* genus were preliminarily screened against α -glucosidase, and *B. garcibarrigae* showed promising results. Thus, aiming to identify bioactive compounds,

this study performed a phytochemical study bioassay-guided by α -glucosidase inhibition, additionally evaluating the antioxidant, anti-glycation activities of extracts and fractions from the stem barks of *B. garcibarrigae*.

METHODOLOGY

Plant material

The collection of plant samples was authorized by the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) of the Brazilian Ministry of the Environment, under the registration 41553-1. Plant material was collected at the Adolpho Ducke Manaus Forestry Reserve (Amazonas) and taxonomically identified, where a voucher specimen was deposited under number 691 by the Herbarium of the National Institute of Amazonia Research (INPA).

Preparation of the plant extract

Stem bark samples of *B. garcibarrigae* were dried at room temperature for 4 days followed by a period of 24 hours at 40 °C in an air circulating stove, then pulverized with a mechanical grinder. The extracts were obtained by maceration using 822 g of stem barks and portions of 700 mL of hexane (total of 6 L), ethyl acetate (total of 4 L) and methanol (total of 4 L), in cycles of 15 min, in an ultrasound bath (Ultrasonic Cleaner Unique®), sequentially named EHBG (2.88 g, 0.35%), EABG (5.67 g, 0.69%), and EMBG (111.46 g, 13.56%).

Total phenolic determination

The test was performed using the Folin-Ciocalteu colorimetric method [12]. Extracts (EHBG, EABG, and EMBG) were mixed with 50 μ L of Folin-Ciocalteu reagent for 8 minutes in room temperature and 240 μ L of sodium carbonate 0.4% was added. After incubation for 3 minutes at room temperature, the absorbance of the reaction mixture was measured at 750 nm. Gallic acid was used as a standard. The experiments were performed in triplicate and the mean results determined.

Instrumental Analysis

A HPLC-MS system consisting of an Accela 600 liquid chromatography system coupled to a TSQ Quantum Access triple-quadrupole (QqQ) mass spectrometer was employed for the chemical analyses (Thermo Fisher Scientific, Waltham, MA, USA). Electrospray ionization in negative mode (ESI) was applied to access the phenolic composition of *B. garcibarrigae*. Mass spectra (MS) were acquired at the m/z range from 50 to 1000, and tandem mass spectra (MS/MS) performed by collisional-induced dis-

sociation of previously isolated precursor ions in the QqQ system. Argon was used as collisional gas. Tentative identifications were performed by manual interpretation of MS/MS spectral data with the literature.

Chromatographic separations were performed on a Kinetex C18 column (2.6 μm , 30 $\times$ 4.6 mm, 100 Å pore size) (Phenomenex, Torrance, CA, USA) column using a binary mobile phase. Solvent A was ultrapure water, and solvent B was methanol. A gradient elution at 28 °C was performed as follows: 0-3 min, 58% B; 3-7 min, 58-100% at a flow rate of 0.4 mL/min. The autosampler temperature was held at 15 °C, and the injection volume was 2 μL . Ionization parameters were capillary voltage 4.5 kV, cone voltage 5 V, sheath gas 25 arb, and auxiliary gas 5 arb. Collision energies were applied as an increasing ramp from 2 to 50 eV.

^1H NMR experiments were recorded in methanol- d_4 (Cambridge Isotope Laboratories, Tewksbury, MA, USA) an INOVA 500 NMR spectrometer (Varian) operating at 500 MHz for ^1H and at 125 MHz for ^{13}C experiments, respectively. TMS (Cambridge Isotope Laboratories) was used as internal standard.

DPPH radical scavenging assay

The DPPH radical scavenging activity was performed according to Molyneux (2004) [13], with modifications. The assay was performed on a 96-well plate using 30 μL of extracts (EHBG, EABG, and EMBG) at 100 $\mu\text{g/mL}$ and mixed with 270 μL of DPPH in ethanol (absorbance equal to 1 at 517 nm). The plate was kept in the dark for 30 minutes, after that the absorbance was measured at 517 nm. Blanks (DMSO) and standard (quercetin) were run simultaneously.

α -Glucosidase inhibition assay

This experiment used α -glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20). Briefly, after the dilution of the extracts and fractions, the reaction was performed on microplate with the prepared enzyme solution (10 U/mL). Then, were obtained the values of the absorbance at 405 nm. With the addition of color reagent, readings were carried out every 5 min until the time of 30 min [14].

Fractionation from the methanolic extract

Fractionation of the EMBG was performed by liquid-liquid partition. For this purpose, 50 g of the extract was solubilized in 500 mL of methanol/water 1:1 and sequentially extracted with 300 mL of hexane and chloroform, and performed in triplicate, respectively. The obtained fractions were dried in a rotary evaporator (IKA® RV10 rotaevaporator basic), providing the following fractions: FHX and FCL. The solvent

residual fraction, hydromethanolic (FHM), was eliminated using a mini spray dryer (MSD 1.0, Labmaq, Brazil).

The most active fraction, FHM (10 g), was fractioned over a polyamide-6-DF (Riedel-de Haen, Seelze, Germany) column chromatography with an isocratic elution of methanol (column of 40 cm high and 6 cm diameter, and 288.4 g of as stationary phase). Fractions were collected into 100 mL flasks and evaluated by thin-layer chromatography (TLC) using silica gel 60 (ALUGRAM® Xtra SIL G, Macherey-Nagel) and ethyl acetate/toluene/formic acid/water 4:0.5:0.5:0.5 as mobile phase, which were grouped according to their similarities, affording 32 fractions.

All fractions were evaluated against α -glucosidase in enzymatic assays. F3 showed the highest inhibitory activity, therefore, it was submitted to preparative chromatography on glass plates with silica gel 60 (70-230 mesh) using chloroform/methanol (9:1) as a mobile phase. The compound was removed from the plates with silica and extracted with ethyl acetate yielding compound 1 (12 mg) and 2 (8 mg).

Epicatechin (1): ¹H NMR (methanol-d₄, 500 MHz) δ H 2.5; 2.8 (dd, H-4); 4.0 (m, H-3); 4.6 (d, H-2); 4.8 (s, H-6); 5.6 (d, H-5'); 5.8 (d, H-2'); 6.75 (m, H-6'). ESI-MS (product ions, 15 eV) m/z: 289 [M-H]⁻, 245, 221, 203, 151, 123, 109.

Quercitrin (2): ¹H NMR (methanol-d₄, 500 MHz) δ H 1,16 (d, J = 1,2 Hz, H-6''); 3,30 (m, H-4''); 3,54 (m, H-2''); 3,63 (m, H-3''); 4,08 (d, J = 1,2 Hz, H-1''); 4,22 (m, H-5''); 6,20 (d, J = 1,8 Hz, H-6); 6,42 (d, J = 1,8 Hz, H-8); 6,80 (d, J = 8,2 Hz, H-5'); 7,55 (dd, J = 2,1 e 8,5 Hz, H-6'); 7,68 (d, J = 2,1 Hz, H-2'). ESI-MS (product ions, 15 eV) m/z: 447 [M-H]⁻, 301 [M-H Rha]⁻, 179, 151, 121.

Antiglycation activity

A mixture of bovine serum albumin (135 μ L), glyoxal, fructose or glucose (135 μ L) and different concentrations of EMBG, FHM, quercitrin and epicatechin (30 μ L) was incubated at 37 °C and kept in the dark, the fluorescence of the samples was measured using an excitation of 340 nm and an emission of 420 nm. Aminoguanidine was used as positive controls and DMSO as negative control [15].

Docking molecular

Docking studies were performed employing the crystalline structures cocrystallized with known inhibitors available on the Protein Data Bank (PDB): α -glucosidase (PDB: 3W37) and receptor for advanced glycation endproducts (RAGE)

(PDB: 3O3U). Water molecules and other molecules used in the crystallization of proteins not required for catalytic activity were removed in Pymol software (Schrödinger, Cambridge, USA), and all ligands were prepared and geometry, as well as energy, was minimized through ChemBioDraw program, and were subjected to energy minimization by the Merck Molecular Force Field (MMFF94).

The procedure of docking of ligands with the receptors has been performed using AutoDock Vina 1.1.2 (The Scripps Research Institute, Jupiter, USA) [16]. Dimensions of grid box was 12x18x12 Å and 14x10x10 Å with grid spacing of 1.0 Å for α -glucosidase and RAGE, respectively.

The results were visualized in the AutoDock Tools and the interactions were evaluated in terms of minimum binding energy (kcal/mol), number of hydrogen bonds, and other interactions formed between the active site residues of the macromolecule and ligand. 2D and 3D images of docking molecular results were obtained using Discovery Studio Visualizer 4.0 software (San Diego, CA, USA).

Statistical analysis

The results are presented as mean $\pm$ standard deviation, where applicable. All the results were compared by the Tukey test, considering values of $p < 0.05$ statistically significant. The median inhibitory concentrations (IC_{50}) were calculated by the linear regression method. All the analyses were performed in GraphPad Prism 6 (San Diego, CA, USA).

RESULTS AND DISCUSSION

Since α -glucosidase inhibitors delay the release of glucose reducing postprandial hyperglycemia, they are an important approach to the treatment of type 2 diabetes [17]. Thus, this work studied the effect of *B. garcibarrigae* extracts on α -glucosidase, as well as their antioxidant potential, which is responsible for the development of long-term diabetic complication, and associates it with the increased risk of mortality and morbidity [18]. The inhibitory effects of the extracts against α -glucosidase were evaluated in comparison to those of acarbose, an antidiabetic drug (table 1).

Table 1. Total phenolic content, antioxidant capacity and inhibitory activity of crude bark extracts from *B. garcibarrigae*.

Sample	Total phenolic content (%)	DPPH	α -glucosidase inhibition
		IC ₅₀ value (μ g/mL)	
EHBG	20.65 $\pm$ 1.43	130.42 $\pm$ 0.23	3.70 $\pm$ 0.61
EABG	28.41 $\pm$ 6.50	10.3 $\pm$ 0.03	3.21 $\pm$ 2.05
EMBG	61.43 $\pm$ 0.50	9.2 $\pm$ 0.23	1.09 $\pm$ 0.32
Quercetin	-	3.4 $\pm$ 0.12	-
Acarbose	-	-	0.86 $\pm$ 0.10

All extracts inhibited α -glucosidase, being that inhibitory activity increased with increasing polarity even as the antioxidant activity. Thus, the activity of the extracts was solvent dependent. Extracts prepared by methanol had higher antioxidant properties, probably due to compounds possessing protonatable functional groups such as -COOH and -OH, such as some polyphenols, which are known antioxidants [19, 20].

Among all extracts, EMBG presented a greater inhibitory capacity and was fractionated. The FHX and FCL fractions were discontinued, because they were inactive against α -glucosidase (9.08 $\pm$ 0.16%, and 11.0 $\pm$ 0.79% of inhibition, respectively). On the other hand, FHM presented an IC₅₀ of 1.02 $\pm$ 0.49 μ g/mL and was fractionated. Thirty-two fractions were obtained and were evaluated for their inhibitory activity, but only F3 and F4 showed promising inhibitory activity, which IC₅₀ values of 91.45 $\pm$ 0.94 and 62.04 $\pm$ 0.65 μ g/mL, respectively. The high activity of EMBG and FHM compared to fractions may be related to the that interactions by additive/non-interactive or synergistic effect of the compounds [21, 22]. In order to identify the compounds, the fraction F4 was subjected to preparative thin-layer chromatography and two compounds were isolated.

The NMR and MS fragmentation are in accordance with previously published data [23, 24] allowed the identification of epicatechin and quercitrin. These compounds had already been identified in other species of the genus *Byrsonima*, such as *B. duckeana* [25] and *B. sericea* [26], but are being reported for the first time in *B. garcibarrigae*.

Thus, due to its expressive antioxidant activity and for exhibit a potential inhibition of α -glucosidase, EMBG, FHM, and the isolated compounds were evaluated for inhibition of AGEs formation. In the body, increased levels of glucose and oxidative stress

induce a non-enzymatic reaction between reducing sugars and proteins, lipids or nucleic acids, which is called glycation, which gives rise to AGEs [27].

Over time, mitochondrial respiratory chain proteins become increasingly glycated and mitochondrial DNA damage occurs, leading to a self-perpetuating cycle of AGE formation and oxidative stress independently of hyperglycemia [28]. For this, AGEs are associated with oxidative stress and inflammation, which are processes involved in the pathogenesis of many chronic diseases, such as diseases [29].

In figure 1 it is possible to observe that in the formation of AGEs via fructose, FHM and EMBG, epicatechin and quercitrin showed better inhibitions when compared to the aminoguanidine, a synthetic inhibitor of glycation. Aminoguanidine, epicatechin, quercitrin, FHM, and EMBG presented IC_{50} values of 29.26 ± 0.13 , 43.92 ± 0.13 , 7.42 ± 0.23 , 2.69 ± 0.14 and 3.68 ± 0.74 $\mu\text{g/mL}$, respectively.

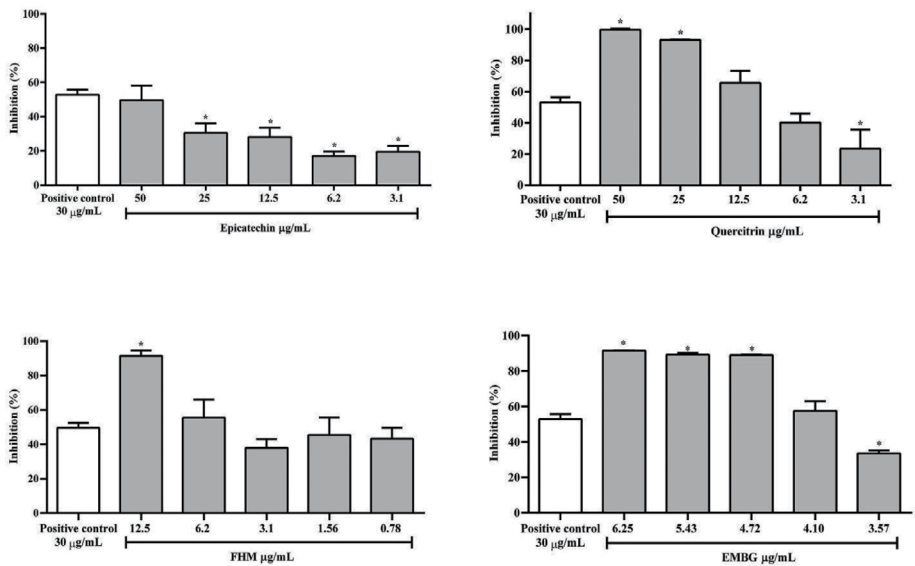


Figure 1. *In vitro* anti-glycation activity of extracts and compounds of *B. garcibarrigae*. The results are presented as mean $\pm$ standard deviation. p value versus Aminoguanidine 30 $\mu\text{g/mL}$ (positive control): * $p < 0.05$.

It is worth mentioning that our work is the first to demonstrate the role of the extracts of *B. garcibarrigae* in the control of non-enzymatic glycation. Our findings suggest that the antioxidant, anti- α -glucosidase and anti-glycation capacities are related of

the synergism between quercitrin, epicatechin and other compounds presents, since FHM was the more potent sample and showed some degree of affinity for the analyzed enzymes *in silico*. Quercitrin was the compound with the highest affinity (lower binding energy) for α -glucosidase which showed two hydrogen bonds with 3 residual amino acids Asp232, Asp357 and Arg552, while epicatechin was the compound with the highest affinity for RAGE which showed hydrogen bonds with 3 residual amino acids Glu44, Trp62 and Asp65 (figure 2). Both compounds showed affinity better than that of the co-crystallized inhibitor and showed affinity with both enzymes (table 2). In addition to the isolated flavonoids, 8 other phenolic compounds were identified in the EMBG (figure 3).

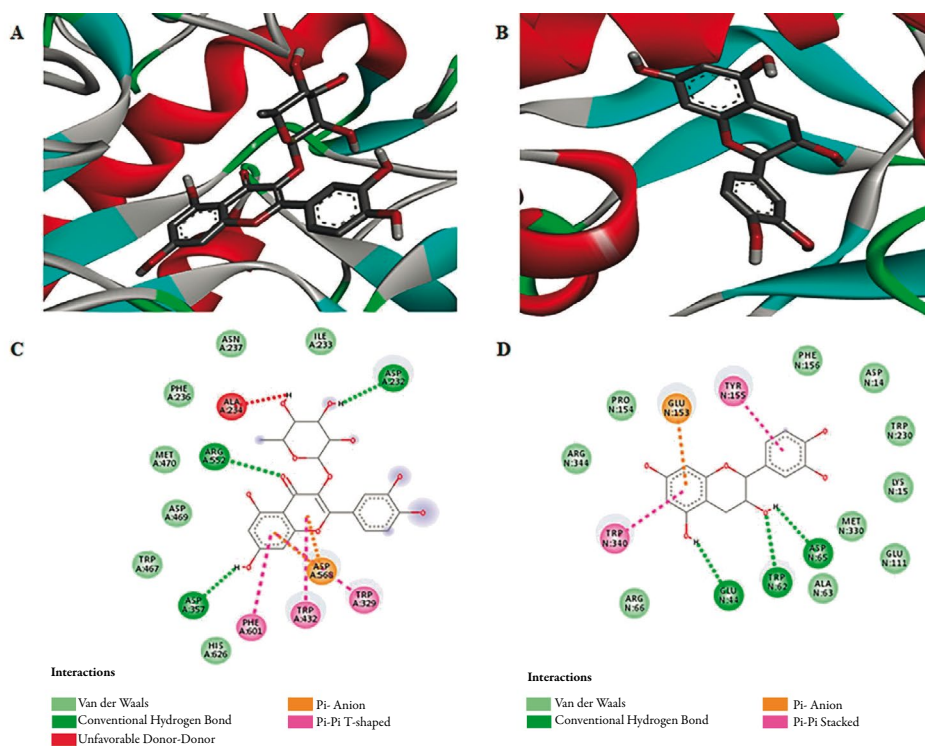


Figure 2. 2D and 3D docking pose of ligands and their amino acid interactions in the active site. Crystal structure of α -glucosidase (PDB: 3W37) in complex with quercitrin (A and C) and RAGE (PDB: 3O3U) in complex with epicatechin (B and D).

Table 2. Energy of interaction (kcal/mol) between phenolic compounds and the enzymes RAGE and α -glucosidase.

Compounds	α -glucosidase (PDB: 3W37)	RAGE (PDB: 3O3U)
Epicatechin	-7.4	-8.7
Quercitrin	-8.2	-9.5
Acarbose	-8.1	-
Maltotriose	-	-9.4

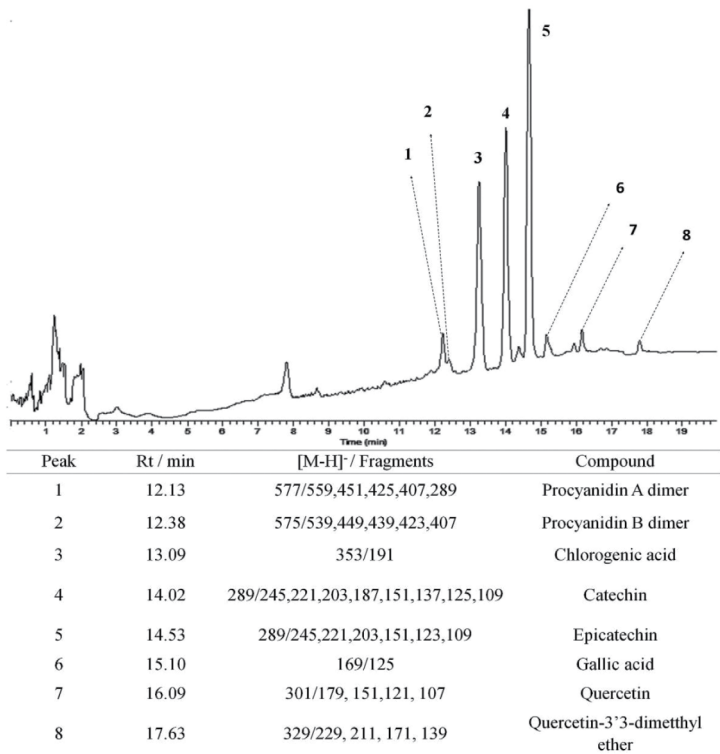


Figure 3. Total ion chromatogram and compounds identified in EMGB using by HPLC-ESI-MS/MS.

Polyphenols have been reported to inhibit AGEs formation, such as chlorogenic acid, which acts by metal chelation and is an insulin-sensitizer, reducing the intestinal glucose absorption and its plasma peak [30]. Furthermore, decreases triglyceride contents in adipose tissue [31].

Other polyphenols such as procyanidins, which are the oligomeric or polymeric forms of epicatechin and catechin, significantly ameliorate postprandial hyperglycemia promoting GLUT4 translocation to the plasma membrane by activating both insulin- and AMPK-signaling pathways [32]. Besides that, epicatechin, catechin and the gallic acid act as α -glucosidase inhibitors [33, 34]. Quercetin and its derivatives exerts a significant hypoglycemic activity might by enhancing insulin sensitivity, promoting glycogen synthesis, inhibiting α -glucosidase activity and decrease the seriousness of numbness, jolting pain, and irritation for patients with type 2 diabetes neuropathy [35, 36].

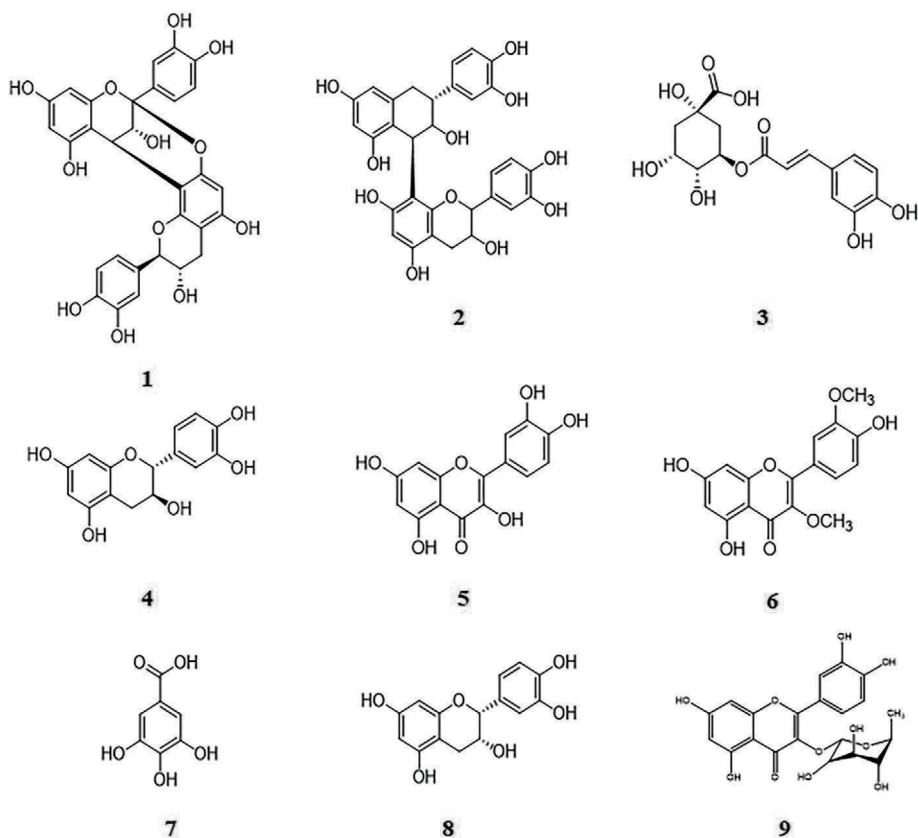


Figure 4. Chemical structures of the phenolic compounds identified in *B. garcibarrigae* by HPLC-ESI-MS/MS (1-8) and NMR (8-9). Procyanidin A dimer (1), procyanidin B dimer (2), chlorogenic acid (3), catechin (4), quercetin (5), quercetin-3'3'-dimethyl ether (6), gallic acid (7), epicatechin (8) and quercitrin (9).

Such findings base the inhibitory activity observed since high levels of phenolic compounds can inhibit enzymes by either interacting or inhibiting specific positions, as well as acting radical free radical scavenging [37, 38]. These evidences support our results and suggest of synergistic or additive effect between phenolic compounds identified (Figure 4) which can would interact with all enzymes analyzed and support a multi-target mechanism determined by the fraction components when compared isolated compounds [39, 40].

In summary, this study showed that *B. garcibarrigae* bark presents potential inhibitory activities against an important enzyme related physiopathological mechanism of type 2 diabetes. It was found that the fraction FHM as a promising source of natural antioxidants, α -glucosidase inhibitors and AGEs-formation inhibitors. Thus, *B. garcibarrigae* has potential to be used in further investigations regarding its activity against diseases such as diabetes.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Research Support Foundation of the State of Amazonas (FAPEAM), Federal University of Amazonas and Analytical Center by the support in this study.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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HOW TO CITE THIS ARTICLE

T. Marques-Machado, C. Queiroz-Valentim, L.D. Rosales-Acho, E. Silva-Lima, H.H. Ferreira-Koolen, F. Guilhon-Simplicio, Bioassay-guided isolation of α -glucosidase inhibitors from *Byrsonima garcibarrigae* Cuatrec., *Rev. Colomb. Cienc. Quim. Farm.*, **51**(3), 1114-1130 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.96982>

Plants with antioxidant activity and their potential use as sun protector agents in Costa Rica

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Received: June 18, 2021

Corrected: February 9, 2021

Accepted: February 17, 2022

SUMMARY

Aims: To carry out a bibliographic review related to plants available in Costa Rica that have demonstrated antioxidant power and a sun protection factor (SPF) suitable to be used in sunscreen products. **Methods:** The bibliographic review was carried out using different descriptors and by consulting different databases. **Results:** Information about antioxidant power about acerola, avocado, carrots, passion fruit, moringa, banana, pumpkin and amaranth, guava, matico, ginkgo, blackberry, mango, coffee was obtained. The information obtained suggests that the mentioned plants could be used for the formulation of sunscreens. **Conclusion:** Sun protection products should be used every day to prevent skin damage; some sunscreens produce allergic reactions, so it is necessary to investigate natural options to be used in sun protection products. Some natural products exhibit SPF values that allow their incorporation as sun protection agents, adjuvants, or enhancers in sunscreens.

Keywords: Antioxidant activity, ultraviolet radiation, natural products, sun protection factor, Costa Rica.

RESUMEN

Plantas con actividad antioxidante en Costa Rica y su potencial uso como protectores solares

Objetivo: llevar a cabo una investigación bibliográfica sobre plantas disponibles en Costa Rica que han demostrado su poder antioxidante y un factor de protector solar (SPF) apto para ser utilizados en protectores solares. **Métodos:** la revisión bibliográfica se realizó utilizando diferentes descriptores y mediante la consulta en diferentes bases de datos. **Resultados:** se obtuvo información sobre el poder antioxidante de las plantas: acerola, aguacate, zanahoria, maracuyá, moringa, banano, calabaza y amaranto, guayaba, matico, ginkgo, mora, mango, café. La información obtenida sugiere que las plantas mencionadas podrían ser utilizadas para la formulación de protectores solares. **Conclusiones:** los protectores solares deben ser utilizados diariamente para prevenir el daño sobre la piel. Algunos de estos productos generan reacciones alérgicas y por esta razón es necesario investigar posibles opciones naturales para ser incorporadas en bloqueadores solares. Algunos productos naturales exhiben valores de SPF que permiten su uso como agentes protectores solares, adyuvantes o potenciadores en los bloqueadores solares.

Palabras clave: Actividad antioxidante, radiación UV, productos naturales, factor de protección solar, Costa Rica.

RESUMO

Plantas com atividade antioxidante na Costa Rica e seu potencial uso como protetores solares

Objetivo: realizar uma pesquisa bibliográfica sobre plantas disponíveis na Costa Rica que demonstraram poder antioxidante e um fator de proteção solar (FPS) adequado para uso em protetores solares. **Métodos:** a revisão bibliográfica foi realizada usando diferentes descritores e consultando diferentes bases de dados. **Resultados:** foram obtidas informações sobre o poder antioxidante das plantas: acerola, abacate, cenoura, maracujá, moringa, banana, abóbora e amaranto, goiaba, matico, ginkgo, amora, manga, café. As informações sugerem que as referidas plantas pode-

riam ser utilizadas para a formulação de protetores solares. **Conclusões:** protetores solares devem ser usados diariamente para prevenir danos à pele. Alguns desses produtos geram reações alérgicas e por isso é necessário investigar possíveis opções naturais para serem incorporadas aos protetores solares. Alguns produtos naturais apresentam valores de FPS que permitem seu uso como protetores solares, adjuvantes ou potencializadores em protetores solares.

Palavras-chave: Atividade antioxidante, radiação UV, produtos naturais, fator de proteção solar, Costa Rica.

INTRODUCTION

In the past, sunscreen was primarily used for quick burn-free tans. Nowadays, sunscreen daily-use is necessary due to the risk generated by direct exposure to ultraviolet (UV) radiation, related to wrinkles, aging, oxidative stress, immunosuppression, erythema, edema, and even skin cancer [1-3]. UV radiation can be divided into three regions: UVA (320-400 nm), UVB (290-320 nm), and UVC (200-290 nm), and it is crucial to be protected from UVA and UVB since these two types of radiation are not entirely filtered by the ozone layer and can cause the damages mentioned above [4].

Topical sunscreens are the most commonly used products to protect the skin from radiation. These products have active ingredients related to the absorption or dispersion of radiation, protecting the skin from direct damage [5].

Natural sources present an attractive option for creating products with protective activity against solar radiation since they can be produced at a low cost and are generally easily found. Plants with antioxidant activity and UV absorption properties are candidates for the formulation of sun protector products [3]. Among the most widely used antioxidant compounds are tocopherols, flavonoids, phenolic acids, indoles, alkaloids, amines, amino acids, and monoterpenes [1]. For an antioxidant-based sunscreen, the extracts must have this fairly high activity and must be stable in the formulation [6]. New natural products' research to formulate and develop sunscreens is closely associated with photosensitivity and dermatitis that synthetic-based sunscreen formulations may cause [7]. The main objective of this work is to carry out a bibliographic review related to plant available in Costa Rica that have demonstrated antioxidant power and a sun protection factor (SPF) suitable to be used in sunscreen products.

NATURAL PRODUCTS WITH ANTIOXIDANT ACTIVITY

Acerola

Acerola or Barbados cherry (*Malpighia emarginata* D.C) is a Central America native fruit and is a high source of vitamin C. Additionally this fruit has other components such as polyphenols, β -carotenes, and folates, which gives it great antioxidant activity [8, 9].

The crude acerola extract was evaluated to study the protection against oxidative stress and cytotoxic damage. A study concluded that acerola has an excellent antioxidant and protective capacity for macromolecules against oxidative damage by increasing antioxidant enzymes' activity and protecting mitochondrial functionality [9].

Another study showed the skin lightening effect of acerola phenol extract in guinea pigs that had been exposed to UV radiation. The results were a lightening of damaged skin and a reduction of melanin in the B16 melanoma cells. It was concluded that this extract's lightening effect might be due to a suppression of melanogenesis by inhibiting tyrosinase activity in melanocytes [10].

Avocado

Avocado (*Persea americana* Mill.) is a plant native to Mexico. It mainly presents phenolic compounds such as catechin in the shell and seed. Also, flavonoids can be found in the peel [11].

A study was carried out in Indonesia using avocado peel ethanolic extracts with concentrations of 5%, 7.5%, and 10% to determine its SPF. No ethanolic extract presented an SPF greater than 15% [12].

Carrot

Carrot (*Daucus carota*) is a root native to the Eastern Mediterranean and Southwestern Asia [13]. It is rich in α and β -carotenes, which, as previously mentioned, are a source of antioxidants with photoprotective properties, that is, with sun protection properties. These properties are related to the extensive system of conjugated double bonds, known as polyene chain, which is the chromophore responsible for absorbing light in the visible region and, consequently, for carotenes' coloration. [14]. A study shows that formulations containing 6% w/v carrot seed oil have SPF values of 6.92 [15].

Passion fruit

Passiflora edulis is native to Brazil's Amazon region; however, it has adapted throughout the tropics and subtropics. The plant has green leaves, stems, a flower with petals, and 4-9 x 4-7 cm oval fruits [16].

The antioxidant capacity and the sun protection factor of the *Passiflora edulis* fruit peel were studied. The peel was extracted with 96, 70, and 45% ethanol and the obtained values were 16.03, 16.23, and 9.06 mg ET/g according to DPPH, respectively. In addition, the 45% extract was used to develop and formulate a cream, which obtained 5.52 SPF [17]. Another study determined the antioxidant capacity and antimicrobial capacity of pulp, leaves, seeds, and rind. The leaves exhibited a higher antimicrobial activity due to the high content of flavonoids, polyphenols, tannins, saponins, proanthocyanidins, among others. [18]. It is important to mention that the photoprotective capacity is dependent on the sunscreen formulation. A study compares the SPF between two formulations, and it indicates that the "mousse formulation" sun protection value doubles in magnitude the liquid base formulation with the following values 15.48 ± 1.60 and 5.88 ± 0.30 , respectively. It also indicates the extract's concentration did not show differences between 0.1 and 0.3% [19]. Obtaining methanolic extracts can be carried out utilizing ultrasound techniques, and it is one of the solvents that shows the best results in terms of antioxidant capacity [20].

Moringa

The plant *Moringa oleifera* Lam. has been used in different parts of the world due to its nutritional and medicinal characteristics [21]. Compounds such as beta-carotene, vitamin C, vitamin E, and polyphenols can be found in this plant's leaves. The biological functions that have been described are a high antioxidant capacity and even anti-inflammatory, anti-cancer, hepatoprotective, and neuroprotective [22, 23].

A study was conducted on sunscreen based on extracts of the moringa seed oil. The SPF was calculated for this formulation, and the obtained value was 1.46. The authors conclude that these extracts can be used as adjuvants in formulations to improve their activity [24], but it would not be viable to use as a base for a formulation with adequate sun protection. Another study in which sunscreen was also performed using moringa seed oil obtained similar results, with an SPF of 1.05 [25].

Banana

The banana is a fruit of the genus *Musa*, belonging to the *Musaceae* family. It is grown in the tropics [26] and is one of the most important crops in Costa Rica. Its peel has been used medicinally to treat some ailments, including burns, snakebites, ulcers, and

diarrhea. Among its components, high levels of fiber and 40 different types of phenols have been identified, including flavonoids, hydroxycinnamic acids, and catecholamines. It has an excellent antioxidant, antimicrobial, and antibiotic capacity [27].

The antioxidant power and sun protection factor of the red banana peel, named *Musa accuminata* L., were investigated. The preparation was carried out by chopping the liquefied peel with distilled water into small pieces to remove the sap. The extraction was carried out with methanol, ethanol, and acetone in reflux for two hours. DPPH method was used to evaluate the antioxidant capacity, and the results were related to tannins, flavonoids, and phenols presence. The extraction with ethanol as solvent showed antioxidant activity with higher values, followed by methanol and acetone, in that order. The sun protection factor's determination through spectrophotometry also showed higher values for ethanol with 16.63, followed by methanol with 16.60 and acetone with 15.42 [28].

Another study compared different species of banana; *Musa acuminata* Colla “purple banana”, *Musa paradisiaca* L. “Gros Michel banana”, “silk banana”, “island banana”, “purple banana” and “apple tree”. The silk banana showed the higher content of phenolic compounds 31.32 ± 0.05 mg EAG/g with an antioxidant potential of 49.13 ± 0.07 mg ET / g (DPPH ●) and 97.98 ± 0.3 mg ET/g (FRAP). The island banana sample showed the highest value for the sun protection factor, with 1.03 ± 0.047 [29].

Pumpkin and amaranth

The seeds of amaranth (*Amaranthus cruentus*) and pumpkin (*Cucurbita seed spp*) contain an oil rich in antioxidants. A study investigated lipid nanoparticles' capacity to encapsulate two UVA and UVB filters. It was determined that nanostructures formulation has a high capacity to inhibit short-lived radicals (93-98%). It was shown that the amaranth oil system with 82% squalene was the most effective for scavenging oxygen free radicals, and the one that demonstrated the higher capacity to inhibit cationic radicals was amaranth oil with 34% squalene. It is concluded that the use of these systems improves the performance of commercial sunscreen [30].

Guava

The leaves of *Psidium guajava* L. trees have compounds such as polyphenols, flavonoids, alkaloids, and saponins, and it has been used to prepare functional beverages and infusions with hypoglycemic activity. High antioxidant activity has been related to a large number of polyphenols contained in guava [31].

An investigation used *Psidium guajava* L (guava), *Musa accuminata* (red plantain), and *Pyrus communis* (pear tree) extracts to formulate a sunscreen. The solvent system used in the formulation was 35% methanol, 35% ethanol, and 30% distilled water to maximize the flavonoids extraction from the mentioned fruits. The SPF value was determined, and the obtained value was 3.90 [32].

In Costa Rica, *Psidium friedrichsthalianum* pulp was studied to determine its antioxidant and anti-inflammatory activity related to phenolic compounds. The ABTS and DPPH test was carried out on different extracts, and it was determined that the extract with ethyl acetate as a solvent had a higher antioxidant activity [33].

Matico

Matico (*Piper elongatum*) is a plant native to Mexico, the Caribbean, and South America. It is present in Costa Rica, and antibacterial, anti-inflammatory, and antioxidant properties have been described [34, 35]. An *in vitro* comparison with commercial creams was carried out to determine the plant's antioxidant capacity and the sun protection factor. The methanolic extract samples of the silver leaves were dried using an oven and a rotary evaporator. Creams containing extract 1% were formulated, and the antioxidant capacity was compared against commercial products by DDPH and ABTS methods. The obtained results were 163.919 μ M equivalent of Trolox by DDPH method and 121.400 μ M equivalent of Trolox by ABTS method. The extract dried by the rotary evaporator method showed a sun protection factor of 5.334; however, the SPF values determined by the Mansur method were not as high as those of the commercial creams, which were 17.489 and 17.554 [34].

Ginkgo

Ginkgo biloba is a native Chinese tree that has been used as a medicinal plant for many years, especially the extract obtained from its leaves [36]. Ginkgo extracts have different compounds such as glucosides and flavone terpenoids, which provide high antioxidant activity [37], and have been used as an anti-aging treatment [38].

The *in vitro* SPF of a dry extract ginkgo was obtained using spectral transmittance and *in vitro* antioxidant activity using DPPH, ABTS, and AAPH. The obtained SPF value was 7.06, and the extract shows the ability to absorb radiation in the 320–400 nm range. In addition, the DPPH, ABTS, and AAPH tests showed an important antioxidant capacity thanks to the plant flavonoids [39].

A sunscreen was formulated using algae, ginkgo extract, and vitamins A, C, and E. The transepidermal water loss technique in mice and the erythema index were used to

evaluate the formulation's performance. This formulation exhibits the ability to protect the skin from erythema caused by UV radiation [40].

Another study showed that ginkgo extract protects against UV-induced damage to mice's skin. Furthermore, the possibility of protecting lamellar bodies is raised. Other benefits are reduction of dryness and irritation of the skin and reduction of erythema [41].

Blackberry

Blackberries are known to have a high antioxidant capacity due to their high content of flavonoids, more specifically, anthocyanins [42, 43]. These compounds appear on plants' surfaces, flowers, and leaves with pink, red, purple, and blue colorations. Topical formulations with blackberry (*Rubus sp*) and raspberry ethanolic extracts' were analyzed; the antioxidant capacity was measured by the DPPH method and SPF determination by spectrometry [42]. Blackberries had an anthocyanin content of 65.58 mg per 100 g of fruit, and raspberry had an anthocyanin content of 29.93 mg per 100 g of fruit. The obtained SPF values were 54.57 with blackberry formulations and 37.32 with raspberry formulations, indicating that both formulations have potential use as a sunscreen since the FDA specifies that the minimum SPF value is 6.0. This study indicates the formulation process, organoleptic characteristics, and stability of formulation [42].

Mango

Mango (*Mangifera indica* L) is a tropical fruit produced worldwide, just like the banana. Mango peel is a source of consumption and contains fiber, antioxidant minerals, carotenoids, enzymes, vitamins, phenolic compounds, and terpenes [44, 45]. The antioxidant capacity has been evaluated using *in vivo* and *in vitro* techniques. *In vitro* evaluation can be carried out by DPPH, FRAP, TEAC, ABTS methods, and some *in vivo* methods: GSH, catalase, LDL, and TBARS methods. A study analyzed Creole mango samples obtained from different places, one from Trujillo and another from Jaén, known as "Kent" mango and "Edward" mango, respectively. It was determined that Creole mango from Jaén had an SPF value ($6,54 \pm 0,02$) higher than the Creole mango sample from Trujillo, which indicates that the location is decisive. The results suggest a correlation between the fruits' antioxidant capacity and the SPF value, so it could be expected that other plants with antioxidant capacity also have SPF [44].

Coffee

Coffee is a source of antioxidants [46]. It is commonly consumed worldwide, mainly in red coffee beans, because green coffee beans are considered waste and are not consumed; therefore, a study was conducted with green coffee. DPPH or ABTS techniques were used to evaluate antioxidant activity attributed to the presence of tocopherols and

linoleic and palmitic acid. *In vitro* studies for the determination of SPF value were carried out by spectrophotometry in the UV region (290–400 nm) and, like squash and amaranth, by different mechanisms, coffee improves the performance of commercial sunblock products by around 20% [47]. The spent coffee grounds are another waste product, and their antioxidant activity and SPF were evaluated. The obtained results show an antioxidant activity of 18.4 and 23.6 mg of DPPH per 1g extract and SPF value in the range of 2.27 to 2.76 for the ethanolic extracts. These results are related to pharmacological and cosmetic properties and promote the spent coffee grounds use, which benefits the environment due to the waste product reduction and recovery [48]. Another study with the same environmental protection approach promotes sunscreens production with used ground coffee. The results indicate that coffee is a plant that enhances the SPF of sunscreen [49].

Green coffee oil (*C. arabica*) is also a linoleic, palmitic, and oleic acid source, absorbing UVB radiation. The unsaponifiable fraction of the oil is rich in pentacyclic terpenes and tocopherols. The DPPH technique evaluated the antioxidant capacity, and the SPF value was determined by spectrometry; the obtained values were 1.85 to 2.78 [50]. Another study carried out the green coffee oil microencapsulation through the spray drying of an oil-in-water emulsion, which increased antioxidant capacity compared to alpha-tocopherol standard and the same coffee oil [51].

CONCLUSIONS

Sun protection is necessary and should be used every day to prevent skin conditions ranging from burns to cancer. However, some sunscreens produce photoallergic reactions, so it is necessary to continue investigating natural options as sun protection agents.

According to the FDA, a compound to be used as a sunscreen must have *in vivo* SPF values higher than 6.0 when only *in vitro* studies are performed. Blackberries, passion fruit, plantain, Ginkgo Biloba, and mango showed promising *in vitro* results. However, many of the plants studied have SPF values lower than 5.0, which indicates that they can be used as adjuvants or enhancers of sunscreen such as acerola, avocado, carrot, moringa, guava, matic, and coffee.

DISCLOSURE STATEMENT

The authors have declared no conflict of interest.

ACKNOWLEDGMENTS

This work was carried out in the Instituto de Investigaciones Farmacéuticas (INIFAR). We want to thank Dr. German Madrigal, the project coordinator.

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HOW TO CITE THIS ARTICLE

R. Arguedas-Herrera, S. Arguedas-Chacón, M.F. Rojas-Salas, M. Chavarría-Rojas, R. Vargas-Zúñiga, G.L. Madrigal-Redondo, Plants with antioxidant activity and their potential use as sun protector agents in Costa Rica, *Rev. Colomb. Cienc. Quim. Farm.*, **51**(3), 1131-1145 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.96627>

Revisión de la fisiología anorrectal y posibles compuestos bioactivos para la erocosmética

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Recibido: 24 de junio de 2021

Revisado: 12 de febrero de 2022

Aceptado: 17 de febrero de 2022

RESUMEN

Objetivo: realizar una revisión bibliográfica sobre la fisiología ano-rectal, el proceso de relajación y contracción del músculo liso del tracto digestivo y posibles materias primas y formulaciones que podrían incorporarse a formulaciones erocosméticas de uso tópico en la región ano-rectal. **Métodos:** la revisión bibliográfica se realizó utilizando diferentes descriptores y mediante la consulta en las bases de datos ScienceDirect, PubMed, Clinical Key, Google Scholar y Google Patents. **Resultados:** inicialmente la revisión de la literatura permite comprender la anatomía y fisiología ano-rectal y los mecanismos que modulan la continencia anal, el tono basal del esfínter anal interno y el reflejo inhibitorio rectoanal (RAIR). Posteriormente, se obtuvo información sobre ejemplos de afrodisíacos naturales y se analizaron formulaciones cosméticas utilizadas como lubricantes anales con el fin de estudiar a profundidad los ingredientes de origen natural e identificar su utilidad, mecanismos de acción tópicos y su función dentro de la formulación. **Conclusiones:** el entendimiento de la fisiología ano-rectal permite el estudio y desarrollo de formulaciones cosméticas con propiedades analgésicas, anestésicas y relajantes, como los lubricantes anales. Productos naturales como la manzanilla, árnica, *Aloe vera* y mentol han sido estudiados para su uso cosmético y tópico como analgésicos, anestésicos o relajantes,

por lo que su utilidad comprobada los hace útiles en el desarrollo de productos erocosméticos destinados para ser utilizados en la región anogenital.

Palabras claves: Relajante anal, lubricante, esfínter anal, manzanilla, aloe vera, árnica.

SUMMARY

Anorectal physiology and possible bioactive compounds for erocosmetics: A review

Aim: To review the anorectal physiology, the process of relaxation and contraction of the smooth muscle of the digestive tract, and possible raw materials and formulations that could be incorporated into erocosmetic formulations for topical use in the anorectal region. **Methods:** The bibliographic review was carried out using different descriptors and by consulting the databases ScienceDirect, PubMed, Clinical Key, Google Scholar and, Google Patents. **Results:** Initially, a literature review was carried out to understand anorectal anatomy and physiology and the mechanisms that modulate anal continence, the internal anal sphincter basal tone, and the recto-anal inhibitory reflex (RAIR). Subsequently, information related to natural aphrodisiacs examples was obtained, and cosmetic formulations used as anal lubricants were analyzed to study the natural ingredients and identify their usefulness, mechanisms of action, and function in the formulation. **Conclusion:** Understanding anorectal physiology allows the study and development of cosmetic formulations with analgesic, anesthetic, and relaxing properties, such as anal lubricants. Natural products such as chamomile, arnica, *Aloe vera*, and menthol have been studied for cosmetic and topical use as analgesics, anesthetics, and relaxants, so the evidence makes them helpful in developing erocosmetic products intended to be used in the anogenital region.

Keywords: Anal relaxant, lubricant, anal sphincter, chamomile, *Aloe vera*, arnica.

RESUMO

Fisiologia anorretal e possíveis compostos bioativos para erocosméticos: uma revisão

Objetivo: revisar a fisiologia anorretal, o processo de relaxamento e contração da musculatura lisa do trato digestivo e possíveis matérias-primas e formulações que

possam ser incorporadas em formulações erocosméticas de uso tópico na região anorretal. **Métodos:** a revisão bibliográfica foi realizada utilizando diferentes descritores e consultando as bases de dados ScienceDirect, PubMed, Clinical Key, Google Scholar e, Google Patents. **Resultados:** inicialmente, foi realizada uma revisão da literatura para entender a anatomia e fisiologia anorretal e os mecanismos que modulam a continência anal, o tônus basal do esfíncter anal interno e o reflexo inibitório retoanal (RAIR). Posteriormente, foram obtidas informações relacionadas aos exemplos de afrodisíacos naturais e analisadas formulações cosméticas usadas como lubrificantes anais para estudar os ingredientes naturais e identificar sua utilidade, mecanismos de ação e função na formulação. **Conclusão:** a compreensão da fisiologia anorretal permite o estudo e o desenvolvimento de formulações cosméticas com propriedades analgésicas, anestésicas e relaxantes, como os lubrificantes anais. Produtos naturais como camomila, arnica, *Aloe vera* e mentol foram estudados para uso cosmético e tópico como analgésicos, anestésicos e relaxantes, de modo que as evidências os tornam úteis no desenvolvimento de produtos erocosméticos destinados ao uso na região anogenital.

Palavras chave: Relaxante anal, lubrificante, esfíncter anal, camomila, *Aloe vera*, arnica.

INTRODUCCIÓN

La defecación y continencia anal son dos funciones importantes del aparato digestivo. Para que ambas funciones se lleven a cabo de manera correcta deben existir condicionamientos anatómicos y fisiológicos que permitan un adecuado transporte a través del colon hasta el recto. Toda esta actividad, al involucrar componentes involuntarios y conscientes, es sumamente compleja [1].

La motilidad de la región rectoanal está relacionada con el mantenimiento de la continencia y abarca los procesos de detección, discriminación, retención y finalmente la expulsión controlada de los productos de desecho de la digestión. Estas funciones requieren una serie de interacciones complejas entre los componentes viscerales y somáticos de la región, coordinados por los mecanismos reflejos y la voluntad consciente [2].

Para estudiar los reflejos peristálticos, la propagación de las ondas propulsivas del recto y la función del esfíncter anal interno (EAI), se han empleado métodos como la manometría ano-rectal (MAR), que ha permitido el análisis de la presión de reposo y la longitud de la zona de elevada presión esfinteriana [2].

Entender la anatomía y fisiología ano-rectal puede ser un punto de partida muy importante para el abordaje de nuevas materias primas y formulaciones erocosméticas. Lo anterior ya que permite dilucidar más fácilmente cuáles productos naturales pueden ser efectivos en los diferentes sitios anatómicos con lo que eventualmente tendrán contacto y una función a nivel fisiológico.

El objetivo de este artículo es realizar una revisión bibliográfica sobre la fisiología anorrectal, el proceso de relajación y contracción del músculo liso del tracto digestivo y posibles materias primas y formulaciones que podrían incorporarse a formulaciones erocosméticas de uso tópico en la región ano-rectal.

METODOLOGÍA

Se realizó una revisión no sistemática y para la búsqueda de información se utilizaron las bases de datos con las que cuenta la Universidad de Costa Rica, como lo son ScienceDirect, PubMed, Clinical Key y otras externas como Google Scholar y Google Patents. En total se revisaron 52 artículos y de estos se excluyeron 16.

Primero se buscaron artículos relacionados con la anatomía y fisiología ano-rectal y los mecanismos que modulan la continencia anal, el tono basal del esfínter anal interno y el reflejo inhibitorio rectoanal (RAIR), se excluyeron los artículos que no contaban con una descripción de la fisiología ano-rectal. Luego se procedió a buscar artículos con ejemplos de afrodisíacos naturales para después consultar la información relevante de cada una de las plantas de manera individual. Además, se analizaron formulaciones cosméticas utilizadas como lubricantes anales con el fin de estudiar a profundidad los ingredientes de origen natural e identificar su utilidad, mecanismos de acción tópicos y su función dentro de la formulación. Dentro de las especificaciones se consultaron artículos que preferiblemente contaran con un estudio científico para respaldar su uso como afrodisíaco y se excluyeron aquellos que solo presentaban información sin una base científica, por ejemplo, opiniones de los autores, sin ningún estudio que respaldara dicha información.

Las palabras claves usadas en la búsqueda fueron: óxido nítrico, inhibición ano-rectal, lubricante, esfínter anal, continencia anal, anestésico anal, relajante anal, manzanilla, árnica, aloe vera, capsaicina.

RESULTADOS

Fisiología rectal: defecación, relajación y fisiopatología

Los mecanismos multifactoriales y complejos son los encargados del mantenimiento de la continencia y la defecación en el cuerpo humano. Dichos mecanismos son controlados por una interacción entre los músculos del colon, el recto y el ano. Estas estructuras se encuentran coordinadas y dependen de controles conscientes e inconscientes [3-5].

El esfínter anal es una estructura cilíndrica de varias capas, en la cual comúnmente destacan dos partes, el esfínter anal interno y el externo, ambos constituidos por distintos tipos de músculo. El esfínter anal interno está compuesto por músculo liso en un estado de contracción continua, el cual representa una barrera natural para la pérdida involuntaria de gas y heces. El esfínter anal externo está constituido por músculo estriado cilíndrico e innervado por el nervio motor rectal inferior, que se encuentra bajo el control voluntario y comprende predominantemente fibras musculares de contracción lenta, capaces de ejercer una contracción prolongada. El esfínter anal externo es crítico para el control voluntario de la defecación cuando se está despierto [3, 6, 7].

Por su parte el esfínter anal interno contribuye con más de un 70% de la presión anal en reposo y es crítico para mantener este canal cerrado y la continencia fecal durante el sueño y la actividad [6]. El mecanismo de continencia automático está formado por un tono mantenido en reposo del esfínter anal interno y magnificado por un reflejo voluntario del esfínter anal externo y su actividad contráctil. [3, 7].

Tono basal

Como fue mencionado anteriormente, el mantenimiento de un tono basal es de suma importancia para sostener la continencia y evitar la expulsión involuntaria de gases y heces. Los principales mecanismos implicados en la continencia son los músculos del esfínter anal, zona de alta presión, el ángulo ano-rectal, la sensibilidad ano-rectal y reflejo inhibitorio rectoanal, el cumplimiento, tono y capacidad rectal, el llenado y vaciado rectal, la motilidad y tiempo de tránsito de colon y recto, los cojines vasculares y el volumen y la consistencia de las heces [3].

Músculos del esfínter anal

Músculo del esfínter anal interno

El músculo del esfínter anal interno tiene un tono de reposo con variaciones cíclicas representadas por las ondas cortas y ultracortas. El hallazgo más común de motilidad del esfínter interno es la presencia de ondas cortas, con frecuencias de 10 a 23 ciclos por

minuto [3]. Estas ocurren independientemente del estado de vigilia o alimentación. Se pueden encontrar ondas ultracortas que disminuyen durante el sueño. La importancia de estas ondas está relacionada con el reflejo de acomodación en la parte superior del esfínter anal interno. Se observa un equilibrio entre las presiones del ano y el recto, permitiendo así el contacto del contenido rectal con la mucosa sensible del canal anal. Este reflejo se produce, entonces, siete veces por hora y, en general, no se percibe [3].

El músculo del esfínter anal interno contribuye con aproximadamente el 50% al 85% del tono de reposo anal. Cuando hay un aumento de la presión intraabdominal (maniobra de Valsalva), existe una mayor actividad eléctrica de estos músculos de soporte, lo que aporta fuerza adicional al mecanismo del esfínter, evitando el paso involuntario del contenido rectal a través del canal anal [3].

Este tono en el músculo del esfínter anal interno posee un componente basal y uno fásico (dependiente del calcio extracelular). Dicho componente fásico es similar al componente con un tono basal sostenido bajo condiciones isométricas, que significa una contracción sostenida superpuesta con cambios de fuerza rítmicos. Luego de probar distintos inhibidores de las uniones GAP y canales de Ca^{2+} voltaje dependientes tipo L (VDCCs), se evidenció la actividad contráctil que requiere de comunicación célula-célula, vía uniones GAP pero no la participación de neuronas [6-11].

También se ha evidenciado la participación de un eje formado por los canales de rianodina (RyR), canales de Cl^- activados por Ca^{2+} (TMEM16A) y los VDCCs, ligados a oscilaciones de Ca^{2+} sincrónicas (SCaOs) y asincrónicas (ACaOs) las cuales generan esos componentes fásico y basal. En términos generales las oscilaciones de Ca^{2+} pueden ser iniciadas por un oscilador citosólico que libere el Ca^{2+} de depósitos internos o un oscilador de membrana que se encarga de abrir y cerrar los VDCC en la membrana plasmática [6-11].

Los osciladores de membrana por medio de difusión pueden liberar Ca^{2+} con una baja velocidad de propagación, pero los osciladores de membrana por medio de un emparejamiento eléctrico pueden generar olas de Ca^{2+} coordinadas de largo alcance que se pueden propagar a áreas mucho mayores con una mayor velocidad [6-11].

Ya se sabe que las SCaOs se pueden propagar a una velocidad y área mucho mayor en comparación con las ACaOs. Se cree que lo primero que sucede es que se libera Ca^{2+} vía los RyRs de depósitos internos que activan los TMEM16A y despolarizan la membrana plasmática, seguido de una propagación eléctrica hacia las células de músculo liso adyacentes vía uniones GAP y finalmente esta despolarización en masa activa los canales VDCC que van a permitir la entrada de Ca^{2+} , el cual activa más RyRs y genera una

liberación de calcio inducida por Ca^{2+} de los depósitos internos. El mecanismo anterior es el encargado de generar el tono sostenido en el esfínter [6-11].

Músculo del esfínter anal externo

El músculo esfínter anal externo también presenta un tono de reposo, incluso durante el sueño. La actividad tónica de este músculo presenta variaciones según las actividades diarias. Este músculo forma un anillo en continuidad con el músculo puborrectal y ambos actúan juntos en el mantenimiento voluntario de la continencia. El canal anal normalmente está cerrado en reposo y durante el sueño debido a la actividad constante del músculo esfínter anal interno y es reforzado por la actividad tónica del esfínter anal externo y puborrectal [3].

Músculos puborrectal y elevador del ano

Los músculos elevadores del ano son responsables del soporte del suelo pélvico y de los órganos pélvicos y abdominales, evitando así un descenso perineal excesivo. Este músculo participa en el mantenimiento de la continencia no solo como un componente importante de la zona de alta presión sino como responsable de la formación del ángulo ano-rectal. Además, también se encuentra en un estado de actividad tónica constante [3].

Zona de alta presión

En el canal anal también se puede encontrar una zona de alta presión que consiste en la longitud del canal anal. En esta zona se observan presiones 30% mayores que las que se encuentran en el recto debido a las propiedades miogénicas de los músculos del esfínter anal interno y externo [3].

Ángulo ano-rectal

Durante muchos años, se ha creído que el ángulo ano-rectal (ángulo formado por la intersección del límite superior del canal anal con la línea media del recto) es el mecanismo más eficaz de control valvular para la continencia anal. Este ángulo, originado principalmente por la fuerza contráctil del músculo puborrectal, realiza la tracción del recto en sentido anterior, presentándose en condiciones normales de reposo, alrededor de los 90 grados. En el caso de la defecación, con la relajación muscular, este ángulo es más obtuso, resultando en una rectificación o alineación del recto con respecto al canal anal [3].

Sensibilidad ano-rectal y reflejo inhibitorio rectoanal (RAIR)

El canal anal, especialmente la zona de transición tiene una gran cantidad de células sensoriales que responden a los estímulos de presión, temperatura y fricción. La distensión de la pared rectal por el contenido intestinal estimula directamente los receptores de presión en la pared del recto [3].

Un aspecto notable de la compleja red neuromuscular del canal anal es el reflejo inhibitorio rectoanal (RAIR). El RAIR se describe manométricamente por la presión de relajación o pérdida de presión del canal anal durante la distensión del esfínter anal y la presión residual o el punto de presión del canal anal más bajo del reflejo [12]. La distensión del recto conduce a la consiguiente relajación del esfínter interno, lo que permite que el contenido rectal acceda al epitelio sensorial especializado que recubre el canal anal superior. Este mecanismo permite el muestreo y la discriminación consciente o subconsciente entre contenidos sólidos, líquidos o gaseosos. Los grados crecientes de distensión rectal conducen a una inhibición completa del esfínter interno. Mientras que el esfínter interno se relaja, el esfínter externo se contrae para mantener la continencia [13, 14].

Cumplimiento, tono y capacidad rectal

El recto tiene la capacidad de adaptarse pasivamente a la distensión. Esta capacidad permite al recto mantener la presión rectal a pesar de los aumentos repentinos de la presión intrarrectal, contribuyendo al mantenimiento de presiones más bajas en comparación con las del canal anal [3].

Llenado y vaciado rectal

El recto normalmente permanece libre de contenido fecal del colon sigmoide por el ángulo rectosigmoideo. El aumento del volumen rectal da como resultado un mecanismo de acomodación progresivo. Aproximadamente la mitad de este volumen refluye inmediatamente hacia el colon sigmoide [3].

Motilidad de colon y recto y tiempo de vaciado y tránsito

La motilidad del recto está representada por ondas de contracción llamadas complejo motor rectal y se observan actividades más intensas después de las comidas. La función de estas ondas rectales aún es incierta, pero se cree que contribuyen a un perfecto vaciado rectal. En el canal anal también están presentes gradientes de onda corta, con mayor frecuencia distales, tendiendo a mantener el contenido dentro del recto. Están relacionados con presiones elevadas en reposo y reflejan la actividad del músculo esfínter anal interno [3].

Cojines vasculares

Las almohadillas vasculares hemorroidales y la mucosa del canal anal ayudan a mantener la continencia fecal. Los cojines tienen la capacidad de expandirse para mantener el canal anal cerrado y prevenir la incontinencia cuando la presión anal disminuye. Estos vasos son útiles para llenar espacios que no pueden ser ocupados por la musculatura y pueden contribuir en aproximadamente el 15% de la presión basal en reposo [3].

Mecanismos de relajación anal

Aunque se ha estudiado extensamente, el mecanismo de la defecación se conoce parcialmente. Se sabe que el acto de defecar requiere la perfecta coordinación de la musculatura del suelo pélvico y del esfínter anal, siendo mediada por los impulsos neuromotores y neurosensoriales. La propulsión de las heces a través de la unión rectosigmoidea que hace que el contenido intestinal llegue al recto parece ser el punto de partida para la estimulación de la evacuación, provocando el deseo de defecar. Los receptores de esta sensación de urgencia de defecar se encuentran en el músculo puborrectal [3-5, 15, 16].

La distensión de las paredes rectales por la presencia del contenido fecal genera un reflejo intrínseco a través del plexo entérico que resulta en la relajación del músculo esfínter interno anal también conocido como reflejo inhibitorio rectoanal. Se cree que RAIR juega un papel importante en el mantenimiento de la continencia, ya que facilita el “muestreo” del contenido rectal en el aparato sensorial especializado del canal anal; esto es lo que permite a una persona distinguir entre contenido sólido, líquido y gaseoso [13]. En este momento, el recto puede acomodar el contenido fecal, debido a una disminución de la presión rectal y su capacidad de distensibilidad, contribuyendo decisivamente a la continencia anal [3, 14].

Se ha demostrado que la amplitud y duración del RAIR dependen de la frecuencia y volumen de distensión rectal. Con el aumento de los volúmenes rectales, la presión residual de RAIR disminuirá y la duración del tiempo de RAIR aumentará. El componente principal que contribuye a la relajación durante el RAIR es el esfínter anal interno y, en segundo lugar, el esfínter anal externo [12].

Este está mediado por un plexo intramural neuronal complejo, específicamente neuronas inhibitoras no adrenérgicas y no colinérgicas. Se ha planteado la hipótesis de que varios neurotransmisores median la relajación, como el péptido intestinal vasoactivo (VIP) y adenosín trifosfato (ATP), pero la evidencia más reciente implica al óxido nítrico (NO). La modulación del RAIR se procesa en centros supraespinales y parasimpáticos [12].

Óxido nítrico (NO) y el péptido intestinal vasoactivo (VIP)

Es importante recalcar que el NO es el neurotransmisor principal involucrado en la relajación de IAS. Se puede decir que este afecta directa o indirectamente los RyRs vía S-nitroilación covalente, liderando a un incremento en la probabilidad de apertura de los RyRs. Así que es probable que el NO exhiba un efecto inhibitorio en el músculo liso del IAS que exprese TMEM16A y que además inhiba los canales VDCC tipo L bloqueando de esta forma las SCaOs [6, 7, 13, 17-19].

El NO puede inhibir por mecanismos dependientes e independientes de GMPc o AMPc a las células del músculo liso del IAS. Su efecto sobre los canales TMEM16A puede estar mediado por la inhibición de los RyRs y de los VDCC tipo L o por modulación directa del canal. Por último, el NO puede inhibir los TMEM16A por medio de la proteína quinasa G (PKG) para bloquear los SCaOs en las células de músculo liso del esfínter anal interno, ya que dicho canal puede interactuar con muchas proteínas [6, 7, 13, 17-19].

Varios informes publicados han demostrado que VIP contribuye en parte a la neurotransmisión inhibitoria de nervios no adrenérgicos no colinérgicos (NANC) en el IAS. El sitio más común de acción del VIP es directamente en las células del músculo liso para causar la relajación del músculo liso mediante la activación de la enzima adenilato ciclasa y la producción de AMPc o GMPc. Además, VIP puede activar el receptor VIP (VIPR) en la terminal nerviosa, sitio donde el VIP activa la óxido nítrico sintasa (nNOS) para producir NO que difunde a las células de músculo liso para producir relajación [18].

En ese momento la continencia se mantiene mediante la contracción refleja del músculo esfínter anal externo. Para que se produzca la evacuación, la presión intraabdominal debe aumentar contrayendo los músculos lumbares, recto del abdomen y diafragma (maniobra de Valsalva). Luego, el músculo puborrectal se relaja, junto con los demás músculos del esfínter, permitiendo un aumento del ángulo ano-rectal, resultando en la rectificación del recto con respecto al canal anal y facilitando la evacuación. Tras la expulsión de las heces, ocurre finalmente el reflejo de contracción del músculo del esfínter externo del ano y el retorno del suelo pélvico a su posición normal [3].

Formulaciones erocosméticas: usos y composición

Posibles materias primas y formulaciones en el área de erocosmética

En la cosmética erótica se encuentran formulaciones como lubricantes, aceites y geles para masajes, perfumes y velas con distintas esencias, pinturas comestibles y otros productos que tienen fines prácticos y muy concretos, ya que su propósito es ayudar a aumentar las sensaciones, retrasar el orgasmo y potenciar las erecciones. En la formulación de estos productos se pueden utilizar materias primas que contribuyan a la relajación o contracción del músculo liso del área ano-rectal.

Basados en los anteriores mecanismos fisiológicos, varios métodos han sido desarrollados para producir relajación del esfínter. En el caso de patologías como la formación de una fisura anal crónica, se describe en el artículo *Uso de trinitrato de glicerol en ungüento para el manejo de la fisura anal crónica en el Hospital Nacional Cayetano Here-*

dia, que los últimos métodos tienen la finalidad de disminuir el tono de relajación anal mediante terapias químicas, sin la desventaja de provocar daño muscular permanente, que conlleva una cirugía [20].

El objetivo es restaurar el tono normal del esfínter lo suficiente para permitir la curación de la fisura anal. Aunque se han analizado las preparaciones de administración oral y aplicación tópica, se ha puesto mayor interés en los agentes tópicos debido a su mayor tasa de curación con pocos efectos colaterales [20].

Una de las medicaciones tópicas más usadas es el trinitrato de glicerol (TNG), que incrementa el NO e induce la relajación del esfínter anal interno. Estudios recientes con TNG mostraron resultados muy prometedores con tasas de éxito de curación de fisuras crónicas de 80 a 86%. A todos los pacientes se les prescribió ungüento de TNG al 0,2% concomitantemente al cumplimiento estricto de las medidas higiénico-dietéticas [20].

Los problemas con TNG oral fueron principalmente los efectos colaterales como cefalea y leves mareos, además de la intolerancia y taquifilaxia como obstáculos mayores para la adherencia al tratamiento [20].

El diltiazem es otro fármaco usado para la relajación del esfínter. Es un bloqueador de los canales de calcio que produce relajación del músculo liso en las arteriolas y en el esfínter anal interno. Ha sido usado en forma oral y tópica para el tratamiento de fístula anal crónica (FAC), y ha demostrado disminuir la presión de relajación del esfínter [20].

Aunque se han usado diferentes dosis para ambas formas de preparación, tanto tópica como oral, la administración al 2 %v/d ha mostrado ser mejor que al 1 %v/d y las preparaciones tópicas sobre el 2% demostraron no tener efectos adversos. En un estudio comparando forma oral y tópica, esta última causó una disminución considerablemente significativa de la presión media de relajación anal y fue asociada con tasas altas de curación [20].

En este estudio, el diltiazem oral fue asociado con un riesgo elevado de efectos colaterales como: cefalea, náuseas y defectos de gusto y olfato. Otros estudios han comparado el diltiazem tópico con TNG, y se han encontrado tasas de curación y recurrencia equivalentes, pero menos efectos colaterales y mejor adherencia o tolerancia al diltiazem. Varios autores han encontrado que el diltiazem puede curar aproximadamente el 50% de los pacientes que tienen falla terapéutica con TNG [20]. En conclusión, los agentes tópicos (TNG y diltiazem) actualmente son usados como terapia de primera línea en patologías fisiológicas del esfínter anal [20].

Composición y materias primas (activos y auxiliares de formulación)

Desde un punto de vista de erocosmética, se han desarrollado diversas formulaciones de uso tópico como dilatador o relajante anal, estas formulaciones se utilizaron como referencia para el análisis de sus componentes y la evaluación de su utilidad como relajante o anestésico del esfínter anal. Algunos de estos ingredientes y sus propiedades son las siguientes:

Aloe vera

Según Surjushe, Vasani *et al.*, el nombre botánico del aloe vera es *Aloe barbadensis* Miller. Pertenecer a la familia *Asphodelaceae* (*Liliaceae*). Crece principalmente en las regiones secas de África, Asia, Europa y América. En India, se encuentra en Rajasthan, Andhra Pradesh, Gujarat, Maharashtra y Tamil Nadu [21]. Cada hoja se compone de tres capas: I) Un gel transparente interior que contiene 99% de agua y el resto está compuesto por glucanos, aminoácidos, lípidos, esteroides y vitaminas, II) la capa intermedia de látex que es la savia amarilla amarga y contiene antraquinonas y glucósidos, III) la capa exterior gruesa de 15 a 20 células llamadas corteza que tiene función protectora y sintetiza carbohidratos y proteínas. Dentro de la corteza están los haces vasculares responsables del transporte de sustancias como agua (xilema) y almidón (floema) [21].

El aloe vera exhibe 75 componentes potencialmente activos con sus respectivas propiedades dentro de los cuales se deben mencionar los siguientes:

Vitaminas: contiene vitamina A (betacaroteno), C y E, que son antioxidantes. También contiene vitamina B12, ácido fólico y colina [21].

Enzimas: contiene 8 enzimas: aliiasa, fosfatasa alcalina, amilasa, bradiquinasa, carboxipeptidasa, catalasa, celulasa, lipasa y peroxidasa. La bradiquinasa ayuda a reducir el exceso de inflamación cuando se aplica a la piel por vía tópica, mientras que otros ayudan en la descomposición de azúcares y grasas [21].

Minerales: aporta calcio, cromo, cobre, selenio, magnesio, manganeso, potasio, sodio y zinc. Son esenciales para el correcto funcionamiento de varios sistemas enzimáticos en diferentes vías metabólicas y pocas son antioxidantes [21].

Azúcares: aporta monosacáridos (glucosa y fructosa) y polisacáridos: (glucanos/polimanosa). Estos se derivan de la capa de mucílago de la planta y son conocidos como mucopolisacáridos. El monosacárido más destacado es el fosfato de manosa y los polisacáridos más comunes se denominan glucananos. También se ha encontrado acemanano, un glucomanano prominente. Recientemente, una glicoproteína con pro-

piedades antialérgicas, llamadas alprogen y nuevos compuestos antiinflamatorios como glucosyl cromona, se ha aislado del gel de aloe vera [21].

Antraquinonas: aporta 12 antraquinonas, que son compuestos fenólicos tradicionalmente conocidos como laxantes. La aloína y la emodina actúan como analgésicos, antibacterianos y antivirales [21].

Ácidos grasos: aporta 4 esteroides vegetales; colesterol, campesterol, β sisosterol y lupeol. Todos estos componentes tienen antiinflamatorios y lupeol también posee propiedades antisépticas y analgésicas [21].

Hormonas: auxinas y giberelinas que ayudan en la cicatrización de heridas y tienen propiedades antiinflamatorias [21].

Otros: proporciona 20 de los 22 aminoácidos necesarios para el ser humano y 7 de los 8 aminoácidos esenciales. También contiene ácido salicílico que posee propiedades antiinflamatorias y propiedades antibacterianas. Lignina, una sustancia inerte, cuando se incluye en preparaciones tópicas, mejora el efecto de penetración de otros ingredientes a través de la piel. Las saponinas, que son sustancias jabonosas, forman aproximadamente el 3% del gel y tienen propiedades limpiadoras y antisépticas [21].

Mecanismos de acción del aloe vera

Propiedades curativas: glucomanano, un manósé rico, polisacárido y giberelina, interactúa con los receptores del factor de crecimiento en el fibroblasto, estimulando así su actividad y proliferación, que a su vez aumenta significativamente la síntesis de colágeno después del uso de aloe vera tópico y oral. El gel de aloe además de aumentar el contenido de colágeno de la herida, también modifica la composición de colágeno e incrementa el grado de reticulación del colágeno. Debido a esto, se produce la contracción acelerada de la herida y aumenta la resistencia a la rotura del tejido cicatricial resultante. También se ha observado un aumento en la síntesis de ácido hialurónico y dermatán sulfato en el tejido de granulación de una cicatriz [21].

Acción antiinflamatoria: el aloe vera inhibe la vía de la ciclooxigenasa y reduce la producción de prostaglandina E2 a partir del ácido araquidónico. Recientemente, el nuevo compuesto antiinflamatorio llamado C-glucosil-cromona se aisló de extractos de gel [21].

Hidratante y antienvjecimiento: los mucopolisacáridos ayudan a retener la humedad en la piel. El aloe estimula los fibroblastos que producen las fibras de colágeno y elastina, lo que hace que la piel se vuelva más elástica y menos arrugada. También tiene efectos cohesivos sobre las células epidérmicas descamadas superficiales al pegarlos juntos, lo que suaviza la piel. Los aminoácidos también suavizan las células cutáneas endurecidas

y el zinc actúa como astringente para cerrar los poros. Sus efectos hidratantes también se han estudiado en el tratamiento de la piel seca asociada con la exposición ocupacional donde los guantes de gel de aloe vera mejoraron la integridad de la piel, disminuye la apariencia de arrugas finas y disminuye el eritema [21].

Efecto antiséptico: el aloe vera contiene 6 agentes antisépticos: lupeol, ácido salicílico, nitrógeno ureico, ácido cinámico, fenoles y azufre. Todos tienen acción inhibitoria sobre hongos, bacterias y virus [21].

Manzanilla

Según Ferreira, Vasques *et al.*, *Chamomilla recutita* es una planta de la familia Asteracea. Sus propiedades antiinflamatorias, su actividad antioxidante, antiespasmódica, antialérgica, antihiperglucemiante y antimicrobiana, justifican su uso reconocido como hierba medicinal. *C. recutita* tiene diferentes metabolitos que incluyen anticumarinas, flavonoides y sesquiterpenos. La apigenina es un flavonoide que se encuentra en mayores cantidades en las flores de manzanilla y aporta las propiedades farmacológicas descritas. Se ha reportado el uso de *C. recutita* para diferentes condiciones de la piel, como eccema e irritaciones [22].

Varios estudios han evaluado el uso de diferentes formulaciones con *C. recutita* en reacciones cutáneas agudas debido a causas físicas, químicas o inmunológicas. Las formulaciones que se utilizaron fueron extractos acuosos y alcohólicos, infundidos y cremas hidroalcohólicas que contenían chamazuleno, el nombre comercial de los productos es Kamilloosan. La *C. recutita* se compone de terpenos (bisabolol y chamazuleno), flavonoides (apigenina y cumarinas) y esteroides. Los terpenos y flavonoides actúan mediante pomada a-bisabolol e inhiben la vía clásica del sistema del complemento e interfieren con el metabolismo del ácido araquidónico al inhibir la síntesis de prostaglandinas araquidónicas vía ciclooxigenasa, que justifica la actividad antiinflamatoria de la planta [22].

El efecto de la manzanilla se comparó contra corticoesteroides y se observó un efecto terapéutico superior o similar de la manzanilla. En otro estudio se evaluó la efectividad de la crema hidroalcohólica con extracto de manzanilla al 10% contra una crema cosmética placebo. En el estudio participaron 44 individuos con pitiriasis alba y, al igual que las lesiones del eccema, se observó una alta tasa de respuesta en todos los individuos tratados con placebo y con crema de manzanilla, pero se observaron diferencias significativas entre los tratamientos [22].

Dos formulaciones semisólidas de extractos enriquecidos con apigenina fueron evaluadas para determinar la tolerabilidad y eficacia clínica en dermatitis de contacto. Al con-

siderar la acción antiinflamatoria, los resultados demostraron que la crema liposomal fue ligeramente más eficaz que la crema no liposomal [22].

En un estudio que evaluó el efecto del extracto de manzanilla frente a placebo en la curación de lesiones por dermoabrasión de tatuajes y se observó una reducción del área exudativa de la herida. Los resultados de los estudios analizados en esta revisión indican un buen potencial terapéutico y una buena tolerancia de la manzanilla en el daño cutáneo, independientemente de la formulación utilizada. Cabe señalar que el uso de prácticas complementarias, como los remedios a base de hierbas, puede evitar algunos de los efectos secundarios de las intervenciones terapéuticas convencionales, contribuyendo también a la satisfacción del usuario [22].

En el estudio *Optimisation of the extraction and processing conditions of chamomile (Matricaria chamomilla L.) for incorporation into a beverage*, para el procesamiento de la manzanilla, se menciona que el secado al horno de manzanilla a bajas temperaturas (es decir, 40 °C) seguido de una extracción acuosa a 90 °C durante 20 min. Este método parece ser eficaz para la preparación de un extracto rico en fenoles con baja turbidez, que podría tener potencial como antiinflamatorio [23].

Árnica

En el artículo *Arnica montana L.*, planta medicinal europea con relevancia, se exponen diferentes aspectos relativos a la especie *Arnica montana* (Asteraceae), una importante planta medicinal de uso ancestral. Debido a que se utiliza para aliviar muchos padecimientos y a que tiene una diversidad de aplicaciones, se destacan sus usos medicinales, cosméticos, ornamentales y en perfumería [24].

Es de resaltar que *A. montana* produce numerosos metabolitos secundarios con actividad biológica, como aceites esenciales, ácidos fenólicos, cumarinas, flavonas, flavonoides, glucósidos, mucílagos, fitosteroles, lactonas sesquiterpénicas, taninos y triterpenos, entre otros [24].

A. montana es una planta con importancia económica en numerosos países debido a su uso como remedio medicinal (en fitoterapia humana y veterinaria y en homeopatía), como materia prima para la elaboración de productos cosméticos y en la fabricación de algunos licores europeos. El uso medicinal del árnica proviene de sus características como astringente y estimulante del corazón y del sistema inmunológico; además, alivia el dolor, actúa como agente antiinflamatorio y es útil en las infecciones bacterianas y fúngicas [24].

Usos medicinales y cosmetológicos: las cabezuelas de las inflorescencias, el vegetal completo o el rizoma son utilizados en la industria cosmética. Se recomienda como

afrodisíaco, analgésico, anticaspa, anticatarral, anticonvulsionante antiedematoso, antiespasmódico, antiinflamatorio, antimicrobiano, antiséptico, diurético, estimulante, estimulante de la circulación sanguínea, inductor de la sudoración, en solución para el lavado de ojos, limitador de infecciones, polvo estornudatorio, reductor de inflamaciones, tónico y ungüento oftálmico [24].

Fitoquímica: se han realizado numerosos análisis químicos de *A. montana*, en especial de las hojas, las inflorescencias y el rizoma, a partir de ellos se han logrado aislar varios compuestos orgánicos y en particular se han identificado aceites esenciales.

En el estudio *Effectiveness and Safety of Arnica montana in Post-Surgical Setting, Pain and Inflammation* se indica que *A. montana* se ha utilizado ampliamente como remedio homeopático para el tratamiento de varias afecciones inflamatorias en el manejo del dolor y en situaciones postoperatorias [25].

En este estudio, *A. montana* resultó ser más eficaz que el placebo cuando se usó para el tratamiento de varias afecciones que incluyen dolor postraumático y postoperatorio, edema y equimosis. Sin embargo, las dosis y preparaciones utilizadas han producido diferencias sustanciales en el resultado clínico. La evidencia acumulada sugiere que *A. montana* puede representar una alternativa válida a los medicamentos antiinflamatorios no esteroides, al menos cuando se tratan algunas afecciones específicas [25].

En el estudio *Arnica montana L. A plant of healing: Review* se ha informado que los extractos de plantas poseen actividad antibacteriana, antitumoral, antioxidante, antiinflamatoria, antifúngica e inmunomoduladora. Una amplia gama de compuestos químicos que incluyen lactonas sesquiterpénicas y sus ésteres de ácido carbónico de cadena corta, flavonoides, carotenoides, aceites esenciales, diterpenos, arnidiol, alcaloides de pirrolizidina, cumarinas, ácidos fenólicos, lignanos y oligosacáridos, etc., se encuentran en diferentes partes de la planta [26].

150 sustancias terapéuticamente activas están presentes en la planta *A. montana*, esta tiene lactonas sesquiterpénicas como helenalina, 11a, 13-dihidrohelenalina y sus ésteres de ácido carbónico de cadena corta (0,3-1% del peso seco en las flores, 0,1-0,5% en hojas, también se han visto flavonoides (0,6-1,7%) por cromatografía capilar electrocinética micelar en forma de glucósidos flavonoides, glucurónidos flavonoides y flavonoides agliconas, además de aceites esenciales, compuestos completamente de grasas, ácidos, derivados de timol, monoterpenos y sesquiterpenos [26]. *A. montana* tiene un potencial antiinflamatorio significativo. El mecanismo molecular de lactonas sesquiterpénicas difiere del mecanismo de los AINEs. Estas lactonas disminuyen significativamente la inflamación mediada por NF kappa beta a medida que atraviesan la piel [26].

El mecanismo de fosforilación y degeneración de IkappaB indica que la subunidad inhibidora de NF-kappa beta estimula a NF-kappa beta. La activación de NF kappa-beta por células T, células B y células epiteliales es inhibido por helenalina que a su vez bloquea kappaB impulsando la expresión génica. Este bloqueo es preciso y se debe a la alteración del complejo NF-kappaB/IkappaB, que inhibe la descarga de IkappaB por la helenalina [26].

Capsaicina

Capsicum consiste en los frutos secos maduros de *Capsicum annum Roxb.* (familia Solanaceae), un pequeño arbusto erecto originario de América tropical, cultivado en América del Sur, China, India y África. *Capsicum* contiene un compuesto activo picante y cristalino conocido como capsaicina, trazas de un alcaloide líquido, colorante rojo y un aceite graso [27].

En la medicina popular, el pimiento se considera afrodisíaco, depurativo, digestivo, estomacal, carminativo, antiespasmódico, diaforético, antiséptico, contrairritante, rubefaciente, estípico y tónico. Popularmente, se ha utilizado para tratar el asma, neumonía, diarrea, calambres, cólicos, dolor de muelas, dispepsia flatulenta sin inflamación, insuficiencia de la circulación periférica, dolor de garganta, faringitis crónica y laringitis; externamente se ha utilizado como una loción o ungüento para tratar la neuralgia, incluido el dolor reumático y artrítico y los sabañones intactos (lesiones por frío). [27].

Las preparaciones tópicas de capsaicina al 0,025 y al 0,075% están disponibles para uso humano y producen analgesia según los resultados de estudios aleatorios doble ciego controlados con placebo, ensayos abiertos e informes clínicos. La capsaicina está reconocida por la FDA de EE. UU. como un contrairritante de uso en productos farmacéuticos analgésicos tópicos de venta libre. Se utiliza como componente en varias preparaciones contrairritantes [27].

La capsaicina tópica produce beneficios en la neuralgia postherpética, neuropatía diabética, síndrome de dolor posmastectomía, dolor neuropático oral, neuralgia del trigémino y trastornos de la articulación temperomandibular, cefalea en racimos, osteoartritis y afecciones dermatológicas y cutáneas. Mientras que el alivio del dolor se observa ampliamente en estos estudios, el grado de alivio suele ser modesto, aunque algunos pacientes obtienen un resultado muy bueno. La capsaicina tópica generalmente no se considera una terapia única satisfactoria para las condiciones de dolor crónico y, a menudo, se considera un adyuvante de otros enfoques [27].

Mentol

Los termo-TRPs, según Bharate, son un subconjunto de la familia de canales catiónicos del potencial de receptor transitorio (TRP) que han sido implicados en la detección de temperatura. Los canales TRPM8 y TRPA1 se activan por enfriamiento, en el caso de TRPM8 se activa mediante enfriamiento inocuo ($<30\text{ }^{\circ}\text{C}$) y contribuye a detectar estímulos de frío desagradables o mediar los efectos de la analgesia por frío, este es un receptor de mentol e icilina (compuestos refrescantes sintéticos y derivados de la menta). TRPA1 (familia Ankyrin) se activa por frío nocivo ($<17\text{ }^{\circ}\text{C}$), icilina y una variedad de compuestos picantes [28].

Las corrientes mediadas por TRPM8 son activadas por varios compuestos refrescantes además del mentol e icilina. Estos incluyen p-mentano carboxamidas sintéticas y compuestos alifáticos, alicíclicos, alcoholes, ésteres, amidas, sulfonas, sulfóxidos, sulfonamidas, heterocíclicos, cetoenaminas, lactamas y óxidos de fosfina [28].

En este estudio, se analizó la química medicinal de varios compuestos refrigerantes como activadores del canal termoTRPM8, también se analizó el potencial de estos compuestos para emerger como agentes terapéuticos [28].

La detección de temperatura está estrechamente relacionada con la compuerta dependiente del voltaje en el canal sensible al frío TRPM8 y el canal sensible al calor TRPV1. Ambos canales se activan con la despolarización y los cambios de temperatura que dan como resultado cambios graduales de sus curvas de activación dependientes del voltaje [28]. La activación de las células nerviosas TRPM8 con compuestos refrigerantes bloquea la transmisión de mensajes de dolor. Y por esta razón, la aplicación de compuestos refrigerantes icilina o mentol en la piel de una persona con dolor crónico ha demostrado ser un tratamiento analgésico eficaz. Todos los termoTRP son canales de Ca^{2+} con compuerta que constan de seis dominios transmembrana (TM1-TM6) flanqueados por grandes dominios citoplásmicos terminales N y C [28].

El mentol es un alcohol terpénico cíclico que se encuentra en las hojas del género *Mentha*, se utiliza en una amplia gama de productos, como confitería, dulces, dentífricos, vaporubs y aromaterapia. Entre los 8 posibles estereoisómeros del mentol, solo el enantiómero (-)-mentol posee el olor a menta y propiedades de enfriamiento intenso. Históricamente, el mentol es un agente refrescante bien conocido. Cuando se aplica a bajas concentraciones en la piel o la boca, el mentol provoca una agradable sensación de frescor, mientras que dosis más altas causan ardor, irritación y dolor [28].

El mentol u otros compuestos refrescantes pueden usarse como efectivos analgésicos. Uno de los estudios muestra que (-)-mentol exhibe propiedades analgésicas a través de

la activación selectiva de receptores k-opioides. El mentol puede actuar directamente sobre reservas presinápticas de Ca^{2+} de neuronas sensoriales para liberar Ca^{2+} resultando en la facilitación de la liberación de glutamato y la modulación de la transmisión neuronal en las sinapsis sensoriales. El mentol también se ha informado que activa el receptor GABAA en neuronas del hipocampo [28].

Ésteres de mentol: (-)-lactato de mentilo se extrae del aceite de menta, tiene un ligero olor a menta y es prácticamente insípido, con un efecto refrescante agradable y duradero. Está disponible comercialmente como una masa sólida blanca de bajo punto de fusión que necesita ser descompuesto y derretido físicamente antes de su uso en alimentos o cosméticos [28].

Estas propiedades físicas hacen que este compuesto sea difícil de trabajar. Por consiguiente, se han hecho intentos para proporcionar el compuesto de una manera más conveniente. Estos esfuerzos llevaron a la invención de su forma cristalina, así como su mezcla con mentol carboxamida, que es estable y está presente en forma líquida. Otros ésteres de mentol tales como mentil-3-hidroxibutirato, acetato de mentilo y succinato de mentilo se han utilizado por sus propiedades de enfriamiento en diversos productos aromatizados y perfumados [28].

Junto con el mentol otros agonistas de TRP se han mostrado prometedores como agentes terapéuticos para el dolor, cáncer, enfermedades respiratorias, etc. El mentol y la icilina se utilizan para aliviar el dolor; por lo tanto, varios otros compuestos refrigerantes que actúan a través de la activación del canal iónico TRPM8 tienen un gran potencial para emerger como tratamiento analgésico [28].

DISCUSIÓN

En la cosmética erótica se utilizan diferentes tipos de formulaciones para fines muy diversos que tienen como principal objetivo mejorar las sensaciones y experiencias relacionadas con la sexualidad del ser humano. Algunas de estas formulaciones están destinadas para ser aplicadas en la región ano-genital y contienen materias primas relacionadas con la contracción y relajación del músculo liso de esta región anatómica. Por esta razón, en el presente artículo se mencionan algunas materias primas naturales que pueden ser utilizadas en formulaciones erocosméticas de uso en la región ano-rectal. El papel de estas materias primas es mejorar las sensaciones de las formulaciones que las contienen, por ejemplo, los lubricantes.

Los lubricantes son cada vez más comunes en la vida sexual del ser humano, como su nombre lo indica, cumplen con una función de lubricación de la piel y mucosas. La

lubricación es definida como la separación de superficies en movimiento para evitar el contacto entre sí, lo que reduce la fricción. Esta característica propia de los lubricantes podría jugar un papel positivo en el desarrollo de la penetración por vía anal. Sin embargo, legalmente, la clasificación de dichos productos es un tema complejo. Para clasificar los lubricantes se deben distinguir y diferenciar algunos conceptos: cosmético, cosmeceútico, medicamento y dispositivo médico [29].

De acuerdo con el *Reglamento técnico centroamericano RTCA 71.03.49:08 sobre productos cosméticos. Buenas prácticas de manufactura para los laboratorios fabricantes de productos cosméticos*; un cosmético se define como una sustancia o mezcla de sustancias fabricadas, vendidas o que se propone para ser utilizadas en la limpieza, mejoramiento o alteración del cutis, piel, cabello o dentadura, incluyendo desodorantes y perfumes. Sin embargo, al cosmético que se le adicionen sustancias medicinales que le confieran propiedades terapéuticas, preventivas o de alivio, debe ser registrado como medicamento [30].

Por su parte, según el *Reglamento para inscripción de propaganda de medicamentos y cosméticos N° 24008-S de Costa Rica*, medicamento se define como toda sustancia o productos naturales, sintéticos o semisintéticos, y toda mezcla de esas sustancias o productos que se utilizan para el diagnóstico, prevención, tratamiento y alivio de las enfermedades o estados físicos anormales, o de los síntomas de los mismos, y para el restablecimiento o modificación de funciones orgánicas en las personas o en los animales. Este concepto incluye los alimentos dietéticos y los alimentos y cosméticos que hayan sido adicionados con sustancias medicinales [31].

Además, la Ley general de salud, N.° 5395 de Costa Rica, para efectos legales y reglamentarios, considera medicamentos a los cosméticos que presentados bajo nombre genérico o registrado tengan actividad medicamentosa o tóxica y se destinen a la preservación o modificación de la apariencia personal mediante la alteración o su influencia en la estructura o función de cualquier organismo o tejido del cuerpo humano [32].

Así mismo, el cosmeceútico (cosmético con acción terapéutica) resulta de la intersección de farmacia y cosmética, por medio de aplicación de técnicas como la cromatografía y espectrometría (HPLC, CG-SM, FTIR) y el empleo de extractos de plantas en ensayos *in vitro* e *in vivo*. Este concepto surgió para describir una categoría emergente de productos dermatológicos que proporcionan beneficios terapéuticos sobre la piel, es decir, se definen como una preparación tópica que se vende como un cosmético, pero que tienen una acción farmacológica [33].

A pesar de lo anterior, en la legislación costarricense no se habla o no reconoce este concepto, aunque en la definición de medicamento se incluye el término de cosmético.

Es importante mencionar que, internacionalmente, la *Food & Drug Administration* (FDA) y *The European Cosmetic and Perfumery Association* (COLIPA) tampoco incluyen dicho término en su marco legal [34].

También es posible clasificar como equipo y material biomédico (EMB) al lubricante utilizado en procedimientos clínicos, ya que este se entiende como cualquier instrumento, dispositivo, equipo, material u otro artículo, utilizado solo o en combinación, incluidos los programas informáticos que intervengan en su buen funcionamiento, destinado por el fabricante a ser utilizado en seres humanos con alguno de los siguientes fines: diagnóstico, prevención, control, tratamiento o alivio de una enfermedad; diagnóstico, control, tratamiento, alivio o compensación de una lesión o de una deficiencia; investigación, sustitución o modificación de la anatomía o de un proceso fisiológico; o regulación de la concepción. Siempre que el producto no ejerza su acción principal por medios farmacológicos, inmunológicos ni metabólicos, aun cuando puedan contribuir a estos medios [35].

Luego de repasar los conceptos anteriores y entender que el lubricante puede entrar en algunas de estas categorías, se puede explicar, ubicar o categorizar este tipo de formulaciones como productos *borderline* por oscilar entre estas, dependiendo de su utilización o composición. Según la Unión Europea (UE) mediante la elaboración de un manual sobre el ámbito de aplicación del reglamento de cosméticos se deben evaluar los reglamentos que rigen estos productos acordes a la individualidad de cada caso, teniendo en cuenta todas las características del producto en cuestión [36].

