

Effects of the butanol-containing fraction from leaves of *Calea prunifolia* H.B.K. on blood pressure and smooth muscle responses of Wistar rats

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SUMMARY

The butanol-containing fraction from leaves of *Calea prunifolia* H.B.K. was obtained in order to examine cardiovascular effects on two distinct rat preparations. The responses elicited in isolated aorta and isolated vas deferens, were examined, as were the changes in blood pressure in anesthetized and conscious Wistar rats. In addition, the effects on angiotensin-converting enzyme activity in plasma and the effects on cytosolic calcium in cardiomyocytes and uterine cells were measured. Results show that the butanol-containing fraction from *C. prunifolia* (0.1–100 µg/mL) relaxes phenylephrine-induced contractions in isolated aorta (CI₅₀: 58 µg/mL), decreases the contractile response induced by noradrenaline in vas deferens, and shows a competitive antagonism profile (pA₂: 4.48, m: 1024). The butanol-containing fraction from *C. prunifolia* also decreases blood pressure in anesthetized rats in a dose-dependent manner (1–100 mg/kg, i.v.), yet is devoid of hypotensive effects in normotensive, conscious rats. In addition, *C. prunifolia* does not modify the hypertensive effects induced by the nitric oxide synthase inhibitor L-NAME, and it also does not affect the angiotensin-converting enzyme plasma activity nor the cytosolic calcium in cardiomyocytes and uterine cells. According to these results, *C. prunifolia* relaxes

smooth muscle and vascular tone, favoring the decrease in blood pressure in rats via mechanisms related to alpha adrenergic inhibition.

Key words: *Calea*, antihypertensive, traditional medicine, vasodilation, vasorelaxant.

RESUMEN

Efecto de la fracción butanólica de las hojas de *Calea prunifolia* H.B.K. sobre la presión arterial y la relajación muscular en ratas wistar

Se evaluó el efecto cardiovascular en ratas inducido por la fracción butanólica de las hojas de *Calea prunifolia* H.B.K., examinando el efecto generado en preparaciones de anillos aislados de aorta y conducto deferente, y los cambios de presión arterial en ratas wistar anestesiadas y despiertas. También se cuantificó el efecto sobre la enzima convertidora de angiotensina (ECA) en plasma y sobre el calcio citosólico en cardiomiocitos y uteromiocitos. Los resultados mostraron que la fracción butanólica de *C. prunifolia* (0,1-100 µg/mL) relajaba anillos de aorta contraídos con fenilefrina (CI_{50} : 58 µg/mL) y disminuía la contracción del conducto deferente inducido por noreadrenalina con un perfil de inhibición competitiva (pA_2 : 4.48, m: 1.024). La fracción butanólica de *C. prunifolia* disminuye la presión sanguínea en ratas anestesiadas en función de la dosis (1-100 mg/kg, i.v.), si bien estuvo desprovista de efectos hipotensores en ratas despiertas. *C. prunifolia* tampoco modificó el efecto hipertensor inducido por el inhibidor de óxido nítrico sintetasa L-NAME, ni los niveles de ECA, ni la concentración de calcio citosólico en cardio y uteromiocitos. De acuerdo con estos resultados, *C. prunifolia* relaja el músculo liso y disminuye el tono vascular favoreciendo la disminución de la presión arterial en ratas por medio de mecanismos vinculados con la inhibición alfa adrenérgica.

Palabras clave: *Calea*, antihipertensivo, medicina tradicional, vasodilatación, vasorrelajante.

INTRODUCTION

Hypertension is a major risk factor for cardiovascular complications such as myocardial infarction, stroke, renal insufficiency, and peripheral vascular disease [1, 2]. Pharmacological therapy, in addition to lifestyle changes that include exercise and a healthy

diet, can reduce the progression of this disorder, but the wide profile of adverse side effects of drugs for hypertension is a key factor that decreases adherence, especially because the disease is asymptomatic during its initial stages [3]. In addition, the cost of medication is increasing progressively [4]. New therapeutic alternatives from natural sources could contribute to the treatment of cardiovascular diseases and ameliorate the economic impact of therapy due to these chronic disorders [5].

