

## Peculiarities of the effect of the ethanol extract of quinquelobate motherwort on the behaviour of rats

Zinaida G. Khabaeva<sup>1a</sup>, Azamat Ch. Chiviev<sup>2b\*</sup>, Valentina S. Gappoeva<sup>1c</sup> & Diana A. Marzoeva<sup>1d</sup>

<sup>1</sup>North Ossetian State University named after K.L. Khetagurov, 362025, 44-46 Vatutin Street, Vladikavkaz, Russia

<sup>2</sup>North Ossetian State Medical Academy, 40, Street Pushkinskaya, 362019, Vladikavkaz, Russia

E-mail addresses: <sup>a</sup>zinahabaeva@mail.ru, <sup>b</sup>a.chiviev@mail.ru, <sup>c</sup>valentina.gappoeva@mail.ru,

<sup>d</sup>marzoeva.diana@yandex.ru.

ORCID IDs: <sup>a</sup><https://orcid.org/0000-0001-7214-7827>, <sup>b</sup><https://orcid.org/0009-0007-0113-0666>,

<sup>c</sup><https://orcid.org/0000-0002-3127-4122>, <sup>d</sup><https://orcid.org/0000-0001-9785-5985>

\*Corresponding author E-mail: a.chiviev@mail.ru

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### SUMMARY

**Introduction:** Modern medicine is actively researching herbal preparations to correct psychoemotional disorders because they have high bioavailability, mild action, and fewer side effects than synthetic analogues. The quinquelobate motherwort, which contains flavonoids, alkaloids, and iridoids, is a promising sedative, and it is being studied in animal models through behavioral tests. **Objective:** The purpose of this research is to evaluate the peculiarities of the neurotropic activity of quinquelobate motherwort extract on experimental behaviour models for laboratory animals. **Materials and methods:** For the manufacture of the drug, dry herbal starting material (leaves) was used, the extraction of which was carried out in a Soxhlet apparatus with ethyl alcohol 70%. To reduce the effect of alcohol itself on the emotional status of animals, it was evaporated in a water bath; the preparation was replenished with distilled water. **Results:** In the black and white chamber test, after administration of the quinquelobate motherwort extract, there was an increase in the time spent in the light compartment by almost 6 times and a simultaneous decrease in the duration of the animals' stay in the dark compartment; the number of exits to the light compartment significantly increased and the number of urinations decreased. In the elevated cruciform maze test, the latent period significantly decreased, which indicates a decrease in the decision-making time for the implementation of an active or passive behaviour strategy; the number of freezing acts significantly increased. In the open field test system, after administration of the tested extract, a significant increase in horizontal motor activity was observed; at the same time, the number of visits to peripheral squares and the time spent in the centre increased. **Discussion:** In accordance with the existing interpretation of such data, the dynamics in behavioural reactions after administration of the herbal preparation indicated a decrease in fear and anxiety responses in all experimental models used, as well as an increase in orientative-trying activity, which collectively indicates the anti-stress effect of the studied extract. **Conclusion:** In addition to the well-known sedative properties of quinquelobate motherwort, its anxiolytic effects — demonstrated through significant antiphobic and antidepressant impact — have been observed in a series of stress-inducing behavioural tests.

**Keywords:** quinquelobate motherwort; laboratory rats; emotional behaviour; open field; elevated cruciform maze; black and white chamber.