Este manual no debe “prescribir” cual marco regulatorio debe ser aplicado; más sí puede sugerir que el producto en cuestión entre o no dentro del ámbito de aplicación de un reglamento específico. Por tanto, en Costa Rica, los productos como los lubricantes aún tienen trabajo de por medio en el área regulatoria con el fin de homogenizar su legislación [36].

CONCLUSIÓN

El adecuado conocimiento de la fisiología anal permite el análisis, estudio y desarrollo de formulaciones cosméticas de uso en esta área anatómica, por ejemplo los lubricantes anales requieren de características específicas con propiedades analgésicas, anestésicas o relajantes. Dado lo anterior, el estudio de formulaciones en el mercado y productos de origen natural es de utilidad para el desarrollo de nuevas formulaciones erocosméticas.

Ingredientes naturales tales como manzanilla, árnica, aloe vera y mentol han sido estudiados y analizados para su uso cosmético y tópico como analgésicos, anestésicos

o relajantes, por lo que su idoneidad comprobada los hace útiles en el desarrollo de productos erocosméticos como lubricantes anales. La legislación de los lubricantes en Costa Rica puede ser variable acorde a la categoría específica en la que se encuentre según su composición y utilización. Es por esto que los lubricantes pueden a su vez ser denominados productos *borderline*.

RECONOCIMIENTO

Este trabajo de investigación se llevó a cabo durante la asistencia realizada para el proyecto del INIFAR: B9402 *Desarrollo de productos erocosméticos a base de recursos naturales y cumpliendo las buenas prácticas de manufactura*.

CONFLICTO DE INTERESES

Todos los autores declaran que no existe ningún conflicto de intereses.

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CÓMO CITAR ESTE ARTÍCULO

S. Monge-Jiménez, M. Piedra-Robles, V. Portuguese-Solano, D. Fernández-Sequeira, M. Chavarría-Rojas, G.L. Madrigal-Redondo, E. Baltodano-Viales, Revisión de la fisiología anorrectal y posibles compuestos bioactivos para la erocosmética, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1146-1170 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.96862>

In vitro cytotoxicity and *ex-vivo* genotoxicity in compounds from *Waltheria viscosissima* A. St. Hil. Brazilian species of the Malvaceae family *sensu lato*

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Received: January 11, 2022

Corrected: February 20, 2022

Accepted: February 17, 2022

SUMMARY

Introduction: Alternative therapies using medicinal plants and herbal medicines have been growing worldwide, as natural treatments have low cost and less toxic effect compared to conventional drugs available. **Aim:** To evaluate *in vitro* cytotoxicity and *ex-vivo* genotoxicity of Brazilian species of the Malvaceae family: *Waltheria viscosissima* A. St. Hil. *In vitro* methods were used to verify the cytotoxic potential through hemolytic and antihemolytic assays and the *ex-vivo* genotoxic analysis. **Results:** The Crude Etanolic Extract (CEE) and Cloroformic Fraction (CF) was obtained in vegetal sample used on this study; the CEE-Wv and CF-Wv products exhibited the lowest cytotoxic potential with hemolysis less than 40% in all concentrations evaluated. A moderate anti-hemolytic effect was described in all samples treated with 500 and 1000 µg/mL, with hemolysis <60 %. In addition, the compounds showed low *ex-vivo* genotoxic effect with a general index of normal cells greater than 84 % at all concentrations. **Conclusion:** The results suggest a low toxic profile of the compounds obtained from species of the Malvaceae family, indicating safe limits for the use of these natural products.

Key-words: Phytotherapy, hemolytic, antihemolytic, genotoxic.

RESUMEN

Citotoxicidad *in vitro* y genotoxicidad *ex-vivo* en compuestos de *Waltheria viscosissima* A. St. Hil. Especies brasileñas de la familia Malvaceae *sensu lato*

Introducción: las terapias alternativas que utilizan plantas medicinales y medicinas a base de hierbas han ido creciendo en todo el mundo, ya que los tratamientos naturales tienen un bajo costo y un efecto menos tóxico en comparación con los medicamentos convencionales disponibles. **Objetivo:** evaluar la citotoxicidad *in vitro* y la genotoxicidad *ex-vivo* de especies brasileñas de la familia Malvaceae: *Waltheria viscosissima* A. St. Hil. Se utilizaron métodos *in vitro* para verificar el potencial citotóxico mediante ensayos hemolíticos y antihemolíticos y el análisis genotóxico *ex-vivo*. **Resultados:** el extracto de etanol crudo (EEC) y la fracción clorofórmica (FC) se obtuvieron en una muestra vegetal utilizada en este estudio; los productos EEC-Wv y FC-Wv exhibieron el potencial citotóxico más bajo con hemólisis menor al 40 % en todas las concentraciones evaluadas. Se describió un efecto antihemolítico moderado en todas las muestras tratadas con 500 y 1000 µg/mL, con hemólisis <60 %. Además, los compuestos mostraron un bajo efecto genotóxico *ex-vivo* con un índice general de células normales superior al 84 % en todas las concentraciones. **Conclusión:** los resultados sugieren un bajo perfil tóxico de los compuestos obtenidos de las especies de la familia Malvaceae, indicando límites de seguridad para el uso de estos productos naturales.

Palabras clave: Fitoterapia, hemolítico, antihemolítico, genotóxico.

RESUMO

Citotoxicidade *in vitro* e genotoxicidade *ex-vivo* em compostos da *Waltheria viscosissima* A. St. Hil. espécie brasileira da família Malvaceae *sensu lato*

Introdução: as terapias alternativas utilizando plantas medicinais e fitoterápicos vêm crescendo em todo o mundo, pois os tratamentos naturais apresentam baixo custo e menor efeito tóxico em relação aos medicamentos convencionais disponíveis. **Objetivo:** avaliar a citotoxicidade *in vitro* e a genotoxicidade *ex-vivo* de espécies brasileiras da família Malvaceae: *Waltheria viscosissima* A. St. Hil. Métodos *in vitro* foram utilizados para verificar o potencial citotóxico por meio de ensaios hemolíticos e antihemolíticos e o teste de genotoxicidade *ex-vivo*. **Resultados:** o extrato de etanol cru (EEC) e a fração clorofórmica (FC) foram obtidos a partir de uma amostra vegetal utilizada neste estudo; os produtos EEC-Wv e FC-Wv exibiram o potencial citotóxico mais baixo com hemólise menor que 40 % em todas as concentrações avaliadas. Foi descrito um efeito antihemolítico moderado em todas as amostras tratadas com 500 e 1000 µg/mL, com hemólise <60 %. Além disso, os compostos mostraram um baixo efeito genotóxico *ex-vivo* com um índice geral de células normais superior a 84 % em todas as concentrações. **Conclusão:** os resultados sugerem um baixo perfil tóxico dos compostos obtidos das espécies da família Malvaceae, indicando limites de segurança para o uso desses produtos naturais.

ticos e anti-hemolíticos e da análise genotóxica *ex-vivo*. **Resultados:** o extrato etanólico bruto (EEB) e a fração clorofórmica (FC) foram obtidos na amostra vegetal utilizada neste estudo; os produtos EEB-Wv e FC-Wv exibiram o menor potencial citotóxico com hemólise inferior a 40 % em todas as concentrações avaliadas. Foi descrito efeito anti-hemolítico moderado em todas as amostras tratadas com 500 e 1000 µg/mL, com hemólise < 60 %. Além disso, os compostos mostraram baixo efeito genotóxico *ex-vivo* com um índice geral de células normais superior a 84 % em todas as concentrações. **Conclusão:** os resultados sugerem um baixo perfil tóxico dos compostos obtidos em espécies da família Malvaceae, indicando limites seguros para o uso desses produtos naturais.

Palavras chave: fitoterapia, hemolítica, antihemolítico, genotóxico.

INTRODUCTION

Alternative therapies using medicinal plants and herbal medicines have been growing worldwide, as natural treatments have low cost and less toxic effect compared to conventional drugs available. In addition, they also have a strong cultural influence. In Brazil, the popular use of medicinal plants is a common activity, involving inherited knowledge about the potential and specificity of plant species in combating diseases [1].

Herbal medicines have their action and effectiveness based on the way that the bioactive compounds present in the formulas and extracts can act. The pharmacological effect of natural products can be enhanced through synergistic interactions between the substances that compose them, optimizing the therapeutic action and reducing the necessary concentrations, then, consequently, minimizing the toxic effects related to these substances [2, 3].

Among the various Brazilian plant species used in natural therapies, the species of the Malvaceae family stand out, both from a chemical and pharmacological point of view. The species *Waltheria viscosissima* A. St. Hil., known as “malva viscosa”, is used in folk medicine as an expectorant, antitussive and antihypertensive [4].

In this context, the execution of studies on Brazilian natural species applied as herbal medicines in the treatment of diseases contributes to the enrichment of the country's genetic and scientific heritage. Thus, the present study aimed to evaluate the *in vitro* cytotoxicity and *ex-vivo* genotoxicity of Brazilian species of the Malvaceae family: *Waltheria viscosissima* A. St. Hil.

METHODOLOGY

Plant material

The aerial parts of *Waltheria viscosissima* were collected in August 2013, in Santa Rita (Paraíba, Brazil-coordinates 07° 06'59" S and 34° 58'52" W). The plant was identified by Professor Maria de Fátima Agra from the Federal University of Paraíba (UFPB) and, later, registered and deposited at the Prof. Lauro Pires Xavier Herbarium (MF Agra number 21709) from UFPB (João Pessoa, Brazil).

The plant material was dried separately in an air oven at 40 °C, pulverized and macerated with 95 % ethanol (5 L) for 72 hours. The extract solution was evaporated using the rotary evaporator under reduced pressure at 40 °C for the production of Crude Ethanol Extract (CEE). Then, the separation was performed by liquid-liquid chromatography, using hexane, dichloromethane (CH₂Cl₂)/chloroform (CHCl₃), ethyl acetate and n-butanol, which resulted in the respective fractions, dichloromethane (DF) and chloroform (CF), in addition to the hydroalcoholic fraction.

The chloroform fraction (CF) was chromatographed in flash silica using petroleum ether, dichloromethane and methanol individually or in binary mixtures. The resulting polar fractions were chromatographed in Sephadex (LH-20), employing methanol and chloroform: methanol (1:1) as the mobile phase. All samples used were provided by the team of Professor Maria de Fátima Vanderlei de Souza (UFPB) and the procedures for obtaining and structural determination were described in previous works [5]. This research was registered in the Sistema Nacional de Gerenciamento de Recursos Genéticos e Conhecimento Tradicional Associado" (SisGen-A568B8A).

In vitro analysis

Samples of oral mucosa cells and human erythrocytes

The tests were carried out in accordance with the World Medical Association Code of Ethics and approved by the Ethics Committee of the Centro Universitario de Patos (protocol number: 3,621,284). Blood samples (A, B and O) and smears of the oral mucosa were collected from healthy young adults, of both sexes, between 18 and 40 years of age.

Hemolytic activity

Human blood (types A, B and O) was mixed with 0.9 % NaCl (1:30) and centrifuged at 2500 rpm for 5 min. The process was repeated twice, and the pellet from the last centrifugation was resuspended in 0.9 % NaCl to produce a 0.5 % red blood cell (RBC)

suspension, free of leukocytes and platelets. 2 mL of RBC suspensions were treated with CEE-Wv and CF-Wv, in concentrations of 50, 100, 500 and 1000 µg/mL. The samples were incubated for 1 h at 22 ± 2 °C and kept in slow and continuous shaking (100 rpm). Subsequently, they were centrifuged at 2500 rpm for 5 min. Hemolysis was quantified by spectrophotometry at the maximum absorbance wavelength (540 nm) [6]. A RBC suspension was used as a negative control (0 % hemolysis) and another suspension, with 1 % Triton X-100, was used as a positive control (100 % hemolysis). Each test was performed in triplicate and the data were expressed in percentages that represent the arithmetic mean of three measures.

Antihemolytic activity

The evaluation of the osmotic fragility of the erythrocytes was performed with the suspension of erythrocytes 0.5 %. The assay used the concentrations of 50, 100, 500 and 1000 µg/mL of the extract CEE-Wv and Fraction CF-Wv, incubated in 2 mL of RBC suspension 0.5 %, for 1 h, at 22 ± 2 °C. The samples were centrifuged at 2500 rpm for 5 min and the supernatant discarded. The RBC was resuspended in a hypotonic 0.24 % NaCl solution for one hour at 22 ± 2 °C. After this period, the samples were centrifuged at 2500 rpm for 5 min and the hemolysis of the supernatant was quantified by spectrophotometry at 540 nm [7]. The RBC suspension was used as a negative control (0 % hemolysis) and another RBC suspension was added with the hypotonic NaCl 0.24 % solution as a positive control (100 % hemolysis).

Ex-vivo analysis

Genotoxic effects on oral mucosa cells

Epithelial cells were collected on both sides of the oral mucosa by means of a cytobrush (endocervical sample/cell collector), considered the most appropriate instrument for obtaining exfoliated cells from this region [8]. The cells were kept in a tube with 5 mL of 0.9 % NaCl (cell preservation medium) until the slides were prepared. Control cells were divided into two groups: treated with hydrogen peroxide (0.0005 %) (positive control) and those that did not receive treatment (negative control).

The cells were washed twice in saline, centrifuged for 10 min at 1500 rpm, and then kept in 5 mL of saline. The supernatant was removed. The samples were washed once more and exposed *ex-vivo* to the CEE-Wv extract and CF-Wv fraction, in different concentrations (50, 100, 500 and 1000 µg/mL), for 30 min. Then, they were centrifuged and the supernatant discarded. Before preparing the smears, the slides were pre-heated to 37 °C and the cells homogenized in a vortex mixer. They were placed on the slides, dried at room temperature and fixed in methanol: acetic acid (3: 1) for 15 min [9]. The slides were kept at room temperature for 12 hours, after which they were

immersed in distilled water for 1 min and stained in Giemsa 2% for analysis under optical microscopy [10]. Cell toxicity was assessed by the presence of cell indicators such as micronucleus, binucleation, karyolysis, cariorrexis and macronucleus [11, 12]. Approximately 1000 cells were analyzed per slide.

Statistical analysis

The experiments were carried out in triplicate and the results expressed in percentages that represent the arithmetic mean of three measures. Data were analyzed using one-way analysis of variance (Anova) and Bonferroni's post hoc test. The tests were performed using the GraphPadPrism software (version 6.0 for Windows, San Diego, CA-USA). The differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Red blood cells are cells highly vulnerable to toxic substances and free radicals. Changes in the environment cause instability and can lead to the rupture of membranes of this anucleated cell type, which also lacks organelles and mitochondria [13]. Analyses of cytotoxic effects *in vitro* through hemolytic and antihemolytic potential show the percentage of hemolysis defined by Rangel *et al.* [6] as low (0 to 40%), moderate (40 to 80%), and high toxicity, when the percentage is above 80%.

The hemolytic analysis of CEE-Wv showed a low level of hemolysis (< 40%) in all evaluated concentrations (50 to 1000 µg/mL). The CF-Wv had a low to moderate index with hemolysis > 40%, at a concentration of 1000 µg/mL. There was variation in cell lysis according to blood type in the evaluation of substances isolated from *W. viscosissima*, in which hemolysis occurred in the order A < O < B. FC-Wv at a concentration of 1000 µg/mL showed moderate hemolysis for blood type B and O, with lysis < 55% and < 52%, respectively. In relation to blood type A, a low rate of cytotoxicity was demonstrated, with lysis < 30% (figure 1).

In relation to other species of *Waltheria*, toxicity studies using extracts of leaves and roots of species *W. indica* indicated the occurrence of low toxicity. In addition, phytochemical analysis indicated the presence of alkaloids, terpenes and flavonoids (tiliroside, quercetin, epicatechin), which have larvicidal activity [14]. Therefore, the cytotoxic content found in the CEE-Wv and CF-Wv products, isolated from *W. viscosissima*, suggests that these species have similarities in the supply of phytochemicals and in their pharmacological applications.

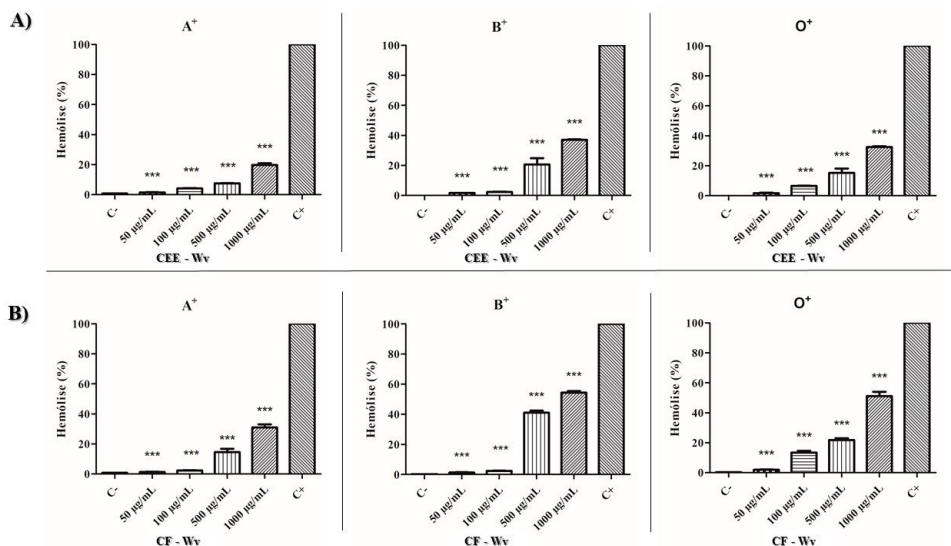


Figure 1. Hemolytic cytotoxic effect of CEE-Wv (A) and CF-Wv (B), products of *W. viscosissima*, against RBC; (C-) Negative control (RBC 0.5%), (C +) Positive control (1% Triton X-100). $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) versus positive control.

The protective effect of the products extracted from the species of the Malvaceae family was verified through the anti-hemolytic potential in red blood cells of the ABO system. The extracts of *W. viscosissima* revealed that the CEE-Wv and CF-Wv showed good potential for stability and protection against hemolysis induced by NaCl 0.24%, at concentrations of 500 and 1000 µg/mL. The lysis index was considered low to moderate (26% to 79%). Blood type B had the lowest rate of hemolysis, and the percentage of lysis occurred in the order $B < O < A$ (figure 2).

The results obtained in the hemolytic and antihemolytic assay showed a slight difference between the substances of the species belonging to the Malvaceae family in the blood types used (A, B and O). However, the variation in the intensity of cell lysis shows a trend of better interaction between the extracts evaluated and the antigenic portion of the red blood cells. The specific monosaccharides on the surface of the erythrocyte membrane are: type A (N-acetylgalactosamine), type B (D-galactose), type AB (has both antigens) and serotype O, which has no antigen on its surface [15].

The evaluation of cellular genotoxicity determines the effect of toxic agents when interacting with DNA, according to their probability of causing chromosomal damage, and structural changes that lead to cell death by necrosis/apoptosis [16]. Tests that analyze the risk of exposure to carcinogenic agents are used to determine the toxico-

logical risk of natural and synthetic substances. In the micronucleus test (comet assay), the exposure of squamous cells from the mucosa, are proposed as a method of assessing chromosomal damage in cells [17, 18].

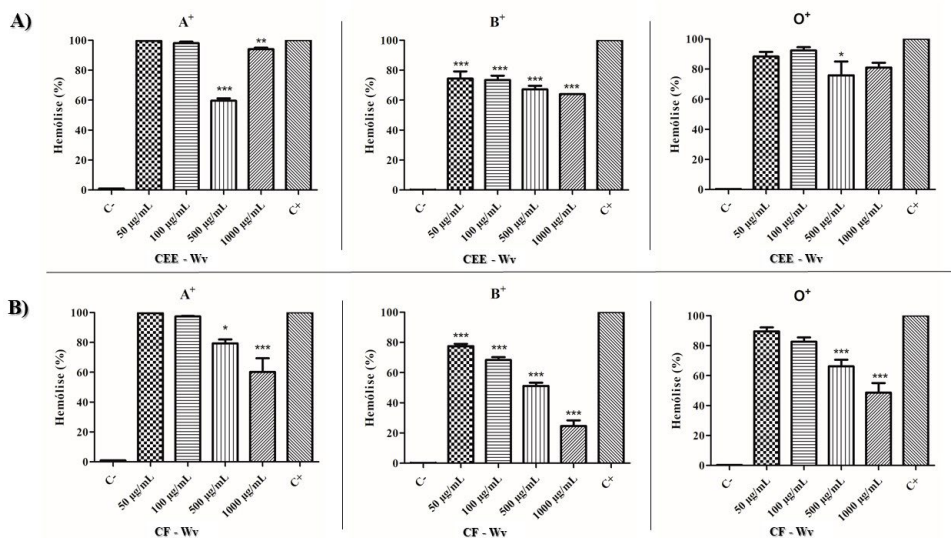


Figure 2. Cytotoxic anti-hemolytic effect of CEE-Wv (A) and CF-Wv (B), products of *W. viscosissima*, against RBC; (C-) Negative control (RBC 0.5 %), (C +) Positive control (1 % Triton X-100). $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) versus positive control.

Oral mucosa cells are important models for assessing DNA damage, as they are one of the first barriers against toxic and mutagenic substances. The analysis of genotoxicity using these cells confers many advantages, such as: primary exposure target, minimally invasive acquisition, monitoring of populations exposed to genotoxic agents, and allows associations between lifestyle [17].

The groups CEE-Wv and CF-Wv induced few cellular changes, when compared to the positive control, and presented a normal cell index $>84\%$ at concentrations of 50 and 1000 $\mu\text{g/mL}$. The main changes presented by these groups were binucleation and macronuclei, cellular modifications compatible with induction of cell division activity. In addition, the CEE-Wv also showed rates of karyolysis compatible with the positive control at a concentration of 1000 $\mu\text{g/mL}$, suggesting induction of cell damage (table 1).

The chromosomal changes that report the presence of macronuclei are more linked to cell activity, in which the process of division can be triggered by some agents, however there is no significant interaction or relationship with DNA. Binucleated cells are originated due to the difficulty in forming the mitotic spindle, caused by the aneugenic

effect of some substances. Therefore, the presence of binucleation is significant to confirm cytological changes, but despite the interference of late events to cell division, no interactions with DNA occur [19, 20]. On the other hand, the occurrence of karyolysis is a nuclear alteration with significant toxicity. According to Tolbert *et al.* [19] and Hollanda *et al.* [17], it is indicative of necrosis and is related to cell death due to cytotoxic events, occurring frequently in inflammatory processes [21].

Table 1. Genotoxic profile of CEE-Wv and CF-Wv.

Group	Micronucleus	Binucleation	Karyolysis	Karyorrhexis	Macronucleus	Normal
C ⁻	2.22 ± 0.11 %	1.88 ± 0.11 %	0.77 ± 0.22 %	0.55 ± 0.22 %	0.01 ± 0.00 %	94.56 ± 0.22 %
C ⁺	4.00 ± 0.88 %	4.33 ± 0.38 %	5.44 ± 0.72 %	5.11 ± 0.67 %	4.55 ± 0.80 %	76.56 ± 3.08 %
CEE-Wv						
1000 µg/mL	1.55 ± 0.22 %	4.66 ± 0.19 %*	5.77 ± 0.88 %*	2.00 ± 0.19 %	1.77 ± 0.11 %	84.22 ± 1.23 %*
500 µg/mL	1.77 ± 0.11 %	4.55 ± 0.48 %*	2.22 ± 0.55 %	1.77 ± 0.44 %	2.55 ± 0.29 %	87.11 ± 0.98 %
100 µg/mL	1.11 ± 0.11 %	2.55 ± 0.40 %	2.11 ± 0.40 %	1.77 ± 0.22 %	1.77 ± 0.22 %	90.67 ± 1.00 %
50 µg/mL	1.11 ± 0.11 %	2.55 ± 0.29 %	1.33 ± 0.19 %	1.22 ± 0.29 %	0.44 ± 0.11 %	93.33 ± 0.19 %
CF-Wv						
1000 µg/mL	1.55 ± 0.11 %	4.66 ± 0.19 %*	1.00 ± 0.03 %	0.77 ± 0.11 %	2.00 ± 0.19 %	90.00 ± 0.33 %
500 µg/mL	0.88 ± 0.11 %	2.33 ± 0.19 %	0.55 ± 0.11 %	0.22 ± 0.11 %	3.11 ± 0.44 %*	92.89 ± 0.67 %
100 µg/mL	0.88 ± 0.11 %	1.66 ± 0.19 %	1.33 ± 0.33 %	0.66 ± 0.19 %	2.11 ± 0.29 %	93.33 ± 0.50 %
50 µg/mL	1.11 ± 0.22 %	1.55 ± 0.11 %	1.22 ± 0.29 %	0.55 ± 0.11 %	2.22 ± 0.29 %	93.33 ± 0.38 %

C⁻: Negative control; C⁺: Positive control; Values are mean ± standard deviation; * p < 0.05 versus positive control.

The products from the species belonging to the Malvaceae family - *W. viscosissima* - revealed to have low *ex vivo* genotoxic potential. The main cellular alterations found were binucleation, macronucleus and karyolysis. In phenolic compounds and flavonoids isolated from other species of the genera described in the present study, anti-tumor and/or antineoplastic activities were observed in the extracts of *W. ovata* [22].

CONCLUSION

The evaluated parameters show that the products of the species *W. viscosissima* showed low cytotoxic and genotoxic potential. The products CEE-Wv and CF-Wv obtained the lowest cytotoxic potential with hemolysis below 40 % in all concentrations evaluated. The moderate anti-hemolytic effect was described in all samples evaluated at concentrations of 500 and 1000 µg/mL, with hemolysis < 60 %. In addition, the compounds evaluated have low *ex-vivo* genotoxic potential, with a general index of normal cells greater than 84 %, even at higher concentrations (1000 µg/mL). The most frequently observed changes were binucleation, macronucleus and karyolysis. The results showed that the natural products analyzed in this study have low toxicity, although a natural component is not free from harm to the organism, since it is used improperly. Thus, studies on toxicity are of great importance, as they can raise awareness about the abuse of medicinal plants.

CONFLICT OF INTEREST

The authors state that there is not conflict of interest.

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HOW TO CITE THIS ARTICLE

A. Pereira de Sousa, M.D. Leite-Ferreira, L. Vilar-Cordeiro, M.d.F. Vanderlei de Souza, H.D. da Silva-Souza, R.d.C. da Silveira e Sá, A. Alves de Oliveira-Filho, *In vitro* cytotoxicity and *ex-vivo* genotoxicity in compounds from *Waltheria viscosissima* A. St. Hil. Brazilian species of the Malvaceae family *sensu lato*, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1171-1182 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100263>

Resistência a compostos quaternário de amônio em patógenos humanos e animais nas últimas décadas: uma revisão sistemática

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Recebido: 8 de setembro de 2021

Revisado: 15 de fevereiro de 2022

Aceto: 21 de fevereiro de 2022

RESUMO

Introdução: compostos quaternários de amônio (QACs) estão disponíveis desde 1908 e são amplamente utilizados como sanitizantes em ambientes diversos. No entanto, a utilização de concentrações subinibitórias de QACs tem sido relacionada com desenvolvimento da resistência microbiana. **Material e métodos:** nesse estudo, a partir de um levantamento teórico em bases de dados da literatura biomédica seguindo as orientações da *Cochrane handbook*, foi realizada uma análise do panorama atual de resistência aos QACs em bactérias de interesse médico e veterinário. Após a seleção dentro dos 1996 artigos encontrados na busca literária, 7 estudos que avaliaram o perfil de susceptibilidade ao cloreto de benzalcônio em bactérias patogênicas foram incluídos. **Resultados:** a análise dos estudos revelou que dentre os potenciais patógenos humanos e animais, a resistência aos QACs é mais investigada em bactérias Gram-negativas tais como *Pseudomonas aeruginosa* e *Acinetobacter baumannii*. Nessas espécies, em alguns isolados foi detectada resistência concomitante aos QACs e antimicrobianos de relevância clínica. Um único estudo mostrou resistência em bactérias Gram-positivas, revelando que o *Enterococcus faecium* apresenta alto potencial de se tornar resistente aos QACs. Dentre os mecanismos de resistência aos QACs, destaca-se a presença do gene *qacE* o qual foi reportado em três dos estudos incluídos. **Conclusão:** apesar da relevância da resistência aos QACs entre bactérias patogênicas, poucos estudos têm sido conduzidos para monitorar

esse evento. Assim, a negligência na vigilância epidemiológica de resistência a esses sanitizantes certamente impactará sua eficácia em um futuro próximo.

Palavras chave: Compostos quaternário de amônio, sanitizante, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecium*, QacE.

SUMMARY

Resistance to quaternary ammonium compounds in human and animal pathogens in recent decades: A systematic review

Introduction: Quaternary ammonium compounds (QACs) have been available since 1908 and are widely used as sanitizers in diverse environments. However, the use of subinhibitory concentrations of QACs has been related to the development of microbial resistance. **Material and methods:** In this study, based on a theoretical survey in biomedical literature databases following the guidelines of the *Cochrane handbook*, an analysis of the current situation of resistance to QACs in bacteria of medical and veterinary interest was carried out. After selection within the 1996 articles found in the literature search, 7 studies that evaluated the profile of susceptibility to benzalkonium chloride in pathogenic bacteria were included. **Results:** The analysis of the studies revealed that among potential human and animal pathogens, resistance to QACs is most investigated in Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. In these species, in some isolates, concomitant resistance to QACs and clinically relevant antimicrobials was detected. A single study showed resistance in Gram-positive bacteria, revealing that *Enterococcus faecium* has a high potential to become resistant to QACs. Among the mechanisms of resistance to QACs, the presence of the *qacE* gene stands out, which was reported in three of the included studies. **Conclusion:** Despite the relevance of resistance to QACs among pathogenic bacteria, few studies have been conducted to monitor this event. Thus, negligence in the epidemiological surveillance of resistance to these sanitizers will certainly impact their effectiveness in the near future.

Key-words: Quaternary ammonium compounds, sanitizer, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecium*, QacE.

RESUMEN

Resistencia a compuestos de amonio cuaternario en patógenos humanos y animales en las últimas décadas: una revisión sistemática

Introducción: los compuestos de amonio cuaternario (QAC) están disponibles desde 1908 y se utilizan ampliamente como desinfectantes en diversos entornos. Sin embargo, el uso de concentraciones subinhibitorias de QAC se ha relacionado con el desarrollo de resistencia microbiana. **Material y métodos:** en este estudio, a partir de un relevamiento teórico en bases de datos de literatura biomédica siguiendo los lineamientos del *Manual Cochrane*, se realizó un análisis de la situación actual de la resistencia a los QAC en bacterias de interés médico y veterinario. Se encontraron 1996 artículos en la búsqueda bibliográfica y en esta revisión se incluyeron 7 estudios que evaluaron el perfil de susceptibilidad al cloruro de benzalconio en bacterias patógenas. **Resultados:** se observó que entre los posibles patógenos humanos y animales, la resistencia a los QAC se investiga más en bacterias Gram negativas como *Pseudomonas aeruginosa* y *Acinetobacter baumannii*. En estas especies, en algunos aislados, se detectó resistencia concomitante a los QAC y a los antimicrobianos clínicamente relevantes. Un solo estudio mostró resistencia en bacterias Gram-positivas, revelando que *Enterococcus faecium* tiene un alto potencial para volverse resistente a los QAC. Entre los mecanismos de resistencia a los QAC, se destaca la presencia del gen *qacE*, reportado aquí en tres estudios incluidos. **Conclusión:** a pesar de la relevancia de la resistencia a los QAC entre las bacterias patógenas, se han realizado pocos estudios para monitorear este evento. Por lo tanto, la negligencia en la vigilancia epidemiológica de la resistencia a estos desinfectantes ciertamente afectará su efectividad en el futuro.

Palabras clave: Compuestos de amonio cuaternario, desinfectante, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterococcus faecium*, QacE.

INTRODUÇÃO

A síntese dos compostos quaternários de amônio (QACs) iniciou em meados de 1890, com a primeira publicação contendo informações sobre atividade antimicrobiana dos QACs datando de 1916 [1]. Contudo somente na década de 1930 é que seu potencial de utilização como sanitizante foi validado [2]. QACs são antimicrobianos de amplo espectro, cuja ação é atribuída à ruptura da membrana celular dos microrganismos. O nitrogênio das moléculas dos QACs é carregado positivamente e atua sobre a mem-

brana citoplasmática de bactérias, fungos (filamentosos e leveduriformes) e vírus envelopados; as quais são carregadas negativamente [3]. Assim, a entrada dos QACs nas células microbianas ocorre por absorção e posteriormente ocorre a reação com as proteínas e os lipídeos presentes, resultando em desorganização da membrana, extravasamento do material intracelular, desnaturação das proteínas e lise da parede celular por enzimas autolíticas. Deve ser ressaltado que a ação destes compostos é mais notória em bactérias Gram-positivas, devido à maior presença de fosfolipídeos e peptidoglicanos na membrana citoplasmática, além de apresentarem parede celular mais espessa, que possibilita a entrada de compostos com alto peso molecular como QACs [4, 5].

Os compostos de amônio quaternário são agentes tensoativos catiônicos. Apresentam estrutura química geral com uma porção catiônica, em que o nitrogênio está ligado covalentemente a quatro grupos alquila ou arila, tornando-o com uma carga final positiva, além de uma porção da molécula aniônica, com carga negativa e formada por cloro ou bromo (figura 1) [6]. O grupo radical alquila posiciona-se entre as moléculas de carbono 8 e 18, e os QACs com maior efetividade são aqueles que possuem de 12 a 16 átomos de carbonos. Algumas variações da molécula, por exemplo, número de átomos de nitrogênio, ramificações da cadeia carbônica e presença de grupos aromáticos, podem potencializar a atividade antimicrobiana destes compostos [7].

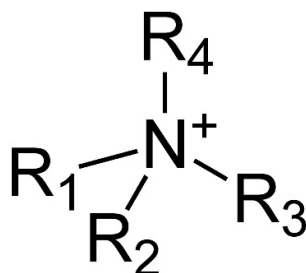


Figura 1. Estrutura geral dos compostos quaternários de amônio (QACs).

QACs são classificados como saneantes e são utilizados para limpeza e desinfecção de domicílios e/ou hospitais. Saneante pode ser definido como qualquer composto, preparação ou substância que tem como funções básicas higienização e desinfecção de vários ambientes tais como domiciliar, públicos e coletivos [8]. Dessa forma alguns QACs como cloreto de benzalcônio, cloreto de cetilpiridínio e brometo de cetiltrimetilamônio, alquilbenzildimetilamônio, dialquildimetilamônio e alquiltrimetilamônio são amplamente utilizados como surfactantes e biocidas em formulações de produtos de limpeza, de higiene pessoal, produtos médicos e formulações de pesticidas [9]. De acordo com Santa Bárbara e colaboradores (2012) [10], dentre os saneantes mais utilizados em ambientes hospitalares estão os QACs e o hipoclorito de sódio.

Assim como observado para a resistência bacteriana aos antimicrobianos, com a ampla utilização de QACs tem sido relatado o desenvolvimento da resistência microbiana a esses compostos. Além disso, resíduos de QACs, os quais exigem condições específicas para sua biodegradação no ambiente, podem ser encontrados em águas residuais de efluentes, cujo efeito cumulativo favorece a resistência bacteriana [11]. É conhecido que a exposição microbiana a quantidades subinibitórias ou contínuas a saneante pode promover a seleção de clones resistentes a estes compostos [12].

O mecanismo de resistência microbiana aos QACs inclui vários processos, tais como perda da osmorregulação da membrana, inibição de enzimas respiratórias, dissipação da força motriz de prótons e estresse oxidativo. Ocasionalmente diversos mecanismos desenvolvem-se concomitantemente durante a adaptação dos microrganismos aos QACs [13]. Um dos principais elementos genéticos de resistência aos QACs é o gene *qac*, o qual pode ser mediado por plasmídeos e codifica uma bomba de efluxo. O gene *qac* codifica proteínas de efluxo da família de resistência a múltiplas drogas (SMR) e são descritos os variantes C, F, G, H, J, Z e E. Em bactérias Gram-negativas, *qacE* e seu variante *qacEΔ1* são os mais frequentes, enquanto *qacA* e *qacB* que codificam proteínas da superfamília de facilitadores principais, são mais encontrados em bactérias Gram-positivas [14, 15]. Mecanismos de resistência mediada por efluxo para os QACs são particularmente preocupantes pois podem conferir co-resistência aos antimicrobianos de uso clínico uma vez que essas proteínas são conhecidamente promíscuas quanto aos seus substratos [13, 16].

Considerando a importância clínica e epidemiológica da resistência aos QACs em bactérias, esse estudo objetivou avaliar através de uma revisão sistemática da literatura a distribuição da resistência a esses agentes em patógenos humanos e animais nas últimas décadas. Além disso, objetivamos avaliar como a resistência aos QACs pode também influenciar o perfil de resistência das bactérias a antimicrobianos de uso clínico. Os resultados descritos aqui poderão contribuir para o conhecimento e implementação de estratégias que visem reduzir a disseminação da resistência aos QACs, o que pode auxiliar na manutenção da sua eficácia como sanitizante químico.

MATERIAL E MÉTODOS

Estratégia de busca

Uma revisão sistemática foi conduzida de acordo com as orientações da *Cochrane Handbook* (*Cochrane Collaboration*, 2011) [17]. As etapas de busca, seleção, extração dos dados de interesse e análise dos resultados observados nos artigos incluídos seguiram as normas do *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*

(PRISMA). Artigos que objetivaram investigar o contexto da resistência aos QACs entre patógenos humanos e animais nas últimas décadas, foram triados a partir de 5 bases de dados biomédicas: Pubmed/Medline, Scopus, Web of Science, Biblioteca Virtual em Saúde (BVS/Lilacs) e Biblioteca Científica Eletrônica Online (SciELO). Os descritores empregados foram definidos pelo *Medical Subject Heading* (MeSH) e Descritores em Ciências da Saúde (DeCS), sendo: “Ammonium Compounds, Quaternary”, “Quaternary Ammonium Compound”, “Ammonium Compound, Quaternary”, “Compound, Quaternary Ammonium”, “Resistance”, “Pathogens”, “Pathogen”.

Os descritores foram combinados empregando o bolearador AND e OR entre eles, sendo inseridos nas abas de busca de cada base segundo as suas características e limitações. Foram estabelecidos como limites artigos em português, espanhol, francês e inglês, sem nenhuma restrição quanto à data de publicação.

Critérios de elegibilidade:

O critério de seleção geral para os estudos obtidos após a busca nas bases de dados previamente definido foi determinado pela estratégia “PVOTS”, como se segue: “**População**”: bactérias Gram-positivas e Gram-negativas patogênicas para homens e/ou animais; “**Variável**”: resistência aos QACs; “*Out come* (desfecho)”: resistência a antimicrobianos de uso clínico; “**Tempo**”: artigos publicados em qualquer ano; “*Study* (estudo)”: estudos observacionais analítico e de vigilância epidemiológica. Para a inclusão dos artigos, foram considerados todos que abordaram a recuperação de bactérias patogênicas apresentando resistência a QACs, seja por mecanismo intrínseco ou adquirido.

Critérios de exclusão

Artigos de revisão, notas, *e-mails*, editoriais, cartas, trabalhos apresentados em eventos científicos e artigos que não apresentavam material original foram excluídos. Além disso, outros artigos foram excluídos com base nos seguintes critérios: (i) isolados que não foram identificados no nível de espécie ou gênero; (ii) estudos que não informaram a origem das amostras das quais os isolados foram obtidos; (iii) trabalhos que não identificaram o país de origem dos isolados; (iv) estudos que não apresentaram o perfil de susceptibilidade microbiana a QACs. No caso de o estudo estar de acordo com os critérios de inclusão, mas o texto original não estiver disponível, o autor correspondente foi contatado por *e-mail* em até 3 vezes (com intervalo de 14 dias entre eles), sendo que o estudo foi excluído se não fosse enviado após o último contato.

Seleção dos estudo e extração de dados

Dois pesquisadores independentes (L.V.B. e M.C.P.) realizaram a busca e seleção dos artigos de acordo com os critérios de inclusão e exclusão estabelecidos. Primeiramente,

foi realizada uma leitura prévia do título, resumo e palavras chave para identificação e pré-seleção dos artigos de interesse. Posteriormente os artigos pré-selecionados foram submetidos a uma leitura completa, tendo em vista a confirmação da inclusão dos mesmos nesta revisão. Discrepâncias encontradas entre os dois pesquisadores foram resolvidas por discussão com um terceiro pesquisador (W.G.L.), chegando a um consenso quanto à inclusão e exclusão dos artigos.

Os artigos selecionados foram submetidos a uma leitura analítica integral para identificar e extrair as variáveis de interesse: referência (primeiro autor e ano de publicação), número de isolados, microrganismos isolados (espécie ou gênero), período de coleta, origem das amostras (humana ou animal), ensaio de suscetibilidade e critérios de interpretação utilizados.

Análise dos dados

Os dados foram resumidos em tabelas para organização das informações extraídas de cada artigo selecionado. Informações referentes à quantidade de microrganismos resistentes aos QACs, ocorrência do mesmo em cada país, além da porcentagem referente a microrganismos QAC-resistentes, foram calculadas de acordo com as informações disponíveis nos trabalhos incluídos.

RESULTADOS E DISCUSSÃO

Após as buscas nas bases de dados até a data de 17 de abril de 2021, um total de 1996 artigos foram encontrados e, após a seleção, 7 estudos foram incluídos nesta revisão. A figura 2 apresenta os detalhes dos artigos encontrados e selecionados considerando os critérios definidos no estudo. Apesar dos saneantes QACs serem amplamente utilizados em todo mundo a partir da década de 1930 [2], este estudo mostra que poucos países desenvolvem pesquisas sobre a evolução da resistência bacteriana a esses compostos.

Neste estudo foi observado que a partir de 2014, dentre os QACs pesquisados, destaca-se a pesquisa com compostos de cloreto de benzalcônio (5/7; 71,4%), seguidos de cetrimida (1/7; 14,3%) e benzetônio (1/7; 14,3%) (tabela 1). De fato, vários autores relatam que o cloreto de benzalcônio é o composto desinfetante e antisséptico mais amplamente utilizado em hospitais. Dessa forma, esse é o QAC para o qual ocorre maior exposição microbiana, o que pode favorecer o desenvolvimento da resistência bacteriana, que deve ser monitorado para preservação da utilização dos mesmos [14, 18-28].

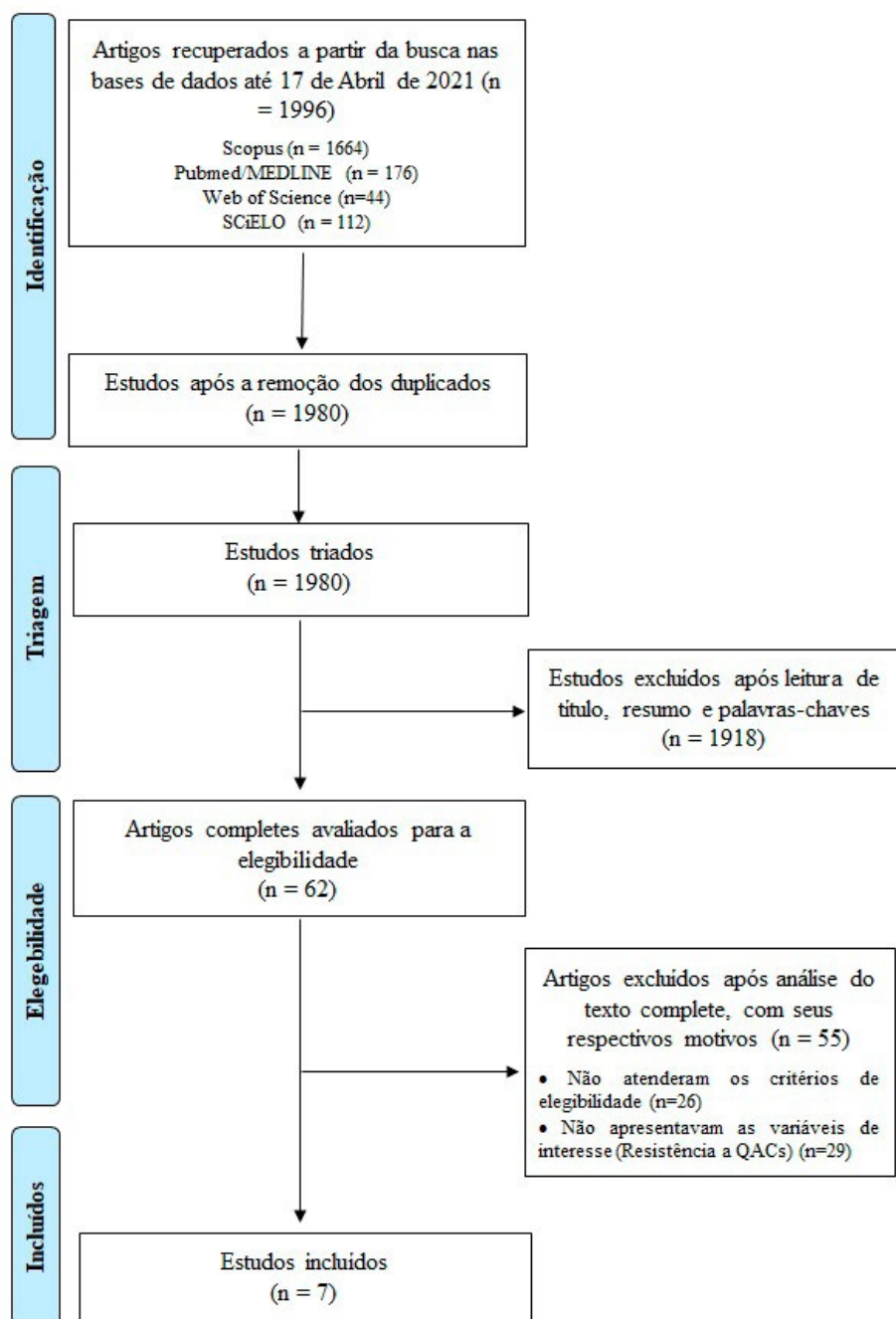


Figura 2. Fluxograma dos artigos selecionados para a revisão sistemática segundo os critérios do PRISMA.

A maioria dos estudos (85 %) incluíram isolados de bactérias Gram-negativas (*Acinetobacter baumannii* e *Pseudomonas aeruginosa*), sendo a maioria destas espécies identificadas por métodos referenciados e indicados na literatura. Importante observar que, a associação de metodologia molecular e fenotípica foi utilizada para identificação microbiológica de *A. baumannii* [14], corroborando a complexidade de definição desta espécie que faz parte do complexo *Acinetobacter calcoaceticus-baumannii* [24].

A. baumannii possui potencial para disseminação e nas últimas décadas ganhou destaque por ser agente de surtos em ambientes hospitalares e pela capacidade de adquirir e desenvolver rapidamente resistência a quase todas as classes de antimicrobianos. É predominante causa de infecções relacionadas à saúde (IRAS) e apresenta grande flexibilidade de sobrevivência ambiental por longos períodos em superfícies bióticas e abióticas [14, 15]. Deve ser ressaltado que nos artigos incluídos nesta revisão, isolados de *A. baumannii* resistentes aos QAC não foram encontrados, mesmo naqueles com fenótipos de multirresistência aos antimicrobianos [15]. De uma certa forma é um dado positivo, porém considerando que a resistência dos microrganismos aos QAC acontece por contato com concentrações subinibitórias por período de tempo relativamente alto, é imperativo o controle e gerenciamento efetivo do perfil de *A. baumannii* [11, 13-15, 25].

Por outro lado, do total de 343 isolados de *P. aeruginosa*, 217 apresentaram resistência ou susceptibilidade reduzida aos QACs, sobretudo ao cloreto de benzalcônio (aproximadamente 50 %). A susceptibilidade e resistência aos QACs em *P. aeruginosa* acontece por mecanismos de efluxo, envolvendo transportadores de cinco superfamílias, o que possibilita a superexpressão simultânea de mais de um sistema de efluxo [26]. Corroborando esse dado, os quatro estudos incluídos nesta revisão que abordaram *P. aeruginosa* também descrevem a resistência aos QACs mediada por bomba de efluxo [18, 19, 26, 27].

Apenas um estudo investigou o perfil de susceptibilidade aos QACs entre Gram-positivos, mais especificamente *Enterococcus faecium*, dos quais 71,4 % (35/49) foram resistentes ao cloreto de benzalcônio (tabela 1). Um dado importante relatado pelos autores [28] é que destes isolados, 33 eram classificados como *Enterococcus* resistentes à vancomicina (VRE), um antimicrobiano de última linha para tratamento de infecções por Gram positivos multirresistentes [29]. Segundo Furtado e colaboradores (2005) [30] tem ocorrido um aumento gradual na resistência à vancomicina em isolados clínicos de *Enterococcus* spp. no Brasil. Esses autores mostraram que em um hospital universitário de São Paulo houve uma progressão da resistência à vancomicina entre *Enterococcus* spp. de 9,5 % no ano 2000, para 14,7 % e 15,8 % nos anos de 2001 e 2002, respectivamente. A espécie *E. faecalis* é a causa da maioria das infecções enterocócicas no geral,

Tabela 1. Principais características dos artigos incluídos na revisão sistemática

Referência	Local e Origem	QAC	Dados dos isolados			Resistência aos QACs	
			Espécie e total de isolados incluído	Método de identificação	Método de avaliação da susceptibilidade	Total resistente (%)	Mecanismo
Beier <i>et al.</i> , 2014 [26]	• EUA • Animal	• Cloreto de benzalcônio • Brometo de etilhexadecil-dimetilamônio • Brometo de hexadeciltrimetilamônio	• <i>Pseudomonas aeruginosa</i> • 175	Automação (Vitek-2)	Microdiluição em caldo	170 (97%)	-
Babaei <i>et al.</i> , 2015 [15]	• Malásia • Humano	• Benzalcônio • Benzerônio	• <i>Acinetobacter baumannii</i> • 122	Teste fenotípicos (AP 20NE)	Microdiluição em caldo	0 (0%)	<i>qacE</i>
Vijaya <i>et al.</i> , 2016 [18]	• Arábia Saudita • Humano	• Cloreto de benzalcônio • Cetrimida	• <i>Pseudomonas aeruginosa</i> • 11	Automação (Vitek-2)	Microdiluição em caldo	2 (18,2%)	-
Alotaibi <i>et al.</i> , 2017 [28]	• Dinamarca • Humano	• Cloreto de benzalcônio	• <i>Enterococcus faecium</i> • 49	Teste molecular (PCR)	Microdiluição em caldo	35 (71,4%)	-
Abdullah and Al-Azzawi, 2018 [27]	• Iraque • Humano	• Cetrimida	• <i>Pseudomonas aeruginosa</i> • 69	Métodos fenotípicos manuais	Microdiluição em caldo	35 (50,7%)	<i>qacE</i>
Osman <i>et al.</i> , 2018 [19]	• Egito • Humano	• Cloreto de benzalcônio	• <i>Pseudomonas aeruginosa</i> • 88	Métodos fenotípicos manuais	Microdiluição em caldo	0 (0%)	-
Ashimi <i>et al.</i> , 2019 [14]	• Malásia • Humano	• Cloreto de benzalcônio • Cloreto de benzetônio	• <i>Acinetobacter baumannii</i> • 100	• Automação (Vitek-2) • Teste molecular (Sequenciamento <i>rpoB</i>)	Microdiluição em caldo	0 (0%)	<i>qacE</i>

enquanto *E. faecium* é mais frequente em infecções adquiridas em ambiente hospitalar, possivelmente associado com as condições mais graves dos pacientes [31]. Esse fato é alarmante e aponta para a necessidade de adoção de medidas de contenção e controle da disseminação desse patógeno entre ambientes de cuidados à saúde.

Deve ser ressaltado que a referência de concentração inibitória mínima (CIM) para classificação do perfil microbiano aos QACs não são encontrados em protocolos tais como *Clinical Laboratory Standards Institute* (CLSI), *European Committee on Antimicrobial Susceptibility Testing* (EUCAST) ou *Brazilian Committee on Antimicrobial Susceptibility Testin* (BRCAST). Assim, os autores dos artigos selecionados utilizaram achados da literatura, por isso faixas de CIM são tão distintas e aqui, a classificação de isolado resistente ou sensível aos QACs seguiu o critério de cada estudo incluído (tabela 1).

Dentre as referências utilizadas para tanto, pode ser citado Sidhu e colaboradores (2002) [32], que consideram resistentes bactérias Gram negativas para as quais a CIM dos QACs é $> 50 \mu\text{g/mL}$, o que foi utilizado por Beier e colaboradores (2014) [26] para classificar *P. aeruginosa* como resistente ao cloreto de benzalcônio (tabela 1). Babaei e colaboradores (2015) [15] relataram que como não há pontos de corte padrão disponíveis para antissépticos contra *A. baumannii*, foi realizado o teste de diluição com concentrações de QAC de 4% a 0,00006% e a suscetibilidade foi interpretada pela turbidez do inoculo comparado ao controle negativo, após incubação a 37°C por 24 horas. Dessa forma verificaram que a CIM de QAC para esta espécie foi muito menor que a concentração utilizada em procedimentos de desinfecção em ambientes hospitalares. No entanto, 73% desses isolados abrigavam o gene *qacE*, alertando para a possibilidade de disseminação bem como de desenvolvimento e acúmulo de mecanismos de resistência que poderiam resultar em ineficácia dos QACs [19]. Por sua vez, A'Shimi e colaboradores (2019) [14] utilizaram uma concentração de QACs que variou de $0,024 \mu\text{g/ml}$ a $50 \mu\text{g/ml}$ com determinação da turbidez medida por leitura em comprimento de onda de 625nm para detecção das CIMs para *A. baumannii*. Com a ausência de padronização para pontos de corte para QACs em bactérias Gram negativas, esses autores realizaram um estudo comparativo com as metodologias referências Babaei e colaboradores (2015) [15] e Vijaya e colaboradores (2016) [18] e observaram concordância dos resultados obtidos, o que após outras pesquisas possibilitará uma padronização dos pontos de corte para QACs.

Resistência microbiana tem sido considerada um grande desafio à humanidade, devido ao seu potencial rápido de disseminação e da indisponibilidade de novos compostos antimicrobianos com ação contra patógenos multirresistentes. De particular preocupa-

ção tem sido a possibilidade de resistência cruzada desenvolvida aos QACs e antimicrobianos de escolha na terapêutica das infecções humanas e animais [19].

Nesta revisão, alguns estudos incluídos abordaram a pesquisa da possível correlação destas resistências. Osman e colaboradores (2018) [19] identificaram 20 isolados com CIM para cloreto de benzalcônio de 2,048 mg/L em isolados de *P. aeruginosa*, os quais foram considerados adaptados a esse composto. Além disso, de acordo com esses autores, o teste de susceptibilidade a antimicrobianos de relevância clínica nestes isolados utilizando o método de disco-difusão mostrou que todos eram resistentes a ceftazidima, cefepime, piperacilina, gentamicina, ampicacina e ciprofloxacina, o que pode estar relacionado com a resistência cruzada. Neste contexto, Vijaya e colaboradores (2016) [18] também relataram a resistência concomitante a cloreto de benzalcônio, ceftrimida e aos antimicrobianos imipenem, meropenem, cefepima, ceftazidima e piperacilina/tazobactam em isolados de *P. aeruginosa*. Também Abdullah e Al-Azzawi (2018) [27] verificaram diferentes taxas de resistência aos antimicrobianos ceftazidima (81 %), cefotaxima (78 %), piperacilina (75 %), ciprofloxacina, tobramicina (74 %), gentamicina (72 %), ampicacina, meropenem (70 %), ofloxacina (66 %) e imipenem (65 %) em seus isolados de *P. aeruginosa*, para os quais alta resistência a ceftrimida (50 %) foram observados. Beier e colaboradores (2014) [26], por sua vez, mostraram uma alta predominância de resistência aos antimicrobianos em *P. aeruginosa* resistente ao cloreto de benzalcônio, com a maioria dos isolados (97,7 %) resistentes a pelo menos 10 antimicrobianos. A prevalência da resistência antimicrobiana entre esses isolados foi de 93,8 %; 93,5 %; 50,3 % e 39,8 %, respectivamente, para β -lactâmicos, sulfonamidas, aminoglicosídeos e quinolonas.

De uma perspectiva *one health*, um conceito de saúde única utilizada a partir de 2008 pela Organização Mundial da Saúde, Organização Mundial da Saúde Animal e Organização das Nações Unidas para Alimentação e Agricultura, conhecer o perfil de susceptibilidade a compostos com ação antimicrobiana é de extrema importância [33]. Isso porque potenciais patógenos expostos aos QACs, por exemplo, em ambientes não clínicos tais como criação de animais e processamento de alimentos, podem alcançar seres humanos e causar infecções, o que poderá ser desafiador devido a possível correlação da resistência aos QACs e antimicrobianos de relevância clínica [34]. Dessa forma, os dados obtidos nesta revisão alertam para a necessidade de mais pesquisas para ampliar o conhecimento dos mecanismos de resistência bacteriana aos QACs bem como da resistência simultânea aos antimicrobianos disponíveis para uso clínico humano e animal, o que poderá contribuir para a preservação da utilização destes compostos.

CONCLUSÃO

Apesar de ser escasso o número de trabalhos sobre resistência bacteriana aos QACs em potenciais patógenos humanos e animais, este estudo indica que a resistência em Gram-negativos é maior, sobretudo em espécies como *P. aeruginosa*. Porém, *E. faecium* QAC-resistentes também foram encontrados o que mostra a possibilidade de desenvolvimento da resistência também em bactérias Gram positivas.

Microrganismos têm sido frequentemente expostos a amplas concentrações de sanitizantes em ambientes diversos, que exercem pressão seletiva e favorecem o desenvolvimento da resistência, possivelmente ao QAC e antimicrobianos de várias classes terapêuticas. No entanto, mais pesquisas devem ser conduzidas no sentido de estabelecer protocolos e padronizações na utilização de QACs, visando monitorar e preservar a eficácia destes compostos bem como de antimicrobianos de relevância clínica.

AGRADECIMENTOS

W.G.L. agradece a Coordenação de Aperfeiçoamento de Pessoal do Nível Superior (Capes) pela bolsa de doutorado. L.V. B. agradece a bolsa concedida ao Programa Institucional de Bolsa de Iniciação Científica (PIBIC/UFSJ) e Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq.

CONFORMIDADE COM OS PADRÕES ÉTICOS

Financiamento: nenhum.

Conflito de interesses: todos os autores relatam que não têm nenhum conflito de interesses.

Aprovação ética: não aplicável.

Contribuição dos autores: todos os autores contribuíram para o desenvolvimento, análise e redação deste artigo.

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COMO CITAR ESTE ARTIGO

L. Valadão-Braga, W.G. Lima, M.C. de Paiva, Resistência a compostos quaternário de amônio em patógenos humanos e animais nas últimas décadas: uma revisão sistemática, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1183-1199 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.98188>

Obtención y evaluación de matrices hidrofílicas para liberación prolongada de metformina

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Recibido: 17 de enero de 2022

Revisado: 3 de marzo de 2022

Aceptado: 12 de marzo de 2022

RESUMEN

Introducción: la diabetes mellitus es un desorden metabólico con alta incidencia a nivel mundial, sin embargo, el tratamiento con tabletas de liberación convencional conlleva la presencia de efectos adversos, así como dosis altas y repetidas. Las formas farmacéuticas de liberación modificada, mejoran el desempeño de los fármacos y el apego a la terapia por parte de los pacientes, por eso son una alternativa útil en el tratamiento de la diabetes. **Objetivo:** fabricar y evaluar matrices hidrofílicas de clorhidrato de metformina empleando proporciones bajas de HPMC con alta viscosidad, para la obtención de comprimidos, que cumplan con los criterios de liberación prolongada de la *Farmacopea* de los Estados Unidos de América (USP). **Métodos:** se fabricaron comprimidos mediante granulación vía húmeda y se caracterizaron mediante diversas pruebas farmacopeicas incluyendo estudios de liberación *in vitro*, con lo que se estudió el mecanismo de liberación mediante el ajuste a modelos matemáticos de Higuchi, Korsmeyer-Peppas y Hixson-Crowell. **Resultados:** la formulación F2 cumplió con las pruebas de calidad farmacopeicas y cumplió con los criterios de aceptación para liberación prolongada USP, mediante difusión tipo Fick. **Conclusiones:** se obtuvieron matrices hidrofílicas para liberación modificada de metformina, empleando proporciones bajas de HPMC como posible plataforma para nuevas formulaciones en el tratamiento de la diabetes.

Palabras clave: Diabetes, metformina, matrices hidrofílicas, HPMC, liberación modificada.

SUMMARY

Obtention and evaluation hydrophilic matrices for prolonged release of metformin

Introduction: Diabetes mellitus is a metabolic disorder with a high incidence worldwide, however, treatment with conventional release tablets entails the presence of adverse effects, as well as high and repeated doses. Modified release dosage forms improve the performance of drugs and adherence to therapy by patients, which is why they are a useful alternative in the treatment of diabetes. **Aim:** To manufacture and to evaluate hydrophilic matrices of metformin hydrochloride using low proportions of HPMC with high viscosity, to obtain tablets that fulfill the prolonged release criteria of United States Pharmacopeia (USP). **Methods:** Tablets were manufactured by wet granulation and characterized by various pharmacopeial tests including in vitro release studies, with which the release mechanism was studied by fitting mathematical models of Higuchi, Korsmeyer-Peppas and Hixson-Crowell. **Results:** Formulation F2 complied with the pharmacopeial quality tests and complied with the acceptance criteria for USP prolonged release criteria, by means of Fick-type diffusion. **Conclusions:** Hydrophilic matrices for modified release of Metformin were obtained, using low proportions of HPMC as a possible platform for new formulations in the treatment of diabetes.

Key-words: Diabetes, metformin, hydrophilic matrices, HPMC, modified release.

RESUMO

Obtenção e avaliação de matrizes hidrofílicas para liberação prolongada de metformina

Introdução: o diabetes mellitus é um distúrbio metabólico com alta incidência mundial, porém, o tratamento com comprimidos de liberação convencional acarreta a presença de efeitos adversos, além de doses altas e repetidas. As formas farmacêuticas de liberação modificada melhoram o desempenho dos medicamentos e a adesão à terapêutica por parte dos pacientes, por isso são uma alternativa útil no tratamento do diabetes. **Objetivo:** fabricar e avaliar matrizes hidrofílicas de cloridrato de metformina utilizando baixas proporções de HPMC com alta viscosidade,

para obtenção de comprimidos que atendam aos critérios de liberação prolongada da United States Pharmacopeia (USP). **Métodos:** os comprimidos foram produzidos por granulação úmida e caracterizados por vários testes farmacopeicos incluindo estudos de liberação *in vitro*, com os quais o mecanismo de liberação foi estudado ajustando-se aos modelos matemáticos de Higuchi, Korsmeyer-Peppas e Hixson-Crowell. **Resultados:** a formulação F2 atendeu aos testes de qualidade farmacopéica e aos critérios de aceitação para liberação prolongada da USP, por difusão do tipo Fick. **Conclusões:** foram obtidas matrizes hidrofílicas para liberação modificada de metformina, utilizando baixas proporções de HPMC como possível plataforma para novas formulações no tratamento do diabetes.

Palavras chave: Diabetes, metformina, matrizes hidrofílicas, HPMC, liberação modificada.

INTRODUCCIÓN

La diabetes mellitus tipo 2 (DM2) es un desorden metabólico con una incidencia creciente a nivel global. Se estima que habrán más de 590 millones de pacientes diagnosticados con DM2 en todo el mundo para el 2035 [1]. La DM2 se considera un desorden metabólico, caracterizado por un aumento de los niveles de glucosa en sangre (hiperglucemia), que es resultado de tres posibles mecanismos: defectos en la secreción de la insulina, defectos en la acción de la misma o la combinación de ambos mecanismos [1]. La DM2 es la forma más frecuente y con mayor impacto sociosanitario, ya que representa alrededor del 90 % de todos los casos de diabetes; su mecanismo va desde una resistencia predominante a la insulina, acompañada con una deficiencia relativa de la hormona, hasta un progresivo defecto en su secreción.

En los pilares del tratamiento de la DM tipo 2 se necesitan dieta y ejercicio, solos o asociados a fármacos orales o insulina. Así, dentro de los fármacos antidiabéticos más conocidos y de primera línea en el tratamiento de los pacientes con DM2 con sobrepeso, se encuentra el clorhidrato de metformina (CM). El CM es un agente anti hiperglucémico oral del tipo biguanida, el cual no estimula la secreción de insulina por las células β -pancreáticas, sino que mejora la sensibilidad a la insulina de los tejidos hepáticos, y de los tejidos periféricos, además de suprimir la gluconeogénesis [2].

El CM de acuerdo al Sistema de Clasificación Biofarmacéutica (SCB) es un fármaco clase III de naturaleza hidrofílica, el cual presenta una absorción en el tracto gastrointestinal lenta e incompleta, que puede presentar una biodisponibilidad oral del 40 al 60 %, además de tener un tiempo de vida media relativamente corto de 1,5

a 4,5 h [3-5]. Es así que las terapias de CM con tabletas o comprimidos de liberación convencional, presentan diversas limitaciones debido a dosis altas y repetidas, ocasionando molestias gastrointestinales como diarrea, náuseas y vómito. Debido a esto, surgió la necesidad de formular comprimidos de liberación modificada. Se ha reportado que las formulaciones de liberación prolongada que puedan mantener los niveles plasmáticos de CM durante 8 a 12 horas, son adecuadas para cubrir la dosificación diaria incluso para dosis altas. Además, mediante el uso de liberación prolongada se logra disminuir los efectos adversos, lo que mejora la aceptación y el cumplimiento de la terapia por parte de los pacientes [2, 6].

Las matrices hidrofílicas han sido ampliamente reportadas para la fabricación de comprimidos de liberación prolongada, pues es una de las tecnologías más simples para obtener de manera eficiente los perfiles de liberación deseados. Las matrices hidrofílicas se obtienen, generalmente, mediante compresión directa o granulación vía húmeda empleando diversos polímeros hidrofílicos, principios activos y otros excipientes, distribuidos de forma homogénea en una red tridimensional. Una de las ventajas de la matrices hidrofílicas es que se pueden emplear formulaciones sencillas y permiten el uso de las tecnologías de compresión que se emplean actualmente en la fabricación de comprimidos, además de que son de bajo costo [7, 8].

Se ha reportado la obtención de matrices a partir de una gran variedad de polímeros hidrofílicos derivados de celulosa como: la metil celulosa (MC), hidroxipropil celulosa (HPC), hidroxietilcelulosa (HEC), carboximetil celulosa (CMC) y hidroxipropil metilcelulosa (HPMC) [7, 8]. El HPMC es un polímero semisintético, no iónico, del tipo éter de celulosa, el cual ha sido ampliamente utilizado como un agente gelificante pH-independiente. El HPMC tiene la capacidad de hincharse, es decir, puede formar una capa gel de alta viscosidad, debido a la relajación las cadenas poliméricas. La capa gel representa una restricción al proceso de difusión del fármaco, lo que permite el control de la liberación durante periodos prologados. Posteriormente, la capa gel se erosiona lentamente hasta disolverse por completo. Así, en las matrices hidrofílicas la liberación se puede modificar mediante las propiedades del polímero como la viscosidad, peso molecular, concentración, etc. [6, 7, 9].

El objetivo de este trabajo fue la formulación y evaluación de matrices hidrofílicas empleando proporciones bajas de HPMC con alta viscosidad, para la obtención de comprimidos, que cumplan con los criterios de liberación prolongada de CM de la *Farmacopea* de los Estados Unidos de América (USP). Además, se analizó la cinética de liberación de las formulaciones empleadas, mediante el uso de modelos matemáticos.

METODOLOGÍA

Materiales

El clorhidrato de metformina DC 95 (CM) se obtuvo de Sinbiotik (Lote CM95-0190619). La hidroxipropil metilcelulosa 90 SH-100000 (HPMC) (lote 6025141), lactosa monohidratada (Lote 20/290198) y estearato de magnesio (Lote 58699) se obtuvieron a través de Shin-Etsu Chemical, Droguería Cosmopolita y Química Lufra, respectivamente. Los disolventes empleados se obtuvieron de J.T. Baker. El fármaco y los excipientes se emplearon tal como se recibieron.

Elaboración de los comprimidos

Se fabricaron comprimidos de CM (1,0 g) mediante granulación vía húmeda de acuerdo a las formulaciones de la tabla 1. Las materias primas se tamizaron (abertura de malla 1,41 mm) y se colocaron en un mezclador en V de acero inoxidable, mezclando durante 10 minutos a 30 rpm (Intertécnica, modelo XV5). Se realizó la humectación manual con agua destilada a temperatura ambiente y se granuló en un granulador oscilante (Erweka FGS AR400). El granulado se secó en una estufa (Impulsora Infisa) a 60 °C, hasta alcanzar la humedad deseada (analizador de humedad Roca DSH-50-10). El granulado seco se tamizó (abertura de malla 1,41 mm) y se añadió el estearato de magnesio como lubricante. Se mezclaron durante 2 minutos a 30 rpm y la mezcla se comprimió en una tableteadora monopunzónica (Korsch) empleando un juego de punzones de acero inoxidable circulares, con cara plana de 10 mm de diámetro.

Tabla 1. Formulaciones de comprimidos de CM.

Componente	Formulación 1 (%)	Formulación 2 (%)
Clorhidrato de metformina	50	50
Lactosa	38	33
HPMC 90 SH-100 000	10	15
Estearato de magnesio	2	2

Caracterización reológica

Se realizó la caracterización reológica del principio activo, los excipientes y los granulados obtenidos para determinar sus propiedades de flujo. Se realizaron determinaciones de densidad aparente y compactada, índice de Hausner, índice de Carr,

velocidad de flujo y ángulo de reposo, de acuerdo a los métodos generales de análisis de la *Farmacopea* de los Estados Unidos Mexicanos (FEUM) [10].

Densidad aparente y densidad compactada

Se pesó una muestra (40 g) y se introdujo sin compactar en una probeta graduada de 100 mL. Se niveló el polvo y se tomó la lectura del volumen sin asentar (V_0). Se cubrió la boca de la probeta y se fijó sobre el soporte. La probeta se levantó a una altura de 10 cm y se impactó 250 veces a ritmo constante. Al finalizar la prueba se tomó la lectura del volumen final por asentamiento (V_f). Se determinaron la densidad aparente y compactada de acuerdo al MGA 1031 método I de la FEUM.

Índice de Carr e índice de Hausner

El índice de Carr y el índice de Hausner se determinaron con la información recabada de la densidad aparente y compactada. Los resultados se interpretaron de acuerdo a la FEUM.

Velocidad de flujo y ángulo de reposo

Una muestra (50 g) se introdujo en un embudo, se dejó fluir libremente, se registró el tiempo y, posteriormente, se midió la altura y diámetro de la base del cono del lecho de polvos. Se calculó la velocidad de flujo (g/s) y el ángulo de reposo (grados); los resultados se interpretaron de acuerdo a MGA 1061 de la FEUM.

Pruebas de calidad en comprimidos

Se evaluó la calidad de los comprimidos obtenidos de las diferentes formulaciones mediante la determinación de la variación de peso, friabilidad, resistencia a la ruptura y tiempo de desintegración, establecidas en la FEUM.

Variación de peso

Se seleccionaron aleatoriamente 10 comprimidos de cada lote desarrollado y se pesaron con exactitud (Balanza analítica, Shimadzu AUX120). Se determinó el peso promedio de cada lote, así como desviación estándar y coeficiente de variación (%).

Friabilidad

Se pesó con exactitud y se determinó el peso promedio de una muestra de 10 comprimidos de cada lote (balanza analítica, Shimadzu AUX120). La muestra se colocó en el tambor de abrasión (friabilizador ELECSA FE30), y se hizo girar a 25 rpm durante 4 min. Finalizado el tiempo, se pesaron nuevamente y se determinó el % de friabilidad de acuerdo al MGA 1041 de la FEUM.

Resistencia a la ruptura

Se determinó la dureza de 10 comprimidos, seleccionados al azar, de cada lote producido. Se colocó cada comprimido de forma diametral entre las dos platinas del durómetro y se ejerció presión hasta la ruptura. Se registraron las lecturas de dureza y se calculó el valor promedio, obtenido en kilopondios (Kp) de acuerdo al MGA 1051 de la FEUM.

Liberación de CM

Se evaluó el perfil de disolución del clorhidrato de metformina, empleando el aparato 2 a $37 \pm 0,5$ °C y 100 rpm (Disolutor Labindia DS8000). El medio de disolución consistió en una solución (1000 mL) de fosfato monobásico de potasio al 0,68 % (m/v) pH 6,8, previamente degasificada [11]. Se tomaron alícuotas de 5 mL del medio de disolución a diferentes tiempos (0, 1, 2, 3, 5 y 7 h), sin reposición del medio. Las alícuotas se filtraron (0,45 μ m), y se analizaron mediante espectrofotometría UV-Vis (Perkin-Elmer 553) a la longitud de onda de máxima absorción ($\lambda_{\text{max}} = 232$ nm), utilizando celdas de cuarzo de 1 cm. La cuantificación de CM se realizó mediante una curva de calibración a partir de una solución Stock, en el intervalo de concentración de 1,009-6,054 μ g/mL. En la prueba de disolución se empleó el criterio de aceptación del método 1 de disolución (711) de la USP conforme a la tabla 2, donde se establecen las tolerancias permitidas, es decir la especificación de la cantidad de CM (%) disuelta en el intervalo de estudio (1,3 y 10 h) [11].

Tabla 2. Tolerancias de cantidad disuelta de Clorhidrato de metformina (método 1 USP) para comprimidos de 500 mg.

Tiempo (h)	Cantidad disuelta (%)
1	20-40
3	45-65
10	No menos de 85

Los resultados obtenidos se ajustaron a diferentes modelos matemáticos para analizar el perfil de liberación empleando DDSolver [12]. El modelo de Higuchi (ecuación 1), es útil para describir la liberación de fármacos a partir de sistemas matriciales. Por otra parte, el modelo de Korsmeyer-Peppas (ecuación 2) permite estudiar el mecanismo de liberación predominante en un sistema mediante el factor n . El valor de n se usa para caracterizar diferentes mecanismos de liberación, de modo que $n = 0,5$ corresponde a la difusión de Fick, los valores de n entre 0,5 y 1,0 corresponden a un transporte no Fickiano (anómalo), $n = 1,0$ corresponde al transporte del caso

II (relajamiento) y $n > 1,0$ refiere a un transporte súper caso II. También, se empleó el modelo de Hixson-Crowell (ecuación 3), que se puede aplicar a formas farmacéuticas como tabletas o comprimidos, en las que la superficie disminuye de forma proporcional y la forma geométrica permanece constante [13, 14].

$$\text{Higuchi} \quad \frac{M_t}{M_\infty} = k_H t^{1/2} \quad \text{Ecuación 1}$$

$$\text{Korsmeyer-Peppas} \quad \frac{M_t}{M_\infty} = kt^n \quad \text{Ecuación 2}$$

$$\text{Hixson-Crowell} \quad W_0^{1/3} - W_t^{1/3} = kt \quad \text{Ecuación 3}$$

RESULTADOS Y DISCUSIÓN

Se determinaron las propiedades reológicas del principio activo y de los excipientes para evaluar su tipo de flujo. La tabla 3 presenta los resultados obtenidos de densidad aparente y compactada, índice de Carr, índice de Hausner, velocidad de flujo y ángulo de reposo. Se observó que el CM presentó propiedades de flujo buenas, de acuerdo al ángulo de reposo (31-35°) e índice de Hausner (1,12-1,18) y propiedades excelentes, de acuerdo con el índice de Carr (5-11). Los excipientes HPMC y lactosa monohidratada presentaron propiedades de flujo de tipo aceptables de acuerdo al índice de Carr y Hausner, así como propiedades buenas a excelentes de acuerdo al ángulo de reposo. La fabricación se realizó mediante granulación vía húmeda, permitiendo obtener gránulos de tamaño de partícula homogéneo y con buenas propiedades reológicas y de compresibilidad. La tabla 4 presenta la caracterización de los granulados obtenidos. Las dos formulaciones presentaron propiedades de flujo y compresibilidad de aceptables a excelentes, lo cual resultó adecuado para la etapa de compresión.

Tabla 3. Resultados de caracterización reológica de materias primas.

Materia prima	Densidad aparente (g/mL)	Densidad compactada (g/mL)	Índice de Carr	Índice de Hausner	Velocidad de flujo (g/s)	Ángulo de reposo (°)
CM	0,580	0,656	11,594	1,131	0,435	31,913
HPMC	0,597	0,800	25,373	1,340	1,190	25,720
Lactosa monohidratada	0,580	0,727	20,290	1,255	0,450	24,827

Tabla 4. Resultados de las pruebas reológicas de los granulados.

Formulación	Densidad aparente (g/mL)	Densidad compactada (g/mL)	Índice de Carr	Índice de Hausner	Velocidad de flujo (g/s)	Ángulo de reposo (°)
F1	0,526	0,615	14,459	1,170	0,886	32,529
F2	0,530	0,635	16,544	1,172	0,953	30,651

Posteriormente, las formulaciones preparadas se comprimieron en una tableteadora monopunzónica (Korsch) y se obtuvieron comprimidos redondos de 10 mm de diámetro, de cara plana, de color blanco con superficie lisa, brillante y libres de partículas extrañas. En los comprimidos se investigó el efecto del HPMC en el tiempo y perfil de liberación del CM en concentraciones de 10 % y 15 %. Los comprimidos obtenidos se caracterizaron mediante diversas pruebas de calidad y los resultados se presentan en la tabla 5. En cuanto a peso promedio y variación de peso, se observó que las dos formulaciones cumplieron con el criterio de aceptación con $CV \leq 5,0 \%$. Los lotes obtenidos, cumplieron con la especificación de friabilidad $<1,0 \%$ y presentaron una dureza adecuada en el intervalo establecido de 8 a 12 Kp, por lo que los comprimidos obtenidos cumplieron con todas las especificaciones.

Tabla 5. Resultados de las pruebas de control de calidad de comprimidos.

Formulación	Peso promedio (g)	Variación de peso (% CV)	Friabilidad (%)	Dureza (Kp)
1	1,036	1,391	0,695	8,629
2	1,024	1,082	0,608	8,875

Para la prueba de liberación, la cuantificación de CM se realizó mediante espectrofotometría UV-Vis. Se construyó una curva de calibración la cual fue lineal en un intervalo de 1,009 a 6,054 $\mu\text{g/mL}$, con un ajuste de R^2 de 0,9968. La prueba de liberación se realizó en una solución de fosfato monobásico de potasio al 0,68 % (m/v) pH 6,8 y 37 °C para simular las condiciones intestinales. La figura 1 presenta los perfiles de liberación de las 2 formulaciones. El perfil de F1 (figura 1a) muestra una liberación inicial rápida obteniendo un % liberado $>40 \%$ en la primera hora, alcanzando el límite de tolerancia máxima (40 %), este efecto Burst se puede atribuir al tiempo necesario para la formación de la capa gel [15]. Posteriormente, se observó una liberación lenta hasta alcanzar el 90 % durante 7 horas. Sin embargo, a partir de las 2 h de disolución, el fármaco disuelto se encuentra fuera del límite superior,

por lo que la F1 no cumplió con la cantidad de fármaco que se debe liberar en los tiempos específicos de acuerdo a la USP. La formulación F2 (figura 1b) presentó una liberación más lenta respecto a F1, debido al incremento de la cantidad de HPMC, alcanzando un porcentaje liberado de ~80 % durante 7 h. En este perfil se logró obtener un recobro del 36,24 % del CM en la primera hora de disolución, y un 62,72 % a las 3 h, que son los tiempos críticos para el cumplimiento de la especificación. Se observó que la formulación F2 se encontró dentro de los límites de aceptación y cumplió con la prueba de disolución.

El uso de polímeros sintéticos, como el HPMC, se ha estudiado como agentes aglutinantes en la granulación vía húmeda de comprimidos, ya que pueden aumentar la cohesividad y mejorar las propiedades de compresión. Generalmente, se recomienda usar concentraciones entre 2 % y 5 % como aglutinante en vía húmeda o seca. Aunque, el HPMC también permite obtener matrices hidrofílicas para liberación controlada de fármacos, para lo cual se recomienda usar concentraciones de 10 hasta 80 %. Sin embargo, se ha reportado que en formulaciones con proporciones de HPMC de alrededor de 30-40 %, ya no se observa un efecto significativo de las propiedades y concentración del polímero en la velocidad de liberación, debido a esto las concentraciones altas del polímero pueden llegar a ser un desperdicio [7]. Diversos autores han reportado el uso de matrices hidrofílicas de CM empleando combinaciones intra y extra granulares de diferentes grados de HPMC, encontrando que el uso de HPMC en una proporción del 26 % pudo mantener la liberación prolongada durante 12 h [2]. Además, otros autores han estudiado la obtención de matrices de CM con diversos polímeros como HPMC, etil celulosa (EC), polivinil pirrolidona (PVP), carragenina, alginato de sodio, y goma arábiga, observando que el HPMC presenta los mejores resultados para prolongar la liberación de forma constante [9]. También, se ha reportado el uso de combinaciones entre diferentes polímeros (HPMC y PVP) o polímeros con diferentes viscosidades (HPMCK4 y HPMCK100M) para lograr controlar la liberación de CM durante 10 h [4]. Otra estrategia que se ha utilizado, es la combinación de polímeros hidrofílicos y materiales hidrofóbicos como el ácido esteárico para controlar la liberación de CM de 8 a 12 h [16]. En este trabajo, la F2 resultó ser una matriz hidrofílica que cumple con los criterios de aceptación de liberación prolongada de la USP, empleando una formulación sencilla con una proporción de HPMC (15 %) menor a los reportados en la literatura que en algunos casos van de 20 a 50 % y no requirió combinaciones de diferentes polímeros [2, 9, 17].

El análisis mediante modelos matemáticos permite describir la liberación de los fármacos, así como los posibles fenómenos físicos que suceden durante la liberación. La aplicación de estos modelos ayuda a elucidar los mecanismos de transporte de masa,

así como la predecir el efecto de los parámetros de diseño en la liberación [18]. En los sistemas matriciales poliméricos, la liberación de los fármacos es controlada por procesos físicos como hinchamiento, disolución del fármaco, difusión, y erosión los cuales dependen de las características del polímero como peso molecular, viscosidad, grado de sustitución, y concentración [19]. Este comportamiento se debe a que, al entrar en contacto con el agua, la matriz se hidrata formando una capa gel, debido a la presencia de agua que actúa como agente plastificante. El agua es capaz de reducir la temperatura de transición vítrea (T_g) de 154-184 °C a 37 °C [7], induciendo el paso del estado vítreo a gomoso. En el estado gomoso hay un incremento en la movilidad de las cadenas poliméricas, así como una expansión de volumen [20]. Se ha reportado que fármacos solubles, como el CM, se liberan principal-

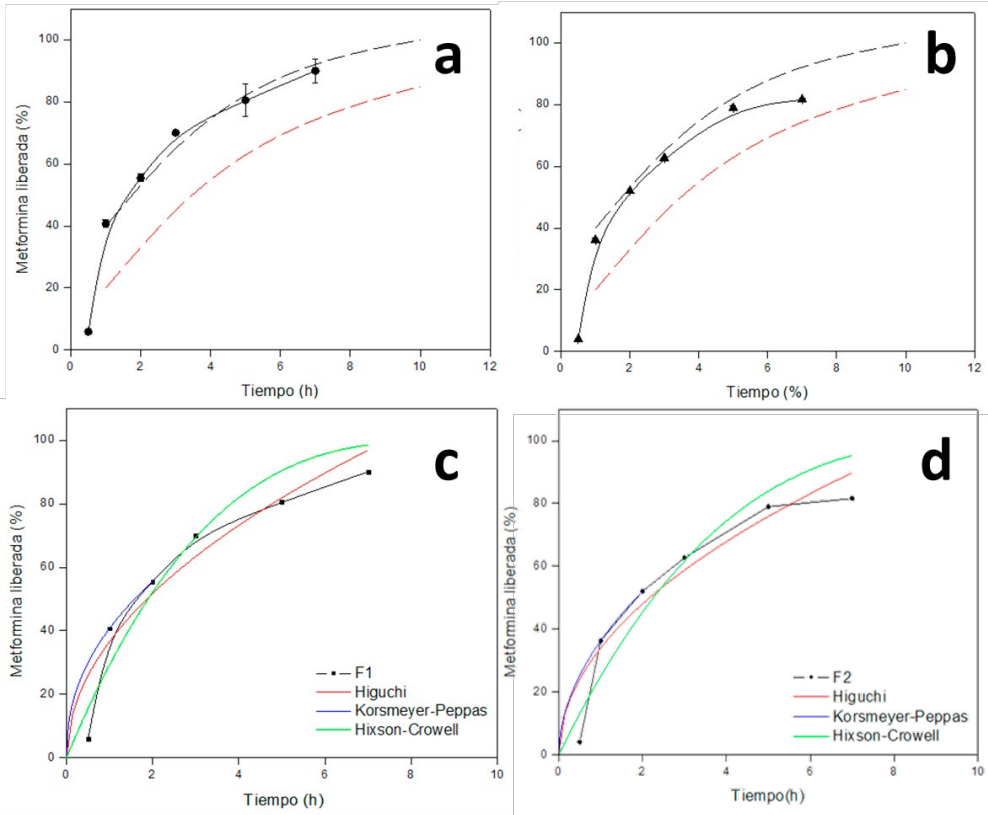


Figura 1. Perfil de liberación de F1 (1a), perfil de liberación de F2 (1b). Los símbolos corresponden a datos experimentales, límite superior (negro) y límite inferior (rojo). El ajuste a modelos matemáticos de F1 (1c) y F2 (1d) de Higuchi (rojo), Hixson-Crowell (verde) y Korsmeyer-Peppas (60 %) (azul).

mente mediante difusión a través dicha capa gel, ya que hay una difusión inicial del medio de disolución al interior de la matriz, disolviendo el fármaco y permitiendo su salida a través de poros o canales en la matriz polimérica. Finalmente, los resultados obtenidos fueron similares a estudios previos reportados en la literatura [9, 21]. Los resultados obtenidos de las 2 formulaciones tuvieron un buen ajuste ($R^2=0,90-0,98$) a los modelos de Higuchi, Korsmeyer-Peppas y Hixson-Crowell (figura 1c y d). Sin embargo, las dos formulaciones presentaron el mejor ajuste ($R^2=0,97-0,98$) al modelo de Korsmeyer-Peppas, en el que el factor $n \leq 0,5$ (0,446-0,521) indica que el mecanismo de liberación predominante es mediante la difusión de Fick, lo cual coincide con el buen ajuste al modelo de Higuchi. Este resultado se debe principalmente al grado de polímero empleado de HPMC SH-100 000, el cual corresponde a un alto peso molecular y alta viscosidad (100 000 cP viscosidad en solución acuosa al 2 %). Generalmente, se ha reportado que las matrices con HPMC de alto peso molecular/alta viscosidad, presentan mayor resistencia a la erosión del polímero, por lo que incluso a bajas proporciones la liberación es lenta y principalmente mediante difusión, especialmente en fármacos de alta solubilidad como CM [7]. En el caso de F2 se observó un ligero incremento en el valor de n y kH , el cual indica el aumento en la restricción para la difusión del fármaco, debido al aumento en la concentración de HPMC. A altas proporciones de polímero, las cadenas se entrelazan formando una estructura con un entrecruzamiento físico, lo cual retarda tanto la entrada de agua como la salida del fármaco por un incremento en la tortuosidad de la matriz [7].

Tabla 6. Parámetros de ajuste a modelos de liberación.

Modelo	Parámetros	F1	F2
Higuchi	R^2	0,961	0,969
	kH	36,607	33,932
Korsmeyer-Peppas	R^2	0,973	0,987
	$WkKP$	40,742	36,236
	n	0,446	0,521
Hixson-Crowell	R^2	0,915	0,906
	kHC	0,109	0,091

CONCLUSIONES

Se fabricaron y evaluaron dos formulaciones de matrices hidrofílicas para liberación prolongada de clorhidrato de metformina empleando HPMC SH-100 000. La formulación F2 con una proporción de 15 % de polímero cumplió con las espe-

cificaciones de la USP para liberación de clorhidrato de metformina en el tiempo estudiado. El fármaco se liberó mediante difusión tipo Fick de acuerdo al ajuste con el modelo de liberación de Korsmeyer-Peppas. La formulación F2 cumple con las especificaciones de liberación y puede emplearse como base para el desarrollo de matrices hidrofílicas con mayor tiempo de liberación.

CONFLICTO DE INTERESES

Los autores manifiestan no tener conflicto de intereses.

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CÓMO CITAR ESTE ARTÍCULO

P. García-Guzmán, G. Calderón-Rodríguez, L. Ortega-Almanza, M.L. Vázquez-Ramírez, L. Schifter-Aceves, Obtención y evaluación de matrices hidrofílicas para liberación prolongada de metformina, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1200-1214 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100434>

Kollicoat MAE® 100P as a film former polymer for nanoparticles preparation

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Received: January 17, 2022

Corrected: March 8, 2022

Accepted: March 16, 2022

SUMMARY

Introduction: Until now, few research works have reported the usefulness of Kollicoat MAE® 100P as a film-former polymer for coating nanocapsules and as a matrix former for nanospheres. **Aim:** To update the current knowledge about the use of Kollicoat MAE® 100P as a film-former polymeric to prepare gastro-resistant nanoparticles. Physicochemical characteristics and functionality of nanoparticles coated with Kollicoat MAE® 100P were reported. **Methodology:** An exhaustive review was performed (from 1980 to 2021) in various scientific databases like Medline, Scopus, EBSCO and Cambridge. **Results:** Kollicoat MAE® 100P is a versatile polymer that can be used to prepare gastro-resistant nanoparticles with actives of natural and synthetic origin. This polymer allows producing homogeneous nanoparticles with sizes smaller than 130 nm, and high z-potential, which confers a great stability to nanoparticle systems. On the other side, nanoparticles coated with Kollicoat MAE® 100P combined with plasticizer exhibit a hard and flexible shell, with excellent thermal stability up to 60 °C that dissolve at pH above 5.5. **Conclusion:** Kollicoat MAE® 100P is a viable, low-cost, and multifunctional alternative for nanoparticle preparation, however, more studies are needed to develop enhanced nanoparticles with better performances.

Key-words: Kollicoat, nanoparticles, acrylates, gastroresistant.

RESUMO

Kollicoat MAE® 100P como polímero formador de filme para a preparação de nanopartículas

Introdução: até hoje, poucas pesquisas têm sido desenvolvidas com o intuito de avaliar a utilidade do Kollicoat MAE® 100P como polímero formador de filme para recobrimento de nanocápsulas e como formador de matriz para preparação de nanoesferas. **Objetivo:** atualizar o estado da arte sobre a utilização de Kollicoat MAE® 100P para a preparação de nanopartículas gastrorresistentes, suas características físico-químicas e a funcionalidade das nanopartículas cobertas com Kollicoat MAE® 100P. **Metodologia:** foi realizada uma revisão exaustiva (de 1980 a 2021) em várias bases de dados como Medline, Scopus, EBSCO e Cambridge. **Resultados:** Kollicoat MAE® 100P é um polímero versátil, que pode ser utilizado para

a preparação de nanopartículas gastrorresistentes carregadas com ativos naturais e sintéticos, com tamanho menor que 130 nm, baixa polidispersão e alto potencial z, o que lhe confere grande estabilidade. O Kollicoat MAE® 100P combinadas com plastificantes adequados, produz cobertas duras e flexíveis, com excelente estabilidade térmica até 60 °C, que dissolvem em pH acima de 5,5. **Conclusões:** Kollicoat MAE® 100P representa uma alternativa viável, de baixo custo e multifuncional para a preparação de nanocápsulas e nanoesferas gastroresistentes. No entanto, outros estudos são necessários para desenvolver nanoformulações baseadas neste polímero com melhor funcionalidade.

Palavras chave: Kollicoat, nanopartículas, acrilatos, gastrorresistente.

RESUMEN

Kollicoat MAE® 100P como polímero formador de película para la preparación de nanopartículas

Introducción: hasta ahora, pocos trabajos de investigaciones han relatado la utilidad de Kollicoat MAE® 100P como polímero formador de película para el recubrimiento de nanocápsulas y como formador de matriz para preparar nanoesferas. **Objetivo:** actualizar el estado del conocimiento sobre las características fisicoquímicas de Kollicoat MAE® 100P, su uso como material formador de películas de cubierta para preparación de nanopartículas gastrorresistentes, y la funcionalidad de las nanopartículas preparadas con este polímero. **Metodología:** se realizó una revisión exhaustiva (de 1980 a 2021) en varias bases de datos como Medline, Scopus, EBSCO y Cambridge. **Resultados:** Kollicoat MAE® 100P es un polímero versátil que puede utilizarse para la preparación de nanopartículas gastrorresistentes usando activos naturales y sintéticos. Este polímero produce nanopartículas menores que 130 nm, bajo índice de polidispersión y potencial z relativamente altos, lo que confiere gran estabilidad a formulaciones de nanopartículas. El Kollicoat MAE® 100P, combinado con un plastificante adecuado, produce una cubierta dura y flexible, con excelente estabilidad térmica a temperaturas hasta 60 °C que se disuelve a pH mayores que 5,5. **Conclusión:** Kollicoat MAE® 100P es un polímero multifuncional, de bajo costo útil para preparar nanopartículas gastroresistentes. Sin embargo, podrían realizarse otros estudios para desarrollar nanopartículas con mejor funcionalidad.