Calea prunifolia H.B.K. (“carrasposa”) is among medicinal plants used in Colombian folk medicine for hypertension [6]. Phytochemical studies on this species revealed the presence of sesquiterpenes derived from acorane, daucane, and caleprunane [7], benzofuran derivatives [8], chromenes [9], and sesquiterpene lactones [10]. More recent analysis of this species further showed polar compounds like the flavonoid glycoside quercetin 3-rutinoside, the quinic derivative caffeoylquinic acid, and a kaurane diterpenoid glycoside [11]. Vasodilator properties seen in tissue assays and antihypertensive effects in anesthetized rats have been described in this species [12]. Previous studies also showed *C. prunifolia* administration led to anti-inflammatory responses [13, 14]. Antihypertensive and vasodilator properties in a similar species, *Calea glomerata*, have also been studied [15].

This study focused on advancing information about the cardiovascular effects of *C. prunifolia* by exploring its effects on conscious rats, examining its possible interactions on angiotensin-converting enzyme and calcium currents, assessing its effects on hypertension induced by nitric oxide deficit, and studying its antiadrenergic properties in vascular and non-vascular smooth muscle.

MATERIALS AND METHODS

Plant material and extraction

The leaves of *Calea prunifolia* were collected in Cunday (Cundinamarca, Colombia) at an altitude of 1300 meters. A voucher specimen was identified and deposited in the Colombian National Herbarium, Natural Sciences Institute, National University of Colombia (COL), and the identity was confirmed by Dr. Edgar Linares (N° 468655/COL000075582). The air-dried leaves of *Calea prunifolia* (1.8 kg) were extracted repeatedly with 96% EtOH at room temperature. After removal of the solvent by evaporation in vacuum, the residue (201 g) was subsequently suspended in water and partitioned successively with CH₂Cl₂ and n-butanol. The n-BuOH extract (39 g) was subjected to silica gel flash column chromatography [11] and pharmacological assays.

Animals

Wistar rats, between 10 and 12 weeks of age and weighing between 250 and 320 g, were provided by the Animalarium of the Pharmacology Department, Paulista School of Medicine, Federal University of Sao Paulo. The animals were maintained under controlled temperature (22 ± 1 °C), with 12 hour light/dark cycles, and water and food consumption ad libitum except on the test day when a fasting time of 12 hours was implemented.

Rat aortic rings assays

The descending thoracic aorta from Wistar rats (n=12) previously anesthetized with ether was dissected and placed in an oxygenated Krebs solution with the following composition (in mM): NaCl, 118.0; KCl, 4.75; CaCl₂, 1.8; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25; glucose, 11; and ascorbic acid, 0.1. Rings of thoracic aorta (4–6 mm in length) were carefully excised and submerged in organ chambers containing 10 mL of Krebs solution of bathing medium maintained at 37 °C and bubbled with 95% O₂ and 5% CO₂ gas mixture (pH=7.4). Rings were mounted by means of two parallel L-shaped stainless-steel holders inserted into the lumen. One holder served as an anchor while the other was connected to a force-displacement transducer (Fort-10, WPI) to measure isometric contractile force recorded by an analog-digital conversion system (lab-trax/8-WPI). The rings were stretched progressively to an optimal basal tension of 2 g, determined by length-tension relationship experiments, and then allowed to equilibrate for 60–75 min, with bath fluid changed every 15 min.

After this incubation time, phenylephrine (1 μM) or KCl (80 mM) was added, and when a plateau of maximal concentration was attained, the butanol-containing fraction from *C. prunifolia* was cumulatively added (0.1, 1, 10, 30, and 100 μg/mL). In some rings, DMSO (0.1%) was added as control. Responses were calculated as a percentage of the maximal contraction induced by phenylephrine or KCl and were computer-fitted to a sigmoidal curve using nonlinear regression to allow for the calculation of EC₅₀ values.

Rat vas deferens assays

Vas deferens from male Wistar rats were isolated and mounted under 1.0 g tension in 10 mL organ baths containing a nutrient solution with the following composition (mM): 138 NaCl, 5.7 KCl, 1.8 CaCl₂·2H₂O, 15 NaHCO₃, 0.36 NaH₂PO₄·H₂O, and 5.5 glucose, prepared in glass-distilled deionized water, bubbled with air and maintained at 32 °C, pH 7.3 [16]. One end of the vas deferens was fixed to the organ chamber, and the other end was attached by means of a silk surgical suture to a force-displacement transducer (model FT 302; CB FT-302, iWORX, NH, USA) connected through a

bridge amplifier to a PowerLab recorder (AD Instruments, Castle Hill, NSW, Australia) coupled to a computer. Contractions were recorded and the data was stored in Chart 4.2.1 software (AD Instruments, Warwick, USA).

