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Research Paper

Hypotensive and vasorelaxant effects induced by the ethanolic extract of the *Mimosa caesalpiniifolia* Benth. (Mimosaceae) inflorescences in normotensive rats

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ABSTRACT

Ethnopharmacological relevance: Caatinga is highly influenced by its seasonality. This species is endemic in the northeastern region, which is rich in plants with pharmacological potential. Many of these plants are used by the population and some of them have confirmed pharmacological properties. *Mimosa caesalpiniifolia* Benth. (Mimosaceae) is a native plant from northeastern Brazil's caatinga, popularly known as sabiá and cascudo. The tea from the inflorescence of this species is used by the population of the semi-arid for the treatment of hypertension, and the utilization of the plant bark for the staunching of bleedings and wound washing in order to prevent inflammation; also, the ingestion of the bark infusion is used in the treatment of bronchitis. However, its pharmacological effects and mechanisms of action have not yet been studied. The aim of the present study was to determine the effect of the ethanolic extract of *M. caesalpiniifolia* on the cardiovascular system in rats.

Material and methods: In a study for the assessment of the hypotensive effect of the extract, the polyethylene catheters were inserted in the aorta artery and inferior vena cava for the measurement of the arterial pressure and heart rate. When intragastric administration was performed, only one catheter was implanted in the abdominal aorta. In studies for the vasorelaxant activity, mesenteric arterial rings (1–2 mm) were used: they were kept in Tyrode's solution (95% O_2 and 5% CO_2) and submitted to tension of 0.75 g/f for 1 h. The results were expressed as mean ± S.E.M., significant to the values of $p < 0.05$.

Results: The administration of the doses through venous pathway (6.25; 12.5 and 25 mg/kg, i.v.) promoted hypotension followed by bradycardia in the higher doses. The pre-treatment with atropine (2 mg/kg, i.v.) interrupted both the hypotension and the bradycardia; with hexamethonium, hypotension was reverted and bradycardia was attenuated. While the administration of tea/flowers (25 mg/kg i.v.) also promoted a following section of hypotension, a slight increase in heart rate was observed. When administered orally, MC-EtOH/flowers (100 mg/kg, v.o.) promoted a decrease in the arterial pressure from 90 min on, without a significant alteration in the heart rate in relation to the control. In the in vitro study, a pharmacological trial was performed with the extracts obtained from parts of the species *M. caesalpiifolia* (leaves, bark, fruit and inflorescences). Among all extracts tested, the ethanolic extract from the inflorescences (MC-EtOH/flowers) presented higher vasorelaxant potency in relation to the other parts of the plant. Henceforth, MC-EtOH/flowers was used in the sequence. In mesenteric preparations pre-contracted with phenylephrine (10^{-5} M), the MC-EtOH/flowers (0.1–750 μg/ml) promoted vasorelaxant effect regardless of the vascular endothelium. MC-EtOH/flowers inhibited the contractions induced by the cumulative addition of phenylephrine (10^{-9}–10^{-5} mol/l) or $CaCl_2$ (10^{-6}–3×10^{-2} M), in a concentration-dependent way. In contractions induced by S(-)Bay K 8644, a Cav-L activator, the MC-EtOH/flowers promoted concentration-dependent relaxation, corroborating previous results.

Conclusion: The tea of flowers of *M. caesalpiniifolia* promotes hypotension and tachycardia, whereas ethanolic extract (MC-EtOH) promotes hypotension and bradycardia involving the participation of the muscarinic and ganglionic pathways, as well as vasorelaxant action involving the Ca^{2+} influx inhibition blockade.