Palabras clave: Biotecnología, nanomedicina, nanomateriales, nanocompuestos.

INTRODUCTION

Nanotechnology, also called the “science of the very small” allows creating nano-sized products and devices (10^{-9} m) with high functionality and specificity. Nanoformulated substances usually have chemical, physicochemical and biological properties different from those in their natural state [1]. In the last years, nanotechnological products have been developed for the treatment of cancer, inflammatory and cardiovascular diseases, diabetes, and obesity, among other conditions [2]. However, the selection of the polymeric material for nanoparticle preparation is a critical phase of this process.

Nanoparticle formulation arises as an effective way to improve solubility and bioavailability of drugs belonging to groups II and IV of the biopharmaceutical classification system (BSC) [3]. The correct selection of the type and quantity of the coating polymer determines important properties such as the site, the release profile, and the drug's physicochemical stability. Coating polymers must be non-toxic, hypoallergenic, and well tolerated by the intended route of administration (biocompatible) [1]. However, the variety of biocompatible film former polymers for nanoparticle preparation is still limited. Among the most used materials for this purpose are synthetic polymers such as those derived from methacrylic acid [4], poly- ϵ -caprolactone, and polylactic acid [5, 6]. Polymers of natural origin such as chitosan, cellulose esters, alginates, and some others are also used [1]. However, synthetic polymers are the most appropriated for nanoparticle preparation. Polymers of natural origin usually produce unstable nanoparticles and make the preparation methodologies less reproducible [5].

Nanocapsules are drugs' carrier systems highly effective. Depending on the type of polymer and the characteristics of the coating layer that it forms, nanocapsules can be vectored towards a specific site (for example the intestine) and the drugs' release can be controlled [5]. Nanocapsules can transport drugs of both chemical-synthetic and natural origins such as plant extracts [7] and isolated and/or purified compounds. However, only some polymers have been shown a wide functionality that allows covering chemical-synthetic and natural-origin drugs, maintaining suitable stability and high functionality.

A versatile polymer with broad functionality that has been little used for nanoparticle preparation is Kollicoat MAE® 100P. This polymer has been used, successfully, to prepare nanoparticles loaded with plant extracts [8], with drugs of chemical-synthetic origin [9], and nanocapsules loaded with a drug isolated from a plant oilresin. Thus, this review aims to update the state of knowledge about the physicochemical characteristics of the Kollicoat MAE® 100P, and its use as a film former polymeric material for preparing gastro-resistant nanoparticles. Physicochemical characteristics and the function-

ality of nanoparticles prepared with Kollicoat MAE® 100P were also reviewed. Thus, an exhaustive review (from 1980 to 2021) in various scientific databases like Medline, Scopus, EBSCO, and Cambridge, was performed.

Nanocapsules

Nanocapsules are nano-sized vesicular particles (figure 1) with a polymeric layer surrounding a nucleus containing the drug [5]. When the nucleus is an oily liquid, they are called nanocapsules, but when the nucleus is a solid lipid, they are called solid lipid nanoparticles [10]. Nanocapsules with an aqueous liquid core are called polymersomes and are prepared using amphiphilic block copolymers [11]. The main characteristic of nanocapsules is the perfect differentiation between the coating shell and the core material, contrary to nanospheres, where the polymeric material and the drug encapsulated form a matrix or lattice where it is impossible to differentiate the shell from the core [5].

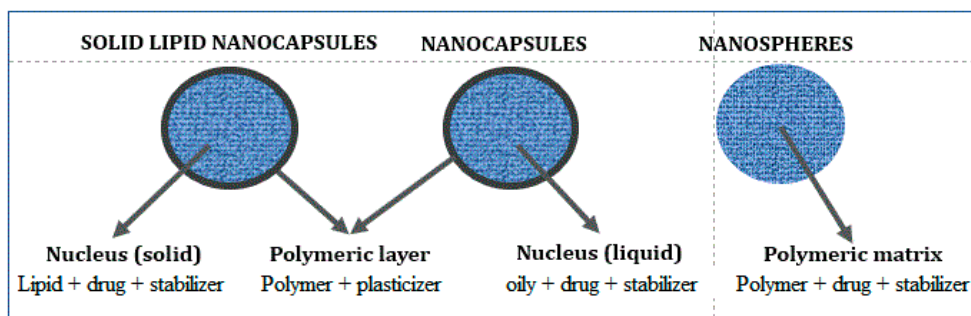


Figure 1. Physical structure of solid lipid nanocapsules, nanocapsules and nanospheres.

The proper selection of the preparation method, the core components, and the coating polymer (and plasticizers) allows preparing the desired nanoparticle [5, 9]. The nucleus of the nanoparticle can contain vegetal extracts, drugs from the chemical-synthetic origin and/or isolated compounds. In all cases, the appropriate selection of the nucleus component and the covering material (polymer and plasticizers) makes it possible to vectorize the drug to a specific site of action and controlling the amount and rate of drug release. The preparation of polymeric nanoparticles from liquid plant extracts (fluid and soft) generally produces nanospheres.

A suitable polymer for nanoparticle preparation must protect the drug from external factors like temperature, light, and O₂. This means that the polymeric layer needs to improve the nanoparticle's stability [7]. Physicochemical properties of polymers (e.g., acid-base behavior, solubility, and lipophilicity) determine nanocapsules stability and the drugs' release characteristics. A precise knowledge of the physic-chemical proper-

ties of the film former polymer allows knowing, in advance, the possible site of release and the possibility of controlling the drug release processes.

Copolymers of methacrylic acid-methacrylate esters are among the most used synthetic materials for the preparation of nanoparticles loaded with drugs of reduced solubility [12]. Within this group of polymers is the class of Kollicoat MAE® copolymers [13], which currently have their greatest application in the preparation of enteric-coated tablets. However, Kollicoat MAE® -grade polymers, despite their excellent properties for nanoparticle preparation, have been little used for this purpose.

Kollicoat classes

There are five classes of Kollicoat on the market: Kollicoat IR, Kollicoat Protect, Kollicoat MAE®, Kollicoat SR, and Kollicoat Smartseal [14]. The chemical composition and utility of these materials are shown in (table 1). Kollicoat IR class are copolymers of vinyl alcohol grafted with ethylene glycol [13]. Kollicoat Protect is a mixture of Kollicoat IR (~60%) and polyvinyl alcohol (40%) and has been claimed for moisture and oxygen protection [13]. Kollicoat SR 30D class was designed for sustained-release formulations being an aqueous dispersion of polyvinyl acetate and polyvinyl pyrrolidone (K30) [15]. By their side, Kollicoat Smartseal 100 P, is a mixture of diethylamino ethyl methacrylate and methyl methacrylate (3:7). Kollicoat Smartseal 30D is a 30% aqueous dispersion of Kollicoat Smartseal 100P. This polymer is insoluble at neutral pH but soluble at pH<5 being suitable for taste masking and moisture protection [13]. Kollicoat MAE® class includes three polymers with similar composition: Kollicoat MAE® 100 55, Kollicoat MAE® 30DP (30% aqueous dispersion of Kollicoat MAE® 100 55), and Kollicoat MAE® 100P (pre-neutralized Kollicoat MAE® 100-55). All these polymers are used in the Pharmaceutical Industry to prepare enteric-coated pellets for hard capsules and tablets [16]. Kollicoat MAE® 100P has shown great utility for preparing gastro-resistant nanoparticles, nonetheless, this functionality has not been claimed in the literature.

Physicochemical characteristics

Kollicoat MAE® 100P (figure 2) is an anionic polymer, with a weak acid behavior having a molecular weight of about 250 000 AMU. This polymer dissolves in solutions of pH> 5.5 [21]. Kollicoat MAE® 100P contains polysorbate 80 as a plasticizer to diminish the friability of the film formed for tablet preparation [14]. However, additional plasticizers must be used to obtain a flexible and more stable coating layer for nanoparticle preparation. This material also contains sodium dodecyl sulfate as an emulsifier. Kollicoat MAE® 100P is partially neutralized (about 6 mol-% of the carboxyl groups) to facilitate its redispersion in water. This characteristic is especially

Table 1. Class of Kollicoat®, composition and their use in pharmaceuticals.

Kollicoat Class	Type	Composition	Use	Reference
Kollicoat IR	IR	Polyethylene glycol-Polyvinyl alcohol ratio of 1:3	-Coating tablets and capsules -Binder in tablets, granules, and pellets -Effervescent tablets -Sustained-release tablets	[15]
	Pro- tect	Kollicoat IR, 59.70 % Polyvinyl alcohol, 40% Silicon dioxide, 0,3 %	-Protective coating of tablets and capsules -Tastes masking	[15]
Kollicoat MAE®	30D	Methacrylic acid-ethyl acrylate co-polymer (1:1), 27.0 % Polysorbate 80, 2.3 % Sodium lauryl sulphate, 0.7 % Water, 70.0 %	-Preparation of enteric tablets, capsules, granules, and crystals -Mask unpleasant tastes -Protect from the effects of high humidity	[15] [17]
	100 55	Methacrylic acid-ethyl acrylate copolymer (1:1), 97.0 % Polysorbate 80, 2.3 % Sodium lauryl sulphate 0.7 %	-Preparation of enteric tablets, capsules, granules, and crystals -Mask unpleasant tastes	[15] [17]
	100P	Methacrylic acid-ethyl acrylate copolymer (1:1), partially neutralized with 6 mol % NaOH, 97.0 % Polysorbate 80, 2.3 % Sodium lauryl sulphate, 0.7 %	-Protect from the effects of high humidity -Nanocapsules film former -Nanoparticles controlled release	[18, 19]
Kollicoat SR	30DP	Polyvinyl acetate, 27.0 % Povidone K30 (Kollidon 30), 2.7 % Sodium lauryl sulphate, 0.3 % Water, 70.0 %	-Sustained release matrix tablets, pellet, and granules, and gelatin hard capsules	[15] [20]
Kollicoat SmartSeal	30DP	Methyl methacrylate and di(ethyl)aminoethyl methacrylate (7:3) copolymer. 30% aqueous polymer dispersion	-Aqueous film coating	[15] [20]
	100P	Methyl methacrylate and di(ethyl)aminoethyl methacrylate (7:3) copolymer (spray dried powder)	-Aqueous, and organic film coating	[15]

useful for preparing nanodispersion of vegetal extracts. Kollicoat MAE® 100P complies with the current monograph of methacrylic acid-ethyl acrylate copolymer (1:1) [22] and is listed as “Partially neutralized methacrylic acid: ethyl acrylate copolymer” in the USP-NF [21].

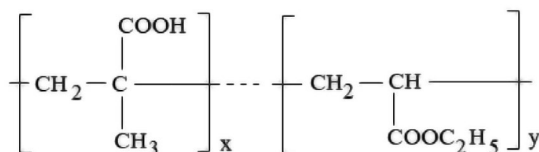


Figure 2. Basic structure of Kollicoat MAE® 100P copolymers formed by methacrylic acid: ethyl acrylate ($x=y$).

Kollicoat MAE® 100P forms aqueous dispersions due to is pre-neutralized with sodium hydroxide, without requiring a further addition of a basic solution [13, 16]. This polymer is soluble in ethanol and acetone (at 35-45 °C) in which forms a slightly viscous solution [16]. This property is used to prepare the organic phase for nanocapsules preparations. For nanocapsules, the organic phase (25-35%) is formed by Kollicoat MAE® 100P, the drug, the organic solvent, and a low HLB surfactant. When the organic phase is added to the aqueous phase (65-75%), which is composed of water plus a nonionic surfactant or another plasticizer, the Kollicoat MAE® 100P insolubilize enclosing the components of the organic phase for forming the nucleus. The change in polarity when the phases are mixes, followed by diffusion of the organic solvent (by stirring or by evaporation under vacuum) get hardened the polymeric shell around the nucleus containing the drug, to form the nanoparticle [23]. This procedure allows preparing nanocapsules loaded with water-insoluble drugs, such as loratadine [9] and α -amylin [24].

Kollicoat MAE® 100P, when dispersed in water (at 20%) form stable particles with a mean diameter of approximately 300 nm [13]. However, the aqueous dispersion (2%) submitted at a high shear of 7000 rpm, for five minutes in an ultraturrax apparatus (IKA T25, UK) produce nanoparticles of ≈ 50 nm (figure 3) with a normal size distribution (Polydispersity index 0.198) [13, 16]. The excellent dispersibility of this partially neutralized polymer in aqueous solutions is an excellent characteristic for nanoparticle preparation that is still little explored.

Kollicoat MAE® 100P forms a brittle film that breaks at an elongation under one percent [13]. For this reason, plasticizers are essential for producing a coating layer with suitable elasticity to achieve good stability. Hydrophilic plasticizers like propylene glycol, triethyl citrate (TEC), as well as non-ionic surfactants (e.g., Brij, polysorbates, and

ethoxylated fatty acid esters), favor the elasticity of the coating layer and improve the stability of the nanocapsules shell. Hydrophilic plasticizers give flexibility and resistance to the coating layer and enhance the dispersion in the aqueous medium, favoring the interaction with the polymeric chain of the Kollicoat MAE® 100P [13].

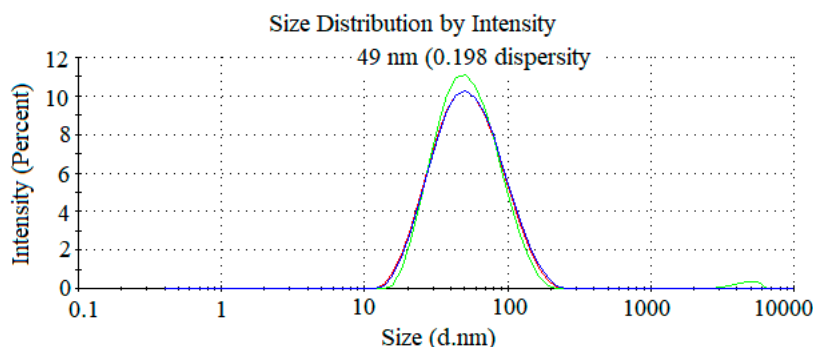


Figure 3. Particle size of 2 % Kollicoat MAE® 100P aqueous nanodispersion (authors' unpublished work).

Nanoformulations with Kollicoat MAE® 100P

The nanodispersion of *Cassia grandis* L. f [19] was developed using the polymer deposition-solvent diffusion method [18]. This methodology allowed obtaining a mean particle size of 75 nm, with a closed polydispersity index of 0.176, and a zeta potential of -11.20 mV. With the same methodology, it was prepared nanoparticles of loratadine, a chemical-synthetic drug [19], which exhibited a mean particle size of 43 nm, with a polydispersity index of 0.250 and -19.30 mV of zeta potential. In the same way, it was prepared nanocapsules loaded with α -amyirin (a hydrophobic drug obtained by isolation and purification from a vegetal oilresin [24]). Nanocapsules loaded with α -amyirin showed particle size of 130 nm, polydispersity index 0.100, and -38.65 mV of zeta potential. The abovementioned nanoformulations showed small particle sizes (between 43 and 130) with normal size distribution and high modular values of zeta potential. These results suggest that Kollicoat MAE® 100P is a versatile polymer, which allows preparing nanoformulation loaded with drugs from diverse origins, resulting nanosystems with homogeneous sizes, which is essential for achieving good stability.

Properties of Kollicoat MAE® 100P loaded nanoparticles

The development of nanoparticles loaded with plant extracts is considered a technological challenge. Plant extracts are complex mixtures of metabolites that present different characteristic of solubility, permeability, and stability [1]. The development of nano-

particles containing plant extracts improves the pharmacological effect of the extracts enhancing the stability and allows optimize their use since lower doses are needed to achieve the desired effect. The use of biocompatible polymers to produce a coating layer to protect metabolites present in the extracts by the formation of nanocapsules or a polymeric matrix (nanospheres), is an innovative and highly effective technology to improve bioavailability and stability of medicinal plant extracts, which, however, is still sub explored.

Prada and co-workers [8, 25] prepared nanoparticles of *Cassia grandis* (a medicinal plant traditionally used for the treatment of Diabetes mellitus, an anemias) coated with Kollicoat MAE® 100P. In that study, three nanoparticle formulations were developed using three different film former polymers (Eudragit® L, PEG 4000, and Kollicoat MAE® 100P). The nanoformulations particle size, polydispersity index, and zeta potential were compared, as well as the stability. Nanoparticles coated with Kollicoat MAE® 100P showed the smaller particle size, a lower polydispersity index, and the higher modular value of Zeta potential. The encapsulation efficiency of the process was greater than 85 % [25] and the loading capacity was 73 %. Kollicoat MAE® 100P coated nanoparticles showed the best performances showing the ability of this polymer to produce nanoparticles loaded with vegetal extracts.

Several studies demonstrated the stability, in the stomach environment, of nanoparticles coated with Kollicoat MAE® 100P [9, 24, 26]. Drugs with erratic absorption in the stomach, or that undergo transformations that reduce their bioavailability need to be formulated, so that their release and absorption occur in the intestine. Loratadine is a drug that belongs to group II of the BSC classification system [26]. This drug is protonated in the acidic environment of the stomach, decreasing its absorption [9]. For this reason, a nanoparticle formulation for intestinal release of loratadine was prepared using Kollicoat MAE® 100P as a coating polymer [9]. Very stable nanoparticles, ≈75 nm in size and with a normal distribution were obtained. An encapsulation efficiency greater than 84% was obtained. This study confirmed the suitability of Kollicoat MAE® 100P as a gastro-resistant shell-forming polymer in the preparation of nanoparticles, a characteristic that had not been reported in the literature.

Effect of temperature on nanoparticles coated with Kollicoat MAE® 100P

In 2006, a study reported the deformation capacity of polymeric films prepared with Kollicoat MAE® 30DP, in the presence and absence of Polysorbate 80, used as a plasticizer [4]. Polysorbate 80 improved the film elasticity, allowing it to withstand deformation forces 5 times greater than those films that did not contain this plasticizer. As a result, the combination of Kollicoat MAE® 30D-Polysorbate 80 produced flexible films with high thermal stability. A study conducted by our working group evaluated

the effect of the temperature (from 20 to 70 °C) on the size and size distribution (polydispersity index) of Kollicoat MAE® 100P nanoparticles loaded with a plant extract [25] and with a synthetic drug [18]. In both cases, Polysorbate 80 was used as the plasticizer. Temperatures between 20 and 60 °C did not modify, significantly, the particle size of both nanoformulations. Temperatures greater than 60 °C produced a 300% increase in size on the nanoparticles loaded with the plant extract (from 200 to 650 nm), while in nanoparticles loaded with the synthetic drug the size remained practically constant (32-35 nm). However, between 20-60 °C, the polydispersity index of both formulations remained low (0.020-0.140 in the plant extract-loaded nanodispersion and between 0.260 and 0.280 in the nanoparticles of the synthetic drug). These studies showed that, despite the particle size deformation produced by the temperature, there was slight variation in the particle size distribution. This fact indicates the high thermal stability of nanoparticles coated with Kollicoat MAE® 100P (between 20 and 60 °C), regarding the nature of the enclosed drug.

It is important to highlight that nanoparticle preparation methods can be of low or high energy input [19]. Some preparation processes and some unitary operations (*e.g.*, ultrasonic stirring) significantly increase the temperature of the liquid medium containing nanoparticles preparation. Under these conditions, it is convenient to use a biocompatible polymer, such as Kollicoat MAE® 100P, which produces thermo-resistant coated films and improves the stability of nanoformulations, regardless of the origin of the nanoformulated drug. However, the strength, elasticity, and stability of the coating layers formed by Kollicoat MAE® 100P depend on the plasticizer.

In an unpublished work of our working group it was evaluated the effect of the carbon chain length of the fatty acid present in the sorbitan ester (non-ionic surfactant) on the particle size and size distribution of nanoparticles coated with Kollicoat MAE® 100P. Four white nanodispersions (without active ingredient) were prepared by the polymer deposition-solvent diffusion method [22] using 750 mg of Kollicoat MAE® 100P in 5 mL of ethanol: acetone mixture 1:1 (organic phase) and 500 mg of each polysorbate in 100 mL of distilled water. In all cases, it was used the same experimental procedure. As the length of the carbon chain of the esterified acid increases, the particle size and polydispersity index decreased (figure 4). This experiment suggested the feasibility of using other sorbitan esters as plasticizers jointly with the Kollicoat MAE® 100P in nanoparticle preparation. Thus, it would be necessary to evaluate the effect of combinations of Kollicoat MAE® 100P with plasticizers of different nature (example: polyethylene glycol, and TEC) and/or the effect of other polymers like polyvinyl alcohol on the physic-chemical characteristics, release profiles, and stability of nanoparticles loaded with active ingredients of diverse nature.

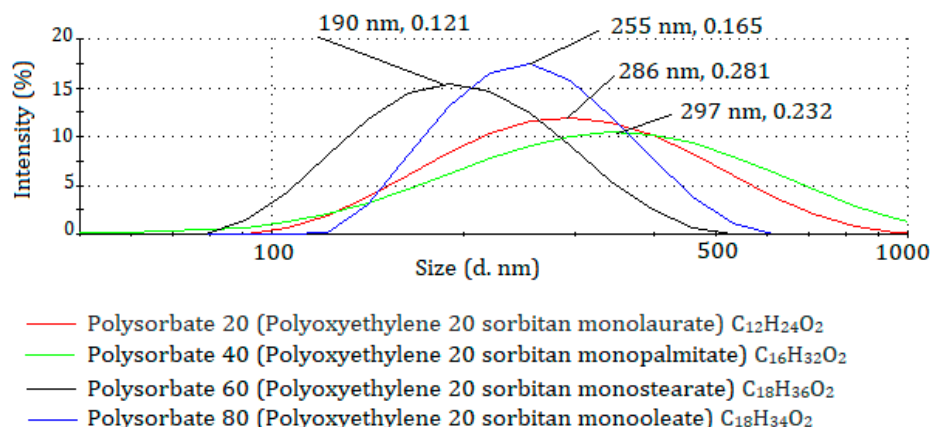


Figure 4. Effect of the carbon chain length of the sorbitan ester fatty acid on the particle size and polydispersity index of nanoparticles coated with Kollicoat MAE® 100P (authors' unpublished work).

Release profile of Kollicoat MAE® 100P -loaded nanoparticles

Nanoparticle formulations produced with Kollicoat MAE® 100P as a coating polymer did not show prolonged release profiles at pH between 6.5-7.4 [9, 26]. The *Cassia grandis* nanoparticles, in simulated intestinal fluid (pH 6.5) with and without pancreatin (pH 6.5 and 7.4, respectively) released 100% of the active ingredient in 70 min [8], while loratadine nanoparticles (in the same fluids) released 100% of the drug in 40 min. These studies confirmed the ability of the nanoparticles produced with Kollicoat MAE® 100P to maintain, for at least 40 min, the drug release at pHs compatibles with the intestinal fluid in fasted and fed condition.

These results suggest the need to conduct other studies combining Kollicoat MAE® 100P with different plasticizers and stabilizers, for controlling, with precision and reproducibility the release of the drug nanoformulated. Nonetheless, broad ranges of drug release were observed with the use of methacrylic acid copolymers combined with some organic compounds, such as ethylcellulose [27].

On the other side, Kollicoat MAE® 100P was used to form a gastro-resistant film in hard gelatin capsules filled with rHuKGF protein. The study determined the absolute bioavailability of this protein in gastro-resistant capsules, comparing it with intravenous administration. After the oral administration, the rHuKGF protein formulated in gastro-resistant capsules showed an absolute bioavailability of 74%. The study demonstrated that the protein was adequately released after the dissolution of Kolli-

coat MAE® 100P, successfully crossing the intestinal membranes to reach the systemic circulation [28].

Pharmacological properties of nanoparticles coated with Kollicoat MAE® 100P

One of the most advantages of nanoparticles is that they can modify the activity of drugs. *Cassia grandis* nanoparticles coated with Kollicoat MAE® 100P showed (*in vitro*) an antioxidant effect more potent than the extract and the gallic acid (used as a control) [8]. On the other side, nanoparticles loaded with *Cassia grandis* extract produced a strong inhibition of the pancreatic lipase, however, the extract did not inhibit pancreatic lipase. Similarly, *Cassia grandis* nanodispersion showed (*in vivo*) a hypoglycemic potency 20-fold higher than the crude extract. The authors suggest that the particle size in nanometric scale and the slow release of the drug from nanoparticles potentiate the pharmacological effect. These results confirm the need for research to improve the release properties of nanoparticle coated with Kollicoat MAE® 100P, in order to obtain a better pharmacological performance, optimizing and rationalizing the use of drugs of any nature that could be nanoformulated.

A gastro-resistant nanocapsules formulation of glibenclamide coated with methacrylic acid-ethyl acrylate copolymer showed a hypoglycemic effect (*in vivo*) five times greater than the commercial form of this drug [29]. This, is one of the most significant characteristics of nanoparticle-based on this kind of polymers, potentiating the pharmacological effect of the drugs.

Another highlighted characteristic of the Kollicoat MAE® 100P is the innocuousness. Synthetic polymers and copolymers based on methacrylic acid tested as drug carriers of different drugs do not exert either toxic effects or changes in the metabolic activity of immune system cells [30]. The innocuity was also observed in immunogenicity studies performed on coated tablets with methacrylic acid. The study showed that the films formed by this polymer do not produce any abnormal response of the immune system [31].

CONCLUSION

Investigations have shown that Kollicoat MAE® 100P is a versatile polymer that can be used for the preparation of gastroresistant nanoparticles with drugs of diverse origins. With this polymer can be prepared nanoparticles with size smaller than 130 nm and low polydispersity indexes, and relatively high z-potentials, which confers a great stability to nanoparticle system. Nanoparticles produced with Kollicoat MAE® 100P combined with a suitable plasticizer exhibit a hard and flexible shell and contribute to sustained

drug release systems. Besides, drugs nanoformulated with Kollicoat MAE® 100P as coating polymer are insoluble at pH<5.5 and presented a good thermal stability at temperatures of up to 60 °C. This review confirmed that Kollicoat MAE® 100P, despite being a well-known polymer in the Pharmaceutical Industry, has still been little investigated for nanoparticles preparations. Kollicoat MAE® 100P represents a viable, low-cost, and multifunctional alternative for the preparation of nanoparticle systems, which requires more studies in its use, to build nanoformulated products with better performances.

ACKNOWLEDGEMENTS

To BASF-SA São Paulo, Brazil for the kindness and gentle support.

DISCLOSURE STATEMENT

Authors declare that there is no competing interest.

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HOW TO CITE THIS ARTICLE

B. Rodrigues-Acacio, V. Bautista-Robles, A. Lafourcade-Prada, J.M. Assis, T. Pereira de Souza, H. Keita, S.F. Neto, E.J. Paredes-Gamero, J.R. Rodríguez-Amado, Kollicoat MAE® 100P as a film former polymer for nanoparticles preparation, *Rev. Colomb. Cienc. Quim. Farm.*, **51**(3), 1215-1231 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100424>

Technological research article / <http://dx.doi.org/10.15446/rcciquifa.v51n3.107382>

A rapid and simple capillary electrophoresis procedure for quantification of vanillylmandelic acid in urine samples

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Received: February 6, 2022

Corrected: March 23, 2022

Accepted: March 26, 2022

SUMMARY

Aim: To develop a one-step vortex-assisted liquid-liquid extraction (VALLE) method, without the need for evaporation and reconstitution steps, to establish a rapid and straightforward treatment procedure based on capillary electrophoresis-diode array detection (CE-DAD) for the determination of vanillylmandelic acid (VMA) in human urine. **Methodology:** Optimization of VALLE and CE-DAD procedures were studied in detail. The effects of various experimental parameters, such as the type of the extraction solvent, sample pH, salt addition, and extraction time were investigated. Also, CE separation conditions including background electrolyte type, concentration, and pH, injection time and separation voltage were optimized as well. **Results:** A successful separation of VMA was achieved in less than 6 min using a basic background electrolyte composed of 60 mmol·L⁻¹ acetate/ACN (acetonitrile) 50% (v/v) (final apparent pH is 5.73). The linear response was obtained over the concentration range from 1.0 to 14 µg·mL⁻¹. The limit of detection and quantification were 0.30 and 1.0 µg·mL⁻¹, respectively. The intra- and inter-day precisions were found to be less than 4.3%. The extraction recoveries of VMA were between 95%-97%. **Conclusion:** The developed method is found to be a simple, rapid, and reliable method for quantitative analysis of urinary VMA.

Keywords: Extraction recovery, capillary electrophoresis, diode-array detection, urine, vortex-assisted liquid-liquid extraction.

RESUMEN

Un procedimiento de electroforesis capilar rápido y simple para la cuantificación de ácido vanililmandélico en muestras de orina

Objetivo: desarrollar un método de extracción líquido-líquido asistido por vórtice (MELLAV) de un solo paso, sin la necesidad de pasos de evaporación y reconstitución, para establecer un procedimiento de tratamiento rápido y sencillo basado en electroforesis capilar con detección por arreglo de diodos (EC-DAD) para la determinación de ácido vanililmandélico (AVM) en orina humana. **Metodología:** se estudió en detalle la optimización de los procedimientos MELLAV y EC-DAD. Se investigaron los efectos de varios parámetros experimentales, como el tipo de solvente de extracción, el pH de la muestra, la adición de sal y el tiempo de extracción. Además, también se optimizaron las condiciones de separación EC, incluyendo el tipo de electrolito de fondo, la concentración y el pH, el tiempo de inyección y el voltaje de separación. **Resultados:** se logró una separación exitosa de AVM en menos de 6 min utilizando un electrolito de fondo básico compuesto por 60 mmol·L⁻¹ de acetato/ACN (acetonitrilo) al 50 % (v/v) (el pH aparente final es 5,73). La respuesta lineal se obtuvo en el rango de concentración de 1,0 a 14 µg·mL⁻¹. El límite de detección y cuantificación fue de 0,30 a 1,0 µg·mL⁻¹, respectivamente. Se encontró que las precisiones intra e interdiarias eran inferiores al 4,3 %. Las recuperaciones de extracción de AVM estuvieron entre 95%-97%. **Conclusión:** el método desarrollado resulta ser un método simple, rápido y confiable para el análisis cuantitativo de AVM en orina.

Palabras clave: Recuperación por extracción, electroforesis capilar, detección por arreglo de diodos, orina, extracción líquido-líquido asistida por vortex.

RESUMO

Um procedimento rápido e simples de eletroforese capilar para a quantificação de ácido vanilmandélico em amostras de urina

Objetivo: desenvolver um método de extração líquido-líquido assistido por vórtice (MELLAV) em uma etapa, sem a necessidade de etapas de evaporação e reconstituição, estabelecer um procedimento de tratamento rápido e simples baseado em eletroforese capilar com detecção de arranjo de diodos (EC-DAD) para a determinação de ácido vanilmandélico (VMA) na urina humana. **Metodologia:** a otimização dos procedi-

mentos MELLAV e EC-DAD foi estudada em detalhes. Os efeitos de vários parâmetros experimentais, como o tipo de solvente de extração, pH da amostra, adição de sal e tempo de extração, foram investigados. Além disso, as condições de separação EC também foram otimizadas, incluindo o tipo de eletrólito de fundo, concentração e pH, tempo de injeção e tensão de separação. Resultados: uma separação AVM bem-sucedida foi obtida em menos de 6 min usando um eletrólito de fundo básico composto de $60 \mu\text{g}\cdot\text{mL}^{-1}$ 50% (v/v) de acetato/ACN (acetonitrila) (pH final aparente é 5,73). A resposta linear foi obtida na faixa de concentração de $1,0$ a $14 \mu\text{g}\cdot\text{mL}^{-1}$. O limite de detecção e quantificação foi de $0,30$ a $1,0 \mu\text{g}\cdot\text{mL}^{-1}$, respectivamente. As precisões intradias e interdias foram inferiores a 4,3%. As recuperações de extração de AVM ficaram entre 95%-97%. Conclusão: o método desenvolvido revela-se um método simples, rápido e confiável para a análise quantitativa de MAV em urina.

Palavras-chave: Recuperação de extração, eletroforese capilar, detecção de matriz de diodos, urina, extração líquido-líquido assistida por vórtice.

INTRODUCTION

Vanillylmandelic acid (VMA, DL-4-hydroxy-3-methoxybenzeneacetic acid with a molar mass of $198.2 \text{ g}\cdot\text{mole}^{-1}$, for the chemical structure of VMA, see figure 1) is the final product of epinephrine and norepinephrine metabolism in the human body [1]. It is well known that the concentration of VMA, as a well-established biomarker, increases in various diseases such as autism [2], neuroblastoma [3], pheochromocytoma [4], and tumors of the neural crest [5], while it may decrease in situations such as depressions [6]. Due to the easy and non-invasive collection, urine samples are frequently used to determine VMA in clinical diagnosis [7]. Normal concentrations of VMA in healthy individuals vary over a wide range in the urine based on the values described in the bibliography, that is from 11.6 to $28.7 \mu\text{mol}\cdot\text{L}^{-1}$ (or 2.3 to $5.7 \mu\text{g}\cdot\text{mL}^{-1}$) [8].

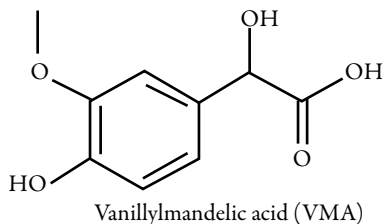


Figure 1. Chemical structure of vanillylmandelic acid (VMA).

Several analytical separation techniques, namely high-performance liquid chromatography (HPLC) with either electrochemical detection (ECD) [9-12], fluorescence detection (FLD) [13-16] or mass spectrometry (MS) [17-24] and gas chromatography coupled to mass spectrometry (GC-MS) [25-27] or flame ionization detector [28] have been reported for the determination of VMA. HPLC-ECD needs laborious sample preparation and extensive analytical time [19]. Although HPLC-tandem mass spectrometry (HPLC-MS/MS) possesses improved selectivity and sensitivity and decrease analysis time, it is costly and needs a qualified and skilled operator. GC-MS offers high identification and separation power, but for most of analytes, it requires a time-consuming derivatization procedure to become volatile, stable, and amenable to the ionization techniques [29]. The use of immunoassay techniques [30, 31] and electrochemical methods [32-34] has also been described in the literature. However, the selectivity and specificity of separation techniques are higher than immunoassay and electrochemical methods, especially in complex biological samples. Capillary electrophoresis (CE) in different separation modes provides superior separation efficiency, short analysis time, small sample and low reagent consumption, and automatability that make it an attractive alternative to HPLC [35]. Therefore, a growing interest in the application of CE to VMA analysis in urine samples can be observed [8, 36-41].

Before the application of the separation technique during quantitative analysis, the sample preparation step is usually needed to be developed and optimized for the selective extraction of analytes of interest from biological samples and to clean-up the sample matrix. Solid-phase extraction (SPE) [42-44] and liquid-liquid extraction (LLE) [38, 41, 45] methods are usually used to pre-treatment urine samples prior to the analysis of VMA. SPE is an efficient sample preparation technique but requires proper sorbent materials and solvents, as well as multiple washing and elution steps, which is tedious and labor-intensive [46]. On the other hand, LLE due to its simplicity and efficiency was described as a good alternative to SPE. Simpler methods such as dilution, centrifugation, and filtration either alone or in combination have also been reported to clean-up urine samples prior to injection to CE [8, 36, 39, 40], but the resulting electropherograms possess many peaks that make it difficult to identify the analyte of interest and also reduce the selectivity of the method. These methods do not eliminate the interferences. In addition, under the influence of the contents of the sample matrix, the performance of the separation column is reduced. Therefore, in the case of CE analysis of VMA, vortex-assisted LLE (VALLE) is often used for the treatment of urine samples [38, 41]. However, VALLE requires evaporation and reconstitution steps, and hence additional time. Furthermore, due to the possibility of analyte

loss in these steps, it is necessary to use a suitable internal standard. The main analytical methods for the determination of VMA in urine along with sample preparation are summarized in table 1.

To diagnose of aforementioned diseases and to monitor response to treatment, the demand for the routine determination of VMA is increasing. Therefore, low-cost and rapid methods are necessary to satisfy the rising demand. CE is a rapid and cost-effective method compared to other mentioned methods. However, using the conventional VALLE method for sample preparation can be a source of error and is time-consuming [8]. In conventional capillary zone electrophoresis (CZE) with aqueous running buffer, direct injection of organic solvent disrupts the separation process due to its very low conductivity. But, the addition of organic solvents to the running buffer composed of a binary solvent allows direct injection of the sample extracted in organic solvents [47]. Therefore, the main goal of the present study was to develop the rapid and simple VALLE method for extraction of VMA from urine samples without the need for additional time-consuming and error-prone evaporation and reconstitution steps. For this purpose, several organic solvents were tested for effective extraction of VMA. Subsequently, the extraction solvent (upper phase, supernatant) was taken and directly injected onto CE-diode array detection (CE-DAD). The method presented here is fast, straightforward, and reliable.

Table 1. A review of main analytical methods for the measurement of VMA in urine.

Analysis method	Sample treatment /reagent	Sample treatment time (min)	Extraction recovery (%)	LOD ($\mu\text{g}\cdot\text{mL}^{-1}$)	LOQ ($\mu\text{g}\cdot\text{mL}^{-1}$)	LR ($\mu\text{g}\cdot\text{mL}^{-1}$)	Method imprecision (RSD %)	Ref.
HPPLC-FLD	LLE/ ethyl acetate; agitation by shaker	> 60	-	-	5.0	5.0-20	4.0	[13]
HPPLC-E-CD	LLE/ ethyl acetate; agitation by shaker	50-80	72	0.81	2.0	2.0 ¹ 5	≤6.0	[11]
HPPLC-FLD	SPE	> 20	98.3	0.12	-	-	7.9	[15]
HPPLC-E-CD	LLE/ ethyl acetate; agitation by shaker	> 30	70	-	-	-	7.5	[12]
HPPLC-FLD	LLE/ diethyl ether; agitation by vortex	> 20	-	0.50	0.20	0.20- 99	6.7	[14]
HPPLC-MS/MS	Centrifugation and dilution (10 times)	<5	-	0.001	0.005	0-50	≤14.6	[22]
HPPLC-MS/MS	Dilution (50 times) and filtration	-	-	0.0002	0.001	0.001 ¹ 0	4.3	[23]
UPLC-MS/MS	Dilution (50 times)	-	-	0.117	0.355	0.25 ¹ 00	≤7.0	[18]
HPPLC-MS/MS	Microextraction by packed sorbent (MEPS)/ eVol®	-	57.5–70.5	-	0.50	0.50 ¹ 00	≤9.6	[17]
HPPLC-MS/MS	Dilution (10 times) and centrifugation	-	-	0.18	0.50	0.50 ¹ 00	≤4.0	[20]
HPPLC-E-CD/UV	SPE	-	100	0.38 (ECD) 2.0 (UV)	2.0 (ECD and UV)	2.0-30 (ECD and UV)	16 (ECD) 3.5 (UV)	[10]

Analysis method	Sample treatment /reagent	Sample treatment time (min)	Extraction recovery (%)	LOD ($\mu\text{g}\cdot\text{mL}^{-1}$)	LOQ ($\mu\text{g}\cdot\text{mL}^{-1}$)	LR ($\mu\text{g}\cdot\text{mL}^{-1}$)	Method imprecision (RSD %)	Ref.
HPLC-MS/MS	Centrifugation and dilution (10 times)	<5	-	0.005	0.025	0.20-50	≤ 3.12	[19]
UPLC-FLD	Centrifugation, filtration and dilution (almost 6 times)	-	-	0.012	0.037	0.01-20	5.78	[16]
HPLC-MS	DLLME/ extraction solvent (dichloromethane) and disperser solvent (ethanol)	33	99	0.005	0.01	0.005-2	≤ 9.8	[24]
GC-FID	1) LLE/ethyl acetate and ether 2) Derivatization by BSTFA/trimethylchlorosilane	>50	79.2	-	-	-	-	[28]
GC-MS	1) LLE/ ethyl acetate 2) Derivatization by BSTFA	-	-	-	-	-	-	[27]
GC-MS	1) LLE/ mixture of ethyl acetate and diethyl ether 2) Derivatization by BSTFA	40	-	0.15	0.45	1.0-50	≤ 9.9	[2]
GC-MS	1) LLE/ ethyl acetate 2) Derivatization by BSTFA	>75	-	-	0.18	-	9.0	[26]

Analysis method	Sample treatment /reagent	Sample treatment time (min)	Extraction recovery (%)	LOD ($\mu\text{g}\cdot\text{mL}^{-1}$)	LOQ ($\mu\text{g}\cdot\text{mL}^{-1}$)	LR ($\mu\text{g}\cdot\text{mL}^{-1}$)	Method imprecision (RSD %)	Ref.
CE-DAD	Centrifugation and filtration	5	-	0.001	0.025	-	-	[36]
CE-ECD	LLE/ ether; agitation by vortex	-	-	0.16	1.0	1.0 ⁻¹ 00	4.9	[38]
CE-UV	PP/ 1 mM formic acid containing 20% ACN and dilution (10 times)	10	-	0.062	-	-	3.1	[39]
CE-ECD	Centrifugation, filtration and dilution	-	-	0.005	0.01	0.01-5.0	3.4	[40]
CE-DAD	LLE/ diethyl ether; agitation by vortex	>20	96.1	0.10	0.50	0.50-50	≤ 9.1	[41]
CE-UV	Centrifugation and dilution (1 time)	5	-	0.006	0.60	0.60 ⁻¹ 9.5	7.0	[8]

HPLC: High Performance Liquid Chromatography, UPLC: Ultra Performance Liquid Chromatography, FLD: Fluorescence Detection, ECD: Electrochemical Detection, MS: Mass Spectrometer, UV: Ultraviolet, GC: Gas Chromatography, FID: Flame Ionization Detector, DAD: Diode Array Detection, CE: Capillary Electrophoresis, LLE: Liquid-Liquid Extraction, SPE: Solid Phase Extraction, PP: Protein Precipitation, BSTFA: Bis (Trimethylsilyl) Trifluoroacetamide, DLLME: Dispersive Liquid-Liquid Microextraction, LR: Linear Range, LOD: Limit Of Detection, LOQ: Limit Of Quantification.

EXPERIMENTAL

Chemicals and reagents

VMA (purity $\geq 98\%$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The reagents used for the preparation of buffer solutions and the samples such as hydrochloric acid (HCl), phosphoric acid, acetic acid, sodium hydroxide (NaOH), sodium chloride (NaCl), sodium dihydrophosphate, sodium tetraborate decahydrate (borax), diethyl ether, 1-octanol, ethyl acetate, acetonitrile (ACN), and methanol (MeOH) were from Merck (Darmstadt, Germany). All chemicals were of analytical grade and used without further purification. Deionized (DI) water was supplied by Shahid Ghazi Pharmaceutical Company (Tabriz, Iran).

Preparation of stock and working solutions

The stock solution of VMA was prepared by accurately weighing 20.0 mg and dissolving in 100 mL of DI water which was stored in a dark container in a freezer (-20°C). This solution was used for the preparation of calibration and quality control (QC) samples. The working solutions were prepared daily by diluting the stock solution as appropriate with pure water or urine.

Preparation of human urine samples

A human urine sample from a healthy volunteer was collected. The study was approved by the Ethics Committee of Tabriz University of Medical Sciences (code of ethics committee: IR.TBZMED.REC.1400.335). VALLE was used to treatment of urine samples, for this purpose, the pH of 1 mL of urine was adjusted to pH 1.0 then 0.2 g NaCl was added and briefly vortexed to the dissolution of NaCl. Subsequently, 1 mL of diethyl ether was added, vortexed for 10 min and centrifuged at 3000 rpm for 5 min. An aliquot of 20 μL of the upper layer was transferred into a 1 mL conical microtube and 20 μL methanol was added and briefly vortexed then an aliquot of 20 μL of the homogenous solution was transferred into the CE microvial to be injected into the CE system through the autosampler.

Capillary electrophoresis instrument

CE separation was carried out using an Agilent CE system equipped with a diode array detector (DAD) (190-600 nm). Data acquisition and control were accomplished using ChemStation (Agilent Technologies, Waldbronn, Germany). Separations were performed on a 36 cm bare fused silica capillary with a 27.5 cm effective length and an internal diameter of 50 μm (Agilent Technology, Waldbronn, Germany). The capillary was preconditioned with 1 M NaOH for 30 min, followed by DI water for 30 min and

background electrolyte (BGE) solution for 30 min. Between runs, the capillary was rinsed with 0.1 M NaOH for 2 min, DI water for 2 min, and BGE solution for 4 min. The BGE solution was 60 mM acetate/ACN (1:1, v/v) buffer with an apparent pH of 5.73. The injection was by pressure (50 mbar) for 3 s. The separations were carried out at +20 kV and the capillary temperature was thermostated at 25 °C. BGE solution was filtered through a 0.45 µm pore size PTFE syringe filter (Chromafil, Germany) and degassed using sonication prior to use. The detection wavelength was 200 nm. UV spectra (200–400 nm) were collected for VMA identification and to check its purity. Also, peak identity is confirmed by spiking of standard solution.

Calculations

Relative recovery (RR) or recovery is calculated as follow:

$$RR\% = \left(\frac{C_{found} - C_{real}}{C_{added}} \right) \times 100 \quad (1)$$

where C_{found} , C_{real} , and C_{aded} are the measured concentration of the analyte in the real sample after the spike of a known amount of the standard, the original concentration of the analyte present in the real sample, and the concentration of the standard spiked in the real sample, respectively. The extraction recovery (ER) indicates how much of the analyte is transferred from the sample solution to the extraction solvent at the end of extraction and is defined as the ratio of the total analyte amount, which was extracted in the organic phase:

$$ER\% = \left(\frac{n_{o,final}}{n_{s,initial}} \right) \times 100 = \left(\frac{V_o}{V_s} \right) \left(\frac{C_{o,final}}{C_{s,initial}} \right) \times 100 \quad (2)$$

Where $n_{s,initial}$, $n_{o,final}$, V_o , V_s , $C_{o,final}$ and $C_{s,initial}$ are the amount of analyte originally present in the sample solution, the amount of analyte transferred to the organic phase, the volume of the organic phase, the volume of the sample solution, the concentration of analyte in the organic phase, and the concentration of analyte in the sample solution, respectively.

RESULTS AND DISCUSSION

This study has two main objectives. First, to optimize the effective and straightforward extraction protocol for VMA from urine samples. For this purpose, three extraction solvents were tested, and then the resultant organic phase was directly injected

onto CE for further analysis. The second concerned the compatibility of the selected extraction method with further determination by the use of CE-DAD. Therefore, the optimization of the simple, rapid, and most efficient extraction procedure with further CE-DAD determination method was carefully accomplished.

Optimization of sample preparation

Sample preparation is a crucial step during the analysis of biological samples such as urine. VALLE is used here to clean up the urine sample. In LLE, the analyte of interest is transferred from an aqueous matrix into an extraction solvent. The extraction efficiency of LLE is improved by the adjustment of ionic strength and pH in the aqueous phase. Since the VMA ($pK_a = 3.11$ [48]) is ionized in the urine ($pH > 4$), it needs to be in molecular form to increase the efficiency of extraction. Therefore, the extraction pH must be optimized. By adding salt to the sample, known as the salting-out effect, the ionic strength of the sample can be changed to increase the extraction efficiency. The contact time between the sample and extraction solvent is also effective. However, the most important option is to select the proper organic solvent in order to achieve maximum recovery. Based on the values reported [41] for extraction of VMA in the previous study, the initial values of 1 for pH, 10 min for vortex agitation, and 0.2 g for NaCl were considered. Then, the extraction yield of 1-octanol, diethyl ether, and ethyl acetate was investigated. Under these conditions, extraction efficiencies in terms of ER were obtained for 1-octanol, diethyl ether, and ethyl acetate 78%, 97%, and 91%, respectively. Diethyl ether was selected for the rest of the experiments. pH values of 1, 2, 4, and 7 were implemented to determine how urine pH affects the extraction efficiency. The maximum extraction yield was obtained in pH 1, at pH values above 4, the extraction yields of VMA were lower as expected. In acidic pH, almost all molecules are neutral, but with increasing pH, they become ionized, which reduces the hydrophobic properties and consequently reduces the extraction efficiency. So pH 1 was considered as optimum. The effect of NaCl addition on the extraction efficiency was investigated. Extraction was performed from aqueous samples in the absence of NaCl and the presence of values of 0.1, 0.2, and 0.3 g of NaCl per 1 mL sample. The amount of extracted VMA increased by increasing the amount of NaCl in the aqueous sample. Its maximum was reached at 0.2 g and remained almost constant for values above this. Finally, the vortex time was examined. For this purpose, 3, 5, and 10 minutes were studied. It was observed that with increasing vortex time, the extraction yield increased, however, at 10 min, only a small amount improved over 5 min (about 5%). So 10 min was chosen as the optimal time. The best working conditions were as follows: NaCl amount, 0.2 g per 1 mL sample; sample volume, 1 mL; vortexing time, 10 min; sample pH, 1 and 1 mL of diethyl ether as an extraction solvent.

Optimization of CE separation

The choice of BGE type is crucial to achieving the satisfactory separation of the analyte of interest in CZE. Due to the acidic nature of the VMA, the best BGE is a buffer with $\text{pH} > 4.11$ ($\text{pH} = \text{pK}_a + 1$ or more). Acetate ($\text{pH} 4.75$) and borate ($\text{pH} 9.2$) buffers enable partial or full ionization of the acidic analytes, respectively [49]. Due to the similar pK_a values of the other catecholamine metabolites found in urine (e.g. homovanillic acid, 5-hydroxyindoleacetic acid, and 3-methoxytyramic acid) to VMA, the use of a slightly acidic acetate buffer allows them to be better separated since the analytes are only partially ionized [50]. So acetate BGE solution was selected for this work. However, since our main goal is to try to inject the extraction solvent directly into the CE, therefore it was necessary to change both the nature of the BGE and extraction solvent to allow direct injection. Consequently, in this study, CE separation was investigated for the analysis of VMA in the organic matrix. Injection of the organic solvents to CE with the fully aqueous BGE solution leads to serious deterioration of CE performance. Subsequently, ACN was picked as a suitable dilution medium [51]. So, a series of acetate /ACN (10-70% (v/v)) were evaluated. BGE and diethyl ether incompatibilities were still observed for values less than 30% of ACN. But the successful separation was achieved for increased ACN concentrations. Besides, improved separation efficiency (up to 50% ACN) was observed. Also, an injection of diethyl ether diluted 1:1 (v/v) with methanol was performed to ensure enough compatibility of the injected sample with the BGE solution [50]. The effect of buffer concentration (20, 40, 60, 80, and 100 $\text{mmol}\cdot\text{L}^{-1}$) as an effective factor on CE separation and resolution was explored and 60 $\text{mmol}\cdot\text{L}^{-1}$ was selected as the optimum acetate buffer concentration. Final optimized BGE which consists of 60 $\text{mmol}\cdot\text{L}^{-1}$ acetate buffer containing 50% (v/v) ACN (final apparent pH; pH after adding 50% (v/v) ACN is 5.73). Different voltage values ranging from +15 to +30 kV were investigated to select the optimized level. Higher separation voltage contributes good resolution, while low separation voltage leads to peak broadening. The voltage of +20 kV was chosen as optimal for acquiring the satisfactory efficiency of the separation and high resolution. In addition, the capillary temperature was fixed at 25 °C to obtain reproducible results. The effect of hydrodynamic injection time on sensitivity and separation efficiency was investigated at varying sampling times (2, 3, 4, 5, and 6 s at an injection pressure of 50 mbar). The results showed that applying a 3 s injection provided the most satisfactory signals. Broadened, overlapping peaks are observed for longer injection times.

Assay validation

The developed VALLE-CE-DAD method for the quantification of VMA in aqueous samples was validated according to the criteria suggested by the FDA, such as linear-

ity, the limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, specificity and ER [52].

Linearity

A calibration curve was acquired by plotting the peak area of the VMA against the nominal concentration of calibration standards analyzed in triplicate under the optimized VALLE-CE-DAD method using a least-squares linear regression model. The linearity of the method was checked by analyzing five calibration standard samples. The assay was found to be linear in the concentration range of 1.0–14 $\mu\text{g}\cdot\text{mL}^{-1}$. From the calibration curve, the linear equation was $y = 17.969x + 0.7211$ ($r^2 = 0.9986$), which demonstrated the excellent linearity of the method in the considered concentration range.

Limits of detection and quantitation

LOD and LOQ were determined based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. The LOD and LOQ were found to be 0.30 and 1.0 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively, which indicates that the developed method is fit for the purpose. Normal concentrations of VMA in urine vary from 2.3 to 5.7 $\mu\text{g}\cdot\text{mL}^{-1}$.

Precision and accuracy

The intra-day precisions were assessed for the developed VALLE-CE-DAD method by measuring five replicate injections at three different concentrations of a standard containing 1.0, 2.0 and 8.0 $\mu\text{g}\cdot\text{mL}^{-1}$ of VMA. The inter-day precision was evaluated at the previously stated concentrations over three consecutive days. Precision was expressed as RSD%. The accuracy of the proposed method was evaluated by measuring the recovery of added known amount of VMA to a real urine sample (standard addition) via the implementation of five replicate extractions at three different concentrations of a sample containing 1.0, 2.0, and 8.0 $\mu\text{g}\cdot\text{mL}^{-1}$ of VMA. Accuracy was expressed as RR and calculated by comparing the measured concentration according to the regression equation with added concentration (equation 1). Intra- and inter-day RSDs were below 4.30%, which indicates good repeatability of the proposed procedure. The RR was in the range of 87–109%, indicating that there is no significant matrix effect.

Selectivity

The selectivity of the developed method was tested by analyzing both the urine samples collected from volunteers and the spiked urine samples to check for interference from peaks at the migration time of the VMA. Figure 2 shows typical electropherograms for the analysis of VMA in real urine and spiked urine samples. It can be seen that the VMA can be determined without any interference from the other endogenous compounds found in urine. Other main peaks present in the blank urine sample may

be related to other acidic metabolites of catecholamines such as homovanillic acid, 3-methoxytyramic acid, and 5-hydroxy-indoleacetic acid, because they are acid, so they can be extracted into the extraction phase under the utilized optimal conditions of VALLE, and also their concentration in urine is much higher than that of the VMA [8]. Principally high concentration acidic compounds can be extracted and detected using here developed treatment method under optimized conditions.

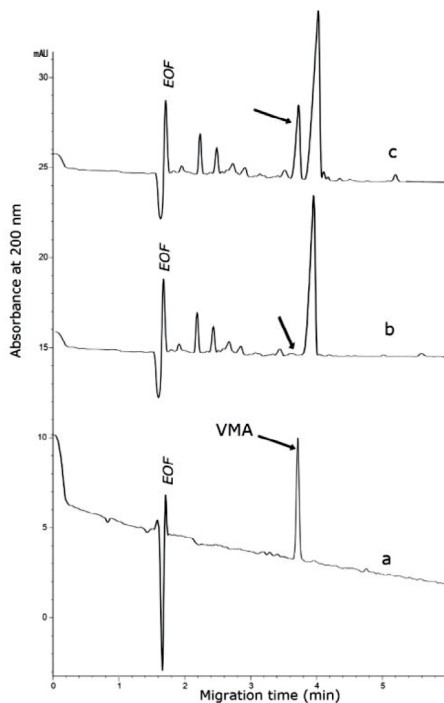


Figure 2. Typical electropherograms of VMA extracted using developed VALLE-CE-DAD under optimized conditions (a) from a standard aqueous solution at concentration of $4 \mu\text{g}\cdot\text{mL}^{-1}$ (b) non-spiked urine sample and (c) urine sample with standard VMA spiked at concentration of $4 \mu\text{g}\cdot\text{mL}^{-1}$.

The extraction recovery

ER of VMA was calculated by comparing the peak area ratio after extraction from the standard at three concentration levels (1 , 2 , and $8 \mu\text{g}\cdot\text{mL}^{-1}$) with the peak area of direct injection of standards containing the same concentrations of VMA (equation 2). Under optimal conditions, the obtained ER for the VALLE method using diethyl ether were between 95-97%. The performed recovery experiments show the high efficiency of the used extraction procedure.

Peak identification and purity check

In order to determine the migration time of VMA, the entire developed method was used to extract VMA from a standard aqueous solution at a concentration of $4\ \mu\text{g}\cdot\text{mL}^{-1}$ (figure 2a) and non-spiked urine sample as well (figure 2b). Figure 2c depicts the electropherogram of the spiked urine sample with standard VMA. Therefore, it is easy to conclude that a marked peak at migration time of 3.7 min is the peak of VMA. In addition, the purity of the peak was examined using the UV spectrum recorded by the DAD, which indicated that the peak was pure and that there was no co-eluted compound (figure 3). Figure 3a shows the selected peak (VMA) spectra. The signals overlap perfectly reaffirming the validity of the background correction. The similarity curve (figure 3b, solid trace) exhibits a profile very similar to and within the threshold curve limits (figure 3b, dotted trace) and, the peak purity ratio (diamond points) is clear within the green band. The purity factor (999.95) is within the calculated threshold limit (998.03).

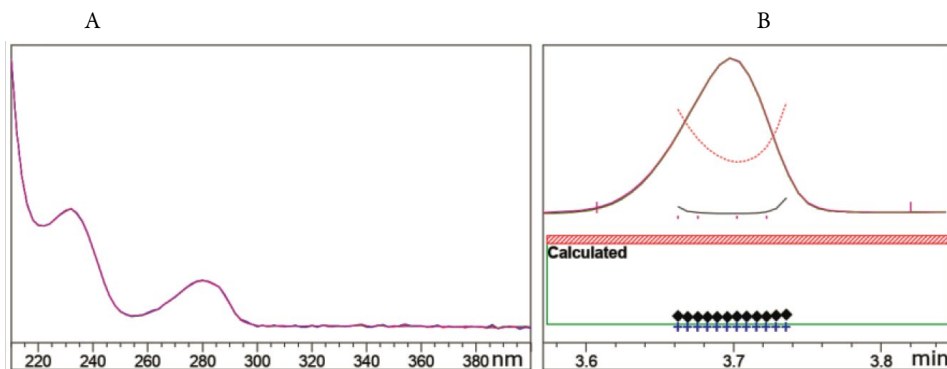


Figure 3. Peak purity evaluation by ChemStation® software. (a) The UV spectra of selected peak at migration time of 3.7 min corresponds to VMA recorded at different wavelengths and (b) the similarity curve (solid trace), threshold curve (dotted trace), and peak purity ratio (diamond points); the placement of the similarity curve below the threshold curve indicates the purity of the peak, which is confirmed by the placement of the peak purity ratio within the green zone and below the red zone.

Real sample analysis

The proposed VALLE-CE-DAD method was applied to determine the VMA in the urine samples from three healthy volunteers. Electropherograms of the urine samples are shown in figure 4. The content of VMA in the tested urine samples was found to be below the LOQ of the method.

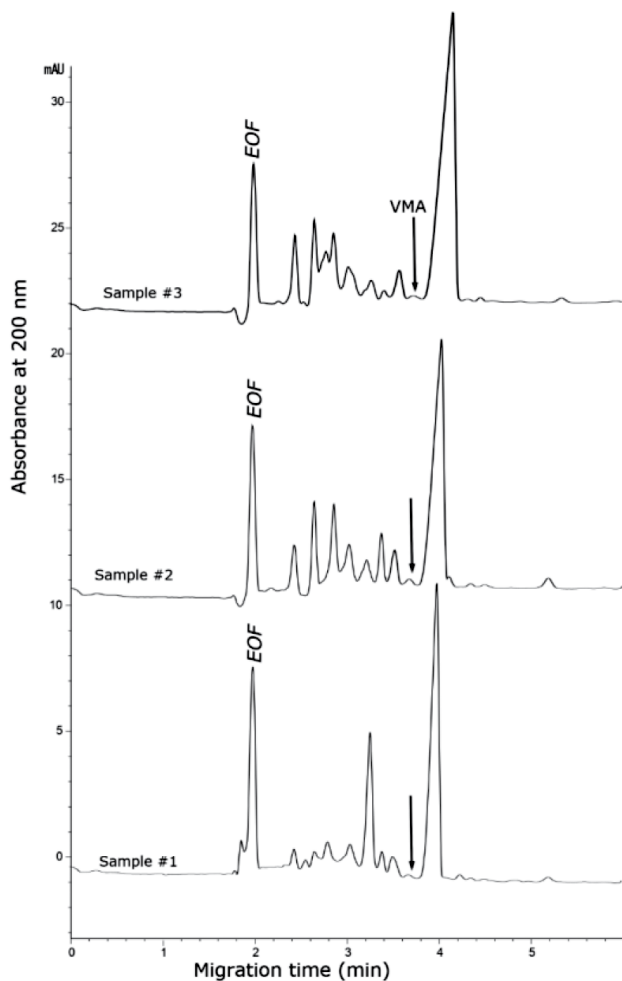


Figure 4. The electropherograms of three urinary sample solutions from healthy volunteers based on VALLE-CE-DAD method under optimized conditions.

VMA is often measured for the diagnosis of neuroblastoma and pheochromocytoma. Commonly VMA is measured using HPLC or GC-MS platforms. These assays have several analytical performance advantages including improved selectivity and sensitivity [20]. However, such procedures are expensive and complicated. SPE [53, 54], dispersive liquid-liquid microextraction [24], microextraction by packed sorbent [17] and dilute-and-shoot approaches [22, 23, 55, 56] have been described for sample clean-

up prior to chromatographic analyses. CE has also been used for quantitation of VMA [8, 36, 38-41], because of its rapid separation, high resolution, small injection volume, and low cost. Sample treatment increases cost, turnaround time, method complexity, and in some cases it may hinder automation. Moreover, sample treatment contributes to method robustness [20]. Prior to CE analyses, simple and fast treatment methods such as centrifugation, dilution and filtration have been used, but these methods suffer from weak selectivity in real samples [8, 36, 39, 40]. Recently, VALLE due to its adequate clean-up used to treat urine samples prior to CE analysis [41]. However, the evaporation and reconstitution steps are time-consuming and complicate the process. In addition, these steps can preclude automation and are also a source of error [8]. We developed a simple and rapid method for quantitative analysis of urinary VMA. Our developed VALLE approach eliminates evaporation and reconstitution and minimizes sample processing. Direct injection of extraction solvent to CE enabled the straightforward analysis of VMA, which represents a distinct advantage over current methods. Compared to previous studies [38, 41] that used the VALLE method (Table 1), the precision of our method has improved ($\text{RSD}\% \leq 4.3$) and the sample clean-up time has also been reduced (15 min). In the developed method, in the absence of the internal standard, RSDs of less than 4.3% have been obtained, which indicates the reliability of the method. The proposed method can be easily automated.

CONCLUSION

CE with features such as higher separation efficiency and faster separation can be a good option for biomedical applications and clinical analyses. The direct injection of organic solvent into CE can be achieved using a binary solvent running buffer. The CE-DAD method for the quantitative analysis of VMA in human urine is described using a simple, rapid, and straightforward VALLE pretreatment. Diethyl ether was used as the extraction solvent for efficient sample clean-up. Direct injection of the diethyl ether + methanol reduces the sample preparation time. Moreover, by eliminating the error prone evaporation and reconstitution steps, the reliability of the method is improved. Besides, under these conditions, there is no need to use the internal standard.

ACKNOWLEDGEMENT

The research protocol was approved and financially supported by Student Research Committee, Tabriz University of Medical Sciences (grant number: 67598).

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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HOW TO CITE THIS ARTICLE

B. Seyfinejad, A. Jouyban, A rapid and simple capillary electrophoresis procedure for quantification of vanillylmandelic acid in urine samples, *Rev. Colomb. Cienc. Quim. Farm.*, **51**(3), 1232-1254 (2022).

Atividade antibacteriana do R-(+)- Limoneno: uma revisão sistemática

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Recebido: 21 de janeiro de 2022

Revisado: 23 de março de 2022

Aceto: 30 de março de 2022

RESUMO

Introdução: as bactérias são organismos unicelulares e procariontes que podem ser causadores de doenças e uma das principais causas de morte em muitos países. O constante desenvolvimento e aumento das bactérias resistentes retratam uma ameaça para a saúde pública, o que conduz a uma necessidade urgente de novos compostos com ação antimicrobiana, onde se destaca os produtos naturais com grande potencial para possíveis agentes antibacterianos, como os terpenos. **Objetivo:** realizar uma revisão sistemática para avaliar o potencial terapêutico do R-(+)-Limoneno contra bactérias patogênicas. **Metodologia:** a pesquisa teve como foco documentos escritos em inglês e português publicados entre 2011 e 2021. Os dados foram coletados na literatura disponível nos Periódicos eletrônicos da CAPES, empregando três bases de dados: Embase, Pubmed e Web of Science. A organização dos estudos e sua gestão até o final da redação foi realizada com o auxílio do software Mendeley. Resultados: foram identificados, inicialmente 1367 artigos, após refinamento da busca, culminou em 15 artigos eleitos para construção da revisão, no qual o R-(+)-Limoneno tanto em sua forma isolada, como associado a outro composto apresentou efeito antibacteriano contra cepas de bactérias, tanto gram positivas, como gram negativas. **Conclusão:** o terpeno R-(+)-Limoneno pode ser considerado como uma substância promissora para o combate de doenças causadas por bactérias patogênicas. No entanto, mais estudos são necessários para aprofundar o mecanismo de

ação, bem como, frente a mais espécies bacterianas isoladas de diferentes origens, a fim de se compreender de forma mais eficaz esse efeito biológico encontrado.

Palavras-chave: Atividade antibacteriana, terpeno, produto natural.

SUMMARY

Antibacterial activity of R-(+)-Limonene: a systematic review

Introduction: Bacteria are unicellular and prokaryotic organisms that can be a cause of disease and one of the main causes of death in many countries. The constant development and increase of resistant bacteria poses a threat to public health, which leads to an urgent need for new compounds with antimicrobial action, in which natural products with great potential for possible antibacterial agents, such as terpenes, stand out. **Aim:** To carry out a systematic review to evaluate the therapeutic potential of R-(+)-Limonene against pathogenic bacteria. **Methodology:** The research focused on documents written in English and Portuguese published between 2011 and 2021. Data were collected from the literature available in CAPES electronic journals, using three databases: Embase, Pubmed and Web of Science. The organization of studies and their management until the end of writing was carried out with the help of Mendeley software. Results: Initially 1367 articles were identified, after refinement of the search, culminating in 15 articles elected for the construction of the review, in which R-(+)-Limonene both in its isolated form and associated with another compound showed antibacterial effect against strains bacteria, both gram positive and gram negative. **Conclusion:** the terpene R-(+)-Limonene can be considered as a promising substance to fight diseases caused by pathogenic bacteria. However, more studies are needed to deepen the mechanism of action, as well as, against more bacterial species isolated from different origins, in order to more effectively understand this biological effect found.

Keywords: Antibacterial activity, terpene, natural product.

RESUMEN

Actividad antibacteriana del R-(+)-Limoneno: una revisión sistemática

Introducción: las bacterias son organismos unicelulares y procarióticos que pueden ser causantes de enfermedades y una de las principales causas de muerte en muchos países. El constante desarrollo e incremento de bacterias resistentes supone una amenaza para la salud pública, lo que conduce a una urgente necesidad de nuevos compuestos con acción antimicrobiana, en los que destacan productos naturales con gran potencial de posibles agentes antibacterianos, como los terpenos. **Objetivo:** llevar a cabo una revisión sistemática para evaluar el potencial terapéutico del R-(+)-limoneno frente a bacterias patógenas. **Metodología:** la investigación se centró en documentos escritos en inglés y portugués publicados entre 2011 y 2021. Los datos fueron recolectados de la literatura disponible en las revistas electrónicas de la CAPES, utilizando tres bases de datos: Embase, Pubmed y Web of Science. La organización de los estudios y su gestión hasta el final de la redacción se realizó con la ayuda del software Mendeley. **Resultados:** inicialmente se identificaron 1367 artículos, luego del refinamiento de la búsqueda, culminando en 15 artículos elegidos para la construcción de la revisión, en los cuales el R-(+)-Limonene tanto en su forma aislada como asociado a otro compuesto mostró efecto antibacteriano contra cepas bacterias, tanto gram positivas como gram negativas. **Conclusión:** el terpeno R-(+)-Limonene puede considerarse como una sustancia prometedora para combatir enfermedades causadas por bacterias patógenas. Sin embargo, se necesitan más estudios para profundizar en el mecanismo de acción, así como, contra más especies bacterianas aisladas de diferentes orígenes, para comprender de manera más efectiva este efecto biológico encontrado.

Palabras clave: actividad antibacteriana, terpeno, producto natural.

INTRODUÇÃO

Bactérias são organismos unicelulares e procariontes que podem ser causadores de doenças e uma das principais causas de morte em muitos países [1, 2]. As mesmas podem ser classificadas de acordo com sua patogenicidade em comensais, que habitam a microbiota normal humana, e em patogênicas, responsáveis por provocar infecções bacterianas [3].

Logo, essas infecções classificam-se de acordo com a proporção do dano causado no organismo do hospedeiro, podendo ser um dano local ou generalizado, nessa situação representada pela septicemia ou bacteremia, além de outras doenças, como a pneumonia, coqueluche, infecções do trato urinário e endocardites [4].

Nesse contexto, com a utilização dos antibióticos o tratamento de doenças infecciosas causadas por bactérias revolucionou e as taxas de morbidade e mortalidade associadas a essas infecções reduziram [5]. Entretanto, o uso indiscriminado e excessivo de antimicrobianos nas comunidades, em ambientes hospitalares ou até mesmo em uso agropecuário colabora diretamente para o desenvolvimento da resistência bacteriana, elevando os custos com tratamentos, além da hospitalização prolongada dos pacientes aumentando ainda mais os índices de morbimortalidade [6].

Uma abordagem cada vez mais popular para lidar com a resistência antimicrobiana (AMR) é One Health (Saúde Única), que corriqueiramente é apresentada como uma preocupação de saúde acerca do conhecimento das relações humano-animal-ambiente. One Health é utilizado como uma estrutura para colaboração entre organizações internacionais como a Organização Mundial de Saúde, a Organização Mundial da Saúde Animal e Organização das Nações Unidas para a Alimentação e Agricultura; além de que, ocupa uma posição de destaque nos documentos de política internacional da AMR [7].

Dessa forma, o constante desenvolvimento e aumento das bactérias resistentes retratam uma ameaça para a saúde pública, o que conduz a uma necessidade urgente de novos compostos com ação antimicrobiana, nesse caso, pode-se destacar os produtos naturais como sendo de grande potencial para possíveis agentes antibacterianos [8, 9], como exemplo os terpenos.

Os terpenos e seus derivados estão associados aos óleos essenciais, os mesmos são considerados compostos voláteis formados por unidades de isopreno (5C), que apontam funções químicas orgânicas, como ácidos, aldeídos, cetonas, fenóis, entre outros [10].

Dentre os diversos terpenos, é possível destacar o Limoneno, um monoterpreno do metabolismo secundário, principalmente, de plantas do gênero *Citrus* [11]. O Limoneno conta com dois enantiômeros, o isômero levógiro S-Limoneno ou (-)-Limoneno ou L-Limoneno, um dos constituintes produzidos por árvores como pinheiros e carvalhos e o isômero dextrógiro R-Limoneno ou (+)-Limoneno ou D-Limoneno, um hidrocarboneto monocíclico naturalmente produzido por plantas e frutas e o principal componente de óleos presentes em cascas de frutas cítricas tais como laranja, limão e bergamota [12, 13]. Além disso, o R-(+)-Limoneno possui propriedades antioxidantes, antimicrobianas, antifúngicas, antiinflamatórias e anticancerígenas [14].

Diante do conhecimento acerca do R-(+)-Limoneno com possíveis propriedades em atividades antimicrobianas, este estudo tem como objetivo realizar uma revisão sistemática para avaliar o potencial terapêutico do R-(+)-Limoneno contra bactérias patogênicas.

METODOLOGIA

A revisão sistemática baseia-se em sintetizar a produção dos conhecimentos científico numa área específica, englobando parte da literatura de forma não tendenciosa, visando agregar, sintetizar e interpretar estudos sobre um determinado assunto, preservando a integridade das fontes de informação com o intuito de fundamentar teoricamente a temática [15].

Elaboração da pergunta

A caracterização da pergunta baseou-se na estratégia PICO, que significa P: população (bactérias), I: intervenção (efeito do R-(+)-Limoneno), C: comparação (bactérias tratadas com antibacterianos) e O: *outcomes*/resultados (potencial antibacteriano). Qual o potencial terapêutico do R-(+)-Limoneno frente a bactérias patogênicas?

Protocolo e coleta de dados

A revisão foi conduzida conforme as recomendações PRISMA-P (Preferred Reporting Items for Systematic reviews and Meta-Analyses Protocols) [16]. A pesquisa foi realizada no período entre novembro e dezembro de 2021 e teve como foco documentos escritos em inglês e português publicados entre 2011 e 2021. Os dados foram coletados na literatura disponível nos periódicos eletrônicos da CAPES, empregando três bases de dados: Embase, PubMed e Web of Science, por meio das seguintes combinações de descritores e operadores booleanos: “antibacterial activity” AND “R-(+)-Limonene” e “antibacterial activity” AND Limonene. A organização dos estudos e sua gestão até o final da redação foi realizada com o auxílio do software Mendeley.

Critério de inclusão e exclusão

Como critério de inclusão adotou-se: artigos originais que estavam de acordo com a temática do estudo envolvendo atividade antibacteriana e o composto R-(+)-Limoneno e com escrita em inglês ou português. Por sua vez, foram excluídos artigos de revisão, monografias, dissertações, teses, artigos duplicados e aqueles que não constituíram o tema do estudo.

Risco de viés

A qualidade dos métodos utilizados e a análise do risco de viés do estudo foram realizadas de acordo com as recomendações PRISMA-P [16]. A avaliação deu prioridade à descrição das informações com clareza, a revisão foi realizada por dois revisores de forma independente e em caso de desacordo foi envolvido um terceiro revisor, evitando qualquer viés potencial e conflito de interesses, mascarando os nomes dos autores e revistas.

Extração dos dados

As informações extraídas dos artigos foram: autor(es), ano, objetivo e os principais resultados obtidos.

RESULTADOS E DISCUSSÃO

Após a busca na literatura foram identificados inicialmente 1367 artigos, destes 280 foram excluídos por estarem duplicados nas bases de dados e outros 143 por serem documentos de revisão, monografias, dissertações ou teses. Desta forma, restaram 944 para leitura de título e resumo, desses 916 artigos foram excluídos por não contemplarem os critérios de inclusão, o que culminou em 28 artigos avaliados para elegibilidade, desses 13 foram excluídos por não realizar experimento com o composto em estudo e por fim 15 artigos foram eleitos para construção da revisão, descrita detalhadamente na figura 1.

Destes artigos observa-se o potencial antibacteriano do R-(+)-Limoneno tanto em sua forma isolada, como associado a outro composto contra diferentes cepas de bactérias, como *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Listeria monocytogenes*, *Shigella flexneri*, *Mycobacterium tuberculosis* e *Xanthomonas oryzae*.

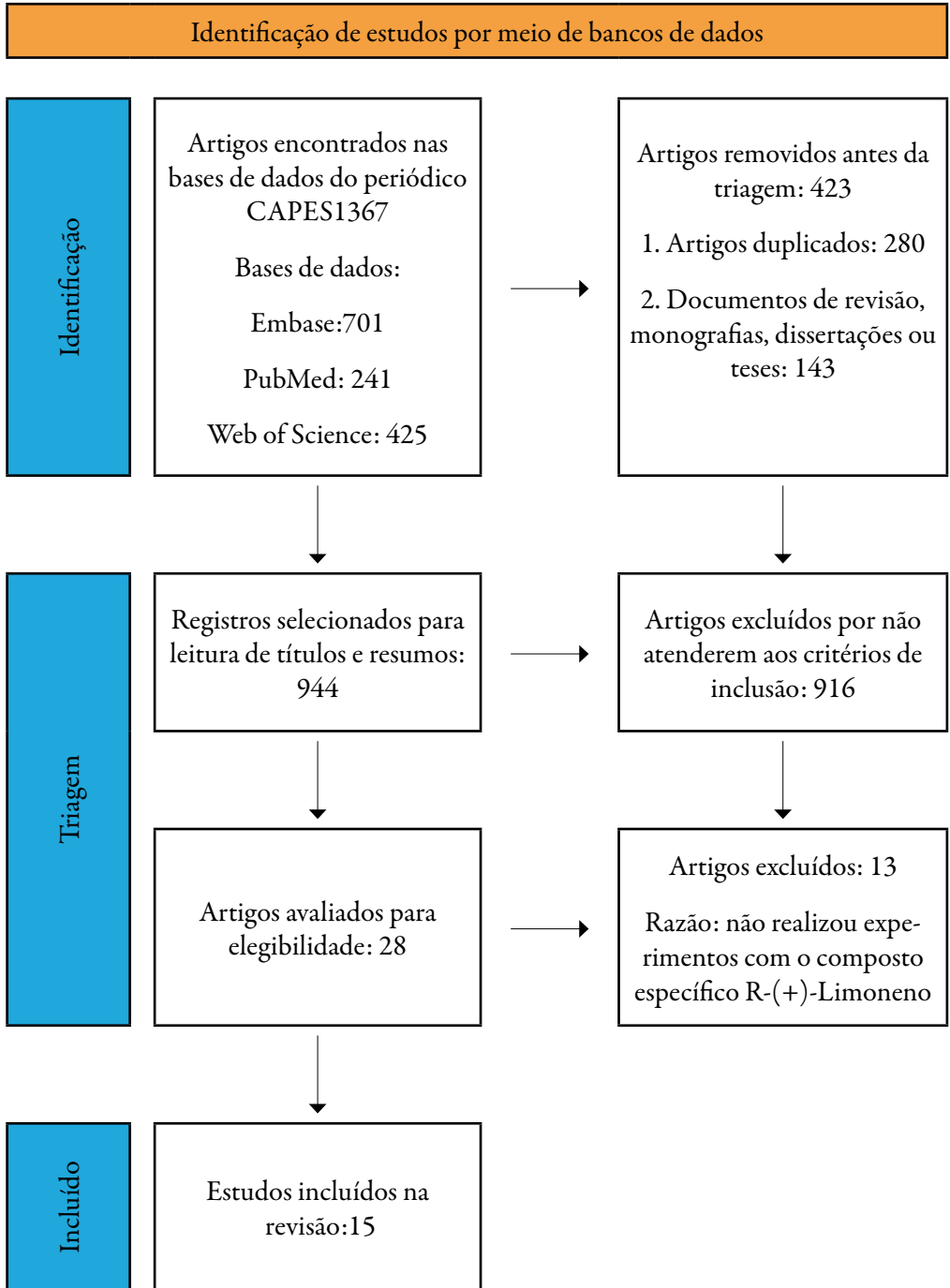


Figura 1. Fluxograma da estratégia de buscas baseado no protocolo PRISMA.

Os resultados principais dos estudos selecionados, como também a caracterização do autor, ano e objetivo estão descritos e sintetizados na tabela 1.

Tabela 1. Caracterização do autor, ano, objetivo e resultados principais incluídos na análise de dados da pesquisa.

Autor/ano /referência	Objetivo	Resultados principais
Costa <i>et al.</i> , 2019 [17]	Avaliar a atividade antibacteriana do D-Limoneno, isolado e complexado com β -ciclodextrina, além de avaliar sua atividade modulatória com diferentes classes antibióticas.	D-Limoneno mostrou uma Concentração Inibitória Mínima (CIM) igual a 256 $\mu\text{g/mL}$ contra <i>Staphylococcus aureus</i> padrão e 512 $\mu\text{g/mL}$ contra <i>Pseudomonas aeruginosa</i> resistente, bem como um efeito sinérgico quando associado à gentamicina.
Yao <i>et al.</i> , 2017 [18]	Demonstrar que quitosana solúvel em água, gelatina de peixe e D-Limoneno podem ser candidatos precursores para preparar material de embalagem de alimentos comestíveis de baixo custo e alto desempenho.	O filme comestível de quitosana de gelatina de peixe contendo a maior concentração de D-Limoneno, inibiu efetivamente o crescimento de <i>Escherichia coli</i> , produzindo halos de cerca de 22 mm.
Silva <i>et al.</i> , 2021 [19]	Determinar a concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM), analisar a terapia combinada com florfenicol e oxitetraciclina e verificar a atividade de inibição de biofilme dos fitoquímicos R-(+)-Limoneno e S-(-)-Limoneno contra algumas bactérias isoladas de jundiá (<i>Rhamdia quelen</i>).	R-(+)-Limoneno apresentou atividade antibacteriana fraca contra <i>Aeromonas hydrophila</i> e nenhuma atividade contra <i>Citrobacter freundii</i> , <i>Raoultella ornithinolytica</i> e <i>Stenotrophomonas maltophilia</i> . No entanto, inibiu fortemente a formação de biofilme de <i>A. hydrophila</i> . R-(+)-Limoneno teve um efeito aditivo quando combinado com florfenicol e um efeito antagonista com oxitetraciclina.
Shao <i>et al.</i> , 2018 [20]	Desenvolver um revestimento comestível incorporando diferentes concentrações de emulsão de R-(+)-Limoneno em <i>Ulva fasciata</i> e avaliar a eficácia antimicrobiana contra inóculos de diferentes bactérias.	Os revestimentos comestíveis com pelo menos 0,15% w/w de R-(+)-Limoneno melhoraram a estabilidade microbiana dos morangos, demonstrou eficácia na descontaminação de patógenos externos como <i>E. coli</i> , <i>Bacillus subtilis</i> e <i>S. aureus</i> .

Autor/ano /referência	Objetivo	Resultados principais
Lan <i>et al.</i> , 2020 [21]	Fornecer uma visão inicial sobre propriedades do filme composto PVA (álcool polivinílico)/CS(quitosana)/DL (D-Limoneno) e a viabilidade alternativa de filmes de embalagem poliméricas naturais para prolongar a vida de produtos frescos e processados.	A adição de D-Limoneno melhorou a atividade antibacteriana dos filmes de PVA e CS contra as duas patologias mais comuns: <i>E. coli</i> e <i>S. aureus</i> .
Su <i>et al.</i> , 2020 [22]	Investigar o efeito de goma arábica (GA) na estabilidade física de emulsões estabilizadas com β -lactoglobulina (β -lg) e, em seguida, comparar a estabilidade de camada única/bicamada sob tratamento UHT e avaliar a eficiência antibacteriana das emulsões de D-Limoneno com ou sem tratamento UHT.	Foi detectado que o valor MIC da emulsão contendo D-Limoneno estabilizadas por membranas de camada única (β -lg) aumentou após o tratamento com UHT contra as bactérias testadas (<i>E. coli</i> , <i>S. aureus</i> e <i>B. subtilis</i>). Já o tratamento UHT utilizando emulsões estabilizadas por membranas de bicamada (β -lg-GA) não tem impacto nas propriedades antibacterianas.
Zhang <i>et al.</i> , 2014 [23]	Estudar os efeitos da nisina sobre a atividade antimicrobiana do D-Limoneno e sua nanoemulsão e desenvolver um novo sistema de entrega antimicrobiana combinando o efeito positivo desses dois agentes antibacterianos ao mesmo tempo.	O D-Limoneno exibiu efeitos inibitórios elevados em relação ao crescimento em todas as cepas de bactérias testadas (<i>E. coli</i> , <i>S. aureus</i> e <i>B. subtilis</i>). A combinação de D-Limoneno e nisina exibiu um sinergismo em relação a <i>S. aureus</i> e <i>B. subtilis</i> , e exibiu um efeito aditivo para <i>E. coli</i> . Os efeitos das nanoemulsões de D-Limoneno em combinação com nisina contra todos os microrganismos exibiu um desempenho antimicrobiano muito mais forte comparado com a nanoemulsão sem nisina e D-Limoneno não processado.

Autor/ano /referência	Objetivo	Resultados principais
Umagiliyage <i>et al.</i> , 2017 [24]	Investigar a atividade antimicrobiana de nanovesículas unilamelares (lipossoma) contendo D-Limoneno contra bactérias causadoras de doenças transmitidas por alimentos (<i>E. coli</i> e <i>Listeria monocytogenes</i>) e fungos apodrecedores de frutas selecionados (<i>Botrytis cinerea</i> e <i>Penicillium criségeno</i>).	A atividade antibacteriana do lipossoma contendo D-Limoneno foi mais elevada contra bactéria gram positiva <i>L. monocytogenes</i> do que na bactéria gram-negativa <i>E. coli</i> nas concentrações de 10 µM e 50 µM. Dessa forma, lipossomas contendo Limoneno foram considerados úteis para reduzir a perda de armazenamento devido à deterioração.
Roy e Rhim, 2020 [25]	Preparar um filme funcional a base de Pullulan/Carrageenan integrando Sulfeto de cobre (CuSNP) e D-Limoneno (DL) como enchimentos para aplicações de embalagem de alimentos ativos e investigar o efeito de CuSNP e Limoneno isoladamente ou em combinação com filmes à base de Pullulan/Carrageenan.	Filmes contendo a combinação CuSNP e DL apresentou um efeito bactericida superficial. Já o D-Limoneno mostrou apenas alguma atividade antibacteriana contra o patógeno <i>E. coli</i> enquanto que o CuSNP mostrou forte redução contra <i>E.coli</i> e leve redução contra <i>L.monocytogenes</i> .
Lan <i>et al.</i> , 2019 [26]	Investigou a influência da adição de D-Limoneno a nanomateriais de PVA eletrofiado e analisou o processamento ultrassônico de fibras eletrofiadas de PVA/D- limoneno em diferentes composições, como também avaliou a atividade antibacteriana contra <i>E. coli</i> e <i>S. aureus</i> .	A adição do composto D-Limoneno elevou a inibição do crescimento dos dois patógenos testados (<i>E. coli</i> e <i>S. aureus</i>), porém o maior efeito antibacteriano foi para <i>E. coli</i> .
Guo <i>et al.</i> , 2020 [27]	Investigar as propriedades estruturais e antimicrobianas das microemulsões à base de polissorbatos (Tween 60 e 80) (PMEs) para encapsulação de R-(+)-Limoneno (LMN).	A diluição L60 apresentou CIM menor para <i>S. aureus</i> em comparação com a CIM de L80 e Limoneno total (LMN) e para as cepas de <i>E. coli</i> e <i>P. aeruginosa</i> a CIM de L60 e L80 teve melhor efeito do que a do LMN.

Autor/ano /referência	Objetivo	Resultados principais
Mehanna <i>et al.</i> , 2020 [28]	Desenvolver um nanoe-mulgel ocular eficaz <i>in-situ</i> à base de R-(+)-Limoneno para aumentar a eficácia das fluoroquinolonas contra a infecção do biofilme ocular associada a cepas de <i>S. aureus</i> resistentes à meticilina (MRSA).	Verificou-se que a CIM do Sistema de Entrega Autonanoemulsificantes (SNEDS) à base de R-(+)-Limoneno sozinho mostrou alta concentração (25 µg/ml), enquanto que a CIM do SNEDS a base de R-(+)-Limoneno carregado com levofloxacina foi menor (3,12 µg/ml), o que também ocorreu para os resultados de CBM contra o mesmo patógeno, onde SNEDS à base de Limoneno carregado com levofloxacina apresentou menor valor (12,5 µg/ml). Em relação à formação de biofilme a inibição é observada apenas com SNEDS a base de Limoneno carregado com levofloxacina.
Salehi <i>et al.</i> , 2021 [29]	Realizar encapsulamento de D- Limoneno (L) em nanoesponjas de β-ciclodextrina (CD-NS) para desenvolver um sistema de liberação controlada para que L obtenha uma atividade antibacteriana adequada para aplicações em alimentos.	A CIM e CBM com melhor resultado encontrado foi do encapsulamento de Limoneno em nanoesponjas (L-NS) para todas as bactérias testadas e o patógeno mais susceptível foi a <i>Shigella flexneri</i> , o que indica que pode inibir completamente o crescimento bacteriano.
Sieniawska <i>et al.</i> , 2018 [30]	Investigar se os terpenos naturais apresentam alguma ação sinérgica com agentes antituberculose contra cepas clínicas de <i>Mycobacterium tuberculosis</i> multirresistentes (MTb).	R-(+)-Limoneno apresentou boa atividade antibacteriana contra todas as cepas testadas (MIC = 128–256 µg/mL) e para a cepa H37Ra apresentou uma inibição de (32 µg/mL). O mesmo também influenciou positivamente a maioria das cepas testadas quando combinado com os antibióticos (etambutol, rifampicina e isoniazida).
Lee <i>et al.</i> , 2016 [31]	Verificar as principais funções biológicas dos terpenos voláteis (α-pineno, limoneno (R e S) e α-copaeno) indutores de mancha bacteriana do arroz, causada por <i>Xanthomonas oryzae</i> (Xoo)	R-(+)-Limoneno só inibiu significativamente o crescimento do Xoo em concentrações mais elevadas, já em ensaios de difusão em disco o R-(+)-Limoneno apresentou pouco ou nenhum efeito na inibição de Xoo.

O composto R-(+)-Limoneno constituinte em diferentes óleos essenciais ou isoladamente apresenta um potencial antibacteriano contra bactérias do grupo dos cocos e bacilos, tanto Gram positivo do gênero *Staphylococcus*, *Bacillus*, *Listeria*, quanto Gram negativo do gênero *Pseudomonas*, *Escherichia*, *Aeromonas*, *Shigella*, *Xanthomonas*. Além disso, também foi identificado efeito antibacteriano contra o gênero *Mycobacterium*.

Segundo Costa *et al.* [17] em seu estudo avaliaram o D-Limoneno através do método de microdiluição em caldo e verificou a concentração inibitória mínima (CIM) e o efeito modulador comparativo do Limoneno sozinho e em associação com antibióticos. Dessa forma, o D-Limoneno apresentou relevante atividade antibacteriana clínica, para bactérias Gram-positivas (SA ATCC 25923) e Gram-negativas (PA 24), bem como um efeito sinérgico quando associado à gentamicina contra *S. aureus* e *E. coli*, para os demais medicamentos testados apresentou efeito antagônico.

Nesse contexto, em uma pesquisa onde foi avaliado o potencial antibacteriano das diluições de D-Limoneno (L60 e L80) foi constatado que as mesmas exibiram uma boa atividade antimicrobiana contra todas as três cepas testadas (*S. aureus*, *E. coli* e *P. aeruginosa*). A combinação de LMN e ϵ -polilisina (EPL) apresentou efeito sinérgico contra todas as bactérias testadas. Dessa forma, observou que o trabalho fornece informações úteis sobre a aplicação dos polissorbatos como carreadores para encapsulamento de óleos essenciais em indústrias farmacêuticas [27].

De forma semelhante, identificou-se que a adição de D-Limoneno a nanomateriais de PVA eletrofiado aumentou a inibição do crescimento de ambos os tipos de bactérias (*E. coli* e *S. aureus*), onde o efeito antibacteriano foi melhor para *E. coli*. O processamento ultrassônico melhorou as propriedades antibacterianas, pois possibilita o aumento do teor de D-Limoneno por unidade de área da fibra, aumentando seu contato com as bactérias e consequentemente aumentando as propriedades antibacterianas [26].

De acordo com Silva *et al.* [19], os fitoquímicos (R-(+)-Limoneno e S-(-)-Limoneno) demonstraram atividade antibiofilme forte contra *Aeromonas hydrophila* e quando combinado com florfenicol, mostraram um efeito aditivo no tratamento da mesma bactéria. O mesmo informa que os fitoquímicos testados podem ser agentes de terapia de combinação promissores com florfenicol para aplicações potenciais em infecções clínicas, porém é necessária a investigação da atividade antibacteriana destes compostos contra bacteriose in vivo, pois in vitro não permite uma boa previsão do efeito in vivo.

Um estudo realizado com a emulsão do D-Limoneno frente várias técnicas, dentre elas, a Concentração Inibitória Mínima (CIM), observou-se que os valores da CIM da emulsão estabilizada com β -lg (camada única) aumentou significativamente após o tratamento com UHT frente as três cepas de bactérias testadas (*E. coli*, *S. aureus* e *B.*

subtilis). Enquanto que, nenhuma diferença da CIM pode ser encontrada entre emulsões de bicamada com ou sem processamento UHT, confirmando o comportamento melhorado das emulsões de bicamada no processamento UHT. Dessa forma, os resultados do trabalho fornecem possibilidade de incorporar óleos essenciais com atividade antimicrobiana em um sistema de entrega de emulsão com uma alta estabilidade físico-química e processabilidade UHT [22].

De forma corroborativa Mehanna, Mneimneh e Abed El Jalil [28] além de outras técnicas, verificou a atividade antimicrobiana, onde pôde analisar a CIM, CBM e a formação de biofilme contra *S. aureus* resistente a meticilina com base no Sistema de Entrega Autonanoemulsificantes (SNEDS) à base de R-(+)-Limoneno carregado ou descarregado com levofloxacina. Desse modo, verificou que tanto para CIM, como para CBM e para formação de biofilme o SNEDS a base de R-(+)-Limoneno carregado com levofloxacina teve melhor efeito e pôde-se observar que esse sistema carregado com levofloxacina é uma alternativa promissora contra bactérias resistentes e formadoras de biofilme.