Cumulative concentration–response curves were made for the adrenergic agonist noradrenaline (NA; 10 nM–300 μ M) in the presence and absence of *C. prunifolia* extract (1, 10, and 100 μ g/mL), to quantify the extract's inhibition of the alpha adrenergic response; this inhibition is expressed as pA_2 and is defined as the calculated negative log concentration of the butanol-containing fraction from *C. prunifolia* needed to increase the effective concentration 50 (EC_{50}) of noradrenaline by twofold. Time-effect curves for KCl (80 mM) to verify the time-course of smooth muscle contraction induced by depolarization were also performed.

Blood pressure in anesthetized rats

Wistar rats (250–350 g) were anesthetized with intraperitoneal (i.p.) sodium pentobarbital (60 mg/kg). Two catheters (PE-50) were implanted in the left carotid artery and right jugular vein to record arterial blood pressure and for drug administration, respectively. The trachea was exposed and cannulated to facilitate spontaneous respiration. At the time of the experiment, blood pressure was monitored using a Letica pressure transducer connected to a PRS 205 amplifier and displayed on one channel of a Letica Polygraph 4000. Heart rate was measured by analysis of the blood pressure data with a CAR 1000 tachograph connected to the same PRS 205 amplifier. Mean aortic pressure was calculated as: diastolic + [(systolic - diastolic)/3]. Software Chart 5 Pro (AD Instruments) was used to view dose–response curves of blood pressure and heart rate. After a stable MAP and HR was obtained, each anesthetized animal received a series of increasing bolus doses of the butanol-containing fraction of *C. prunifolia* (1, 5, 10, 20, 30, 50, and 100 mg/kg), or physiological saline solution as control (0.05 mL/100 g), via the intravenous catheter, with each dose administered after a time interval of 5 min.

Blood pressure in non-anesthetized rats

Systolic blood pressure (SBP) in non-anesthetized rats was measured using non-invasive tail-cuff plethysmography [16]. Animals acclimated during a two-week period were randomly assigned to the following treatment groups (n=6 per group): *C. prunifolia*, *C. prunifolia* plus L-NAME, vehicle (distilled water) plus L-NAME, prazosin plus vehicle, and vehicle only. The dosage regimen was: *C. prunifolia* 0.5 g/kg/d, p.o. (according to preliminary trials); prazosin, 3 mg/kg/d, p.o.; and L-NAME, 10 mg/kg, i.p. These treatments were applied every 48 h for six weeks. Administration volume was 0.1 mL/100 g of weight. Blood pressure was measured two times a week.

Determination of angiotensin-converting enzyme activity

At the fifth week of treatment, about 0.5 mL of blood was taken from the tail artery of rats treated with *C. prunifolia* extract and rats treated with vehicle (as control). Following collection with a heparinized syringe, plasma was separated by centrifugation (10 min, 2500 r/min, 4 °C) and stored at -20 °C. Plasma angiotensin-converting enzyme (ACE) activity was determined using the indirect fluorometric method [16]. The method is based on the hydrolysis of hypuril-l-histidine-l-leucine by plasma ACE, producing the dipeptide l-histidine-l-leucine (His-Leu), which was quantitatively coupled to the fluorescence probe orthophthaldialdehyde measured at $\lambda_{\text{ex}} = 320$ nm and $\lambda_{\text{em}} = 420$ nm. ACE activity (nmol/mL/min) was determined by interpolation of a standard curve of His-Leu.

Determination of cytosolic free calcium

Cytosolic free calcium was measured in cultured myocardium cells of newborn rats and cultured myometrium cells of adult rats, based on the methodology described by Vallin and Head [17, 18]. Muscle cells were rapidly removed and incubated in Tyrode solution containing 0.2% collagenase (Sigma, type I) for 30 min at 37 °C. After incubation, the muscles were transferred to a Tyrode solution containing 0.5 mg/mL BSA and washed in normal solution. Dissociation of intact muscle fibers was obtained by gentle agitation of the solution with a Pasteur pipette.