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1. Introduction

Mimosa caesalpiniifolia Benth belongs to the family Mimosaceae, an arboreous plant that grows naturally in the caatinga and in the cerrado in northeastern Brazil, being popularly known as sansão-do-campo, unha-de-gato, sabiá or cascudo (Oliveira et al., 2007, Albuquerque et al., 2007). Brazil is the main distribution center for the genus *Mimosa*, with around 340 species; 60% of those are endemic in different regions, out of which 189 species are referred to in the cerrado (Grether, 2000; Silva and Secco, 2000). The medicinal use of this species is about the utilization of the plant bark for the staunching of bleedings and wound washing in order to prevent inflammation; also, the ingestion of the bark infusion is used in the treatment of bronchitis (Carvalho, 2007), and the steam bark and flowers is used by the population of the semi-arid for the treatment of hypertension (Albuquerque et al., 2007). As it has been demonstrated that the genus *Mimosa* contains flavonoids (Souza et al., 2008), a possible vasodilatation effect of *M. caesalpiniifolia* can be attributed to this phenolic compound, which is known by its cardiovascular effects, such as hypolipidemic (Dong et al., 1998), hypoglycemiant (Dong et al., 2002) and hypotensive (Zhang et al., 2009) effects. Thus, considering the popular use in the treatment of hypertension, the present study aims at assessing the hypotensive and vasorelaxant activity induced by the ethanolic extract obtained from parts (stem, leaves, fruit and inflorescence) of *M. caesalpiniifolia*, as well as its possible mechanisms involved, using in vivo and in vitro models.

2. Materials and methods

2.1. Plant material

The leaves, stem bark, fruits and inflorescences from *M. caesalpiniifolia* were collected in May 2009 in Teresina city, Piauí state, Brazil. A voucher specimen was identified and deposited in the Graziela Barroso Herbarium from the Federal University of Piauí (number TEPB 26,824).

2.2. Extract preparation

Dried and powered plant materials was extracted exhaustively with ethanol 98% at 1:7 plant material/solvent (w/v) five consecutive times at room temperature, which was then sonicated in ultrasound (30 min/day). The solvent was removed under reduced pressure using rotary evaporator and lyophilized to yield dried ethanolic extract (MC-EtOH) of leaves (21%, w/w), stem bark (5%, w/w), fruits (8%, w/w) and flowers (inflorescences) (11%, w/w).

2.3. HPLC-UV/vis analysis of MC-EtOH extracts and ESI(−)-MS analysis of MC-EtOH/flowers

MC-EtOH extracts (20 mg) were submitted to solid-phase extraction C_{18} (500 mg, Phenomenez, Torrance, CA, USA) eluted with 5 mL of MeOH–H_2O (8:2, v/v). The eluted fraction was evaporated under N_2 flow, then added with 2 mL of MeOH–H_2O (8:2, v/v) and filtered (0.45 μm, PTFE, Millipore). MC-EtOH was analyzed by means of a HPLC Shimadzu system (SPD-10A UV–vis detector) equipped with C18 column (4.0 × 250 mm^2, Perkin Elmer) with 0.01% trifluoroacetic acid in water (A) and acetonitrile (B) following this gradient: 15% of B at 0 min, 65% of B at 40 min and 85% of B at 60 min. The flow rate was 1 mL/min, at room temperature (25 °C) and an injection of 20 μL of samples. The chromatograms were monitored at 340 nm.

MC-EtOH was analyzed by direct infusion in a LTQ-Orbitrap mass spectrometer equipped with ESI in negative mode (ESI (-)-LTQ-Orbitrap-MS, Thermo Fischer Scientific). The MS full mode parameters were as follows: spray voltage 3.30 kV; sheath gas 8 (arbitrary units); capillary voltage −46 V; capillary temperature 300 °C; and tube lens −115, 14 V. The multi-stage analysis (MS^n) mode on the ion trap analyzer was acquired by CID fragmentation using normalized collision energy 35.0. The data were processed using the XCalibur software (version 2.0. Thermo Fischer Scientific).