Nessa conjuntura, em uma pesquisa realizada com o R-(+)-Limoneno frente cepas de *Mycobacterium tuberculosis* resistente (MTb), o mesmo apresentou boa atividade antibacteriana, como também em associação com antibiótico demonstrou efeito positivo. Portanto, sendo considerado promissor para o tratamento contra (MTb) [30].

No contexto dos estudos, Lee *et al.* [31] demonstraram que a estereoespecificidade do composto pode ser um fator determinante de funções biológicas, pois apenas o enantiômero específico, (S)-Limoneno e não (R)-Limoneno, apresentou atividade antibacteriana forte contra *Xoo*. Os resultados demonstram a possibilidade de que apenas o enantiômero (S)-Limoneno pode ser usado como uma alternativa de produto químico ecológico para controlar a doença na fase de muda.

No entanto, pesquisas revelam que o D-Limoneno apresenta elevados efeitos inibitórios em relação aos microrganismos *E. coli*, *S. aureus* e *B. subtilis* e a combinação desse composto com nisina apresentou efeitos sinérgicos e aditivos frente as cepas testadas, como também efeitos antagônicos e de indiferenças foram observados, porém a combinação de D-Limoneno com a nisina resultou em um alto efeito antimicrobiano. Desse modo, pôde concluir que a aplicação combinada fornece um método antimicrobiano mais eficaz para a produção, processamento, serviço e consumo de alimentos e bebidas [23].

Por meio de diferentes metodologias, Yao *et al.* [18] demonstraram que os filmes comestíveis de gelatina-quitosana de peixe suplementado com o D-Limoneno revelou forte atividade antibacteriana contra *E. coli*. Como também, a incorporação do mesmo no filme de gelatina-quitosana de peixe melhorou a resistência à penetração de água e luz e a ductilidade.

Dessa forma, utilizar o R-(+)-Limoneno evidencia potenciais vantagens como antimicrobiano natural dentro de revestimento comestível, atuando como conservantes e melhorando a segurança, qualidade e propriedades nutricionais dos alimentos, pois foi investigada a eficácia das emulsões do R-(+)-Limoneno na diminuição do crescimento de microorganismos através da difusão de seus componentes ativos voláteis e constatou que os revestimentos de R-(+)-Limoneno mostrou eficácia na descontaminação dos patógenos externos *E. Coli*, *B. subtilis* e *S. aureus*, como também preservou a aparência dos morangos por mais tempo, estendendo sua vida útil [20].

Nesse cenário, Lan *et al.* [21] demonstraram que a adição de D-Limoneno melhorou a atividade antibacteriana dos filmes contendo PVA/CS contra: *E. coli* e *S. aureus*. Além disso, o composto PVA/CS/DL-5% prolongou a vida útil da manga apresentando uma taxa de decomposição inferior a 20% após 10 dias de armazenamento à temperatura ambiente. Desse modo, este estudo indicou que os filmes de PVA/CS incorporados com DL podem ser promissores como um material de embalagem ecológico para preservação de alimentos, devido à sua considerável biodegradabilidade, propriedades mecânica e atividade antibacteriana.

Em estudo com o D-Limoneno encapsulado em lipossoma demonstrou uma boa estabilidade física e forte atividade antimicrobiana *in vitro* contra as cepas testadas e o mesmo reduziu a perda de armazenamento do mirtilo em um terço, no período de nove semanas. Logo, D-Limoneno encapsulado em lipossoma como um revestimento comestível, pode ser uma boa alternativa de tratamento pós-colheita para estender a vida de armazenamento de bagas em curta temporada [24].

Conforme, Roy e Rhim [25] os filmes contendo CuSNP e DL em sua formação mostrou alguma atividade antibacteriana contra as cepas testadas *E. coli* e *L. monocytogenes*, porém a atividade antibacteriana de CuSNP foi mais eficaz em comparação com DL, onde o mesmo mostrou ser eficaz apenas contra *E. coli*. Portanto, os filmes apresentam efeito antimicrobiano contra os agentes patogênicos alimentares e pode ser usado para controlar o crescimento do microorganismo em alimentos embalados, porém são necessárias pesquisas adicionais.

Em ensaios de avaliação da CIM e CBM do D-Limoneno livre (L) e D- Limoneno carregado com nanoesponjas (L-NS) contra quatro bactérias *E. coli*, *Shigella flexneri*, *S. aureus* e *Enterococcus*. Os valores da CIM e CBM para L-NS foram significativamente menores em comparação com L livre sendo indicativo que nanoesponjas com ciclodextrinas (CD-NS) pode elevar o potencial efeito antibacteriano de L. O maior efeito da atividade antimicrobiana e a liberação controlada de L encapsulado de CD-NS, sugerem que os mesmos têm um potencial como agente antimicrobiano para uso em embalagens de alimentos ou formulações de alimentos [29].

CONCLUSÃO

Analisando as evidências de todos os estudos mencionados, pode-se concluir que o terpeno R-(+)-Limoneno pode ser considerado como uma substância promissora para o combate de doenças causadas por bactérias patogênicas. No entanto, mais estudos são necessários para aprofundar o mecanismo de ação, bem como, frente a maior quantidade de espécies bacterianas isoladas de diferentes origens, a fim de se compreender de forma mais eficaz esse efeito biológico encontrado.

CONFLITO DE INTERESSES

Os autores declaram não haver conflito de interesses.

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COMO CITAR ESTE ARTIGO

M. de Souza-Alves, M.A. Araújo de Medeiros, A. Alves de Oliveira-Filho, Atividade antibacteriana do R-(+)-Limoneno: uma revisão sistemática, *Rev. Colomb. Cienc. Quím. Farm.*, 51(2), 1255-1272 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100584>

Modeling the solubility of ketoprofen in mono-solvents at various temperatures

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Received: February 11, 2022

Corrected: March 23, 2022

Accepted: March 30, 2022

SUMMARY

Aim: To report a correlative model to calculate the solubility of ketoprofen in mono-solvents at various temperatures. **Methodology:** Previously reported solubility data of ketoprofen in a number of mono-solvents at various temperatures are re-analyzed using a recently developed model employing Abraham, Hansen and Catalan parameters as input values. The accuracy of the model is evaluated by computing the standard deviation of residuals (SDRs) and compared with those of previous models. **Results:** The new model provided very accurate correlation for the solubility of ketoprofen in the investigated mono-solvents at various temperatures. The obtained SDR is 2.20 where the SDRs of previously reported models are 1.54 to 2.56. The new model correlates whole solubility data using a single model, whereas other models correlate the solubility data in each mono-solvent using a separate set of model constants. **Conclusion:** The trained model provided reasonably accurate results for ketoprofen by using Abraham, Hansen and Catalan parameters of the mono-solvents as input values.

Keywords: Solubility, van't Hoff model, mono-solvents, correlation, temperature.

RESUMEN

Modelación de la solubilidad del ketoprofeno en mono-solventes a varias temperaturas

Objetivo: reportar un modelo correlativo para calcular la solubilidad del ketoprofeno en mono-disolventes a varias temperaturas. **Metodología:** los datos de solubilidad de ketoprofeno reportados anteriormente en varios mono-solventes a varias temperaturas se analizan nuevamente utilizando un modelo desarrollado recientemente que emplea los parámetros de Abraham, Hansen y Catalan como valores de entrada. La precisión del modelo se evaluó calculando la desviación estándar de los residuos (DER) y se compara con los modelos anteriores. **Resultados:** el nuevo modelo proporcionó una correlación muy precisa de la solubilidad del ketoprofeno en los mono-solventes investigados a varias temperaturas. El DER obtenido es 2,20 mientras que los DER de los modelos reportados anteriormente variaron de 1,54 a 2,56. El nuevo modelo correlaciona todos los datos de solubilidad utilizando un solo modelo, mientras que otros modelos correlacionan los datos de solubilidad en cada mono-solvente utilizando un conjunto separado de constantes. **Conclusión:** el modelo entrenado proporcionó resultados razonablemente precisos para la solubilidad del ketoprofeno utilizando los parámetros de Abraham, Hansen y Catalan de los mono-solventes como valores de entrada.

Palabras clave: Solubilidad, modelo de van't Hoff, mono-solventes, correlación, temperatura.

RESUMO

Modelando a solubilidade do cetoprofeno em monossolventes em várias temperaturas

Objetivo: relatar um modelo correlativo para calcular a solubilidade do cetoprofeno em monossolventes em várias temperaturas. **Metodologia:** os dados de solubilidade relatados anteriormente para o cetoprofeno em vários monossolventes em várias temperaturas são reanalisados usando um modelo desenvolvido recentemente usando os parâmetros de Abraham, Hansen e Catalan como valores de entrada. A precisão do modelo foi avaliada pelo cálculo do desvio padrão dos resíduos (DP) e comparada com modelos anteriores. **Resultados:** o novo modelo forneceu uma correlação altamente precisa da solubilidade do cetoprofeno nos monossolventes

investigados em várias temperaturas. O DER obtido é de 2,20 enquanto o DER dos modelos relatados anteriormente variou de 1,54 a 2,56. O novo modelo correlaciona todos os dados de solubilidade usando um único modelo, enquanto outros modelos correlacionam os dados de solubilidade em cada monossolvente usando um conjunto separado de constantes. **Conclusão:** o modelo treinado forneceu resultados razoavelmente precisos para a solubilidade do cetoprofeno usando os parâmetros Abraham, Hansen e Catalan dos monossolventes como valores de entrada.

Palavras-chave: Solubilidade, modelo de van't Hoff, monossolventes, correlação, temperatura.

INTRODUCTION

Ketoprofen (2-(3-benzoylphenyl)-propionic acid) is a potent analgesic and anti-pyretic drug prescribed as a painkiller. It is a low soluble in water and possesses a chiral center, but it is used as a racemic mixture. The solubility of pure S-ketoprofen is more than that of the racemate [1]. Ketoprofen is a class II drug, low solubility and high permeability, in the biopharmaceutical classification system with a partition coefficient ($\log P$) of approximately 3.12 [2]. Its aqueous solubility at 37 °C is 0.45 mg/mL was increased to 34.88 mg/mL after converting as a sodium salt [3]. The solubility of ketoprofen was also increased by addition of ethanol [4] and propylene glycol [5] as the pharmaceutical cosolvents. Solubility of ketoprofen in organic solvents are available from the literature [6] but shows variability up to 300%. As an example, the molar solubility of ketoprofen in ethanol at 298.2 K were reported as 1.038 (or 0.0701 in mole fraction) [7], 0.957 (or 0.0640 in mole fraction) [8], 0.672 (or 0.0441 in mole fraction) [9] and 1.746 [10]. Possible reasons for such discrepancies in the experimental solubilities arise from; drug purity, solvent purity, lack of equilibration, temperature, analysis method, laboratory technique, typographical error, polymorphism or enantiomeric forms of the drug [11]. Soto *et al.* [12] reported the solubility data of ketoprofen in eight monosolvents at various temperatures.

Aqueous solubility of drugs could be calculated using a number of published models including the general solubility equation (GSE) of Yalkowsky [13], the linear solvation energy relationship (LSER) approach of Abraham [14], a combined two parameters model [15] and some other models/software which were reviewed in a previous publication [16]. The solubility of drugs in organic solvents could also be computed using some published models including an adopted version of GSE for calculating the solubility of drugs in octanol [17], a model proposed by Dearden *et al.* to calculate

the solubility of solutes in cyclohexane [18], the Abraham solvation model [19] and the KAT-LSER model [20]. All these models were briefly reviewed in a recent work [21] and calculate the solubility of drugs in a given solvent or the solubility of a drug in various solvents at an isothermal condition. Efficient crystallization process of drugs is required in the pharmaceutical industry, since the small amount of impurities may cause severe health problems. Selection of an appropriate solvent and finding a suitable temperature range are crucial to provide an efficient crystallization method and the trial and error approach is commonly used in practice, since there is no a good model to simulate these conditions so far. The aim of this short communication is to report a new model for correlating the solubility of ketoprofen in the mono-solvent systems at various temperatures. The model provides useful information for calculating the solubility of ketoprofen in different solvents at various temperatures, which are in demand in many industrial applications.

METHODS AND MATERIALS

The reported experimental solubility data of ketoprofen in eight mono-solvents, *i.e.* methanol, ethanol, 2-propanol, 1-butanol, acetonitrile, ethyl acetate, 1,4-dioxane and toluene at seven temperatures ranging from 273.15 to 303.15 K [12] is re-analyzed in this work. Soto et al. correlated the solubility data using some mathematical models and reported the accuracy of the calculations using the standard deviation of residuals (*SDR*). The *SDR* values were computed by:

$$SDR = \left[\frac{\sum (x_2^{cal} - x_2)^2}{N - 1} \right]^{1/2} \quad (1)$$

where N is the number of experimental data points, x_2^{cal} is the calculated solubility and x_2 is the experimental solubility of ketoprofen in the mono-solvent systems.

The van't Hoff equation is widely used for correlating the solute solubility of a given drug in a certain mono-solvent at temperatures varying in a narrow range. The model is presented as:

$$\ln x_2 = \alpha + \frac{\beta}{T} \quad (2)$$

in which, α and β are the model constants. The α and β terms represent the entropic and enthalpic changes in the solution. In the proposed model it has been assumed that these changes could be correlated using a combination of Abraham solvation parameters (AP_i), Hansen solubility parameters (HP_i) and Catalan parameters (CP_i) [21]. The numerical values of AP_i , HP_i and CP_i for the investigated mono-solvents are listed in table 1. The general form of the proposed model is:

$$\ln x_2 = \left(\alpha_0 + \sum_{i=1}^5 \alpha_{i,Ap} AP_i + \sum_{i=1}^3 \alpha_{i,HP} HP_i + \sum_{i=1}^4 \alpha_{i,CP} CP_i \right) + \left(\frac{\beta_0 + \sum_{i=1}^5 \beta_{i,Ap} AP_i + \sum_{i=1}^3 \beta_{i,HP} HP_i + \sum_{i=1}^4 \beta_{i,CP} CP_i}{T} \right) \quad (3)$$

where α and β terms are the model constants.

Table 1. The Abraham, Hansen and Catalan parameters of the investigated solvents.

Solvent ⁻						
Abraham →	<i>c</i>	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>
Acetonitrile	0.41	0.08	0.33	-1.57	4.39	3.36
n-Butanol	0.17	0.40	-1.01	0.06	-3.96	4.04
1,4-Dioxane	0.10	0.35	-0.08	-0.56	-4.83	4.17
Ethyl acetate	0.33	0.37	-0.45	-0.70	-4.90	4.15
Ethanol	0.22	0.47	-1.04	0.33	-3.60	3.86
Isopropanol	0.10	0.34	-1.05	0.41	-3.83	4.03
Methanol	0.28	0.33	-0.71	0.24	-3.32	3.55
Toluene	0.14	0.53	-0.72	-3.01	-4.82	4.55
Hansen →	δ_D	δ_P	δ_H			
Acetonitrile	11.59	12.95	16.34			
n-Butanol	16.00	5.70	15.80			
1,4-Dioxane	19.00	1.80	7.40			
Ethyl acetate	15.80	5.30	7.20			
Ethanol	15.80	8.80	19.40			
Isopropanol	12.97	10.35	15.68			
Methanol	15.10	12.30	22.30			
Toluene	18.00	1.40	2.00			

(Continued)

Solvent ^a						
Abraham →	<i>c</i>	<i>e</i>	<i>s</i>	<i>a</i>	<i>b</i>	<i>v</i>
Catalan →	<i>SP</i>	<i>SdP</i>	<i>SA</i>	<i>SB</i>		
Acetonitrile	0.65	0.97	0.04	0.29		
n-Butanol	0.67	0.66	0.34	0.81		
1,4-Dioxane	0.74	0.31	0.00	0.44		
Ethyl acetate	0.66	0.60	0.00	0.54		
Ethanol	0.63	0.78	0.40	0.66		
Isopropanol	0.63	0.81	0.28	0.83		
Methanol	0.61	0.90	0.61	0.55		
Toluene	0.78	0.28	0.00	0.13		

Source: Jouyban *et al.* [21].

The accuracy of the proposed model was compared with those of previously applied models by Soto *et al.* [12] employing the *SDR* values. Soto *et al.* used an empirical three-parameter model represented as:

$$\ln x_2 = \frac{c_1}{T^2} + \frac{c_2}{T} + c_3 \quad (4)$$

the Buchowski-Ksiazczak equation:

$$\ln \left[1 + \frac{\lambda(1-x_2)}{x_2} \right] = \lambda h \left[\frac{1}{T} - \frac{1}{T_m} \right] \quad (5)$$

and an activity coefficient (γ_2) based model of Svrd-Rasmuson [22]:

$$\ln \gamma_2 = \frac{c_4}{T} \left(1 - e^{-\left(\frac{c_5}{T} - \frac{c_5}{T_m} \right)^{c_6}} \right); \quad \{c_6 \geq 2\} \quad (6)$$

In Eqs. (4)-(6), c terms, λ and h , are the model constants, T_m is the melting point of ketoprofen.

RESULTS AND DISCUSSION

The significant ($p < 0.05$) parameters obtained from the regression analysis of ketoprofen solubility data in the investigated mono-solvents at various temperatures is:

$$\ln x_2 = (35.789 - 14.658c + 17.622e - 1.959a - 0.064b - 2.048\delta_D - 8.735SA) + \left(\frac{4552.248 + 4429.264c - 5751.595e + 1859.422a - 431.710\delta_p - 5582.527SB}{T} \right) \quad (7)$$

in which, c , e , a and b are the AP_i parameters, δ_D and δ_p are HP_i , SA and SB are CP_i parameters. The significant contribution of an independent variable on the correlation/prediction capability of a model is evaluated by using t-test in which the equality of the model constant with zero is tested for the independent variable under investigation. The model constant is considered significant when the p values is less than 0.05. Equation (7) correlated the solubility data of ketoprofen in the investigated mono-solvents with the $R > 0.999$ and F value of 1870 ($N=56$). The F test examines the significance of a correlation of the dependent variable with the included independent variables in the built model and is a function of the number of cases (i.e. N or the number of investigated solubility data points in this work) and the number of independent variables. The F value is considered significant when p is less than 0.05. The SDR_s for the calculated solubility data for each mono-solvent along with the SDR_s for the above mentioned models were listed in table 2. The overall SDR values for equations (2), (4), (5) and (6) are 2.04, 2.15, 2.56 and 1.54, respectively, whereas the corresponding value for equation (7) is 2.20. It is obvious that all these overall SDR values could be considered as acceptable error ranges. It should be added that the proposed model was trained for all investigated solvents whereas other models should be trained for each mono-solvent using separate model constants.

Table 2. The standard deviation of residuals ($SDR \times 1000$) of various equations for correlating ketoprofen in the investigated mono-solvents at various temperatures.

Solvent	Eq. (2)	Eq. (4) ^a	Eq. (5) ^a	Eq. (6) ^a	Eq. (7)
Acetonitrile	0.88	0.242	0.758	0.177	0.88
n-Butanol	2.61	2.975	3.136	0.744	3.63
1,4-Dioxane	2.48	3.406	4.123	1.489	2.48
Ethyl acetate	2.37	1.408	2.366	0.293	2.08
Ethanol	2.02	2.649	2.726	1.385	2.09
Isopropanol	1.87	2.398	2.439	2.418	2.22
Methanol	3.61	3.817	4.509	5.749	3.70
Toluene	0.50	0.299	0.445	0.093	0.52
Overall	2.04	2.15	2.56	1.54	2.20

^aTaken from Ref. [12].

CONCLUSION

Concerning the above mentioned results, it is evident that the proposed model is a promising one for the mathematical representation of the solubility data of ketoprofen dissolved in mono-solvent systems at various temperatures. The model has the advantage of accurate solubility correlation capability in the mono-solvents at various temperatures. The data is required in the process design and scale up calculations in the chemical/pharmaceutical industries. Some drugs possess polymorphic forms, however, there is no independent variable to represent the characteristics of the polymorphs of a drug, so the proposed model could not be used to calculate the solubility of polymorphic forms and this could be considered as a disadvantage for the model.

ACKNOWLEDGEMENT

This work was supported by the Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran under grant number of 66495.

DISCLOSURE STATEMENT

The author declares that he has no conflict of interest.

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HOW TO CITE THIS ARTICLE

A. Jouyban, Modeling the solubility of ketoprofen in mono-solvents at various temperatures, *Rev. Colomb. Cienc. Quim. Farm.*, 51(3), 1273 -1283 (2022).

Scientific research article / <http://dx.doi.org/10.15446/rcciquifa.v51n3.107385>

The theoretical description for omeprazole electrochemical determination, assisted by the composite poly(1,2,4-triazole)-Ag₂O₂

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Received: July 21, 2021

Corrected: April 2, 2022

Accepted: April 5, 2022

SUMMARY

Introduction: Omeprazole is one of the most used proton-blocking molecules applied in the gastric ulcer treatment. Its blocking effect is achieved by the presence of both acidic and basic nitrogen heteroatoms in its composition. Nevertheless, due to its collateral effects, which may be moderate to severe, the development of its electrochemical determination is really actual. **Methods:** In this work, we describe theoretically the possibility of omeprazole electrochemical determination, assisted by the composite, containing the silver (I, III) oxide as an active substance and the polymer of 1,2,4-triazolic derivative as a mediator. In this case, the omeprazole molecule is gradually oxidized yielding firstly phenolic and then quinonic derivative. The pyridinic nitrogen is oxidized later. **Results:** The electroanalytical process is described by a trivariant equation-set, analysis of which confirms the efficiency of the composite of poly(1,2,4-triazole) with Ag₂O₂. The electrochemical oscillations are

also possible, being realized beyond the detection limit. **Conclusion:** The composite of poly(1,2,4-triazole) with Ag_2O is suitable for omeprazole electrochemical determination both *in vivo* and *in vitro*.

Keywords: Omeprazole, electrochemical sensors, silver (I, III) oxide, conducting polymers, stable steady-state.

RESUMO

A descrição teórica da detecção eletroquímica de omeprazol, assistida pelo compósito poli(1,2,4-triazol)- Ag_2O_2

Introdução: o omeprazol é um dos bloqueadores de protons mais usados no tratamento de úlceras gástricas. O seu efeito de bloqueio se dá pela presença dos heteroátomos de nitrogênio de carizes ácido e básico. Entretanto, vistos os seus efeitos colaterais, que podem ser moderados a severos, o desenvolvimento de um método da sua determinação eletroquímica é realmente atual. **Métodos:** neste trabalho, nós descrevemos teoricamente a possibilidade da determinação eletroquímica de omeprazol, assistida pelo compósito, que contém o óxido de prata (I, III) como substância ativa e o polímero 1,2,4-triazólico como mediador. Neste caso, a molécula do omeprazol se oxida gradualmente, rendendo primeiro o seu derivado fenólico, depois, o quinônico. O nitrogênio piridínico se oxida depois. **Resultados:** o processo eletroanalítico se descreve por um conjunto de equações diferenciais trivariante, cuja análise confirma a eficácia do compósito de poli(1,2,4-triazol) com Ag_2O_2 . As oscilações eletroquímicas também são possíveis, realizando-se além do limite de detecção. **Conclusão:** o compósito de poli(1,2,4-triazol) com Ag_2O é idóneo para a detecção eletroanalítica de omeprazol tanto *in vivo* como *in vitro*.

Palavras-chave: Omeprazol, sensores eletroquímicos, óxido de prata (I, III), polímeros condutores, estado estacionário estável.

RESUMEN

Descripción teórica de la detección electroquímica de omeprazol, asistida por el compuesto poli(1,2,4-triazol)- Ag_2O_2

Introducción: el omeprazol es uno de los bloqueadores de protones más comunes en el tratamiento de úlceras gástricas. Su efecto de bloqueo se da por la presencia

de los heteroátomos de nitrógeno de tipos ácido y básico. Entretanto, sus efectos colaterales pueden variar de moderados a severos, y el desarrollo de un método de su determinación electroquímica es realmente actual. **Métodos:** en este trabajo, nosotros describimos teóricamente la posibilidad de la determinación electroquímica de omeprazol, asistida por el compuesto, que contiene el óxido de plata (I, III) como sustancia activa y el polímero 1,2,4-triazólico como mediador. En este caso, la molécula de omeprazol se oxida gradualmente, rindiendo primero su derivado fenólico, después, el quinónico. El nitrógeno piridínico se oxida ulteriormente. **Resultados:** el proceso electroanalítico se describe por un conjunto de ecuaciones diferenciales trivariante, cuyo análisis confirma la eficiencia del compuesto de poli(1,2,4-triazol) con Ag_2O_2 . Las oscilaciones electroquímicas también son posibles, realizándose lejos del límite de detección. **Conclusión:** el compuesto de poli(1,2,4-triazol) con Ag_2O es idóneo para la detección electroanalítica de omeprazol *in vivo* e *in vitro*.

Palabras clave: Omeprazol, sensores electroquímicos, óxido de plata (I, III), polímeros conductores, estado estacionario estable.

INTRODUCTION

Nowadays, the frequency of verticular and chronic gastritis and gastritic ulcers occurrences has been grown slightly [1-4] in both urban and rural areas. Gastritis and ulcers may be treated by the H₂-blocking molecules, detaining the proton transfer. One of this molecules is omeprazole (6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfenyl]-1H-benzimidazole, M= 345 g/mol, PubChem Number=4594, CAS N: 73590-58-6, Fig. 1).

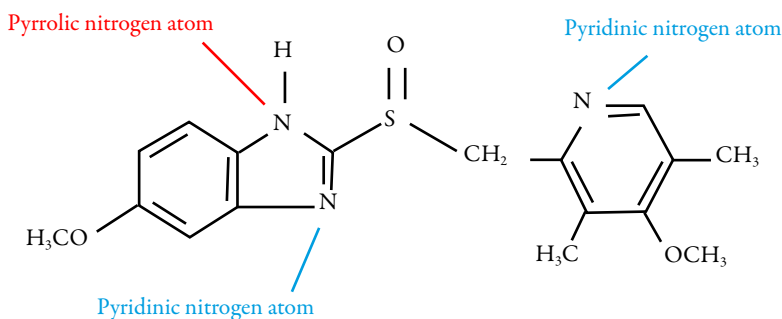


Figure 1. Omeprazole molecule.

Its blocking effect is achieved by the formation of its sulphenamide metabolite, forming complex with both $H^+K^+ATPase^+$ and gastric mucosa carbonic anhydrase [3, 4], inhibiting the proton transfer into the gastric lumen. This effect is possible, due to the presence of both pyrrolic and pyridinic nitrogen atoms, in its composition. Nevertheless, may present various collateral effects, like headache, vertigo, nausea, diarrhea and flatulence, which may cause discomfort [5-8]. Moreover, the concentration control is also important for successful treatment of patients with liver and kidney insufficiency or older people. Thus, the search of a precise, exact, rapid and sensitive method for its quantification is a really actual problem [9-12].

Many electrochemical methods have been proposed for omeprazole [13-15] electrochemical determination. Nonetheless, the bare electrodes may not be suitable, due to high overvoltage needed to commence the electrochemical reaction, thus the electrode must be chemically modified by a hybrid composite, possessing both the active substance and mediator.

This composite may contain inorganic oxidant nanoparticles as active substance, stabilized by organic conducting polymer [16-22], acting as a mediator.

Nevertheless, the organic electrooxidation processes (including the electropolymerization) tend to be accompanied by electrochemical instabilities. These instabilities include the oscillatory changes in electrochemical potential and monotonic instabilities [23-25] and influence the sensing properties of the composite.

Thus, in order to investigate the parameter values, correspondent to the most efficient monomer and polymer formation, like also verify the possibility and probability of the electrochemical instabilities in this system, an *a priori* theoretical observation of the electrochemical determination.

In this work, we analyze the omeprazole electrochemical determination, assisted by the composite of silver (I, III) oxide nanoparticles, stabilized by poly (1,2,4-triazole). In order to achieve it, we realize the specific goals:

- Suggestion of the mechanism of the reaction consequence, leading to the appearance of analytical signal.
- Development of the balance equation mathematical model, correspondent to the electroanalytical system.
- Analysis and interpretation of the model in terms of the electroanalytical use of the system.

- The seek for the possibility of electrochemical instabilities and for the factor, causing them.
- The comparison of the mentioned system 's behavior with the similar ones, involving omeprazole electrochemical determination [26-28].

SYSTEM AND ITS MODELING

The mechanism of the Ag_2O_2 assisted electrochemical determination may be schematically depicted as on the Fig. 2.

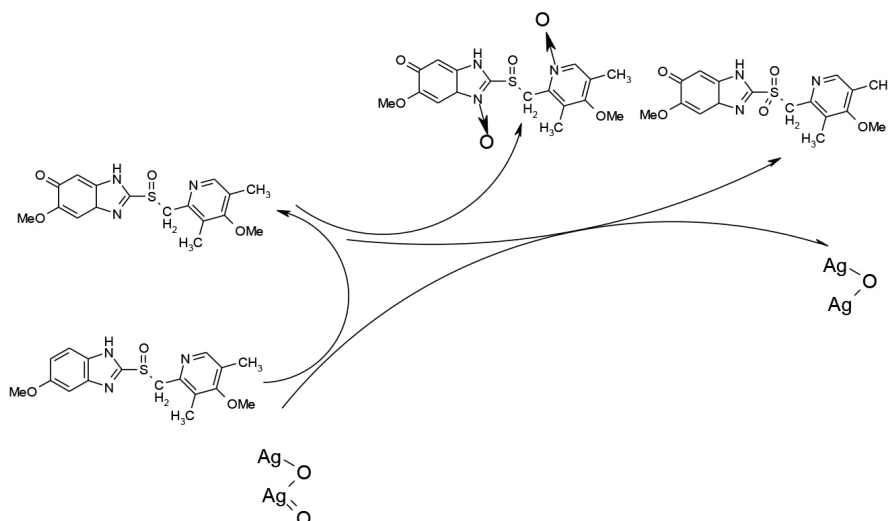


Figure 2. The mechanism for omeprazole electrochemical determination, assisted by a Ag_2O_2 -Poly (1,2,4-triazole) composite.

On the first stage, the omeprazole is oxidized, yielding the quinonic derivatives [13-15, 26-28]. Then, the resulting quinone is oxidized by either nitrogen or sulfur atom, yielding N-oxide and sulfone, respectively. Therefore, in order to describe the behavior of the electroanalytical system, we introduce three variables:

- c : omeprazole concentration in the pre-surface layer;
- q : quinonic form concentration in the pre-surface layer;
- s : silver (I) oxide surface coverage degree.

To simplify the modeling, we suppose that the reactor is intensively stirred, so we can neglect the convection flow. Also we assume that the background electrolyte is in excess, so we can neglect the migration flow. The diffusion layer is supposed to be of a constant thickness, equal to δ , and the concentration profile in it is supposed to be linear.

It is possible to show that the electroanalytical process will be described by the three-dimensional equation set, exposed as:

$$\begin{cases} \frac{dc}{dt} = \frac{2}{\delta} \left(\frac{\Delta}{\delta} (c_0 - c) - r_1 \right) \\ \frac{dq}{dt} = \frac{2}{\delta} (r_1 - r_{21} - r_{22}) \\ \frac{ds}{dt} = \frac{1}{S} (r_1 + r_{21} + r_{22} - r_3) \end{cases} \quad (1)$$

Herein, Δ is the diffusion coefficient, c_0 is the omeprazole bulk concentration, S is the silver (I) oxide maximal surface concentration and the parameters r are the correspondent reaction rates, expressed as:

$$r_1 = k_1 c (1 - s)^3 \quad (2)$$

$$r_{21} = k_{21} q (1 - s)^2 \quad (3)$$

$$r_{22} = k_{22} q (1 - s) \quad (4)$$

$$r_3 = k_3 s \exp \frac{2F\varphi_0}{RT} \quad (5),$$

in which the parameters k are the correspondent rate constants, F is the Faraday number, R is the universal gas constant and T is the absolute temperature.

In this system, the electrochemical determination of omeprazole is gradual, due to the electrochemical oxidation of the benzenic ring to quinonic derivative, followed by N-oxide and sulfone formation. Nevertheless, as all of the additional chemical stages do not influence the DEL capacitance and ionic force, the electroanalytical system will resemble the described in [26-28], as shown below.

RESULTS AND DISCUSSION

In order to describe the behavior of the system with omeprazole electrochemical determination, assisted by a composite Ag_2O_2 -poly(1,2,4-triazoles), we analyze the equation set (1) by means of linear stability theory and bifurcation analysis. The steady-state Jacobian matrix members will be exposed as:

$$\begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} \quad (6),$$

in which:

$$a_{11} = \frac{2}{\delta} \left(-\frac{\Delta}{\delta} - k_1 (1-s)^3 \right) \quad (7)$$

$$a_{12} = 0 \quad (8)$$

$$a_{13} = \frac{2}{\delta} \left(3k_1 c (1-s)^2 \right) \quad (9)$$

$$a_{21} = \frac{2}{\delta} \left(k_1 (1-s)^3 \right) \quad (10)$$

$$a_{22} = \frac{2}{\delta} \left(-k_{21} (1-s)^2 - k_{22} (1-s) \right) \quad (11)$$

$$a_{23} = \frac{2}{\delta} \left(-3k_1 c (1-s)^2 + 2k_{21} q (1-s) + k_{22} q \right) \quad (12)$$

$$a_{31} = \frac{1}{C} \left(k_1 (1-s) \right) \quad (13)$$

$$a_{32} = \frac{1}{C} \left(k_{21} (1-s)^2 + k_{22} (1-s) \right) \quad (14)$$

$$a_{33} = \frac{1}{C} \left(-3k_1 c (1-s)^2 - 2k_{21} q (1-s) - k_{22} q - k_3 \exp \frac{2F\phi_0}{RT} + jk_3 s \exp \frac{2F\phi_0}{RT} \right) \quad (15)$$

Contrarily to the similar systems [26-28], the oscillatory behavior in this system, despite of remaining possible, is of less probability. It is explained by the absence of DEL influences of chemical stages, present in the similar systems. Thus, the unique main-diagonal element, which may be possible (and, consequently, responsible to the positive callback, causing the Hopf bifurcation), is $+jk_{3,s}\exp\frac{2F\phi_0}{RT}$ if $j>0$, describing the cyclic DEL capacitance changes during the electrochemical stage. This factor is common for the great majority of the electroanalytical systems and is realized in sufficient manner to cause significant oscillations far beyond the detection limit. The oscillations are expected to be frequent and of small amplitude.

Yet if the mentioned element is negative, the *steady-state stability* is warranted. Really, applying the Routh-Hurwitz criterion to the Jacobian matrix and introducing new variables, having it rewritten as (16):

$$\frac{4}{\delta^2 S} \begin{vmatrix} -\kappa - \Xi & 0 & \Lambda \\ \Xi & -\Sigma & P - \Lambda \\ \Xi & \Sigma & -P - \Lambda - \Omega \end{vmatrix} \quad (16),$$

and opening the brackets and applying the condition $\text{Det } J < 0$, salient from the criterion, we obtain the steady-state stability requirement (17):

$$-\kappa(2\Sigma\Lambda + \Sigma\Omega) + 2\Lambda\Xi\Sigma < 0 \quad (17)$$

Contrarily to the similar systems [26-28], which are mostly diffusion-controlled, this system will be kinetically-controlled, due to the relative strong destabilizing influences on the first chemical stage.

Nonetheless, it defines the vast parameter region of the steady-state stability. From the electroanalytical point of view, it is correspondent to the linear dependence between the electrochemical parameter and the drug concentrations, describing an efficient diffusion-controlled electroanalytical process, even more stable than in similar systems [26-28].

Thus, it is possible to conclude that the Ag_2O_2 -Poly (1,2,4-triazoles) composite is efficient electrode modifier for the omeprazole electrochemical determination.

The monotonic instability, correspondent to the detection limit, from the electroanalytical point of view, is also probable, if the destabilizing and stabilizing influences are equal. Its conditions will be described as:

$$-\kappa(2\Sigma\Lambda+\Sigma\Omega)+2\Lambda\Xi\Sigma=0 \quad (18)$$

The same model may be used for the electrochemical determination of omeprazole, assisted by silver (I, III) oxide, stabilized by conducting polymer of other monomers. Yet the use of cobalt (IV) oxide in some conditions excludes the N-oxide formation, annihilating the r_{21} . In the rest, the model will be similar to described above.

CONCLUSIONS

From the system with the electrochemical determination of omeprazole on a Ag_2O_2 – Poly(1,2,4-triazole) composite it was possible to conclude that:

- Contrarily to the similar systems involving omeprazole electrochemical detection, this electroanalytical system will be kinetically-controlled.
- The linear dependence between the electrochemical parameter and concentration of the pesticides is realized in a broad topological parameter region. The composite is efficient electrode modifier for omeprazole electrochemical determination.
- The oscillatory behavior in this system is less probable than in the similar systems, being caused uniquely and exclusively by the DEL influences of the electrochemical stage.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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HOW TO CITE THIS ARTICLE

V.V. Tkach, M.V. Kushnir, Y.G. Ivanushko, V.G. Ostapchuk, S.P. Melnychuk, S.C. de Oliveira, V.V. Parchenko, I.I. Aksyonova, V.M. Odyntsova, P.I. Yagodynets', Z.O. Kormosh, O.V. Luganska, The theoretical description for omeprazole electrochemical determination, assisted by the composite poly (1,2,4-triazole) – Ag₂O₂, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1284-1295 (2022).

Artigo de revisão / <http://dx.doi.org/10.15446/rcciquifa.v51n3.101663>

Impactos ambientais relacionados ao descarte de medicamentos no Brasil: uma revisão bibliográfica e documental

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Recebido: 22 de março de 2022

Revisado: 8 de março de 2022

Aceto: 15 de abril de 2022

RESUMO

Introdução: a elevação da renda de parcelas significativas da população brasileira tem contribuído para o aumento do consumismo de diversos produtos, favorecendo a geração dos resíduos. O Brasil tem alcançado cerca de 22 bilhões de dólares por ano pelo consumo de medicamentos, e a grande demanda da indústria farmacêutica tem gerado inúmeras consequências diante do descarte inadequado. **Objetivo:** identificar as principais causas e consequências do descarte de medicamentos, e verificar as formas de manejo já existentes no Brasil. **Metodologia:** foi feita uma revisão bibliográfica e documental, com pesquisa nas bases de dados: Google acadêmico, SciELO, Capes periódicos, PubMed e Ncbi. Além disso, foram realizadas buscas em sites de órgãos públicos e privados do país, e em leis e legislações voltadas ao assunto. As análises foram classificadas em três enfoques: causas, consequências e manejos já adotados no Brasil, relacionados ao descarte de medicamentos. O critério para seleção foi a busca por artigos publicados entre 2010-2020, não sendo adotado para as alternativas de manejo. **Resultados e discussão:** maioria da população desconhece os meios de descarte correto de medicamentos e os impactos negativos que estes causam, e parte da legislação brasileira estabelece normas para o descarte correto apenas para estabelecimentos de saúde, indústrias, farmácias e hospitais, não atingindo a população como um todo. **Conclusão:** o estudo identificou causas e

consequências ao nível individual e coletivo, a necessidade de eficácia das diretrizes existentes, a ausência de fiscalização e de programas de conscientização ambiental voltados à população, frente ao descarte irregular dos fármacos.

Palavras-chave: Resíduos, fármacos, meio ambiente.

SUMMARY

Environmental impacts related to drug disposal in Brazil: a bibliographic and documentary review

Introduction: The increase in income of significant portions of the Brazilian population has contributed to the increase in consumerism of various products, favoring the generation of waste. Brazil has reached about 22 billion dollars per year for the consumption of medicines, and the great demand of the pharmaceutical industry has generated numerous consequences in the face of improper disposal. **Objective:** To identify the main causes and consequences of the disposal of medicines, and to verify the forms of management that already exist in Brazil. **Methodology:** A bibliographic and documental review was carried out, with research in the following databases: Google academic, SciELO, Capes journals, PubMed and Ncbi. In addition, searches were carried out on websites of public and private agencies in the country, and on laws and legislation related to the subject. The analyses were classified into three approaches: causes, consequences and managements already adopted in Brazil, related to the disposal of medicines. The selection criterion was the search for articles published between 2010 and 2020, not being adopted for the management alternatives. **Results and discussion:** Most of the population is unaware of the means of correct disposal of medicines and the negative impacts they cause, and part of the Brazilian legislation establishes norms for the correct disposal only for health establishments, industries, pharmacies and hospitals, not reaching the population as a whole. **Conclusion:** The study identified causes and consequences at the individual and collective level, the need for effectiveness of existing guidelines, the absence of inspection and environmental awareness programs aimed at the population, in the face of irregular disposal of drugs.

Keywords: Waste, drugs, environment.

RESUMEN

Impactos ambientales relacionados con el desecho de medicamentos en Brasil: una revisión bibliográfica y documental

Introducción: el aumento de los ingresos de la población brasileña ha contribuido al aumento del consumismo de diversos productos, favoreciendo la generación de residuos. Brasil ha alcanzado cerca de 22 millones de dólares por año en el consumo de medicamentos y la gran demanda de la industria farmacéutica ha generado numerosas consecuencias por la disposición inadecuada de los desechos. **Objetivo:** identificar las principales causas y consecuencias de la disposición de medicamentos y verificar las formas de gestión que ya existen en Brasil. **Metodología:** se realizó una revisión bibliográfica y documental, con pesquisa en las siguientes bases de datos: Google académico, SciELO, revistas Capes, PubMed y Ncbi. Además, se realizaron búsquedas en sitios web de organismos públicos y privados del país, y sobre leyes y legislación relacionada con el tema. Los análisis se clasificaron en tres enfoques: causas, consecuencias y gestiones ya adoptadas en Brasil relacionadas con el desecho de medicamentos. El criterio de selección fue la búsqueda de artículos publicados entre 2010-2020, no siendo adoptado para alternativas de gestión. **Resultados y discusión:** la mayor parte de la población desconoce la forma correcta de desechar los medicamentos y los impactos negativos que provocan, y parte de la legislación brasileña establece reglas para la eliminación correcta solo para establecimientos de salud, industrias, farmacias y hospitales, no para la población en su conjunto. **Conclusión:** el estudio identificó causas y consecuencias a nivel individual y colectivo, la necesidad de efectividad de las directrices existentes, la ausencia de programas de inspección y concientización ambiental dirigidos a la población, frente al descarte irregular de drogas.

Palabras-clave: Residuos, drogas, medio ambiente.

INTRODUÇÃO

Ao longo do século XX o avanço da urbanização provocou uma pressão significativa em diversos setores da economia brasileira, em especial nos serviços relacionados ao abastecimento de água, esgotamento sanitário e coleta de resíduos dos serviços de saúde. Aliado a isso, com o passar dos anos houve a elevação da renda de parcelas significativas da população, e isso têm contribuído para o aumento do consumismo de diversos produtos, favorecendo a geração dos resíduos aos mesmos associados [1].

O Brasil tem alcançado cerca de 22 bilhões de dólares por ano pelo consumo de medicamentos. A comercialização de medicamentos genéricos tem sido a maior do país e cresceu 12,3% em 2015, atingindo um total de vendas de 467,3 milhões de unidades. Esses dados demonstram um número elevado, que por consequência provoca aumento da geração de resíduos de fármacos [2, 3].

Denomina-se medicamento qualquer produto sólido ou líquido composto por um princípio ativo, passível de descarte, tornando-se consequentemente um resíduo. Sendo obtido ou elaborado, com finalidade profilática, curativa, paliativa ou para fins de diagnóstico [4]. São produtos originalmente fabricados para resolver problemas de saúde humana ou animal, mas que podem causar efeitos biológicos e ecotoxicológicos ao meio e à saúde dos seres humanos [5].

Por meio da Resolução nº 358/2005, o Conselho Nacional do Meio Ambiente (Conama), disciplina o tratamento e a disposição final dos Resíduos de Serviço de Saúde (RSS), sendo estes classificados em cinco grupos: A (biológico), B (químico), C (radioativo), D (comum) e (perfurocortante). Resíduos medicamentosos são enquadrados no grupo B e diante de suas características tóxicas, e reativas, apresentam um risco ambiental de influência na saúde pública [6-8].

A grande demanda da indústria farmacêutica tem gerado um alto consumo e inúmeras consequências diante do descarte inadequado, como contaminação do solo, das águas e intoxicações medicamentosas. Porém, mesmo com o reconhecimento desses efeitos, ainda não há regulamentação específica para o descarte de medicamentos no território brasileiro. Isso têm despertado crescente preocupação entre pesquisadores devido ao aumento da ocorrência desses resíduos [5, 9-13].

Aspectos relacionados ao descarte são deveras importantes quando dados estimam que 20% dos medicamentos adquiridos são descartados em lixo comum [14]. Esse tipo de destinação contribui na liberação de resíduos químicos que causam riscos à sociedade. Estudos demonstram que várias substâncias existentes nos fármacos são resistentes ao processo de tratamento, permanecendo no meio ambiente por longos períodos, e acarretando sérios riscos socioeconômicos e ambientais [4, 15].

É reconhecido que o mercado farmacêutico está altamente concentrado em regiões desenvolvidas, tanto em produção, quanto em consumo. Em 2010, a Europa, EUA e Japão atingiram 79% do mercado mundial. Por outro lado, Ásia e Austrália, que são responsáveis por 85% da população mundial, atingiram no mesmo ano 21% do mercado de fármacos. Quanto ao consumo mundial, a Nova Zelândia, EUA e Inglaterra são vistos como os maiores consumidores [16].

No âmbito da União Europeia, que lideram as atividades de seus Estados na implementação de sistemas de coleta de resíduos de medicamentos, alguns países são reconhecidos por apresentarem variadas iniciativas de políticas públicas voltadas para a destinação correta de resíduos de medicamentos através da implantação de programas de logística reversa e destinação correta [17].

Já o Brasil, ocupa a sétima posição entre os maiores mercados consumidores de medicamentos no cenário mundial. Espera-se que futuramente o país ocupe a quarta posição, ficando atrás apenas dos três maiores consumidores [18]. Estima-se que sejam descartados um total entre 10,3 e 19,8 mil toneladas de medicamentos por ano, e em torno de 6,7 mil toneladas para municípios com mais de 100 mil habitantes [19].

No país, embora exista regulamentação sobre a destinação de medicamentos utilizados pelos hospitais e centros de saúde, a legislação é considerada escassa. Vários Estados e municípios possuem legislação própria específica sobre o gerenciamento dos resíduos de saúde, porém as que estão em vigor não possui clareza e muitas vezes são conflitantes, impossibilitando a adoção de normas práticas e eficazes em todo o país [20].

A ausência de informação da população quanto ao descarte correto de medicamentos ainda é comum. De acordo com Ferreira, as questões relacionadas a este assunto e a geração de resíduos devem ser amplamente discutidas e estudadas na saúde pública, para que exista maior conscientização pelo impacto ambiental desses resíduos e diminuição de sua geração [21].

Tendo em vista o consumo elevado de fármacos e a influência desses componentes no ambiente, o trabalho tem o objetivo avaliar estudos que abordem sobre o descarte de medicamentos, com o intuito de identificar a atual situação brasileira e promover uma orientação socioeducativa sobre as causas, consequências e manejo desses resíduos. Além disso, pretende-se detectar problemas e demonstrar propostas para a melhoria da execução de leis e programas voltados para o descarte consciente de fármacos.

METODOLOGIA

O presente trabalho trata-se de uma pesquisa do tipo bibliográfica e documental, que apresenta um estudo de revisão através de pesquisa nas bases de dados: Google acadêmico, SciELO, CAPES periódicos, PubMed, e NCBI, sendo utilizado como descritores: causas do descarte de fármacos, resíduos de medicamentos, consequências ambientais, manejo de medicamentos no Brasil, leis e programas de descarte de medicamentos no Brasil, classes de medicamentos e impactos ambientais. Além disso, foi realizada busca em sites de órgãos públicos e privados que apresentassem relação com o tema nos seus objetivos, diretrizes e normas.

O critério adotado para seleção foi a busca por artigos publicados entre 2010-2020, sendo realizada a divisão dos resultados da pesquisa em três enfoques: causas, consequências e manejos já adotados no Brasil relacionados ao descarte de medicamentos. Não foi adotado o critério seletivo de anos para as alternativas de manejo, por incluir leis e resoluções.

No geral, foram selecionados 89 artigos, onde destes 32 foram descartados por não tratar sobre o que se buscava ou por apresentar referencial fora do período estipulado. Então, 57 artigos recentes foram utilizados, juntamente com 16 sites selecionados. Destes, 9 são voltados a resoluções sobre o assunto abordado.

RESULTADOS E DISCUSSÕES

Causas do descarte e acúmulo inadequado de medicamentos

O interesse do homem por medicamentos tem uma origem remota e vem desde a antiguidade quando era comum o uso de vegetais para tratar de enfermidades, e de elementos de origem animal e mineral, que eram utilizados na preparação de pílulas, pomadas, supositórios, entre outros [22].

O acúmulo de medicamentos nas residências é conhecido no país como “farmácia caseira”, e esta abundância tem ocorrido por vários motivos, dentre eles: o avanço da cultura da automedicação, a divulgação midiática, a facilidade de adquirir amostras-grátis, e a impossibilidade de fracionamento [23]. Para Brasil *et al.* [24], as causas para as sobras de medicamentos são variáveis, e a principal delas é o não fracionamento dos medicamentos, que fazem com que as pessoas levem para suas casas uma quantidade maior que a necessária. Outros fatores também têm influência, como a ocorrência de interrupção do tratamento, a falta de orientação sobre local de descarte e a ausência de estruturas adequadas para receber esses resíduos.

Porém, os estudos de Schmid *et al.* [25] demonstram que o problema vai além de questões culturais, pois o padrão atual de prescrições faz com que as pessoas adotem seus próprios critérios para solucionar os problemas de saúde que para eles são considerados simples.

Diante da ocorrência desses fatores, a Associação da Indústria Brasileira de Pesquisa relata que o mercado farmacêutico brasileiro tem avançado cada vez mais e cresceu 11% no ano de 2018, chegando a R\$ 90 bilhões. Porém, estima-se que ocorra movimentação de US\$ 39 bilhões a US\$ 43 bilhões até o ano de 2023, chegando a ser comercializadas em torno de 238 milhões de doses de medicamentos [26].

A globalização e a facilidade no transporte de mercadorias a preços cada vez mais competitivos alterou os padrões de consumo, assim como a relação entre o consumidor, e os produtos [27]. O avanço tecnológico do comércio possibilitou aos consumidores a aquisição de medicamentos a preços mais baixos e a disponibilidade de maior variedade de fármacos, bem como uma maior acessibilidade aos mesmos de forma fácil e imediata [28].

Um levantamento realizado em 2018 no Brasil, demonstrou que o relaxante muscular (Dorflex) e o medicamento para tratar tromboembolismo venoso profundo (Xarelto) apresentou as vendas significativamente mais expressivas, em comparação com o Japão, chegando a ser 95% maior. Em termos de faturamento, o mercado nacional tem se apresentado equilibrado, considerando os medicamentos de referência, os similares, os isentos de prescrição e os genéricos. Acredita-se que com esse avanço o país suba no ranking mundial [26].

Dados do Conselho Federal de Farmácia demonstram que no país existe uma farmácia para cada 2700 habitantes, número muito próximo ao dos países europeus. Porém, a recomendação da Organização Mundial da Saúde é de que haja uma farmácia para cada 8000 habitantes [29].

De acordo com João [21], a indústria farmacêutica apresenta administração inadequada de estoques de medicamentos e isso também é um dos motivos de gerar acúmulo e perda por vencimento, isso tem relação ao conceito de assistência farmacêutica, sendo responsabilidade do farmacêutico a garantia de que seja executada de maneira correta. Certas ações industriais também contribuem de forma considerável à geração de resíduos sólidos e contaminação do meio ambiente, pois faz parte do processo gerar resíduos durante e após a produção, apresentando ou não o recolhimento e devolução após o consumo [30].

Pesquisas demonstram que em torno de 50% das medicações são vendidas de forma ilegal e sem prescrição médica, tendo como causas o aumento de farmácias, o consumo descontrolado de medicamentos e o fácil acesso à compra [31]. Aproximadamente, 76,4% da população brasileira faz ou já fez em algum momento da vida uso de medicamentos por indicação de familiares, amigos ou vizinhos. Na mesma seara, cerca de 32% aumentam a dose por conta própria, que em algum momento podem formar sobras e serem desperdiçados [32].

O aumento do consumo populacional, da expectativa de vida, do uso de medicamentos e da propaganda, são processos que a têm contribuído para o aumento do descarte de fármacos em locais impróprios como pias, vasos sanitários e lixo doméstico [21, 33, 34].

Santos [35] aborda o quanto a falta de informação tem sido um dos maiores problemas em meio a esta situação. Diante disso, a educação no âmbito ambiental é um meio essencial para o esclarecimento da população sobre efeitos tóxicos dessas substâncias e implicações que o descarte impróprio pode causar no ambiente e na saúde.

Consequências ambientais dos fármacos

O descarte dos medicamentos em lixo e/ou esgoto doméstico pode causar sérias implicações para a saúde pública, uma vez que os compostos farmacêuticos contaminam os reservatórios de água e os aquíferos, trazendo danos aos sistemas ecológicos. Dados da Organização Mundial de Saúde (OMS) mostram que vários resíduos ou metabólitos de fármacos estão presentes em fontes de água potável [36].

Diversos países como o Brasil, apresentam a ocorrência de medicamentos nos sistemas de água devido à ausência de padronização do descarte final dos medicamentos. Estima-se que no país cerca de 20% dos medicamentos adquiridos são lançados na rede de esgotamento sanitário ou no lixo doméstico, podendo resultar em problema ambiental devido a contaminantes tóxicos provenientes destes resíduos [37]. Dados do Instituto Brasileiro de Geografia e Estatística (IBGE) de 2016, revelam que apenas 33,5% dos domicílios são atendidos com sistema de coleta de esgoto, e apenas 52,2% dos municípios brasileiros têm serviço por rede geral de esgoto [38].

Apesar das modernas tecnologias de tratamento adotadas nas Estações de Tratamento de Esgoto (ETE), os fármacos não são completamente eliminados. Em pesquisas já foram identificadas a presença de compostos farmacológicos em ETE. Isso implica dizer que as atuais estações de tratamento de esgoto não conseguem eliminar totalmente poluentes de origem farmacêutica, algumas melhorias ou modificações devem ser estudadas para que ocorra uma total remoção destes compostos de modo a atenuar riscos à saúde pública [39, 40].

A poluição ambiental proveniente do uso de medicamentos ocorre de maneira silenciosa. Os riscos estão associados ao descarte incorreto e podem provocar variados impactos no ambiente de acordo com os componentes de cada classe (tabela 1).

Tabela 1. Lista das classes de medicamentos e seus impactos ambientais.

Classes de medicamentos	Impactos no ambiente
Analgésicos	- Desequilíbrio ambiental. - Ameaça ecossistemas [41].

Antibióticos	- Interfere no desenvolvimento de espécies de micro-organismos que fazem parte da flora bacteriana do solo, inibindo algumas e provocando o aumento em outras. - Atinge o ambiente pela ação dos seus princípios ativos [42].
Anti-inflamatórios	- Atingem águas do mar, apresentando risco aos organismos aquáticos, e contaminação em peixes. - São atraídos para tecidos gordurosos da biota e favorecem o processo de bioacumulação na cadeia alimentar. - Causam alteração nos cloroplastos de algas, interferindo na fotossíntese [43-45].
Antidepressivos	- Infectam águas residuárias. - Resistem aos processos de estações de tratamento de esgoto. - Afetam o solo agrícola [46-48].
Antimicrobianos	- Afetam os ecossistemas. - Causam a morte de bactérias do solo. - Prejudicam a capacidade do solo em degradar contaminantes. - Causam bioacumulação em plantas e organismos pequenos [49].
Hormônios	- Estrógenos atingem os corpos hídricos, águas superficiais e sistemas de esgoto [50, 51].
Anti-hipertensivos	- Betabloqueadores foram encontrados em estações de efluente, águas superficiais e corpos hídricos. - Causam problemas à biota aquática, alterações fisiológicas em mexilhões-de água-doce da espécie <i>Dreissena polymorph</i> [52, 53].
Medicamentos Veterinários	- Invermectinas- Apresentam maior resistência à biodegradação e biotransformação, e são encontrados em água residuais [54].

De acordo com Arrubla *et al.* [55], os anti-inflamatórios vêm apresentando elevadas taxas de consumo pela população. Este aumento pode ter relação com o número de prescrições médicas inadequadas ou pode estar associado ao uso indiscriminado através da automedicação. De qualquer forma, a frequência e o volume de medicamentos utilizados relacionam-se ao aumento de suas concentrações em efluentes.

Além disso, o desenvolvimento a resistência bacteriana também tem se tornado um problema de saúde pública e ambiental. Em torno de 55% dos microrganismos, apresentam resistência a pelo menos um antibiótico devido à interrupção do tratamento e diante do desuso tem como finalidade o descarte. A detecção de genotoxicidade tem sido comum e vista como um dos impactos mais frequentes em rios afetados pelos contaminantes, bem como a ocorrência de alterações no desenvolvimento de plantas aquáticas, micro-organismos e insetos [42].

De acordo com estudos, fármacos são compostos biologicamente ativos e não se biodegradam com facilidade, fazendo com que esses resíduos farmacêuticos apresentem durabilidade no ambiente [56]. Arrifin e colaboradores confirmam que ao entrar em contato com os sistemas dos variados organismos vivos podem gerar impactos adversos na fisiologia e no comportamento, incluindo o bem-estar dos seres, problemas reprodutivos, distúrbios respiratórios e até mesmo o câncer [57].

Segundo Polastri [58], no meio terrestre os medicamentos estão entre os tipos de resíduos perigosos que possui persistência ambiental, ou seja, com grande permanência de certas substâncias no ambiente. Dependendo da composição, temperatura, fatores ambientais e tipo de solo que se encontram, pode ocorrer a danificação da sua constituição e provocar poluição. Suas análises são confirmadas em pesquisa de Silva *et al.* [42], que identifica os quanto compostos farmacêuticos, podem causar grave desequilíbrio na microbiota do solo, favorecendo o crescimento de algumas espécies e outras não.

Um dos grandes responsáveis pela contaminação do solo são os fármacos veterinários, que são enquadrados na categoria de poluentes emergentes. Vale ressaltar que a classificação dos resíduos gerados pela saúde animal principalmente em áreas rurais, não é realizada com o mesmo rigor de resíduos de saúde hospitalares [59].

Medicamentos como as Ivermectinas, apresentam importantes vias de entrada e disseminação no ambiente, pois, a metabolização no organismo do animal depende das propriedades físico-químicas da substância, do modo de aplicação, da espécie animal e do tempo de tratamento. Caso o tratamento seja interrompido acaba ocorrendo a excreção dos compostos de origem [60].

Pesquisas afirmam que quando existe combinação entre fármacos de diferentes classes ou não, ocorre uma potencialização do dano ambiental. Efeitos ecotoxicológicos encontrados em peixes apresentam relação, principalmente com a elevação do peso corporal, aumento do volume hepático e desregulação endócrina. Já em algas, ainda não foram identificados efeitos genotóxicos de forma direta, mas já foi verificado que o cloroplasto é afetado negativamente [61].

Fatores associados a gestão de resíduos sólidos também têm gerado consequências negativas. Dados da Associação Brasileira de Empresas de Limpeza Pública e Resíduos Especiais (Abrelpe) demonstram que lixões e aterros controlados ainda representam 40,5% de ocorrência no Brasil, são encontrados em todas as regiões, e recebem mais de 80 mil toneladas de resíduos por dia. Tal fato influencia no índice de poluição ambiental e nos impactos negativos à saúde, apresentando uma relação com o esgotamento sanitário [62]. Para Ramos *et al.* [8] assuntos como esses despertam o interesse na

comunidade científica, já que se trata de eventos podem contaminar o solo e demais recursos hídricos em suas diferentes formas.

Em muitas cidades brasileiras ainda é comum a existência de lixões a céu aberto, e isso contribui para que catadores de lixo encontrem medicamentos vencidos ou inapropriados e realizem o uso dos mesmos para tratar de enfermidades próprias. Atitudes como essa podem causar ocasionar reações adversas e graves, como intoxicações e outros problemas de saúde [63, 64].

As informações permitem perceber a ausência de responsabilidade social, participação popular e movimentações que contribuam na ampliação da efetividade das ações de educação ambiental voltadas ao consumo e descarte consciente [65].

Para Michael *et al.* [66], a falta de conformidade com as diretrizes nacionais é um dos maiores motivos do descarte de medicamentos, pois aumenta o risco potencial de contaminação do meio ambiente e a possibilidade de ingestão de resíduos farmacêuticos tóxicos por humanos e animais. Isso demonstra importância da análise das regulamentações existentes e verificação da eficácia das normas já propostas relacionadas ao descarte incorreto de compostos farmacêuticos.

Programas e resoluções elaboradas para o manejo de resíduos de medicamentos no Brasil

No que se refere à implantação do descarte correto de medicamentos, as medidas relevantes adotadas no Brasil ressaltam iniciativas da Anvisa por meio da Resolução da Diretoria Colegiada (RDC) n.º 306/2004 que regulamenta o gerenciamento de resíduos de serviços de saúde, e a Resolução n.º 358/2005 do Ministério do Meio Ambiente, que aborda o tratamento e a disposição final dos resíduos dos serviços de saúde [67, 68].

Além disso, a RDC n.º 44/2009 da Anvisa, disponibiliza um sistema de boas práticas farmacêuticas para o controle sanitário do funcionamento, dispensação e comercialização de produtos e da prestação de serviços farmacêuticos em farmácias e drogarias. No entanto, essas legislações não impõem às farmácias e às drogarias a obrigatoriedade da coleta dos medicamentos. Então, a política nacional de resíduos sólidos, instituída pela lei n.º 12305/2010 e regulamentada pelo decreto n.º 7404/2010, dispôs no Congresso Nacional o Projeto de lei n.º 595/2011 que tem como objetivo obrigar farmácias, drogarias e postos de saúde a receberem da população os medicamentos vencidos ou não utilizados, obtendo aprovação [68, 69].

Nos termos do art. 13, parágrafo único, a lei 12305/2010 implanta o método de logística reversa como um instrumento de desenvolvimento econômico e social caracterizado pelo conjunto de ações destinadas a viabilizar a coleta e a restituição dos resíduos

sólidos ao setor empresarial, para reaproveitamento, em seu ciclo, em outros ciclos produtivos, ou outra destinação final ambientalmente adequada [70].

Já a lei n.º 13021, promulgada em 8 de agosto de 2014, dispõe sobre o exercício e a fiscalização das atividades farmacêuticas, onde o artigo 1 cita que farmacêuticos e proprietários devem sempre agir e promover o uso racional de medicamentos [71]. Porém, o que é comumente visto em prática é a ausência do cumprimento da legislação, e da falta de orientação relacionada a automedicação e descarte dos fármacos, uma vez que, os estabelecimentos apresentam uma atenção prioritária em atingir um grande número de vendas.

Diante das propostas já realizadas pelas resoluções federais, Estados brasileiros chegaram a propor leis para tornar obrigatória a coleta dos resíduos farmacêuticos em domicílios. Como exemplo mais específico, está o plano estadual de logística reversa de medicamentos, realizado pelo Estado do Paraná que cumpre a lei n.º 17211/2012, dispõe sobre a responsabilidade da destinação dos medicamentos em desuso no Estado do Paraná e seus procedimentos [72]. O plano, que tem apoio do Conselho Regional de Farmácia do Paraná envolve os diversos âmbitos da cadeia farmacêutica, sendo acompanhado e fiscalizado pelo Poder Público [73].

Na Paraíba, existe a lei Estadual n.º 9646 que desde 2011 determina que “as drogarias e farmácias, incluindo as de manipulação, são obrigadas a instalar pontos para recebimento dos medicamentos já comercializados, que se encontram vencidos ou impróprios para o consumo, em locais visíveis” [74]. Na capital do Estado, existe a lei Municipal n.º 12949, que segue as diretrizes da lei estadual e foi promulgada em 2014. Além desses Estados como Amazonas, Rondônia, Rio grande do Norte, Acre, Mato Grosso do Sul, Rio de Janeiro, São Paulo, dispõem de leis estaduais. Porém, ainda são raros os estabelecimentos que colocam em prática essas normas em todo o território do Estado, e ainda não existe uma fiscalização [75].

Há países com iniciativas de resolver esses problemas, tais como, México, Canadá, Colômbia e Portugal que apresentam programas para recolhimento de medicamentos vencidos. Em Portugal ocorre o envolvimento do Estado e a participação das indústrias para implantação de programas. As indústrias farmacêuticas se associaram à rede de distribuição de medicamentos e farmácias e assim os distribuidores e as farmácias recebem destaque na logística de recolhimento e na conscientização populacional. Essas iniciativas, demonstram que todos são responsáveis no gerenciamento dos resíduos gerados [76].

A eficácia de experiências internacionais contribuiu na identificação de diretrizes básicas e úteis para a implantação de programas específicos no recolhimento de medicamentos vencidos no Brasil. Em certas localidades do território brasileiro já foram

adotados alguns programas através de iniciativas privadas sobre o descarte de medicamentos, tendo como exemplos os dispostos na (tabela 2).

Tabela 2. Programas de manejo do descarte de medicamentos no Brasil e suas respectivas características.

Programas	Características
Programa descarte consciente	- Reconhecido como o maior programa do país para coleta de medicamentos vencidos e em desuso, foi criado pelo Brasil Health Service (BHS) em parceria com a Rede Droga Raia e Medley. - Realiza o recolhimento de medicamentos através de uma máquina de sistema computadorizado, havendo registro da quantidade de medicamentos descartados [77].
Programa descarte consciente com destino certo	- Criado pela Panvel (Rede de farmácias da região Sul). - Dispõe de estações coletoras em várias lojas, garantindo o registro e o correto acondicionamento dos medicamentos e está presente em cidades do Rio Grande do Sul, Santa Catarina, Paraná e São Paulo [78].
Programa farmácia solidária	- Tem como objetivo reaproveitar as sobras de medicamentos que ainda estejam em condições de uso, evitando o desperdício e realizando a distribuição a população carente. - Presente no Estado do Rio Grande do Sul [79].
Poluição tem cura	- Proposto por uma farmácia de manipulação denominada Cia-fórmulas, onde medicamentos de uso humano ou veterinário podem ser levados, e a empresa é responsável pela destinação final [80].
Projeto descarte correto de medicamentos	- Proposto pela Eurofarma e o Grupo Pão de Açúcar. Atua recolhendo medicamentos vencidos, embalagens primárias e materiais perfurocortantes como agulhas e ampolas, através de coletores expostos nestes locais. Funciona na região de Araraquara SP [81].

A maior parte dos programas foram formulados pela indústria farmacêutica, sendo identificada uma preocupação maior com o descarte no Estado do Rio Grande do Sul, que no geral apresenta a atividade de três programas em seu território. Isso destaca que as ações existentes foram iniciativas isoladas de municípios ou Estados e **ainda é ausente um** Programa Nacional de Recolhimento de Medicamentos Vencidos no Brasil.

Além de programas, existem sites como o Ecycle, que permite o acesso de localização de postos de coleta de diversos resíduos, incluindo medicamentos. O site tem como objetivo criar conteúdo e disponibilizar serviços de ampliação da consciência dos indi-

víduos em suas ações, em busca de uma relação mais equilibrada e harmoniosa consigo mesmo, e com o meio ambiente, buscando minimizar e eliminar as substâncias sabidamente nocivas à saúde dos sistemas biológicos e ecológicos em sua preservação [82].

Visando melhorias sobre o assunto, em junho de 2020 o presidente da república regulamentou o § 1º do caput do art. 33 da lei n.º 12305 (política nacional de resíduos sólidos). O novo decreto estabelece que farmácias e drogarias disponibilizem pontos de coleta de medicamentos vencidos. Drogarias e farmácias terão de disponibilizar e manter pelo menos um ponto fixo de recebimento de remédios vencidos a cada 10 000 habitantes. Diante das regras, capitais e municípios com população superior a 500 000 habitantes deverão ter os coletores em até dois anos. Já o prazo para municípios com mais de 100 000 moradores é de cinco anos. A fiscalização ficou sob responsabilidade do Ministério do Meio ambiente [69].

Apesar de existirem boas iniciativas com relação ao gerenciamento de resíduos, no Brasil ainda faltam políticas de implementação na área ambiental: como consequência tem-se a poluição de rios e corpos d'água pelo lançamento de efluentes, esgotos urbanos e industriais sem tratamento correto; degradação de ecossistemas e áreas naturais de relevância ecológica; depósito e destinação final inadequada de lixo urbano; e abandono de bens integrantes do patrimônio cultural brasileiro [83].

As questões políticas e sociais existentes no país são fatores de grande relevância e que apresentam certa dificuldade para serem alteradas, a disponibilidade de técnicas é importante para implantação de um eficiente programa de gerenciamento de resíduos. Todavia, “a maneira como o descarte de medicamento é efetuada pelo consumidor final é decorrente de uma lacuna na legislação, pois o Brasil tem baixa infraestrutura, faltam aterros sanitários e incineradores licenciados” [84]. Ainda são necessárias ações públicas para possibilitar a maneira correta para descartar os medicamentos.

Um ponto importante na legislação sobre resíduo de fármacos está no fato de que a abordagem muitas vezes muda de acordo com o ramo de atividade da empresa geradora de resíduos. O segmento industrial segue diferentes regulamentações, pois o caminho feito pelos resíduos de medicamentos gerados desde a fabricação até a entrega ao usuário fragmenta-se na legislação, cabendo ao gerador do resíduo buscar a regulamentação que considerar melhor, de acordo com o tipo de atividade que desempenha [85].

Conclusão

O estudo demonstrou que existem diferentes causas e consequências relacionadas ou descarte de medicamentos ao nível individual e coletivo, obtendo um destaque para a

ausência de informações na população sobre os benefícios do retorno dos resíduos, e riscos ambientais que o descarte irregular pode causar.

As regulamentações existentes no país apresentam boas iniciativas relacionadas ao descarte, porém ainda são insuficientes para resolver a problemática dos resíduos advindos dos produtos fármacos. Além disso, a formulação do novo decreto de 2020, agrega novas exigências à lei n.º 12305 (PNRS), apresenta-se como um bom passo para que futuramente seja constituído o Programa Nacional de Recolhimento de Medicamentos Vencidos no Brasil, essencial à qualidade de vida populacional e ambiental do país.

CONFLITO DE INTERESSES

Os autores declaram não haver conflitos de interesse.

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COMO CITAR ESTE ARTIGO

A.C. Paulino de Queiroz, M.S.d.A. da Silva-Souza, J. Batista-Alves, Impactos ambientais relacionados ao descarte de medicamentos no Brasil: uma revisão bibliográfica e documental, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1296-1319 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.101663>

Scientific research article / <http://dx.doi.org/10.15446/rcciquifa.v51n3.107549>

Hepatoprotective activity of lycopene in experimental paracetamol-induced liver injury in rats

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Received: February 3, 2022

Corrected: April 13, 2022

Accepted: April 20, 2022

SUMMARY

Aim: To evaluate the hepatoprotective effects of lycopene pretreatment in paracetamol-induced liver damage (PILD). **Methods:** Wistar rats were administered oral lycopene (4 mg/kg/day) by gastric lavage for 8 days. Subsequently, 3 g/kg paracetamol was administered on day 8. After 24 and 72 h, animals were euthanized, and intracardiac blood samples were collected to measure levels of aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase, and alkaline phosphatase (ALP). In addition, the liver was harvested for histological analyses. **Results:** Negative and positive control groups (treated with saline or paracetamol on day 8, respectively) were compared with lycopene- and lycopene-paracetamol-treated (lycopene+paracetamol on day 8) groups. Notably, we observed that 24 h after PILD, lycopene treatment significantly reduced serum transaminase (ALT/AST) levels when compared with those in the saline-treated group. **Conclusion:** Lycopene improved liver recovery following PILD. Although lycopene exhibits antioxidant action and has been indicated for liver diseases, its use must be cautiously undertaken, especially considering the liver pathology involved, as results may vary for each underlying factor.

Keywords: Lycopene, paracetamol, ALT, AST.

RESUMO

Atividade hepatoprotetora do licopeno na lesão hepática experimental induzida por paracetamol em ratos

Objetivo: avaliar os efeitos hepatoprotetores do pré-tratamento com licopeno no dano hepático induzido por paracetamol (PILD). **Metodologia:** ratos Wistar receberam licopeno oral (4 mg/kg/dia) por lavagem gástrica por 8 dias. Posteriormente, 3 g/kg de paracetamol foi administrado no dia 8. Após 24 e 72 h, os animais foram eutanasiados e amostras de sangue intracardiaco foram coletadas para medir os níveis de aspartato aminotransferase (AST), alanina transaminase (ALT), gama-glutamil transferase, e fosfatase alcalina (ALP). Além disso, o fígado foi colhido para análises histológicas. **Resultados:** os grupos de controle negativo e positivo (tratados com solução salina ou paracetamol no dia 8, respectivamente) foram comparados com os grupos tratados com licopeno e licopeno-paracetamol (licopeno+paracetamol no dia 8). Notavelmente, observamos que 24 h após PILD, o tratamento com licopeno reduziu significativamente os níveis séricos de transaminase (ALT/AST) quando comparados com os do grupo tratado com solução salina. **Conclusão:** o licopeno melhorou a recuperação do fígado após PILD. Embora o licopeno exiba ação antioxidante e tenha sido indicado para doenças hepáticas, seu uso deve ser realizado com cautela, principalmente considerando a patologia hepática envolvida, pois os resultados podem variar para cada fator subjacente.

Palavras-chave: Licopeno, paracetamol, ALT, AST.

RESUMEN

Actividad hepatoprotectora del licopeno en la lesión hepática experimental inducida por paracetamol en ratas

Objetivo: evaluar los efectos hepatoprotectores del pretratamiento con licopeno en el daño hepático inducido por paracetamol (PILD). **Métodos:** a las ratas Wistar se les administró licopeno oral (4 mg/kg/día) mediante lavado gástrico durante 8 días. Posteriormente, se administró paracetamol 3 g/kg el día 8. Después de 24 y 72 h, los animales fueron sacrificados y se recolectaron muestras de sangre intracardiaca para medir los niveles de aspartato aminotransferasa (AST), alanina transaminasa (ALT), gamma-glutamil transferasa, y fosfatasa alcalina (ALP). Además, se cosechó el hígado para análisis histológicos. **Resultados:** los grupos de control negativo y positivo (tratados con solución salina o paracetamol el día 8, respecti-

vamente) se compararon con grupos tratados con licopeno y licopeno-paracetamol (licopeno+paracetamol el día 8). En particular, observamos que 24 h después de PILD, el tratamiento con licopeno redujo significativamente los niveles de transaminasa sérica (ALT/AST) en comparación con los del grupo tratado con solución salina. **Conclusión:** el licopeno mejoró la recuperación del hígado después de PILD. Aunque el licopeno exhibe acción antioxidante y ha sido indicado para enfermedades hepáticas, su uso debe realizarse con cautela, especialmente considerando la patología hepática involucrada, ya que los resultados pueden variar para cada factor subyacente.

Palabras clave: Licopeno, paracetamol, ALT, AST.

INTRODUCTION

It is well-established that the liver exhibits endocrine and exocrine functions, and hepatocytes are responsible for bile production (exocrine secretion) and the formation of several endocrine products [1]. In addition, hepatocytes convert harmful substances into non-toxic materials excreted in the bile [2]. Accordingly, the liver is susceptible to the harmful actions of these agents, given the potent metabolism of substances, including therapeutic agents [3, 4].

Toxic hepatitis is characterized by liver damage caused by inhalation, ingestion, or parenteral administration of pharmacological or chemical agents, which remains a critical issue in current clinical practice, accounting for approximately 0.2% of all hospital admissions and 2-3% of hospitalizations attributed to adverse drug effects [5, 6].

Metabolic cell injury can be mediated via various cell injury mechanisms, including covalent binding to cellular structures, lipid peroxidation, oxidative reactions, and glutathione depletion [7-10]. Cell damage may result in mitochondrial alterations, changes in the cytoskeleton structure, or altered ion homeostasis [7-10]. Depending on the extent of mitochondrial involvement and the balance between activating and inhibiting factors associated with intracellular signaling pathways, cells may undergo necrosis or apoptosis; the former mediates inflammatory mechanisms [7-10].

Paracetamol, also known as acetaminophen or N-acetyl-p-aminophenol, is an active metabolite of phenacetin, an analgesic derived from coal tar [11-13]. It remains one of the most widely used analgesic and antipyretic drugs. In the United States (US) and the United Kingdom, paracetamol is the major cause of fulminant liver failure, especially in cases of accidental and intentional overdosage [11-13]. In the 1990s, it was the main drug implicated in deaths reported at toxicity centers in the US [11-13].

Paracetamol undergoes detoxification by phase II drug-metabolizing enzymes in the liver, mediated via glucuronidation and sulfation; a small portion is metabolized by cytochrome P-450, subjected to N-hydroxylation to form a toxic intermediate compound, N-acetyl-p-benzoquinone imine (NAPQI), which is initially conjugated to glutathione and excreted [14]. Toxic doses of paracetamol saturate the glucuronidation and sulfation pathways, and the cytochrome P-450 pathway is critical for drug biotransformation, promoting increased NAPQI formation [14]. Thus, glutathione reserves in the liver are depleted, and the reaction with sulfuric groups of liver proteins is enhanced, interrupting the flow of mitochondrial calcium and resulting in liver cell necrosis [15]. As mitochondrial metabolism is altered, hepatotoxicity also occurs via the formation of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), reactive nitrogen species (RNS), including nitric oxide and peroxynitrite ($ONOO^{\cdot}$), and products of peroxidation reactions [16, 17]. The reaction is amplified by Kupffer cell activation, cytokine secretion, and free radical production, resulting in apoptosis and centrilobular necrosis in zone 3 [18]. Necrosis is known to occur at this location, given that zone 3 hepatocytes possess the highest concentration of cytochrome P-450, where drug conversion into active metabolites occurs [18].

Lycopene is a potent antioxidant, affording cellular protection by reacting with peroxide radicals and molecular oxygen [19]. *In vitro* and *in vivo* tests suggest that carotenoids are excellent antioxidants that scavenge and inactivate free radicals [20]. The radical scavenging action is reportedly proportional to the number of conjugated double bonds present in carotenoid molecules [20]. The mechanism through which carotenoids protect biological systems from free radicals depends on energy transfer from the excited oxygen to the carotenoid molecule, during which energy is dissipated through rotations and vibrations of the carotenoid in the solvent medium [21]. Lycopene is reported to exhibit the highest capacity to scavenge oxygen free radicals, possibly due to the presence of two unconjugated double bonds, conferring greater reactivity [22]. The consumption of pure tomatoes or products that promote high lycopene concentrations in the blood can be inversely correlated with the risk of heart attack, prostate cancer, and cancers involving other tissue types [23, 24].

Previous studies have shown that the ingestion of tomatoes and derived products enhances the antioxidant system and inhibits lipid peroxidation in humans [25]. In addition, it has been reported that oral lycopene administration for two weeks can inhibit lipid peroxidation in liver tissues of rats [26].

Animal models are valuable for evaluating lycopene as a potential chemopreventive agent, as well as for assessing its protective function against other disorders or the

deleterious actions of toxic agents in different tissues [27]. Accordingly, studies were undertaken to evaluate the action of lycopene in the liver.

In the present study, we aimed to evaluate the effectiveness of lycopene as a hepatoprotective agent against experimental liver damage induced by paracetamol in Wistar rats.

MATERIALS AND METHODS

Animals and experimental design

Herein, we utilized 40 albino Wistar non-isogenic male rats (*Rattus norvegicus*). The animals were provided by the Campus II Animal House of Campinas Catholic University at 50 days of age.

Experimental animals were maintained in the Animal House of the Laboratory of Surgical Technique and Experimental Surgery at the Life Sciences Center, Campinas Catholic University, under controlled illumination and ventilation and access to solid Nuvilab ration and water until 60 days of age. The present study was approved by the Animal Ethics Committee of Campinas Catholic University, according to CI CEUA n.º 003/2012 and protocol number 2 012 070 332.

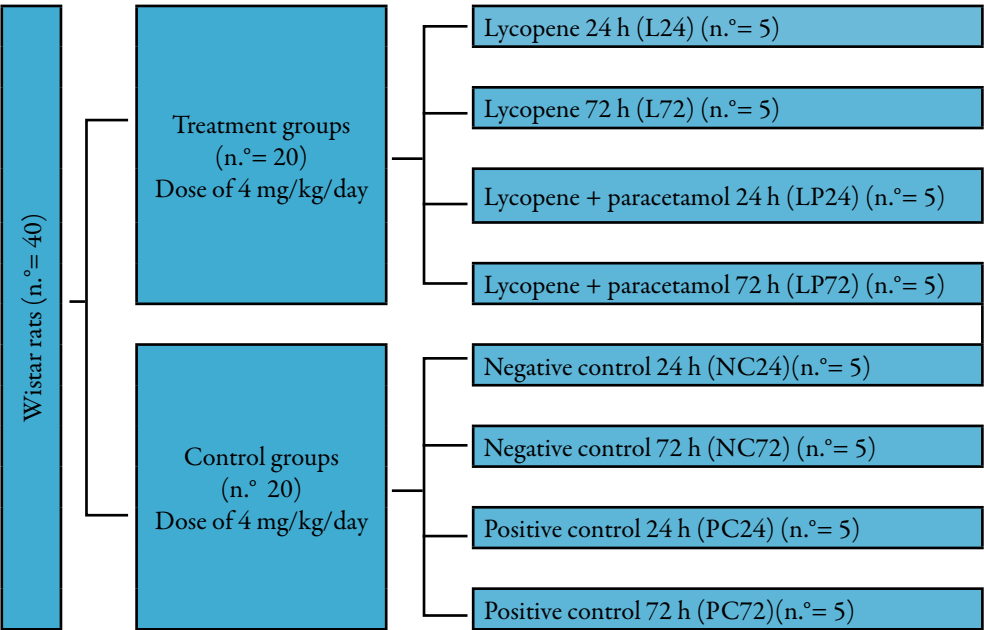


Figure 1. Experimental design.

The animals were divided into two groups (20 animals per group): treatment and control groups. The treatment group was divided into 4 subgroups: lycopene 24 h (L24) and lycopene 72 h (L72) (n=5/group) were orally administered a lycopene suspension for 8 days at a dose of 4 mg/kg/day and sacrificed at 24 and 72 h, respectively, after the last administration; lycopene + paracetamol 24 h (LP24) and lycopene + paracetamol 72 h (LP72) (n=5/group) were orally administered a lycopene suspension for 8 days (4 mg/kg/day), with paracetamol solution (Pharma Nostra lot 09124425G) orally administered on day 8. The rats were sacrificed after 24 and 72 h, respectively.

In addition, the control group was divided into 4 groups: negative control 24 h (NC24) and negative control 72 h (NC72) (n=5/group) received only water for 8 days. These animals were euthanized after the last administration at 24 and 72 h, respectively; positive control 24 h (PC24) and positive control 72 h (PC72), (n=5/group) were orally administered water for 8 days, with a paracetamol solution (3 g/kg) administered on the final day. The rats were sacrificed after 24 and 72 h, respectively. The dose of paracetamol was established as reported by Oyagbemi and Odetola [28]. The lycopene dose was determined according to a study by Bahcecioglu *et al.* [29]. Treatment was administered via the oral route, that is, intragastric gavage. Lycopene was diluted in water prior to oral administration.

Subsequently, animals were anesthetized using a ketamine solution (100 mg/kg intraperitoneal) 24 or 72 h after paracetamol administration. Then, the thoracic region was incised to access the left ventricle of the heart.

Blood was collected to measure hepatic transaminase and alkaline phosphatase (ALP) levels. Subsequently, anesthesia was deepened for euthanizing the animal, and the liver tissue was harvested. For histological analysis, the liver was fragmented and fixed in 10% buffered formalin. In addition to the liver, the kidneys, heart, and lungs were harvested. Fragments weighing approximately 200 mg were frozen for subsequent maceration. Ketamine was selected as the anesthetic agent, as it lacks hepatotoxicity and does not interfere with the experimental findings.

Histologic processing

Liver tissue specimens underwent standard histological processing using histological paraffin (Synth[®]), obtaining 5- μ m thick sections using a Leica RM2245 Rotatory Microtome. The slides were stained with hematoxylin-eosin (HE), Picrosirius red, or periodic acid Schiff (PAS), and images were captured digitally to obtain results using a photomicroscope (Nikon Eclipse E200) coupled to a camera (Nikon Coolpix 4500). For each group, the type and intensity of hepatic lesions were evaluated by observing the presence of steatosis, inflammatory infiltrate, fibrosis, and necrosis.

Measurement of liver transaminases and alkaline phosphatase

After collection, blood samples were centrifuged at 3000 rpm for 5 min to separate serum from other components. Serum samples were then used to estimate levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), indicators of hepatocellular damage. Analyses were performed using LaborLab® enzyme test kits (kinetic-colorimetric method) and measured using a UV Varian spectrophotometer, according to the manufacturer's specifications. For analysis of biochemical parameters, $n=4$ was used.

Data analysis

Histological analyses of the liver were performed using a specific methodology for each parameter. For evaluating necrosis, the number of centrilobular veins (clv) affected by the lesion was determined (vcl) in HE-stained slides (a section of 2 liver fragments from different lobules), and the results are expressed in vcl/cm². For counting, the TPS Dig® 1.30 software was used. For assessing fibrosis, 5 micrographs of the centrilobular area and 5 micrographs of the portal area were randomly obtained from Picrosirius red-stained slides (a total of 50 micrographs/group) at 480× magnification and analyzed using AreaMed® software, measuring the area compromised by fibrosis (collagen fibers). To evaluate the distribution of glycogen and other 1,2-glycols, qualitative assessment of slides stained using PAS was performed, comparing treated animals to NC and PC groups. Micrographs were obtained to present the results.

Statistical analyses of morphometry and levels of AST, ALT, and ALP were performed using Graph Pad Prism® 3.0. Differences between groups were compared using ANOVA, followed by the Bonferroni test. Statistical significance was set at $P < 0.05$.

RESULTS

As shown in Figure 2, animals in the NC and lycopene groups showed normal liver tissue; however, numerous inflammatory infiltrates, with or without necrosis, were detected in the PC and LP groups. In the PC24 and PC72 groups, a greater predominance of this type of injury was observed, especially in the latter group, as confirmed by the number of centrilobular regions affected (figures 2 and 3).

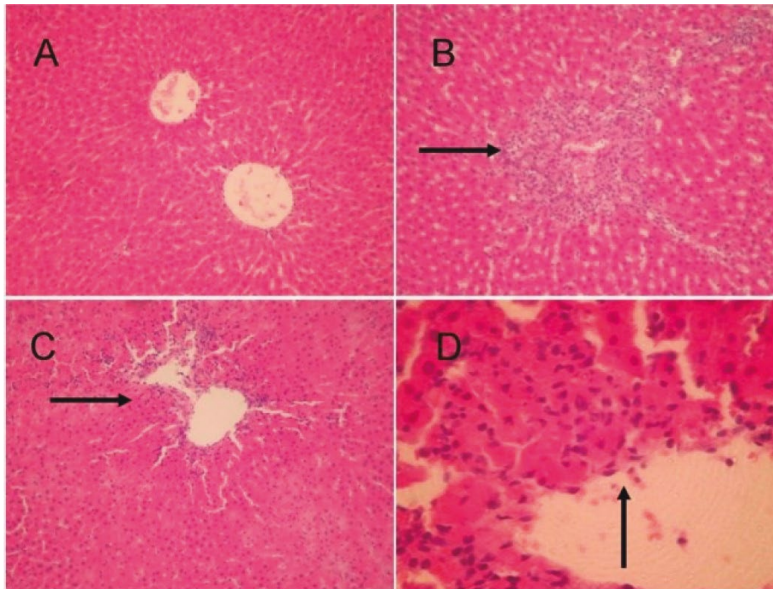


Figure 2. Micrographs showing histopathological changes, especially necrosis and inflammatory infiltration. A: negative control 72 h (150 $\times$). B: positive control 72 h (150 $\times$). C: lycopene+paracetamol (150 $\times$). D: lycopene+paracetamol (600 $\times$). Arrows indicate regions with inflammatory infiltrate and necrosis. Hematoxylin-eosin staining.

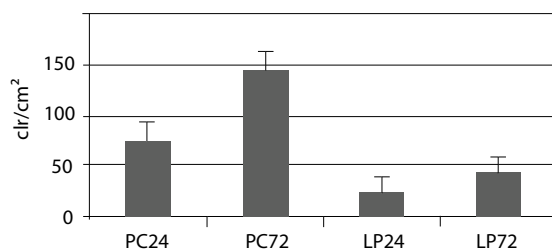


Figure 3. The qualitative analysis of inflammatory/necrotic foci in the centrilobular region of treated and negative control groups. The lycopene (LP) groups show a decreased incidence of injury. Differences between groups were analyzed using ANOVA, followed by the Bonferroni test, with $P < 0.05$ deemed significant. PC24, positive control 24 h; PC72, positive control 72 h; LP24, lycopene+paracetamol 24 h; LP72, lycopene+paracetamol 72 h.

On examining Picrosirius red-stained slides, we observed increased collagen in acinar zones 1 and 3; thus, quantification was performed in these regions. All groups exhibited modest collagen deposition, approaching that observed in the NC group (figures 4, 5 and 6).

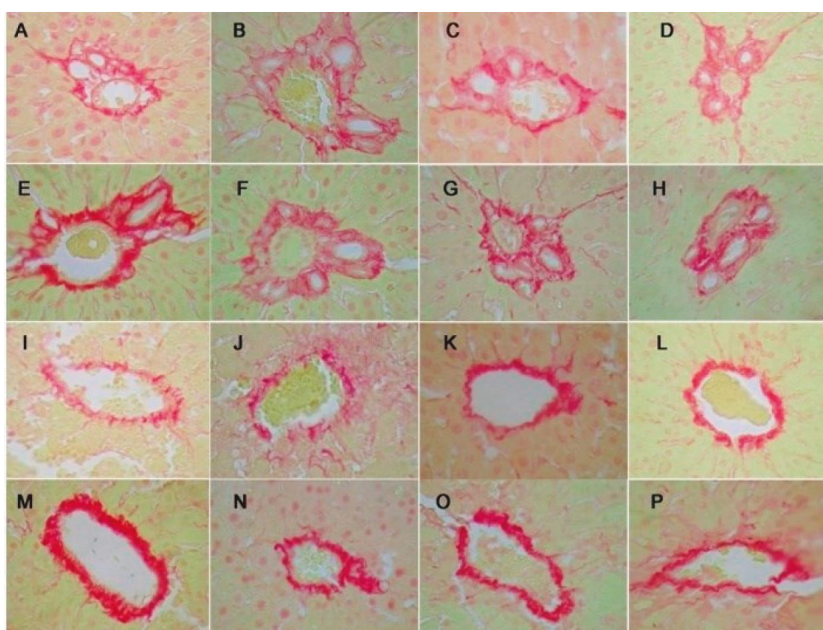


Figure 4. Acinar zone 1. A: 24 h positive control; B: 72 h positive control; C: 24 h negative control; D: 72 h negative control; E: lycopene 24 h; F: lycopene 72 h; G: lycopene+paracetamol 24 h; H: lycopene+paracetamol 72 h; Acinar Zone 3 – I: 24 h positive control; J: positive control 72 h; K: 24 h negative control; L: 72 h negative control; M: lycopene 24 h; N: lycopene 72 h. O: lycopene+paracetamol 24 h; P: lycopene+paracetamol 72 h. Sirius Red Zone, 600×.

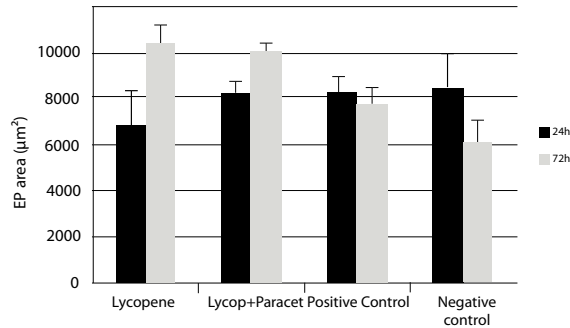


Figure 5. Results of the quantification of collagen fibers in the portal region (acinar zone 1). Small variation in values can be observed, with no significant difference between groups. ANOVA followed by Bonferroni test; P values <0.05 were considered significant. EP

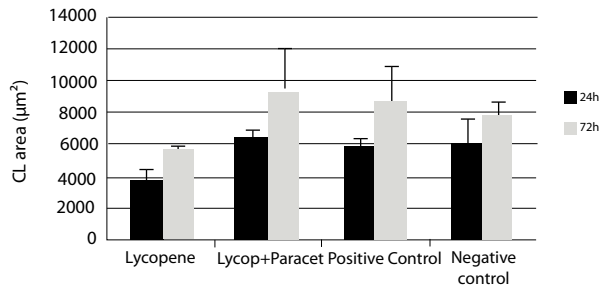


Figure 6. Acinar zone 3. Small variations in values can be observed, with no significant difference between groups. ANOVA followed by Bonferroni test; P values <0.05 were considered significant. CL, centrilobular

On analyzing PAS-stained sections, we observed slight or intense deposition in PAS-positive inclusions in the acinar zone 1 region (portal space), as shown in Fig. 7.