Calcium measurements were performed on suspensions of collagenase-dispersed muscle fibers using the fluorescent dye acetoxymethyl ester of fura-2 (Fura-2/AM, Sigma). Cell suspensions were loaded for 2 h at room temperature with fura-2/AM (2 μ M) and pluronic acid F127 (0.01%) in 2.5 mL Tyrode solution, while continuously stirring in the dark. After loading, cells were washed with Tyrode solution and incubated for 15 min at 37 °C for complete de-esterification of the probe. Loaded cells were alternatively excited at 340 and 380 nm, and emission fluorescence was monitored at 510 nm using a Photon Technology International spectrofluorimeter (Lawrenceville, NJ, USA). At the end of each experiment, calibration data was obtained by adding 50 μ M digitonin (Sigma) for the determination of maximum fluorescence (F_{max}). Minimal fluorescence (F_{min}) was obtained by addition of 2 mM MnCl_2 followed by 10 mM EGTA (pH 8.5). Calcium measurement was calculated using the equation: $[\text{Ca}^{2+}]_i = K_d (F - F_{\text{min}}) / (F_{\text{max}} - F)$. K_d is the apparent dissociation constant for Ca^{2+} (224 nM). KCl (70 mM) was added in order to increase cytosolic free calcium which was measured in the presence and absence of Verapamil (reference agent, 100 μ M) and the butanol-containing fraction from *C. prunifolia* (100 μ g/mL) in experiments with at least three replications.

Data analysis

Results are expressed as mean values \pm the standard error of the mean (SEM). One-way analyses of variance followed by Dunnett comparison test were applied to *in vivo* and *in vitro* experiments. Differences in blood pressure and isolated organ bath concentration–response curves were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test, or by unpaired student t-test, with a criterion set for statistical significance at $p \leq 0.05$. Concentration–response curves in isolated aortic rings and vas deferens were fitted to nonlinear regression curves. EC_{50} values were expressed as geometric means and 95% confidence limits (CL); pA_2 value in isolated vas deferens was obtained as the negative log concentration of *C. prunifolia* needed to increase twice the noradrenaline EC_{50} [19]. Data were analyzed with Excel worksheet[®] (MS) and the GraphPad[®] statistical program.

RESULTS

Isolated aortic rings

Stimulation of aortic rings with 80 mM KCl and 1 μ M PE resulted in a sustained contraction (2345 ± 74 and 1261 ± 31 mg, respectively). The addition of the butanol-containing fraction from *C. prunifolia* caused similar concentration-dependent relaxant responses (Figure 1). Its median effective doses (g/mL) IC_{50} , were $8 [5-14] \times 10^{-5}$ and $5 [4-8] \times 10^{-5}$ against KCl and PE respectively. Maximal relaxant responses (E_{max}) were 54 ± 5 and 61 ± 7 , respectively.

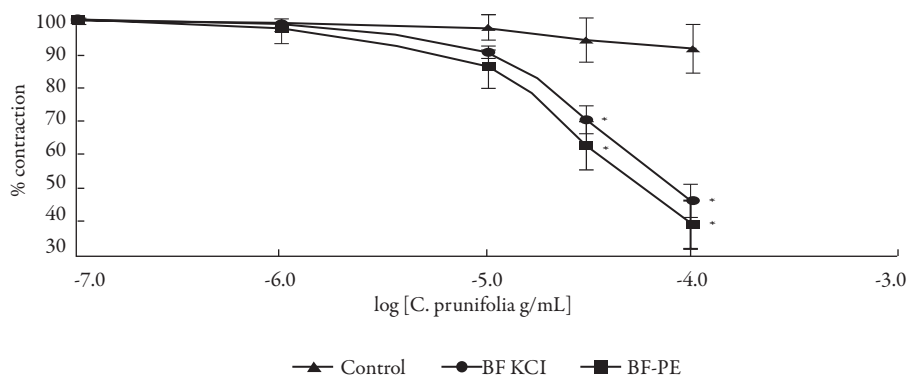


Figure 1. Cumulative dose–response curves of the butanol-containing fraction (BF) from *C. prunifolia* (10^{-7} – 3×10^{-4} g/mL) in intact aorta rings from Wistar rats contracted with: phenylephrine (PE, 10^{-6} M) or KCl (80 mM). Some rings were exposed to DMSO (0.1%) as control. Results are expressed as the mean \pm S.E.M., * $p \leq 0.05$ versus control.