## RESUMEN

### Peculiaridades del efecto del extracto etanólico de agripalma quinquelobato en el comportamiento de ratas

**Introducción:** La medicina moderna investiga activamente las preparaciones herbales para corregir trastornos psicoemocionales debido a su alta biodisponibilidad, acción suave y menos efectos secundarios que los análogos sintéticos. La agripalma quinquelobato, que contiene flavonoides, alcaloides e iridoides, es un sedante prometedor y se está estudiando en modelos animales mediante pruebas de comportamiento. **Objetivo:** El propósito de esta investigación es evaluar las peculiaridades de la actividad neurotrópica del extracto de agripalma quinquelobato en modelos experimentales de comportamiento en animales de laboratorio. **Materiales y métodos:** Para la fabricación del fármaco, se utilizó materia prima herbácea seca (hojas), cuya extracción se realizó en un aparato Soxhlet con alcohol etílico al 70 %. Para reducir el efecto del alcohol en el estado emocional de los animales, se evaporó al baño maría; la preparación se reabasteció con agua destilada. **Resultados:** En la prueba de la cámara blanca y negra, tras la administración del extracto de agripalma quinquelobato, se observó un aumento de casi 6 veces en el tiempo de permanencia en el compartimento iluminado y una disminución simultánea de la duración de la estancia de los animales en el compartimento oscuro; el número de salidas al compartimento iluminado aumentó significativamente y el número de micciones disminuyó. En la prueba del laberinto cruciforme elevado, el período de latencia disminuyó significativamente, lo que indica una disminución del tiempo de decisión para la implementación de una estrategia de comportamiento activo o pasivo; el número de actos de inmovilización aumentó significativamente. En el sistema de prueba de campo abierto, tras la administración del extracto probado, se observó un aumento significativo de la actividad motora horizontal; al mismo tiempo, aumentó el número de visitas a los cuadrados periféricos y el tiempo de permanencia en el centro. **Discusión:** De acuerdo con la interpretación existente de estos datos, la dinámica de las reacciones conductuales tras la administración del preparado herbal indicó una disminución de las respuestas de miedo y ansiedad en todos los modelos experimentales utilizados, así como un aumento de la actividad orientadora, lo que, en conjunto, indica el efecto antiestrés del extracto estudiado. **Conclusión:** Además de las conocidas propiedades sedantes de la agripalma quinquelobato, sus efectos ansiolíticos, demostrados mediante un significativo efecto antifóbico y antidepresivo, se han observado en una serie de pruebas conductuales de inducción de estrés.

**Palabras clave:** agripalma quinquelobato; ratas de laboratorio; comportamiento emocional; campo abierto; laberinto cruciforme elevado; cámara en blanco y negro.

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## RESUMO

### Peculiaridades do efeito do extrato etanólico da erva-mãe quinquelobato sobre o comportamento de ratos

**Introdução:** A medicina moderna pesquisa ativamente preparações fitoterápicas para corrigir distúrbios psicoemocionais, pois apresentam alta biodisponibilidade, ação suave e menos efeitos colaterais do que análogos sintéticos. A erva-mãe quinquelobato, que contém flavonoides, alcaloides e iridoides, é um sedativo promissor e está sendo estudada em modelos animais por meio de testes comportamentais. **Objetivo:** O objetivo desta pesquisa é avaliar as peculiaridades da atividade neurotrópica do extrato da erva-mãe quinquelobato em modelos experimentais de comportamento com animais de laboratório. **Materiais e métodos:** Para a fabricação do fármaco, utilizou-se material vegetal seco (folhas), cuja extração foi realizada em aparelho Soxhlet com álcool etílico 70%. Para reduzir o efeito do álcool no estado emocional dos animais, este foi evaporado em banho-maria; a preparação foi completada com água destilada. **Resultados:** No teste de câmara preta e branca, após a administração do extrato de erva-mãe quinquelobato, houve um aumento no tempo gasto no compartimento de luz em quase 6 vezes e uma diminuição simultânea na duração da permanência dos animais no compartimento escuro; o número

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de saídas para o compartimento de luz aumentou significativamente e o número de micções diminuiu. No teste do labirinto cruciforme elevado, o período latente diminuiu significativamente, o que indica uma diminuição no tempo de tomada de decisão para a implementação de uma estratégia de comportamento ativo ou passivo; o número de atos de congelamento aumentou significativamente. No sistema de teste de campo aberto, após a administração do extrato testado, foi observado um aumento significativo na atividade motora horizontal; ao mesmo tempo, o número de visitas a quadrados periféricos e o tempo gasto no centro aumentaram. **Discussão:** De acordo com a interpretação existente de tais dados, a dinâmica nas reações comportamentais após a administração da preparação à base de ervas indicou uma diminuição nas respostas de medo e ansiedade em todos os modelos experimentais usados, bem como um aumento na atividade de tentativa de orientação, o que indica coletivamente o efeito antiestresse do extrato estudado. **Conclusão:** Além das conhecidas propriedades sedativas da erva-mãe quinquelobata, seus efeitos ansiolíticos — demonstrados por meio de impacto antifóbico e antidepressivo significativo — foram observados em uma série de testes comportamentais indutores de estresse.