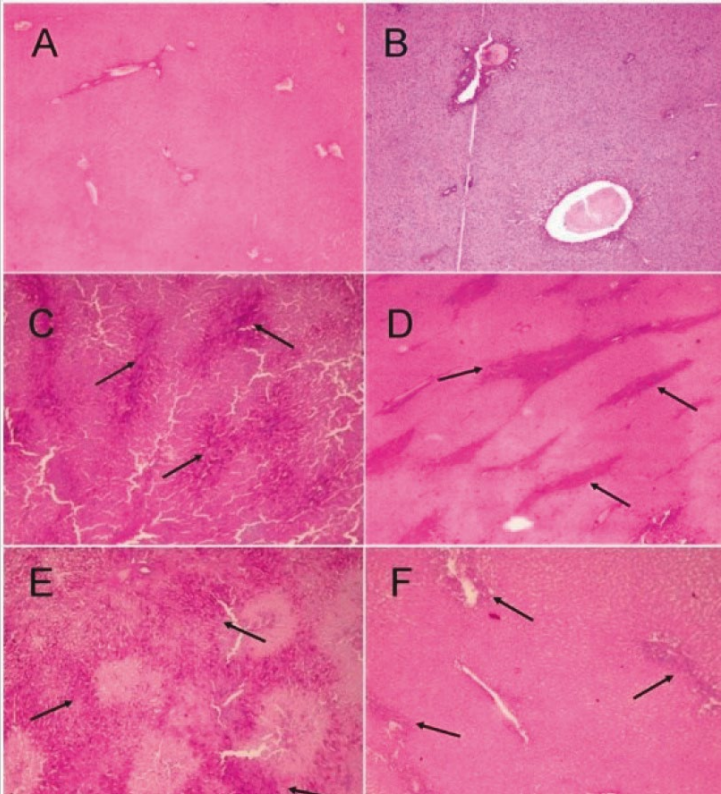


Figure 7. Micrographs of acinar zone 3 showing the distribution of glycogen and other 1,2-glycols. A: 24 h negative control. B: negative control 72 h. C: 24 h positive control. D: positive control 24 h. E: lycopene+paracetamol 24 h. F: lycopene+paracetamol 72 h. Arrows indicate regions with PAS-positive inclusions. Periodic Acid Schiff (PAS), 60 $\times$.

In lycopene-treated groups, positive PAS inclusions were preserved, exhibiting 20% lesion reduction in the LP24 group and 60% in the LP72 group, thus indicating the hepatoprotective activity of lycopene (Table 1).

Table 1. Semiquantitative analysis of the deposition of PAS-positive inclusions in acinar zone 1. Note the significant reduction in inclusions in the LP72 group compared to the PC 72 group.

Group	Number of animals			Reduction in relation to PC
	Positive PAS Deposition in Zone 1			
	No deposition (-)	Mild/moderate deposition (+)	Intense deposition (++)	
NC24	5	0	0	-
NC72	5	0	0	-
PC24	0	2	3	-
PC72	1	2	3	-
LP24	1	0	3	20%
LP72	4	1	0	60%

PAS: Periodic Acid Schiff; PC: positive control; NC24: negative control 24 h; NC72: negative control 72; PC24: positive control 24 h; PC72: positive control 72; LP24: lycopene+paracetamol 24 h; LP72: lycopene+paracetamol 72 h.

Pretreatment with lycopene greatly impacted ALT and AST levels. As shown in Figure 8, serum AST and ALT levels were significantly reduced in the LP24 group when compared with those in the PC24 group. No significant changes in ALP and gamma-glutamyltransferase (GGT) levels were detected.

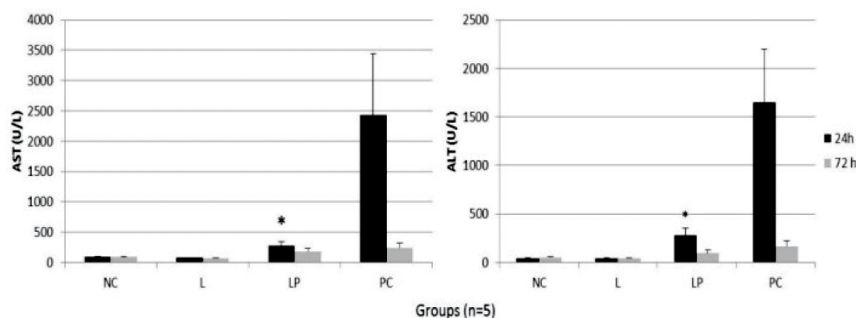


Figure 8. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. NC: negative control group; L: lycopene; LP: lycopene+paracetamol; PC: positive control group. ANOVA followed by Bonferroni test. *P < 0.05 compared to the PC24 group. Reference values for rats: ALT, 51 U/L; AST, 81 U/L [30].

DISCUSSION

Free radicals (ROS) are naturally and continuously generated during energy production, and most organisms possess protective mechanisms against these oxidants [31]. Maintaining a balance between oxidant and antioxidant species within intracellular and extracellular environments is critical for optimal metabolism. It is well-known that energy is obtained from the metabolic breakdown of dietary macronutrients; however, this degradation can generate ROS and RNS, also called free radicals, potentially damaging lipids, proteins, and DNA.

Under normal conditions, there exist mechanisms to neutralize excess ROS or RNS, thus protecting against an imbalance of excessive oxidants, often referred to as “oxidative stress” [31]. Oxidative stress contributes to various disorders and chronic diseases, such as cancer, cardiovascular disease, osteoporosis, diabetes, and cataracts. Thus, antioxidants can eliminate free radicals and prevent damage [31]. However, these mechanisms become ineffective under conditions inducing exacerbated production and activity, resulting in cell and, consequently, tissue damage. Therefore, the search for new molecules or substances with antioxidant properties, such as lycopene, remains crucial to combat diseases and injuries triggered by ROS, and in the present study, paracetamol intoxication.

Histological analysis was performed with three main objectives: (1) observation and quantification of necrosis and inflammatory infiltration using HE staining, (2) quantification of collagen fibers to determine fibrosis using Picrosirius red staining, and (3) qualitative analysis of glycogen and other 1,2-glycols using PAS staining.

On assessing the first parameter, we observed that paracetamol, at the dose employed, caused necrosis and hepatic inflammatory infiltration in all animals in the 24 and 72 h groups, with the latter group exhibiting severe lesions. Lycopene reduced inflammatory infiltrate and necrotic foci; however, these findings were not significant when compared with the PC group (figures 2 and 3). A previous report has reported that lycopene protected hepatocytes in lesions caused by D-galactosamine/lipopolysaccharide-induced lesions, as determined by morphological analysis [32]. Thus, histopathological studies can serve as direct evidence indicating the efficacy of lycopene as a potential hepatoprotectant.

Picrosirius red-stained sections enable the quantification of collagen fibers to identify fibrosis. Following computational analysis of obtained images (figures 3, 4, and 5), we detected no significant difference between groups in terms of acinar zone 1 or 3 when compared with the lycopene-treated group. This result can be explained by the low

intensity of collagen fibers observed and measured in the PC group, corroborating the findings of several previous reports, which did not detect the presence of fibrosis in paracetamol-induced toxic hepatitis [18].

On assessing the third listed histological parameter, we observed that animals treated with high-dose paracetamol exhibited an increase in PAS-positive inclusions in the portal region and a decrease in these inclusions in the centrilobular region when compared with the NC group. A paracetamol-induced decrease in hepatic glycogen was noted, possibly via an irreversible association with a reactive metabolite or the inhibition of mitochondrial energy metabolism, deemed an indicator of drug-mediated hepatotoxicity [33]. Although a quantitative analysis was not performed, it was clearly observed that lycopene pretreatment promoted the homogeneous distribution of glycogen. The decrease in glycogen in acinar zone 1 was expected, as the P450 complex enzymes are found in greater quantity in this region, and consequently, where paracetamol-induced histopathological lesions occur. Notably, we documented a 60 % reduction in glycogen depletion in the LP72 group when compared with the PC group (Figure 7 and Table 1), thus indicating the efficacy of this phytopharmaceutical in preserving cytoplasmic glycogen in hepatocytes.

Considering liver transaminases, lycopene exhibits hepatoprotective potential. AST is a well-known enzyme found in high concentrations in the cardiac muscle, liver, and skeletal muscle and, specifically, in the kidneys and pancreas. In liver cells, AST is located in the cytoplasm (40%) and mitochondria (60%). ALT or glutamic-pyruvic transaminase is an enzyme predominantly found in the liver, at moderate concentrations in the kidneys, and in smaller amounts in the cardiac and skeletal muscle. In liver cells, ALT is located in the cytoplasm (90%) and mitochondria (10%). Tissue damage or diseases affecting the liver parenchyma will induce the release of these enzymes into the bloodstream, raising serum levels of AST and ALT, thus indicating hepatocellular disease.

Previous studies have evaluated AST and ALT levels in patients with acute and chronic liver disease. The AST/ALT ratio provides useful clinical information regarding the cause and severity of the underlying liver disease in these patients. Advantages of using these parameters include easy estimation and interpretation at a low cost, allowing widespread application [34]. Elevated serum levels of ALT and AST can be attributed to damage liver structure, given that these enzymes are located in the cytoplasm and are released into the bloodstream after cell damage [35].

Thus, we observed that serum AST and ALT levels were lower in the LP24 and LP72 groups than in the PC group (treated with paracetamol). In line with the findings of the

present study, several previous reports have confirmed the hepatoprotective action associated with decreased serum AST and ALT levels following toxic hepatitis [18, 32, 36].

ALP reflects pathological changes in the bile duct [37]. Thus, elevated serum ALP levels in rats with induced hepatitis can be associated with a disturbance in the secretory activity, metabolite transport, or the synthesis of certain enzymes altered during other hepatotoxic conditions [34]. Based on our findings, we observed no discernible change in the levels of this enzyme, even in the PC group. The absence of elevated ALP levels in the PC group (and in other groups) was in agreement with the histological findings, given that no changes were observed in bile ducts on light microscopy examination, thus indicating that paracetamol-induced hepatotoxicity is cytolytic and not cholestatic [18].

GGT is an enzyme known to be involved in the transport of amino acids and peptides across cell membranes, protein synthesis, and regulation of tissue glutathione levels. This enzyme is primarily found in the liver and kidneys. In the liver, GGT is located in the canaliculi of liver cells and epithelial cells lining bile ducts; thus, along with ALP, changes in serum levels of GGT indicate biliary involvement, demonstrating more sensitivity and persistent effects. We noted no change in GGT levels in any examined groups, thus indicating that paracetamol intoxication does not cause biliary damage.

Lycopene is a fat-soluble, red pigment carotenoid found to occur in certain plants and microorganisms, where it helps protect against ultraviolet B rays. Notably, lycopene is synthesized by plants and microorganisms but not animals [38]. This carotenoid possesses 40 carbon atoms ($C_{40}H_{56}$), containing 11 conjugated and 2 unconjugated double bonds, predisposing lycopene to isomerization and degradation upon exposure to light, excessive heat, and air [38].

The mechanism of lycopene absorption has been well-characterized. Lycopene ingested in natural form is poorly absorbed. Processed tomatoes or tomatoes that induced isomerization of the *trans* to *cis* configuration exhibit increased bioavailability. In addition, given its fat-soluble component, lycopene absorption can be improved by ingesting oils. Reportedly, its concentration in human tissues exceeds that of all other carotenoids. Lycopene is mainly distributed to adipose tissues, as well as organs such as adrenal glands, liver, or testes [39].

Lycopene has been described as the most important antioxidant of the carotenoid group. It also has an important anti-inflammatory effect and acts as a scavenger of free radicals, thus reducing induced cell damage [40]. Numerous studies using *in vivo* models of radiation exposure have demonstrated that lycopene protects against radiation toxicity via its antioxidant mechanism [41]. *In vitro* models have revealed that the use of lycopene prior to radiotherapy can reduce the effects of radiation-induced oxidative

damage in rats [41]. In addition, lycopene has hepatoprotective action against D-galactosamine/lipopolysaccharide-induced damage [32].

Lycopene can afford hepatoprotective benefits by preventing mercury-induced oxidative stress [42]. Literature have reported that tomato pulp, which contains high doses of lycopene, has hepatoprotective activity against carbon tetrachloride-induced intoxication [43]. However, no study has reported the non-hepatoprotective effects of lycopene.

CONCLUSION

Based on the findings of the present study, we concluded that lycopene could afford hepatoprotective action following paracetamol-induced acute intoxication, as shown by the histological decrease in necrosis and inflammatory foci, preservation of glycogen and other 1,2-glycols in acinar zone 3, and reduced serum levels of ALT and AST.

DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

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HOW TO CITE THIS ARTICLE

R.M. Bonilha-Dezena, G.H. da Silva, G.M. Silva-Gonçalves, Hepatoprotective activity of lycopene in experimental paracetamol-induced liver injury in rats, *Rev. Colomb. Cienc. Quim. Farm.*, **51**(2), 1320-1340 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.107549>

Determination of phenolic bioactive compounds and evaluation of the antioxidant and hemolytic activities in the methanolic extracts of *Tradescantia zebrina*

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Received: March 8, 2022

Corrected: April 22, 2022

Accepted: April 28, 2022

SUMMARY

Introduction: *Tradescantia zebrina* is a species used mainly in South America, with few reports about its chemical composition and bioactivity. **Aim:** To determine phenolic bioactive compounds, for the first time in the literature, and the antioxidant and hemolytic activities of extracts (dried and *in natura*) of this species. **Methods:** The extracts were obtained by maceration in methanol and the total phenolic and flavonoid contents were expressed in mg equivalent of gallic acid (EAG)/g and quercetin (EQ) of extract, respectively. The antioxidant potential was evaluated by DPPH• and ABTS•+ radical capture methods. The identification and quantification of phenolic bioactive compounds was performed by high performance liquid chromatography coupled to a diode detector (HPLC-DAD) and, the hemolytic activity assay was performed by diffusion in blood agar, using the disk antibiogram technique. **Results:** The total phenolic and flavonoid contents in the extracts (*in natura* and dried) were 67.68 and 233.94 mg EAG/g and 29.70 and 10.99 mg EQ/g of concentrated extract, respectively. Phenolic acids (caffeic,

ellagic, ferulic, *p*-coumaric and protocatechuic), flavonoids (chrysin and rutin) and a phenolic aldehyde (vanillin) were determined by high performance liquid chromatography-diodearray detector (HPLC-DAD). The antioxidant activities were confirmed by inhibition of DPPH• (44.08%) and ABTS•+ (46.99%) radicals for *in natura* samples, respectively. **Conclusion:** The tested methanolic extracts of *Tradescantia zebrina* did not present toxicities when tested for hemolytic activity after 72 hours. Therefore, *Tradescantia zebrina* is a potential source of bioactive substances.

Keywords: *Tradescantia zebrina*, antioxidants, phenolic compounds, HPLC-DAD, biological activity.

RESUMEN

Determinación de compuestos bioactivos fenólicos y evaluación de las actividades antioxidante y hemolítica en los extractos metanólicos de *Tradescantia zebrina*

Introducción: *Tradescantia zebrina* es una especie utilizada principalmente en Sudamérica, hay pocos reportes sobre su composición química y bioactividad. **Objetivo:** determinar compuestos bioactivos fenólicos, por primera vez en la literatura, y las actividades antioxidante y hemolítica de extractos (secos e *in natura*) de esta especie. **Métodos:** los extractos se obtuvieron por maceración en metanol y los contenidos de fenoles y flavonoides totales se expresaron en mg equivalentes de ácido gálico (EAG)/g y quercetina (EQ) de extracto, respectivamente. El potencial antioxidante fue evaluado por los métodos de captura de radicales DPPH• y ABTS•+. La identificación y cuantificación de compuestos bioactivos fenólicos se realizó por cromatografía líquida de alta resolución acoplada a detector de diodo (HPLC-DAD) y el ensayo de actividad hemolítica se realizó por difusión en agar sangre, utilizando la técnica de antibiograma de disco. **Resultados:** los contenidos de fenoles y flavonoides totales en los extractos (*in natura* y secos) fueron 67,68 y 233,94 mg EAG/g y 29,70 y 10,99 mg EQ/g de extracto concentrado, respectivamente. Los ácidos fenólicos (cafeico, elágico, ferúlico, *p*-cumárico y protocatequiico), los flavonoides (crisina y rutina) y un aldehído fenólico (vainillina) se determinaron mediante cromatografía líquida de alta resolución-detector de diodos (HPLC-DAD). Las actividades antioxidantes

fueron confirmadas por la inhibición de los radicales DPPH• (44,08%) y ABTS•+ (46,99%) para muestras *in natura*, respectivamente. **Conclusión:** los extractos metanólicos de *Tradescantia zebrina* probados no presentaron toxicidades cuando se probaron para la actividad hemolítica después de 72 horas. Por lo tanto, *Tradescantia zebrina* es una fuente potencial de sustancias bioactivas.

Palabras clave: *Tradescantia zebrina*, antioxidantes, compuestos fenólicos, HPLC-DAD, actividad biológica.

RESUMO

Determinação de compostos fenólicos bioativos e avaliação das atividades antioxidante e hemolítica em extratos metanólicos de *Tradescantia zebrina*

Introdução: *Tradescantia zebrina* é uma espécie utilizada principalmente na América do Sul, há poucos relatos sobre sua composição química e bioatividade. **Objetivo:** determinar compostos bioativos fenólicos, pela primeira vez na literatura, e as atividades antioxidante e hemolítica de extratos (secos e *in natura*) desta espécie. **Métodos:** os extratos foram obtidos por maceração em methanol e os teores de compostos fenólicos e flavonóides totais foram expressos em mg equivalente de ácido gálico (EAG)/g e quercetina (EQ) de extrato, respectivamente. O potencial antioxidante foi avaliado pelos métodos de captura do radical DPPH• e ABTS•+. A identificação e quantificação de compostos bioativos fenólicos foi realizada por cromatografia líquida de alta eficiência acoplada a detector de diodo, na região do ultravioleta e o ensaio de atividade hemolítica foi realizado por difusão em ágar sangue, pela técnica de antibiograma em discos. **Resultados:** os teores de fenóis totais e flavonoides nos extratos (*in natura* e seco) foram 67,68 e 233,94 mg EAG/g e 29,70 e 10,99 mg EQ/g de extrato concentrado, respectivamente. Ácidos fenólicos (caféico, elágico, ferúlico, p-cumárico e protocatecúico), flavonoides (crisina e rutina) e um aldeído fenólico (vanilina) foram determinados por cromatografia líquida de alta eficiência-detector de diodo (CLAE-DAD). As atividades antioxidantes foram

confirmadas pela inibição dos radicais DPPH• (44,08%) e ABTS•+ (46,99%) para amostras *in natura*, respectivamente. **Conclusão:** os extratos metanólicos de *Tradescantia zebrina* testados não apresentaram toxicidade quando testados para atividade hemolítica após 72 horas. Portanto, *Tradescantia zebrina* é uma fonte potencial de substâncias bioativas.

Palavras-chave: *Tradescantia zebrina*, antioxidantes, compostos fenólicos, HPLC-DAD, atividade biológica.

INTRODUCTION

The use of medicinal plants for the prevention, treatment, and cure of various diseases is as old and universal as the history of humanity. *Tradescantia* is a genus of herbaceous plants found mainly in South America, being abundant and easily accessible in Brazil, more specifically in the state of Bahia. Reports of the bioactivity of the species *Tradescantia zebrina*, popularly known as “egenda”, “trapoeraba”, “viuvinha”, “lambari” or sea wave, are distant from each other, as there is not enough data in the scientific literature regarding its chemical composition, biological activities, effectiveness, and therapeutic safety. The plant is traditionally used in Mexico as a drink capable of treating various diseases, including cancer [1, 2].

Phenolic compounds are secondary metabolites of plants and are subclassified into flavonoids (polyphenols: flavones, flavanones, flavanols, isoflavones, anthocyanins, etc.) and non-flavonoids (phenolic acids, tannins, coumarins and lignans) [3]. Currently, phenolic compounds have been the substances of greatest interest to science, due to their beneficial characteristics for health, such as antioxidant, antimicrobial and anti-neoplastic activities [4-8].

Few studies have evaluated total phenolic and flavonoid levels in *Tradescantia zebrina* [1, 9-11]. To guarantee the phytochemical profile of a species or its extracts, a chromatographic *fingerprint* is essential to ensure the quality and reproducibility of the chemical composition, concentration of chemical substances, and, subsequently, therapeutic efficacy. Therefore, high-performance liquid chromatography (HPLC) is an alternative analytical tool for the separation, identification, quantification, and quality control of medicinal plants [12-16].

Although very important, the use of medicinal plants can present risks, since many plants can have a variable chemical composition and relative toxicity [17]. The possibility of

hemolysis is one of the most relevant problems in the use of plants since the saponins or alkaloids present can cause risks to consumption. The *in vitro* hemolysis test has been used frequently in studies that evaluate the toxicity of medicinal plants [18, 19].

This study aims to expand the knowledge about the species *Tradescantia zebrina*, contributing to the chemical and biological prospection of substances with antioxidant and non-hemolytic potential. For the first time, bioactive phenolic compounds were identified and quantified in methanolic extracts from samples (dried and *in natura*) in this species, promoting greater pharmacological interest and its traditional use

MATERIALS AND METHODS

Plant material

Tradescantia zebrina specimens were collected in Aporá/Bahia/Brazil within the same terrain (11.6605° S, 38.0803° W) at autumn (May 2021), in the full moon. The dried samples (whole plant) were collected, cleaned of dirt and earth remains, and dried in the shade until they became friable. Then, the material was ground in a knife mill (OMDR100, Oster®, São Paulo, Brazil) with a power of 150 W. The process was carried out in 3 cycles of comminution to powder and then they were stored until the extracts were prepared. The *in natura* plant was collected, cleaned, scraped, and weighed for the preparation of extracts.

The botanical identification of the species was carried out by comparison with the characterization described in taxonomic reviews and scientific botanical literature (botanical atlases and photos of the species) and, from traditional native knowledge. A branch of the species was used to identify the macromorphological aspects and preparation of the exsiccate, observing division, leaf blade shape, blade base, type of enervation, presence of petiole, sheath, and stipule, and also the morphological characteristics of the flowers.

Extraction of samples

The extracts obtained from dried samples were performed by maceration in methanol and a re-maceration with solvent exchange in 24 hours and, the extract from fresh samples (*in natura*) of *Tradescantia zebrina*, was obtained by dynamic maceration in methanol, using a blender for 4 hours. The crude extracts were concentrated in a rotary evaporator (Quimis®, São Paulo, Brazil) under vacuum (40 mbar) at a temperature below 35 °C. After complete evaporation, the adhered material was reconstituted with approximately 3.0 mL of methanol to wash the collection flask, until complete drying. The concentrated extracts (dried and *in natura*) were placed in clean and identified

ependorfs and stored in the freezer until the standardized extracts were prepared at a concentration of $1 \text{ mg}\cdot\text{mL}^{-1}$. The unconcentrated extract was standardized at a concentration of $75\,000 \text{ mg}\cdot\text{L}^{-1}$, being used in a maximum of 3 days. Extracts were filtered and stored at 4°C when not in use. All analyses were conducted in triplicates.

Determination of total phenolic and flavonoids contents

To quantify the total phenolic compounds, the methodology proposed by Bonoli *et al.* (2004) and the contents of total phenolic compounds were expressed in mg gallic acid equivalent (GAE)/g of extract. All analyses were conducted in triplicates [20].

The determination of total flavonoid levels was performed in triplicate, according to the method described by Woisky & Salatino in 1998 and the total flavonoid levels were expressed in mg quercetin equivalent (EQ)/g of extract the [21].

In vitro assays for evaluating the antioxidant activity

DPPH radical scavenging activity: It was performed according to Brand-Williams *et al.* (1995) with some modifications [22].

ABTS radical scavenging activity: It was performed according to Nenadis *et al.* (2004) with some modifications [23].

Chromatographic analysis

To extract the phenolic compounds, about 10 mg of the crude extract of *Tradescantia zebrina* was solubilized in 1 mL of methanol (HPLC grade). Analyzes were performed in HPLC with a Prominence-i LC-2030C 3D Plus liquid chromatography system (Shimadzu[®], Kyoto, Japan), equipped with a diode array detector (DAD) (model SPD-20A), high-pressure quaternary (model LC-20AD), heating oven (model CTO-20A), automatic sampler (model SIL-20A) and communication module (CBM-40Life), all treatment and acquisition of samples were controlled by the LCsolutions software (Shimadzu[®], Kyoto, Japan).

Chromatographic separation was performed using a Lichrospher[®] C18 column ($5 \mu\text{m}$, $4.0 \times 250 \text{ mm}$) from Sigma-Aldrich[®] (USA), at 40°C and flow rate of $1.0 \text{ mL}\cdot\text{min}^{-1}$ with analytical solvents for binary elution mixture consisting of (A) ultrapure water acidified with 1% glacial acetic acid ($\text{v}\cdot\text{v}^{-1}$) and (B) HPLC grade methanol according to gradient program: 0-10 min, 100% A; 10-20 min, 30% A and 70% B; 20-21, 25% A and 75% B; 21-25, 100% A. Prior to injection ($20 \mu\text{L}$) into the HPLC-DAD system, extract solutions were filtered through a PTFE syringe filter ($0.45 \mu\text{m}$) [12, 24].

A sample of each extract (dried and *in natura*) was fortified with a 0.005 mg·mL⁻¹ solution containing a mix of 17 phenolic standards for identification and quantification from its spectrum (chromatogram). Identification and qualitative analyzes were performed by comparison with standard spectra at each retention time (Sagdic *et al.*, 2011). The standards of phenolic compounds that were tested show absorbances at the following wavelengths: Caffeic acid (330 nm), Chlorogenic acid (330 nm), Ellagic acid (260 nm), Ferulic acid (330 nm), Gallic acid (272 nm), *p*-coumaric acid (310 nm), Protocatechuic acid (260 nm), Sinapic acid (330 nm), Syringic acid (272 nm), Trans-cinnamic acid (280 nm), Catechin (280 nm), Chrysin (272 nm), Kaempferol (360 nm), Naringenin (280 nm), Quercetin (360 nm), Rutin (360 nm) and Vanillin (280 nm). The identified analytes were quantified using external calibration curves (0.1 - 2.5 mg·L⁻¹) and the results were expressed in mg·g⁻¹ of extract.

The proposed method was validated according to Brazil in 2017 and the main analytical parameters normally found for method validation are: linearity, precision, limits of detection (LOD) and of quantification (LOQ) [25]. Linearity was evaluated according to the coefficient of determination (R^2) of the analytical curves. Precision was demonstrated by the dispersion of the results, calculating the relative standard deviation (RPR) of the measurements. The limits of detection (LOD) and quantification (LOQ) were calculated based on the parameters of the analytical curves, relating the standard deviation of the intersection (*s*) and the slope of the curve (*S*).

Hemolytic activity test

The hemolytic activity assay was performed according to CLSI in 2015 with modifications, by diffusion in blood agar using the disc antibiogram technique [26]. Filter paper discs were impregnated with samples (1 mg·mL⁻¹ of dried extract) of *Tradescantia zebrina*. After impregnation, the discs were distributed on the plates and incubated at 35 °C for 24, 48 and 72 hours. As a positive control, a solution of the detergent Triton X-100 1 % (v·v⁻¹) was used. Methanol (0.1%) and saline solution (20%) were used. After incubation, the plates were inspected for the presence of halos of hemolysis between 24 and 72 hours.

Statistical analysis

Data are given as mean $\pm$ SD of three measurements for each experiment, using Graph-Pad InStat (Graph software Inc., V 3.05, Ralph Stahlman, Purdue University, Lafayette, IN). IC₅₀ were calculated by nonlinear regression analysis with Graph Pad Prism version 5 for Windows (Graph Pad Inc., USA).

RESULTS AND DISCUSSION

Macroscopic analysis, total phenolic and flavonoids contents, and antioxidant activities

The macroscopic characteristics of the specimens analyzed were in agreement with the family Comelinaceae [27]. The analysis of the architecture of the leaves showed a parallel pattern, presence of hermaphroditic inflorescences and protection by bracts recorded for the species. It was possible to identify a stoloniferous stem with creeping growth, with the presence of nodes from which adventitious roots emerged and the formation of new branches.

Tradescantia zebrina is a species that offers easy adaptation in any space, in addition to developing throughout the year, both outdoors and in greenhouses [28]. The environmental influences that most interfere with its growth and development are photoperiod, radiation and temperature [29]. Therefore, the place and time of planting are essential to obtain a high yield of the species and substances of secondary metabolism. Phenolic acids and flavonoids have several functions for plant growth and metabolism (defense against insects, fungi, viruses and bacteria and protection against ultraviolet radiation). In addition, these compounds show antioxidant, antimicrobial, anti-inflammatory and antineoplastic activities, as well as in the prevention of neurodegenerative diseases [30-34].

In the literature, some studies have indicated the presence of phenolic compounds in *Tradescantia zebrina*, as well as its antioxidant potential [1, 2, 35]. Table 1 presents the comparative average values (found in this study and in the scientific literature) for phenolic compounds and total flavonoids and, also for the antioxidant activity of extracts of *Tradescantia zebrina*.

The results indicated variations in the contents of phenolic compounds and total flavonoids, in the dried and *in natura* extracts of the *Tradescantia zebrina* specimens studied when compared with a few data from the literature. A reduction in the concentration of phenolics and total flavonoids was observed in the dried sample, probably due to the drying process of the plant, associated with the process of rotaevaporation of the crude extract. The drying temperature can decrease the polyphenol content of plant extracts, as well as their antioxidant activity [37]. The results indicated concentrations of total phenolic compounds and flavonoids higher than those found by Cheah *et al.* in 2017 for dried methanolic extracts of *Tradescantia zebrina*, in Malaysia [11].

Table 1. Comparative mean values (found in this study and in the scientific literature) for total phenolics and flavonoids and, antioxidant activity for extracts of *Tradescantia zebrina*.

Studies/country	Total Phenolics (mg EAG/g) (sample type)	Total flavonoids
Present study/Bahia, Brazil	67.68 ± 0.12* (dried) 233.94 ± 0.71* (<i>in natura</i>)	10.99 ± 0.08* (dried) 29.70 ± 0.04* mg EQ/g (<i>in natura</i>)
Cheah et al. in 2017 [11]/ Kuala Lumpur, Malaysia	33.50 ± 2.58* (dried)	9.40 ± 1.06* mg ECAT/g (dried)
Olivo-Vidal et al. in 2020 [10]/Tabasco, Mexico	70.20 ± 1.20** (<i>in natura</i>)	16.80 ± 0.50 mg ECAT/g** (<i>in natura</i>)
Baghalpour et al. in 2021 [9]/ Alborz, Iran	0.08 ± 0.14**	-
To in 2020 [36]/Kuala Lumpur, Malaysia	694.86 ± 54.46 (stem)** (<i>in natura</i>) 510.70 ± 31.45 (leaf)** (<i>in natura</i>)	496.22 ± 16.56 mg ECAT/g (stem)** (<i>in natura</i>) 410.27 ± 8.34 mg ECAT/g (leaf)** (<i>in natura</i>)
Tan et al. in 2014 [1]/Kuala Lumpur, Malaysia	6.21 ± 0.40* (<i>in natura</i>)	17.10 ± 2.80 mg ER/100 g (<i>in natura</i>)
Studies/country	Antioxidant activity (%) (DPPH)	Antioxidant activity (%) (ABTS)
Present study/Bahia, Brazil	44.08 (ExC: 45.00 mg mL ⁻¹)*	46.99 (ExC: 0.80 mg mL ⁻¹)*
Cheah et al. in 2017 [11]/ Kuala Lumpur, Malaysia	18.10 (ExC: 0.10 mg mL ⁻¹)*	-
Olivo-Vidal et al. in 2020 [10]/Tabasco, Mexico	84.00 (ExC: 100 mg mL ⁻¹)**	16.00 (ExC: 100 mg mL ⁻¹)**
To in 2020 [36]/Kuala Lumpur, Malaysia	26.19 (stem) (ExC: not informed)** 18,78 (leaf) (ExC: not informed)**	-

EAG: gallic acid equivalents; ECAT: catechin equivalents; EQ: quercetin equivalents; ER: rutin equivalents; *: methanolic extract; **: ethanol extract; ExC: extract concentration; -: not available.

The results obtained for the *in natura* samples were also superior to those found by Tan *et al.* in 2014 in methanolic extracts of this species, in Malaysia [1] and, by Olivo-Vidal *et al.* in 2020 [10] and Baghalpour *et al.* in 2021 [9], who determined phenolic compounds in ethanolic extracts of this species in Mexico and Iran, respectively. However, were inferior (about 10 times) to those found by To (2020), in ethanol extracts (stem and leaf) of *Tradescantia zebrina*, in Malaysia [36].

Thus, secondary metabolites represent a chemical correlation between the species and the environment where they are planted. Environmental stimuli can alter the biosynthesis of different compounds, such as seasonality, collection location, the incidence of solar radiation, rainfall, plant interactions with microorganisms, insects and/or other plants, circadian cycle, nutrition, time and time of collection and, sample preparation method [38, 39].

The DPPH• radical was inhibited (44.08%) in 45 g of plant (*in natura*) extracted in 1 liter of methanol. To prepare the drink “Agua de Matalí”, popular among Mexicans according to the cultural tradition typical of Tabasco, about 100-150 g of plant is used infused in 1 liter of boiling water ($\pm$ 1 liter) and, after cooling, it is added with lemon juice and honey, serving ice cold or hot [40]. Thus, the consumption of soft drinks, in this proportion, promotes the intake of antioxidant compounds in the diet.

Olivo-Vidal *et al.* (2020) [10] compared the contents of phenolic compounds and total flavonoids, as well as the determination of antioxidant activity by the methods of DPPH, ABTS and by the antioxidant power capable of reducing Iron III (FRAP) with extracts (dynamic maceration in 80% ethanol; aqueous maceration at 22 °C; and aqueous infusion, in the proportion of 1 g of leaf for 10mL of solvent) prepared with fresh leaves of *Tradescantia zebrina*. The authors indicated that the aqueous extract, obtained by maceration, showed a greater ability to capture the radical ABTS•+ and also a greater dosage of total flavonoids (27.8 mg ECAT/g). However, the total phenolic compounds showed lower concentrations (51 mg EAG/g) when compared to the ethanolic extract (70.2 mg EAG/g); and this, in turn, had lower flavonoid levels (16.8 mg ECAT/g) and higher DPPH sequestration activity (84%). The extract obtained by infusion showed better results by the FRAP method, being equivalent to the extract obtained by aqueous maceration, in total phenolic contents.

The results of the present study, when compared to those of Cheah *et al.* (2017) [11], in Malaysia, showed higher concentrations of phenolic compounds and total flavonoids for the methanolic extract of *Tradescantia zebrina*. Also, were comparable with those of Olivo-Vidal *et al.* in 2020, in Mexico [10]. This may be due to seasonal interference, the planting process, and the collection and preparation of samples, as the authors

reported that only the leaves were used to obtain the extracts. Also, it is noteworthy that the Mexican plant was acquired at a local fair and the conditions of time and storage of the specimens after collection until analysis were not informed.

The phytochemical assessment investigated by Tan *et al.* (2014) [1] highlighted the presence of phenolic compounds and the antioxidant activity of the *Tradescantia zebrina* species when compared to others of the *Tradescantia* family, *Tradescantia spathacea* var. *variegata*, *Tradescantia spathacea* “Hawaiian Dwarf” and *Tradescantia pallida*. Among these species, *Tradescantia zebrina* showed the highest antioxidant activity (about nine times greater) than the other species studied. This observation was also confirmed by Cheah *et al.* (2017) [11] and Baghalpour *et al.* (2021) [9].

Chromatographic analysis

To validate the proposed method, a solution containing 17 phenolic compounds ($0.005 \text{ mg}\cdot\text{mL}^{-1}$) was prepared and, from the chromatograms, the analytical parameters were obtained (Table 2). Then, the method was applied to analyze the methanolic extracts of the samples (dried and *in natura*) of *Tradescantia zebrina*.

Table 2. Analytical validation parameters for the proposed method by HPLC-DAD.

Analyte	Regression equation	R ²	LOD ($\text{mg}\cdot\text{L}^{-1}$)	LOQ ($\text{mg}\cdot\text{L}^{-1}$)
Caffeic acid	$y = 149675x - 15785$	0.9999	0.04	0.13
Chlorogenic acid	$y = 65056x + 5481.7$	0.9973	0.14	0.41
Ellagic acid	$y = 180471x - 6206.8$	0.9992	0.13	0.39
Ferulic acid	$y = 106019x - 10833$	0.9998	0.06	0.17
Gallic acid	$y = 69058x - 987.92$	0.9988	0.16	0.48
ρ -Coumaric acid	$y = 173234x - 16450$	1	0.03	0.09
Protocatechuic acid	$y = 132697x - 8605$	0.9997	0.08	0.24
Sinapic acid	$y = 107178x - 10354$	0.9998	0.06	0.18
Syringic acid	$y = 73260x - 8065.3$	0.9999	0.04	0.11
trans-Cinnamic acid	$y = 156741x - 16963$	1	0.03	0.08
Catechin	$y = 11344x + 3841.1$	0.9979	0.21	0.63
Chrysine	$y = 23736x - 10191$	0.9903	0.44	1.34
Kaempferol	$y = 23417x - 4108.7$	0.9925	0.40	1.20
Naringenin	$y = 63685x - 6429.1$	0.9995	0.10	0.29

(Continued)

Analyte	Regression equation	R ²	LOD (mg·L ⁻¹)	LOQ (mg·L ⁻¹)
Quercetin	$y = 45981x - 10078$	0.9985	0.18	0.54
Rutin	$y = 31435x - 3903$	1	0.01	0.04
Vanillin	$y = 92846x - 620.8$	1	0.02	0.07

The identification of phenolic compounds by HPLC-DAD was based on the comparison between the peaks obtained from the respective analytical standards and the extracts, regarding retention times and UV spectra. To confirm the presence of phenolic compounds, the samples (dried and *in natura*) were fortified with the standard mixing solution (5.00 µg·mL⁻¹) and the chromatograms obtained were compared (Fig. 1).

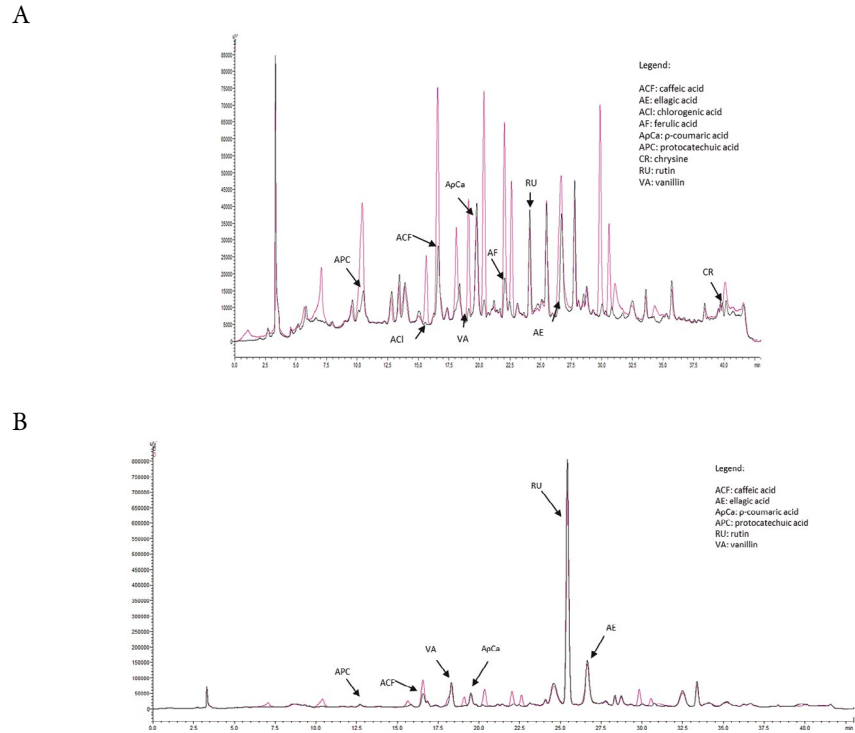


Figure 1. Chromatograms obtained from pure methanolic extract (black line) and enriched with analytical standards (5 µg·mL⁻¹) of phenolic compounds (pink line) from samples of *Tradescantia zebrina* (A – dried and B – *in natura*) by HPLC-DAD.

It was possible to identify nine phenolic compounds for the dried extract: six phenolic acids (caffeic, ellagic, chlorogenic, ferulic, *p*-coumaric and protocatechuic), two flavonoids (chrysin and rutin) and one a phenolic aldehyde (vanillin) and, of these, eight analytes were quantified (Table 3). Ellagic acid, caffeic acid, and rutin showed higher concentrations. Chlorogenic acid, identified in the extract from its analytical standard, presented amounts below the detection limit (LOD). For extracts *in natura* a change in the chromatogram profile was observed (Fig. 1B). It was possible to identify six phenolic compounds for the dried extract: four phenolic acids (caffeic, ellagic, *p*-coumaric and protocatechuic), one flavonoid (rutin) and one phenolic aldehyde (vanillin) and, of these, five analytes were quantified (Table 4).

Table 3. Phenolic compounds quantified from the dried methanolic extract of *Tradescantia zebrina* by HPLC-DAD.

Analyte	Wavelength	Retention time	Integrated area	Conc. (mg·L ⁻¹)	Conc. (µg·g ⁻¹)
Caffeic acid	330	16.636	257002	1.82	182.25
Chlorogenic acid	330	15.566	5636	nd	nd
Ellagic acid	260	26.717	521488	2.92	292.40
Ferulic acid	330	22.136	134203	1.37	136.80
<i>p</i> -Coumaric acid	310	20.394	95896	0.65	64.85
Protocatechuic acid	260	10.512	103298	0.84	84.33
Chrysine	272	40.168	17318	1.16	115.90
Rutin	360	25.495	107323	3.54	353.83
Vanillin	280	19.139	38708	0.42	42.36

nd: not determined; Conc: concentration.

A high intensity peak was obtained, with a larger integrated area, for the flavonoid rutin, with a concentration much higher than the sample enriched with the analytical standard. After calculating the purity of this substance (from the integrated area of the peak), a value of 70% was obtained. Therefore, it was decided not to predict the actual content of this flavonoid, since other compounds (present in *in natura* sample, not contained in the standard solution used in this study) may be eluting together with rutin, by absorbing radiation at 360 nm.

Table 4. Phenolic compounds quantified from the *in natura* methanolic extract of *Tradescantia zebrina* by HPLC-DAD.

Analyte	Wavelength	Retention time	Integrated area	Conc. (mg L ⁻¹)	Conc. (µg g ⁻¹)
Caffeic acid	330	16.580	427860	2.96	296.41
Ellagic acid	260	26.649	2253336	12.52	1,252.03
<i>p</i> -Coumaric acid	310	20.232	87347	0.60	59.92
Protocatechuic acid	260	10.433	38817	0.36	35.74
Rutin	360	25.434	2534792	nd	nd
Vanillin	280	19.147	15829	0.18	17.72

nd: not determined; Conc: concentration.

The nature and amount of chemical compounds identified in plants can vary according to several factors [41]. No data were found in the scientific literature on the identification and quantification of phenolic compounds in *Tradescantia zebrina* by HPLC-DAD. Therefore, this study fills a gap because, for the first time, it demonstrated the relevance of this species to science, confirming the chemical, biological, and therapeutic potential of *Tradescantia zebrina*.

Hemolytic activity test

In tests of biological and, especially, toxicological activities of plant extracts, there is a need to evaluate hemolytic activity. With the rupture of red blood cells, the iron released from the heme group is harmful and can cause damage to essential organs, such as the liver and kidneys, resulting in hemoglobinemia [42, 43]. When this value exceeds 100 mg%, hemoglobin begins to be filtered by the kidneys and, when excessively high (above 3000 mg%), it can promote kidney damage with hemoglobinuria [19, 44].

From the results obtained in the hemolytic activity test through the diffusion of the extract in blood agar, no sample showed formation of a hemolytic halo after 72 hours (Fig. 2). This confirms no toxicity to the erythrocyte membrane, from the methanolic extracts of samples (dried and *in natura*) of the species. Only, there was the formation of a halo of 2 cm of hemolysis with the use of the positive control, a solution of the detergent Triton X-100 1 % (v.v⁻¹). Therefore, it is suggested that, preliminarily, *Tradescantia zebrina* does not indicate toxicological risks for use in living organisms, despite the saponins present in the plant.

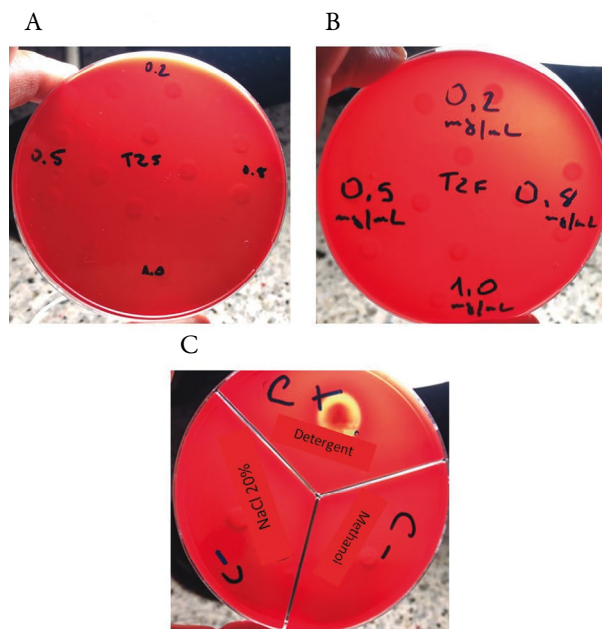


Figure 2. Evaluation of the presence of hemolysis halos, in plates, with controls (positive and negative), after 72 hours in methanolic extracts of *Tradescantia zebrina*. A: dried extract; B: *in natura* extract and C: negative and positive controls.

CONCLUSION

The methanolic extracts of samples (dried and *in natura*) of *Tradescantia zebrina* showed considerable levels of phenolic compounds and antioxidant activities. From a preliminary fingerprint obtained by HPLC-DAD, the presence of phenolic compounds in this species was confirmed, for the first time described in the literature. It was possible to identify eight phenolic compounds in the samples, being five phenolic acids (caffeic, ellagic, ferulic, *p*-coumaric and protocatechuic), two flavonoids (chrysin and rutin) and one a phenolic aldehyde (vanillin). The proposed and validated chromatographic method was linear, with good precision and accuracy and presented low detection and quantification limits, confirming its sensitivity and applicability. The absence of toxicity observed in the hemolytic activity assays suggests the performance of additional studies to determine the therapeutic properties of this species.

Therefore, from the observation of the botanical and morphological aspects, from the chemical and biological prospection, it is possible to conclude that *Tradescantia zebrina*

is a species of interest, as a potential source of bioactive substances. Gaps are still present in this knowledge and will serve as a starting point for the continuity of these studies.

DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

The authors are grateful for State University of Bahia (UNEB), Coordination for the Improvement of Higher Education Personnel-Brazil (CAPES), Brazilian National Council for Scientific and Technological Development (CNPq) and Research Group: “Biopharmaceutics and Drugs”.

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HOW TO CITE THIS ARTICLE

V.C. Silva, B.E.A. Magalhães, T.B.S. Magalhães, E.T. Guimarães, A.S. Guedes, M.D. Mota, W.N.L. dos Santos, B.A.V. Cerqueira, A. de Freitas Santos Júnior, Determination of phenolic bioactive compounds and evaluation of the antioxidant and hemolytic activities in the methanolic extracts of *Tradescantia zebrina*, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1341-1361 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.101403>

Scientific research article / <http://dx.doi.org/10.15446/rcciquifa.v51n3.100452>

Evaluation of the antifungal activity and mode of action of citral against *Cladophialophora carrionii*.

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Received: January 17, 2022

Corrected: April 28, 2022

Accepted: May 4, 2022

SUMMARY

Introduction: *Cladophialophora carrionii* is one of the most frequent etiologic agents of human chromoblastomycosis, a chronic cutaneous disease. Such fungal infections are difficult to treat and underlines the need for new antifungal agents. Citral is a monoterpene with known pharmacological properties, including antimicrobial activity. **Aims:** To investigate the antifungal activity of citral against strains of *C. carrionii*. **Methodology:** The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) were determined by broth micro-dilution techniques. Citral was tested to evaluate its effects on *C. carrionii* mycelia growth and germination of fungal conidia. Next, it was investigated the possible citral action on cell walls (sorbitol assay) and cell membranes (ergosterol binding assay). **Results:** The MIC₅₀ and MFC₅₀ of citral were respectively 128 µg/mL and 512 µg/mL. The study shows that citral displayed *in vitro* antifungal potential against strains of *C. carrionii*, where it was capable of inhibiting both its mycelial growth and germination of conidia for *this fungus*, whilst affecting the structure of fungal

cell membranes. Citral's mechanism of action involves binding to ergosterol. Further study is needed to completely describe its effects before clinical use as a therapeutic antifungal agent.

Keywords: Antifungal, Chromoblastomycosis, Citral, *Cladophialophora carrionii*, Monoterpene.

RESUMEN

Evaluación de la actividad antifúngica y forma de acción de citral frente a *Cladophialophora carrionii*.

Introducción: *Cladophialophora carrionii* es uno de los agentes etiológicos más frecuentes de la cromoblastomycosis humana, una enfermedad cutánea crónica. Tales infecciones fúngicas son difíciles de tratar y subrayan la necesidad de nuevos agentes antifúngicos. Citral es un monoterpeneo con propiedades farmacológicas conocidas, incluida la actividad antimicrobiana. **Objetivo:** investigar la actividad antifúngica de citral contra cepas de *C. carrionii*. **Metodología:** la concentración inhibitoria mínima (CIM) y la concentración fungicida mínima (CFM) se determinaron mediante técnicas de microdilución de caldo. Citral se probó para evaluar sus efectos sobre *C. carrionii* crecimiento de micelios y germinación de conidios fúngicos. A continuación, se investigó la posible acción citral sobre las paredes celulares (ensayo de sorbitol) y las membranas celulares (ensayo de unión al ergosterol). **Resultados:** La CIM₅₀ y CFM₅₀ de citral fueron respectivamente 128 µg/mL y 512 µg/mL. El estudio muestra que citral muestra potencial antifúngico *in vitro* contra cepas de *C. carrionii*, donde fue capaz de inhibir tanto su crecimiento micelial como la germinación de conidios para este hongo, a la vez que afectaba a la estructura de las membranas celulares fúngicas. El mecanismo de acción de Citral implica la unión al ergosterol. Se necesitan estudios adicionales para describir completamente sus efectos antes del uso clínico como agente antifúngico terapéutico.

Palabras clave: Antifúngico, chromoblastomycosis, citral, *Cladophialophora carrionii*, monoterpeneo.

RESUMO

Avaliação da atividade antifúngica e modo de ação do citral contra *Cladophialophora carrionii*.

Introdução: *Cladophialophora carrionii* é um dos agentes etiológicos mais frequentes da cromoblastomíase humana, uma doença cutânea crônica. Tais infecções fúngicas são difíceis de tratar e sublinham a necessidade de novos agentes antifúngicos. Citral é um monoterpreno com propriedades farmacológicas conhecidas, incluindo atividade antimicrobiana. **Objetivos:** investigar a atividade antifúngica do citral contra cepas de *C. carrionii*. **Metodologia:** a concentração inibitória mínima (CIM) e a concentração fungicida mínima (CFM) foram determinadas por técnicas de microdiluição. Citral foi testado para avaliar seus efeitos em crescimento micelar e germinação conidial de *C. carrionii*. Em seguida, foi investigada a possível ação do citral na parede celular (ensaio de sorbitol) e membrana celular (ensaio de ligação ergosterol) fúngicas. **Resultados:** a CIM₅₀ e CFM₅₀ do citral foram respectivamente 128 µg/mL e 512 µg/mL. O estudo mostra que o citral exibiu potencial antifúngico *in vitro* contra *C. carrionii*, onde era capaz de inibir tanto seu crescimento micelar quanto a germinação conidial, ao mesmo tempo em que afetava a estrutura da membrana celular fúngica. O mecanismo de ação do citral envolve a ligação ao ergosterol. Mais estudos são necessários para descrever completamente seus efeitos antes do uso clínico como um agente antifúngico terapêutico.

Palavras-chave: Antifúngico, cromoblastomicose, citral, *Cladophialophora carrionii*, monoterpreno.

INTRODUCTION

Cladophialophora carrionii are saprobic dematiaceous fungi, associated with opportunistic human and animal infections. This fungus is one of the relatively most common causative agents of chromoblastomycosis, a chronic, progressive, polymorphic implantation mycosis of skin and subcutaneous tissue, causing hyperproliferation leading to verrucous nodular clinical features, and is histologically characterized by muriform cells. Infection occurs after the etiologic agent gains entrance through a traumatic lesion [1].

The disease is seen worldwide, but most reports are from tropical and subtropical areas, with greater prevalences in Africa and Latin America [2]. The lesions associated with the disease are polymorphic or hyperkeratotic and in therapy must be differentiated

from those associated with other infectious or autoimmune disorders [3]. This infection is very difficult to treat. Various therapies are commonly applied, such as amphotericin, which binds to the fungal ergosterol and creates pores in the fungal membrane leading to the leakage of intracellular contents, and voriconazole an ergosterol biosynthesis inhibitor. However, there is no standard treatment [1].

The increased incidence of these fungal infections, especially dangerous hospital-acquired infections and infections in immunocompromised patients, has underlined the need for new antifungal treatments. Since there are limited antifungals options and some strain are able to exhibit antifungal resistance, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants [4].

Plants and their derivatives are known to be important in pharmacological research; this is due to their great potential as a source for a variety of biologically active components used in drug development. Among these products are the terpenes, a class of phytoconstituents formed by the 5-carbon alkene isoprene (C_5H_8) [5].

Citral (3,7-dimethyl-2,6-octadienal) is a natural mixture of two geometric isomers: geranial (*trans*-citral) and neral (*cis*-citral), which are acyclic α,β -unsaturated monoterpene aldehydes that occur naturally in many essential citrus fruit oils and in other herbs or spices [6]. Citral has become a flavoring substance of great importance and a heavily used raw material for the pharmaceutical, food, perfume, and cosmetics industries [7].

Various biological activities such as anti-inflammatory, anti-tumor, antiprotozoal and spasmolytic have been reported for citral [8-10]. Besides its low toxicity ($LD_{50} > 1000 \text{ mg/kg}$), antimicrobial activity was exhibited as demonstrated through *Trichophyton mentagrophytes* hyphae growth inhibition, where it was observed that the cell membrane and organelles were irreversibly damaged by citral, as well as a potent *in vitro* activity against *Candida* spp. [11-13].

Given the above, the aim of this study was to determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of citral, and to also investigate its mechanism of action against *C. carrionii* regarding its mycelial growth, conidial germination, cell wall formation, and interactions involving ergosterol.

MATERIALS AND METHODS

Micro-organisms

The strain *C. carrionii* URM-2871 used in the antifungal assay was obtained from the fungal collection of the Mycology Department (URM), Biological Sciences Center, Federal University of Pernambuco, Brazil and randomly selected *C. carrionii* strains (LM-0212 and CQ-02) were obtained from the Micro-organisms Collection of the Mycology Laboratory, at the Department of Pharmaceutical Sciences, Health Sciences Center, Federal University of Paraíba, Brazil. The samples were maintained on Sabouraud dextrose agar (SDA) (DIFCO®) under refrigeration (4 °C).

Stock inoculations (suspensions) of *C. carrionii* were prepared from 7-14 days old SDA (Difco Lab., USA); the cultures were grown at room temperature (28 °C to 30 °C). Fungal colonies were covered with 5 mL of sterile saline solution (0.9%), and then, gently agitated with vortexes. Next, the fungal elements in saline solution were transferred to sterile tubes. The inoculum was standardized according to the 0.5 McFarland scale (10^6 CFU/mL). The final concentration confirmation was done by counting the micro-organisms in a Neubauer chamber [14-16].

Chemicals

The product tested was the monoterpene Citral, obtained from (Sigma Aldrich, Brazil) as well as Amphotericin B and Voriconazole. The monoterpene was dissolved in 2% tween 80 and DMSO (dimethylsulfoxide). The antifungal standards were dissolved in DMSO, and sterile distilled water to obtain solutions of 2048 µg/mL for each substance. The concentration of DMSO did not exceed 0.5% in the assays.

Culture media

The culture media employed to test the biological activity of the products were SDA, purchased from Difco Laboratories (Detroit, MI, USA), and RPMI-1640-L-glutamine (without sodium bicarbonate) (Sigma-Aldrich, Sao Paulo, SP, Brazil). Both were prepared and used according to the manufacturers' instructions.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Broth microdilution assays were used to determine the MICs of monoterpene citral, amphotericin B and voriconazole against *C. carrionii* (URM-2871, LM-0212 and CQ-02). RPMI-1640 was added to all the wells of 96-well plates. Two-fold serial dilutions of the agents were prepared to obtain concentrations varying between 4 µg/mL

and 1024 µg/mL. Finally, 10 µL aliquots of the inoculum suspension were added to the wells, and the plates were incubated at 28 °C for 5 days. Negative controls (without drugs) were used to confirm conidia viability and sensitivity controls (for DMSO and Tween 80) were also included in the studies. At 5 days, there were visual observations of fungal growth. The MIC was defined as the lowest concentration capable of visually inhibiting fungal growth by 99.9%. The results were expressed as the arithmetic mean of the three experiments [15, 16].

The MFC was determined by microdilution method to verify if the inhibition was reversible or permanent [17]. Aliquots of 20 µL (from the wells without microbial growth detected in the MIC procedure) were transferred to 96-well plates previously prepared with 100 µL of RPMI-1640. The plates were aseptically sealed followed by mixing on a plate shaker (300 rpm) for 30 seconds, incubated at 28 °C and read at 5 days of incubation. Tests were performed in duplicate and the geometric mean values were calculated. MFC was defined as the lowest citral concentration in which no visible growth occurred when subcultured on the 96-well plates containing broth without antifungal products.

After determination of the MIC and MFC, one strain (*C. carrionii* URM 2871) was selected to continue the citral antifungal activity study.

Effects on mycelia growth

Analyses of the interferences of citral, voriconazole and amphotericin on *C. carrionii* URM-2871 mycelia growth were determined using poisoned substrate technique (dilution in solid medium) by daily measuring of radial mycelial growth on SDA by adding products in an amount adjusted to provide final concentrations similar to the MIC, MIC × 2, and MIC × 4 previously found in order to assess if its effect occurs in a dose-dependent manner. For this, 2 mm plugs taken from a 10-day-old mold culture cultivated on SDA slants at 28 °C were placed at the center of the sterile SDA Petri dishes containing the test drugs. At different intervals (0, 2, 4, 6, 8, 10, 12, and 14 days) of incubation at 28 °C, the mold's radial mycelial growth was measured (mm) with calipers. The controls in this assay revealed the mold's radial growth on SDA without adding drugs. Each assay was performed twice and the results were expressed as the average of the two repetitions [18, 19].

Conidial germination assay

Citral, voriconazole and amphotericin B were tested to evaluate the effects on the germination of *C. carrionii* URM-2871 fungal conidia. Flasks containing MIC, MIC × 2 and MIC × 4 of citral, voriconazole, amphotericin and a control with distilled water

were used. In sterile test tubes, 500 μL of RPMI-1640 plus citral were evenly mixed with 500 μL of fungal conidia suspension and immediately incubated at 28 °C. Samples of the mixture were taken after 48h of incubation for analysis. The whole experiment was performed in duplicate, where the number of conidia was determined in a Neubauer chamber, and the spore germination inhibition percentage at each time point was calculated by comparing the results obtained in the test experiments with the results of the control experiment. The analysis was conducted under an optical microscope (Zeiss Primo Star) [20].

Sorbitol assay effects

The assay was performed using medium with and without sorbitol (control), to evaluate possible mechanisms involved in the antifungal activity of the test product on the *C. carrionii* URM-2871 cell wall at the starting concentration of 1024 $\mu\text{g/mL}$. The sorbitol was added to the culture medium in a final concentration of 0,8M. The assay was performed by microdilution method in 96-well plates in a “U” (Alamar, Diadema, SP, Brazil) [15, 16]. The plates were sealed aseptically, incubated at 28 °C and readings were taken at 5 days. Based on the ability of sorbitol to act as an osmotic protective agent of the fungal cell wall, the higher MIC values observed in the medium with sorbitol added (as compared to the standard medium) suggests that the cell wall is a possible target for the product tested [12, 21, 22]. The assay was performed in duplicate and expressed as the geometric mean of the results.

Ergosterol binding assay: MIC value determination in the presence of ergosterol.

To assess if the product binds to fungal membrane sterols, an experiment was performed according to the method described by Escalante *et al.* (2008) [23], with some modifications. Ergosterol was prepared as described by Leite *et al.* (2014) [12]. The MIC of citral, against *C. carrionii* URM 2871 was determined by using broth microdilution techniques at the starting concentration of 1024 $\mu\text{g/mL}$ [15, 16], in the presence and absence of exogenous ergosterol added to the assay medium, in different lines on the same microplate. Briefly, a solution of citral was two-fold diluted with RPMI-1640 (100 μL) containing ergosterol added at a concentration of 400 $\mu\text{g/mL}$. 10 μL of the yeast suspension (0.5 McFarland) was added to each well. The same procedure was realized for amphotericin B, whose interaction with membrane ergosterol is already known, serving as a control drug. The plates were sealed and incubated at 28 °C. The plates were read after 5 days of incubation and the MIC was determined as the lowest concentration of test agent inhibiting visible growth. The assay was carried out in duplicate and the geometric mean of the values was calculated. The binding assay reflected the ability of the compound to bind with ergosterol.

Statistical analysis

The results are expressed as mean $\pm$ S.E. Differences between the means were statistically compared using the Student's t-test. The values were considered significant with $p < 0.05$.

RESULTS AND DISCUSSION

The results for citral's antifungal activity against *C. carrionii* were determined using the MIC and MFC in broth microdilutions. The MIC of citral varied between 128 and 256 $\mu\text{g/mL}$. The MIC₅₀ (minimum fungicidal concentration for 50% of strains tested, calculated via arithmetic mean), was 128 $\mu\text{g/mL}$. Amphotericin B and voriconazole retained a lesser MIC₅₀ than the phytoconstituent at 16 $\mu\text{g/mL}$ MIC (Table 1).

Table 1. MIC and MFC of citral, amphotericin B and voriconazole against *C. carrionii*.

Micro-organisms	Citral ($\mu\text{g/mL}$)		AMB ($\mu\text{g/mL}$)		VCZ ($\mu\text{g/mL}$)		Control strains*
	MIC	MFC	MIC	MFC	MIC	MFC	
<i>C. carrionii</i> URM-2871	128	512	16	64	16	16	+
<i>C. carrionii</i> LM-0212	256	1024	>1024	ND	>1024	ND	+
<i>C. carrionii</i> CQ-02	128	256	16	64	8	16	+

Legend: AMB, Amphotericin B; VCZ, Voriconazole; MIC, Minimum Inhibitory Concentration; MFC, Minimum Fungicidal Concentration; ND, Not determined; *, Micro-organism growth in RPMI-1640, DMSO (5%), and Tween 80 (2%), without antifungal agents or monoterpenes.

The MFC of citral varied between 256 and 1024 $\mu\text{g/mL}$. The MFC₅₀ (minimum fungicidal concentration for 50% of strains tested) was 512 $\mu\text{g/mL}$. The MFC₅₀ for amphotericin B and voriconazole were 64 $\mu\text{g/mL}$ and 16 $\mu\text{g/mL}$, respectively (Table 1).

The results for the control (Tween 80 and DMSO) showed no fungal growth inhibition; fungal growth in the medium without drug added was detected (viability control). In accordance with the results above, the *C. carrionii* URM-2871 strain was selected for further testing.

In previous studies, it was observed that *Melissa officinalis* essential oil (at 256 µg/mL) has antifungal activity, inhibiting the mycelial growth and conidial germination of *C. carrionii* [24]. *M. officinalis* essential oil is characterized by monoterpene compounds and citral is the principal component present [25].

The antimicrobial activity of citral has been confirmed. More recently, studies have shown that citral exhibits antimicrobial effect against *C. sakazakii* strains, with MICs ranging from 0.27 to 0.54 mg/mL [26]. Khan and Ahmad (2013) [27] showed that citral has effective antifungal activity against azole-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum*. In recent studies, citral showed *in vitro* antifungal potential against strains of *C. albicans* [12] and *C. tropicalis* [13].

In the present study, citral showed activity against *C. carrionii* URM-2871. The product is therefore considered actively antifungal in accordance with the parameters defined by Sartoratto *et al.* (2004) [28]: strong activity (MIC<0.50 mg/mL), moderate activity (0.6<MIC<1.50 mg/mL) and weak activity above 1.50 mg/mL.

This study also verified citral's action against *C. carrionii* mycelial growth and spore germination. The effect of differing concentrations of the test drug (MIC, MIC × 2 and MIC × 4) on mycelia growth was determined by measuring radial mycelial growth and the results are shown in Figure 1. Concerning *C. carrionii*, it can be seen that citral at its MIC concentration (128 µg/mL) was not capable of inhibiting mycelial growth, but at MIC × 2 (256 µg/mL) and MIC × 4 (512 µg/mL) concentrations, normal mycelia growth was inhibited when compared to the control (mycelia diameter being 99.9%). The control strains showed a constant rate of mycelial growth over the time evaluated, indicating good antifungal effect for citral (Fig.1A).

The voriconazole test against *C. carrionii* URM-2871 showed significant inhibition of mycelial growth at all concentrations tested (Fig. 1B). With amphotericin B, inhibition of mycelial growth was only observed at a higher concentration, MIC × 4 (64 µg/mL) (Fig. 1C).

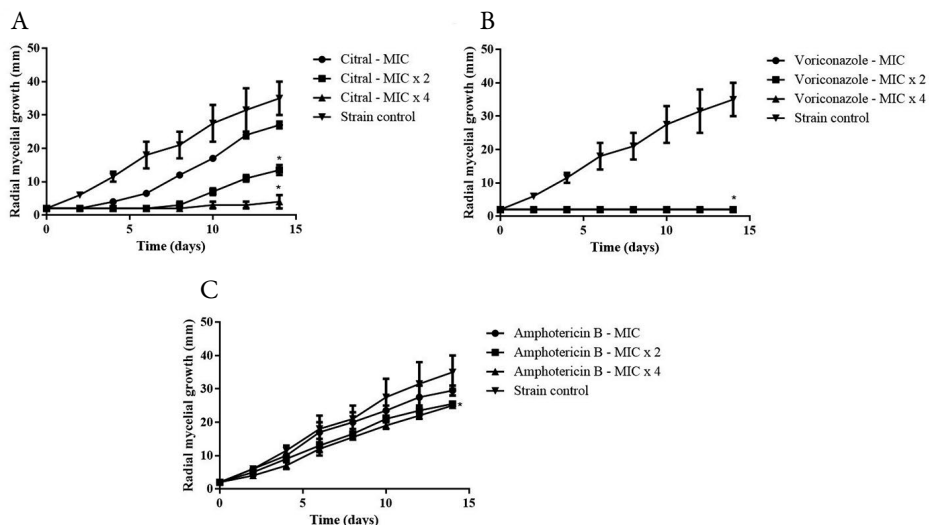


Figure 1. Radial mycelial growth produced by *C. carrionii* URM-2871 in the absence (control), and presence of citral (A), voriconazole (B) and amphotericin B (C). * $p < 0.05$ compared to control.

The results show that citral at its MIC $\times$ 2 and MIC $\times$ 4 concentrations, was more potent than amphotericin B at its respective MIC $\times$ 2 and MIC $\times$ 4 concentrations ($p < 0.05$). However, the action was shown to be less potent ($p < 0.05$) when compared with respective MIC and MIC $\times$ 2 concentrations of voriconazole.

The production of hyphae and consequent mycelium formation are important virulence factors for fungal filaments as well as serving as relevant protective factors which demands higher doses of antifungal agents to exert inhibitory effect. In an infection, longitudinal growth of the hyphae facilitates penetration into the inner layers of the skin, while lateral growth exacerbates the damage [29]. Hyphae are more difficult to phagocytize and can induce apoptosis in macrophages, since they often form inside the macrophage after phagocytosis [30].

These results corroborate with the data obtained in previous studies of investigation of the antifungal potential of citral in inhibiting the mycelial growth of pathogenic and non-pathogenic fungi [31, 32]. A previous study reported that citral at concentrations of 50 and 100 $\mu\text{g/mL}$ can inhibit *Trichophyton mentagrophytes* growth in PDA by 8.5% and 99.9%, respectively [11].

More recently, OuYang *et al.* (2016) [33] observed that citral inhibited the mycelial growth of *Penicillium digitatum*, with the MIC of 1.78 mg/mL in a dose-dependent

manner. Given the importance of mycelial growth to the development of mycoses, the inhibition of *C. carrionii* mycelial growth caused by citral (as observed in the present study), proving superior to amphotericin B in its respective concentrations, is a significant contribution to the search for new natural products with antifungal activity.

The study of the conidia germination has great implications for clinical practice, because it is possible to develop new therapeutic approaches that block the infection at its onset [34]. From this perspective, the effect of citral on the germination of *C. carrionii* URM-2871 conidia was investigated. The effects of different concentrations (MIC, MIC $\times$ 2 and MIC $\times$ 4) of citral, voriconazole and amphotericin B on the germination of conidia are shown in Fig. 2.

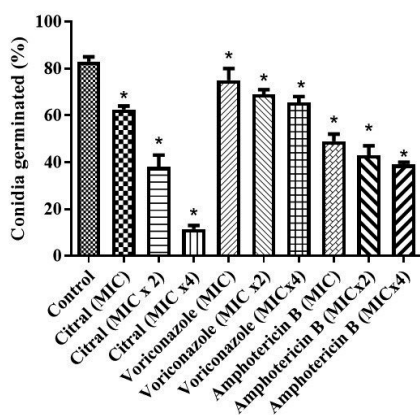


Figure 2. *C. carrionii* URM-2871 conidial germination percentage in the absence (control) and presence of citral, voriconazole and amphotericin B. * $p < 0.05$ compared to control.

In the three test concentrations (MIC, MIC $\times$ 2 and MIC $\times$ 4), citral displayed significant inhibitory action against *C. carrionii* as compared to the control. The tested antifungals standards (voriconazole and amphotericin B), also showed significant inhibition on conidia germination. However, the results shows that citral at its MIC $\times$ 4 concentration was more potent than amphotericin B and voriconazole at their respective MIC $\times$ 4 concentrations ($p < 0.05$).

These results are in accordance with previously published work, as the one conducted by Li *et al.* (2014) [35], who described the effect of citral on *Magnaporthe grisea*. In this study, it was found that germination in a concentration-dependent manner was significantly inhibited by citral and a similar trend was observed for mycelial growth, demonstrating the efficacy of citral for inhibition of pathogenic fungal growth.

Conidia represent the most common mode of asexual reproduction; they play an important role in natural fungal propagation and are structurally resistant. It is important to note that the conidia are not merely quiescent cells; a basal level of RNA and protein synthesis is required for survival of the spores [21]. Conidia are distributed in large quantities in the atmosphere and some have the ability to cause disease in humans, animals and plants [36]. Thus, it is important to quantitatively evaluate the power of a product to interfere with fungal spore germination [30].

The great challenge when developing new antifungal drugs is in the similarity between fungal cells and human cells. Thus, the targets for a new antifungal's action must be unique or at least sufficiently different from the host [37]. Based on this, two important fungal structures become targets for detecting antifungal agents; the fungal cell wall and the ergosterol present in the plasma membrane.

Ergosterol is one of the principal sterol components in the fungal membrane and plays the same role in fungal membranes that cholesterol plays in the mammalian cell. Generally, a decrease in ergosterol content results in osmotic disturbances with disruption of cell growth and proliferation [32, 38]. Many drugs available for clinical use interact directly with ergosterol, causing damage to the fungal cell membrane.

Considering possible fungal cell membrane interference of citral, the compound was tested to investigate its ability to form complexes with ergosterol (Table 2). Whether the effects of citral on the fungal cell are due to ergosterol binding in the membrane can be verified if they interact directly. In the presence of exogenous ergosterol in the culture medium, decreased binding of the product to the ergosterol of the membrane occurs. Thus, the product's MIC tends to increase in the presence of exogenous ergosterol, needing a much higher concentration to interact with ergosterol in the fungal membrane [12].

The MIC of citral against *C. carrionii* increased sixteen times in the presence of ergosterol at 400 µg/mL. Amphotericin B, the positive control that has a known interaction with ergosterol showed a 341-fold higher MIC in the presence of ergosterol. These results suggest that the mechanism of citral's antifungal action involves direct interaction with ergosterol, which leads to the disruption of the fungal membrane and loss of intracellular contents.

To investigate the action of the product on the fungal cell wall, was performed an assay with sorbitol (Table 2). Sorbitol is an osmotic protective used to stabilize fungi protoplasts. Specific fungal cell wall inhibitors share a distinctive characteristic where their antifungal effects are reversed in mediums containing sorbitol [22]. Cells protected with sorbitol can grow in the presence of fungal cell wall inhibitors, whereas growth

is inhibited in the absence of sorbitol. This effect is detected by increases in the MIC value as observed in medium with sorbitol as compared to the MIC value in medium without sorbitol (standard medium).

Table 2. MIC values ($\mu\text{g/mL}$) of drugs in the absence and presence of sorbitol (0.8 M) and ergosterol (400 $\mu\text{g/mL}$) against *C. carrionii* URM-2871.

Drugs	Sorbitol		Ergosterol	
	Absence	Presence	Absence	Presence
Citral	128	128	128	2048
Amphotericin B ^a	-	-	6	2048

Legend: ^a, Positive control; -, Not tested.

In this work, the MIC values of citral in both experiments (in mediums with and without sorbitol) were identical, suggesting that citral does not act by inhibiting fungal cell wall synthesis, but probably by affecting another target.

These results are in agreement with those reported by Miron *et al.* (2014) [39], who evaluated the antifungal activity of citral against seven opportunistic pathogenic yeasts and four dermatophyte species; no changes were observed in the MICs of this monoterpene in the sorbitol protection assay.

The lipophilic nature of terpenoids enables them to preferentially enter the lipid membrane, which results in an increased membrane fluidity and eventually to an increase in membrane permeability [38].

According to Harris (2002) [40], citral appears to act predominantly on the fungal cell membrane, affecting its structure, blocking its synthesis and causing cell death; inhibiting spore germination, proliferation and cellular respiration.

The action of citral on the cell membrane has been widely studied. In a recent study Tao *et al.* (2014) [32] showed that citral considerably impaired ergosterol biosynthesis in *Penicillium italicum* cells, significantly decreasing lipid levels; suggesting that the plasma membrane may well be an important citral antifungal target.

Zhou *et al.* (2014) [31] evaluated the antifungal activity of three volatile compounds: citral, octanal and α -terpineol against *Geotrichum citri-aurantii*. It was found in the study that citral was able to significantly inhibit mycelial growth. Antifungal activity was attributed to cell membrane disruption and to the consequent loss of cellular components.

Another study also showed that citral at a concentration of 200 µg/mL irreversibly damaged cell organelles and the cell membrane of *Trichophyton mentagrophytes* [11].

More recently OuYang *et al.* (2016) [33] suggested that citral might exhibit its anti-fungal activity against *P. digitatum* by down-regulation of ergosterol biosynthesis. The positive results of citral in the “Ergosterol Affinity Assay” and other reports on the subject strongly suggest that the mechanism of action of this monoterpene is related to ergosterol-binding and a subsequent destabilization of fungal cell membranes.

The test product presents as a relevant and promising antifungal agent which may be considered as an alternative prototype for production of a new and future antifungal drug, thus contributing to the existing arsenal of products which have proven antifungal activity against *C. carrionii*. Investigations of this nature are important since they provide clearer expectations for future pharmacological studies, with a view to a better understanding of citral's mode of action, its toxicity and possible therapeutic applications.

ACKNOWLEDGMENTS

The authors are grateful to the Federal University of Paraíba (UFPB) and to David Harding who edited the English version of the paper. This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Universidade Federal da Paraíba (UFPB).

CONFLICT OF INTEREST

All authors report that they do not have any conflicts of interest.

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HOW TO CITE THIS ARTICLE

C. Pinheiro de Menezes, A.L. Alves de Lima-Perez, J. Pereira de Sousa, K.R.d.L. Porto-Ramos, H.D. Neto, A. Alves de Oliveira-Filho, Edeltrudes de Oliveira Lima, Evaluation of the antifungal activity and mode of action of citral against *Cladophialophora carrionii*., *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1362-1379 (2022).
<http://dx.doi.org/10.15446/rcciquifa.v51n3.100452>

Scientific research article / <http://dx.doi.org/10.15446/rcciquifa.v51n3.100819>

Influence of germination on the bioactive compounds, antioxidant capacity and thermic characteristics of *Phaseolus vulgaris* L. and *Cajanus cajan* seeds

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Received: February 3, 2022

Corrected: June 14, 2022

Accepted: June 21, 2022

SUMMARY

Aim: To evaluate the effect of germination on total phenols, antioxidant capacity, trace elements, and thermal behavior of the seeds of Pigeon peas (*Cajanus cajan* L.) and Chacha and Recline varieties of the common bean (*Phaseolus vulgaris* L.). **Methodology:** The evaluation of the total phenols was done using Folin Ciocalteu's spectrophotometric method; the antioxidant capacity through the DPPH y ABTS^{•+} radicals; the macro and microelements using the spectrophotometric optical emission coupled inductively with plasma method ICP-OES; and the thermic behavior through differential scanning calorimetry (DSC). **Results:** The germination process influenced the total polyphenol content, antioxidant capacity, and macro and microelements in each specie that was studied. For example, for the Recline variety seedlings, the phenol content increased by 57.75% in comparison to the seed. The antioxidant capacity against DPPH and ABTS^{•+} was greater in the seedlings in the following order: Chaucha variety>Pigeon pea>Recline variety. For the seeds, the macro and micro-element content had the following order: K>Mg>Ca>Na y Fe>Zn>Cu>Cr; for the seedlings, the greatest decrease was in K,

Cr and the greatest increase was in Ca and Mn; the Na:K relationship was less than one. Finally, the thermic behavior, in reference to the gelatinization enthalpy (ΔH) was greater for the seed than the seedlings.

Keywords: Trace elements, *Cajanus cajan*, *Phaseolus vulgaris*, Differential scanning calorimetry, Sprout.

RESUMEN

Influencia de la germinación sobre los compuestos bioactivos, capacidad antioxidante y características térmicas de semillas de *Phaseolus vulgaris* L. y *Cajanus cajan*

Objetivo: evaluar el efecto de la germinación sobre los fenoles totales, capacidad antioxidante, oligoelementos y comportamiento térmico de las semillas de guandú (*Cajanus cajan* L.) y frijol común (*Phaseolus vulgaris* L.) de las variedades Chaucha y Recline. **Metodología:** la evaluación de los fenoles totales se realizó mediante el método espectrofotométrico de Folin Ciocalteu; la capacidad antioxidante a través de los radicales DPPH y ABTS^{o+}; los macro y microelementos utilizando la emisión óptica espectrofotométrica acoplada inductivamente con plasma método ICP-OES; y el comportamiento térmico mediante calorimetría diferencial de barrido (DSC). **Resultados:** el proceso de germinación influyó en el contenido de polifenoles totales, capacidad antioxidante y macro y microelementos en cada especie estudiada. Por ejemplo, para las plántulas de la variedad reclinada, el contenido de fenoles aumentó en un 57,75 % en comparación con la semilla. La capacidad antioxidante frente a DPPH y ABTS^{o+} fue mayor en las plántulas en el siguiente orden: variedad Chaucha > guandú > variedad Recline. Para las semillas, el contenido de macro y micro elementos tuvo el siguiente orden: K>Mg>Ca>Na y Fe>Zn>Cu>Cr; para las plántulas, la mayor disminución fue en K y Cr, y el mayor incremento fue en Ca y Mn; la relación Na:K fue menor que uno. Finalmente, el comportamiento térmico, en referencia a la entalpía de gelatinización (ΔH) fue mayor para la semilla que para las plántulas.

Palabras clave: Elementos traza, *Cajanus cajan*, *Phaseolus vulgaris*, calorimetría diferencial de barrido, brote.

RESUMO

Influência da germinação nos compostos bioativos, capacidade antioxidante e características térmicas de sementes de *Phaseolus vulgaris* L. e *Cajanus cajan*

Objetivo: avaliar o efeito da germinação nos fenóis totais, capacidade antioxidante, oligoelementos e comportamento térmico das sementes do feijão boer (*Cajanus cajan* L.) e das variedades Chacha e Recline do feijão comum (*Phaseolus vulgaris* L.). **Metodologia:** a avaliação dos fenóis totais foi feita pelo método espectrofotométrico de Folin Ciocalteu; a capacidade antioxidante através dos radicais DPPH y ABTS^{°+}; os macro e microelementos utilizando a emissão óptica espectrofotométrica acoplada indutivamente com o método de plasma ICP-OES; e o comportamento térmico por calorimetria exploratória diferencial (DSC). **Resultados:** o processo de germinação influenciou o teor de polifenóis totais, capacidade antioxidante, macro e microelementos em cada espécie estudada. Por exemplo, para as mudas da variedade reclinada, o teor de fenóis aumentou 57,75% em relação à semente. A capacidade antioxidante contra DPPH e ABTS^{°+} foi maior nas mudas na seguinte ordem: variedade Chaucha>feijão boer>variedade reclinada. Para as sementes, os teores de macro e microelementos tiveram a seguinte ordem: K>Mg>Ca>Na y Fe>Zn>Cu>Cr; para as mudas, a maior diminuição foi em K, Cr e o maior aumento foi em Ca e Mn; a relação Na:K foi menor que um. Por fim, o comportamento térmico, referente à entalpia de gelatinização (ΔH) foi maior para a semente do que para a plântula.

Palavras-chave: Oligoelementos, *Cajanus cajan*, *Phaseolus vulgaris*, calorimetria exploratória diferencial, rebentos.

INTRODUCTION

Legumes (Fabaceae/Leguminosae) are considered the second most important crop in human nutrition, after cereals (Gramineae). Leguminosae seeds constitute an essential part of the human diet, they are an excellent source of protein, bioactive compounds, minerals, and vitamins, in comparison to cereals which are more widely represented; this is the reason that it's known as "poor man's meat" [1]. The *Phaseolus* L. genre is made up of 76 species throughout the world, with those most widely represented being the: tepary bean (*P. acutifolius* A. Gary), runner bean (*P. coccineus* L.), lima bean (*P. lunatus* L.), year bean (*P. palyanthus* Greenman) and the common bean

(*P. vulgaris* L.) [2]. The last of which has a high nutritional and nutraceutical value; thus, it is considered to be a valuable nutrient source. Beans contain carbohydrates, vitamins, soluble fiber, minerals, phenolic compounds, phenolic acid, flavonoids and proanthocyanidins; all with antioxidant properties [3]. They also contain non-nutritional components and toxic compounds such as saponins, lecithin, condensed tannins, trypsin inhibitors and phytic acid, all of which interfere with the digestion of proteins and minerals [4]. At the same time, beans present phytochemical compounds, which provide additional health benefits, given that they possess preventative effects against cancer, due to having antimutagenic and antiproliferative properties [5, 6]. In addition, they are an important source of polyphenols, with effects that have been proven to protect the oxidation of low-density lipoproteins (LDL) [7]. The Pigeon pea (*Cajanus cajan*) is a species of high nutritional value and occupies a very important place in the diets of many countries around the world [8]. This Leguminosae is eaten as a vegetable (green unripe seeds) or as a legume (dry seeds) [9].

In the seedlings, the nutraceutical properties are improved, which modify the content of different metabolites; in particular the peptides and amino acids [8]. This process can cause changes in the phytochemical compounds and their functional properties due to cellular respiration and biochemical metabolism [7]. For example, the nutritional values of the seeds can be improved due to the degradation of the principal macronutrients such as carbohydrates, proteins, and organic acids; favoring the increase of simple sugars and amino acids. At the same time, the non-nutritional factors such as protease and lecithin are reduced [10]. The research that has been done up to now to determine the antioxidant potential and the polyphenol and mineral contents, specifically for the common bean and Pigeon pea, shows scarce information. Based on this framework, the objective that was proposed was to evaluate the total phenols, antioxidant capacity, macro and micro-elements, and the thermic behavior of the seeds and seedlings of Pigeon peas (*C. cajan*) and common beans (*P. vulgaris* L.) of the Chaucha and Recline varieties.

MATERIALS AND METHODS

Raw Material

Chaucha and Recline varieties of the common bean (*P. vulgaris* L.) and Pigeon peas (*C. cajan*) were acquired from the seeds laboratory of the Faculty of Agronomy at Universidad Nacional Agraria de la Selva (Peru).

Germination process

The methodology recommended by Pakaj *et al.* (2014) [11] and Cardador-Martinez *et al.* (2020) [12] was followed, where the seeds were disinfected by immersion in ethanol at 70% for one minute, then soaked in distilled water at a proportion of 1:10 (m/v) for twelve hours. The water they were soaked in was eliminated and the seeds were spread out on sterile trays and stored in the dark at room temperature for five days; to avoid microbial growth, they were washed with sterile distilled water every twenty-four hours. The sprouts were dried at 50°C until they reached the constant weight, then they were ground, placed into triple laminated bags, stored in the dark, and refrigerated until they were later analyzed.

Preparation of the extract

The method started with a mix of 1 g of the sample with methanol at 80%, at a total volume of 20 mL, with an agitation rate of 250 rpm for 24 hours at room temperature. The suspension was centrifuged at 10 000 rpm, 10 minutes at 4 °C (Hettich, Mikro R22, Germany) and then the supernatant was filtered using a PVDF 0.45 µm filter and refrigerated at 4 °C until its analysis [13].

Total Phenols

These were determined by using the Folin-Ciocalteu's spectrophotometer [14] with minimal modification; gallic acid was used as the standard ($10\text{--}100\text{ }\mu\text{g}\cdot\text{mL}^{-1}$). In brief, 100 µL of the extract was mixed with 500 µL of Folin-Ciocalteu reagent (0.2 N), in a 1.5 mL polystyrene tray and it was left to react in a room temperature environment for two minutes. A saturated sodium carbonate solution (400 µL; 7.5%) was added and left to rest for two hours at room temperature and the absorbency reading was done at 740 nm. The total phenols was expressed as milligrams of equivalent gallic acid per gram of dry sample ($\text{mg EAG}\cdot\text{g}^{-1}$).

Antioxidant Capacity

Capacity for capturing DPPH radicals

50 µL of the extract was mixed with 950 µL of the DPPH methanolic solution (100 µM) in a 1.5 mL polystyrene tray and they were incubated in the dark at room temperature for fifteen minutes. Its absorbance at 517 nm was measured using a UV-Vis spectrophotometer (Thermo Scientific, Genesys 10, U.S.A.). The activity for the elimination of DPPH radicals was expressed as Trolox millimole equivalents per gram of the dry sample ($\text{mmol ET}\cdot\text{g}^{-1}$) [15].

Capacity for capturing ABTS^{•+} radicals

The activity for the elimination of ABTS^{•+} radicals was measured according to the method described by Re *et al.* (1999) [16], with some modifications. ABTS was dissolved in water at a concentration of 7 mM, the radical was formed by making the ABTS solution react with potassium persulfate at 2.45 mM and the prepared mix was left to rest in the dark at room temperature for sixteen hours. The solution of ABTS^{•+} radicals was diluted with ethanol until an absorbency of 0.70 was reached at 734 nm (Thermo Scientific spectrophotometer, Genesys 10, U.S.A.). The mix was 10 μ L of extract with 990 μ L of ABTS^{•+} radical and the reaction time was a fifteen minutes period, where at the end an absorbency reading was done. The results were expressed in millimole trolox equivalents per gram of the dry sample (mmol ET·g⁻¹).

Analysis of the micro and macro elements

Preparation of the digestion

A sample of 0.5 g in powder was weighed in a flask and 10 mL of nitric acid solution (65%) with perchloric acid (98%) was added at a relationship of 4:1(v/v). The mix was led to digest through heating, starting at 50 °C and reaching 280 to 300 °C. The complete digestion was reached at 3 h when white-colored vapor appeared. The digested sample was cooled to room temperature, filtered using Whatman N° 20 paper, and flushed with distilled water in a 25 mL flask [17, 18].

Instruments

The digested samples were analyzed with an inductively coupled plasma optical emission spectrometry (ICP OES) (Horiba brand, Ultima Expert model). Argon gas 5.0 was used (99.99 % purity, Praxair, Peru). The operational conditions were: argon gas-plasm flow of 12 L/min, nebulizer flow velocity of 2 L/min, pump velocity of 30 rpm, stabilization time of 15 s, three trials for each measurement and observation of the radial plasma in view of the total plasma. The results were expressed in mg·100 g⁻¹ of the dry sample [19].

Differential scanning calorimetry (DSC) analysis

The thermic behavior of the flour was found using a calorimeter (SETARAM, Labsys Evo Robot, France). The method recommended by Alzate *et al.* (2013) [20], was followed, with slight modifications. The sample was sifted with a 60 mesh (250 μ m/ 0.250 mm) and was hydrated with distilled water until a suspension of 80% humidity was reached. Forty milligrams of the flour-water suspension were weighed and loaded into an aluminum crucible (75 μ L); the crucibles were hermetically sealed and left to rest for one hour at room temperature. For the differential scanning calorimetry (DSC)

analysis, the equipment was calibrated with indium and an empty aluminum crucible was considered to be the reference. The velocity for heating the samples was 2 °C/min at an interval of 25-120 °C, with a nitrogen atmosphere. The onset temperature (T_o), the peak temperature (T_p), the completion temperature (T_c) and the gelatinization enthalpy (ΔH_{gel}) were automatically calculated with CALISTO software.

RESULTS AND DISCUSSION

Total phenol content in the seeds and sprouts

According to the scientific evidence, the phenolic compounds have beneficial effects at a physiological, antioxidant and therapeutic level; the total phenol content of the seeds and sprouts (Table 1) shows a significant statistical difference ($p \leq 0.05$) between the samples. For the seeds, the phenol content varied between 0.466 (Chaucha variety) and 0.136 mg EAG·g⁻¹ (Recline variety); the results were inferior to those reported by James *et al.* (2020) [9] in red beans, 221.05 mg EAG·100 g⁻¹ and *Cajanus cajan*, 196.33 mg EAG·100 g⁻¹. However, they were similar to those reported by Lee *et al.* (2018) [21] in 209 varieties of the common bean from Korea, where the average was 1.3 mg EAG·g⁻¹ (0.55 to 2.23 mg GAE·g⁻¹). However, Gan *et al.* (2017) [10] concluded that for common beans, the phenols can vary between not detectable (ND) and 4871 mg EAG·100g⁻¹, with the red bean having the greatest value. It is worth pointing out that the variation in the total phenol content depends on the species of the bean, extraction method [22], climatological conditions, crop management and storage conditions, which affect the nutritional quality of the seeds [4].

Concerning sprouts, the total phenol content increased for the Chaucha by 57.7% and the Pigeon pea by 39.4%, but for the Recline, it decreased by 13.3%. With respect to the black bean sprouts, the phenols increased by 99% and there was no difference in the drying method [12]. Meanwhile, Yang *et al.* (2018) [3] reported 4.00 mgEAG·g⁻¹ for the black bean seed and 6.74 mgEAG·g⁻¹ for the sprout, with an increase of 40.6%; and according to Torres *et al.* (2018) [8], for the Pigeon pea, the number of total polyphenols increased by 110%. This is related to the reactivation of the polyphenol oxidase during germination, which intervenes in the synthesis of the polyphenol compounds. According to James *et al.* (2020) [9], the increase in the total phenols could be a result of the solubility of the condensed tannins when the seeds are soaking and the migration of the phenol compounds to the external layer, as a result of the germination observed during the darkening of the germinated seeds.

Table 1. Results of the total phenols and antioxidant capacity in the seeds and sprouts.

	Sample	Total phenols (mg EAG·g ⁻¹)	DPPH (mmol ET·g ⁻¹)	ABTS ⁰⁺ (mmol ET·g ⁻¹)
Seed	Chaucha Variety	0.466±0.003 ^b	2.766±0.008 ^b	1.996±0.031 ^b
	Recline Variety	0.136±0.003 ^d	0.290±0.000 ^c	0.066±0.003 ^d
	Pigeon Pea	0.270±0.000 ^c	0.623±0.006 ^d	0.593±0.267 ^{cd}
Sprout	Chaucha Variety	1.103±0.008 ^a (+57.7)	3.993±0.014 ^a (+30.7)	3.766±0.081 ^a (+47.7)
	Recline Variety	0.120±0.000 ^d (-13.3)	0.263±0.008 ^c (-10.2)	0.090±0.005 ^d (+26.6)
	Pigeon Pea	0.446±0.003 ^b (+39.4)	1.450±0.040 ^c (+57.0)	1.086±0.031 ^c (+45.3)

Values represent: average ± SEM; repetitions: n=3; values from the same column with different superindices are significant ($p \leq 0.05$); (+/-) = % increase /decrease. (*) mgEAG·g⁻¹ of sample; (**) mmol ET·g⁻¹ of sample.