Isolated vas deferens

Cumulative concentration–response curves with the adrenergic agonist NA (10^{-8} – 3×10^{-4} M) gave an EC_{50} of 4.9×10^{-6} M [3.6 – 6.9] (Figure 2). The butanol-containing fraction from *C. prunifolia* (0.01–0.1 and 1 mg/mL) shifted the noradrenaline dose–response curve to the right in agreement with a competitive profile, giving a pA_2 and slope (m) values of 4.7 and 1.02 (SE: 0.51 and 0.55), respectively.

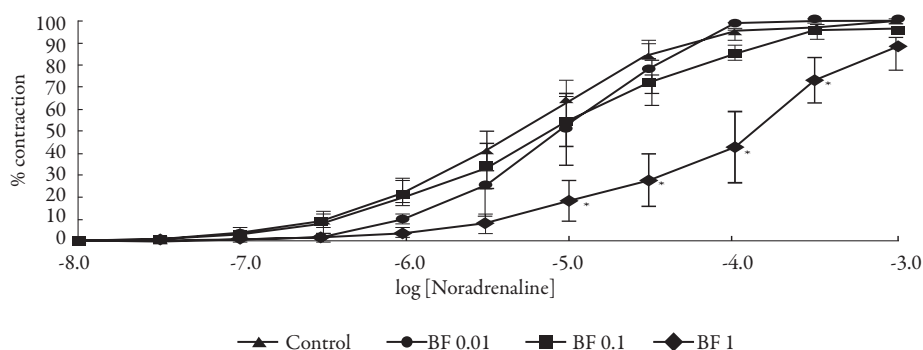


Figure 2. Cumulative dose–response curves of maximal contraction induced by noradrenaline (10^{-8} – 3×10^{-4} M) in vas deferens from Wistar rats in the presence or absence of the butanol-containing fraction from *C. prunifolia* (0.01, 0.1, 1 mg/mL). Results are expressed as the mean \pm S.E.M., * $p < 0.05$ versus control.

Blood pressure in anesthetized rats

In anesthetized, normotensive Wistar rats ($n=12$) the mean value of the baseline arterial pressure was 93 ± 4 mmHg. The dose–response curve of the butanol-containing fraction from *C. prunifolia* (1–100 mg/kg, i.v.) shows the resulting decreased blood pressure in a dose–dependent manner, with mean values of 32 ± 7 mmHg (Figure 3).

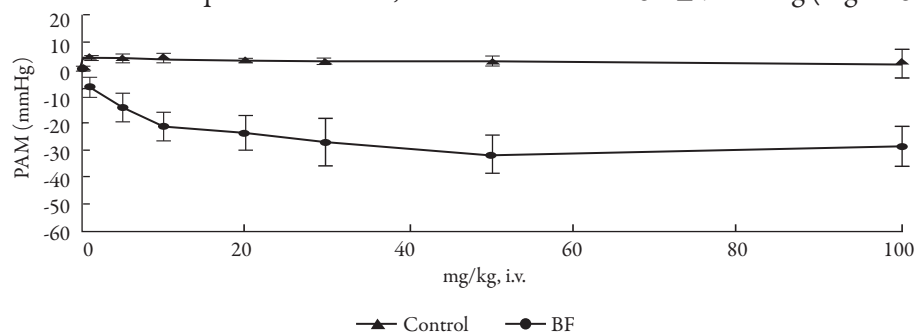


Figure 3. Cumulative dose–response curves of the butanol-containing fraction from *C. prunifolia* (1–100 mg/kg, i.v.) or physiological saline solution as control in anesthetized Wistar rats. Results are expressed as the mean \pm S.E.M., * $p < 0.05$ versus control.