**Palavras-chave:** erva-mãe quinquelobata; ratos de laboratório; comportamento emocional; campo aberto; labirinto cruciforme elevado; câmara preta e branca.

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## 1. INTRODUCTION

Numerous disorders of the central nervous system affecting the psychoemotional state of an individual and triggering various somatic manifestations — such as insomnia, headaches, and emotional instability — highlight the need for the development of modern targeted medicines. On the other hand, the emergence of the 'drug disease/syndrome' has enhanced interest in herbal medicines. The use of innovative technologies in the production of herbal remedies, including the selection of plant starting materials, processing methods, isolation and use of secondary plant metabolites, etc. significantly improve the quality of modern medicinal products. Phytotherapy is currently used both in the treatment and in prevention of various diseases, contributing to an increase in the adaptive and protective-compensatory capabilities of the body [1, 2].

Drugs that actively influence the central nervous system hold a special place among phyto-preparations. These neurotropic compounds modulate various processes of the brain, affecting both mental and motor activities with diverse action profiles. Among the wide range of drugs with neurotropic effects, plant-based medicines occupy a large niche. This is due to their high bioavailability to the human body, possible complex organ-protective effect, minimal number of adverse reactions, as well as the relative cheapness of herbal starting materials in contrast to synthetic ones [3-5].

Sedatives of plant origin are widely used for correction of psychoemotional conditions of various genesis; they can be sold in pharmacies without prescriptions and do not require medical advice. The quinquelobate motherwort (*Leonurus quinquelobatus*) meets these requirements, possessing, as it is believed, sedative and less pronounced antidepressant properties [6]. It is a perennial herbaceous plant of the *Labiaceae* family with a wide natural area; its herb-age and leaves are used for practical purposes. The wide range of the plant's chemical components (flavonoids, alkaloids, iridoids) determines the variety of its optimizing and medicinal effects on the human body. In particular, the neurotropic effects of the motherwort are due to the presence of iridoids that determine the bitterness of drugs made on its basis (marubin, leonuride, garpagide, ajugol, ayugoside, etc.). In its chemical structure, *Leonurus quinquelobatus* has tannins, saponins, sugars, vitamins of group C and A, flavonoids, essential oils [7, 8].

The physiological effects of sedation on the nervous system are well known to be medi-

ated by enhancing either excitation or inhibition processes. Depending on the drug's mechanism of action, this can lead to different functional outcomes, ranging from drowsiness to increased alertness, with various intermediate tonic effects possible. The existing data on the possibility of using quinquelobate motherwort as a component of a complex plant preparation creates the need to quantify manifestations of its pharmacological activity *in vivo*. The high validity of physiological behavioural tests and the authors' skills in using such test models had determined the choice of an experimental approach to assess the immediate effects of motherwort on animal behavioural reactions on three test systems: black and white chamber (BWC), elevated cruciform maze (ECM) and open field (OF).

## 2. MATERIALS AND METHODS

The purpose of this research is to determine the peculiarities of the neurotropic activity manifestation of the ethanol extract of quinquelobate motherwort on behavioural models for rodents.

The studies were performed on 30 albino Wistar rats (males, weight 180-220 g). Animal care in a vivarium, the conditions of their adaptation to the beginning of the experiment and the experiment procedure itself met the requirements for work with animals in accordance with Order No. 199H of the Ministry of Healthcare of the Russian Federation dated 01.04.16 On Approval of Rules of Good Laboratory Practice [9]. The experiment procedure took place under standard conditions for maintaining the constancy of environmental factors in the laboratory environment, including illumination, sound insulation, conditions, feeding patterns (balanced food appropriate for rodents), etc.