Antioxidant capacities of the seeds and sprouts

Inhibition capacity for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The antioxidant capacity of the legumes in study varied between 0.290 and 2.766 mmol ET·g⁻¹ sample of the Chaucha and Recline varieties, respectively. Regarding 209 common beans in Korea, an activity of trapping the DPPH IC₅₀ radical between 62.3 and 643.9 was reported, with an average of 105.0 [21] and a DPPH antioxidant activity of 11.6 to 19.2 µmol ETrolox·g⁻¹ of dry weight [4]. The reported variation can be justified by the fact that the phenol-compounds and the antioxidant activity of the seeds, which have different colors (red, black, brown, white beans, etc.) are influenced by the cultivators [6].

The results for the sprouts revealed a significant statistical difference. When comparing the seed and the sprout of the Chaucha variety, there was an increase of 30.7% and for the Pigeon pea, it was 57.0%. This behavior was also demonstrated by James *et al.* (2020) [9] evaluating the antioxidant capacity against the DPPH free radical for the red bean seed. It was 28.35% and by the third day of germination, 60.12%, with an increase of 52.84%, as well as, in the Pigeon pea seed which was 32.95% and germinated was 57.84%, with an increase of 43.03%. The DPPH of the mung bean seed was 0.11±0.00 mg Trolox·g⁻¹, whereas the germinated was 1.41±0.11 mg Trolox·g⁻¹, with an increase of 92.19% [11]. Germination, as a domestically developed process in many countries, improves the nutritional and functional deficiencies of the Leguminosae, increasing the antioxidant activity [22]. In the case of Pigeon pea, the activity of trap-

ping the radicals after germination increased by 64%, possibly due to the changes that occur in the distribution of the secondary metabolites; the mobilization of the reserve proteins which are stored in the protein bodies of the cotyledons [8]. For Pigeon pea sprouts, the antioxidant capacity for DPPH increased by 63.52% [23]. The germination process modifies the antioxidant activity, measured by the capacity for capturing the DPPH free radicals, provoking an increase [24].

Capacity for inhibiting the 2,2-azinobis (3-ethylbenzothiazoline-6- sulfonic - acid) (ABTS^{•+})

Concerning seeds, the antioxidant capacity for the ABTS^{•+} varied between 0.066 and 1.996 mmol ET·g⁻¹ for the Recline and Chaucha varieties, respectively. For the ABTS^{•+} radical, the degree of discoloration correlated to the antioxidant activity, which was revealed with the white beans and varied from 27 µmol to 43 µmol Trolox·g⁻¹; this content was very low in comparison to the red bean (0.19-0.40 mmol Trolox·g⁻¹) [6].

The effect of the germination, regarding the Chaucha variety seed, had an increase of 47.7%, the Recline variety had 26.6% and the Pigeon pea had 45.3%. Pankaj *et al.* (2014) [11] reported an increase of 92.40% for the mung bean seeds. Germination is a technique of natural bioprocessing which improves the bioactive components, the antioxidant activity and the functional properties of the food grains [24]. The antioxidant capacity of the common beans can be affected by the method of thermic processing, fermentation and germination [3]. During germination, the quantity of phenolic compounds in the Leguminosae increase due to the presence of more hydroxyl groups, which can act in reducing free radicals, which results in an improved antioxidant activity [24].

Macro and microelement content of seeds and sprouts

The content of macro-elements for the bean seeds (Table 2), the Ca was within the range of 69.95 to 121.36 mg·100 g⁻¹; sodium (Na), it was between 50.01 and 53.16 mg·100 g⁻¹; potassium (K), it was between 891.2 and 1175.4 mg·100 g⁻¹ and for magnesium (Mg), it was between 157.7 and 160.83 mg·100 g⁻¹. For the Chaucha and Recline varieties, the values that were found concur with those reported by Grela *et al.* (2017) [25] for common beans (cv. Mela) with a Ca of 0.98 g·kg⁻¹, K of 8.47 g·kg⁻¹ and Mg of 1.47 g·kg⁻¹. Morales-Morales *et al.* (2016) [26] reported a Ca of 1.2 and K of 19.7 g·kg⁻¹ for black-eyed peas; Espinoza-Garcia *et al.* (2016) [27] reported Ca values from 148.9 to 100.1, Na from 63.9 to 85.1, K from 846.1 to 946.6, and Mg 113.7 a 125.9 mg·100 g⁻¹, for sixty-seven native bean populations in Mexico. The greatest content of macroelements for the beans was potassium. This mineral is the principal cation in intracellular liquid and participates in the function of regulating the acid-base equilibrium, muscle contraction, correct cellular membrane function and the maintenance

of volume of fluids. For the Pigeon pea seeds, the greatest content of macro-elements was K at 1140.1 and the least was Na with 52.13 mg·100 g⁻¹. The results for Ca, K, Na and Mg were superior to those reported by Asouzu and Umerah (2020) [28], where the macro element values for the Pigeon pea seeds were Mg 68.24, K 2.10, Na 6.10 and Ca 7.28 mg·100 g⁻¹. The same authors indicated that Na and K are necessary for maintaining the osmotic equilibrium of body fluids, the pH of the body, regulating the irritability of muscles and nerves, controlling the absorption of glucose and improving the normal retention of proteins during growth.

For the Chaucha and Recline variety sprouts, it was shown that there was an increase in the Ca of 47.4% and 51.5%, for the Na it was 8.1% and 3.5%, but for Mg, the Chaucha variety registered an increase of 4.8%, while the Recline variety, a decrease of 0.08%. The K decreased for the Chaucha variety by 16.2% and the Recline variety by 9.8%; this behavior was cited by Devi *et al.* (2015) [29] for black-eyed pea sprouts where the Ca content increased from 5.9 % to 9.9 %, which can be attributed to the salts from Ca which are present in the water that is used during the germination. Santos *et al.* (2020) [30], in the germination of twelve varieties of lentils, obtained an increase of Zn, Ca and K; moreover, the germination of Leguminosae is associated with the reduction of phytates, and these are tied to minerals that form insoluble and unavailable compounds. For the Pigeon pea, the values that were found for Ca, Na, K and Mg were inferior to those reported by Torres *et al.* (2018) [8] for Pigeon pea sprouts, which had a Ca of 186.9±7.9 mg·100 g⁻¹, K of 956.6±26.4 mg·100 g⁻¹ and Mg of 137.9±5.6 mg·100 g⁻¹. On the other hand, it is worth clarifying that the germination also provoked a greater increase, with respect to the seeds of Ca at 31.1% and a decrease of K at 10.4%. The Na: K relationship for the seeds and sprouts varied between 0.04 and 0.33. The results found were the opposite of those reported by Audu and Aremu (2011) [31] in their work with pinto beans, where the Na: K relationship was found to be greater than one; this indicates that the consumption of the bean can generate arterial hypertension problems. At the same time, it is known that a diet low in sodium (Na) and high in potassium (K), is highly recommended as a strategy for lowering arterial pressure and reducing the risk of cardiovascular disease [32].

The micro-elements (Table 2), for the bean seeds, the results of the Zn and Fe contents were within the range, Mn was slightly superior and Cu slightly inferior to those reported by Morales-Morales *et al.* (2016) [26] for black-eyed peas which had a Zn of 83.2 mg·kg⁻¹ and a Fe of 36.3 mg·kg⁻¹. Moreover, Fe is an essential component of diverse heme enzymes, non-heme enzymes and transporters; Zn is an essential component of diverse dehydrogenase, protease and peptidase and, Fe is an important constituent of hemoglobin and participates in numerous biochemical roles within the body. Celmeli *et al.* (2018) [2] found that for common and modern varieties of beans the Zn ranged

from 17.81 to 37.90 mg·kg⁻¹ and the greatest Fe content was 133.64 mg·kg⁻¹, for local varieties. The microelements of bean sprouts, an increase of 30.2% occurred in Zn for the Chaucha variety. For the Fe and Mn, the Recline variety showed a decrease of 1.6% and 13.7%; the Cu increased for the Chaucha variety, as well as the Recline variety; and concerning the Cr, there was a decrease of 75% and 77.7%, respectively. According to Devi *et al.* (2015) [29], an increase from 0.4 to 1.4% was reported for the Fe, the value of which was lower when compared to this study. According to Santos *et al.* (2020) [30], in the germination of twelve varieties of lentils, a decrease in the Fe and Mn were found. Just as it is known that germination is an individual biological process for all seeds, it is multi-enzymatic with the preparation to bring about all of the metabolic activity that is required to generate a new plant. One of the most important enzymes in the process is phytase, which is activated during soaking and continues during the germination; it is the enzyme in charge of hydrolyzing the phytic acid, which keeps the nutrients (mineral ions, proteins, and carbohydrates) to one side for when the seed needs them [33]. For the Pigeon pea, the Fe, Mn and Cu contents reported were slightly superior to those cited by Torres *et al.* (2018) [8].

The Zn that is necessary for the correct growth and maintenance of the human body is found in various systems and biological reactions, and it is necessary for immunological, coagulation, and thyroid function, among others [28].

The Pb content in the seeds varied from 0.16 to 0.07 mg·100 g⁻¹, the Cd from 0.01 to 0.03 mg·100 g⁻¹, and the Pb and Cd decreased for the sprouts; only for Pigeon peas sprouts did it slightly increase. The Pb, it did not surpass the limits reported by Glavač *et al.* (2017) [34] in plants. The European Commission considers the limits of Pb and Cd in plants to be 1.0 mg·kg⁻¹ and 5.0 mg·kg⁻¹, respectively. The presence of Pb in food is a problem for health and food security, thus norms have been established with maximum allowable limits according to Casteblanco (2018) [35]. Cd is a trace metal with no essential biological functions and it is toxic to plants, animals, and humans at low concentrations [36].

Table 2. Macro and microelement contents in Leguminosae seeds and sprouts.

		Seeds				Sprouts		
		Chaucha variety	Recline variety	Pigeon pea	Chaucha variety	Recline variety	Pigeon pea	
Macro-elements (mg·100 g ⁻¹)	Ca	69.95±0.64 ^e	121.36±0.35 ^d	168.63±2.95 ^b	133.13±0.71 ^c (+47.4)	250.64±2.67 ^a (+51.5)	244.96±1.43 ^a (+31.1)	
	Na	53.16±0.41 ^b	50.01±0.64 ^c	52.13±0.17 ^{bc}	57.85±0.52 ^a (+8.1)	51.86±0.85 ^{bc} (+3.5)	55.95±0.53 ^a (+6.8)	
	K	1175.4±14 ^a	891.2±42 ^d	1140.1±1.2 ^{ab}	1010.3±24 ^c (-16.2)	811.3±27 ^d (-9.8)	1032.2±1.7 ^{bc} (-10.4)	
	Mg	157.70±0.63 ^{bc}	160.83±0.49 ^{ab}	155.00±1.60 ^c	165.76±1.81 ^a (+4.8)	160.7±1.23 ^{abc} (-0.08)	164.20±0.83 ^a (+5.6)	
	Na:K	0.04	0.05	0.33	0.05	0.06	0.05	
	Zn	2.03±0.01 ^c	2.71±0.03 ^c	2.23±0.01 ^d	2.91±0.01 ^{ab} (+30.2)	2.84±0.04 ^b (+4.5)	2.96±0.01 ^a (+24.6)	
Micro-elements (mg·100 g ⁻¹)	Fe	13.51±0.11 ^c	14.16±0.06 ^c	13.73±0.04 ^c	15.03±0.14 ^b (+10.1)	13.93±0.08 ^c (-1.6)	15.86±0.31 ^a (13.4)	
	Mn	2.35±0.04 ^d	2.65±0.03 ^c	2.56±0.09 ^{cd}	8.85±0.05 ^a (+73.4)	2.33±0.03 ^d (-13.7)	4.22±0.01 ^b (+39.3)	
	Cu	0.66±0.01 ^c	0.90±0.00 ^b	0.73±0.00 ^c	0.69±0.01 ^d (+4.3)	0.97±0.01 ^a (+7.2)	0.91±0.00 ^b (+19.7)	
Heavy metals (**)	Cr	0.21±0.01 ^a	0.16±0.00 ^b	0.11±0.00 ^{cd}	0.12±0.01 ^c (-75)	0.09±0.00 ^d (-77.7)	0.08±0.00 ^d (-37.5)	
	Pb	0.16±0.00 ^a	0.08±0.00 ^b	0.07±0.00 ^{bc}	0.14±0.01 ^a	0.06±0.00 ^c	0.09±0.00 ^b	
	Cd	0.03±0.00 ^a	0.01±0.00 ^c	0.02±0.00 ^b	0.01±0.0002 ^c	0.01±0.00 ^d	0.01±0.00 ^c	

*The values represent: average ± SEM; repetitions: (n=3); values within the same row with different superindices are significative (p ≤ 0.05); (+/-) = % increase /decrease. (**) (mg·100 g⁻¹).

Thermic behavior of the seeds and sprouts

The gelatinization of starch measured by differential scanning calorimetry (DSC) represents every one of the endothermic events, which take place during the process of the transition phase. These thermal properties of the flours in grains depend on the humidity and the endothermic transition of the thermogram DSC, which reflects the gelatinization of the flours when excess water is added. In the study, the gelatinization temperature and the enthalpic changes (ΔH) of the flour from the seeds as well as from the sprouts were examined (Table 3). For the flour from the seeds, the gelatinization from thermal transition starts with a slight endothermic peak above the baseline of the thermogram. The lowest process is initiated at 62.29 °C (Chaucha variety) and the highest at 77.48 °C (Pigeon pea), which is known as the onset temperature (T_0). The peak temperature (T_p) is the temperature where the highest values of heat absorption are recorded for the seeds, which was between 68.13 °C (recline variety) and 81.28 °C (Pigeon Pea). The completion temperature (T_c) for the gelatinization of the seeds was in the range of 72.38 °C (Recline variety) and 86.93 °C (Pigeon pea), and it represents the temperature at which the process is finalized. The behavior found was similar to that reported by Maninder *et al.* (2007) [37] between the flour from *P. sativum* (FPF), which was low (T_0 : 59.45 °C; T_p : 65.5 °C; T_c : 74.1 °C), and Pigeon pea (PPF) (T_0 : 75.6 °C; T_p : 82.0 °C; T_c : 87.2 °C). It indicates that the difference in the gelatinization temperatures can be attributed to the differences in size, shape and distribution of the starch granules in the flours, as well as the internal disposition of the fraction within the starch granule. According to Xu *et al.* (2019) [38], the temperatures of thermic transition to the flour, from grains, are influenced by the amylose and protein content, and the distribution of amylopectin ramified chains.

The energy necessary to complete the process that is known as gelatinization enthalpy, which is calculated as the area under the curve of the gelatinization peak (ΔH_{gel}), varied for the seeds: Chaucha variety 1.019, Recline variety 0.643 and Pigeon pea 0.714 J·g⁻¹. Moreover, it allows for the structural and chemical changes, which influence the quality of the products derived from this material to be analyzed. The results were superior to those reported by Sanchez-Arteaga *et al.* (2015) [39]; the ΔH in six defatted beans varied from 0.34 ± 0.03 (black-eyed) to 0.71 ± 0.06 J·g⁻¹ (pinto). The variation in the thermic profile of the bean also depends on the amylose content, the distribution of the amylopectin ramified chain, the complex lipid's amylose chain, and the proteins present in them [40].

Table 3. Temperature and Gelatinization Enthalpy of the Seeds and Sprouts.

	Sample	(To) °C	(Tp) °C	(Tc) °C	ΔHgel (J/g)
Seed	Chaucha variety	62.29±0.41 ^b	77.09±0.49 ^b	81.09±1.87 ^{ab}	1.019±0.06 ^a
	Recline variety	65.67±0.20 ^b	68.13±0.06 ^d	72.38±0.01 ^b	0.643±0.014 ^{bc}
	Pigeon pea	77.48±1.37 ^a	81.28±0.24 ^a	86.93±0.29 ^a	0.714±0.07 ^b
Sprout	Chaucha variety	63.05±0.40 ^b	74.20±0.17 ^c	80.22±3.47 ^{ab}	0.442±0.06 ^{bcd}
	Recline variety	62.48±0.09 ^b	68.37±0.20 ^d	74.46±0.05 ^b	0.308±0.00 ^d
	Pigeon pea	79.64±0.44 ^a	82.48±0.36 ^a	86.52±0.02 ^a	0.355±0.00 ^{cd}

Data represent (mean ± SEM). Different lower-case letters indicate statistically significant differences intraspecies (p < 0.05). T_o, T_p, T_c and ΔH is the onset temperature, peak temperature, completion temperature, and enthalpy, respectively.

All of the flour from the seeds and sprouts (Fig. 1) showed only one endothermic transition from 50 to 100 °C, which reflects the gelatinization. For the Chaucha variety sprouts, the T_o was 63.05 °C, the T_p was 74.20 °C and the T_c was 80.22 °C; for the Recline variety T_o was 62.48 °C, T_p was 68.37 °C and T_c was 74.46 °C; and for Pigeon pea T_o was 79.64 °C, T_p was 82.48 °C and T_c was 86.52 °C. As can be seen, the three samples had different values of T_p; and T_p is considered the temperature at which 50% of the molecules have suffered a thermic transition. The differences observed in the thermic profiles are possibly associated with the variation in the chemical composition, thermal properties, and quality of the protein fraction [39].

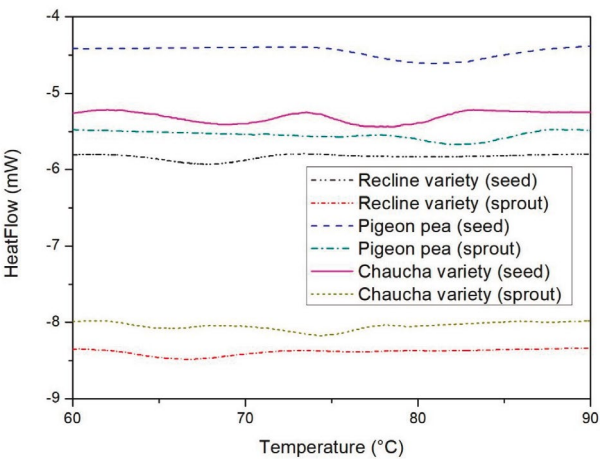


Figure 1. Thermal transitions of *C. cajan* and *P. vulgaris* L. seed and sprout through DSC.

For the sprouts, the ΔH diminished, in comparison to the seed. Jimenez *et al.* (2019) [41] reported similar effects in quinoa sprouts, probably due to the hydrolysis of the starch; with a greater amylose/amylopectin relationship, due to greater degradation of the amylopectin during germination. Xu *et al.* (2019) [38] indicated that the ΔH can be utilized to predict the energy necessary for the decomposition of the intermolecular hydrogen bond in the starch granules. In garbanzo beans and peas this diminishes by the third day of germination, which indicates that there is a reduction in necessary energy to convert the chemical composition of the flour from an orderly to a disorderly form. During germination, partial hydrolysis of the starch occurs due to the activation of enzymes that affect the hydrogen bonds of the starch, making the detachment easier when heated.

CONCLUSION

The sprouts are considered functional food due to the increase in the content of phenols, antioxidants, and minerals, thus, it is necessary to study the technological properties associated with functionality of these Legumes.

CONFLICT OF INTEREST

All authors report that they do not have any conflicts of interest.

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HOW TO CITE THIS ARTICLE

E.S. Ordoñez, D. Reátegui, T. Menacho-Mallqui, Influence of germination on the bio-active compounds, antioxidant capacity and thermic characteristics of *Phaseolus vulgaris* L. and *Cajanus Cajan* seeds, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1380-1398 (2022). <http://dx.doi.org/10.15446/rcciquifa.v51n3.100819>

Clinical research article / <http://dx.doi.org/10.15446/rcciquifa.v51n3.102279>

Pharmacotherapeutic follow-up of patients on warfarin in primary care: randomized clinical trial

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Received: May 4, 2022

Corrected: July 5, 2022

Accepted: July 11, 2022

SUMMARY

Aim: To evaluate the impact of pharmacotherapeutic follow-up on bleeding, time in therapeutic range (TTR), thrombotic events, general adverse events, hospitalizations, drug interactions and average number of medications used in patients taking warfarin in the Brazilian public healthcare system. **Methods:** A randomized clinical trial was conducted with individuals divided into two groups (intervention group [pharmacotherapeutic follow-up] and control group) who received at-home visits over an eight-month period. **Results:** 38 individuals (21 in the intervention group and 17 in the control group) concluded the study. Fewer number of cases of bleeding was found in the group that received pharmacotherapeutic follow-up, but no significant association was found between these variables. No significant association was found between pharmacotherapeutic follow-up and TTR. The intervention group had a greater frequency in the therapeutic range for capillary INR but not for laboratory INR. Reductions were found in the intervention group with regards to general adverse events, the use of medications and drug interactions, whereas no reduction was found in hospitalizations. **Conclusions:** Based on the findings of the present study, pharmacotherapeutic follow-up did not exert an influence on bleeding or

TTR. However, reductions were found in adverse events and drug interactions, which can contribute to the rational use of medicines and could result in lower care costs for patients requiring blood thinners.

Keywords: Warfarin, pharmaceutical care, primary health care.

RESUMO

Acompanhamento farmacoterapêutico de pacientes em uso de varfarina na atenção primária: ensaio clínico randomizado

Objetivo: Avaliar o impacto do acompanhamento farmacoterapêutico sobre sangramento, tempo de intervalo terapêutico (TTR), eventos trombóticos, eventos adversos gerais, internações, interações medicamentosas e número médio de medicamentos utilizados em pacientes em uso de varfarina no sistema público de saúde brasileiro. **Métodos:** Foi realizado um ensaio clínico randomizado com indivíduos divididos em dois grupos (grupo intervenção [acompanhamento farmacoterapêutico] e grupo controle) que receberam visitas domiciliares durante um período de oito meses. **Resultados:** Trinta e oito indivíduos (21 no grupo intervenção e 17 no grupo controle) concluíram o estudo. Um menor número de casos de sangramento foi encontrado no grupo que recebeu acompanhamento farmacoterapêutico, mas não foi encontrada associação significativa entre essas variáveis. Não foi encontrada associação significativa entre seguimento farmacoterapêutico e TTR. O grupo intervenção apresentou maior frequência na faixa terapêutica para INR capilar, mas não para INR laboratorial. Foram encontradas reduções no grupo intervenção em relação aos eventos adversos gerais, uso de medicamentos e interações medicamentosas, enquanto não houve redução nas internações. **Conclusões:** Com base nos achados do presente estudo, o acompanhamento farmacoterapêutico não exerceu influência sobre sangramento ou TTR. No entanto, foram encontradas reduções nos eventos adversos e interações medicamentosas, o que pode contribuir para o uso racional de medicamentos e pode resultar em menores custos assistenciais para pacientes que necessitam de anticoagulantes.

Palavras-chaves: Varfarina, atenção farmacêutica, atenção primária à saúde.

RESUMEN

Seguimiento farmacoterapéutico de pacientes en tratamiento con warfarina en atención primaria: ensayo clínico aleatorizado

Objetivo: Evaluar el impacto de la monitorización farmacoterapéutica sobre el sangrado, el tiempo de intervalo terapéutico (TTR), los eventos trombóticos, los eventos adversos generales, las hospitalizaciones, las interacciones medicamentosas y el número medio de medicamentos utilizados en pacientes que utilizan warfarina en el sistema de salud pública brasileño. **Métodos:** Se realizó un ensayo clínico aleatorizado con sujetos divididos en dos grupos (grupo de intervención [seguimiento farmacoterapéutico] y grupo control) que recibieron visitas domiciliarias durante un período de ocho meses. **Resultados:** Treinta y ocho sujetos (21 en el grupo de intervención y 17 en el grupo de control) completaron el estudio. Se encontró un menor número de casos de sangrado en el grupo que recibió seguimiento farmacoterapéutico, pero no se encontró asociación significativa entre estas variables. No se encontró asociación significativa entre seguimiento farmacoterapéutico y TTR. El grupo de intervención tuvo mayor frecuencia en rango terapéutico para INR capilar, pero no para INR de laboratorio. Se encontraron reducciones en el grupo de intervención con respecto a los eventos adversos generales, el uso de medicamentos y las interacciones medicamentosas, mientras que no hubo reducción en las hospitalizaciones. **Conclusiones:** Con base en los hallazgos del presente estudio, el seguimiento farmacoterapéutico no influyó en el sangrado ni en la RTT. Sin embargo, se encontraron reducciones en los eventos adversos y las interacciones medicamentosas, lo que puede contribuir al uso racional de los medicamentos y puede resultar en menores costos de atención para los pacientes que requieren anticoagulantes.

Palabras clave: Warfarina, atención farmacéutica, atención primaria de salud.

INTRODUCTION

Warfarin is the most widely prescribed oral anticoagulant in primary care due to its proven effectiveness and low cost. However, it is a potentially hazardous drug due to the narrow therapeutic window and variable response [1], which can lead to adverse reactions and preventable hospital admissions [2, 3].

A single-center cohort study involving patients on warfarin found a higher incidence of bleeding compared to other studies, which was associated with exposure to warfa-

rin-related drug interactions [4] demonstrating the need for interventions to minimize such adverse events and the promotion of the safer use of this anticoagulant.

Follow-up through pharmaceutical care could improve the quality of treatment with warfarin [5, 6] and could be an effective strategy for preventing, identifying and solving problems related to pharmacotherapeutic treatment [7]. Studies conducted in other countries have demonstrated the benefits of the engagement of the pharmacist in oral anticoagulation with warfarin [8, 9]. In Brazil, few studies have addressed the follow-up of patients on blood thinners [10], especially studies on at-home pharmacotherapeutic follow-up.

The aim of the present study was to evaluate the impact of pharmacotherapeutic follow-up on bleeding, time in the therapeutic range, thrombotic events, general adverse events, hospitalizations, drug interactions and average number of medications used in patients taking warfarin in the Brazilian public healthcare system.

MATERIAL AND METHODS

Study design

A randomized, parallel, controlled, clinical trial was conducted comparing the results of pharmacotherapeutic follow-up in patients on warfarin in the public healthcare system to a control group without pharmacotherapeutic follow-up. The flowchart of the randomization procedure in accordance with the *Consolidated Standards of Reporting Trials* (CONSORT) is shown in Figure 1.

This investigation is linked to a study by the *Universidade Regional do Noroeste do Estado do Rio Grande do Sul* entitled “Evaluation of the effectiveness of a protocol for patients on anticoagulants in the public healthcare system of the municipality of Ijuí/RS” (approved through process number: 1.850.054/2016 and PPSUS/FAPERGS 002/2017). Data were collected through monthly visits over an eight-month period between September 2018 and April 2019.

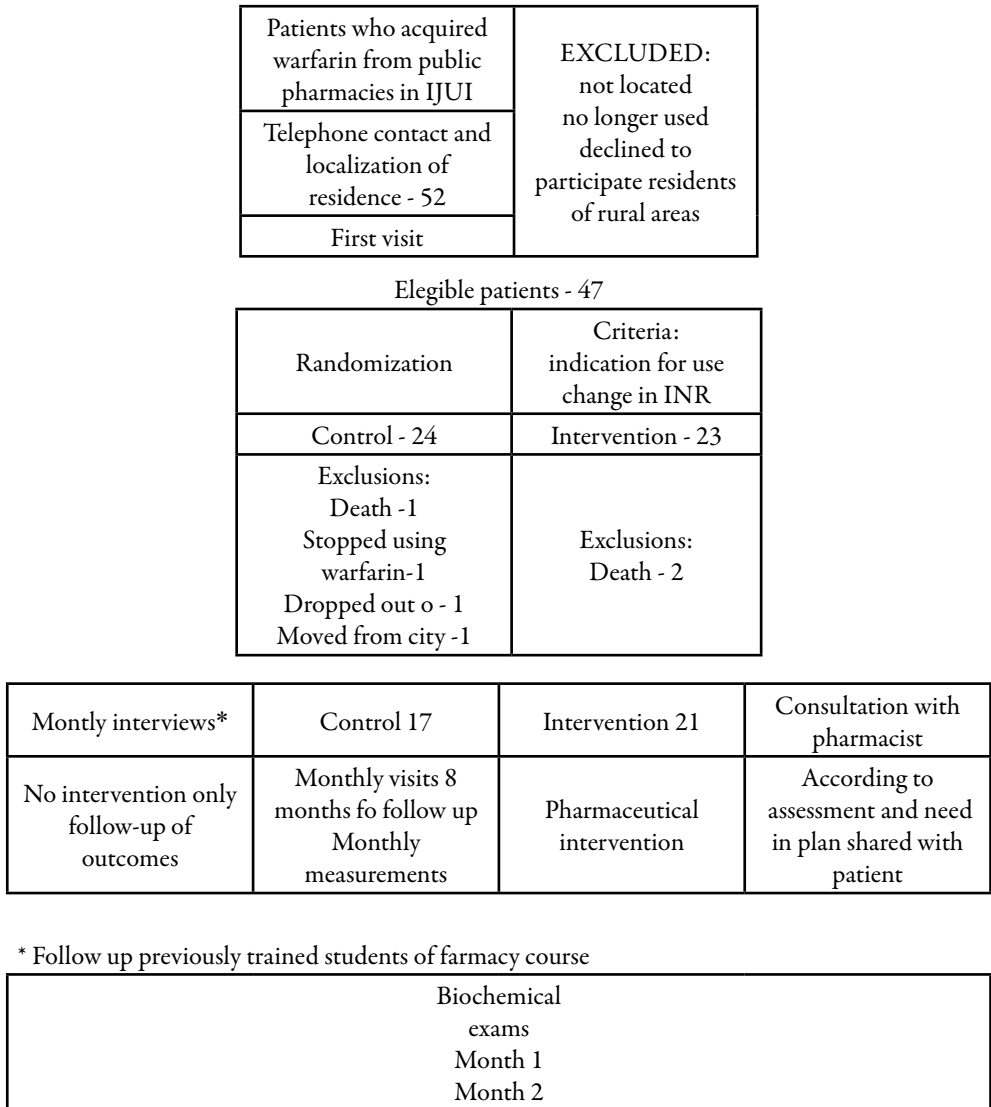


Figure 1. Flowchart of randomization procedure

Inclusion and exclusion criteria

Patients were recruited from the list of patients on warfarin who acquired the medication at primary care units in the municipality of Ijuí, Brazil. The inclusion criteria were male and female adults (18 years of age) on warfarin for chronic diseases, residents of

urban areas, having been seen at a primary care unit in the municipality and having received at least seven follow-up visits during the study.

Sample size and randomization

Fifty-two patients were identified, 47 of whom were eligible for the study and randomized to a control group (n=24) and intervention group (n=23). The randomization of the patients was performed using Microsoft Excel Software 2016. To minimize bias, the groups were stratified according to the indication for the use of warfarin and alterations in the INR. These data were obtained during the initial interview. The allocation sequence was generated by the researchers, as blinding was not performed. Both groups underwent an initial and final evaluation using the same protocol. The procedures for the control and intervention groups are described below.

Control group (CG): monthly at-home visits by previously trained researchers with no pharmaceutical intervention.

Intervention group (IG): pharmaceutical intervention by a previously trained researcher during monthly at-home visits. After the initial evaluation, a care plan was designed with the participation of the patient and pharmaceutical interventions were performed in accordance with the needs of each patient. The care plan was designed following the recommendations of the *Health Ministry for Pharmaceutical Care in Primary Health Care* [11]. An agreement was forged with the patient on the actions to be performed and the definition of the therapeutic goals.

Data collection

At the participants' homes, the researchers administered questionnaires for the collection of data. Questionnaire 1 addressed socioeconomic aspects, pharmacotherapy and comorbidities. Questionnaire 2 was used for monthly follow-up. The data were used to characterize the population of warfarin users based on sociodemographic and pharmacotherapeutic data, perform follow-up and guide counseling.

Measures

- a. *INR – laboratory:* determined by an outsourced laboratory with at-home collections from the participants in September 2018 and April 2019. Patients with INR results within the reference values according to the reason for warfarin use were considered to be within the adequate therapeutic range [12].
- b. *INR – capillary:* determined using the Roche® Coagucheck device during visits in October, December and February.

- c. *Bleeding, thrombosis, hospitalizations*: The incidence of these events was verified through a direct question posed to the participant during each visit (self-report). All hospitalizations were considered and not only those related to warfarin use.
- d. *Time in therapeutic range (TTR)*: calculated considering laboratory and capillary INRs. The calculation for each period was performed by the difference between two consecutive INR values and the target range [13].
- e. *Adverse events*: Using a list of the most common symptoms that may be caused by medications in primary care [14] the following statement was made to the participants: "I am now going to read you a list of common problems and I would like you to tell if you are feeling or have felt any of them in the past month."
- f. *Drug interactions*: Using the Drug Interactions tool from Micromedex[®] Solutions, warfarin-related interactions were identified and classified as 1) increased risk of bleeding and 2) increased risk of thrombosis.

Outcomes

The primary outcomes were the minimization of the incidence of bleeding and an increase in the TTR. The secondary outcomes were the minimization of thrombotic and other events and reductions in hospitalizations, drug interactions and the number of medications.

Statistical analysis

All analyses were conducted using the *Statistical Package for the Social Sciences* (SPSS Inc., Chicago, IL, USA), version 23.0. The normality of the data was tested using the Kolmogorov-Smirnov test. Continuous data were expressed as mean and standard deviation or median and interquartile range. Categorical data were expressed as absolute and relative frequency. Associations between two or more qualitative variables were investigated using either Pearson's chi-square test or Fisher's exact test. For quantitative variables, comparisons of means were performed using the Student's t-test for independent parametric variables and the Mann-Whitney and Kruskal-Wallis tests for independent nonparametric variables. The significance level was set at 5%.

RESULTS AND DISCUSSION

Fifty-two individuals were identified, five of whom were excluded for not meeting the eligibility criteria and 47 were randomized to the different groups. Thirty-eight individuals (21 in the IC and 17 in the CG) concluded the study Table 1).

Among the patients who concluded the study, mean age was 67.42 ± 13.74 years. The other characteristics of the sample are displayed in Table 1. The main reason for the use of warfarin was the prevention of thrombosis. Time on warfarin ranged from one to 20 years. The most frequent comorbidity was hypertension in both groups. The only significant difference between the two groups regarded obesity ($p=0.010$), which was more frequent in the IG (Table 1).

Table 1. Characterization of individuals in control and intervention (pharmacotherapeutic follow-up) groups on warfarin in public healthcare system. Ijuí, RS, Brazil, 2018

		Groups		P
		Control n=17	Intervention n=21	
		n (%)	n (%)	
Sex	Male	7 (41.2)	7 (33.3)	0.618&
	Female	10 (58.8)	14 (66.7)	
Marital status	Single	2 (11.8)	2 (9.5)	0.789#
	Married	7 (41.2)	12 (57.1)	
	Separated/divorced	4 (23.5)	3 (14.3)	
	Widowed	4 (23.5)	4 (19.0)	
Skin color	White	16 (94.1)	17 (89.5)	0.543#
	Brown	1 (5.9)	2 (10.5)	
Schooling	Illiterate	0 (0.0)	3 (14.3)	0.146#
	Incomplete/complete primary school	9 (56.2)	9 (42.9)	
	Incomplete/complete high school	6 (37.6)	3 (14.3)	
	Complete higher education	1 (6.2)	1 (4.8)	
Morbidity/ Risk factors	Diabetes mellitus	5 (29.4)	7 (33.3)	0.796
	Dyslipidemia	10 (58.8)	9 (42.9)	0.328
	Hypertension	16 (94.1)	20 (95.2)	0.701
	Coronary artery disease	12 (70.6)	18 (85.7)	0.230
	Chronic kidney failure	2 (11.8)	0 (0.0)	0.193
	Obesity	2 (11.8)	11 (52.4)	0.010*
	Vascular disease	7 (41.2)	4 (19.0)	0.128
	Sedentarism	1 (5.9)	2 (9.5)	0.581
	Smoking	10 (58.8)	11 (52.4)	0.691

Legend: # - Fisher's exact test; & - chi-square test; * $p < 0.05$.

Fewer cases of bleeding occurred in the IG but no significant association was found with pharmacotherapeutic follow-up ($p=0.389$). Throughout the entire eight-month study, eight patients in the CG (53.3%) and seven in the IG (46.7%) had at least one episode of bleeding.

Table 2 displays the data on the incidence of bleeding and thrombosis throughout the study. Patients in the IG with episodes of bleeding received the following interventions: sent to undergo laboratory INR exam, sent to physician at reference primary care unit, counseling on non-pharmacological measures, review of pharmacotherapy, and creation of medication posology calendar, pictorial labels/instructions and medication storage organizer to assist in administration of medications. Adjustment of the warfarin dose was suggested for six patients and the dose was altered in one patient after contact with the primary physician, corresponding to a 16.7% acceptance rate of this pharmaceutical intervention.

Table 2. Bleeding and thrombosis in control and intervention (pharmacotherapeutic follow-up) groups of patients on warfarin in public healthcare system. Ijuí, RS, Brazil, 2019

Month	R	Bleeding			Thrombosis		
		Yes n (%)	No n (%)	<i>P</i>	Yes n (%)	No n (%)	<i>p</i>
01	<i>Control</i>	5 (29.4)	12 (70.6)	0.052	1 (5.9)	16 (94.1)	0.459
	<i>Intervention</i>	1 (4.8)	20 (95.2)		0 (0.0)	20 (100)	
02	<i>Control</i>	3 (17.6)	14 (82.4)	0.420	2 (11.8)	15 (88.2)	0.204
	<i>Intervention</i>	2 (10.0)	18 (90.0)		0 (0.0)	20 (100)	
03	<i>Control</i>	2 (12.5)	14 (87.5)	0.333	0 (0.0)	17 (100)	-
	<i>Intervention</i>	5 (23.8)	16 (76.2)		0 (0.0)	21 (100)	
04	<i>Control</i>	2 (11.8)	15 (88.2)	0.604	0 (0.0)	17 (100)	0.299
	<i>Intervention</i>	3 (14.3)	18 (85.7)		2 (9.5)	19 (90.5)	
05	<i>Control</i>	1 (5.9)	16 (94.1)	0.387	1 (5.9)	16 (94.1)	0.701
	<i>Intervention</i>	3 (14.3)	18 (85.7)		1 (4.8)	20 (95.2)	
06	<i>Control</i>	2 (11.8)	15 (88.2)	0.419	0 (0.0)	17 (100)	-
	<i>Intervention</i>	1 (4.8)	20 (95.2)		0 (0.0)	21 (100)	
07	<i>Control</i>	4 (23.5)	13 (76.5)	0.112	3 (17.6)	14 (82.4)	0.081
	<i>Intervention</i>	1 (4.8)	20 (95.2)		0 (0.0)	21 (100)	
08	<i>Control</i>	1 (6.7)	14 (93.3)	0.627	0 (0.0)	15 (100)	0.364
	<i>Intervention</i>	1 (4.8)	20 (95.2)		0 (0.0)	21 (100)	

Note: Some patients in control group did not receive at-home visits in months 1, 2, 5 and 8; Fisher's exact test for all analyses; * $p < 0.05$.

TTR results ranged from 47.1 to 41.2% in the CG and 38.1 to 28.6% in the IG, but this difference did not achieve statistical significance ($p=0.901$). No significant differences between groups were found with regards to median TTR ($p=0.271$), bleeding ($p=0.392$) or thrombosis ($p=0.409$).

Table 3 displays the percentages of patients according to the therapeutic range of laboratory and capillary INR. Patients with laboratory INR below the target range predominated in both groups. Moreover, no statistically significant difference between groups was found regarding capillary INR, but a greater frequency of normal values was found in the three analyses conducted in the IG.

Table 3. Frequencies according to therapeutic range of INR^a in control and intervention (pharmaco-therapeutic follow-up) groups of patients on warfarin in public healthcare system. Ijuí, RS, Brazil, 2019

			INR ^a			p
			L ^b	N ^c	H ^d	
	Month	Groups	n (%)	n (%)	n (%)	
INR Laboratory 01	01	<i>Control</i>	10 (58.8)	7 (41.2)	0 (0.0)	0.154
		<i>Intervention</i>	11 (52.4)	6 (28.6)	4 (19.0)	
INR Laboratory 02	08	<i>Control</i>	9 (52.9)	8 (47.1)	0 (0.0)	0.601
		<i>Intervention</i>	12 (57.1)	8 (38.1)	1 (4.8)	
INR Coaguchek 01	01	<i>Control</i>	6 (37.5)	3 (18.8)	7 (43.8)	0.841
		<i>Intervention</i>	8 (40.0)	5 (25.0)	7 (35.0)	
INR Coaguchek 02	02	<i>Control</i>	4 (30.8)	4 (30.8)	5 (38.5)	0.755
		<i>Intervention</i>	6 (28.6)	9 (42.9)	6 (28.6)	
INR Coaguchek 03	04	<i>Control</i>	4 (28.6)	6 (42.9)	4 (28.6)	0.340
		<i>Intervention</i>	3 (15.8)	13 (68.4)	3 (15.8)	

Legend: a – INR = international normalized ratio; b – low; c – normal; d – high; * $p < 0.05$, Fisher's exact test for all analyses.

All adverse events investigated were significantly less frequent in the IG. The most frequent adverse event in the IG was headache, with 11 patients (52.4%) reporting this problem at the initial evaluation and three (30%) reporting at the end of the follow-up ($p=0.02$) (Table 4).

Table 4. Adverse events in control and intervention (pharmacotherapeutic follow-up) groups of patients on warfarin in public healthcare system at onset and end of study. Ijuí, RS, Brazil, 2019

	Initial			Final			p ^b
	CG	IG	p ^a	CG	IG	p ^a	
Adverse event	n (%)	n (%)		n (%)	n (%)		
Headache	3 (17.6)	11 (52.4)	0.027*	3 (30)	3 (30)	0.301	0.002*
Itching	3 (17.6)	4 (19.0)	0.912	2 (20)	0	0.034*	0.001*
Sleep problem	7 (41.2)	5 (23.8)	0.252	4 (40)	0	0.07*	0.001*
Gastrointestinal problem	1 (5.9)	3 (14.3)	0.401	1 (10)	0	0.141	0.02*
Mood swings	4 (23.5)	2 (9.5)	0.239	4 (40)	0	0.002*	0.04*
Dizziness	6 (35.3)	3 (14.3)	0.130	4 (40)	0	0.002*	0.02*
Incontinence	4 (23.5)	1 (4.8)	0.089	3 (30)	0	0.008*	0.05*
Muscle pain	10 (58.8)	6 (28.6)	0.05*	5 (50)	3 (14.3)	0.034*	0.04*
Fatigue	10 (58.8)	5 (23.8)	0.252	4 (40)	0	0.002*	0.01*

Legend: a = intergroup analysis; b = intra-group analysis comparing intervention group at onset and end of study. Fisher's exact test for all analyses; * p < 0.05.

Table 5 describes the interventions performed in the IG. A mean of 9.4 ± 1.36 interventions were performed per patient. The mean was 9.8 ± 1.21 among those with episodes of bleeding and 9.2 ± 1.42 ($p=0.321$) among those with no episodes of bleeding. The following interventions were performed for all patients and are therefore not displayed in the table: 1) patient/caregiver counseling on treatment in general; 2) patient/caregiver counseling on specific health conditions and 3) patient/caregiver counseling on overall health. No significant associations were found between the interventions and the occurrence of bleeding during the study period. The recommendation for laboratory exams occurred for all patients with episodes of bleeding and five (45%) were sent to the physician after telephone contact with an indication for an adjustment of the dose. The provision of material (medication organizer) was more frequent for individuals with episodes of bleeding.

Table 5. Pharmaceutical interventions and occurrence of bleeding in intervention group of patients on warfarin in public healthcare system. Ijuí, RS, Brazil, 2019.

Intervention		Yes	Bleeding No	<i>p</i>
INFORMATION AND COUNSELING				
Counseling on non-pharmacological measures	<i>Yes</i>	10 (55.6)	8 (44.4)	0.462
	<i>No</i>	1 (33.3)	2 (66.7)	
Counseling on self-monitoring	<i>Yes</i>	3 (60.0)	2 (40.0)	0.550
	<i>No</i>	8 (50.0)	8 (50.0)	
Counseling on access to medications	<i>Yes</i>	6 (46.2)	7 (53.8)	0.392
	<i>No</i>	5 (62.5)	3 (37.5)	
Counseling on storage of medications	<i>Yes</i>	8 (53.3)	7 (46.7)	0.633
	<i>No</i>	3 (50.0)	3 (50.0)	
CHANGE OR SUGGESTION FOR CHANGE IN THERAPY				
Suspension of medication	<i>Yes</i>	2 (100)	0 (0.0)	0.262
	<i>No</i>	9 (47.4)	10 (52.6)	
Replacement of medication	<i>Yes</i>	1 (100)	0 (0.0)	0.524
	<i>No</i>	10 (50.0)	10 (50.0)	
Change of medication dose	<i>Yes</i>	1 (50.0)	1 (50.0)	0.738
	<i>No</i>	10 (52.6)	9 (47.4)	
MONITORING				
Recommendation for laboratory monitoring	<i>Yes</i>	11 (57.9)	8 (42.1)	0.214
	<i>No</i>	0 (0.0)	2 (100)	
REFERRAL				
Sent to physician	<i>Yes</i>	5 (50.0)	5 (50.0)	0.835
	<i>No</i>	6 (54.5)	5 (45.5)	
PROVISION OF MATERIAL				
Medication posology list or calendar	<i>Yes</i>	9 (56.2)	7 (43.8)	0.450
	<i>No</i>	2 (40.0)	3 (60.0)	
Pictorial labels/instructions	<i>Yes</i>	2 (40.0)	3 (60.0)	0.450
	<i>No</i>	9 (56.2)	7 (43.8)	
Log for self-monitoring	<i>Yes</i>	4 (80.0)	1 (20.0)	0.185
	<i>No</i>	7 (43.8)	9 (56.2)	
Medication organizer or device to assist in adherence	<i>Yes</i>	4 (66.7)	2 (33.3)	0.367
	<i>No</i>	7 (46.7)	8 (53.3)	

Note: Interventions “sent to nurse, physiotherapist, nutritionist, social support service and structured educational program” were performed for only one patient each, none of whom had episodes of bleeding. Fisher’s exact test for all analyses; * $p < 0.05$.

Three patients in the CG and seven in the IG were hospitalized during the study period. No significant association was found between these variables.

In the CG, the mean number of medications among the participants was 7.41 ± 3.14 at the onset of the study and 9.41 ± 3.79 at the end of the study ($p=0.004$). These figures were respectively 8.00 ± 3.16 and 8.33 ± 3.02 in the IG ($p=0.540$). The individuals in the CG presented 8.88 ± 5.9 drug interactions at the onset of the study and 10.47 ± 6.87 at the end of the study. These figures were respectively 9.40 ± 5.93 and 9.05 ± 6.37 in the IG. Thus, a significant increase in the occurrence of potential drug interactions occurred in the CG ($p=0.002$), whereas little change occurred in the IG ($p=0.340$). The number of drug interactions that increase the risk of bleeding and the number that increase the risk of thrombosis were more frequent in the CG ($p=0.044$ and $p=0.05$, respectively).

The main findings of the present study were the smaller number of cases of bleeding in the group that received pharmacotherapeutic follow-up, but with no significant association between these variables. Moreover, no significant association was found between an improvement in TTR and pharmacotherapeutic follow-up. The intervention group had a greater frequency in the therapeutic range for capillary INR but not laboratory INR and with no significant association for either measure. A reduction in thrombotic and other events as well as the minimization of medications and drug interactions were found in the intervention group IG.

Fewer cases of bleeding occurred in the IG but no significant association with follow-up was found. According to the literature, the prevention of bleeding requires educational measures, the promotion of the rational use of medicines, monitoring of the INR and the maintenance of values in the therapeutic range [15], which are similar to the actions performed in the present study. However, some factors may have interfered with achieving more promising results, such as the use of polytherapy, the occurrence of drug interactions, older patients and other factors that were not measured, such as the weekly dose, which is directly related to the occurrence of this event [16].

No association was found between receiving pharmacotherapeutic follow-up and an increase in TTR values in patients in anticoagulation therapy. However, previous studies have found such an association [5, 6, 17]. This divergence may be due to the fact that these studies were conducted in the hospital setting and/or anticoagulation clinics linked to cardiology services, involved multidisciplinary care teams and had larger samples, different follow-up methods and a greater frequency of INR measurements.

One should bear in mind that patients in primary care have difficulty in gaining access to the requisition of INR exam and giving the results to the physician. In one study the majority of patients (73.5%) performed one to five INR exams/year and approximately

half of the patients did not return to the physician to show the results [18]. In the present study, the exams were covered in the research, but the limited budget impeded the execution of a greater number of analyses, which would have been ideal. It should be pointed out that, although monitoring was less frequent in comparison to other studies, it was superior to the frequency of patient analyses in the public healthcare system of the municipality [18].

Warfarin is among the medications most associated with preventable hospitalizations [3]. The number of hospitalizations was greater in the IG than the CG. However, the analysis was not limited only to hospitalizations related to the use of warfarin, which constitutes a limitation of the present study. Moreover, the source of these data were the patients themselves, some of whom were unable to state the reason for hospitalization and this information was not available at the primary care unit.

The occurrence of thrombotic events is less frequent than bleeding but has considerable clinical importance. Cases of thrombosis were more frequent in the CG, but the difference between groups did not achieve statistical significance. Another study achieved the minimization of these events through pharmacotherapeutic follow-up [6]. Risk factors associated with thromboembolic disorders found in the present study included hypertension, smoking, diabetes, dyslipidemia and an advanced age. Thus, the prevention of adverse events involving warfarin should be a priority to ensure patient safety.

Pharmacotherapeutic follow-up minimized drug interactions and reduced the occurrence of adverse events. Most of the patients in the present study were polymedicated and each medication had potential side effects, which underscores the importance of preventive measures and the monitoring of patients exposed to potential interactions. The adverse events addressed in this study may be indirectly associated with warfarin, but other causes cannot be discarded considering the presence of comorbidities, other medications and, consequently, drug interactions. Moreover, the literature reports a greater frequency of warfarin-related adverse events at hospitals [19] as the follow-up of patients is facilitated and more regular in these settings.

The number of interventions performed in the IG was greater among the patients with bleeding, although no significant association was found between the variables. Considering the complexity of warfarin therapy, the high need for interventions and the lack of information found in the present study may be important factors to the increase in adverse events. Thus, the development of counseling strategies is essential.

The adjustment of the warfarin dose was only performed on one patient due to resistance on the part of physicians in primary care. Although the presence of a pharmacist

is a strongly recommended safety strategy in other countries [5] it is not common practice in Brazil.

Regarding the characterization, the results of the present study are similar to those reported in the literature, with a predominance of older people, women and low schooling [4, 20, 21] as well as the indication for the use of an oral anticoagulant [20, 22]. Regarding the duration of warfarin use, a study states that patients starting treatment (less than six months) have more adverse effects and lower TTR values [23]. In the present investigation, time of use ranged from one to 20 years, which may have contributed to the lack of significant differences between groups. It is noteworthy that dropouts were minimal, with some patients in the CG and none in the IG dropping out, which indicates satisfaction with regards to follow-up, as demonstrated in previous studies [24, 25].

The present study has limitations that should be considered. The sample size was too small to enable adequate inferential statistics. Some variables were not controlled, such as adherence to treatment, weekly dose and patient satisfaction with the follow-up. Some information, such as the reason for use and hospitalizations, may not have been reported precisely by the participants. Moreover, the frequencies of the INR tests and visits were pre-established and not based on changes in the exam results.

CONCLUSION

The intervention by the pharmacist resulted in a reduction in adverse events compared to the control group as well as a reduction in the number of drug interactions. However, no associations were found between pharmacotherapeutic follow-up and bleeding or time in the therapeutic range among patients on warfarin in the public healthcare system.

Although barriers remain regarding the clinical participation of pharmacists in primary care, especially for patients on blood thinners, the present results can help guide future studies with different approaches to these patients. The public health system has frailties and these patients require special attention from the health team. Home care service is accepted well by these patients and can contribute to better care quality.

DISCLOSURE STATEMENT

All authors report that they do not have any conflicts of interest.

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HOW TO CITE THIS ARTICLE

A. Schneider, P. Lorenzoni-Nunes, K.R. Uhdich-Kleibert, C.d.F. Colet, E. Roseli-Winkelmann, Pharmacotherapeutic follow-up of patients on warfarin in primary care: randomized clinical trial, *Rev. Colomb. Cienc. Quím. Farm.*, 51(3), 1399-1417 (2022). DOI: <http://dx.doi.org/10.15446/rcciquifa.v51n3.102279>

Calificación de un sistema de HPLC-IR para la cuantificación de carbohidratos de diversas composiciones

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Recibido: 15 de junio de 2022

Revisado: 6 de julio de 2022

Aceptado: 13 de julio de 2022

RESUMEN

Introducción: los carbohidratos son las biomoléculas más abundantes en la naturaleza. Su importancia va desde aspectos nutricionales hasta aplicaciones industriales. Entre la inmensa variedad de carbohidratos se encuentran los fructooligosacáridos considerados prebióticos; los azúcares invertidos, de importancia industrial por su alto poder edulcorante; las dextranas, con diversas aplicaciones, pero cuya presencia en jugos de caña provoca afectaciones en la producción azucarera. Las investigaciones y aplicaciones relacionadas con carbohidratos requieren la obtención de datos fiables sobre las cinéticas de síntesis y degradación de los mismos. Uno de los métodos de cuantificación de los carbohidratos es mediante un equipo de cromatografía líquida de alta resolución (HPLC) acoplado a un detector de índice de refracción (IR). **Objetivo:** calificar un equipo de HPLC acoplado a un detector de índice de refracción (IR) para la validación del método de cuantificación de carbohidratos. **Metodología:** la calificación del HPLC-IR se realizó según la *Guía para la calificación del equipamiento - Anexo 1* de 2011. Se evaluó la exactitud del flujo de la bomba, la precisión y linealidad del inyector manual, la exactitud y estabilidad de la temperatura del horno, así como la linealidad y relación señal/ruido en el detector IR. **Resultados:** este trabajo demostró que el sistema HPLC-IR se desempeña de manera consistente de acuerdo con las especificaciones del fabricante, de esta forma se aseguró el rendimiento correcto del equipo en condiciones reales de

funcionamiento. **Conclusión:** la calificación satisfactoria del instrumento analítico permitió la validación del método de cuantificación de carbohidratos mediante esta tecnología.

Palabras clave: Calificación, HPLC, índice de refracción, validación, carbohidratos, FOS.

SUMMARY

Qualification of an HPLC-IR system for the quantification of carbohydrates of various compositions

Introduction: Carbohydrates are the most abundant biomolecules in nature. Its importance goes from nutritional aspects to industrial applications. Among the immense variety of carbohydrates are the fructooligosaccharides considered prebiotics; inverted sugars, of industrial importance due to their high sweetening power; dextrans, with various applications, but whose presence in cane juice affects sugar production. Research and applications related to carbohydrates require obtaining reliable data on their synthesis and degradation kinetics. One of the carbohydrate quantification methods is by means of a high-performance liquid chromatography (HPLC) equipment coupled to a refractive index (IR) detector. **Objective:** To qualify a HPLC equipment coupled to a refractive index (IR) detector to validate the carbohydrate quantification method. **Methodology:** The qualification of the HPLC-IR was carried out according to the *Guide for the Qualification of the Equipment - Annex 1* of 2011. The accuracy of the flow of the pump, the precision and linearity of the manual injector, the accuracy and stability of the temperature of the oven, as well as the linearity and signal/noise ratio in the IR detector were determined. **Results:** This work showed that the HPLC-IR system performs consistently according to the manufacturer's specifications, thus ensuring the correct performance of the equipment under real operating conditions. **Conclusion:** The satisfactory qualification of the analytical instrument allowed the validation of the carbohydrate quantification method using this technology.

Keywords: Qualification, HPLC, refractive index, validation, carbohydrates, FOS.

RESUMO

Qualificação de um sistema HPLC-IR para a quantificação de carboidratos de várias composições

Introdução: os carboidratos são as biomoléculas mais abundantes na natureza. Sua importância vai desde aspectos nutricionais até aplicações industriais. Dentre a imensa variedade de carboidratos estão os frutooligosacarídeos considerados prebióticos; açúcares invertidos, de importância industrial devido ao seu alto poder adoçante; dextrans, com diversas aplicações, mas cuja presença no caldo de cana afeta a produção de açúcar. Pesquisas e aplicações relacionadas a carboidratos requerem a obtenção de dados confiáveis sobre sua síntese e cinética de degradação. Um dos métodos de quantificação de carboidratos é por meio de um equipamento de cromatografia líquida de alta eficiência (HPLC) acoplado a um detector de índice de refração (IR). **Objetivo:** qualificar um equipamento de HPLC acoplado a um detector de índice de refração (IR) para a validação do método de quantificação de carboidratos. **Metodologia:** a qualificação HPLC-IR foi realizada de acordo com o *Guia para a qualificação do equipamento - Anexo 1* da 2011. A precisão do fluxo da bomba, a precisão e linearidade do injetor manual, a precisão e estabilidade da temperatura do forno, bem como a linearidade e relação sinal/ruído no detector IR. **Resultados:** este trabalho demonstrou que o sistema HPLC-IR funciona consistentemente de acordo com as especificações do fabricante, garantindo assim o correto desempenho do equipamento em condições reais de operação. **Conclusão:** a qualificação satisfatória do instrumento analítico permitiu a validação do método de quantificação de carboidratos utilizando esta tecnologia.

Palavras-chave: Qualificação, HPLC, índice de refração, validação, carboidratos, FOS.

INTRODUCCIÓN

En la actualidad la cromatografía líquida de alta resolución (HPLC) ha llegado a ser una de las técnicas de laboratorio más importantes como herramienta analítica para separar y detectar compuestos químicos, dada su sensibilidad y fácil adaptación a las determinaciones cuantitativas exactas. Esta técnica permite la separación, identificación y cuantificación de los azúcares presente en muestras orgánicas [1].

La Conferencia Internacional de Armonización (conocida por sus siglas en inglés ICH) planteó la necesidad de validación de los métodos analíticos con el objetivo de demostrar que es adecuado para el propósito previsto, así como ofrecer robustez y confianza a los datos obtenidos [2].

La validación de una técnica analítica es la recopilación de evidencia científica de que un procedimiento es adecuado para el uso previsto. Un procedimiento validado con instrumentos analíticos calificados proporciona la confianza de que el procedimiento generará prueba de datos de calidad aceptable [3].

La calificación de instrumentos analíticos (AIQ) es un requisito previo para la validación de cualquier método y, por lo tanto, debe considerarse como una base vital para la integridad y la calidad de los datos [4]. Se han establecido etapas para la calificación del equipamiento: calificación de diseño (DQ), calificación de instalación (IQ), calificación de operación (OQ) y calificación de desempeño (PQ). La DQ es la parte donde el diseño y las características técnicas de un instrumento están predefinidos tomando en cuenta las especificaciones de requisitos del usuario (URS). La IQ establece que el instrumento se recibe tal como fue diseñado y que está correctamente instalado. La OQ de un equipo para HPLC se lleva a cabo de forma modular con la intención de garantizar que los módulos específicos del sistema y todo el sistema funcionan de acuerdo con las especificaciones definidas. La PQ demuestra que un instrumento se desempeña de manera consistente de acuerdo con las especificaciones definidas por el usuario y es apropiado para el uso previsto, de esta manera se asegura el rendimiento satisfactorio de un instrumento en condiciones reales de funcionamiento [3, 5, 6].

En nuestro laboratorio la cuantificación de carbohidratos se realiza por HPLC acoplado a un detector de índice de refracción (HPLC-IR). Esta técnica ha permitido cuantificar diversos carbohidratos tales como fructooligosacáridos [7], dextranas [8] o azúcares invertidos [9, 10]. Sin embargo, debido a los años de explotación del equipamiento se requirió un mantenimiento general del mismo, lo cual implica una recalificación para su puesta en marcha. La calificación debe realizarse a los diferentes componentes del sistema HPLC-IR de manera que puedan lograrse buenos resultados en la validación del método analítico, pues se necesita demostrar previamente que el sistema trabaja de forma satisfactoria de acuerdo a los requisitos especificados. El objetivo de este trabajo fue la calificación del equipo de HPLC acoplado a un detector de IR para la validación del método analítico de cuantificación de carbohidratos.

METODOLOGÍA

Equipos

Se empleó un cromatógrafo para líquidos Merck Hictachi® Lachrom (Alemania), equipado con una bomba de fase móvil (modelo L-7100), horno para columna (modelo L-7350) y un detector de índice de refracción Knauer Differential-Refractometer (modelo 2300) (Alemania). Se utilizó una columna Aminex HPX 42-C (BioRad, Richmond, Estados Unidos) con precolumna Carbo C (BioRad, Richmond, Estados Unidos), inyector con un lazo de 20 μ L y jeringuilla Hamilton® de 50 μ L. La salida analógica del detector se encuentra conectada a un dispositivo NI USB-6008 (interfaz de adquisición de datos de National instrument) que provee 8 canales analógicos de entrada, con un interfaz USB de alta velocidad conectada a un ordenador que permite el registro de la señal mediante el uso del *software* AdqUSB4. Los datos registrados son exportados al *software* ezData (www.chemilab.net), para integrar y calcular el tiempo de retención, altura, altura media del pico y área bajo la curva.

La calificación de la instalación (IQ) se realizó mediante pruebas cualitativas y cuantitativas de los componentes del equipo (módulos). Se verificaron el inyector, la bomba, el detector, el horno para la columna, los *hardware/software*, el cableado, el chasis, los sensores, controles e interruptores, el *software*/pantalla, las señales sonoras, las alarmas y la documentación del equipo.

Para la calificación de operación (OQ) se evaluó cada módulo del equipo HPLC de forma individual, se basó en las especificaciones de diseño del fabricante (bomba, inyector manual, horno de columna, detector y *hardware software*).

La calificación de desempeño (PQ) evaluó parámetros para determinar el rendimiento del instrumento en condiciones reales de funcionamiento. Se tomó como criterio de aceptación lo planteado en la *Guía para la calificación del equipamiento* (anexo 1) [5]. Los cálculos matemáticos se realizaron en el programa Excel 2010 y en el software MATLAB (versión R2015a).

Determinación de la exactitud del flujo en la bomba

Se evaluaron tres velocidades de flujo (0,2 mL/min, 0,3 mL/min y 0,5 mL/min), comprendidas en el rango de operaciones del equipo a escala analítica, se purgó el sistema y puso en funcionamiento asegurando que no había burbujas. Se utilizó agua desgasificada para HPLC como fase móvil. El flujo de salida fue colectado durante 4 min en una probeta graduada y calibrada (Oficina Territorial de Normalización de Villa Clara, www.otn.vcl.cu), el tiempo se midió con un cronómetro calibrado (Oficina Territorial

de Normalización de Villa Clara, www.otn.vcl.cu). Se repitió cinco veces esta operación y se determinó el promedio de cada volumen. La desviación (D_f) se calculó como el valor porcentual de la diferencia absoluta entre la media del flujo medido (f) y el flujo fijado en la bomba (F) dividido por el flujo fijado en la bomba ambos en mL/min (ecuación 1). El criterio de aceptación fue de $D_f \pm 5\%$.

$$D_f = \left(\frac{f - F}{F} \right) * 100 \quad (\text{Ec. 1})$$

Determinación de la precisión y linealidad del inyector manual

La evaluación se realizó con agua desgasificada para HPLC como fase móvil a un flujo de 0,5 mL/min. Para la preparación de la solución de referencia se utilizó sacarosa (Sigma-Aldrich, USA). A partir de la solución de sacarosa a una concentración de 40 mg/mL se realizaron inyecciones para cada volumen seleccionado (5, 10, 15 y 20 μ L) (2 analistas, 2 días cada uno y 3 réplicas por analista). Se registró el tiempo de retención, el área y la altura del pico en cada perfil cromatográfico. Se evaluó la precisión intra-ensayos (repetibilidad) y la precisión inter-ensayos que contempla la variabilidad debida al error experimental y a los analistas. La precisión se determinó mediante el coeficiente de variación (CV) de las áreas y de las alturas de los picos obtenidos en cada cromatograma (ecuación 2). El criterio de aceptación fue un $CV \leq 1\%$.

$$CV \left(\frac{DS}{media} \right) * 100 \quad (\text{Ec. 2})$$

Donde DS es la desviación estándar.

La linealidad se calculó con la media de las alturas medidas para cada volumen de inyección, se obtuvo la ecuación de regresión y el coeficiente de determinación (R^2). El criterio de aceptación utilizado para autoinyectores es un $R^2 \geq 0,999$.

Exactitud de la temperatura del horno para columna

Se evaluaron tres temperaturas (40 °C, 60 °C y 85 °C) que comprenden el rango de operaciones del equipo de HPLC. Se colocaron los sensores de temperatura (UTT10K termopares calibrados, UNI-T, China) en diferentes posiciones del horno (parte anterior, media y posterior). El horno se ajustó a la temperatura deseada, una vez equilibrado el sistema, se realizaron tres mediciones durante 15 min. La desviación de la temperatura (D_T) se calculó como la diferencia absoluta entre la media de la temperatura medida (t) y la temperatura fijada (T) (ecuación 3). El criterio de aceptación fue de $D_T \pm 2$ °C.

$$D_T = t - T \quad (\text{Ec. 3})$$

La estabilidad se calculó para cada temperatura como la diferencia entre la temperatura más alta y más baja de las tres lecturas en una misma posición. Como criterio de aceptación se consideró una diferencia $D_T \leq 1^\circ\text{C}$.

Calificación del detector de índice de refracción (Knauer)

Se utilizó como fase móvil agua desgasificada para HPLC a un flujo de 0,5 mL/min. Se inyectaron 25 μL de la solución de referencia de sacarosa a cuatro concentraciones diferentes (5, 10, 20 y 40 mg/mL), el experimento se realizó por triplicado. Se registró el tiempo de retención, el área y la altura del pico en cada perfil cromatográfico. Para el cálculo de la linealidad se determinó la media de las alturas medidas para cada concentración, se obtuvo la ecuación de regresión lineal y se calculó el coeficiente de determinación (R^2). El criterio de aceptación fue un $R^2 \geq 0,995$.

Para el cálculo del ruido de la línea de base se utilizó como fase móvil agua desgasificada para HPLC a un flujo de 0,5 mL/min. Se inyectaron 3 réplicas de la solución blanco (fase móvil) durante un tiempo de ejecución donde el sistema fue estable. Se midió el ruido de la línea base durante 20 min. Como criterio de aceptación se consideró una altura media de las tres réplicas $< 1000 \mu\text{V}$. Para calcular la relación señal/ruido (S/R) se inyectó tres veces la solución de referencia de sacarosa a una concentración de 5 mg/mL y se calculó la media de las réplicas (ecuación 4). El criterio de aceptación fue una relación $S/R \geq 10$.

$$SN/R = 2H/h \quad (\text{Ec. 4})$$

Donde H es la altura del pico para cada perfil cromatográfico y h es la altura del ruido de la línea base.

RESULTADOS Y DISCUSIÓN

La calificación de los diferentes componentes del sistema HPLC-IR es un aspecto de vital importancia para la validación del método analítico de cuantificación de carbohidratos.

Calificación de la instalación

Para la IQ del equipo para HPLC se verificó que el lugar de instalación estuviera libre de polvo, gases corrosivos y grandes campos magnéticos. Se corroboró que las dimensiones y estabilidad de las mesetas eran las adecuadas. La temperatura del local se mantuvo en $20 \pm 2^\circ\text{C}$, la humedad relativa $50 \pm 5\%$ y la alimentación eléctrica fue de $220 \pm 5 \text{ V}$. Con la calificación de instalación se verificó además que los distintos módu-

los del sistema estaban correctamente instalados según los requisitos especificados por los proveedores y el entorno era el adecuado para su funcionamiento.

La farmacopea americana para la calificación de instrumentos analíticos categoriza a los equipos para HPLC en el grupo C [3]. Este grupo incluye instrumentos y sistemas analíticos computarizados, donde los requisitos del usuario para la funcionalidad, los límites operativos y de rendimiento son específicos para la aplicación analítica.

La calificación del equipo para HPLC-IR se realizó mediante pruebas de funcionamiento y de rendimiento específicas, que incluyen la calificación de operación a nivel modular y la verificación de parámetros para evaluar el desempeño del equipo en tiempo real. Se tuvieron en cuenta los criterios planteados por Kaminski con el objetivo de disminuir los tiempos de operaciones y el costo del proceso [4].

Calificación de la bomba

En un sistema para HPLC, la bomba constituye una de sus partes más importantes, de su buen funcionamiento dependen la calidad de separación y la precisión del ensayo analítico, sobre todo en un método cuantitativo [11]. La evaluación de la bomba se realizó en un rango de operaciones que cubre los flujos más utilizados a escala analítica. La tabla 1 muestra los resultados de la exactitud del flujo isocrático en la bomba L-7100.

Tabla 1. Evaluación de la exactitud del flujo en la bomba HPLC modelo L-7100.

Flujo fijado (mL/min)	Media flujo medido (mL/min)	Desviación (%)
0,20	0,204	1,875
0,30	0,295	-1,750
0,50	0,491	-1,800

En todos los casos la bomba funcionó con exactitud dentro de los límites de incertidumbre de la medición [4,5]. Estos resultados demuestran que el instrumento mantiene un flujo estable y sostenible, el regulador de presión cumple con las condiciones programadas y no existen fugas de líquido causado por problemas con los sellos de los pistones o conexiones. La bomba en estudio cumple con las especificaciones establecidas y adecuadas a las características del equipo.

Calificación del inyector manual

La figura 1 muestra los perfiles cromatográficos obtenidos al evaluar la precisión del inyector manual. Se utilizaron diferentes volúmenes de una solución de referencia de

sacarosa a una concentración de 40 mg/mL. En la tabla 2 se resumen los resultados del área y de la altura obtenidos para los picos detectados.

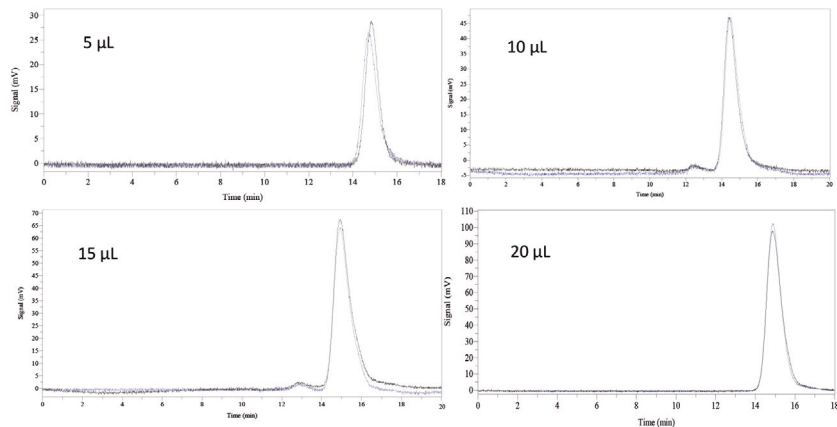


Figura 1. Perfiles cromatográficos obtenidos en la evaluación de la precisión del inyector manual, con 5, 10, 15 y 20 µL de volumen de inyección de la solución de referencia de sacarosa a una concentración de 40 mg/mL. Los valores mostrados representan la media de tres réplicas para cada volumen, por cada analista, en un día de ensayo.

Tabla 2. Evaluación de la precisión del inyector manual

Cálculos basados en la altura de los picos				
Volúmenes (µL)	5	10	15	20
Media (altura)	25,50	49,69	68,63	96,10
DS	0,02	0,02	0,45	0,14
CV (%)	0,08	0,04	0,66	0,15
Criterio de aceptación ≤ 1%	Cumple	Cumple	Cumple	Cumple
Cálculos basados en el área de los picos				
Volúmenes (µL)	5	10	15	20
Media (área)	25,42	51,56	65,76	95,03
DS	0,11	0,02	0,16	0,52
CV (%)	0,45	0,04	0,25	0,54
Criterio de aceptación ≤ 1%	Cumple	Cumple	Cumple	Cumple

El comportamiento de los picos obtenidos en el estudio de la precisión del inyector manual fue similar para cada una de las réplicas evaluadas con los diferentes analistas y días de aplicación (figura 1), lo que se refleja en un coeficiente de variación menor que el 1 %, tanto para el área como para la altura del pico (tabla 2). Este resultado, similar al reportado por Quiñones-García *et al.*, 2015 [12], demostró que es posible minimizar los errores potenciales asociados a la inyección manual de las muestras si se realizan réplicas y se calcula el valor medio de la respuesta, con ello se consigue la precisión instrumental requerida.

Al referirnos a un inyector manual, donde la mayor parte de la operación recae sobre el operario, la evaluación de este parámetro también demostró la calificación de los analistas. La farmacopea americana plantea que los analistas son los responsables de la operación de los instrumentos analíticos y la calidad de los datos [3].

La altura de los picos es un parámetro que se afecta con pequeñas variaciones, pero brinda mayor información de la calidad de la separación y resolución cromatográfica. El área bajo la curva, por su parte, permite calcular concentraciones desconocidas de analitos de interés. Este parámetro es de mayor importancia teniendo en cuenta que el objetivo de este trabajo es la calificación del equipo para HPLC para la validación de la cuantificación analítica de carbohidratos.

La linealidad del inyector es un parámetro que evalúa la capacidad del instrumento de obtener resultados que sean directamente proporcionales a los volúmenes de la solución de referencia inyectados. La figura 2 muestra un coeficiente de determinación cercano a los valores reportados en la literatura como estándares para autoinyectores [5].

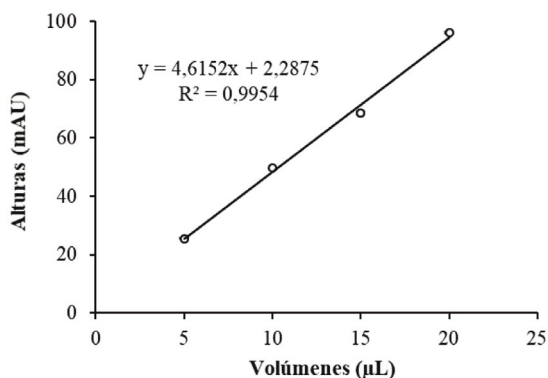


Figura 2. Evaluación de la linealidad del inyector manual. Los valores obtenidos representan la media de tres réplicas para cada volumen $\pm$ la desviación estándar.

Calificación del horno para columna

Por su importancia, la exactitud de la temperatura del horno para columna se considera uno de los parámetros a evaluar para demostrar la idoneidad del sistema. La tabla 3 resume los resultados obtenidos.

Tabla 3. Evaluación de la exactitud de la temperatura del horno de columna.

T (°C)	Posición	t (°C)	D _T (°C)	Criterio aceptación $\pm 2^{\circ}\text{C}$
40	anterior	38,0	-2,0	Cumple
	media	38,7	-1,3	Cumple
	posterior	40,3	0,3	Cumple
60	anterior	58,0	-2,0	Cumple
	media	59,0	-1,0	Cumple
	posterior	61,3	1,3	Cumple
85	anterior	83,0	-2,0	Cumple
	media	85,7	0,7	Cumple
	posterior	87,0	2,0	Cumple

Convenciones: T: temperatura fijada, t: media de la temperatura medida y D_T: desviación de la temperatura.

La desviación de la temperatura demuestra que a medida que aumenta la temperatura fijada en el horno hay una menor exactitud de la misma en las diferentes posiciones de la cámara del horno. La temperatura en la posición anterior (posición del inyector) es inferior a la fijada y en la posición posterior (salida del aire caliente) es superior. Este patrón está asociado a la necesidad del horno de mantener la temperatura constante en la cámara. No obstante, la desviación de la temperatura está en el rango del criterio de aceptación ($\pm 2^{\circ}\text{C}$). La variable estabilidad de la temperatura en los puntos de medición cumple con el criterio de aceptación de una variabilidad $\leq 1^{\circ}\text{C}$ [5]. Es importante evidenciar un buen funcionamiento del horno, pues se encarga de regular y mantener la temperatura dentro de la columna, este parámetro influye directamente en la retención y selectividad de la misma [13].

Calificación del detector

Otro componente del sistema HPLC de vital importancia para la validación de un método analítico es el detector, en este trabajo se evaluó el detector IR asociado a la cuantificación de muestras de carbohidratos.

La linealidad del detector evalúa la capacidad del ensayo de obtener resultados que sean directamente proporcionales a las concentraciones de las soluciones de referencia inyectadas. La figura 3 muestra los resultados obtenidos a las diferentes concentraciones ensayadas, donde el coeficiente de determinación cumple con el criterio de aceptación establecido de un $R^2 \geq 0.9950$ [4, 5]. El IR evaluado es capaz de emitir una señal con una linealidad adecuada para el análisis y cuantificación de carbohidratos.

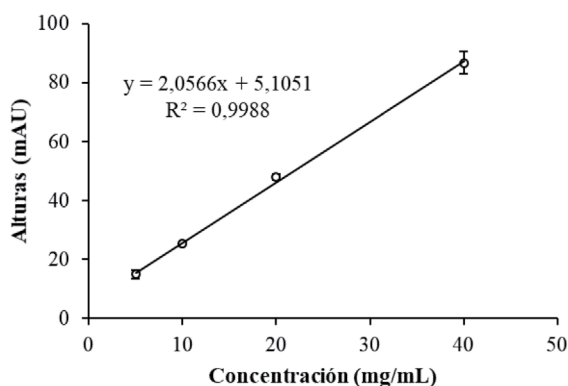


Figura 3. Evaluación de la linealidad del detector de índice de refracción. Los valores obtenidos representan la media de tres réplicas para cada concentración $\pm$ la desviación estándar.

En la figura 4 aparece el perfil cromatográfico resultante de la evaluación de la relación señal/ ruido del detector en un período de 20 min. El parámetro ruido de la línea de base se evaluó de satisfactorio por mostrar valores $< 1000 \mu V$ en condiciones dinámicas de operación.

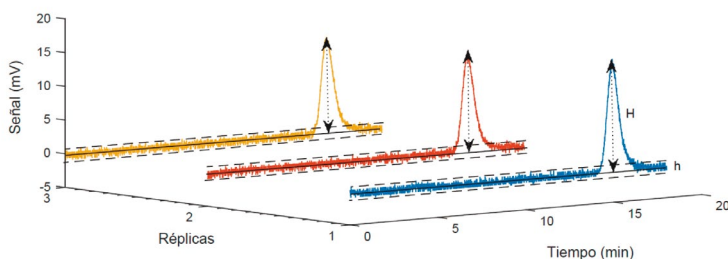


Figura 4. Perfil cromatográfico resultante de la evaluación de la relación señal/ruido del detector IR.

Donde H es la altura del pico del perfil cromatográfico y h es la altura del ruido de la línea base. Para esta evaluación se aplicó una solución de sacarosa a una concentración de 5 mg/mL.

La tabla 4 muestra los datos de la relación señal/ruido obtenidos de la inyección de la solución de referencia (sacarosa 5 mg/mL) realizada por tres analistas. Se evidenció que la relación S/R fue superior al límite inferior del criterio de aceptación para la concentración evaluada [5].

Tabla 4. Evaluación de la relación señal/ruido.

Relación señal/ruido	S/R (analista 1)	S/R (analista 2)	S/R (analista 3)
Concentración 5 mg/mL	18,49	16,27	15,78
Criterio de aceptación (≥ 10)	Cumple	Cumple	Cumple

Los parámetros de calificación del detector IR se consideran adecuados, pues cumplen con los estándares internacionales [5]. Los valores obtenidos, (relación señal/ ruido y linealidad) satisfacen los requerimientos necesarios para la validación del método de cuantificación de carbohidratos. Según la Conferencia Internacional de Armonización es posible utilizar varios enfoques para determinar el límite de detección y cuantificación de un analito dependiendo si el procedimiento es instrumental o no instrumental [2]. En este caso, la calificación del HPLC-IR demostró que los cálculos del límite de detección y cuantificación pueden estar basados en la relación S/R. El límite de detección se calculó para la relación $S/R \geq 3$, mientras que el límite de cuantificación se calculó para la relación $S/R \geq 10$ [2].

Una calificación de desempeño continua evita tiempo de trabajo extra, mejora la certeza analítica y, por lo tanto, la calidad general de los datos. La calificación proporciona no solo una instantánea del desempeño del sistema, sino también un historial de rendimiento continuo, basado en el uso de los gráficos de control. Esto permite al usuario contar con una lista de verificación sencilla con la posibilidad de calificar el instrumento antes de su utilización. La determinación de los tiempos de retención o las áreas de picos debe realizarse cada vez que se ejecuta el método, brinda una información rápida y confiable del sistema, demuestran que los diferentes módulos del sistema HPLC-IR se desempeñan de manera consistente de acuerdo con las especificaciones definidas por el fabricante.

CONCLUSIONES

La calificación del sistema HPLC-IR mostró la exactitud del flujo de la bomba, la precisión y linealidad del inyector manual, la exactitud y estabilidad de la temperatura del horno, así como la linealidad y relación señal/ruido en el detector IR según las normas ICH y USP. Este trabajo demostró que los diferentes módulos del sistema HPLC-IR se desempeñan de manera consistente de acuerdo con las especificaciones definidas por cada fabricante para los diferentes módulos, por lo que resultan apropiados para el uso previsto de cuantificación de carbohidratos de diversas composiciones, estructuras y grados de polimerización como son los FOS, los azúcares invertidos y las dextranas.

AGRADECIMIENTOS

Los autores agradecen al Centro de Ingeniería Genética y Biotecnología de Sancti Spíritus.

CONFLICTO DE INTERESES

El trabajo aquí descrito no ha sido publicado con anterioridad. Los autores declaran no tener conflicto de intereses y aceptan la publicación del artículo en esta revista. Los autores de este trabajo declaran presentar una participación igualitaria en la concepción, ejecución y redacción de la investigación.

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CÓMO CITAR ESTE ARTÍCULO

I. Veitía-Coba, J.D. Brizuela-Cardoso, J.M. Fernández-Torres, D. Martínez-García, E. Pérez-Cruz, Calificación de un sistema de HPLC-IR para la cuantificación de carbohidratos de diversas composiciones, *Rev. Colomb. Cienc. Quím. Farm.*, 51(3), 1418-1433 (2022). DOI: <http://dx.doi.org/10.15446/rcciquifa.v51n3.102515>

Artículo de investigación clínica / <http://dx.doi.org/10.15446/rcciquifa.v51n3.102229>

Abordaje de la antibioticoterapia prescrita en pacientes pediátricos de una unidad de salud, Tungurahua-Ecuador

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Recibido: 4 de mayo de 2022

Revisado: 8 de julio de 2022

Aceptado: 16 de julio de 2022

RESUMEN

Introducción: las infecciones respiratorias superiores constituyen la principal enfermedad de la infancia, los pacientes menores de 5 años reciben mayor prescripción antibiótica y, por ende, aumenta el riesgo de resistencia antimicrobiana.

Objetivo: abordar la antibioticoterapia prescrita en pacientes pediátricos de una unidad de salud en Tungurahua-Ecuador. **Metodología:** se analizaron 91 historias clínicas de pacientes entre 1 a 6 años, que recibieron asistencia ambulatoria durante los meses de julio a octubre del 2019, y que en su prescripción según su diagnóstico, requirieron antibioticoterapia. Se determinaron enfermedades de mayor prevalencia y antibióticos más prescritos durante el estudio, comparando la correspondencia entre la prescripción emitida y la indicada en el protocolo terapéutico del Ministerio de Salud Pública de Ecuador. **Resultados:** las infecciones del tracto respiratorio superior alcanzaron un 83,52% de los diagnósticos encontrados y entre ellas la amigdalitis fue la más frecuente. Los macrólidos con un 50,55% fueron los fármacos más prescritos. Al estimar la correspondencia entre los prescritos durante el período de investigación y los indicados en el documento oficial, se encontró que la prescripción de los antibióticos de segunda elección con 38,46% superaron a los de primera elección que solo alcanzaron el 14,29% y estos a su vez, fueron superados por la prescripción

de otros antibióticos con un 47,25%. **Conclusiones:** la antibioticoterapia empleada para las infecciones respiratorias pediátricas no se ajustó con los fármacos de primera y segunda elección, establecidos en el protocolo terapéutico oficial, así, es necesario revisar y actualizar dichos documentos, considerando las realidades epidemiológicas y la resistencia antimicrobiana.

Palabras clave: Pacientes pediátricos, antibioticoterapia, protocolos terapéuticos, infecciones respiratorias.

SUMMARY

Approach to prescribed antibiotic therapy in pediatric patients of a health unit, Tungurahua-Ecuador

Introduction: Upper respiratory infections are the main childhood disease, patients under 5 years of age receive more antibiotic prescription, and therefore, the risk of antimicrobial resistance increases. **Aim:** To address the antibiotic therapy prescribed in pediatric patients from a health unit in Tungurahua-Ecuador. **Methodology:** 91 clinical histories of patients between 1 and 6 years old, who received outpatient care during the months of July to October 2019, and who in their prescription according to their diagnosis, required antibiotic therapy were analyzed. The most prevalent diseases and most prescribed antibiotics during the study were determined, comparing by correspondence between the prescription issued and the one indicated in the therapeutic protocol of the Ministry of Public Health of Ecuador. **Results:** Upper respiratory tract infections reached 83.52% of the diagnoses found and among them, tonsillitis was the most frequent. Macrolides with 50.55% were the most prescribed drugs. When estimating the correspondence between the prescriptions during the research period and those indicated in the official document, it was found that the prescription of second-choice antibiotics with 38.46% exceeded those of first choice, which only reached 14.29% and these, in turn, were surpassed by the prescription of other antibiotics with 47.25%. **Conclusions:** the antibiotic therapy used for pediatric respiratory infections was not adjusted with the first and second choice drugs, established in the official therapeutic protocol, thus, it is necessary to review and update these documents, considering the epidemiological realities and antimicrobial resistance.

Keywords: Pediatric patients, Antibiotic therapy, Therapeutic protocols, Respiratory infections.

RESUMO

Abordagem à antibioticoterapia prescrita em pacientes pediátricos de uma unidade de saúde, Tungurahua-Ecuador

Introdução: as infecções respiratórias superiores são a principal doença infantil, os pacientes com menos de 5 anos recebem mais prescrição de antibióticos e, portanto, o risco de resistência antimicrobiana aumenta. **Objetivo:** abordar à antibioticoterapia prescrita em pacientes pediátricos de uma unidade de saúde em Tungurahua-Ecuador. **Metodologia:** foram analisadas 91 histórias clínicas de pacientes entre 1 e 6 anos, que receberam atendimento ambulatorial durante os meses de julho a outubro de 2019, e que em sua prescrição de acordo com seu diagnóstico, necessitaram de antibioticoterapia. Foram determinadas as doenças mais prevalentes e os antibióticos mais prescritos durante o estudo, comparando a correspondência entre a prescrição emitida e a indicada no protocolo terapêutico do Ministério de Saúde Pública do Ecuador. **Resultados:** as infecções do trato respiratório superior atingiram 83,52% dos diagnósticos encontrados e dentre eles a amigdalite foi o mais frequente. Os macrolídeos com 50,55% foram os medicamentos mais prescritos. Ao estimar a correspondência entre as prescrições do período da pesquisa e as indicadas no documento oficial, constatou-se que a prescrição de antibióticos de segunda escolha com 38,46% superou a de primeira escolha, que atingiu apenas 14,29%, estes foram superados pela prescrição de outros antibióticos com 47,25%. **Conclusões:** a antibioticoterapia utilizada para infecções respiratórias pediátricas não foi ajustada com as drogas de primeira e segunda escolha, estabelecidas no protocolo terapêutico oficial, sendo necessário revisar e atualizar esses documentos, considerando as realidades epidemiológicas e resistência antimicrobiana.

Palavras-chave: Pacientes pediátricos, antibioticoterapia, protocolos terapêuticos, infecções respiratórias.

INTRODUCCIÓN

Con frecuencia, los niños, una parte importante de la población, reciben prescripción de antibióticos, empleándose hasta en el 60% de las ocasiones como farmacoterapia para las infecciones del tracto respiratorio superior, que constituyen la principal enfermedad de la infancia [1]. Dichas infecciones en muchas ocasiones suelen ser de origen viral, aun cuando, se presentan con un exudado nasal mucopurulento tras el período

catarral, por lo que, no debe considerarse una sobreinfección bacteriana si estos síntomas son de evolución benigna y autolimitada, no requiriendo para ello, la antibiotico-terapia [2, 3].

El uso irracional de antibióticos para infecciones respiratorias puede alcanzar hasta un 50% de los casos tratados [4], incrementando el gasto sanitario y generando el desarrollo de resistencia bacteriana, que constituye en la actualidad un problema de salud mundial y particularmente importante en pediatría, ya que son los niños menores de 5 años, los pacientes con mayor prescripción antibiótica [5, 6]. Además, algunos de los patógenos más comunes, causantes de las infecciones pediátricas, como los neumococos, *Haemophilus influenzae* y *Staphylococcus pyogenes*, exhiben elevadas tasas de resistencia a las más importantes familias de antibióticos, como betalactámicos y macrólidos [7].

A nivel mundial, se estima que entre el 85-95% de los antibióticos se prescriben en el ámbito comunitario: consultas de atención primaria, consultas privadas y servicios de urgencias [8].

Los errores de prescripción constituyen el tipo de error de medicación más frecuente en el ámbito hospitalario y en esta población vulnerable son potencialmente nocivos, considerándose que el número de errores de medicación que producen una lesión grave o la muerte de los pacientes pediátricos es significativamente más alto (31%) que cuando se compara con el de los adultos (13%) [9]. Una investigación cualitativa determinó que la prescripción de antibióticos para niños con enfermedades agudas, constituían prescripciones innecesarias, pues se prestaba menos atención a los factores clínicos y los médicos se centraban, en una prescripción “por si acaso” cuando no estaban seguros de los resultados clínicos y teniendo más probabilidades de recetar, si percibían la presión de los padres [10].

Según la Organización Panamericana de la Salud, en Ecuador las defunciones de niños menores a 5 años por infecciones respiratorias agudas alcanzaron el 7,6% durante el 2016, siendo cada vez más difícil para la población pediátrica, disponer de moléculas con actividad farmacológica que reemplacen aquellas, para las cuales los patógenos han desarrollado resistencias, por ello resulta fundamental no iniciar un tratamiento antibiótico hasta que existan argumentos a favor de una infección bacteriana [11, 12].

En tal sentido, se planteó en este estudio, realizar un abordaje sobre la prescripción de antibióticos a pacientes pediátricos, que acudieron durante el período julio - octubre 2019, al servicio de consulta externa de una unidad de salud en Tungurahua, Ecuador.

METODOLOGÍA

Esta investigación fue de carácter descriptivo, observacional, no experimental y retrospectivo, correspondiente a la revisión de historias clínicas de pacientes pediátricos entre 1 a 6 años, que recibieron asistencia ambulatoria en una unidad de salud de segundo nivel de la provincia de Tungurahua, durante los meses de julio a octubre 2019, y que, en su prescripción de acuerdo con su diagnóstico, requirieron el uso de antibioticoterapia.

La investigación se inició con la selección y revisión de las historias clínicas de los pacientes pediátricos, recolectando los datos relacionados con la anamnesis y farmacoterapia indicada, durante la consulta ambulatoria. Para el análisis, se determinaron las enfermedades de mayor prevalencia y los antibióticos más prescritos durante el periodo de estudio, comparando la correspondencia entre la prescripción emitida y la indicada en el protocolo terapéutico del Ministerio de Salud Pública de Ecuador (MSP), a fin de identificar el cumplimiento del uso racional de los medicamentos en las patologías descritas, y que constituye el objetivo principal de este documento oficial [13].

Se analizaron 91 historias clínicas, registrando los datos para su tabulación en una base de datos en Microsoft Excel 2016 y procesados estadísticamente con el programa SPSS® (Statistical Product and Service Solutions) versión 2019.

Por no manipularse muestras biológicas en el estudio, no fue necesario solicitar el aval ante un comité de bioética en experimentación humana. Para la revisión de la información desde las historias clínicas de los pacientes, se contó con la autorización de la unidad de salud, garantizando la confidencialidad de la información.

RESULTADOS Y DISCUSIÓN

Durante el período de estudio, se identificaron cinco diagnósticos (tabla 1), siendo las infecciones respiratorias superiores, las reportadas con mayor frecuencia y entre estas, la más prevalente, resultó ser la amigdalitis. Estos resultados fueron comparables con la literatura donde se señala que, las infecciones respiratorias pediátricas se presentan entre seis y ocho veces al año [14, 15]. De igual manera en la tabla 1 se muestran los medicamentos de primera y segunda elección indicados en el protocolo terapéutico del MSP, para cada una de las enfermedades identificadas en esta investigación.

Tabla 1. Prevalencia de enfermedades en los pacientes pediátricos y medicamentos de elección según protocolo MSP- Ecuador para cada diagnóstico.

Enfermedad	Historias clínicas		Medicamentos de elección según protocolo MSP
	N.º	(%)	
Amigdalitis	35	38,46	1. Penicilina benzatínica* 2. Azitromicina*
Hipertrofia adenoidea	18	19,78	1. Amoxicilina+ Ac. Clavulánico 2. Cefalexina
Enfermedad diarreica aguda (EDA)	15	16,48	1. Sales de rehidratación oral 2. Ampicilina 3. Cotrimoxazol
Otitis media aguda (OMA)	12	13,19	1. Amoxicilina 2. Amoxicilina+ Ac. Clavulánico 3. Azitromicina
Bronquitis	11	12,09	1. Amoxicilina 2. Salbutamol
Total	91	100,00	

*Medicamentos de elección para amigdalitis de tipo estreptocócica.