Blood pressure in non-anesthetized rats

The mean systolic arterial pressure value at basal conditions and week 1 ranged from 99 to 104 mmHg ($n=30$) without significant differences between groups. The increase in arterial pressure induced by L-NAME was significant from week 2 to week 5, attaining values of 142 ± 0.7 mmHg. Normotensive control Wistar rats maintained values at about 122 ± 1.8 mmHg during this interval time. Prazocin (reference agent) decreased systolic pressure values to 110 ± 1.7 mmHg. The butanol-containing fraction from *C. prunifolia* did not decrease systolic pressure in L-NAME-exposed rats ($140\text{--}148 \pm 6.8$ mmHg) nor in normotensive rats ($117\text{--}120 \pm 2.2$ mmHg; Figure 4).

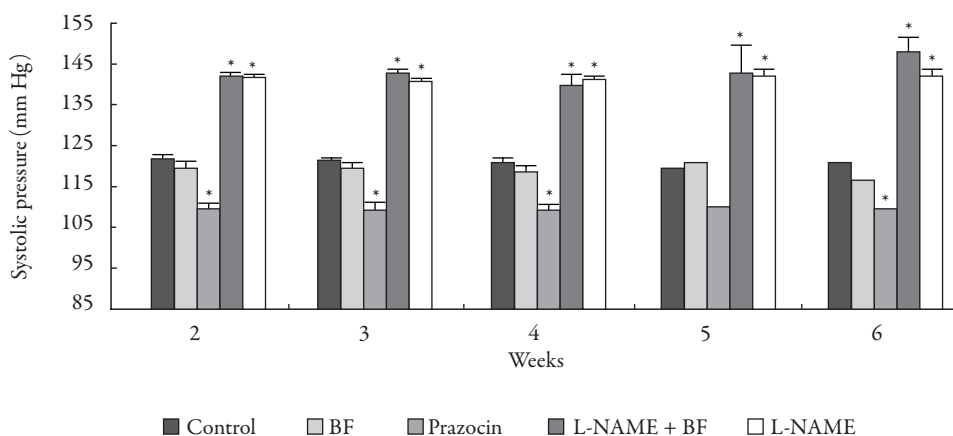


Figure 4. Values of systolic blood pressure in Wistar rats treated with: (1) physiological saline solution (control group, 0.1 mL/100 g), (2) the butanol-containing fraction of *C. prunifolia* (0.5 g/kg/day, p.o.), (3) Prazocin (reference agent, 3 mg/kg, p.o.), (4) L-NAME (10 mg/kg, i.p.), (5) L-NAME (10 mg/kg, i.p.) plus the butanol-containing fraction of *C. prunifolia* (0.5 g/kg/day, p.o.). Results are expressed as the mean \pm S.E.M after one week of treatment, * $p \leq 0.05$ versus control.

ACE activity and cytosolic free calcium assays

The plasma ACE activity was not significantly different between control rats (56.62 ± 1.9 mol/min/mL) and the rats treated with the butanol-containing fraction from *C. prunifolia* (58.39 ± 8.69 nmol/min/mL; Figure 5). The percentages of resting intracellular calcium concentrations ($[Ca^{2+}]_i$) determined in enzymatically dissociated myocardium and myometrium cells were 7.2 ± 0.8 and $5.3 \pm 1.0\%$ respectively. KCl increased these concentrations to 35.3 ± 3.0 and $25.1 \pm 1.4\%$. Verapamil (reference agent, 100 μ M) significantly decreased the calcium concentrations induced by KCl to 17.8 ± 2.3 and $10.7 \pm 0.9\%$, whereas the butanol-containing fraction from *C. prunifolia* (100 μ g/mL) did not: 31.1 ± 0.7 and $23.9 \pm 0.6\%$ (Figure 6).