The object of the study is an ethanol extract of quinquelobate motherwort made in a Soxhlet extractor. To do this, the dry herbal starting material was crushed and poured into a cartridge of filter paper. The filled cartridge was placed in the apparatus and extracted with a 70%-solution of ethyl alcohol for 14-15 cycles. To avoid the influence of ethyl alcohol on the emotional status of animals, ethanol was evaporated in a water bath; the volume of the evaporated extractant was then replenished with distilled water. The animals received the tested phytosolution by voluntary consumption. To do this, each rat was individually trained to drink from a syringe, and later, at a fixed time, the animals consumed 1 ml of the extract [10]. All experiments were performed at the same time (in the period from 12:00h to 16:00h).

The emotional status of the animals was evaluated before and after administration of the solution (60 minutes after administration) on three behavioural models: BWC, ECM and OF. Testing and interpretation of the obtained results were carried out according to the methods described in previously published studies [10, 11]. Based on the literature on the stress associated with behavioural models and the results of our own research, the entire cycle of experimental studies was conducted under a scheme that incorporates the following sequence of test systems: BWC – ECM – OF (from least to most stressful). A digital video system No. VS1304 was used to record the behaviour of animals during testing.

Statistical analysis of the data was carried out using the Excel and SPSS software according to the Student's t-criterion [12]. The differences were considered significant at  $p \leq 0.05$ .

### 3. RESULTS

At the first stage, the behavioural characteristics of the albino laboratory rats were analysed before and after the administration of the extract in the black and white chamber test (Table 1). The test allows us to evaluate the anxiety level in test animals and identify the possible anxiolytic effect of the drug under study. In the emotional status of intact animals, we observed anxiety-related responses caused by the stress of the experimental model. Those animals preferred to be in the dark compartment of the chamber and rarely peeked out from there; urination and defecation were recorded during the test period. After administration of the prescribed dosage of dealcoholized motherwort extract, there was an increase in the time spent in the light compartment of the chamber by almost 6 times and a simultaneous significant decrease in the duration of the animals' stay in the dark chamber ( $p < 0.001$ ). Along with these changes, there was a significant increase in the time and number of exits from the dark compartment of the system ( $p < 0.05$ ) and a decrease in the number of urinations and defecations ( $p < 0.01$ ).

**Table 1.** Features of the effect of herbal extract of the quinquelobate motherwort on the behaviour of rats in the black and white chamber test

Behavioural characteristics	Control (intact) n=15		Experiment n=15
	Statistical indicators		
	M±m	p	M±m
Latent period of entering the dark compartment, with	11.04±3.55	$p > 0.05$	11.83±3.1
Number of peeks from the dark compartment, pcs	2.10±0.74	$p > 0.05$	3.3±0.55
Duration of peeks from the dark compartment, s	5.81±3.10	$p < 0.001$	32.6±4.7
Number of exits from the dark compartment, pcs	0.75±0.11	$p < 0.05$	1.7±0.32
Time spent in the light compartment, s	19.8±3.22	$p < 0.001$	125.86±15.14
Time spent in the dark compartment, s	272.71±11.4	$p < 0.001$	130.57±9.53
Number of defecations, pcs	0.16±0.1	$p < 0.05$	0±0
Number of urinations, pcs	1.88±0.32	$p < 0.01$	0±0

Source: compiled by the authors

Note: hereafter M is the arithmetic mean, m is the sampling error; p is the result significance level.

These facts collectively suggest a reduction in anxiety levels and a decrease in autonomic nervous system reactions to stress following the administration of *Leonurus quinquelobatus* extract.

Table 2 shows the characteristics of animal behaviour in the elevated cruciform maze test before and after administration of the extract. As can be seen from the data in the table, the latent period significantly decreased, which indicates a decrease in the decision-making time for the implementation of an active or passive behaviour strategy. The number of freezing acts has also significantly increased which indicates a possible sedative effect of the motherwort extract. The change in other parameters was not statistically significant.