2.4. Solutions and drugs

The composition of Tyrode's solution used was (mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 10.0; and glucose, 5.6 mM (Tanaka et al., 1999). KCl 20, 60 and 80 mM of Tyrode's solution were prepared by equimolar replacement of Na^+ for K^+. The solution was nominally free of calcium, i.e., there was not the addition of $CaCl_2$. The drugs used were L-(-)-phenylephrine hydrochloride, acetylcholine, S(-)-BayK8644, sodium nitroprusside (SNP), cremophor, atropine, hexamethonium (Sigma-Aldrich, St. Louis, MO, USA), sodium thiopental (Cristália) and sodium salt of heparin (Roche). In order to prepare stock solutions of the drugs, all substances were dissolved in distilled water and diluted to the appropriate concentrations, except for S (-)-BayK8644, which was dissolved in 1 mM ethanol. The extract (MC-EtOH) was dissolved in Tyrode's solution for the (10 mg/mL) in vitro protocols and saline solution (100 mg/mL) for in vivo protocols using Cremophor (0.1% v/v) as the eluent. All solutions were stored at 0 °C for 24 h after preparation. In folk medicine, the decoction is of a handful (10–15 g) in a liter of water. It is drunk until the symptoms disappear (Agra et al., 2007). The tea/flowers was prepared on the day of the experiment, which used the equivalent to the popular use: 10 g of dried flowers in 1000 ml of boiling water. The same administration was carried out at room temperature at a dose of 25 mg/kg i.v equivalent to MC-EtOH.

2.5. Animals

Male Wistar rats (250–350 g) were used for all experiments. Animals were kept under conditions of controlled temperature (24 ± 1 °C) and 12-h light/dark cycle. They had free access to food (PURINA, Brazil) and tap water ad libitum. All experimental procedures were approved by the Animal Research Ethics Committee of the Federal University of Piauí, Brazil (CEEA no. 008/12). Procedures regarding the euthanasia of the animals were in accordance to the Resolution number 1000 (2012) of the Federal Council of Veterinary Medicine, Brazil.

2.6. Pharmacological tests

2.6.1. Preparation of rat superior mesenteric artery rings

The superior mesenteric arteries were removed and cleaned from the connective tissue and fat. Mesenteric rings (1–2 mm) were obtained and suspended by cotton threads in organ baths containing 10 ml of Tyrode's solution, at 37 °C, and gassed with carbogenic mixture (95% O_2 and 5% CO_2). Rings were stabilized with a resting tension of 0.75 gf for at least 1 h. During this time, the solution was changed every 15 min (Adaramoye et al., 2009). The isometric tension was recorded by means of a force transducer coupled to a data acquisition system (AECAD 1604, AQCAD 2.0.5., AVS Projects, São Paulo, SP, Brazil). When necessary, the endothelium was removed by gently rubbing the intimal layer with moistened cotton strings. The endothelium integrity was verified through relaxation to ACh (10^{-5} M) in rings pre-contracted by Phe

(10^{-5} M). In endothelium-intact studies, preparations were discarded when ACh-induced relaxation was lower than 70%.

2.6.2. Screening of the effect of MC-EtOH (bark, fruit, leaves and flowers) in the mesenteric artery isolated from rats pre-contracted with phenylephrine rings

After stabilization (60 min), rings with and without endothelium were pre-contracted with phenylephrine (10^{-5} M) in different preparations and were tested by adding cumulative concentrations of the MC-EtOH extracts (bark, leaves, fruits and flowers; 0.1–750 μg/ml) in the tonic contraction phase to obtain a concentration–response curve. The extract that showed higher vasorelaxant effect was selected for the study.