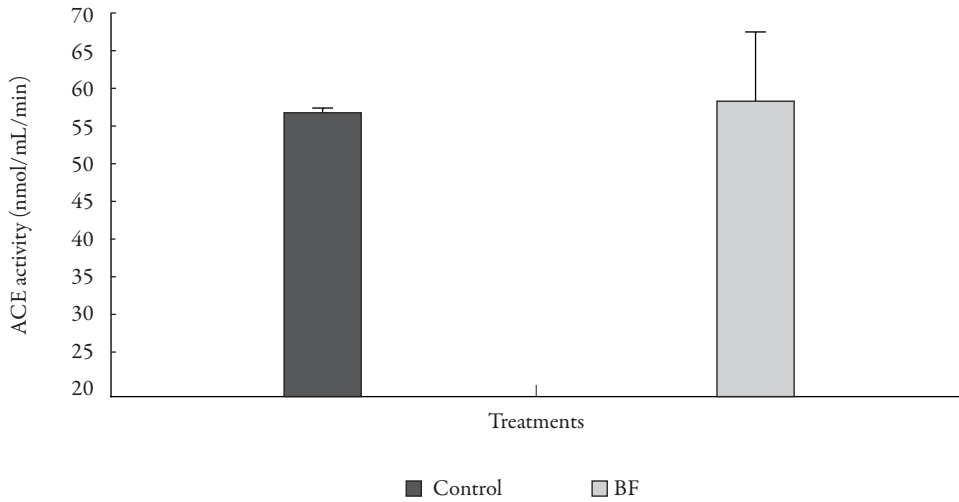


Figure 5. Angiotensin-converting enzyme activity (nmol/mL/min) in Wistar rats previously treated with the butanol-containing fraction from *C. prunifolia* or physiological saline solution as control. Results are expressed as the mean \pm S.E.M.

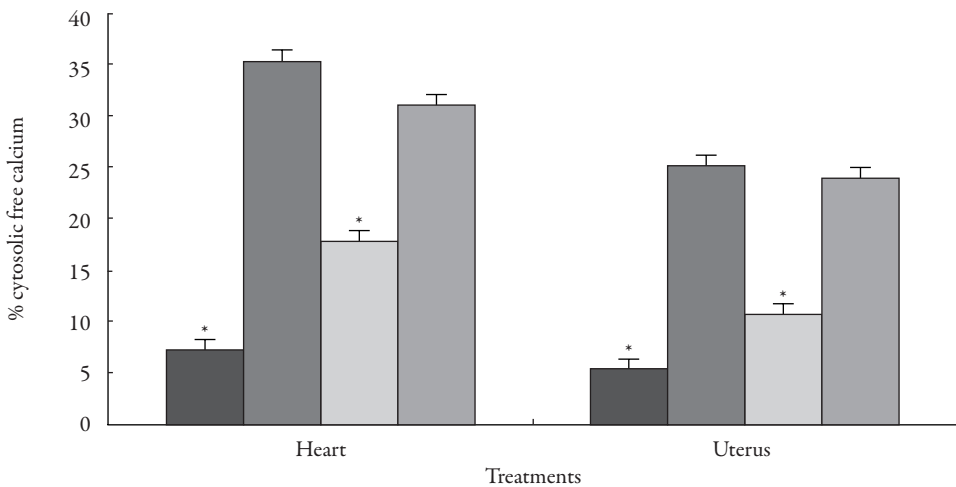


Figure 6. Cytosolic free calcium determination (% of maximal fluorescence) in cultured myocardium cells of newborn rats and cultured myometrium cells of adult rats in the absence (control) or presence of KCl (70 mM), KCl plus verapamil (reference agent, 100 μ M), or KCl plus the butanol-containing fraction from *C. prunifolia* (100 μ g/mL). Results are expressed as the mean \pm S.E.M., * $p < 0.05$ versus the KCl group.

DISCUSSION

The results of this study show that the butanol-containing fraction from *C. prunifolia* relaxes smooth muscle cells in rat isolated aortic rings and isolated vas deferens and decreases blood pressure in anesthetized rats without modifying blood pressure in conscious normotensive rats nor rats exposed to the nitric synthase inhibitor, L-NAME. In addition, *C. prunifolia* extract does not affect the activity of angiotensin-converting enzyme from plasma of rats and also does not affect the cytosolic free calcium currents of rat cultured ventricular and myometrium cells. The results from these experiments suggest that, in muscle tissue, *C. prunifolia* displays relaxant properties related to antiadrenergic actions without significantly affecting *in vivo* nitric oxide pathways, angiotensin-converting enzyme activity, or calcium currents.