La antibioticoterapia indicada al inicio de la enfermedad suele ser empírica, ya que generalmente el microorganismo responsable, se desconoce; sin embargo, existen datos clínicos que orientan sobre la posible etiología de la infección, debiendo limitarse el espectro antibacteriano a cubrir, a las bacterias más probablemente implicadas que, en el caso de la amigdalitis, es el estreptococo la bacteria más común, responsable de la infección [16,17].

Resulta fundamental, la responsabilidad en el momento de la prescripción, pues la inadecuada distinción entre la amigdalitis bacteriana y vírica, puede conllevar al uso irracional de antibióticos y sus consecuencias, mientras que, una amigdalitis estreptocócica no tratada con antibiótico o tratada de forma incompleta puede diseminarse a otras partes del organismo y causar problemas más graves como otitis o sinusitis [18].

El tratamiento de elección para la amigdalitis aguda, descrito en diversos manuales de farmacoterapia a nivel mundial y de Ecuador, resulta ser la penicilina benzatínica, principalmente por la rara resistencia del estreptococo a este antibiótico y por ser el único que ha demostrado su eficacia en la reducción de la incidencia del reumatismo articular

agudo (RAA); aun cuando, el tratamiento se administra en una dosis única, debe disponerse de los recursos inherentes a una administración parenteral [13, 19]. En este sentido, resulta una alternativa la amoxicilina por vía oral y para el caso de pacientes alérgicos a la penicilina, se indican los macrólidos, especialmente la azitromicina [20].

En la tabla 2 se recogen los antibióticos prescritos con mayor frecuencia durante el estudio y para cada una de las enfermedades anteriormente identificadas.

Tabla 2. Antibióticos de mayor prescripción durante el estudio y por enfermedad.

Antibiótico	Frecuencia prescripción	(%)	Prescripción en									
			Amigdalitis		Hipertrofia adenoidea		EDA		OMA		Bronquitis	
			n	%	n	%	n	%	n	%	n	%
Azitromicina	33	36,26	19	20,88	6	6,59	3	3,30	0	0,00	5	5,49
Amoxicilina	14	15,38	2	2,20	5	5,49	1	1,10	5	5,49	1	1,10
Claritromicina	13	14,29	6	6,59	2	2,20	1	1,10	1	1,10	3	3,30
Amoxicilina+ Ac. clavulánico	11	12,09	2	2,20	2	2,20	0	0,00	6	6,59	1	1,10
Cotrimoxazol	10	10,99	0	0,00	0	0,00	10	10,99	0	0,00	0	0,00
Penicilina benzatínica	8	8,79	5	5,49	3	3,30	0	0,00	0	0,00	0	0,00
Cefalexina	2	2,20	1	1,10	0	0,00	0	0,00	0	0,00	1	0,00
Total	91	100,00	35	38,46	18	19,78	15	16,48	12	13,19	11	1,10

Durante esta investigación, las diferentes enfermedades diagnosticadas fueron en su mayoría las infecciones del tracto respiratorio superior (83,52%), las cuales debieron ser tratadas con antibióticos betalactámicos, tal como lo establecen los protocolos terapéuticos oficiales; sin embargo, se identificaron como antibióticos más prescritos al grupo de los macrólidos en un total de 50,55%, que correspondieron un 36,26% a azitromicina y 14,29% a claritromicina. Estos resultados fueron equivalentes a estudios reportados a nivel mundial, en los que se han comunicado un aumento en el consumo de estos fármacos, ubicándose como el segundo grupo de antibióticos más prescritos en la edad pediátrica [21].

Entre las razones que pueden justificar el aumento en el empleo de este tipo de antibióticos e inclusive con mayor frecuencia que los betalactámicos, indicados como primera elección en infecciones respiratorias, puede mencionarse que, los azálidos además de su actividad antimicrobiana, poseen un efecto inmunomodulador y antiinflamatorio que les otorga un posible rol en el tratamiento de enfermedades inflamatorias respiratorias [22]. De igual manera, algunos de estos antibióticos como la azitromicina, presentan características farmacocinéticas que permiten administrarlos en esquemas posológicos simplificados, lo que por conveniencia resulta útil para la administración en pacientes pediátricos, como se establece en el protocolo terapéutico del MSP Ecuador [13, 23].

Teniendo en cuenta la información presentada en la tabla 1, sobre los antibióticos de primera y segunda elección establecidos en el protocolo terapéutico del MSP para cada una de las enfermedades identificadas en este estudio, se estimó la correspondencia entre los prescritos durante el período de investigación y los indicados en el documento oficial, resultados que se presentan en la tabla 3.

Tabla 3. Antibioticoterapia de primera y segunda elección según el protocolo del MSP y porcentaje empleado para cada enfermedad durante el estudio.

Enfermedad	Antibiótico								Total empleados	
	1° elección	N.º %		2° elección	N.º %		No establecidos	N.º %		N.º %
Amigdalitis	Penicilina benzatinica	5	14,28	Azitromicina	19	54,29	Otros	11	31,42	35 100
Hipertrofia adenoidea	Amoxicilina+ Ac. Clavulánico	2	11,11	Cefalexina	0	0,00	Otros	16	88,89	18 100
EDA	Ampicilina	0	0,00	Cotrimoxazol	10	66,67	Otros	5	33,33	15 100
OMA	Amoxicilina	5	41,67	Amoxicilina+ Ac. Clavulánico	6	50,00	Otros	1	8,33	12 100
Bronquitis	Amoxicilina	1	9,10	---	---	---	Otros	10	90,90	11 100
Total		13	14,29		35	38,46		43	47,25	91 100

Los resultados anteriormente expuestos permitieron determinar que, solo en la otitis media aguda (OMA) se cumplió en cierta medida con lo establecido en el protocolo MSP, ya que, el tratamiento de primera elección, amoxicilina, alcanzó un 41,67%, cercano al 50% obtenido por las prescripciones de amoxicilina + ac. clavulánico, que fue la segunda opción de tratamiento. Por su parte en otras enfermedades como, en la hipertrofia adenoidea y la bronquitis, la farmacoterapia empleada en ambos casos fue de 88,89% y 90,90%, respectivamente, y se fundamentó en otros antibióticos distintos a la primera y segunda elección de tratamiento, indicados en el protocolo MSP, no cumpliéndose así con lo establecido. Puede observarse entonces, que la prescripción de los antibióticos de segunda elección con 38,46% superó a los de primera elección que solo alcanzaron el 14,29% y estos a su vez, fueron superados por la prescripción de otros antibióticos (con un 47,25%) que no son descritos, ni como de primera o segunda elección en los protocolos terapéuticos oficiales.

Una de las razones que podría explicar por qué en la OMA, se ha empleado con más frecuencia amoxicilina + ac. clavulánico que solo la amoxicilina, es que los β -lactámicos conforman los antibióticos más utilizados para el tratamiento de enfermedades infecciosas en la práctica clínica pediátrica, por lo que, su uso indiscriminado ha contribuido a la aparición de mecanismos de resistencia de las bacterias grampositivas y gramnegativas, prefiriéndose entonces, la utilización de la combinación con el ácido clavulánico, pues presenta un efecto posinhibidor de β -lactamasas mayor, favoreciendo el efecto posantibiótico de amoxicilina [24].

Por último, en la tabla 4 se muestran situaciones registradas en las historias clínicas que permitieron identificar, algunos pacientes con segundas consultas y con sintomatología similar al diagnóstico inicial, particularmente para la amigdalitis (cuatro casos) y la OMA (un caso). Así se encontró que, para estas infecciones ya se habían prescrito antibioticoterapia; sin embargo, se realizó una nueva indicación antibiótica.

Tabla 4. Situaciones de segunda consulta con sintomatología similar al diagnóstico inicial, que conllevaron a nueva prescripción antibiótica durante el periodo de estudio.

Diagnóstico inicial	Antibiótico prescrito según diagnóstico inicial	Duración tratamiento inicial (días)	Tiempo en el que ocurre segunda consulta con síntomas del diagnóstico inicial (días)	Antibiótico prescrito en segunda consulta	Duración tratamiento segunda consulta (días)
OMA	Amoxicilina + Ac. clavulánico	7	15	Azitromicina	7
Amigdalitis	Azitromicina	3	8	Azitromicina	5
	Azitromicina	5	15	Amoxicilina + Ac. clavulánico	7
	Claritromicina	7	8	Azitromicina	5
	Penicilina benzatínica	1	8	Azitromicina	5

Estos resultados demostraron que, para la amigdalitis, tres de los cuatro casos reportados correspondieron a antibióticos del grupo de los macrólidos y uno para la penicilina benzatínica, aun cuando fue esta última, el fármaco de primera elección para esta enfermedad. Es importante señalar que, las causas que pudieron dar paso a estas situaciones pueden ser múltiples y muy complejas de determinar con la escasa información disponible en las historias clínicas; no obstante, es relevante observar cómo, de forma general, las segundas consultas en todos los casos se sucedieron en 1 o 2 semanas posteriores a la primera consulta y que de igual forma se indicaron antibióticos por segunda ocasión que, en algunos casos, debieron prescribirse desde el inicio [25].

CONCLUSIONES

El análisis de la prescripción de antibióticos en pacientes pediátricos permitió corroborar que las infecciones respiratorias superiores son frecuentes en este grupo etario, para su tratamiento se emplea la antibioticoterapia que en muchas ocasiones (47,25%) no se ajustó con los fármacos de primera y segunda elección, establecidos en el protocolo terapéutico oficial del MSP-Ecuador. En tal sentido, queda en evidencia la necesidad

de revisar y actualizar en forma continua dichos documentos, a fin de que puedan adaptarse a las realidades epidemiológicas y al dinámico panorama actual de la resistencia antimicrobiana.

Aunque el objetivo de esta investigación no fue catalogar como adecuada o inadecuada la prescripción de antibióticos, los resultados permitieron confirmar la inminente necesidad de tomar conciencia sobre el uso racional de estos fármacos sobre todo en pacientes pediátricos y más aún, de contar con protocolos oficiales que constituyan verdaderas guías farmacoterapéuticas.

CONFLICTO DE INTERESES

Los autores no declaran conflicto de intereses.

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CÓMO CITAR ESTE ARTÍCULO

A. Rincón-Alarcón, J. Quispillo-Moyota, S. Cando-Punguil, V. Rodríguez-Vinueza, Abordaje de la antibioticoterapia prescrita en pacientes pediátricos de una unidad de salud, Tungurahua -Ecuador, *Rev. Colomb. Cienc. Quím. Farm.*, 51(3), 1434-1447 (2022). DOI: <http://dx.doi.org/10.15446/rcciquifa.v51n3.102229>

Artigo de pesquisa clínica / <http://dx.doi.org/10.15446/rcciquifa.v51n3.103123>

A incidência da polifarmácia em pacientes atendidos pelo *Programa Melhor em Casa* (MemC)

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Recebido: 15 de junho de 2022

Revisado: 9 de julho de 2022

Aceto: 16 de julho de 2022

RESUMO

Objetivo: avaliar a incidência da polifarmácia em pacientes em atendimento domiciliar pelo *Programa Melhor em Casa*. **Material e métodos:** foram avaliados 28 prontuários de pacientes em atendimento pelo MemC na cidade de Goiátuba-GO, no período de dezembro de 2020 a julho de 2021. Foi avaliado o perfil do paciente e as variáveis idade, sexo e medicamentos utilizados. **Resultados e discussão:** a maioria dos pacientes são do gênero feminino, 64,3% (n=18) em oposição a 35,7% do gênero masculino (n=10). Durante a pesquisa 5 pacientes faleceram (17,24%). A idade média dos pacientes foi de $67,3 \pm 19,0$ anos; desse total 28,57% (n=8) possuem idade entre 18 e 59 anos; e, os outros 71,43% (n=20) idades acima de 60 anos, caracterizando-se como idosos. A média de medicamentos utilizados foi de $5,7 \pm 3,3$ medicamentos/paciente, sendo maior entre os pacientes idosos, $6,0 \pm 3,5$. **Conclusão:** a utilização exacerbada de medicamentos pode provocar aparecimento de reações adversas que, por vezes, podem ser confundidas com novos sintomas. A terapia farmacológica não deve ser a única alternativa do paciente, ela deve estar associada com acompanhamento nutricional, físico, psicológico.

Palavras-chave: atendimento domiciliar, envelhecimento, tratamento farmacológico, qualidade de vida.

SUMMARY

The incidence of polypharmacy in patients assisted by the *Programa Melhor em Casa* (MemC)

Aim: To evaluate the incidence of polypharmacy in patients attended at home by the *Programa Melhor em Casa Program*. **Material and methods:** 28 medical records of patients attended by MemC in the city of Goiatuba-GO, from December 2020 to July 2021 were evaluated. The patient profile and the variables age, sex and medications used were evaluated. **Results and discussion:** the majority of patients were women, 64.3% (n=18) compared to 35.7% of men (n=10). During the study 5 patients died (17.24%). The mean age of the patients was 67.3 ± 19.0 years; of this total, 28.57% (n=8) were between 18 and 59 years old; and, the other 71.43% (n=20) were over 60 years old, characterizing them as elderly. The mean number of drugs used was 5.7 ± 3.3 drugs/patient, being higher among elderly patients, 6.0 ± 3.5 . **Conclusion:** The exacerbated use of drugs can lead to the appearance of adverse reactions that can sometimes be confused with new symptoms. Pharmacological therapy should not be the only alternative for the patient, but should be associated with nutritional, physical and psychological support.

Keywords: home care, aging, pharmacological treatment, quality of life.

RESUMEN

Incidencia de la polifarmacia en los pacientes asistidos por el *Programa Melhor em Casa* (MemC)

Objetivo: evaluar la incidencia de la polifarmacia en los pacientes atendidos a domicilio por el Programa Melhor em Casa. **Material y métodos:** se evaluaron 28 historias clínicas de pacientes atendidos por MemC en la ciudad de Goiatuba-GO, desde diciembre de 2020 hasta julio de 2021. Se evaluó el perfil del paciente y las variables edad, sexo y medicamentos utilizados. **Resultados y discusión:** la mayoría de los pacientes son mujeres, el 64,3% (n=18) frente al 35,7% de hombres (n=10). Durante el estudio murieron 5 pacientes (17,24%). La edad media de los pacientes fue de $67,3 \pm 19,0$ años; de este total, el 28,57% (n=8) tenían entre 18 y 59 años; y, el otro 71,43% (n=20) tenían más de 60 años, caracterizándose como ancianos. El número medio de fármacos utilizados fue de $5,7 \pm 3,3$ fármacos/paciente, siendo

mayor entre los pacientes de edad avanzada, $6,0 \pm 3,5$. **Conclusión:** el uso exacerbado de fármacos puede provocar la aparición de reacciones adversas que a veces pueden confundirse con nuevos síntomas. La terapia farmacológica no debe ser la única alternativa para el paciente, sino que debe ir asociada a un acompañamiento nutricional, físico y psicológico.

Palabras clave: atención domiciliaria, envejecimiento, tratamiento farmacológico, calidad de vida.

INTRODUÇÃO

O cuidado domiciliar é uma prática remota, na Europa da Idade Média essa prática era amplamente utilizada, mesmo antes da institucionalização dos hospitais [1]. Em 1947, a primeira unidade de assistência domiciliar surgiu nos Estados Unidos e teve como motivações iniciais descongestionar o hospital e proporcionar aos pacientes e familiares um ambiente psicológico mais favorável [2].

Em comparação com as instituições em um ambiente regulamentado, o atendimento domiciliar oferece aos indivíduos maior liberdade. No Brasil, provavelmente, a primeira experiência de cuidado domiciliar tenha sido a desenvolvida pelo Serviço de Assistência Médica Domiciliar e de Urgência- Samdu, criado em 1949 [3].

A atenção domiciliar (AD), no âmbito do Sistema Único de Saúde (SUS), foi instituída pela Portaria nº 2029 de 24 de agosto de 2011 [4] sendo revogada e alterada no mesmo ano pela Portaria nº 2527 de 27 de outubro de 2011 [5]. Após sofrer alterações a Portaria nº 2527/2011 foi revogada pela Portaria nº 963/2013 [6].

Atualmente, a Portaria nº 825 de 25 de abril de 2016 traz as definições atualizadas e as equipes habilitadas para o atendimento domiciliar. A AD é indicada para pessoas que, estando em estabilidade clínica, necessitam de atenção à saúde em situação de restrição ao leito ou ao lar de maneira temporária ou definitiva ou em grau de vulnerabilidade [7].

O *Programa Melhor em Casa* (MemC) iniciou-se em novembro de 2011 pelo Governo Federal com o objetivo de ampliar o atendimento domiciliar pelo SUS [8]; promovendo assistência gratuita e multiprofissional em domicílio [9]. Existem bons motivos para o atendimento domiciliar, uma vez que o lar é um lugar emocional, com memórias e conforto, e para algumas pessoas sair de casa pode ser perturbador e deprimente [10]. A atenção domiciliar, em alguns casos, é considerada a oferta mais oportuna para tratamento, palição, reabilitação e prevenção de agravos, tendo em vista a ampliação de autonomia do usuário, família e cuidador [7].

O atendimento domiciliar é voltado à humanização da medicina e dos atendimentos em saúde, considerando a integralidade do indivíduo [11]. Esse programa possui abordagem multiprofissional; as equipes do programa MemC são formadas por médicos, enfermeiros, técnicos em enfermagem, fisioterapeutas, assistentes sociais, fonoaudiólogos, nutricionistas, terapeutas ocupacionais, odontólogos, psicólogos e farmacêuticos [12].

O Brasil possui 5570 municípios [13] e o *Programa Melhor em Casa* está em 732 municípios brasileiros, com mais de 1,6 mil equipes multiprofissionais ativas e já alcançou mais de 28,9 milhões de procedimentos [14]. Pela quantidade de municípios, a cobertura do MemC representa apenas 13,14% do território nacional. Isso porque não é qualquer município que está apto para aderir ao *Programa Melhor em Casa*. O município deve possuir uma população maior ou igual a 20 000 (vinte mil) habitantes e possuir serviço de hospitalização ou hospital de referência dentro da sua região de saúde e serviço móvel de urgência (SAMU) ou serviço equiparante [15].

O envelhecimento aumenta a prevalência de diversas afecções, principalmente as de caráter crônico e em condições de sobrecarga como, por exemplo, doenças, acidentes e estresse emocional. Como consequência, dessas doenças crônicas ligadas ao envelhecimento, observa-se o aumento da quantidade de medicamentos necessários. Algumas vezes os idosos passam a ter uma alimentação deficitária, além de se tornarem mais sedentários e desenvolverem transtornos mentais. No entanto, pacientes complexos representam diferentes segmentos da população e requerem abordagens que enfoquem as necessidades específicas [16].

O medicamento é um produto farmacêutico com finalidade profilática, curativa, paliativa ou para fins de diagnóstico [17]. Esses produtos são amplamente utilizados pela população tanto para uso contínuo, como no caso de tratamento de doenças, uso esporádico, como alívio de dores, e até mesmo auxiliar de diagnóstico, como é o caso dos contrastes. A tomada de medicamentos envolve sequência de etapas –prescrição, comunicação, dispensação, administração e acompanhamento clínico– o que a torna um ato complexo e vulnerável às iatrogenias, particularmente em idosos [18].

A polifarmácia é definida como o uso simultâneo e rotineiro de cinco ou mais medicamentos, incluindo os medicamentos prescritos e/ou medicamentos tradicionais e complementares usado por um paciente [19]. E grande parte dos idosos que tem acesso a serviços de saúde fazem uso da polifarmácia. Em um estudo realizado por Ramos *et al.* [20] concluíram que o risco de polifarmácia aumenta significativamente na presença de diabetes, problemas cardíacos, hipertensão, doenças reumáticas e pulmonares. Além de implicar em um risco aumentado de problemas relacionados a medicamentos, como interações e reações adversas a medicamentos, subutilização de tratamentos necessários, baixa adesão e mortalidade parcialmente evitável [21].

Em 2012, foi estabelecido a Relação Nacional de Medicamentos Essenciais (RENAME) no âmbito do SUS que compreende a seleção e a padronização de medicamentos indicados para atendimento de doenças ou de agravos no âmbito do SUS [22]. Ela foi elaborada atendendo aos princípios da universalidade, efetividade, eficiência, comunicação, racionalidade e serviços farmacêuticos qualificados. Esses princípios conferem que os usuários do SUS terão acesso igualitário à medicamentos eficientes e de forma racional e humanizada.

Para a Organização Mundial da Saúde (OMS, no inglês WHO) [23], medicamentos essenciais são aqueles que satisfazem às necessidades de saúde prioritárias da população, os quais devem ser selecionados por critérios de eficácia, segurança, conveniência, qualidade e custo favorável. A RENAME cumpre papel estratégico nas políticas de saúde, ao relacionar os medicamentos utilizados no âmbito do SUS:

“A atualização permanente da Rename, como instrumento promotor do uso racional e lista orientadora do financiamento e acesso a medicamentos no âmbito da Assistência Farmacêutica, torna-se um grande desafio para os gestores do SUS, diante da complexidade das necessidades de saúde da população, da velocidade da incorporação tecnológica e dos diferentes modelos de organização e financiamento do sistema de saúde [...]

A disponibilidade ambulatorial de medicamentos no Sistema Único de Saúde (SUS) ocorre por meio dos Componentes da Assistência Farmacêutica, sendo: Componente Básico, Componente Estratégico e Componente Especializado, que possuem características, forma de organização, financiamento e elenco de medicamentos diferenciados entre si, bem como critérios distintos para o acesso e a disponibilização dos fármacos [...]

O Componente Básico da Assistência Farmacêutica (Cbaf) inclui os medicamentos que tratam os principais problemas e condições de saúde da população brasileira na Atenção Primária à Saúde [...]

O Componente Estratégico da Assistência Farmacêutica (Cesaf) destina-se ao acesso dos medicamentos e insumos destinados aos agravos com potencial de impacto endêmico e às condições de saúde caracterizadas como doenças negligenciadas, que estão correlacionadas com a precariedade das condições socioeconômicas de um nicho específico da sociedade [...]

O Componente Especializado da Assistência Farmacêutica (Ceaf) é uma estratégia de acesso a medicamentos, no âmbito do SUS, para doenças crônico-degenerativas, inclusive doenças raras, e é caracterizado pela busca da garantia da

integralidade do tratamento medicamentoso, em nível ambulatorial, cujas linhas de cuidado estão definidas em Protocolos Clínicos e Diretrizes Terapêuticas (PCDT) publicados pelo Ministério da Saúde” [24].

Osorio-de-Castro *et al.* [25] apontam que os gerentes dos sistemas de saúde e os prescritores devem se atentar as listas de medicamentos essenciais (LME), à educação farmacoterapêutica do prescritor para uso racional e diretrizes sustentáveis para doenças prioritárias. A padronização de medicamentos e produtos para saúde é o início da assistência farmacêutica, cuja elaboração desencadeia uma cascata de benefícios que facilitam e melhoram a gestão de todas as atividades que os envolvam [26].

A Relação Municipal de Medicamentos Essenciais (REMUME) deve ser elaborada para atender às necessidades específicas da população de cada município, tendo a RENAME como modelo [27]. Juntamente com a padronização, a seleção e aquisição de medicamentos devem ser realizados de forma responsável. A seleção admite medicamentos eficazes e seguros de acordo com as necessidades de uma população específica e, se bem conduzida, resulta em ganhos terapêuticos e econômicos, seja para instituições públicas ou privadas [26].

O objetivo do presente artigo foi avaliar a incidência da polifarmácia em pacientes em atendimento domiciliar pelo *Programa Melhor em Casa*.

MATERIAL E MÉTODOS

O projeto foi aprovado pelo Comitê de Ética da Universidade Estadual de Goiás, sob o número 4.348.814. Foram avaliados 28 pacientes em atendimento pelo MemC na cidade de Goiatuba-GO, no período de dezembro de 2020 a julho de 2021. Os pacientes e responsáveis foram informados sobre o objetivo da pesquisa e solicitado aos mesmos a assinatura do Termo de Consentimento e Livre Esclarecimento (TCLE) ou o Termo de Assentimento Livre Esclarecido (TALE), sendo o último assinado na impossibilidade do próprio paciente assinar.

A pesquisa foi realizada através da análise dos prontuários, onde foi avaliada o perfil do paciente e as variáveis idade, sexo e medicamentos utilizados. Essa análise foi realizada na sala da coordenação do *Programa Melhor em Casa*, onde os prontuários são acondicionados.

Foi realizada análise estatística. Os resultados foram expressos como média $\pm$ desvio padrão. Para os testes estatísticos foi adotado um nível de significância de 5% e todos foram considerados como bilaterais. Para a comparação das medidas numéri-

cas, quando a distribuição é normal foram aplicados testes-t. Para a comparação das medidas categóricas foi utilizado o teste exato de Fisher. A associação entre as medidas numéricas foi analisada com o coeficiente de correlação de Spearman.

RESULTADOS E DISCUSSÃO

Foram avaliados 28 pacientes. Na tabela 1 está apresentado o perfil dos pacientes que, em sua maioria, são do gênero feminino 64,3% (n=18) em oposição a 35,7% do gênero masculino (n=10). Durante a pesquisa 5 pacientes faleceram (17,24%). A idade média dos pacientes foi de $67,3 \pm 19,0$ anos; desse total 28,57% (n=8) possuem idade entre 18 e 59 anos; e, os outros 71,43% (n=20) idades acima de 60 anos, caracterizando-se como idosos. A média de medicamentos utilizados foi de $5,7 \pm 3,3$ medicamentos/paciente, sendo maior entre os pacientes idosos, $6,0 \pm 3,5$.

Tabela 1. Características sociodemográficas e clínicas dos pacientes atendidos pelo *Programa Melhor em Casa*, na cidade de Goiatuba-GO.

Medida	Total (n = 28)	Idade	
		< 60 (n = 8)	>= 60 (n = 20)
Idade (anos)	$67,3 \pm 19,0$	$42,6 \pm 13,1$	$77,2 \pm 9,6$
Sexo			
Masculino	10 (35,7%)	2 (25,0%)	8 (40,0%)
Feminino	18 (64,3%)	6 (75,0%)	12 (60,0%)
Qtd. medicamentos	$5,7 \pm 3,3$	$4,8 \pm 2,6$	$6,0 \pm 3,5$

Legenda. n: número de pacientes. Qtd: Quantidade.

Para verificar se houve a associação entre idade e quantidade de medicamentos, foi calculada a correlação de Spearman. Não foi verificada associação estatisticamente significativa entre idade e quantidade de medicamentos, $p = 0,784$.

Verificou-se que um pouco mais da metade dos participantes, 57% ($n = 16$), está classificado como polifarmácia (Tabela 2). A idade média observada desses pacientes, $70,4 \pm 12,5$ anos, é um pouco maior que os que usam menos medicamentos, $63,1 \pm 25,3$. A polifarmácia é consequência das comorbidades, quanto mais comorbidades, mais medicamentos utilizados. As patologias mais frequentemente encontradas foram Hipertensão Arterial, Acidente Vascular Cerebral (AVC) e Diabetes. Durante a análise dos dados, foram encontrados que entre os pacientes avaliados foram registrados o uso de 78 fármacos diferentes.

Tabela 2. Perfil dos pacientes conforme classificação de polifarmácia.

Medida	Total ($n = 28$)	Paciente polifarmácia	
		Não ($n = 12$)	Sim ($n = 16$)
Idade (anos)	$67,3 \pm 19,0$	$63,1 \pm 25,3$	$70,4 \pm 12,5$
Sexo			
Masculino	10 (35,7%)	3 (33,3%)	7 (36,8%)
Feminino	18 (64,3%)	6 (66,7%)	12 (63,2%)
Qtd. comorbidades	$4,5 \pm 2,0$	$4,0 \pm 1,7$	$4,9 \pm 2,1$

Legenda. n: número de pacientes. Qtd: Quantidade.

Foram encontrados 78 fármacos diferentes nos prontuários. Entre os fármacos prescritos e encontrados nos prontuários, o líder foi a losartana potássica, com prescrição para 12 (doze) pacientes. A losartana potássica é um Antagonista de Receptor de Angiotensina II, utilizado como anti-hipertensivo e vasodilatador [28]. A losartana potássica é o medicamento mais consumido por idosos e o mais vendido no Brasil [29]. As principais classes terapêuticas prescritas foram antiagregantes plaquetários (7,33%), anti-hipertensivos (25,33%), antipêêmico (6,66%), hipoglicemiantes (5,33%) e antidepressivos (8%), que corresponderam à 52,65% dos medicamentos prescritos (Tabela 3).

Tabela 3. Prevalência do consumo de medicamentos pelos pacientes, segundo os prontuários analisados.

Classe terapêutica	Quantidade	%
Anti-hipertensivo	38	25,33
Antidepressivo	12	8,00
Antiagregante plaquetário	11	7,33
Antilipêmico	10	6,67
Hipoglicemiante	8	5,33
Vitaminas ou Suplementos	6	4,00
Ansiolítico	6	4,00
Neurolépticos	5	3,33
Inibidor da Bomba de Prótons	4	2,67
Analgésicos	3	2,00
Anticoagulante	3	2,00
Outros	44	29,33

Entre os 78 fármacos prescritos, 48 fármacos (61,53%) estão presentes na RENAME, e desses, apenas 17 fármacos (21,79%) estão presentes na REMUME do município em estudo [30]. Inúmeras vezes os medicamentos tomam-se uma parcela importante do rendimento familiar, prejudicando a qualidade de alimentação, lazer e adimplência da família.

A presença de comorbidades proporciona o uso de medicamentos e, como consequência pode desencadear a polifarmácia. Akgün *et al.* [31] alertam que nem todos os medicamentos são clinicamente adequados e alguns podem até contribuir para causar danos; exemplificando, os medicamentos hipoglicêmicos, mesmo que necessários, podem causar diminuição da atividade por hipoglicemia e ganho de peso [32]. A revisão periódica de medicamentos utilizados por idosos deve fazer parte intrínseca da prática clínica [18].

Os efeitos terapêuticos de certos medicamentos podem diminuir com a multimorbidade e fragilidade e podem justificar uma reavaliação dos benefícios potenciais em comparação com seus danos [31]. Com o avanço da idade, os aspectos farmacocinéticos (absorção, distribuição, metabolismo e excreção) do paciente sofrem alteração, contribuindo com o aparecimento de efeitos tóxicos. Em um estudo realizado por Auvinen *et al.* [33], apontam que mais de 80% dos pacientes em atendimento domiciliar usaram medicamentos que afetam a função renal. Há a necessidade de estratégias que possam

orientar os médicos clínicos sobre como proporcionar os benefícios do tratamento medicamentoso para esses pacientes, mas ao mesmo tempo evitar consequências negativas [34]. Assim:

“Com o avanço da idade, a quantidade de água no corpo diminui, reduzindo o volume de distribuição dos medicamentos hidrofílicos [...] O aumento da gordura corporal, aumenta o volume de distribuição de drogas lipossolúveis e, portanto, a meia-vida do fármaco. O tempo para atingir o estado de concentração é prolongado. A absorção intestinal do medicamento é menor devido à diminuição de seu movimento. Há diminuição da função hepática e de drogas metabolizadas e excretadas pelo fígado” [35].

A documentação insuficiente e a troca de informações relacionadas à medicação entre os profissionais de saúde são uma causa potencial de erros de prescrição e reações adversas ao medicamento [36]. É importante que em cada visita ao paciente, os medicamentos sejam analisados e avaliados a necessidade de continuarem sendo administrados. Yildirim e Kilinç [35] afirmam que quanto menor a quantidade de medicamentos, menor será o número de interações medicamento-medimento e medicamento-dieta:

“Identificar Problemas Relacionados aos Medicamentos (PRM), duplicações de medicamentos, interações medicamentosas, suspeita de reação adversa ao medicamento, monitoramento de drogas terapêuticas, medicamentos desnecessários e manejos não farmacológicos inadequados” [37].

A aquisição de medicamentos pode consumir grande parte dos recursos financeiros do paciente, fazendo com que inúmeras vezes medicamentos considerados mais importantes sejam prioritários no momento da compra. A terapia farmacológica deve ser adotada com o menor número de fármacos possível; e os fármacos de ação prolongada devem ser preferidos. Lin *et al.* [37] demonstraram que um programa colaborativo médico-farmacêutico direcionado a pacientes geriátricos de polifarmácia proporciona economia de custos.

No estudo, 57% dos pacientes podem ser considerados em uso de polifarmácia, e esse hábito pode aumentar a quantidade de interações medicamentosas e reações adversas aos medicamentos. Outro ponto importante é que medicamentos de venda livre e fitoterápicos mais comuns, geralmente, não são relatados ao médico [35]. Os resultados contabilizados consideraram apenas os medicamentos listados no prontuário médico do paciente, podendo esse número ser maior caso seja considerado os medicamentos utilizados esporadicamente, por indicação de terceiros e a automedicação.

A terapia farmacológica pode consumir grande parte da renda familiar, principalmente quando a terapia prescrita não está na Relação Municipal de Medicamentos Essenciais (REMUME), causando impactos na alimentação, lazer, adimplência no comércio, entre outros.

Dos 16 pacientes em uso de polifarmácia, 12 pacientes (63,2%) são mulheres e 7 pacientes (36,8%) são homens. Entre os idosos, 87,5% dos pacientes estão em uso de polifarmácia, entre eles 10 são mulheres (71,42%), enquanto que 4 são homens (28,58%). O aumento da prevalência da polifarmácia entre as mulheres também foi encontrado por Romano-Lieber *et al.* [38], com valores de 36,6 e 26,9%, respectivamente, para mulheres e homens idosos.

Visto que a polifarmácia está presente na maioria dos pacientes, é importante a atuação do farmacêutico no âmbito da atenção à saúde, exercendo ações de educação, orientação e intervenções. O farmacêutico é o profissional de saúde mais adequado da atenção primária para gerenciar as atividades objetivadas à melhoria do acesso e promoção do uso racional de medicamentos [26].

Conceição *et al.* [29] explicam que o farmacêutico identifica os problemas relativos aos medicamentos, efeitos colaterais, interações medicamentosas, uso incorreto de medicamentos, além de elaborar e implementar o plano de atenção farmacêutica. O farmacêutico clínico pode direcionar a revisão para problemas clinicamente relevantes e o médico pode fazer alterações na medicação do paciente na reunião de equipe quando todas as informações estiverem disponíveis [33].

CONCLUSÃO

A terapia farmacológica, sem dúvidas, é uma das ferramentas utilizadas mundialmente para controle de patologias, melhora de sintomas e, conseqüente melhoria da qualidade de vida do indivíduo. Entretanto, a utilização exacerbada desses medicamentos pode provocar aparecimento de reações adversas que, por vezes, podem ser confundidas com novos sintomas.

Outro fator que deve ser levado em consideração é o acompanhamento do paciente por diversas especialidades médicas, que pode aumentar o número de medicamentos prescritos. É importante orientar aos pacientes e cuidadores, que a cada consulta, seja informada ao médico assistente os medicamentos em uso, uma vez que pode ser necessário a substituição ou alteração de dose do fármaco.

A terapia farmacológica não deve ser a única alternativa do paciente, ela deve estar associada com acompanhamento nutricional, físico, psicológico. Todavia, uma vez que ela for necessária, deve ser feita levando-se em conta a REMUME, para que o paciente tenha acesso ao medicamento sem ter o comprometimento financeiro familiar. A REMUME jamais acolherá a totalidade dos medicamentos necessários da população, mas deve ser padronizada, selecionada e adquirida levando-se em consideração a população atendida. Realizar levantamentos epidemiológicos se faz necessário para programar a seleção em compra de medicamentos. O farmacêutico deve fazer parte das equipes multiprofissionais sempre que envolvam a utilização de medicamentos.

AGRADECIMENTOS

Agradecemos à Deus por nos permitir ampliar o conhecimento, à família por nos apoiar e à orientadora por nortear a nossa trajetória. A Universidade Estadual de Goiás, a FAPEG e a Capes pelo incentivo à pesquisa.

CONFLITOS DE INTERESSE

Os autores não relatam nenhum conflito de interesse.

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COMO CITAR ESTE ARTIGO

L.C. Alves de Sousa, B.R. Ferreira, K. Rodrigues-Tassara, J. Ballico de Moraes, I. Jubé-Wastowski, A incidência da polifarmácia em pacientes atendidos pelo *Programa Melhor em Casa* (MemC), *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1448-1463 (2022). DOI: <http://dx.doi.org/10.15446/rcciquifa.v51n3.103123>

Serum adipokines as biomarkers for the therapeutic monitoring of patients with inflammatory bowel diseases (IBDs) treated with infliximab: a systematic review and meta-analysis

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Received: March 22, 2022

Corrected: July 13, 2022

Accepted: July 18, 2022

SUMMARY

Introduction: Inflammatory bowel diseases (IBDs) are idiopathic inflammations of the colon, which can often undergo remission with the use of specific anti-TNF antibodies such as the infliximab. Although that the therapeutic monitoring can provide valuable insight into the possible etiology of unfavorable outcomes and allow for an appropriate management strategy for these patients, currently none technique or biomarker have proved ideal for the evaluation of therapeutic benefits of anti-TNF antibodies in IBDs. **Aim:** To summarise current knowledge on the role of serum adipokines as potential biomarkers for the therapeutic monitoring of patients with IBDs under infliximab. **Methods:** A systematic review was carried out in the PubMed/MEDLINE, Cochrane Library, Scopus and *Biblioteca Virtual em Saúde* databases. Next, the meta-analysis was performed with the mean values of serum adipokine levels in patients with IBDs before and after the use of infliximab using the Review Manager (RevMan) * 5.3 software. **Results:** Three studies that together included 58 patients diagnosed with IBDs and treated with inflix-

imab at 5 mg/kg were selected. According to the quantitative analysis, serum leptin levels were significantly increased after the use of infliximab (p -value=0.01; Heterogeneity: $I^2=61\%$), which is correlated with the clinical remission of the disease. In addition, the circulating adiponectin (p -value=0.006; Heterogeneity: $I^2=0\%$) and resistin (p -value=0.009; Heterogeneity: $I^2=93\%$) concentrations were both reduced after the clinical remission of IBD with the use of infliximab. **Conclusion:** Serum leptin levels are significantly increased, while circulating adiponectin and resistin is reduced among IBD patients after the use of infliximab.

Keywords: Therapeutic monitoring, Infliximab, Leptin, Adiponectin, Resistin, Inflammatory Bowel Diseases.

RESUMO

Adipocinas séricas como biomarcadores para monitoramento terapêutico de pacientes com doenças inflamatórias intestinais (DIIs) tratados com infliximabe: revisão sistemática e metanálise

Introdução: as doenças inflamatórias intestinais (DIIs) são inflamações idiopáticas do cólon, que muitas vezes podem sofrer remissão com o uso de anticorpos anti-TNF específicos, como o infliximabe. Embora o monitoramento terapêutico possa fornecer informações valiosas sobre a possível etiologia de desfechos desfavoráveis e permitir uma estratégia de manejo adequada para esses pacientes, atualmente nenhuma técnica ou biomarcador se mostrou ideal para a avaliação dos benefícios terapêuticos dos anticorpos anti-TNF em DIIs. **Objetivo:** resumir o conhecimento atual sobre o papel das adipocinas séricas como potenciais biomarcadores para o monitoramento terapêutico de pacientes com DII em uso de infliximabe. **Métodos:** foi realizada revisão sistemática nas bases de dados PubMed/MEDLINE, Cochrane Library, Scopus e Biblioteca Virtual em Saúde. Em seguida, foi realizada a meta-análise com os valores médios dos níveis séricos de adipocinas em pacientes com DII antes e após o uso de infliximabe, utilizando o software Review Manager (RevMan) * 5.3. **Resultados:** três estudos que juntos incluíram 58 pacientes diagnosticados com DII e tratados com infliximabe na dose de 5 mg/kg foram selecionados. De acordo com a análise quantitativa, os níveis séricos de leptina aumentaram significativamente após o uso de infliximabe (valor de $p=0,01$; Heterogeneidade: $I^2=61\%$), o que está correlacionado com a remissão clínica da doença. Além disso, as concentrações circulantes de adiponectina (valor de $p=0,006$; heterogeneidade: $I^2=0\%$) e

resistina (valor de $p=0,009$; heterogeneidade: $I^2=93\%$) foram ambas reduzidas após a remissão clínica da DII com o uso de infliximabe. **Conclusão:** os níveis séricos de leptina estão significativamente aumentados, enquanto a adiponectina e a resistina circulantes estão reduzidas entre os pacientes com DII após o uso de infliximabe.

Palavras-chave: Acompanhamento terapêutico, infliximabe, leptina, adiponectina, resistina, doenças inflamatórias intestinais.

RESUMEN

Adipocinas séricas como biomarcadores para el seguimiento terapéutico de pacientes con enfermedades inflamatorias intestinales (EII) tratados con infliximab: revisión sistemática y metanálisis

Introducción: las enfermedades inflamatorias intestinales (EII) son inflamaciones idiopáticas del colon, que en muchas ocasiones pueden entrar en remisión con el uso de anticuerpos anti-TNF específicos, como el infliximab. Aunque el seguimiento terapéutico puede proporcionar información valiosa sobre la posible etiología de los desenlaces desfavorables y permitir una estrategia de manejo adecuada para estos pacientes, actualmente ninguna técnica o biomarcador ha demostrado ser ideal para evaluar los beneficios terapéuticos de los anticuerpos anti-TNF en las EII. **Objetivo:** resumir el conocimiento actual sobre el papel de las adipocinas séricas como biomarcadores potenciales para el seguimiento terapéutico de pacientes con EII que utilizan infliximab. **Métodos:** Se realizó una revisión sistemática en las bases de datos PubMed/MEDLINE, Cochrane Library, Scopus y Virtual Health Library. Luego, se realizó un metanálisis con los valores medios de los niveles de adipoquinas séricas en pacientes con EII antes y después del uso de infliximab, utilizando el software Review Manager (RevMan)[®] 5.3. **Resultados:** se seleccionaron tres estudios que en conjunto incluyeron 58 pacientes diagnosticados de EII y tratados con infliximab a dosis de 5 mg/kg. Según el análisis cuantitativo, los niveles de leptina sérica aumentaron significativamente después del uso de infliximab (valor de $p = 0,01$; Heterogeneidad: $I^2 = 61\%$), lo que se correlaciona con la remisión clínica de la enfermedad. Además, las concentraciones circulantes de adiponectina (valor de $p = 0,006$; hete-

roogeneidad: $I^2 = 0 \%$) y resistina (valor de $p = 0,009$; heterogeneidad: $I^2 = 93 \%$) se redujeron después de la remisión clínica de la EII con el uso de infliximab. **Conclusión:** los niveles de leptina sérica aumentan significativamente, mientras que la adiponectina y la resistina circulantes se reducen entre los pacientes con EII después del uso de infliximab.

Palabras clave: Seguimiento terapéutico, infliximab, leptina, adiponectina, resistina, enfermedades inflamatorias intestinales.

INTRODUCTION

Inflammatory bowel diseases (IBDs) are chronic and idiopathic disorders that essentially cover Crohn's disease (CD) and ulcerative colitis (UC). IBDs are considered immune-mediated diseases without a definitive cure, since they have a chronic, progressive, and recurrent character, resulting from an inadequate immune response in genetically susceptible individuals [1-3]. Clinically, IBDs are characterized by intense inflammation of the intestinal mucosa, which evolves into structural and functional changes in the large and/or small intestine [1, 3]. Recently, a population-based systematic review showed that during the second half of the twentieth century, there was a significant increase in the incidence of IBDs in North America and Europe. Since 1990, this rate has been shown to be unstable or decreasing in these regions, but its prevalence continues to increase [4]. Currently, IBDs affect 1.3% of the American population, thus totalling 3.1 million cases [5]. Conversely, IBDs in developing countries still have a low prevalence, but with increasing incidence, so the total number of cases is expected to increase in the coming years in this region [4].

The treatment of IBDs is usually aimed at controlling acute phase episodes and, in the long term, disease remission. Pharmacological therapy is usually started with the use of corticosteroids, aminosalicylates, and immunomodulators [1, 2]. However, in cases of moderate to severe IBDs, which do not respond well to the first therapeutic options, the use of so-called biologics drugs is indicated [6, 7]. Infliximab was the first medication (prototype) of this class to be approved against IBDs, which is indicated for the treatment of adults and children from 6 years old who live with moderate to severe CD or UC [1, 2, 8]. It is a chimeric human-murine monoclonal IgG1 antibody that is capable of strongly binding to tumour necrosis factor (TNF). After binding to TNF, infliximab prevents it from interacting with its receptors, consequently resulting in the inhibition of pro-inflammatory pathways that trigger acute and recurrent episodes of IBDs [8]. Infliximab has a half-life of approximately 14 days, is adminis-

tered intravenously (iv) by weight-based dosing, and is typically dosed every 4–8 weeks following an initial loading period (0–2–6 weeks) [9]. However, the development of anti-drug antibodies (ATIs) directed against the Fab fragment of infliximab can be observed in patients with IBDs, which is an important mechanism involved in the loss of response. The ATIs interfere with the biological activity by inhibiting the binding of TNF inhibitors to both serum and membrane-bound TNF alpha (TNF- α) molecules and by creating immune complexes that are eliminated by the reticuloendothelial system [6, 9]. Thus, the therapeutic monitoring of TNF inhibitors may, in many cases, provide insight into the mechanism of the evolving loss of response, as well as suggest a possible salvage strategy [10].

However, the therapeutic monitoring of infliximab efficacy in unresponsive IBDs is often complicated by the need for invasive procedures, such as endoscopy and recurrent biopsies [7, 9, 11]. Furthermore, the monitoring of ATI levels has important drawbacks, one being the inability to detect ATIs in the presence of infliximab in the serum by Enzyme-Linked Immunosorbent Assay (ELISA). Moreover, in the detriment of additional detection methods such as homogenous mobility shift assay and radioimmunoassay to be used in clinical practise, none demonstrate superior diagnostic value in direct comparison with ELISA techniques [10]. Thinking about this gap, several studies focused on developing new non-invasive, precise, sensitive, specific, and accessible biomarkers to be used in the therapeutic monitoring of infliximab, which led to the emergence of some serological and coprological candidates [9]. Unfortunately, all candidate biomarkers have several limitations, and none have proved relevant for the evaluation and therapeutic management of IBDs treated with infliximab [11].

Adipokines, which are peptides and bioactive proteins produced and secreted by adipose tissue, stand out as potential biomarkers because they have been shown to play an active role in relapse and even remission of IBDs [12, 13]. The ambiguous role of these adipokines is due to the fact that they may have a pro- or anti-inflammatory effect depending on their serum levels, type of adipokine, and type of receptor to which they activate [14]. Among these adipokines, adiponectin is characterised by its anti-inflammatory effects; therefore, its production is inversely proportional to the lipid content inside the adipocytes [15]. In turn, leptin, resistin, and visfatin are characterised by their known pro-inflammatory effects, and the production of these adipokines is proportional to the content of lipids and the degree of inflammation in adipocytes [16]. Studies have shown that serum levels of resistin and visfatin are increased in patients with CD or CU when compared to healthy controls [17, 18]. A recent meta-analysis by our group revealed that serum leptin levels, in turn, did not differ between patients with IBD compared to healthy patients. However, leptin levels were significantly higher in patients with CD than in patients with UC because of creeping fat (i.e. a

locally restricted hyperplasia of the mesenteric fat adjacent to the inflamed segments of the intestine), which is a pathognomonic feature of CD [19]. In turn, the effects of IBDs on serum adiponectin levels are controversial, as some studies show an increase in serum adiponectin [17], while others have found a reduction in its circulating concentration [20].

Due to its relationship with intestinal inflammation, it is suggested that serum levels of adipokines can be explored in the therapeutic monitoring of patients with IBDs, assisting in the management of treatment in these patients. Currently, despite much evidence associating changes in serum concentration of several adipokines to the efficacy of infliximab, the results are still controversial, especially considering the ambiguous effects of many of these peptides [21-23]. Thus, we aim to summarise current knowledge on the role of serum adipokines as potential biomarkers for the therapeutic monitoring of patients with IBD under the use of infliximab through a systematic review of the biomedical literature and meta-analysis of the recovered data. This study, therefore, seeks to elucidate the role and potential of these adipokines in the therapeutic management of patients with IBD who benefit from the use of this anti-TNF antibody.

METHODS

Study type

This is a systematic review and meta-analysis conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [24]. To assess the employability of serum adipokine levels in the therapeutic monitoring of patients with IBDs treated with infliximab, the **PICOS** strategy was used, as follows: “**P**opulation”, patient with a diagnosis of IBD (CD; UC; or indeterminate IBD); “**I**ntervention”, use of infliximab; “**C**ontrol”, patients before the use of infliximab; “**O**utcomes”, serum levels of leptin, adiponectin, visfatin, or resistin; “**S**tudy”, observational before-and-after studies.

Search strategy

A systematic search was carried out in the PubMed/MEDLINE, Cochrane Library, Scopus, and *Biblioteca Virtual em Saúde* (VHL) databases. To determine the descriptors used in the search strategies, the Medical Subject Heading (MeSH) terms were employed to define the keywords in English and the *Descritores Virtual em Saúde* to define the keywords in Portuguese and Spanish. The terms “Leptin” OR “Adiponectin” OR “Nicotinamide Phosphoribosyltransferase” OR “Resistin” were combined using the AND Boolean with the terms “Inflammatory Bowel Diseases” OR “Colitis,

Ulcerative” OR “Crohn’s Disease”. In all combinations, the term “Infliximab” was also used, as in the following examples: “Leptin” AND “Inflammatory Bowel Diseases” AND “Infliximab”. The detailed search strategy used in each of the selected databases is shown in the supplementary materials.

The reference list of all included studies, as well as of prominent narrative reviews in the field of interest, was also evaluated for the selection of other possible articles that fit the inclusion criteria in the study. In addition, with the help of the Web of Science platform, all articles that cited the included papers were also screened. After the search, only articles written in Portuguese, Spanish, and English were selected, with no restrictions on the date of publication.

Inclusion and exclusion criteria

The inclusion of the studies was carried out according to the criteria defined in the PICOS strategy, described above. Thus, studies that do not fit this strategy were excluded, such as reviews, notes, e-mails, editorials, and letters to the editor. Other exclusion factors were as follows: (i) articles that did not describe the therapeutic scheme applied in association with infliximab, (ii) articles that did not describe the doses and routes of administration of infliximab, and (iii) studies that included, in a combined therapeutic protocol, other biologic drugs in addition to infliximab.

Selection of studies

For the selection of studies, the EndNote® software was used to remove duplicates and organise the articles found in a table, including author, year, title, periodic, keywords, and abstract. Next, the titles, abstracts, and keywords were analysed by two independent authors (P.A.C. and W.G.L.) in order to identify relevant studies. In cases where the article was in accordance with inclusion criteria but the full text was not available, the corresponding author was contacted by e-mail three times (with 14-day intervals between them), and the articles were included if they were received in response to the last point of contact at the latest. Any discrepancy between the two evaluators at this stage was resolved by discussion with the judge (S.O.A.F.). The pre-selected articles were then thoroughly analysed to confirm their relevance and potential inclusion in this systematic review and meta-analysis.

Data analysis

For critical interpretation, all data of interest obtained were summarised and organised in tables. The selected articles were submitted to a full analytical reading to identify and extract the variables of interest, which include the following: reference (first author and year of publication), characteristics of the population studied (age group,

sex, region of the study, number of patients included), type of IBD, and serum levels of adipokines (concentration and technique used). For the meta-analysis, the mean and standard deviation values of serum adipokines (ng/mL), determined in patients before and after the use of infliximab, were extracted. Studies that defined serum adipokine levels as nonparametric data and the median and 25th percentile were used for the meta-analysis. Some of the studies included in our review used other units (such as pg/L) to report the level of some cytokines. In these cases, the units were converted to ng/mL in order to standardise all data. Values in pg/mL were multiplied by 1,000 and converted to ng/mL.

Quality assay

The quality of the included studies was independently assessed by two reviewers (P.A.C. and W.G.L.) using the Newcastle-Ottawa Quality Assessment Scale to examine the selection of participants and study design, comparability of groups, ascertainment of exposure, and outcome processing [25]. Included studies were categorised into three quality groups: high quality (received 7 to 9 stars), moderate quality (received 4 to 6 stars), and low quality (received 3 to 0 stars). Furthermore, the risks of bias of studies were determined using the tool described by Munn et al [26]. This tool includes 10 items for critical assessment of the methodological quality of observational studies. For each criterion, the study was attributed “yes” or “no” or “not applicable” or “unclear”. The total number of “yes” answers per study was tallied. Herein, the higher the number of “yes” means the lower the risk of bias of the study. Because a limited number of articles were available, the results of the quality assessment were not used as an inclusion/exclusion criterion.

Statistical analysis

The meta-analysis was performed with the mean values of serum adipokine levels in patients with IBD before and after the use of infliximab. Review Manager (RevMan)® 5.3 software was used to analyse data from individual studies, which were then combined using a random-effects model to estimate the combined mean difference of the variable of interest (adipokine levels) and their confidence intervals. The heterogeneity of primary data, in turn, was analysed by the I^2 test; we considered $I^2 > 50\%$ as a criterion for substantial heterogeneity. In all procedures, the level of significance was set at 5%.

RESULTS

Search and selection of studies

As shown in Fig. 1, 85 articles were identified during the search in the selected databases, 49 in Scopus, 22 in the Virtual Health Library, nine in PubMed/MEDLINE, and five in the Cochrane Library. With the exclusion of duplicate records using the EndNote® software, 72 studies were conducted to review the titles, abstracts, and keywords. After this stage, 10 relevant studies were selected for full-text read and evaluated according to the previously defined eligibility criteria. At the end, there were three studies that followed for critical reading and data extraction of interest [21-23]. Of these, only one was excluded from quantitative studies, as it did not present comparable data to the others [21]. The remaining two studies were included in the quantitative analysis and used to conduct a meta-analysis of the relationship between serum adipokine levels and the efficacy of infliximab in the treatment of patients with IBD.

Study characteristics

The main characteristics of the studies included in the systematic review are summarised in Table 1. Of the studies, two were prospective cohorts (2/3; 66%) [21-23] and one was a retrospective cohort (1/3; 44%) [22]. All studies were carried out in European countries, namely, Belgium [21], Germany [22], and Greece [23]. Overall, 58 patients were analysed in these studies, of which 55 were diagnosed with CD and three with UC. No cases of indeterminate IBD have been reported. The overall mean age of the patients was 26.7 years, and the majority were male (32/58; 55%). All patients were treated with 5 mg/kg of infliximab, but the administration regimens differed between the studies (Table 1).

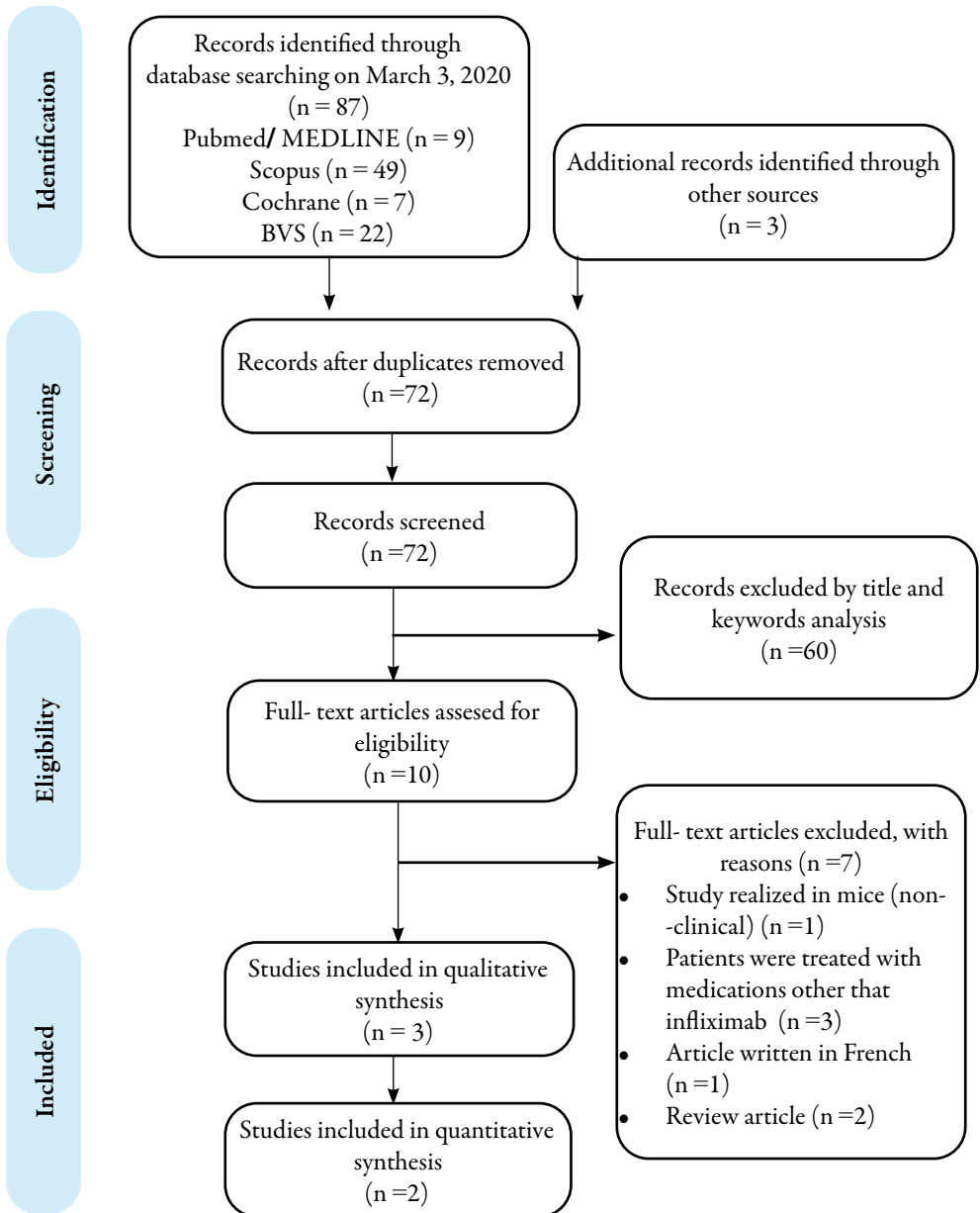


Figure 1. Flowchart of article selection for systematic review and meta-analysis according to PRIS-MA criteria [24].

Table 1. Main characteristics of the included studies

Study	Study design and follow-up	Country	Patients included (n)	Type of DII (DC or UC)	Age, Years	Sex, %	Infliximab treatment	Outcomes associated to infliximab treatment	Number of stars
Franchimont <i>et al.</i> , 2005 [21]	Prospective cohort Single center	Belgium	20	Active CD [CD activity index (CDAI) > 150]	26.5 ± 9.4 ^a	40	.5 mg/kg .Patients received either a three-dose administration regimen (0, 2, and 6 wk) (n = 14) or a single-dose induction (n = 6)	.Leptin levels were significantly higher after IFX administration .IFX significantly induced clinical remission in all patients at 4 wk; .The increase in serum leptin was negatively correlated with the decrease in CRP at 1 wk .Leptin levels increased in patients treated with IFX who were entering into clinical and biological remission	7

Study	Study design and follow-up	Country	Patients included (n)	Type of DII (DC or UC)	Age, Years ^a	Sex, % [§]	Infliximab treatment	Outcomes associated to infliximab treatment	Number of stars
Frivolt <i>et al.</i> , 2018 [22]	Retrospective cohort Single center	Germany	18	CD	15.0±1.5	45	.5 mg/kg . Patients received infliximab at 0, 2, and 6 weeks, followed by a maintenance treatment every 8 weeks.	.Resistin significantly decreased under IFX .Leptin significantly increased in CD patients under .Adiponectin significantly increased after the first IFX infusion but decreased after 14 weeks to levels lower than at week 2	9
Karmiris <i>et al.</i> , 2007 ^b [23]	Prospective cohort Single center	Greece	20	UC (3) and CD (17)	UC (43.3) CD (37.9) Overall mean: 38.7	UC (33) CD (41) Overall: 48	.5 mg/kg . Patients received infliximab at 0, 2, and 6 weeks, followed by a maintenance treatment every 8 weeks.	.Resistin significantly decreased under IFX .Adiponectin and leptin levels were no changed after IFX infusion .IFX induced significant increase of BMI in all patients	6

Note: ^aData refer to the mean age of the groups or the age group employed. [§]Data were proportional to women. ^aAge at diagnosis (yr). ^bIntra-assay precision was evaluated.

Methodological quality and risk of bias

The methodological quality of the included studies was generally considerable, being judged to be moderate [23] and high [21, 23] according to the criteria established in the Newcastle-Ottawa scale. The studies were signed with 9 [22], 7 [21], or 6 [23] stars, since only the studies by Frivolt *et al.* [22] clearly present the limitations and potential sources of bias and inaccuracy. None of the selected studies described the intra- and inter-trial variations related to the assessed adipokine dosages.

Overall, the included studies had a risk of bias due to not accounting for possible confounding factors and/or failure to perform subgroup analyses, except the study by Franchimont *et al.* [21], which is suitable for all these criteria (Table 2). With regard to the population analysed, none of the studies included a satisfactorily large group of patients to infer a cause-effect relationship with great statistical robustness, and furthermore, the sample size calculation is not discussed in any of the studies selected. However, the studies by Frivolt *et al.* [22] and Karmiris *et al.* [23] clearly describe the method used to recruit included patients (Table 2).

Association between circulating leptin levels and the use of infliximab

To evaluate the association between serum leptin levels and clinical remission of IBDs after infliximab use, 38 patients (35 patients with CD and three patients with CU) were pooled for this meta-analysis. As shown in Fig. 2, treatment with infliximab was associated with a significant increase in circulating leptin level (IV, -0.73; 95% CI, -1.30 to -0.16; $p = 0.01$). The heterogeneity, indicated by the I^2 statistic, was relatively high ($I^2 = 61\%$), suggesting that factors from the included population may be associated with the result observed. However, as only two articles were selected, a subgroup analysis could not be performed to determine the possible sources of heterogeneity.

Franchimont *et al.* [21] revealed that an increase in serum leptin levels accompanied the clinical remission of IBDs after the use of infliximab, suggesting that this adipokine is a potential indicator of disease remission. In addition, the study also correlated the increase in serum leptin levels after the use of infliximab with a significant reduction in plasma C-reactive protein, which is an important biomarker of disease activity (Table 1).

Table 2. Risk of bias of observational studies included according to criteria adopted by Munn *et al.* (2014).

Reference	Was the sample representative of the target population?	Were study participants recruited in an appropriate way?	Was the sample size adequate?	Were the study subjects and the settings described in detail?	Was the data analysis conducted with sufficient coverage of the identified sample?	Were objective, standard criteria used for the measurement of the condition?	Was the condition measured reliably?	Was there appropriate statistical analysis?	Are all important confounding factors/subgroups/differences identified and accounted for?	Were subpopulations identified using objective criteria?	Total number of "yes"
Franchimont <i>et al.</i> , 2005 [21]	Unclear	Unclear	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	7
Frivolt <i>et al.</i> , 2018 [22]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Not applicable	7
Karmiris <i>et al.</i> , 2007 [23]	Unclear	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Not applicable	6

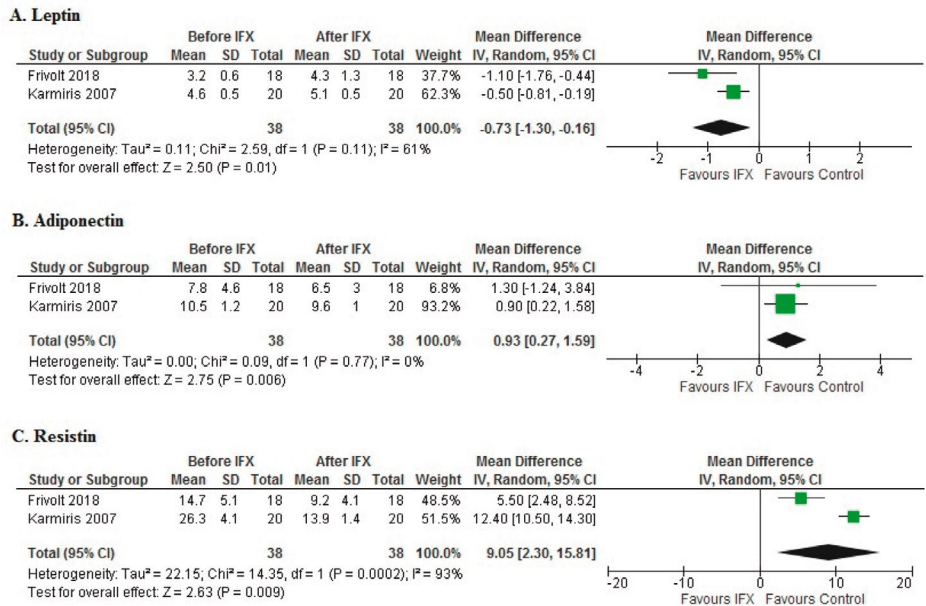


Figure 2. Meta-analysis of circulating levels of leptin, adiponectin, and resistin among patients with IBD treated with infliximab at 5 mg/kg. CI, confidence interval; IV, mean variation; SD, standard deviation.

Association between circulating adiponectin levels and the use of infliximab

Infliximab treatment was negatively and significantly related to serum adiponectin levels (IV, 0.93; 95% CI, 0.27-1.59; $p = 0.006$). The heterogeneity of the population included in this meta-analysis was low ($I^2 = 0\%$), indicating good robustness in the association shown.

Association between circulating resistin levels and the use of infliximab

For this meta-analysis, 38 patients with IBDs (35 patients with CD and three patients with CU) were included. As shown in Fig. 2, serum resistin levels were significantly reduced after clinical remission of IBDs with the use of infliximab (IV, 9.05; 95% CI, 2.30-15.81; $p = 0.0002$). However, the heterogeneity of this population was substantial, as revealed by the I^2 statistic ($I^2 = 93\%$). In the absence of an adequate number of studies, subgroup analysis cannot be performed to assess possible sources of heterogeneity. Interestingly, the two studies included in this meta-analysis [21, 23] used the same therapy protocol with infliximab, and both found an association between the decrease in serum resistin levels and the clinical remission of IBDs associated with anti-

TNF therapy (Table 1). Therefore, it is suggested that the therapeutic regimen does not consist of a source of heterogeneity in this case.

DISCUSSION

IBDs are diseases that present a clinical picture characterized by idiopathic inflammation of the colon, which can often undergo remission with the use of specific biologic drugs such as infliximab [8, 9]. However, one of the biggest challenges for patients with IBD under the use of infliximab is therapeutic monitoring and management of treatment effectiveness, since many of the procedures are currently invasive and cannot be performed with frequency [2, 9]. Currently, new serological and faecal biomarkers have been studied, but none have been shown to be ideal for the therapeutic management of IBDs due to their specificity limitations [11]. In this context, certain adipokines may have great potential for the therapeutic monitoring of patients with IBDs treated with infliximab, because their serum levels have been shown to be strongly influenced by the use of anti-TNF agents. However, current studies are contradictory in terms of the precision, sensitivity, and specificity of serum adipokine levels for this purpose [17, 22]. Thus, we aimed to synthesise the available evidence on the possible use of circulating adipokines as biomarkers in the therapeutic monitoring of patients with IBDs using infliximab.

Two cohorts and one case-control that included 58 patients (mean age, 26.7 years) diagnosed with IBDs (55 with CD and three with CU) and treated with infliximab at 5 mg/kg were selected. According to the quantitative analysis of the main outcomes of each study, serum leptin levels were significantly increased after the use of infliximab (IV, -0.73; 95% CI, -1.30 to -0.16; $p = 0.01$), which is correlated with the clinical remission of the disease. In addition, the circulating adiponectin (IV, 0.93; 95% CI, 0.27-1.59; $p = 0.006$) and resistin (IV, 9.05; 95% CI, 2.30-15.81; $p = 0.009$) levels were reduced after clinical remission of IBD with the use of infliximab. To the best of our knowledge, this is the first systematic review and meta-analysis that highlights the potential of serum leptin, adiponectin and resistin levels as biomarkers for monitoring the clinical remission of IBDs induced by infliximab use.

The influence of infliximab on serum adipokines levels may, at least in part, be justified by its effect on TNF- α . The mesenteric adipose tissue of patients with IBDs is characterised by the involvement of an important inflammatory process associated with increased tissue concentrations of TNF- α [12, 27]. Infliximab is able to bind to soluble (sTNF) and transmembrane (tmTNF) forms of TNF- α , which is a key mediator in the inflammatory cascade associated with the activity of CD and UC [28]. In this direc-

tion, it is expected that infliximab may also inhibit the inflammatory process associated with mesenteric adipose tissue, which is known to regulate circulating adipokine levels in patients with IBDs [12, 29]. In vitro studies showed that the exposition of TNF- α (10 ng/ml) by 48 h decreased leptin production by 30-50% and gene expression by 80–90% in adipocytes from human abdominal subcutaneous adipose tissue [30]. Similarly, leptin release from subcutaneous adipocytes was inhibited by 17.7%, 21.6%, and 37.1% by 1, 10, and 100 ng/mL TNF- α , respectively, after 48 h in culture, indicating a dose-dependent effect [31]. In addition, Bokarewa *et al.* [32] showed that stimulation of human peripheral blood mononuclear cells (PBMCs) with TNF- α (10 ng/mL) resulted in upregulation of resistin mRNA and increased resistin levels in the supernatants after 48 h of stimulation. Taken together, these results suggest that the action of TNF- α on the mesenteric adipocytes of patients with IBD is associated with the inhibition of expression, synthesis, and release of leptin. Moreover, the activity of TNF- α on macrophages into mesenteric adipose tissue induces an increase in the expression and synthesis of resistin. In this direction, it is expected that the use of infliximab will reverse the effects of TNF- α on mesenteric adipose tissue, allowing an increase in leptin content and a reduction in resistin, which is in accordance with the findings of this meta-analysis.

Leptin is an appetite-depressing (anorexigenic) peptide that has a direct relationship with body fat mass and has several metabolic functions associated with the regulation of glucose and lipid homeostasis [33]. However, in addition to its function as an anti-obesity factor, leptin may play an important role in acute and chronic inflammatory conditions. In patients with acute inflammation, such as sepsis, leptin concentrations are increased up to three-fold from baseline levels [34]. In contrast, under conditions of chronic inflammation, like IBDs, leptin release is inhibited [22]. In fact, most studies showed that the circulating leptin levels were found to be decreased in patients with IBDs compared with healthy controls [13, 18, 35]. The decreased serum leptin levels of patients with IBD could be partially attributed to an elevated circulating TNF- α , which has already been shown to inhibit the expression, synthesis, and release of leptin in vitro [30, 31]. In this direction, suggesting that the chronic inhibitory effect of TNF- α on leptin release is blocked by anti-TNF antibodies, justifying the increased leptin levels shown in this review after the use of infliximab. Therefore, monitoring serum leptin levels is a good indicator of the efficacy of anti-TNF therapy and can thus be a valuable tool in the therapeutic monitoring of patients with IBDs treated with infliximab. Furthermore, the increase in leptin levels after infliximab use might also be associated with increased appetite and weight gain when patients enter remission [22]. However, because several studies indicate that leptin does not seem to influence food behaviour in humans to the same extent as in mice [33, 36, 37], this mechanism may be less relevant.

Resistin (or “resistance to insulin”) is a peptide proposed as a link between obesity and diabetes, which was named for its ability to resist (interfere with) insulin action [38]. The expression patterns of resistin differ considerably between species, as the murine resistin is expressed primarily by adipocytes, while, in human resistin, is predominantly expressed by PBMCs and macrophages [39]. Thus, although resistin is postulated to contribute to insulin resistance in mice, human resistin plays a major regulatory role in the inflammatory response [17, 39, 40]. In line with this, human resistin stimulates the secretion of pro-inflammatory cytokines such as TNF- α , IL-6, IL-12, and monocyte chemoattractant protein (MCP)-1 in PBMCs, macrophages, and hepatic stellate cells via the nuclear factor- κ B (NF- κ B) pathway *in vitro* [32, 41]. Furthermore, serum resistin levels have been found to be elevated in inflammation-related diseases such as type 2 diabetes mellitus, coronary atherosclerosis, chronic kidney disease, rheumatoid arthritis, and/or sepsis [41]. In IBD patients, serum resistin levels were found to be elevated, and in the creeping fat from CD patients, resistin mRNA is overexpressed, suggesting that this pro-inflammatory adipokine may play an important role in the pathophysiology of IBDs (especially in CD) [35]. Interestingly, TNF- α , but not other pro-inflammatory cytokines such as IL-6 and IL-1 β , induced resistin mRNA expression, suggesting that this adipokine is markedly elevated in IBD patients as a response to the sustained increase in TNF- α levels in these patients [32]. Infliximab treatment, in turn, inhibits the local and systemic effects of TNF- α in patients with IBDs, causing a significant decrease in circulating resistin levels, as shown in this review. Consistent with our study, Wistar rats with colitis induced by repeated intracolonic trinitrobenzene sulphonic acid (TNBS) instillations showed a significant increase in resistin mRNA in mesenteric adipose tissue after infliximab administration (5 mg/kg intraperitoneally), which was associated with the restoration of adipocyte morphology and PPAR- γ expression [42]. In fact, it has been shown that the expression and release of resistin in PBMCs are downregulated by PPAR- γ [32, 41, 43], indicating that infliximab increases resistin levels by activating this pathway in macrophages from mesenteric adipose tissue of patients with IBDs.

Adiponectin is the most abundant peptide secreted by adipocytes and has been extensively studied for its involvement in obesity and associated morbidities such as cardiovascular disease, metabolic syndrome, and type 2 diabetes [44]. There is much evidence that adiponectin is a potent anti-inflammatory agent associated with the inhibition of pro-inflammatory cytokines (e.g. TNF- α , IL-6, IL-1 β , and chemokine) and to the induction of anti-inflammatory ones (e.g. IL-10), downregulation of adhesion molecule expression (e.g. ICAM-1 and VCAM-1), antagonism of toll-like receptors and their ligands (e.g., LPS), and inhibition of pro-inflammatory pathways (e.g. NF- κ B) [45]. However, the relationship between serum adiponectin levels in patients with

IBD is controversial [46]. This is because some studies have shown high levels of this adipokine in patients with active IBD, indicating a negative feedback mechanism on the ongoing inflammatory process [17, 47, 48], while others point to reduced circulating adiponectin levels as a triggering mechanism of disease activity [20]. In addition, the study by Frivolt *et al.* [22] indicates that the effects of infliximab on serum levels of adiponectin is significantly influenced by the temporary effect of this biologic drug. In this study, the authors showed that adiponectin increased two weeks after the use of infliximab to a level higher than that observed before treatment. However, after the fourteenth week, the values decreased considerably and did not differ from the baseline values before treatment, even if the clinical remission of the disease maintained. Thus, the marked early increase of the potent anti-inflammatory adiponectin may contribute to the rapid response to infliximab in IBD, but this adipokine is not a good biomarker to assess the long term therapeutic benefit of the drug.

This meta-analysis has some limitations. First, the number of articles included was small, which reflects the limited number of studies on this topic. Due to this limitation, it was not possible to perform subgroup analysis to determine the possible sources of heterogeneity for each of the studied outcomes. Second, the difference in the dosing period for adipokines after the start of infliximab use between studies may have interfered with the association shown, especially in the case of adiponectin where temporality is a relevant factor. Third, as a result of the small number of patients included in this review, the extrapolation of the associations found in other populations is limited. Fourth, only three of the patients included in the selected studies were diagnosed with UC, which makes it difficult to say that the result obtained for this meta-analysis is considerable for this group. Fifth, none of the selected studies described the intra- and inter-trial variations related to the adipokine dosages, thus increasing the bias in the results. Finally, it is emphasised that the associations shown in the meta-analysis do not imply causality and are always sensitive to residual confounding factors, especially in this case where the included studies present the observational design.

CONCLUSION

In summary, this systematic review and meta-analysis indicates that serum leptin levels are significantly increased, while circulating adiponectin and resistin is reduced among IBD patients after the use of infliximab. This effect is coupled with the activity of infliximab on TNF- α , which a cytokine is known to inhibit the expression, synthesis, and release of leptin by the mesenteric adipose tissue of patients with IBD and stimulate the expression and synthesis of adiponectin and resistin. However, it was possible to verify that clinical studies aimed at evaluating the use of adipokines as biomarkers in the

therapeutic monitoring of patients with IBD undergoing treatment with infliximab are still scarce, especially when it comes to patients with UC. In this direction, large randomised and controlled clinical trials need to be conducted to define the real role of these adipokines in the therapeutic monitoring of patients with CD or CU treated with anti-TNF agents such as infliximab.

ACKNOWLEDGMENTS

W.G.L. is grateful to Coordenação de Aperfeiçoamento de Pessoal do Nível Superior (CAPES) for a Ph.D. fellowship.

COMPLIANCE WITH ETHICAL STANDARDS

Funding: None

Conflict of Interest: All authors report that they do not have any conflicts of interest.

Ethical approval: Not applicable.

Author contributions: All authors contributed to the development, analysis, and drafting of this article.

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HOW TO CITE THIS ARTICLE

W.G. Lima, P. Alcântara-Cândido, A.d.P. Sabino, V.N. Cardoso, S.O. Antunes-Fernandes, Serum adipokines as biomarkers for the therapeutic monitoring of patients with inflammatory bowel diseases (IBDs) treated with infliximab: a systematic review and meta-analysis, *Rev. Colomb. Cienc. Quím. Farm.*, **51**(3), 1464-1492 (2022). DOI: <http://dx.doi.org/10.15446/rcciquifa.v51n3.107328>

SUPPLEMENTARY FILE

Serum adipokines as biomarkers for the therapeutic monitoring of patients with inflammatory bowel diseases treated with infliximab: a systematic review and meta-analysis

Search strategy

1. PubMed

Search (((((((((((Leptin[Text Word] OR Ob Gene Product[Text Word] OR Ob Protein[Text Word] OR Obese Gene Product[Text Word] OR Obese Protein[Text Word])))) OR “Leptin”[Mesh])) OR ((“Adiponectin”[Mesh] OR ((Adiponectin[Text Word] OR ACRP30 Protein[Text Word] OR Adipocyte Complement-Related Protein 30-kDa[Text Word] OR Adipocyte, C1q[Text Word] AND Collagen Domain Containing Protein[Text Word] OR Adipose Most Abundant Gene Transcript 1[Text Word] OR apM-1 Protein[Text Word])))) OR ((“Nicotinamide Phosphoribosyltransferase”[Mesh] OR ((Nicotinamide Phosphoribosyltransferase[Text Word] OR NAMPT Protein[Text Word] OR NAMPTase[Text Word] OR NMN Pyrophosphorylase[Text Word] OR Pre-B-Cell Colony-Enhancing Factor[Text Word] OR Visfatin[Text Word])))) OR ((“Resistin”[Mesh] OR ((Resistin[Text Word] OR Adipocyte Cysteine-Rich Secreted Protein FIZZ3[Text Word])))) OR ((“Visfatin”[Mesh] AND ((Inflammatory Bowel Diseases[Text Word] OR Bowel Diseases, Inflammatory[Text Word])))) OR ((“Colitis, Ulcerative”[Mesh] OR ((Colitis, Ulcerative[Text Word] OR Colitis Gravis[Text Word] OR Idiopathic Proctocolitis[Text Word] OR Inflammatory Bowel Disease Ulcerative Colitis type[Text Word] OR Ulcerative Colitis[Text Word])))) OR ((“Crohn Disease”[Mesh] OR ((Crohn Disease[Text Word] OR Colitis, Granulomatous[Text Word] OR Crohn’s Disease[Text Word] OR Crohn’s Enteritis[Text Word] OR Enteritis, Granulomatous[Text Word] OR Enteritis, Regional[Text Word] OR Ileitis, Regional[Text Word] OR Ileitis, Terminal[Text Word] OR Ileocolitis Inflammatory Bowel Disease 1[Text Word] OR Regional Enteritis[Text Word])))) AND ((“Infliximab”[Mesh] OR ((Infliximab[Text Word] OR Inflectra[Text Word] OR Infliximab-abda[Text Word] OR Infliximab-dyyb[Text Word] OR MAb cA2[Text Word] OR Monoclonal Antibody cA2[Text Word] OR Remicade[Text Word] OR Renflexis[Text Word]))))

2. Cochrane library

ID	Search Hits	
#1	MeSH descriptor: [Leptin] this term only	975
#2	Leptin OR Ob Gene Product OR Ob Protein OR Obese Gene Product OR Obese Protein	6090
#3	#1 OR #2	6090
#4	MeSH descriptor: [Adiponectin] this term only	838
#5	Adiponectin	3475
#6	#4 OR #5	3475
#7	MeSH descriptor: [Nicotinamide Phosphoribosyltransferase] explode all trees	45
#8	Nicotinamide Phosphoribosyltransferase OR NAMPT Protein OR NAMPTase OR NMN Pyrophosphorylase OR Pre-B-Cell Colony-Enhancing Factor OR Visfatin	209
#9	#7 OR #8	209
#10	MeSH descriptor: [Resistin] this term only	113
#11	Resistin OR Adipocyte Cysteine-Rich Secreted Protein FIZZ3	478
#12	#10 OR #11	478
#13	#3 OR #6 OR #9 OR #12	9411
#14	MeSH descriptor: [Inflammatory Bowel Diseases] this term only	461
#15	Inflammatory Bowel Diseases OR Bowel Diseases, Inflammatory	2015
#16	#14 OR #15	2015
#17	MeSH descriptor: [Colitis, Ulcerative] explode all trees	1415
#18	Colitis, Ulcerative OR Colitis Gravis OR Idiopathic Proctocolitis OR Inflammatory Bowel Disease OR Ulcerative Colitis type OR Ulcerative Colitis	4758
#19	#17 OR #18	4758
#20	MeSH descriptor: [Crohn Disease] this term only	1431

ID	Search Hits	
#21	Crohn Disease OR Colitis, Granulomatous OR Crohn's Disease OR Crohn's Enteritis OR Enteritis, Granulomatous OR Enteritis, Regional OR Ileitis, Regional OR Ileitis, Terminal OR Ileocolitis Inflammatory Bowel Disease 1 OR Regional Enteritis	5127
#22	#20 OR 21	260746
#23	#16 OR #19 OR #22	265135
#24	MeSH descriptor: [Infliximab] this term only	662
#25	Infliximab OR Inflectra OR Infliximab-abda OR Infliximab-dyyb OR MAb cA2 OR Monoclonal Antibody cA2 OR Remicade OR Renflexis	2567
#26	#24 OR #25	2567
#27	#13 AND #23 AND #26	7

3. Scopus

(TITLE-ABS-KEY ("Leptin" OR "Adiponectin" OR "Nicotinamide Phosphoribosyltransferase" OR "Resistin") OR INDEXTERMS ("Leptin" OR "Adiponectin" OR "Nicotinamide Phosphoribosyltransferase" OR "Resistin")) AND (TITLE-ABS-KEY ("Inflammatory Bowel Diseases" OR "Colitis, Ulcerative" OR "Crohn's Disease") OR INDEXTERMS ("Inflammatory Bowel Diseases" OR "Colitis, Ulcerative" OR "Crohn's Disease")) AND (TITLE-ABS-KEY ("Infliximab") OR INDEXTERMS ("Infliximab"))

4. Biblioteca virtual em Saúde (Portal Regional da BVS)

(mh:("Leptin")) OR (tw:("Leptin" OR "Leptina" OR "Gene Product, Ob" OR "Gene Product, Obese" OR "Ob Gene Product" OR "Ob Protein" OR "Obese Gene Product" OR "Obese Protein")) OR (mh:("Adiponectin")) OR (tw:("Adiponectin" OR "Adiponectina" OR "ACRP30 Protein" OR "Adipocyte Complement Related Protein 30 kDa" OR "Adipocyte Complement-Related Protein 30-kDa" OR "Adipocyte, C1q and Collagen Domain Containing Protein" OR "Adipose Most Abundant Gene Transcript 1" OR "apM 1 Protein" OR "apM-1 Protein")) OR (mh:("Nicotinamide Phosphoribosyltransferase")) OR (tw:("Nicotinamide Phosphoribosyltransferase" OR "Nicotinamida Fosforribosiltransferasa" OR "Nicotinamida Fosforribosiltransferase" OR "Colony-Enhancing Factor, Pre-B-Cell" OR "NAMPT Protein" OR

“NAMPTase” OR “NMN Pyrophosphorylase” OR “Phosphoribosyltransferase, Nicotinamide” OR “Pre B Cell Colony Enhancing Factor” OR “Pre-B-Cell Colony-Enhancing Factor” OR “Visfatin”)) OR (mh:(“Resistin”)) OR (tw:(“Resistin” OR “Resistina” OR “Adipocyte Cysteine Rich Secreted Protein FIZZ3” OR “Adipocyte Cysteine-Rich Secreted Protein FIZZ3”)) AND (mh:(“Inflammatory Bowel Diseases”)) OR (tw:(“Inflammatory Bowel Diseases” OR “Enfermedades Inflamatorias del Intestino” OR “Doenças Inflamatórias Intestinais” OR “Bowel Diseases, Inflammatory” OR “Inflammatory Bowel Disease”)) OR (mh:(“Colitis, Ulcerative”)) OR (tw:(“Colitis, Ulcerative” OR “Colitis Ulcerosa” OR “Colite Ulcerativa” OR “Colitis Gravis” OR “Idiopathic Proctocolitis” OR “Inflammatory Bowel Disease, Ulcerative Colitis Type” OR “Ulcerative Colitis”)) OR (mh:(“Crohn Disease”)) OR (tw:(“Crohn Disease” OR “Enfermedad de Crohn” OR “Doença de Crohn” OR “Colitis, Granulomatous” OR “Crohn’s Disease” OR “Crohn’s Enteritis” OR “Crohns Disease” OR “Enteritis, Granulomatous” OR “Enteritis, Regional” OR “Granulomatous Colitis” OR “Granulomatous Enteritis” OR “Ileitis, Regional” OR “Ileitis, Terminal” OR “Ileocolitis” OR “Inflammatory Bowel Disease 1” OR “Regional Enteritis” OR “Regional Ileitides” OR “Regional Ileitis” OR “Terminal Ileitis”)) AND (mh:(“Infliximab”)) OR (tw:(“Infliximab” OR “Inflectra” OR “Infliximab-abda” OR “Infliximab-dyyb OR “MAb cA2” OR Monoclonal Antibody cA2” OR “Remicade” OR “Renflexis” OR “cA2, Monoclonal Antibody”))

Abstracts of the IV RedIF Congress 2022. Porto Alegre, Brazil, October, 5th to 7th, 2022.

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PHARMACOKINETIC STUDY OF A COUMARIN EXTRACTED OF *ZANTHOXYLUM TINGOASSUIBA* IN TWO ANIMALS MODELS

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6-methoxyseselin is a coumarin present in the roots of *Zanthoxylum tingoassuiba* and has many pharmacological properties, such as antinociceptive, anti-inflammatory, and immunomodulatory activities [1, 2]. These effects make it a potential drug candidate. Thus, a preclinical pharmacokinetic study becomes indispensable to evaluate the behavior of this substance in biological systems. Two studies were performed in rats and mice to evaluate the pharmacokinetic parameters, following a previously validated bioanalytical method. In rats (n=3), an intravenous dose of 15 mg/kg was administered, and in mice (n=40), the inhalation and intravenous routes were used with doses of 50 and 15 mg/kg, respectively. Blood samples were collected at the times 0.083, 0.25, 0.5, 0.75 (only in mice), 1, 1.5, 2, 4, 6, 8 and 10 (only in rats) hours after the administration. The samples were treated and analyzed by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD). The bioanalytical method shows good linearity ($r^2 > 0.99$) and stability for up to 30 days. The PK parameters were estimated by PKAnalix® (Lixoft, France). The values of elimination constant, half-life, clearance, the volume of distribution, and mean residence time were, respectively: 0.41 h⁻¹, 1.7 h, 3.9 L/h/kg, 9.6 L/kg, and 2.29 h. In mice, the values were: 0.3 h⁻¹, 2.31 h, 9.47 L/h, 3.16 L and 2.31 h (intravenous); 0.23 h⁻¹, 2.99 h, 1.94 L/h, 8.36 L and 4.66 h (inhalation). Most parameters did not change significantly between the administration route or animal models, except for the volume of distribution. To our knowledge, this is the first work to study the pharmacokinetics of 6-methoxyseselin in rats and mice.

Ethical approval

All procedures were reviewed and approved by the Committee on Ethics in the Use of Animals (CEUA-IGM) of Oswaldo Cruz Foundation (Fiocruz/ BA) (013-2021).

Financial support and acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)-Finance Code 001

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POPULATION PHARMACOKINETIC MODELING OF CARBAMAZEPINE IN PATIENTS AT A UNIVERSITY HOSPITAL

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Carbamazepine (CBZ) treats epilepsy and other neurological and psychiatric disorders [1]. Therapeutic monitoring can monitor drugs whose doses are not directly related to plasma concentrations, such as CBZ. Pharmacometrics tools have been used in this monitoring through pharmacokinetic and pharmacodynamic modeling and simulations, such as population pharmacokinetic modeling (PopPK). To analyze concentrations at the sites of action and, when necessary, adjust the dosage [2]. The aim is to develop the PopPK model for patients admitted to the University Hospital Teacher Edgard Santos (HUPES) and used CBZ. Data have been extracted from electronic medical records between 2012 and 2016, using plasma concentrations quantified in HUPES. The Microsoft Office Excel resource was used for statistical analysis, with the ANOVA test followed by Student's t-test comparing the data ratios. Values of $p < 0.05$ were considered statistically significant. We obtained 50 patients treated with CBZ, ages 7 to 87 years. Data were divided into subgroups based on the results: underdose, overdose, and normal dose, concerning the therapeutic window. Where among the patients using CBZ, 20 patients (40%) had a normal concentration ($>4\mu\text{g/mL}$ and $<10\mu\text{g/mL}$), 17 patients (34%) had an underdose and 13 patients (26%) had an overdose. We can reaffirm the importance of CBZ quantification and monitoring with the initial results. A population pharmacokinetic model will be developed to evaluate the data using the software Monolix® (Lixoft, France).

Ethical approval

Research Ethics Committee of the Professor Edgar Santos University Hospital/
UFBA - N° 2.780.692.

Financial support and acknowledgements

University Hospital Teacher Edgard Santos (HUPES) and Bahia Research Support
Foundation (Fapesb).

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COMPARISON OF WAGNER-NELSON AND MECHANISTIC DECONVOLUTION METHODS: APPLICATION OF IVIVR FOR LAMOTRIGINE IR

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In vitro-in vivo relationship (IVIVR) can be a key factor for the earlier successful along the formulation development phase and in future stages of the post-registration of generic drugs [1]. For the construction of an IVIVR, *in vitro* data from dissolution profiles and *in vivo* data from plasma profiles are required. From the plasma concentration data, the percentage of the fraction absorbed or dissolved *in vivo* can be determined, this technique is called deconvolution [2]. Thus, the aim of this work was to compare traditional Wagner-Nelson and Mechanistic deconvolution forms and establish an IVIVR for lamotrigine (LTG) IR, an antiepileptic drug and class 2 in the Biopharmaceutical Classification System (BCS). The dissolution data were obtained from flow-through cell apparatus using biorelevant medium. An oral PBPK was developed and verified using GastroPlus® software. Data deconvolution and IVIVR were performed using the IVIVCPlus® module. The IVIVR models were analyzed by the slope of the regression line, the determination coefficient (R^2), and visually. The models were validated internally and externally. Mean percentage prediction error (PE%) of a maximum of 15% was accepted [3]. The verifications for both methods showed PE% below 15%. However, the mechanistic absorption method presents smaller errors than the Wagner-Nelson. Although the Wagner-Nelson method is more traditional, the mechanistic method was able to predict *in vivo* dissolution data in a pure and more accurate way. So, it can be the method chosen for other applications of LTG IVIVR as to develop new formulations and to establish a virtual bioequivalence.

Financial support and acknowledgements

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes -Brasília, Brazil) and Araucária Foundation (SETI/Paraná State) (061/2021 - SUS2020131000109). The authors thank Dr. Sandra Suarez-Sharp for her support and scientific discussions, Simulations Plus Inc. (Lancaster, CA, USA), and Prati-Donaduzzi Pharmaceutical Company (Toledo, PR, Brazil).