2.6.3. Effect of the MC-EtOH/flowers on the hemodynamic parameters in non-anaesthetized rats

The measurements of the mean arterial pressure and heart rate were performed as described by Oliveira et al., 1996, with slight modifications. Under sodium thiopental anesthesia (45 mg/kg, i.p.), the lower abdominal aorta and vena cava were canulated via the left femoral artery and vena using a polyethylene catheter. Thereafter, a catheter was filled with heparinized saline solution and led under the skin to emerge between the scapulae. When administered orally, the catheter was inserted only into the artery. Arterial pressure was measured after 24 h by connecting the arterial catheter to a pre-calibrated pressure transducer (Statham P23 ID; Gould, Cleveland, OH, USA) coupled to an amplifier (AECAD 1604 Model, AVS Projetos, São Paulo, Brazil) and connected to a computer equipped with the AQCAD 2.02 software (AVS Projetos, São Paulo, Brazil). The data were sampled at a frequency of 500 Hz. For each cardiac cycle, the computer calculated the mean arterial pressure (MAP). After the stabilization of the hemodynamic parameters, SNP, a donor of nitric oxide (Castro et al., 2011), was used (10 μg/kg i.v.) as positive control. A successive injection of MC-EtOH/flowers (6.25; 12.5 and 25 mg/kg, i.v.) was well separated by a time interval long enough to allow full recovery of the basal parameter (30 min). In order to elucidate the possible mechanisms underlying the hypotensive response, MC-EtOH/flowers was administered to animals pre-treated with one of the following drugs: a non-selective antagonist of muscarinic receptor (atropine, 2 mg/kg) (Mitchelson, 1984) and a ganglionic blocking agent (hexamethonium, 20 mg/kg) (Borodinova et al., 2013). In another set of experiments, the tea/flowers was administered through venous route at the dose of 25 mg/kg. In intragastric administration, the animals were divided into two groups of five animals each. After 30 min, the first group received saline (0.3 ml) over cremophor (vo) and then received a second dose of (100 mg/kg v.o.) MC-EtOH/flowers. The values of MAP and HR were recorded at 0, 10, 30, 60, 90, 120, 150, 180, 210 and 240 min after administration. After protocols, all animals were killed with sodium thiopental anesthesia (100 mg/kg; i.p.).

2.6.4. Investigation for the effect of MC-EtOH/flowers on Phe-induced concentration–response curves in endothelium-denuded preparations

After stabilization period, the effect of MC-EtOH on Phe-induced contractions in endothelium-denuded rings was assessed (Oliveira et al., 2006). Cumulative concentration–response curves for Phe (10^{-9}–10^{-5} M) were obtained before and after pre-incubation separately with MC-EtOH (81, 243 and 500 μg/ml) for 30 min. The results were expressed as percentages of the maximal response for Phe-induced response and curves were statistically compared.

2.6.5. Investigation for the effect of MC-EtOH/flowers on $CaCl_2$-induced concentration–response curves in endothelium-denuded preparations

After the stabilization period, the effect of MC-EtOH on $CaCl_2$-induced contractions in endothelium-denuded rings was assessed (Oliveira et al., 2006). Cumulative concentration–response curves for $CaCl_2$ (10^{-6}–3×10^{-2} M) were obtained in endothelium-denuded rings exposed nominally without Ca^{2+} solution with KCl 60 mM before and after pre-incubation separately with MC-EtOH/flowers (81, 243, 500 and 750 μg/ml) for 30 min. The results were expressed when percentages of the maximal response for $CaCl_2$-induced response and curves were statistically compared.

2.6.6. Effect of the MC-EtOH/flowers on contractions induced by S-(-) Bay K 8644 and KCl 80 mM in mesenteric artery rings isolated from rats

After checking the absence of the vascular endothelium, the mesenteric preparations were pre-incubated with a depolarizing solution from 20 mM KCl for 20 min in order to obtain a better response to the agent used, S(-)-BayK8644. After the administration of S(-)-BayK8644 (10^{-6} M), a Cav-L activator (Catterall et al., 2005), a sustained contraction was obtained. In the tonic phase of contraction, MC-EtOH/flowers were administered cumulatively. The ability of MC-EtOH/flowers to attenuate the sustained contraction in the rings induced by KCl 80 mM was also examined similarly. The vasorelaxant potency of MC-EtOH/flowers was evaluated by comparing the pD_2 values obtained with different preparations of isolated rat mesenteric artery rings.

2.6.7. Vasorelaxant effect of the MC-EtOH/flowers in the presence of Tetraethylammonium (TEA) 3 mM

Endothelium-denuded mesenteric artery rings were pre-incubated for 30 min with TEA (3 mM), a non-selective blocker for potassium channel. After 30 min of this procedure, contraction with phenylephrine was administered (10^{-5} M) (40 min) in mesenteric artery rings. In the sustained phase of contraction, MC-EtOH/flowers was added cumulatively (0.1–750 μg/ml). The response obtained was compared to the control in the absence of TEA (3 mM) (Rocha and Bendhack, 2009).