**Table 2.** Features of the effect of herbal extract of the quinquelobate motherwort on the behaviour of rats in the elevated cruciform maze test

Behavioural characteristics	Control (intact) n=15	Experiment n=15	
	Statistical indicators		
	M±m	p	M±m
Latent period, s	2.79±0.29	p>0.05	0.23±6.78
Number of visits to open maze arms, pcs	1±0	p>0.05	1.0±0.4
Number of visits to closed maze arms, pcs	2.75±0.64	p>0.05	2.0±0.4
Duration of visits to open maze arms, s	9.95±5.46	p>0.05	74.67±10.2
Duration of visits to closed maze arms, s	282.34±9.71	p>0.05	158.3±32.4
Duration of the acts of freezing behaviour, s	68.89±19.35	p>0.05	164.5±21.1
Number of the acts of freezing behaviour, pcs	3.75±0.73	p<0.05	6.5±0.4
Number of rears, pcs	1.75±0.21	p>0.05	2.5±0.6
Number of defecations, pcs	0±0	p>0.05	0±0
Number of urinations, pcs	1±0.35	p<0.05	0±0

Source: compiled by the authors.

Table 3 shows the results obtained on the open field test system. This model allows us to evaluate the motor activity of rats, expressed in the number of intersections of squares and racks; research activity – by the number of examinations of holes in the bottom of the arena. Among the three models used in this study, the open field model induces the most pronounced stress. This is due to both the size of the chamber and its illumination, as a brightly lit open space is an inherently extreme stimulus for rats, triggering psychomotor and autonomic responses characteristic of fear and stress reactions (Table 3, control group of animals).

**Table 3.** Features of the effect of herbal extract of the quinquelobate motherwort on the behaviour of rats in the open field test

Behavioural characteristics	Control (intact) n=15	Experiment n=15	
	Statistical indicators		
	M±m	p	M±m
Latent period of exits to the periphery, s	2.99±0.49	p>0.05	2.4±0.16
Horizontal motor activity, pcs	20±2.93	p<0.05	33.5±3.8
Vertical motor activity, pcs	1.75±0.41	p<0.05	2.50±2.06
Number of grooming acts, pcs	1±0.35	p<0.05	3.0±0.8
Duration of grooming acts, s	15.19±5.15	p<0.05	27.7±1.06
Number of the acts of freezing behaviour, pcs	6±0.5	p>0.05	4.0±0.5
Duration of the acts of freezing behaviour, s	104.68±25.91	p>0.05	67.95±12.12
Number of defecations, pcs	0.25±0.21	p<0.05	0±0
Number of urinations, pcs	0.25±0.21	p<0.05	0±0
Number of well inspections, pcs	1±0.35	p<0.05	1.5±0.4
Number of crossed central squares, pcs	2.75±0.41	p<0.05	4.0±1.6
Number of crossed peripheral squares, pcs	17.25±2.76	p<0.05	29.5±2.76
Time spent at the centre, s	0.25±0.21	p<0.05	0.5±0.4

Source: compiled by the authors.

After administration of the tested herbal preparation, the animals showed an increase in horizontal and vertical motor activity ( $p<0.05$ ); the number of grooming acts and its duration

( $p < 0.005$ ), as well as the time spent in the centre ( $p < 0.05$ ), increased. The number of the inspected wells and the number of the crossed central and peripheral squares significantly increased ( $p < 0.05$ ). At the same time, the number of defecations and urinations significantly decreased ( $p < 0.05$ ).

#### 4. DISCUSSION

Drugs with a strong anxiolytic effect are known to manage psychoemotional tension by reducing anxiety and fear (antiphobic effect), and associated symptoms, while also providing a sedative effect [13, 14]. It is believed that the main effects of anxiolytic drugs are achieved by reducing the excitability of subcortical emotionogenic structures of the brain, including the limbic system and the diencephalic brain (thalamus, hypothalamus). The main neurochemical effect is realized through the inhibitory GABAergic system, less pronounced effect – through the noradrenergic, dopaminergic and serotonergic systems. Some anxiolytics are capable of a sedative (calming) effect due to their impact on the reticular activating system of the brain. Various herbal preparations have neurotropic effects ambiguous in terms of their nature and severity.