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ADULT AND PEDIATRIC PHYSIOLOGICALLY BASED BIOPHARMACEUTICS MODEL TO EXPLAIN LAMOTRIGINE IMMEDIATE RELEASE DISSOLUTION AND ABSORPTION PROCESS

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Physiologically based biopharmaceutics modeling (PBBM) is a viable tool to identify formulation-physiology interactions and predict the clinical impact of variations in formulation parameters [1]. PBBM has an immense potential benefit in speeding up the development of new drugs, ensuring quality, and reducing unnecessary animal and human testing, particularly in children where data is lacking due to ethical issues [2]. Thus, the aim of this work was to develop and verify an adult PBBM model for lamotrigine (LTG) and extrapolate to pediatrics to discuss the impact of dissolution on LTG pharmacokinetics (PK). The dissolution data were obtained from flow-through cell apparatus using biorelevant medium. An oral physiologically based pharmacokinetic model (PBPK) was developed using GastroPlus™ software. The predicted and observed PK profiles were compared to assess the predictability of the model and PBBM model was developed by incorporating dissolution data by calculation of the z-factor. LTG PBPK profiles showed percentage prediction error (PE%) less than 25%. The PBBM model, with dissolution data incorporated, improve the adult PK prediction with t_{max} %PE from 52% to 13%. For pediatric model, it was necessary to adjust the volume of distribution by optimizing the log P and it was assumed the fed state given the specific characteristics of this population. Employing pediatric dissolution condition, the %PE for AUC, C_{max} and t_{max} were between -12.68-27.07%. So, the dissolution process seems to be an important step for LTG PK. Additionally, the LTG PBBM model can be used in formulation development studies.

Financial support and acknowledgements

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes-Brasília, Brazil) and Araucária Foundation (SETI/Paraná State) (061/2021 - SUS2020131000109). The authors thank Simulations Plus Inc. (Lancaster, CA, USA), and Prati-Donaduzzi Pharmaceutical Company (Toledo, PR, Brazil).

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VALIDATION OF METHODOLOGY FOR QUANTIFICATION OF MEFLOROQUINE FOR PK/PD MODELING

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Malaria is an acute febrile infectious disease caused by intracellular protozoa of the genus *Plasmodium*, which, despite being treatable and preventable, still generates a high mortality rate in the world [1]. Mefloquine is one of the antimalarials used in association for its treatment. However, despite the advantages described, there are still limitations in its use, mainly because of the parameters that guide the dosage. That said, the first step in performing PK/PD modeling is validating a methodology for quantifying mefloquine in plasma, microdialysate, and culture medium. Validation took place according to FDA [2] requirements, using a C18 column (5 µm, 250 × 4.6 mm), mobile phase composed of methanol, acetonitrile, and monobasic potassium phosphate buffer (35:25:40, v/v), pH 3.9, and 0.05 M, with a flow rate of 1 mL/min, with detection at 284 nm, using HPLC. The method is linear in the concentration range 0.75 - 24 µg/mL, with precision and accuracy of the quality controls (0.9, 4, and 20 µg/mL) within 5% and 15%, for the analytical and bioanalytical methods, respectively, except for the lower limit of quantification (0.75 µg/mL) which is acceptable up to 20%. The quantification method proved to be selective, linear, precise, and accurate for measuring mefloquine in plasma and microdialysate samples from healthy and *P. berghei*-infected rat after oral administration of 55 mg of mefloquine.

Ethical approval

All procedures were reviewed and approved by the Committee on Ethics in the Use of Animals (CEUA) of School of Veterinary Medicine at the Federal University of Bahia (89/2019).

Financial support and acknowledgements

We thank FAPESB for the scholarship support.

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NANO-ARCHITECTURE APPROACHES TO IMPROVE THE ANTIMALARIAL ERYTHROCYTE PENETRATION: A STUDY OF POPPK/PD MODELING IN *PLASMODIUM BERGHEI* INFECTED RODENTS

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Our research group has been dedicated to improving the efficacy of antimalarial drugs by changing the nanocarrier architecture and have shown promising results, especially for quinine (QN) [1-3]. However, the studies are lacking to distinguish the biological effects of anionic (NC1) and cationic (NC2) coated nanocapsules containing QN. Our aim was to describe the PopPK/PD modeling of free QN, NC1, and NC2 in *Plasmodium berguei* infected rodents. From QN plasma profile and erythrocytes partition coefficient of QN, both in *P. berguei* infected rats, QN drug concentrations in whole blood were calculated and PopPK using Monolix-SuiteTM 2020R1 (Simulation Plus, USA). Peter's suppressive test was assessed in mice [3] and then, interspecies translation were performed. A QN whole blood in mice [4] were modeled on Scientist and the hybrid constants were used to recalculate the populational parameters using the covariates from PopPK model. With this results, PopPKPD modeling were performed using Monolix. A two-compartment model with linear elimination described QN whole blood data in rats. NC1 and NC2 reduce CL and intercompartmental CL. NC2 reduced V2, as well as the weight influenced this parameter. A modified maximum inhibition model was able to describe the reduction of parasitemia in all treatments. NC1 and NC2 have been shown to decrease the IC50 by 100 and 500-fold, respectively. Nanoencapsulation of QN potentiated drug interaction with infected erythrocytes. In summary, results of PopPK and PopPK/PD indicated cationic nanocapsules are promising strategies to treat malaria.

Financial support and acknowledgements

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and 19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) #309401/2020-8). We want to thank to Simulation Plus for MonolixSuite™ license.

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IS CLINDAMYCIN A POTENTIAL TREATMENT FOR PROSTATITIS?

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The presence of *Cutibacterium acnes* has been associated with chronic prostatitis, this situation has the potential to favor the appearance of prostatic tumors [1]. The effectiveness of clindamycin in the treatment of infections by this bacterium has been highlighted [2-4]. Male Wistar rats were divided into two groups (plasma, $n = 8$; prostate, $n = 8$) for pharmacokinetic studies. Clindamycin was administered at 80 mg/kg iv *bolus*. Carotid artery was cannulated for blood collection in the plasma group. For the prostate group, a microdialysis probe was implanted in the prostate gland. Collections were made over an 8-hour period. The pharmacokinetic parameters were determined by a non-compartmental approach (NCA). NCA resulted in the following values (plasma and prostate, respectively): elimination rate constant (h^{-1}): 0.33 ± 0.02 and 0.46 ± 0.07 ; half-life (h): 2.09 ± 0.15 and 1.55 ± 0.25 , peak concentration (mg/L) 35.82 ± 10.51 and 11.53 ± 3.24 , area under the curve (h mg/L) 58.53 ± 14.78 and 19.40 ± 4.40 , mean residence time (h): 2.51 ± 0.17 and 1.89 ± 0.17 . Half-life values show that clindamycin is removed more quickly from the prostate than from the bloodstream. The concentrations in prostatic tissue were lower than the total plasma concentrations. In both cases clindamycin reached the minimum inhibitory concentration against *Cutibacterium acnes* [5]. Clindamycin could be suggested as an effective therapeutic option for studies on the treatment of chronic prostatitis.

Ethical approval

The experimental protocols involving animals were approved by the Ethics Committee on the Use of Animals of the University of Caxias do Sul (017/2018).

Financial support and acknowledgements

We are grateful for the financial support received from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through a PIBIC grant during the project.

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PHARMACOKINETICS OF A NANOSTRUCTURED CLOXACILLIN FORMULATION IN GOATS NATURALLY INFECTED WITH *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

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The treatment of caseous lymphadenitis with antimicrobials is ineffective due the limited intracellular penetration [1]. However, the use of a nanostructured antibiotic may contribute to the effectiveness of therapy and microbiological cure, as demonstrated in studies with nanoencapsulated cloxacillin benzathine (CLOX-NS) against *Staphylococcus aureus* in bovine mastitis (patent WO/2011/150481) [1,2]. The aim was to construct a pharmacokinetic (PK) model of CLOX-NS in goats. Plasma PK data were obtained from an *in vivo* PK study previously performed in goats experimentally infected with *Corynebacterium pseudotuberculosis* [3]. With the concentration values over time a PK model was established in Monolix Suite 2020R1 software [4]. The PK model had an extravascular route of administration, with lag time, first order absorption, three compartments and linear elimination, and weight was considered a covariate. The estimated parameters were lag time ($T_{lag} = 0.0018h$), absorption constant ($K_a = 1.19 h^{-1}$), transit constants between compartments ($K_{12} = 9.58 h^{-1}$; $K_{21} = 1.28 h^{-1}$; $K_{13} = 9.77 h^{-1}$; $K_{31} = 0.8 h^{-1}$), elimination constant ($K = 104.9 h^{-1}$) and central compartment volume ($V = 2.61 mL$). There was a correlation between weight and the parameters V ($R = 0.94$) and K ($R = -0.53$). The established PK model of CLOX-NC in goats is the first step for development of simulations to determine probability of target attainment (PTA) based on pharmacokinetic/pharmacodynamic integration.

Ethical approval

This study was approved by the Embrapa Institutional Animal Care and Use Committee under the protocol number 011/2014.

Financial support and acknowledgements

This study received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 448011/2014-0) and Undergraduate Scholarship Research Program from UFLA.

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K-PD MODELS APPLICATIONS, LIMITATIONS AND PERSPECTIVES: A SYSTEMATIC REVIEW

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Frequently on clinical trials and pre-clinical studies data on certain drug plasma concentration measurements can be sparse, unavailable or economically unfeasible, consequently PK/PD modeling can become non representative or require complex alternatives [1]. In this context K-PD models are useful, since they don't require PK data. Our aim is to assess the applications and limitations on the use of K-PD models on pre and clinical trials. To achieve it, the systematic review used "Cochrane handbook for Systematic reviews of interventions" as a guide and Rayyan app was used to conduce a double-blind review during selection phase. The research was conducted on PubMed, Cochrane Reviews, Web of science and Embase, using the keywords: "K-PD", "model", "clinical", "semi-mechanistic". In total 131 articles were found, only 37 were kept after all selection steps. The results showed the wide application K-PD models, varying from toxicity pre-clinical studies with animal envenomation [2] to a helpful decision tool on phase II and III clinical trials [3]. From all 37 articles only 9 were published by industry and 28 by universities and research centers. The main limitation detected was for non-linear elimination drugs, which can require more complex and adequate equations [4], in addition to more deep understanding PK and PD characteristics from the drug. Only 1 article with nonlinear elimination were found. In conclusion our findings asses that K-PD models are useful tools with wide application, however require robust knowledge to carry out the proper equations and drug biological characteristics to achieve a model clinically relevant.

Ethical approval

Do not apply.

Financial support and acknowledgements

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)-UFBA

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PHARMACOKINETIC EVALUATION OF FREE AND NANOENCAPSULATED CURCUMIN IN RATS

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Curcumin (CUR) is a natural compound with anti-inflammatory, antioxidant, antitumoral and antimicrobial activities [1]. CUR has low solubility and permeability, and nanotechnology is a promising alternative for improving delivery. Then, our research group nanoencapsulated CUR through functionalizing nano-capsules (NC) surface [2]. The aim of this work was to evaluate the PK of CUR nanoformulation after 2 mg/kg i.v. bolus administration in male Wistar rats (n=6/group). The groups evaluated were free CUR (F-CUR), NC1 (negative surface), and NC2 (positive surface). The quantification of CUR was performed by HPLC-PDA previously validated method. Non-compartmental analysis was evaluated by Pkanalix® from MonolixSuite™ 2020R1 (Simulation Plus, USA). Formulations presented nanometric size and zeta potential compatible with coating. At the same dose, AUC_{0-∞} was 49 ± 4 , 276 ± 91 and $119 \pm 56 \mu\text{g.h.ml}^{-1}$ for F-CUR, NC1 and NC2, respectively. Compared to F-CUR, NC2 reduced $t_{1/2}$, in view of Vd_{ss} and Cl_{Tot} was decreased in almost 7 and 2-fold, respectively. CUR from NC1 showed similar behavior, except in the Cl_{Tot} . The NC showed modified the PK of CUR.

Ethical approval

All experiments were performed after approval of the experimental protocol by the Ethical Committee for Animal Use of Federal University of Pampa (UNIPAMPA - Protocol CEUA 036/2018) and according to the guidelines of the Council for Control of Animal Experiments (CONCEA).

Financial support and acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and #19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) grant #309401/2020-8. We want to thank to Simulation Plus for MonolixSuite™ license.

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INTEGRATION OF PK/PD FOR DOSE OPTIMIZATION OF CLOXACILLIN SODIUM AGAINST *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* FOR *IN VIVO* STUDIES IN GOATS

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The pharmacokinetic model allows for predicting the plasma concentration of a drug and thus estimating its therapeutic or toxic effect [1, 2]. We aimed to determine the first dose of intramuscular (IM) cloxacillin sodium (CLXS) against *Corynebacterium pseudotuberculosis* for *in vivo* study in goats. For this, a pharmacokinetic model with pharmacokinetic/pharmacodynamic (PK/PD) integration was constructed from data obtained from two plasma pharmacokinetic studies of CLXS, in goats (intravenous administration) and sheep (IM administration) [3-5]. After establishing the model, dose regimens were simulated to maintain the plasma concentration above the minimum inhibitory concentration (MIC = 4 µg/mL), for at least 40% of the time between doses interval [6]. The model was constructed using Lixoft® software, integrating the sheep's absorption constant (Ka) with the goat's volume of distribution (Vd) and clearance (Cl). The PK model was extravascular, with no delay, first-order absorption, one compartment, and linear elimination. The Ka (0.93 h⁻¹), Vd (0.32 L/kg), and Cl (0.28 kg/h) were determined, and then, two therapeutic regimens were simulated, one with intervals between administrations of 8 (q8h) and another of 12 hours (q12h), both with doses ranging from 10 to 200 mg/kg. According to the simulations, the doses of 10 mg/kg (q8h) and 30 mg/kg (q12h) reached concentrations higher than the MIC for 43 and 42% of the time between dose intervals, respectively. Through this study, it was possible to determine doses of CLXS with potential antibacterial effects to initiate *in vivo* studies.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

This study was funded by a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 448011/2014-0), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG - APQ-01168-21), EMBRAPA (SEG 20.20.03.002.00.00) and Undergraduate Research Scholarship Program of UFLA.

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POPULATIONAL PHARMACOKINETICS MODELING OF FREE AND NANOENCAPSULATED CURCUMIN IN MALE WISTAR RATS

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Curcumin (CUR) nanocapsules (NC) were developed by our research group and showed increase anti-inflammatory and neuroprotective effects [1-3]. Thus, the objective of this work was to be modeling PopPK from free (F-CUR) and nano-encapsulated CUR. NC1 (anionic NC) and NC2 (cationic NC) were prepared by nanoprecipitation method at 0,6 mg/ml concentration [1]. For the PK studies, healthy male Wistar rats were used. PK of free CUR (F-CUR), NC1 and NC2 were performed by administering 2 mg/kg i.v. bolus (n=6/group). Blood samples were collected and quantified in HPLC-PDA. A sequential modeling approach was used to describe CUR plasma concentration versus time profiles in rats using MonolixSuite™ 2020R1 (Simulation Plus, USA). For all groups structural model was a two-compartmental distribution and linear elimination. Besides that, model showed good precision of parameter estimates, with constant residual error and normal distribution. For NC1 and NC2 model, after fixed the F-CUR parameters, the k_r (CUR release from NC constant) was added to model (derivate from CUR *in vitro* release test [1]). Then, F-CUR had similar V_1 and V_2 while V_2 NC1 and V_2 NC2 increased significantly. Between nanoformulations cationic NC2 increased 3 and 2-fold the values of V_2 and Q , respectively. These results demonstrate the NC are promising as CUR drug delivery System.

Ethical approval

All experiments were performed after approval of the experimental protocol by the Ethical Committee for Animal Use of Federal University of Pampa (UNIPAMPA - Protocol CEUA 036/2018) and according to the guidelines of the Council for Control of Animal Experiments (CONCEA).

Financial support and acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and #19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) grant #309401/2020-8. We want to thank to Simulation Plus for MonolixSuite™ license.

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DETERMINATION OF A PROTOCOL FOR INTRAMUSCULAR TRAMADOL WITH POTENTIAL ANALGESIC EFFECT IN ZEBRAFISH (*DANIO RERIO*)

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The zebrafish (*Danio rerio*) is widely used in studies of nociceptive stimuli, so is necessary to determine analgesic protocols in this species [1,2]. Human pharmacokinetic/pharmacodynamic (PK/PD) model allowed quantifying the analgesic effect after tramadol treatment [3]. For this reason, this study aimed to construct a PK/PD model to estimate tramadol doses by intramuscular route (IM) with potential analgesic effect in zebrafish. The model was constructed from the PK model of tramadol in zebrafish integrated with the PK/PD model in humans [3-5]. The established PK model was with extravascular administration, with no delay, first-order absorption, one-compartment distribution and linear elimination. The average values of the parameters absorption constant ($K_a = 3.18 \text{ h}^{-1}$), central compartment volume ($V = 0.067 \text{ mL}$) and elimination constant ($K = 3.18 \text{ h}^{-1}$) were calculated. For the PK/PD integration, the parameters Hill coefficient, zero effect ($E_0 = 110.32 \text{ } \mu\text{A}$), maximum effect ($E_{\text{max}} = 78.56 \text{ } \mu\text{A}$) and effective-concentration 50 ($\text{EC}_{50} = 17.25 \text{ ng/mL}$) of the human tramadol model were used. IM protocols of tramadol were estimated to maintain the analgesic effect above 60% of the maximum effect throughout the interval between doses. Starting at the dose of $20 \text{ } \mu\text{g/animal}$, the maximum analgesic effect was achieved for 78 minutes, while doses of 30 and $40 \text{ } \mu\text{g/animal}$ increased the duration of the effect by 12 and 24 minutes, respectively. Thereby, protocols starting at $20 \text{ } \mu\text{g/animal}$, every 78 minutes, are promising for the evaluation of the analgesic effects of tramadol *in vivo*.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

This study was funded by a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Undergraduate Research Scholarship Program of UFLA.

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PBPK MODELING AS AN ALTERNATIVE METHOD OF EXTRAPOLATION INTERSPECIES THAT REDUCE THE USE OF ANIMALS: A SYSTEMATIC REVIEW

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The use of animals in biomedical research is a practice that continues to prompt ethical and scientific debate due to animal suffering, reliability and limitations for extrapolation of data to humans [1, 2]. An alternative to traditional methods used for interspecies extrapolation is Physiologically Based Pharmacokinetic (PBPK), this approach simulates the anatomical structure of species using a computational model in which organs or tissues are represented as compartments interconnected by arterial and venous flows and are described by differential equations to predict the change in the concentration of the evaluated molecule [3]. The aim of this systematic review was to analyze published articles that used PBPK models for interspecies extrapolation in drug development and toxicological Assessment. For this, a systematic search was performed in PubMed, using the following search terms: "PBPK" and "Interspecies extrapolation", the revision was performed followed PRISMA guidelines. It was determined that the main source to obtain the anatomical and physiological parameters required for the development of the models were previous publications and rodents were the most used species; *in vivo* studies were mainly used for model validation. Additionally, 76% of the PBPK models were developed to extrapolate pharmacokinetic parameters to humans and the main application of the models was in health risk assessment. PBPK modeling is an alternative that allows the integration of *in vitro* and *in silico* data and parameters reported in the literature to predict the pharmacokinetics of chemical substances, reducing in large quantity the use of animals in research.

Financial support and acknowledgements

Acknowledgments to the Universidad Nacional de Colombia and the Federal University of Bahia for making possible the project "Beyond Research" and allowing the development of the present review. To the Sistema Nacional de Bibliotecas (SINAB) of the Universidad Nacional de Colombia and the professors involved for their guidance and support.

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PHYSIOLOGY-BASED PHARMACOKINETIC MODELING OF METHYLPHENIDATE FOR THE TREATMENT OF ATTENTION DEFICIT HYPERACTIVITY DISORDER IN ADULTS

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Physiology-Based Pharmacokinetic (PBBPK) modeling is a mathematical approach that offers a unique modality to predict age-specific pharmacokinetics based on physiological parameters [1]. Attention Deficit Hyperactivity Disorder (ADHD) is a common neurodevelopmental disorder in childhood and may persist into adulthood, addressing approximately 2.5 to 5% of this group [2]. Methylphenidate (MPH) is a psychotropic stimulant drug and the first pharmacological choice for treating ADHD [3, 4]. But the various problems associated with its use require further investigation into its pharmacokinetics (PK) in treating ADHD, especially in human adults [5, 6]. A PBBPK model for MPH was developed in the PK-SIM® software (version 8) utilizing *in silico*, *in vitro* drug metabolism, and *in vivo* pharmacokinetic parameters. Adult male and female individuals (18-65 years) were built considering CES1 as predominant metabolizing enzyme, half-life = 3.5 h, Cl = 10.5 L/h/kg, Vss = 2.23 L/kg. Regarding compound properties, MPH presents logP = 1.47, low protein binding = 15%, MW = 233.31 g/mol, experimental solubility = 1255 mg/L, pKa = 9.09 (strongest base) and no halogens in its structure. Only the oral extended-release (Concerta® 18 mg) formulation was considered for the model development. Once finished, the PBBPK model will be beneficial in guiding optimal dosing regimens for MPH in the adult population. Financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)-Finance Code 001.

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PROGESTERONE INTRAVAGINAL DEVICE PHARMACOKINETIC MODEL

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Progesterone-releasing intravaginal devices are used to control the cattle estrous cycle in the protocol of Fixed-time artificial insemination (FTAI), which is helpful for rationalizing livestock management [1]. The aim of this study is to create a pharmacokinetic model of progesterone-releasing intravaginal devices in cattle to support development of new protocols and intravaginal devices. The model used data from different intravaginal devices commercially available in Nellore (CIDR® 1,9g; Primer® 0,5g; Proclelar® 0,75g; Syncrogest® 1g) and Holstein animals (CIDR® 1,38g; PRID® 1,55g; Proclelar® 0,75g; Repro sync® 2g; Cue Mate® 1,56g; DIB® 0,5g; Syncrogest® 1g) [2]. It was developed with Lixoft® software, applying the constants calculated from Monolix suit in PK analix 2020R1 [3]. The PK model was extravascular, with delay, zero order, one compartment and Michaelis-Menten kinetics. The values of the parameters were determined in absorption duration (T_{k0}) 11,84 mg.h⁻¹, lag time (T_{lag}) 2,78 h, Michaelis-Menten constant (K_m) 4,09 mg.L⁻¹, maximum velocity (V_m) 11,87 mg.h⁻¹ and volume of the central compartment (V) 482,09 L⁻¹. Therefore, the model was able to estimate plasma concentrations of progesterone reliably with a homogeneous dispersion of the predicted value line, within the 90 % interval, even with a diverse data from two races with different devices and concentrations. From this work it is possible to establish new protocols and devices for FTAI.

Ethical approval

Since this is a silico study, no animals were used.

Financial support and acknowledgements

This study was funded by a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - 313757/2019-4), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG - PPM 00691/17), EMBRAPA and Undergraduate Research Scholarship Program of UFLA.

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PHARMACOKINETIC MODEL OF FLORFENICOL IN NILE TILAPIAS (*Oreochromis niloticus*) SUBMITTED TO DIFFERENT REARING TEMPERATURES

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One of the most important factors for tilapia breeding is water temperature, since temperature variation influences the kinetics of antibiotics, therefore it is essential to optimize doses, as it may cause adverse effects and generate residues in animal products [1]. This study aimed to develop a pharmacokinetic (PK) model of florfenicol in Nile tilapia (*Oreochromis niloticus*) subjected to different rearing temperatures to estimate the plasma concentration of the products authorized in Brazil. For this purpose, a PK model was created from previously published data [2]. The plasma concentrations were estimated from treatments with doses of 10, 15 and 20 mg/Kg administered every 24 hours for 10 days, at temperatures of 18, 21, 26, and 30°C, using Monolix and Simulix Suite 2020R1 [3]. The parameters of lag time ($R = -0,882$), absorption ($R = 0,867$), and clearance ($R = 0,807$) were correlated with temperature. It can be observed that animals kept at 30°C have a median plasma concentration of half that of animals raised at 18°C treated with the same doses. This PK model serves as a basis for developing a pharmacokinetic/pharmacodynamic model for optimizing doses of florfenicol based on the rearing temperature of Nile tilapia.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

This study was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological

Development (CNPq), the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), and the Undergraduate Research Scholarship Program of UFLA.

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ASSOCIATION BETWEEN PLASMA IMATINIB LEVELS AND RESPONSE T₀ TO THE TREATMENT OF CHRONIC MYELOID LEUKEMIA IN PATIENTS FROM MANAUS, BRAZIL

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Imatinib mesylate (MI) is the drug of choice for the treatment of Chronic Myeloid Leukemia (CML). However, despite the majority of the results obtained with this therapy being positive, some patients still present a suboptimal therapeutic response or still develop some type of resistance [1]. Therefore, the aim of this study was to evaluate IM plasma levels in CML patients treated at a referral unit in Manaus and correlate them with variables that might interfere with these levels. Data from 52 patients were obtained through a standardized questionnaire containing clinical, sociodemographic,

lifestyle and use of other medication information, as well as an estimate of therapeutic adherence. Additionally, blood collection was performed to measure the plasma concentration of the drug using the HPLC-UV technique. Molecular studies were done to identify the presence of polymorphism in the ABCG2 C421A membrane transporter. Among the results obtained, most patients were male with a mean age of 52 ± 12.3 years (95% CI 49.0-55.9). There was a high variation in drug concentrations in the range from 0 to 4694 ng/mL, with a mean of 1558.59 ± 989.79 ng/mL (95% CI 1283.0-1834.1). Approximately two thirds of patients were classified in the drug level range considered therapeutic. Furthermore, there was a correlation between plasma concentration and higher molecular response. Additionally, most individuals had the normal genotype for the ABCG2 C421A polymorphism but further studies should be performed to reveal the role of this variable in the outcome of the disease in this population.

Ethical approval

The study was approved by the Research Ethics Committee at the Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas, Manaus, AM, Brazil (approval number 2.540.371).

Financial support and acknowledgements

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) for providing research grants.

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PBPK MODEL FOR PREDICTING DDI BETWEEN CHLOROQUINE AND COLCHICINE IN HEALTHY AND INFECTED PATIENTS

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New alternatives for the treatment of meloxicam should be explored due to resistance, such as drug repositioning [1] and encapsulation in nanoparticulate systems. How Chloroquine (CQ) [2] is a CYP inhibitor and Colchicine (CC) [3] is a CYP substrate, the study of synergism between the drugs for the treatment of malaria is important. As a pre-formulation study, the objective was to evaluate drug interactions (DDI) of CQ and CC for further co-encapsulation in nanocapsules. Physiologically based pharmacokinetic (PBPK) modeling was performed with the PK-Sim 9.0 software. The models for CQ and CC were independently optimized, using data in the literature [4,5,6] with oral administration (dose 300 mg CQ and 1 mg CC) in healthy and infected (altered hematocrit) patients. Subsequently, DDI was evaluated between CC and CQ. The models presented predicted error and mean fold error below 2%, demonstrating that the built model was able to describe the observed data. DDI of CQ and CC were analyzed, separately, with CQ inhibiting CYP3A4, CYP2D6, and P-gp to analyze if they may impact in PK of CC. There were no changes in CC AUC, T_{max}, and C_{max} for any assessment when CQ was inhibited. We also separately evaluated the impact on CQ PK when CC showed inhibition of CYP3A4 and P-gp, similarly, there were no changes in CQ AUC, T_{max}, and C_{max}. PBPK model was capable of characterizing the PK of CQ and CC in healthy patients and sick patients and demonstrated that there was no drug interaction under the conditions tested when administered, simultaneously.

Financial support and acknowledgements

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and 19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) #309401/2020-8).

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ELEVATED VANCOMYCIN THROUGH LEVELS IN PATIENTS ADMITTED TO AN UNIVERSITY HOSPITAL FROM MANAUS, BRAZIL

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Vancomycin is a key antibiotic in the therapy of Gram-positive infections caused mainly by *Staphylococcus* sp. Several studies have reported that inadequate dosage is associated with therapeutic failure, bacterial resistance and toxicity [1]. Therefore, therapeutic drug monitoring (TDM) of this agent is essential to ensure the achievement of clinical results and minimize adverse effects according to the literature. Thus, this study aimed to evaluate the through serum levels of vancomycin in patients admitted to an university hospital. An analytical-observational cross-sectional study was carried out from January to October 2020 to attain this goal. Medical records including sociodemographic, clinical and laboratorial data were collected and the plasma levels of vancomycin were achieved using a previously validated HPLC-UV method [2]. In total, 50 patients were included in this investigation. Most patients were male (60%) and the mean age observed was 50.76 ± 19.47 years. The mean through vancomycin concentration was 40.34 ± 24.21 mg/L. Furthermore, approximately half of the patients had serum levels considered to be toxic (> 30 mg/L). The association of higher levels in patients on polypharmacy regimen and with acute renal failure was also observed. The results obtained here call attention to the importance of TDM in order to adjust drug therapy of vancomycin.

Ethical approval

The study was approved by the Research Ethics Committee at the Universidade Federal do Amazonas, Manaus, AM, Brazil (approval number 3.858.967).

Financial support and acknowledgements

The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing research grants.

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THERAPEUTIC DRUG MONITORING OF PHENOBARBITAL IN SALIVA IN EPILEPTIC DOGS

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In veterinary medicine, therapeutic monitoring of phenobarbital (PB) in patients under antiepileptic treatment is performed routinely in blood samples. This method is invasive and, in some patients, impractical due to the difficulties involved in its handling. In this work, we investigated the usefulness of saliva as monitoring fluid. Fourteen epileptic dogs under treatment with PB were included. Serum and saliva samples were extracted simultaneously at steady state and concentration were analyzed using Monolix® 2021R1 software (Lixoft SAS, Antony, France). Saliva/serum fraction (f), apparent clearance (CL/F) and covariate analysis were performed. A monocompartmental disposition model was assumed and typical estimates for absorption rate constant (ka) and apparent volume of distribution (Vd/F) fixed based on bibliographic information [1,2]. A positive correlation between PB concentrations in saliva and serum was found. A monocompartmental model with autoinductive elimination CL was found to better describe PB data [3,4]. Seizure control and age were found to significantly affect f, while CL was significantly affected by WT and age.

Equation best describes CL and f were:

$$CL = CL_{pop} (WT/20)^{3/4} (AGE/5)^{\beta_{AGE}}$$

$$f = f_{pop} (AGE/5)^{\beta_{AGE}} f_{AGEc}^{\beta_{f\_CONTROL\_2}} \text{ (if CONTROL=2)}$$

Being CONTROL=2 no responsive patients, 20 the average WT in kg, and 5 the average age in years of the studied population

Discussion and Conclusion

There is a good correlation between saliva and serum concentrations of PB being saliva a suitable fluid for therapeutic monitoring of PB in dogs. The difference in f between responders and refractory patients could be due to an overexpression of efflux transporters in the salivary gland of unresponsive patients.

Ethical approval

This research was approved by the Committee of Ethics in the Use of Animals (CEUA-FVET) of the School of Veterinary Medicine-Protocol number 799

Financial support and acknowledgements

Financial Support: funds from the Pharmacology and Therapeutics unit

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USE OF PK/PD MODELING TO MINIMIZE THE ENVIRONMENTAL IMPACT OF THE USE OF DORAMECTIN 3.5% AGAINST *RHIPICEPHALUS MICROPLUS* IN CATTLE

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The use of antiparasitic drugs contributes significantly to the increase of productivity in cattle breeding, and optimization of the dosing regimen is essential for greater efficacy and reducing residue elimination in the environment [1]. The aim of this study was to develop a pharmacokinetic/pharmacodynamic (PK/PD) model of 3.5 % doramectin protocols against *Rhipicephalus microplus* in cattle. For this purpose, previously published PK and PD data were used [2]. Plasma concentrations and efficacy against ticks were estimated from single dose (700 µg/Kg on day 0), two-dose (350 µg/Kg on days 0 and 14) and four-dose (175 µg/Kg on days 0, 14, 28 and 42) regimens, using Monolix and Simulix Suite 2020R1[3]. The PK model had extravascular route of administration, lag time, first-order absorption, one-compartment distribution and linear elimination; the PD model had direct response, Emax for drug action, baseline = 0 and sigmoidicity. Means of estimated parameters were: lag time (Tlag = 2.49 d), absorption rate constant (Ka = 0.089 d⁻¹), clearance (Cl = 356 mL d⁻¹), volume of the central compartment (V = 2036.8 mL), maximum effect (Emax = 100 %), concentration producing 50 % of Emax (EC50 = 4.88 µg/mL) and Hill's coefficient (gamma = 1.29). The model was able to estimate a protocol that increases the residual effectiveness period by 75 % without increasing the environmental impact.

Ethical approval

No animals were used in this *in silico* study.

Financial support and acknowledgements

The study received financial support from the Coordination for the Improvement of Higher Education Personnel (CAPES), National Council for Scientific and Technological Development (CNPq - 448011/2014-0), Brazilian Agricultural Research Corporation (Embrapa) and Undergraduate Scholarship Research Program from Federal University of Lavras (UFLA).

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DEVELOPMENT OF DRUG-DRUG INTERACTION MODELS IN VITRO FOR MACROCYCLIC LACTONES AND THEIR UNDERSTANDING

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Combination anthelmintic formulations have gained traction in the role of drugs in the control of parasitic nematode in recent years. While the use of pharmacometrics modeling is well-established

in the drug formulations development process, previously there is an increasing need for a better quantitative biological understanding of the drug-drug interaction (pharmacodynamic). In this study, we assessed the synergism of two anthelmintic drugs, eprinomectin (EPM) and ivermectin (IVM), widely used in veterinary medicine. In the animal model *Caenorhabditis elegans* [1], we used an infrared motility assay to measure EPM and IVM effects on worm movement over time. Different EPM/IVM concentration ratios were used and synergy scores determined with two different methodology to determine the drug exposure (AUC vs. Count at last time). The study discussing four modeling efforts in the field, applying highest Single Agent (HSA), Loewe additivity model (Loewe), Bliss model (BLISS) and zero Interaction Potency (ZIP) models [2]. The results the Synergy Score values were lower than ± 0.5 for all models, clearly indicating that there is no synergy between both macrocyclic lactones. This study show that it is more relevant to prioritize the exposure time of each individual drug than to combine them to improve their effects. The results highlight the utility of *C. elegans* to address preliminary pharmacodynamics studies, particularly for drug-drug interactions. Models *in vitro* can be further integrated to facilitate preclinical to clinical translation and help researchers better understand the mechanisms of drug-drug interactions to help achieve a more favorable therapeutic.

Ethical approval

The study does not require approval.

Financial support and acknowledgements

This work was supported by CSIC - Universidad de la República and FOCEM - Fondo para la

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SIMULATION OF VARIABLE RATE INFUSION REGIMENS FOR PROPOFOL IN DOGS

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Variable rate infusion (RCI) aims to adapt the infusion rate for constant plasma concentration over time. Pharmacokinetic (PK) models coupled with RCI systems has been increasingly used in veterinary medicine [1]. The objective of this study was to develop a PK model of propofol for simulations of different regimens of bolus induction associated with RCI in dogs. Raw data of propofol plasma concentration over time in dogs was used for the development of the PK model [2]. Induction and maintenance bolus protocols with RCI were simulated in order to maintaining plasma concentration within an established target for magnetic resonance imaging (3 - 6 ug/mL) and dental procedures (2.5 - 4.7 ug/mL). Monolix and Simulix Suite 2020R1 was used for modeling and simulation [3]. The established PK model had no delay, three-compartment distribution and linear elimination. Protocols able to keep consistent plasma concentration over time had 2, 3 and 4.5 mg/Kg bolus induction, and initial rates of 200, 300 and 450 ug/Kg/min, with rate reductions of 10, 15 and 25 ug/Kg/min every 20 minutes, respectively. For MRI, 3 - 300 - 15 protocol kept the mean plasma concentration within the target, as well as the 2 - 200 - 10 protocol demonstrated data within the target for dental procedures. The PK model was able to estimate plasma concentration of propofol in dogs, and enabled the investigation of rate adjustments to maintain propofol plasma concentration within the established range.

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

This study was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq), the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), and the Undergraduate Research Scholarship Program of UFLA.

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MODELING & SIMULATION OF TRAMADOL IN RED-EARED TURTLE (*TRACHEMYS SCRIPTA SCRIPTA*)

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In reptiles and avians, the administration site can impact the pharmacokinetic profile and the therapeutic effect due to renal and/or hepatic portal deviation [1,2]. Thus, modeling & simulation (M&S) helps to predict the pharmacokinetics and effect in these animals. The objective was to build a pharmacokinetic/pharmacodynamic (PK/PD) model to simulate therapeutic protocols of tramadol, after administration on the forelimb and hindlimb, in the *T. scripta scripta* species. The data of plasma

concentration and antinociceptive effect were obtained from literature, from which it was constructed a PK/PD model, and protocols were simulated at a dose of 10mg/kg at different intervals of administration [12 (q12h), 24 (q24h) and 48 (q48h) hours] and administration routes (hindlimb or forelimb) [2]. For M&S, the Lixoft® 2021R1 package was used [3]. The PK model was of extravascular administration, without delay, with first-order absorption, three compartments and linear elimination, while the PD model was with a turnover response, stimulation-producing action and with sigmoidity. The protocol of 10mg/kg (q12h) maintained the antinociceptive effect for a longer time, in both administration routes. The forelimb had a higher plasma concentration, but the

hindlimb had a greater effect, which may be related to the hepatic portal shunt, which results in the metabolism of tramadol into O-demethyltramadol hydrochloride, its active metabolite. Because of the M&S, it was possible to perceive the effect of the hepatic port system on the metabolism of tramadol, and consequently, the difference in the effect depending on the route of administration [4]

Ethical approval

Since this is an *in silico* study, no animals were used.

Financial support and acknowledgements

This study was funded by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Undergraduate Research Scholarship Program from Federal University of Lavras (UFLA).

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IN VITRO RELEASE AND POPULATION PHARMACOKINETIC MODELING OF FREE AND NANOENCAPSULATED MELOXICAM IN MALE WISTAR RATS

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Computational modeling through simulations proves to be a promising alternative in the drug research, development and innovation (RD&I) phases. The aim of this study was to characterize a pharmacokinetic model (PK) in male *Wistar* rats (n=10/group) treated with free meloxicam (F-MLX) and encapsulated in cationic nano-capsules (NCM). The pharmacokinetics of F-MLX and NCM were performed at a dose of 5 mg/kg iv and plasma concentrations were quantified on HPLC-PDA. Concentration versus time profiles were modeled using a population pharmacokinetic (popPK) approach in MonolixSuite™ 2020R1 (Simulation Plus, USA). *In vitro* release experiments using the dialysis bag method in pH 7.4 phosphate buffer medium were performed, aiming to describing release of MLX in the blood. Based on this, the release constant (kr) was calculated (DDSolver) and added to the NCM model. A two-compartment model with linear elimination, combined1error and normal distribution described the F-MLX. The values obtained were fixed to NCM modeling, together with the kr value previously calculated. The model that best described it was the three-compartment model with linear elimination, combined1error and normal distribution. The F-MLX and the NCM had a similar Volume of Distribution ($V_1=0.028$, $V_{1NC}=0.03$; $V_2=0.027$ and $V_{2NC}=0.033$ mL, respectively). However, for NCM, there was a third compartment, with a relatively small intercompartmental clearance ($Q_{3NC}=0.0095$ L/h), and a relatively large V_{3NC} (0.21 mL), when compared to the other compartments. These results demonstrate that it was possible to describe the behavior of nanoencapsulated MLX using *in vitro* and *in vivo* data.

Ethical approval

Protocol was approved by the Ethical Committee for Animal Use of UNIPAMPA (Protocol N°030/2021).

Financial support and acknowledgements

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and #19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) grant #309401/2020-8. We want to thank Simulation Plus for MonolixSuite™ license.

DEVELOPMENT AND VALIDATION OF A MICROSAMPLING STRATEGY TO OPTIMIZE ANTITUBERCULOSIS THERAPY

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Tuberculosis (TB) is caused by the *Mycobacterium tuberculosis* complex and the Directly Observed Therapy Short-course (DOTS), recommended by World Health Organization, includes first-line anti-tuberculosis drugs such as isoniazid (INH), rifampicin (RFM), pyrazinamide (PZA) and ethambutol (ETB) [1,2]. At present, the standard method of blood sampling for therapeutic drug monitoring (TDM) is venipuncture; unfortunately, it can cause discomfort in TB patients. Dried blood spots (DBS) are a minimally invasive method for the collection of whole blood [3]. The aim of this study was to develop a UPLC-MS/MS method for quantification of anti-tuberculosis drugs in plasma and DBS to demonstrate its clinical application in TDM. Separation and detection conditions were optimized to quantify the four anti-tuberculosis drugs in both samples. For plasma, the method was linear from 0.5-25 µg/mL for RFM, 0.5-12 µg/mL for ETB, 0.5-20 µg/mL for INH and 5-120 µg/mL for PZA. For DBS, the method was linear from 0.375-24 µg/mL for RFM, INH and ETB and 2.031-130 µg/mL for PZA. The methods developed were proved to be precise (inter- and intra-assay), selective and accurate; matrix effect was demonstrated to be within the established limits. Short- and long-term stability, as well as freeze-thaw cycles for plasma and DBS were determined. The method was applied in the monitoring of the anti-TB drugs in 9 TB patients. Finally, anti-TB drugs plasma concentrations were related to DBS concentrations by linear regression to determine the concordance between both measures. The plasma results are clinically useful for decision-making in dose adjustments.

Ethical approval

The present work was approved by “Comité de ética en investigación del Hospital Central Dr. Ignacio Morones Prieto” with registration 67-21 and “Comité de ética en Investigación y Docencia de la Facultad de Ciencias Químicas (CEID-FCQ)” with code CEID2021-012-S.

Financial support and acknowledgements

This work was promoted by the Technological Research Council of Science for a master’s fellowship (781907).

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A MODEL FOR THE CHANGES IN THE PHARMACOKINETICS OF QUERCETIN-3-O-RUTINOSIDE IN AN EXTRACT OF CALYCES FROM *PHYSALIS PERUVIANA* IN RATS

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Quercetin-3-*O*-rutinoside (rutin) is the marker compound of a hydroethanolic extract of calyces from *P. peruviana* L. with hypoglycemic activity [1]. The pure flavonoid belongs to class IV of the biopharmaceutical classification system, but the complex mixture of compounds in the extract increases its solubility [2] and produces pharmacokinetics alterations in animals [3]. The aim of this work was to develop a population pharmacokinetic model (popPK) describing the changes in the pharmacokinetics of rutin due to the extract in rats. Blood plasma concentrations of rutin and quercetin (representing rutin metabolites) were obtained from rats administrated with at least two independent doses of pure rutin or extract intravenously and orally ($n = 5$), using a validated bioanalytical method. The popPK modeling was carried out in Monolix 2019R1. Blood plasma concentration-time profiles of i.v. rutin were fitted to a model of two compartments with linear elimination. Only quercetin was detected from p.o experiments, and their concentrations were fitted to a model with double absorption. The analysis of covariates showed that the extract increases the volume of distribution and the elimination rate of rutin, but also increases the absorption rate and decreases the time for the second absorption of their metabolites, in a dose-dependent manner. These observations were consistent with the non-compartmental and statistical analysis of the data. The popPK modeling approach let to better describe the complex pharmacokinetics of rutin and their metabolites and the variability due to the extract dose. The models could be used in new researches of the group.

Ethical approval

The study was approved by the ethics committee of the science faculty (Act 06, 2015, project 40831, 22 June 2015).

Financial support and acknowledgements

This research received no financial support from agencies.

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EFFECTS OF SEX ON PHARMACOKINETIC OF MELOXICAM IN RATS: A POPPK STUDY

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The pharmacological response may vary in men and women due to their anatomical, physiological and hormonal differences. Studies related to the influence of gender on the pharmacokinetics (PK) of drugs allow us to understand this variability in the pharmacotherapeutic response [1]. MLX, a non-steroidal anti-inflammatory drug used to treat pain, inflammation, and rheumatoid arthritis, has been widely used by both sexes [2]. In studies with male and female rats, there are several discussions about PK and the influence on sex is still controversial for MLX [3]. The aim of this study was to evaluate the influence of sex on the PK of MLX after 5 mg/kg, iv dosing in male and female rats. Plasma data from the male (n=11) and female (n=12) rats amounting to 319 observations were analyzed using the MonolixSuite™ 2020R1 (Simulation Plus, USA). The best-fit structural model is the two-compartment model with linear elimination. Inter-individual variability and correlation between Clearance (Cl) and Volume of distribution of the central compartment (V1) were entered. In covariate analysis, the female gender in Intercompartmental Clearance (Q) was statistically significant. The values for the fixed effects agree, as well as the coefficient of variation for all parameters and the visual predictive check with the observed data. Then, the final PopPK model was able to describe MLX PK for both sexes and demonstrated that females impact the drug plasma clearance.

Ethical approval

The experimental protocol was approved by the Ethics Committee on the Use of Animals of the UNIPAMPA (030/2021).

Financial support and acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and #19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) grant #309401/2020-8. We want to thank to Simulation Plus for MonolixSuiteTM license.

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PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF A NOVEL TIZANIDINE HYDROCHLORIDE SYRUP FOR PEDIATRIC USE

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Tizanidine hydrochloride (TZ) is a muscle relaxant and is an effective and safe drug for decreasing spasticity associated with spastic cerebral palsy in children. The problem is that TZ is sold only in tablets¹. To bypass it, a syrup containing TZ for children was developed². The aim of this work was to predict TZ plasma concentrations in children through a physiologically-based pharmacokinetic (PBPK) model after the administration of this novel TZ syrup. Plasma drug concentration data in adults after oral administration of TZ tablets (4mg and 8mg) were collected and used to build the PBPK model. After syrup formulation inclusion, the model was scaled to children accounting for age-related changes. Thus, TZ plasma concentrations were predicted through the model in children aged from 0 to 2 years and from 2 to 12 years. Pharmacokinetics (PK) parameters were evaluated. The model predicted TZ plasma concentration in adults was well performed and the average fold error (AFE) fits the 2-fold acceptance criteria in both scenarios (1.79 and 1.19). Eldest children's clearance (CL) and volume of distribution (Vd) were 2.65 ± 0.48 L/h/kg and 7.22 ± 0.74 L/kg, respectively. For the youngest, estimated values were 2.33 ± 0.74 L/h/kg and 6.64 ± 1.13 L/kg. Although, the area under the curve (AUC) for this group (6157.21 ± 12097.82 ng.min/mL) shows more variability in comparison to the eldest (2029.30 ± 707.71 ng.min/mL). In conclusion, TZ syrup seems to be safe for eldest children. On the other hand, more studies are necessary for the youngest population.

Ethical approval

Not applicable.

Financial support and acknowledgements

Financial Support from Coordination for the Improvement of Higher Education Personnel (CAPES). Acknowledgments to Coordination for the Improvement of Higher Education Personnel (CAPES) and Hospital de Clínicas de Porto Alegre (HCPA).

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PHARMACOKINETICS OF METHADONE IN ADULTS PATIENTS UNDERGOING CARDIAC SURGERY WITH EXTRACORPOREAL CIRCULATION

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Methadone has characteristics that make it attractive for postoperative pain management after cardiac surgery. The use of cardiopulmonary bypass (CPB) involves changes that can alter pharmacokinetics and clinical response to drugs [1]. The aim of this work was to describe the pharmacokinetics of an intravenous bolus dose of methadone in adult patients undergoing cardiac surgery with CPB. We prospectively studied 18 patients between 45 and 75 years scheduled for cardiac surgery with CPB. A single bolus of 0.2 mg/kg methadone was administered in anesthetic induction. Then arterial blood samples were taken during 24 h from each patient. Methadone concentrations plasma analysis was performed using a spectrofluorometric method. Population pharmacokinetic analysis was performed with the program NONMEM. One- and two-compartmental models were used to describe the time profile of methadone plasma concentrations. All 18 patients completed the study. A two-compartment structural model produced a better fit of the data than a one-compartment model. (Decrease in the OFV of 297 points, $p < 0.01$) The influence of CPB on methadone PK was better described by an increment in elimination clearance during CPB. Incorporation of this new parameter greatly improved the fit of the model with a decrease in OFV of 43 points. ($p < 0.01$). The use of CPB alters the pharmacokinetics of methadone. These changes were adequately characterized by an increase in elimination clearance during CPB using a 2-compartment model with linear elimination. The clinical impact of the observed CPB changes in methadone pharmacokinetics are probably negligible with current dosing scheme.

Ethical approval

The study was registered at clinicaltrials.gov NCT05075265.

We obtained Clinical Ethics Committee approval and patient informed consent before starting the study. ID protocol 200323005.

Financial support and acknowledgements

This work was financed with grant funds granted by the Chilean Society of Anesthesiology.

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DEVELOPMENT AND VALIDATION OF A PHYSIOLOGICALLY BASED BIOPHARMACEUTICS MODELING TO ASSESS THE BIOEQUIVALENCE OF FUROSEMIDE ORAL PRODUCTS IN DOGS

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Furosemide is widely used in cardiological therapies in canines [1], however there is a lack of knowledge regarding the dose-exposure-response relationship and the impact of multi-source drug products with no bioequivalence evaluation. In this work, we aimed to predict the relative bioavailability between two furosemide oral local products available for use in dogs by means of physiologically based biopharmaceutics modeling (PBBM), integrating formulation-related parameters estimated through *in vitro* dissolution testing in a PBPK model [2,3]. Further, the model predictions were validated with a bioequivalence study in healthy dogs (n=6). A previously reported furosemide PBPK model for humans [4] was adapted to dogs in PK-Sim® and MoBi® [5] using literature pharmacokinetic data in plasma and urine after intravenous and oral administration [6,7]. The model was optimized including a concentration-dependent increase in renal blood flow, affecting furosemide renal excretion. Dissolution profiles from two local products obtained in biorelevant conditions were fitted with a gomPERTZ model and included in the PBPK model to describe drug dissolution. Single-dose furosemide C_{max} and AUC for both formulations were predicted with a relative bias below 30%. The predicted Test/Reference GMR in dogs (n=1000) were 0.156 for AUC_{0-inf} (90%CI 0.102-0.249) and 0.146 for C_{max} (90%CI 0.105-0.202), while the observed results were 0.186 for AUC_{0-inf} (90%CI 0.132-0.260) and 0.203 for C_{max} (90%CI 0.129-0.320). The PBBM approach predicted adequately furosemide pharmacokinetics and the relative product performance in dogs.

Ethical approval

The protocol for the *in vivo* determination of plasma concentrations of furosemide in dogs was approved by the Ethics Commission for the Use of Animals (CEUA) of the Honorary Commission for Animal Experimentation (CHEA) under protocol number 1174 of the period 2021-2022.

Financial support and acknowledgements

Postgraduate students María Luisa Rodríguez and Cristian Valiante receive funding from the CAP (Postgraduate Academic Committee) postgraduate scholarship (UdelaR) and the economic rate for postgraduate students of the Basic Sciences Development Program (PEDECIBA).

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POPULATION PHARMACOKINETICS OF MOXIFLOXACIN IN PATIENTS FROM A TERTIARY CARE CENTER IN MUMBAI, INDIA

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Moxifloxacin (MFX) is one of the drugs recommended by the World Health Organization to be included in the regimen to treat multi-drug resistance tuberculosis (MDR-TB) and India is the country with the highest absolute burden with 27% [1]. The aim of the study was to describe the pharmacokinetics of MFX in an Indian population with MDR-TB. A total of 234 concentrations from 39 MDR-TB Indian patients were analyzed. Twenty four patients were female, with median weight, fat-free mass, and age of 56 (range 35–103) kg, 40 (26–73) kg, and 28 (17–46) years, respectively. The pharmacokinetics of MFX was best described by a two-compartment model with first-order elimination and transit compartment absorption. The allometry was implemented using fat-free mass and weight. The typical values for clearance, intercompartmental clearance, central and peripheral volume were 10.6 L/h, 4.45 L/h, 90.7 L, and 50.8 L, respectively. Between subjects variability on clearance was 31.2% described by an exponential function. The unexplained variability was 3.96% (heteroscedastic component) and 0.16 mg/L (homoscedastic component), additionally an extra additive error was estimated for unobserved data in 0.35 mg/L. The simulations showed that a MFX dose of 800 mg/24h is adequate if the minimum inhibitory concentration (MIC) is 0.5 mg/L. However, for a MIC of 0.25 mg/L a dose of 400 mg/24h is enough to achieve the therapeutical target of area under the curve of free MFX/MIC > 53[2] in more than 90% of the simulated patients.

Ethical approval

Institutional Review Boards at the PD Hinduja National Hospital and Medical Research Centre (884-14-ZFU) & Johns Hopkins University School of Medicine (IRB00076738).

Financial support and acknowledgements

Data was collected as part of the Regional Prospective Observational Research for Tuberculosis (RePORT) India Consortium as a part of P.D. Hinduja Hospital funded RePORT India Parent Protocol. This project has been funded in whole or in part with Federal funds from the Government of India's (GOI) Department of Biotechnology (DBT), Department of Science and Technology, South Africa MRC, the United States National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Office of AIDS Research (OAR), and distributed in part by CRDF Global. Study investigators were supported NIH NIAID (K23AI135102, R21AI122922, and UM1AI069465), the UJMT Fogarty Global Health Fellows Program (D43 TW009340), and the Johns Hopkins University School of Medicine Clinician Scientist Career Development Award. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the DBT, the DST, the SA MRC, the NIH, or CRDF Global.

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IMPLEMENTATION OF A PILOT THERAPEUTIC DRUG MONITORING PROGRAM FOR ANTIBIOTICS IN PEDIATRIC PATIENTS

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Antibiotics are among the most utilized drugs in pediatrics. Nonetheless, there is a lack in pharmacokinetics (PK) information for this population, and dosing criteria may vary between healthcare centers [1]. Physiological variability associated with maturation in pediatrics makes it difficult to reach a consensus on adequate dosing, and it is accentuated in more vulnerable groups, such as critically ill or oncology patients [2]. Therapeutic drug monitoring (TDM) is a useful practice that allows dose optimization and attainment of antibiotic-specific PK/pharmacodynamic (PD) targets [3]. The aim of this study was to implement a TDM program for antibiotics in pediatric patients on a local hospital in which this service is not available. A liquid chromatography coupled to tandem mass spectrometry method for the quantification of clindamycin, fluconazole, linezolid, meropenem, metronidazole, piperacillin and vancomycin in plasma was developed and validated based on FDA guidelines. A total of 23 pediatric patients aged 2-16 years were monitored either with an optimized sampling scheme or through opportunistic sampling. PK parameters were estimated through a Bayesian approach using the Abbottbase PKS software. The most frequent diagnostic was neutropenic fever and pneumonia in immunocompromised oncology patients (49%), followed by others such as appendicitis (17%); 43 TDM events were registered and 27 (63%) of them required dose adjustments as follows: 14 patients were underdosed, 4 were overdosed and 9 patients needed infusion rate adjustments. These results show high variability in target attainment throughout pediatric patients with standard dosing regimens, showcasing the need for TDM in especially vulnerable subpopulations.

Ethical approval

The study was submitted and approved by the Hospital's Research and Ethics Committee (Registration number: 69-21).

Financial support and acknowledgements

The present study was financed by Mexico's *Secretaría de Educación Pública* Through the government program: *Programa para el Desarrollo Profesional Docente* (Oficio número: 511-6/2020-8585).

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EXTERNAL EVALUATION OF POPULATION PHARMACOKINETIC MODELS FOR TACROLIMUS IN URUGUAYAN KIDNEY TRANSPLANT PATIENTS

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Tacrolimus (FK) is a calcineurin inhibitor commonly used as part of immunosuppressive therapy in renal transplant patients to prevent graft rejection. Due to its high intra- and inter-individual variability as well as its narrow therapeutic index, FK therapeutic drug monitoring (TDM) is indispensable. In this context, model-informed precision dosing (MIPD) can lead to safer and more effective treatments [1], and several population pharmacokinetic (popPk) models have been reported for FK and are available for prospective implementation. However, the large variability among these models in the predicted exposure of typical patients prevents its application with no previous validation. The aim of this work was to conduct an external evaluation of popPk models of FK in kidney transplanted adults using retrospectively collected TDM data from 39 hospitalized patients (998 observations) to assess the *a priori* and *a posteriori* predictive performance using the covariate information and Bayesian forecasting, respectively. A mean relative bias (rBias) between +/- 20% with the 95%CI including zero was considered clinically acceptable. The relative root mean square error (rRMSE) [2], was computed to assess precision. Other metrics for accuracy and precision were also considered. A total of 28 published models were evaluated showing significant variability in the predictive performance. Bayesian forecasting showed to significantly improve the model's accuracy and precision. Nine models [3-11], proved to meet the requirements to be considered suitable for potential dosages adjusts of FK in our population. Those models included CYP3A5 polymorphism, hematocrit, post-operative-day, and prednisone dose as relevant covariates.

Ethical approval

This work is part of the Project “Desarrollo de Modelos predictivos y farmacogenéticos en pacientes trasplantados renales en tratamiento con Tacrolimus” which was evaluated and accepted by the Ethics Committee of the Hospital de Clínicas Dr Manuel Quintela (Approved 03/2021).

Financial support and acknowledgements

This work is supported by the Agencia Nacional de Investigación e Innovación and by Comisión Sectorial de Investigación Científica Investigación + Desarrollo (CSIC I+D).

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PHYSIOLOGICALLY BASED BIOPHARMACEUTICS MODELING TO PREDICT BIOEQUIVALENCE RESULTS: A PROOF-OF-CONCEPT STUDY

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Omeprazole is a widely used proton pump inhibitor commercialized as capsules containing enteric-coated microgranules due to its liability at acidic conditions. The drug-induced increase in gastric pH can affect the enteric-coating performance under multiple-dosing, affecting the oral bioavailability. The aim of this work was to evaluate a physiologically based biopharmaceutic modeling (PBBM [1]) approach to predict the relative bioavailability achieved by omeprazole multi-source products after single and multiple doses. *In vitro* dissolution testing was performed in I-USP apparatus including an acidic stage followed by a neutral stage. Acidic stages with pH 1.2 and 4.5 were used to simulate the gastric pH after single and multiple doses, respectively. Resistance of the enteric coating at increasing pH (3 to 5) was tested in IV-USP apparatus. Omeprazole pH-dependent degradation kinetics were also characterized *in vitro*. This information was quantitatively included in a semi-PBPK omeprazole model, developed using Simulx® [2] by adapting and extending a previously published model [3]. Significant product-related differences in dissolution rates and enteric-coating performance were found. Relative bioavailability after single and multiple oral doses for multi-source products was predicted and validated in a bioequivalence study performed between the most different products in healthy subjects (n=12). Interestingly, the model predictions were found to accurately describe observations in men, since we observed a sex-by-formulation interaction in the bioequivalence outcome. The semi-PBPK model was further refined to characterize the physiologically based mechanism behind this interaction, finally achieving accurate predictions for both sexes. This work stands as a proof-of-concept for the use of PBBM in bioequivalence applications.

Ethical approval

The bioequivalence clinical trial was approved by the ethics committee of Faculty of Chemistry of the Universidad de la República, Uruguay.

Financial support and acknowledgements

The study was supported and financed by the National Agency for Research and Innovation (ANII).

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IN SILICO PHARMACOKINETIC PREDICTIONS FOR THE DESIGN OF A NOVEL GASTRORETENTIVE RELEASE SYSTEM FOR SILDENAFIL CITRATE

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In silico simulations have been suggested to reduce time and effort in the pharmaceutical development of drug products [1]. Currently, this approach has been successfully predicting *in vivo* performance of drugs and could be useful in the design of novel modified-release dosage forms (MR)[2]. Gastroretentive drug delivery systems (GRDS) are MR designed to prolong their residence time in the upper gastrointestinal tract [3-4]. Thus, this work proposes a novel GRDS for sildenafil citrate and evaluates the proposed formulations by *in silico* pharmacokinetic (PK) modeling and simulations with GastroPlus™. A compartmental model with mechanistic absorption was built. The predictive power for the pharmacokinetic parameters of C_{max} , T_{max} and AUC was proven. An *in silico* simulation study of a single dose of 60 mg in five formulations (F1-F5) was carried out and compared with the adopted target profile, the administration of 20 mg of immediate-release (IR) three times a day. It was possible to verify that formulations F2 and F4, which have a higher dissolution rate than the others, present AUC_{0-inf} and AUC_{0-t} very close to 100% in this comparison with the IR target profile. However, the absorption rate measured by the C_{max} parameter was below 80% for formulations F2, F3, and F5, indicating that the delay in drug release by the formulation also leads to a delay in the absorption process. Thus, it is concluded that gastroretentive systems are a promising alternative for controlling the dose release of drugs such as sildenafil, which have a narrow absorption window due to their solubility characteristics.

Acknowledgements

The authors thank Prati-donaduzzi Pharmaceutical Industry for its data support and, Simulations Plus for the academic license.

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CLOZAPINE-LOADED NANOCAPSULES IMPROVE ANTIPSYCHOTIC ACTIVITY IN RATS: BUILDING A PK/PD MODEL TO DISCRIMINATE NANOCARRIERS IN THE DEVELOPMENT STEP

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Here, we used a popPKPD modeling to discriminate the pharmacokinetics and pharmacodynamics of different coated-nanocapsules (NCs) containing clozapine (CZP), an antipsychotic with low solubility and serious adverse effects. A sequential model building approach (MonolixSuite™-2020R1-Simulation Plus-USA) was conducted integrating *in vitro* CZP release, plasma profiles after administration of different NCs (NC1, NC2, and NC3), and percentage of head movements in stereotyped model induced by amphetamine in rats [1]. A two-compartment model with linear elimination described CZP plasma data after i.v. administration of CZP solution. These parameters were fixed to subsequently analyze CZP plasma data for NCs. Two additional compartments were inserted to describe NC1 and NC2 distribution (anionic surface), while for NC3 (cationic surface) was included a third compartment. For all NCs, the rate constant for CZP release (k_r) was calculated from *in vitro* data using a first-order model with Fmax. We found that nanoencapsulation promotes an increase in the Vd for all groups, particularly NC3. An indirect turnover model for drug inhibition was found to characterize the PK/PD of CZP. In this analysis, one important limitation was the absence of PK data after intraperitoneal administration. Under the assumption that this route provides complete CZP bioavailability, results show a formulation-dependent plasma IC50, with 20-, 100-,

and 700-fold reduction relative to CZP solution (NC1, NC2, and NC3, respectively). This indicates that the dissimilar distribution achieved by NCs can alter the plasma exposure-response relation for CZP. The proposed popPK/PD model proved to be a useful tool for nanoformulation development.

Financial support and acknowledgements

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and 19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) #309401/2020-8). We want to thank to Simulation Plus for MonolixSuite™ license.

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APPROXIMATION TO THE POPULATION PHARMACOKINETIC MODEL OF A SELF-EMULSIFYING DELIVERY SYSTEM OF AN EXTRACT FROM *PHYSALIS PERUVIANA*

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The hydroalcoholic extract of *Physalis peruviana* presents poor oral bioavailability since it has low solubility and permeability according to previous investigations. A self-emulsifying delivery system (SEDDs) was formulated in order to improve the bioavailability of the extract, mainly the flavonoid rutin, its major therapeutic compound [1, 2]. The objective of this work is to perform the population pharmacokinetic modeling of the extract formulated with the SEDDs that describes the pharmacokinetic behavior of the extract in Wistar rats. The modeling was performed by the software Monolix 2021R1 (Lixoft®, Paris, France) and the model was chosen based on the observation of graphs, Akaike information criteria (AIC) values and accuracy of the estimated parameters. The final PopPK model has dual compartment, double absorption order 1 and order 0, a lag time for the second absorption and Michaelis Menten elimination [3]. Besides to the high bioavailability of the extract formulated in the SEDDS, the main difference with the unformulated extract is the Michaelis Menten elimination. That results could be explained since the high plasma levels of rutin (from extracts) after oral administrations SEDDs, cause changes in metabolic processes rate such as transport to the intestinal lumen and active tubular secretion can be saturated [4-6].

Ethical approval

The study was approved by the Ethics Committee of the Science Faculty of Universidad Nacional de Colombia (Act 06, 2015, project 40831, 22 June 2015).

Financial support and acknowledgements

To the Universidad Nacional de Colombia and lo Lixoft for the license of the Monolix software.

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PHYSIOLOGICALLY BASED BIOPHARMACEUTICS MODEL APPLICATIONS TO ANVISA: A BRIEF CHARACTERIZATION CAN EVIDENCE SOME REGULATORY TRENDS AND NEEDS

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Physiologically based biopharmaceutics modeling (PBBM) applications are increasing for all regulatory agencies [1]. PBBM has been used by pharmaceutical companies in distinct scenarios as a guide for drug development, ensuring quality and reducing clinical trials, and as support for regulatory decisions [2]. The aim of this work was to investigate and characterize the PBBM-related applications to ANVISA, from 2020 to 2021, considering two offices (CETER and GQMED). The inclusion criteria were applications including PBBM analysis to support chemistry, manufacturing, and controls (CMC) post-approval changes and bioequivalence assessment. Applications including physiologically based pharmacokinetic modeling (PBPK), population pharmacokinetic modeling (PopPK), and modeling and simulation (M&S) for other purposes were not considered. The results reveal a total of 4 applications, 2 per year, which encompass drugs classified according to Biopharmaceutic Classification System (BCS) as class I/III (n=2), class II (n=1), and class IV (n=1). All applications aimed to support bioequivalence assessment, half of them requesting biowaiver for register of lower (n=1) or higher (n=1) strengths and, the other half, biowaiver for manufacturing site change (n=2). The decision outcome was 1 application withdraw from the sponsor, 2 denials and 1 approval by ANVISA. All applications were subject to regulatory questions and for the ones denied, the sponsor could not provide the data and justification necessary. The trend of rapid increase in PBBM applications necessitates the exploration of ANVISA current regulations to accommodate this demand and to provide guidance on M&S good practices for industries.

Financial support and acknowledgements

Borges, Fernandes, and Mendes thank ANVISA for the availability and authorization to work with this research.

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STUDY OF AMIKACIN POPPK IN HOSPITALIZED PATIENTS WITH DIMINISHED CREATININE CLEARANCE

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Amikacin (AMK) is an aminoglycoside antibiotic widely prescribed to treat life-threatening infections, with concentration-dependent activity and a narrow therapeutic index, which complicates the establishment of an optimal dosing regimen [1-3]. The drug disposition in target populations allows clinicians to optimize drug regimen during therapy [4]. This study aimed to evaluate population pharmacokinetics (popPK) of patients with diminished creatinine clearance (CrCl) treated with AMK at the University Hospital of Santa Maria (HUSM), Brazil. Peak and trough steady-state blood samples were collected and plasmatic AMK concentration was quantified with liquid chromatography-tandem mass spectrometry [5]. PopPK analysis was performed using MONOLIXTM v.2020R1 (Lixoft, France). Adult patients (n=16) with CrCl < 60mL/min were enrolled in this study. A one-compartment model provides the best results in terms of goodness of fit plot, accuracy of parameter estimation and model stability. The following parameters were estimated by the popPK model: volume of the central compartment ($V = 19.6$ L; RSE% 20.6); total body clearance ($CL = 1.03$ L/h; RSE%17.3); standard deviation of the random effects $\omega_V = 54$ %, $\omega_{CL} = 63$ %; a proportional error model was adopted, with parameter being $b = 0.32$. The parameters estimated in our population are similar with other previously published in the literature [1, 2]. In the present study, our popPK model was able to characterize the PK profile of the patients. Although a further study with more patients should be conducted and covariate effect analyzed in this population. The obtained results would be useful information on the dosing optimization and individualization in this patient's population.

Ethical approval

The study was approved by the University's Research Ethics Committee (CAAE: 83200618.7.0000.5346).

Financial support and acknowledgements

Universal MCTIC/CNPq 2018; FIPE-UFSM; PIBIC-CNPq; FAPERGS; CAPES.

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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING TO EVALUATE THE DDI BETWEEN BUPROPION AND RIFAMPICINE IN PATIENTS WITH TUBERCULOSIS

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Pharmacological therapy for Tuberculosis (TB) is accomplished reduction transmission of *Mycobacterium tuberculosis* and patient cure. One of the factors to poor prognosis of TB is the tabagism. Bupropion (BUP) is a drug used to treat tabagism [1] and to be successful treatment is required to ensure adequate plasma concentrations of BUP and its metabolite hydroxy bupropion (OHBUP), however when a CYP2B6 inductor rifampicin (RIF) is used together in the TB treatment, a probable interaction would happen. In this sense, a PBPK has been employed to rate drug-drug interactions between BUP and OHBUP and RIP in patients with tabagism and TB. The model was developed using PK-SIM® software using literature reports. The average fold error was between 0.5-2 for all PK parameters evaluated for all drugs. DDI was simulated using virtual population with tabagism and TB, for 3 months, according to Brazilian guidelines [2], and RIF did not influence the BUP and OHBUP concentrations. Then, the DDI modeling has exhibited a significant performance of model to prediction interaction among the RIF, BUP and OHBUP. The obtained results report that concomitant administration of bupropion and hydroxy bupropion supply required concentrations in smoking patients with TB contributing to their prognostics.

Financial support and acknowledgements

This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and #19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) grant #309401/2020-8.

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A METHOD TO ASSESS MODEL-BASED BIOEQUIVALENCE

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Model-based bioequivalence (MBBE) encompasses the use of nonlinear mixed effect (NLME) models in bioequivalence (BE) analysis, supporting the estimation of pharmacokinetic endpoints to assess the relative biopharmaceutical performance between drug products. The application of MBBE is being evaluated as a valuable tool to overcome limitations on study design. In turn, specific statistical methods are being developed and compared to the traditional BE approach. Previously, we implemented a MBBE method to estimate the relative bioavailability between two formulations with different release properties (immediate-release vs extended-release) and showed a superior performance versus the traditional approach by overcoming limitations in the sampling design [1]. In this method, concentration-versus-time profiles are initially simulated from an estimated NLME model considering parameter uncertainty and pharmacokinetic metrics (C_{max}, AUC) are computed. The CI_{90%} for the test/reference geometric ratio of means for each metric is then obtained through ANOVA on the log-transformed metrics. Here, we compare the performance of this method versus a previously reported method [2] and the traditional BE approach. A two-compartment population pharmacokinetics model including a formulation effect on the bioavailable dose equal to 1.25 was used to estimate the type I error rate of the methods under different scenarios studying the impact of interindividual variability (10,10,5% and 50,50,50% in *k_a*, CL and V_c, respectively), interoccasion variability (0% and 10% in *k_a*, CL and V_c), residual unexplained variability (10 and 20%) and study design (sampling, balanced/unbalanced and number of subjects). MBBE methods showed an appropriate and controlled type I error rate under the evaluated scenarios.

Financial support and acknowledgements

This work was supported by the CAP (*Comisión Académica de Posgrado, Udelar*) with a grant to AS as a graduate student. PEDECIBA-Química and CSIC (*Comisión Sectorial de Investigación Científica, Udelar*) granted funds to support AS Ph.D studies.

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MECHANISTIC BASED PK/PD MODEL DESCRIBES CHANGES ON CORTICAL DOPAMINE CONCENTRATIONS FOLLOWING QUETIAPINE ADMINISTRATION TO SCHIZOPHRENIA PHENOTYPED RAT

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Schizophrenia (SCZ) is a severe mental disorder associated with a dysregulation in brain dopaminergic circuits, which presents a high therapeutic variability [1]. Quetiapine (QTP) is an antipsychotic that increases catecholamine levels as dopamine (DA) in prefrontal cortex, playing a role in SCZ treatment [2]. In the present work, we aimed to develop a pharmacokinetic/pharmacodynamic (PK/PD) model to describe extracellular DA concentrations in naïve and schizophrenic animals. A neurodevelopmental animal model of schizophrenia was implemented previously [3] in Wistar rats offspring, and confirmed with prepulse inhibition (PPI) test. Dopamine baseline and concentrations after QTP 5 mg/kg i.v. *bolus* dosing to the naïve and schizophrenic phenotype (SPR) male and female rats were determined by microdialysis on medial prefrontal cortex (mPFC) using CMA 12 probes (3 mm-CMA, µDialysis®). The quantification of DA in microdialysate was performed by LC-ESI-MS/MS [4]. DA concentrations at baseline and over the course of the study were lower in SPR animals compared to naïve animals with gender related differences as well. The PK/PD model was developed in NONMEM 7.4 (Icon®). The predicted brain unbound levels of QTP were generated from a previous developed semi-mechanistic popPK model [5]. DA concentrations were best described

with a model considering that the QTP-D2 receptor complex inhibits DA post synaptic re-uptake. Our results indicate that the pharmacodynamics of QTP is altered in SCZ, and that the differences seen in the *in vivo* DA profiles between naïve and SPR animals cannot be attributed exclusively to altered pharmacokinetics associated to the disease.

Ethical approval

This study was approved by the Ethics Committee in Animal Use from the Federal University of Rio Grande do Sul (UFRGS/CEUA #31001).

Financial support and acknowledgements

This study was financed in part by CNPq/Brazil (Process 421767/2016-2) and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. The authors also thank CNPq and CAPES for the individual grant.

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ACUTE *KLEBSIELLA PNEUMONIAE* LUNG INFECTION IN RODENTS DECREASE CIPROFLOXACIN TOTAL CLEARANCE

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Therapeutic drug monitoring commonly uses antimicrobials plasma concentrations as surrogate of biophase pharmacologically active concentrations. This study aims to develop a population pharmacokinetic (POPPK) model to characterize ciprofloxacin (CIP) plasma concentrations on acute *Klebsiella pneumoniae* lung infection in rodents. Drug plasma concentrations in healthy rodents were obtained from a previous study [1]. The POPPK model was developed using NONMEM® (version 7.4.3) with FOCE+I and R software was used for graphical visualization. Plasma data from healthy (n = 6) and acutely infected (n = 6) groups following 20 mg/kg i.v. *bolus* dosing, quantified by a validated LC-MS/MS method [2], were described as a three-compartment model with first-order elimination. Interindividual variability was identified on central clearance (22.6%) and on the volume of the second peripheral compartment (14.5%). Infection was added as a covariate on clearance, which showed a 25% reduction on acutely infected group (CL = 0.738 L/h/kg) in comparison to healthy one (CL = 0.991 L/h/kg). The increased CIP plasma concentrations in *K. pneumoniae* acute infection could mislead the clinician to overestimate the antimicrobial lung effect. The real impact of this infection on CIP free lung concentrations remains to be evaluated.

Ethical approval

UFRGS' Ethics Committee in Animal Use #36515.

Financial support and acknowledgements

Financial Support from CNPq/Brazil and doctoral scholarship from CAPES/Brazil.

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EXTERNAL EVALUATION OF SINGLE AND MULTI-MODEL METHODS FOR MODEL-INFORMED PRECISION DOSING OF VANCOMYCIN IN CRITICALLY ILL PATIENTS

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Vancomycin is a widely used antibiotic to treat Gram-positive bacterial infections in critically ill patients. The variable pathophysiological condition of these patients directly impacts drug pharmacokinetics, hindering the achievement of successful therapies. Vancomycin AUC-guided dosing through model informed precision dosing (MIPD) is recommended for therapeutic drug monitoring (TDM) [1]. Several vancomycin population pharmacokinetics (PopPK) models have been reported in critically ill patients and could be prospectively implemented; however the external evaluation of their predictive performance is required. In addition to using a single PopPK model, multi-model methods for MIPD: model selection algorithm (MSA) and model averaging algorithm (MAA), have been reported to improve the predictive performance at the individual level [2]. In this work, we explored and compared these approaches by retrospectively evaluating 10 reported PopPK models with vancomycin TDM data from 31 critically ill patients. Relative bias (rBias) and relative root mean squared error (rRMSE) were calculated by a priori prediction and including measured vancomycin concentrations. The *a posteriori* individual AUCs were estimated using best fit single models, MSA and MAA, and the efficacy/safety interpretation were compared versus using trough concentrations (C_t). MSA, MAA, and two single models met the acceptance criteria (rBias<25%) [3]. Multi-model methods achieved better precision. Overall, AUC criteria using MIPD

methods indicated that ~50% of patients achieved safe and efficacious exposure, versus ~20% according to the traditional approach, being more (~50%) the patients with subtherapeutic Ct. In conclusion, as previously reported, the use of Ct would lead to increased vancomycin doses and therefore higher risk of drug-induced toxicity.

Ethical approval

The clinical study was approved by the Institutional Ethics Committee (*Comité Institucional de Ética en Investigación en Salud, CIEIS, Hospital Córdoba, Argentina*) with registration number 346.

Financial support and acknowledgements

There was no specific financial support for conducting this work.

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POPULATION PHARMACOKINETICS OF TENOFOVIR/ EMTRICITABINE AMONG TRANSWOMEN AT HIV RISK ON ORAL PREP AND FEMINIZING HORMONE THERAPY: ANALYSIS OF POTENTIAL INTERACTIONS ON PREP

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Introduction: Potential interactions between feminizing hormone therapy (FHT) and preexposure prophylaxis (PrEP) may be a barrier to PrEP use among transwomen (TGW). We aimed to assess the impact of FHT on PrEP pharmacokinetics (PK) among TGW, by population-pharmacokinetic analysis. **Methods:** TGW were assigned to receive PrEP only (noFHT) or FHT (sFHT; oestradiol valerate 2–6mg plus spironolactone 100–300mg) plus PrEP for 12 weeks, after which they could start any FHT. PK assessments occurred at Weeks 12 and 30–48 (plasma samples prior and 0.5, 1, 2, 4, 6, 8, and 24h after dose). Concentration-time data were analyzed using SAEM algorithm on Monolix®. **Results:** The population-pharmacokinetic model was developed using data from Week12. Concentration-time data from 30 participants (noFHT: 12, sFHT: 18) were analyzed (266 observations). A two-compartment model with first-order absorption and lag-time was used to describe tenofovir and emtricitabine PK. In tenofovir model, interindividual variabilities of k_a , V_c/F , and CL/F were estimated. Body mass index (BMI) was associated with V_c/F and CL/F ; a correlation between these two parameters was also observed. Considering emtricitabine model, interindividual variabilities were attributed to lag-time, k_a , CL/F , and CL_2/F . A correlation between CL/F and CL_2/F was observed; age and BMI were associated with emtricitabine CL/F . **Discussion:** The population-pharmacokinetic models indicated no impact of FHT or oestradiol

levels on tenofovir or emtricitabine PK. The influence of BMI and age on PrEP PK was already reported by previous studies [1,2]. **Conclusions:** Our results indicate no interaction of FHT on tenofovir and emtricitabine PK, further supporting PrEP use among TGW using FHT

Ethical approval

Evandro Chagas National Institute of Infectious Diseases (INI)-FIOCRUZ Institutional Review Board (CAAE: 08405912.9.1001.5262).

Financial support and acknowledgements

CNPq; FAPERJ; CAPES Finance Code 001; Brazilian Ministry of Health (SVS-MS).

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PLASMA PIPERACILLIN CONCENTRATIONS IN MEXICAN PATIENTS FROM NEONATAL INTENSIVE CARE UNIT (A PILOT STUDY)

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The physiological differences and the organic immaturity of the neonates, contribute to a high piperacillin pharmacokinetic (PK) variability [1,2]. This affects the clinical efficacy of the antibiotic treatment and increases the probability of developing drug resistance and nephrotoxicity. The present work aims to describe the plasma piperacillin concentrations (CPIP) in Mexican neonates with severe infections on 100 mg/Kg treatment every 8 or 12 hours. A total of 34 CPIP from 15 neonatal intensive care unit (NICU) patients were determined by a liquid chromatography/tandem mass spectrometry (LC/MS/MS). The overall median value (range) postnatal age, gestational age, postmenstrual age, body weight, body-surface area, serum creatinine and glomerular filtration rate (GFR) were 11 (8-27) days, 33.5 (28-40) weeks, 35.2 (30-41.3) weeks, 1815 (995-3190) grams, 0.15 (0.10-0.21) cm², 0.47 (0.32-1.05) mg/dL and 34 (14-65) mL/min/1.73 m² respectively. Four neonates achieved the PK-PD target defined as CPIP >20 mg/L for more than 75% of the dosing interval (75% fT>MIC). Seven patients were below to the PK-PD target and had CPIP in a range from 2.5 to 16.4 mg/L at 6-or-9 hours post-infusion. Four neonates experienced potential toxicity CPIP>150 mg/L. In patients with life-threatening infection the rapid pathophysiological fluctuations can impact the pharmacokinetics of antibiotics and difficult to achieve the PK-PD target [3]. In this study the dosing regimen was not adequate for some patients. To identify the possible association between the covariates and CPIP it is necessary to develop a PK model for piperacillin in neonates to optimize antibiotic therapy in this special population.

Ethical approval

The present study was approved by the Comité en Ética e Investigación del Hospital Central “Dr. Ignacio Morones Prieto” with the registration code: CONBIOETICA-24-CEI-001-20160227.

Financial support and acknowledgements

This project is supported by Mexico’s Consejo Nacional de Ciencia y Tecnología through the student grant number: 745752.

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POPULATION PHARMACOKINETIC MODELING (POP PK) OF FREE AND NANOENCAPSULATED MELOXICAM IN MALE WISTAR RATS

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Meloxicam (MLX) is a non-steroidal anti-inflammatory drug used in the therapy of osteoarthritis, arthritis and muscle discomfort [1]. MLX has high intestinal permeability, however, its low dissolution is a limitation for its use. Thus, the objective of this work was to model PopPK from free and nanoencapsulated MLX. Then, anionic polymeric nanocapsules loaded with MLX (NCM) were formulated by the interfacial deposition method of preformed polymer at a concentration of 1mg/mL and the PK of free MLX and NCM was evaluated in male Wistar rats after iv administration of 5 mg/kg (n=8 animals/group). Blood samples were collected and quantified on HPLC-PDA. The dataset with preclinical observations was transferred to MonolixSuite™ 2020R1 (Simulation Plus, USA), and the final model was two-compartment model with linear elimination, combined error 1. Covariate analysis showed that the NCM affected the Clearance (Cl), the central (V1) and peripheral (V2) Volume of Distribution parameters with positive values of beta. Therefore, from the data modeling, it was possible to observe the nanocapsules containing meloxicam positively influenced Cl, V1 and V2.

Ethical approval

The experimental protocol was approved by the Ethics Committee on the Use of Animals of the UNIPAMPA (030/2021).

Financial support and acknowledgements

This study was financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES), Rio Grande do Sul Science Foundation (FAPERGS), grants #88881.506652/2020-01 and 19/2551-0001970-0, National Counsel of Technological and Scientific Development (CNPq) #309401/2020-8). We want to thank to Simulation Plus for MonolixSuiteTM license.

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VALIDATION OF AN LC-ESI-QTOF-MS METHOD TO MEASURE CEFEPIME IN THE PLASMA AND PERITONEAL FLUID OF RATS

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Introduction: Cefepime (CEF) can be administered in secondary peritonitis to treat sepsis. It is a fourth-generation cephalosporin used in the treatment of severe hospital-acquired infections. **Methods:** Chromatographic separation was performed using a Shimadzu Shim-pack CLC-ODS C₁₈ column (250 mm x 4.6 mm), a C₁₈ pre column, (4 mm, 5 µm, Phenomenex) and isocratic elution. Gallic acid was used as the internal standard (IS). The mobile phase consisted of (A) ultrapure water and (B) acetonitrile (80:20, v/v) at 0.8 mL/min. Quantification was performed using a mass spectrometer with electrospray ionization in positive mode to monitor ions with m/z 481.1322 (CEF) and m/z 171.0288 (IS). The method was validated employing FDA guidance. The applicability of the method was evaluated in Wistar rats. Plasma and peritoneal microdialysate samples were collected for 4 hours. Pharmacokinetic parameters for CEF were calculated using non-compartmental analysis. Results and discussion: The optimization of the analytical conditions allowed the chromatographic separation of CEF and IS, with retention times of 3.9 min and 4.5 min, respectively. All analytical performance parameters were approved. Pharmacokinetic parameters for CEF were: half-life = 1.10 ± 0.04 and 0.94 ± 0.07 hours; area under the curve = 137.12 ± 25 h.mg/L and 46.53 ± 14.28 h.mg/L for plasma and peritoneal microdialysate, respectively. Conclusion: The technique was validated and successfully applied in a pilot pharmacokinetic study for estimating the free concentration of CEF in the peritoneal microdialysate of Wistar rats for the first time.

Ethical approval

The experimental protocols involving animals were approved by the Ethics Committee on the Use of Animals of the University of Caxias do Sul (007/2020).

Financial support and acknowledgements

This study didn't receive financial support.

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IMPLEMENTATION OF THERAPEUTIC DRUG MONITORING OF 5-FLUOROURACIL IN GASTROINTESTINAL AND COLORECTAL CHILEAN CANCER PATIENTS

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Introduction: 5-fluorouracil (5-FU) is the cornerstone of chemotherapy for the treatment of colorectal and gastrointestinal cancer. It has a wide interindividual variability when is dosage based on body surface area (BSA), with 2/3 patients not achieving optimal therapeutic ranges. Therapeutic drug monitoring (TDM) of 5-FU has emerged as a high valuable tool for optimize dosage and improve the safety and efficacy. The aim of this study is to evaluate the clinical and pharmacokinetic impact of 5-FU TDM in Chilean patients with colorectal and gastrointestinal cancer. **Methods:** One-compartment lineal population model implemented in the Abbott base PKS System® software was used for individual pharmacokinetic parameter Bayesian estimates. Plasma concentrations of 5-FU were measured by High-Performance Liquid Chromatography. The Area Under the Curve (AUC) between 20 - 30 mcg x h/mL was considered as the therapeutic objective. Safety was evaluated by comparing severe adverse effect related to BSA or TDM dosage. **Results:** Thirty-two patients were recruited with Servicio Salud Valdivia Committee Ethical Approval. Only 28% of the patients dosed by BSA were within therapeutic range, while TDM reached 89% dosage within AUC-objective, with AUC differences between patients up to 4 times. The PK parameters of 5-FU estimated were: volume of distribution (V_d) = 22.1 ± 3.2 L and clearance (Cl) = 218.9 ± 63.8 L/h. Severe adverse effect was more frequent in BSA (4.27%) vs TDM-dosage (3.12%). **Conclusions:** 5-FU shown a high PK variability, while TDM a promissory tool for dose individualization with potential to improve clinical outcomes.

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BIOPHARMACEUTICAL STUDIES OF FLAVONOIDS EPICATECHIN AND NARINGENIN

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Flavonoids are widely distributed in herbal species [1] used in traditional medicine, because their many pharmacological effects. Solubility studies clarify biopharmaceutical properties [2]. The aim of this study was to evaluate the experimental equilibrium solubility (ES) of flavonoids in aqueous media to be applied in drug development. The HPLC-UV methodologies for the flavonoids epicatechin and naringenin were developed and validated. The ES studies were performed for compendial media (phosphate buffer pH 6.8; HCl 0.1 M pH 1.2) and biorelevant media (FaSSGF pH 1.6 and FaSSIF pH 6.5). The theoretical solubility of each flavonoid was assumed added plus 30% of the amount of the standard to guarantee the excess of solute. The shake-flask technique was performed at 37 °C and 250 rpm until equilibrium solubility to be achieved [3]. The samples were centrifuged, and the supernatant quantified. For epicatechin, the theoretical solubility is 5.59 mg/mL for acidic pH, 5.73 mg/mL for neutral pH, and the experimental solubility in phosphate buffer and HCl 0.1 M were 6.81 mg/mL and 6.47 mg/mL, respectively. In FaSSGF and FaSSIF the results were 6.77 mg/mL and 6.51 mg/mL, respectively. The naringenin was not stable in acidic media, then, only neutral pH media studies were performed. The theoretical solubility is 0.79 mg/mL for a pH of 7.4 and the experimental solubilities were 1.28 mg/mL for phosphate buffer and 0.31 mg/mL for FaSSIF. The experimental solubility showed higher values compared to theoretical. The accuracy on aqueous solubility of natural compounds is crucial to guide drug development and understanding of pharmacokinetic profile.

Financial support and acknowledgements

A special acknowledgement to the PALAFITO laboratory, as well as the Araucaria Foundation for the offering of scholarships and to Gaiola, Lippa and Bittencourt.

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PHARMACOMETRIC STUDY OF NEUROEPO IN A NON-HUMAN PRIMATE MODEL *MACACA FASCICULARIS*

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Erythropoietin (Epo) is a glycosylated hormone considered the main growth factor in erythroid cells with cytoprotective action on non-hematopoietic tissues such as central nervous system (CNS).[1] NeuroEPO, is a nasal pharmaceutical solution of recombinant Epo with low sialic acid with proven neuroprotective effect at the preclinical and clinical level. [2, 3] A single dose of intranasal NeuroEPO (5750 IU* kg⁻¹) was administered to a population of non-human primates (*macaca fascicularis*) and the C(EPO) vs t profile in plasma of peripheral venous blood and lumbar cerebrospinal fluid (LCF) was determined by sparse sampling. A non-compartmental pharmacokinetic analysis with Phoenix-WinNonlin, 8.3.4.295, (Certara) led to drug targeting efficiency (> 100%) and a direct transport percentage (90,47%) that supported the high NeuroEPO biodistribution in CNS involving nerve pathways. A turnover model accounting for endogenous production in LCF and exogenous dosing was developed using non-linear-mixed-effects models (NONMEM7.4). The inhibitory feedback on Epo synthesis significantly improved the model (p<0.005). Four fixed effects were estimated with FOCEI, namely distribution volume (V=3760±481 mL), endogenous synthesis rate (K_{syn}=53.4±4.50 IU/h), elimination constant (K_{out}=3.60±0.25 h⁻¹) and Epo concentration leading to a 50% inhibition of K_{syn} (IC₅₀=0.00241±0.00048 IU/mL). The absorption rate constant was fixed (K_a=4 h⁻¹). Interindividual variability was included on V and residual error was modeled as proportional. The stability of the model was proved by means of a non-parametric bootstrap method. Good agreement between model predictions

and observed concentrations was found and vpc confirmed the predictive capability of the model. A mPBPK, is needed to leverage the pharmacokinetics of NeuroEPO in CNS.

Ethical approval

The study was designed and conducted in accordance with international ethical criteria related to non-clinical research in non-human primates and was approved by the corresponding Ethics Committee of the Center for Laboratory Animal Studies.

Financial support and acknowledgements

The study was financed by the Center for Molecular Immunology, producer of NeuroEPO and promoter of preclinical and clinical studies.

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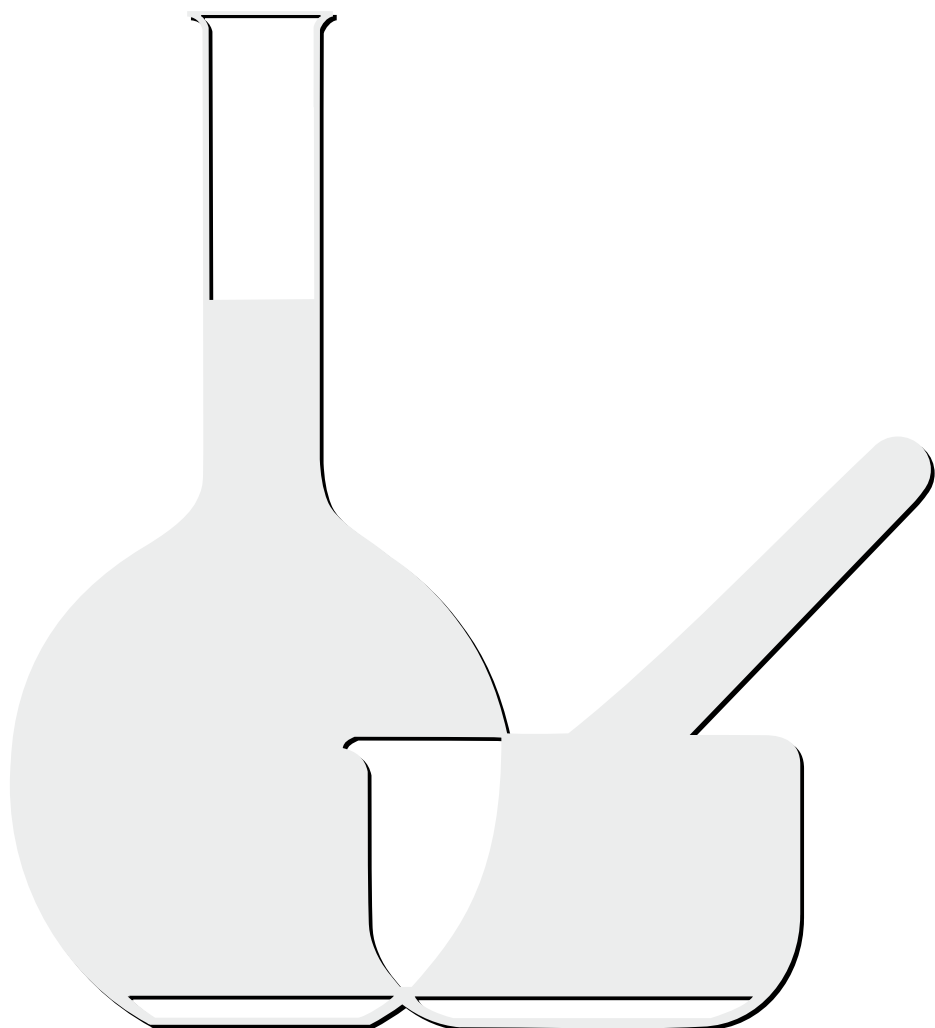
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EM m/z (% intensidad relativa). Ej.: em m/z (%): 340 (M^+ , 100), 295 (10), 134 (26) ...

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6. Lipidat, Lipid thermotropic phase transition database, Ohio State University, URL: <http://www.lipidat.chemistry.ohio-state.edu>, consultado en septiembre de 2001.

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*Revista Colombiana
de Ciencias Químico-Farmacéuticas, 51(3)*
se terminó de editar en Proeditor,
en diciembre de 2022.
Bogotá, D. C., Colombia.