3. Statistical analysis

Results are expressed as the mean values ± S.E.M. (standard error of means) of four to eight animals. The statistical evaluation was carried out using the unpaired Student's *t*-test. In all analyses, a p value < 0.05 was considered as statistically significant. All procedures were performed by using Graph Pad Prism 5.0™ (Graph Pad Software, Inc., San Diego, CA, USA).

4. Results

4.1. Chemical analysis

HPLC fingerprinting analyzes of MC-EtOH showed a metabolite profile with differentiated level of phenolic compounds (Fig. 1), using absorbance at 340 nm, typical of flavonoids. The phytochemical analysis through HRMS and MS/MS revealed the presence of phenolic acids and flavonoid derivatives (Table 1; Fig. 2), according to previous phytochemical studies of *M. caesalpiniifolia* flowers which showed gallic acid, methyl galate and quercetin.

4.2. Vasorelaxant effect of MC-EtOH (bark, fruit, leaves and flowers) on the mesenteric artery isolated from rats pre-contracted with phenylephrine rings

The MC-EtOH extracts induced vasorelaxant effect dependent on the concentration and independent on the vascular endothelium

in mesenteric arteries pre-contracted with phenylephrine (Fig. 3). The ethanolic extract of flowers (MC-EtOH/flowers) showed higher vasorelaxant potency and therefore was used in this study sequence. After the experiments, the preparations were washed for 60 min. A contraction was induced by phenylephrine to check for the reversal of the relaxation induced by MC-EtOH extract, having in all preparations, reversing contractile response.

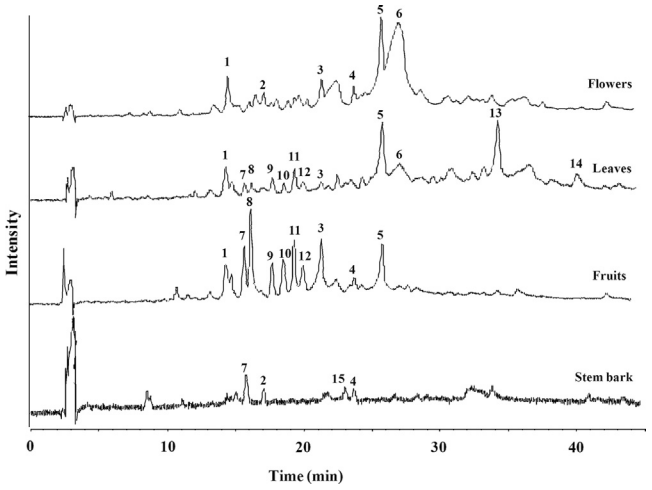

Fig. 1. HPLC-UV Fingerprinting of the ethanolic extracts of *M. caesalpiniifolia* recorded at 340 nm.

4.3. Hypotensive effect

4.3.1. Effect of the Mc-EtOH/flowers and tea/flowers on the hemodynamic parameters before and after the muscarinic and ganglionic blockade

Administration of the Mc-EtOH/flowers (6.25; 12.5 and 25 mg/kg, i.v.) in non-anaesthetized rats caused hypotension (-9.98 ± 0.79; -28.55 ± 6.56; -38.87 ± 6.09) as it is shown in the original register in Fig. 4 and bradycardia (9.47 ± 1.00; -19.12 ± 7.33; -36.68 ± 5.75). The hypotensive effect was practically abolished after the administration of atropine (0.51 ± 0.29; -4.73 ± 1.73; 4.21 ± 2.12; Fig. 5A) and reverted after the hexamethonium (17.39 ± 5.77; 18.22 ± 5.51; 12.21 ± 6.30; Fig. 6A). In the doses 12.5 and 25 mg/kg, we observed accentuated bradycardia. After the blockade with atropine, bradycardia was practically abolished (1.29 ± 0.61; 3.55 ± 1.00; 2.09 ± 0.62; Fig. 5B) and attenuated after hexamethonium (-2.83 ± 0.46; -7.83 ± 0.87; -7.50 ± 1.97; Fig. 6B). The infusion of tea/flowers (25 mg/kg, i.v.) caused hypotension (-10.35 ± 0.10) and heart rate (7.57 ± 1.74) Fig. 7A and B.