Motherwort tincture belongs to the category of the most commonly used drugs with a pronounced sedative effect. It is believed to regulate the functional state of the central nervous system by reducing nerve cell excitability, offering a calming effect and, depending on the dose, a hypnotic effect. We analysed the features of behavioural and vegetative reactions of animals in stressful situations after administration of the tincture of *Leonurus quinquelobatus*, taking into account its sedative effect as well as possible targeted anxiolytic action. It is believed that the open field, elevated cruciform maze and dark/light chamber tests are important for assessing anxiety, which is an endophenotype of depression and is important for antidepressant screening [12]. The series of experimental behavioural models made it possible to identify a set of physiological reactions of rats that are most clearly manifested under the influence of a herbal preparation. The experimental models themselves, due to the features of their construction (large, brightly lit, open spaces, heights), were stress-inducing for animals, causing manifestations of fear, anxiety, discomfort, etc.

In general, the set of animal reactions evaluated according to these test systems can be grouped into three behavioural blocks: locomotor or motor activity (horizontal motor activity, latent period of the first movement), exploratory and searching activity (rears and dippings from the open maze arms, inspection of wells), and emotional reactivity (time, number of visits of open and closed maze arms, time at the centre, the number and duration of freezing behaviour acts, frequency of urination and defecation). Such behavioural and vegetative reactions were observed to varying degrees across all three experimental models used for testing. For instance, in the BWC test, after administration of the drug, the duration of staying in the dark / light compartments of the chamber changed significantly: the duration of peeking and coming out of the dark part increased, the time spent in the light part increased (accordingly, the time in the dark compartment decreased). At the same time, a decrease in urination and defecation was observed. This dynamics of animal behaviour in a stressful situation can be regarded as a manifestation of a significant antiphobic effect mediated by the action of the quinquelobate motherwort extract [15]. Naturally, the decrease in the fear reaction was accompanied by other anti-stress manifestations (reduction of anxiety and uneasiness). This fact is supported by the fact of changes in vegetative reactions (urination, defecation). In the ECM test, after administration of the herbal preparation, the changes were less pronounced, however,

the latent period of being in the centre decrease, which, according to the accepted test interpretation of behaviour, is regarded as an increase in the speed of decision-making in a stressful situation [16]. According to the OF test, significant differences before and after administration of the phytopreparation were recorded in almost all parameters, both in the behaviour of the animals and in their vegetative reactions. An increase in the frequency of horizontal (sum of crossed squares) and vertical motor activity indicates an increase in locomotor activity; an increase in the number of inspected wells, sniffing of the wells and vertical motor activity indicate an increase in the exploratory and searching reactions [17]. Such behaviour caused less frequent urination and defecation and lowered the number of freezing acts in rats. These changes are the result of shifts in the animals' emotional state, marked by reduced anxiety, fear, and depression caused by the situation of the experimental model that is inadequate for animals. This dynamics in the behavioural and vegetative reactions of albino laboratory rats due to the intake of dealcoholized ethanol extract of *Leonurus quinquelobatus* serve as a confirmation of a pronounced anxiolytic effect of the drug. The increase in locomotor, exploratory and searching activity and the nature of the dynamics of vegetative reactions after administration of the drug are indicative of the animals' recovery from stress caused by the abnormal conditions of the experimental setup.

## 5. CONCLUSION

The quinquelobate motherwort extract induced both sedative and anxiolytic effects in rats, as evidenced by various behavioural and physiological responses across all three stress-inducing behavioural models. The most significant reactions of the anti-stress activity of ethanol extract from *Leonurus quinquelobatus* were found in the models of BWC and OF. After administration of the studied drug, the behaviour of animals in the BWC test system significantly changed: rats started preferring the open test spaces (reduction of anxiety and fear), and peeking out of the dark compartment of the chamber became more frequent (exploratory activity). In the OF test, an increase in locomotion and exploratory and searching activity was observed in rats due to the tested phytopreparation. An increase in the number of freezing acts in the ECM can be considered as a sedative effect of the motherwort extract. In all three test systems, the administration of the herbal preparation led to changes in the animals' vegetative status, indicating a reduction in emotional excitability. Behavioural reactions indicating a decrease in decision-making time, regardless of the active or passive strategy of behaviour (latent period), allow us to speak about the preservation of sufficient performance capabilities of cortical cells when exposed to a herbal medicinal product with a pronounced sedative effect.

## CONFLICT OF INTEREST

All authors report that they do not have any conflicts of interest.

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