C. prunifolia relaxed rat thoracic aorta rings previously exposed to either KCl, a depolarizing agent that elicits the opening of voltage-dependent calcium channels [20], or phenylephrine, an alpha agonist [21]. This suggests that *C. prunifolia* activity could, at least in part, be due to vasodilator effects likely caused by the inhibition of Ca^{2+} influx through voltage-dependent calcium channels [22]. In addition, *C. prunifolia* displayed a competitive profile in rat vas deferens contracted with noradrenaline. Taken together, these results suggest that *C. prunifolia* exerts vasorelaxant properties on smooth muscle via mechanisms that relate to alpha and calcium channel inhibition. Although *C. prunifolia* did not decrease blood pressure in non-anesthetized rats, its vasorelaxant properties in smooth muscle might account for the beneficial effects ascribed to this species in traditional folk medicine [6].

It is noticeable that the butanol-containing fraction from *C. prunifolia* elicited hypotensive effects in anesthetized rats in addition to its relaxant properties in aortic rings contracted with PE and KCl and vas deferens contracted with noradrenaline. However, the extract did not inhibit calcium currents in cardiomyocytes or uterine cells. This response may be due to a higher sensitivity to calcium influx in smooth muscle cells than in cardiomyocytes and myometrial cells, and by the accumulating evidence suggesting that the alpha antiadrenergic effect of *C. prunifolia* linked to its antioxidant mechanisms is more directly related to the NO/GMPc pathway rather than to calcium channels blocking properties [23].

While constituents responsible for the vasorelaxant effects of *C. prunifolia* have not yet been completely elucidated, bioflavonoid compounds, especially rutin, may play a key role in the cardiovascular effects caused by this species [11]. Previous works have shown that rutin, as do many polyphenols, possesses antioxidant properties that would explain several biological effects, such as vasodilation related to NO/GMPc pathways

and cardio-protective properties [24, 25]. However, rutin lacks an alkyl group at position 7 of ring *A*, a feature that enhances the free radical scavenging activity of flavonoids, which in addition to a free catechol moiety at ring *B* and a free hydroxyl in position 3 in ring *C*, would attenuate the free scavenger profile of rutin [26]. Therefore, rutin might not have potent antioxidant properties, and this would help to explain why *C. prunifolia* is not so effective in decreasing blood pressure in *in vivo* experiments.

Rutin is a widely consumed flavonoid ascribed with several pharmacological activities including antioxidant, anti-inflammatory, anti-diabetic, anti-adipogenic, neuroprotective, and hormone therapy. However, the mechanism of this flavonoid after its consumption is unclear [27]. More pharmacological studies have been conducted using *in vitro* bioassays than *in vivo*, because the biotransformation process *in vivo* can affect rutin efficacy, and rutin administration would be required at high concentrations in order to reach target organs [28]. Given that the butanol-containing fraction from *C. prunifolia* did not result in effects after administration, absorption and biotransformation problems should be considered.

A previously described key factor that affects the biotransformation of rutin is gut microflora that mediates the hydrolysis of rutin to its metabolites (quercetin, aglycone, and other phenol derivatives), resulting in little leftover dietary rutin for absorption, and consequently results in a minor biological effect [29]. Although the presence of the glycoside radical, rutinose disaccharide, indicates the first step of hydrolysis, some authors have found that the moiety more readily absorbed is in the form of aglycone [30]. Therefore, the question of whether the effective biological activity of rutin is due to the molecular structure of the respective aglycone needs investigation.

Bearing in mind that rutin is not the only active metabolite present in *C. prunifolia*, but that some kind of synergistic activity may exist among the principal constituents of this species [11], and considering that an adequate consumption of a rutin-rich diet may decrease the risk of chronic diseases including hypertension [31], there are elements to suppose that *C. prunifolia* would benefit individuals with chronic disorders like hypertension.

In conclusion, the butanol-containing fraction from *C. prunifolia* results in vasorelaxant effects on rat smooth muscle and hypotensive effects in anesthetized rats. These effects are likely related to mechanisms that lead to alpha adrenergic inhibition. Given that one of its primarily described constituents is rutin, this bioflavonoid may play a pivotal role in the relaxant effect seen, surely due to its free radical scavenging properties. As the active metabolites of this species interact in a synergistic way, there are elements to support the ethnobotanical use of this species.

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CONFLICT OF INTEREST

There were no conflicts of interest in carrying out this work.

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