4.3.2. Effect of MC-EtOH/flowers administered orally on the arterial pressure and heart rate in rats

After a period of stabilization of the hemodynamic parameters of the basal values of the normotensive animals (MAP = 104 ± 4, HR = 360 ± 18), 100 mg/kg of MC-EtOH/flowers was administered

Table 1
Chemical compounds identified by ESI(−)-MS and MS^n experiments in the ethanolic extract of *M. caesalpiniifolia* inflorescences.

$[M-H]^-$	$[MS^2]$	Formula	Chemical costituents
169.0140	[169]: 125	$C_7H_6O_5$	Gallic acid
183.0120	[183]: 125	$C_8H_8O_5$	Methyl gallate
297.0406	[297]: 267, 251, 227, 175	$C_{17}H_{14}O_5$	5-Hydroxy-4′,7-dimethoxy-flavone
301.0352	[301]: 179, 151	$C_{15}H_{10}O_7$	Quercetin
463.0877	[463]: 301	$C_{21}H_{20}O_{12}$	Quercetin-*O*-hexoside
593.1512	[593]: 575, 561, 547, 502, 447, 429, 411, 383, 357, 285	$C_{27}H_{30}O_{15}$	Vicenin-2
609.1464	[609]: 301	$C_{27}H_{30}O_{16}$	Rutin

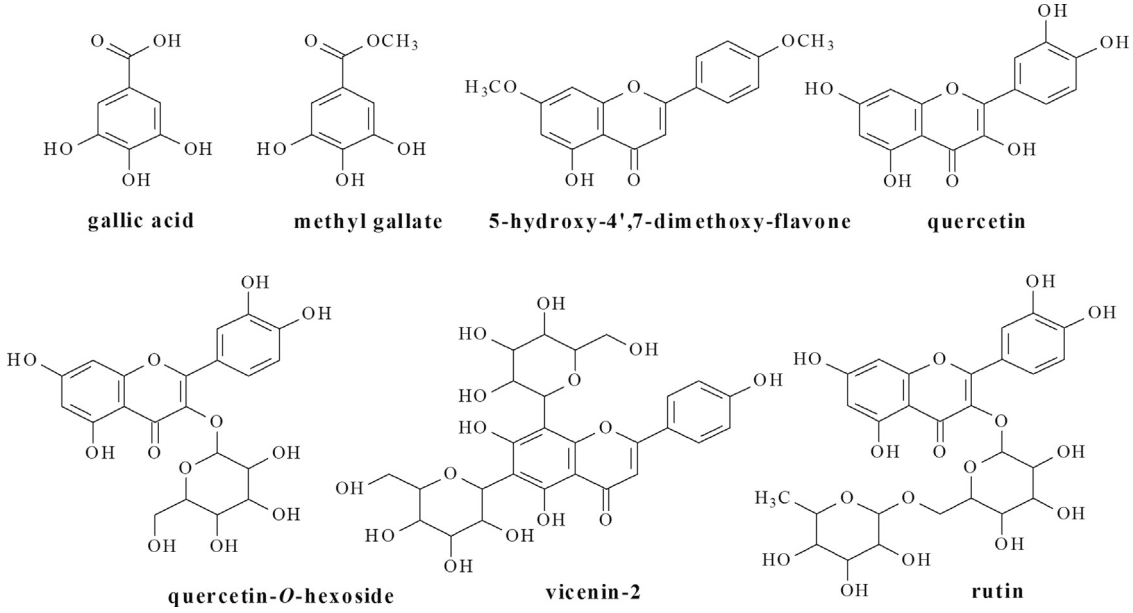

Fig. 2. Chemical structures identified by ESI(−)-MS in the ethanolic extract of *M. caesalpiniifolia* flowers.

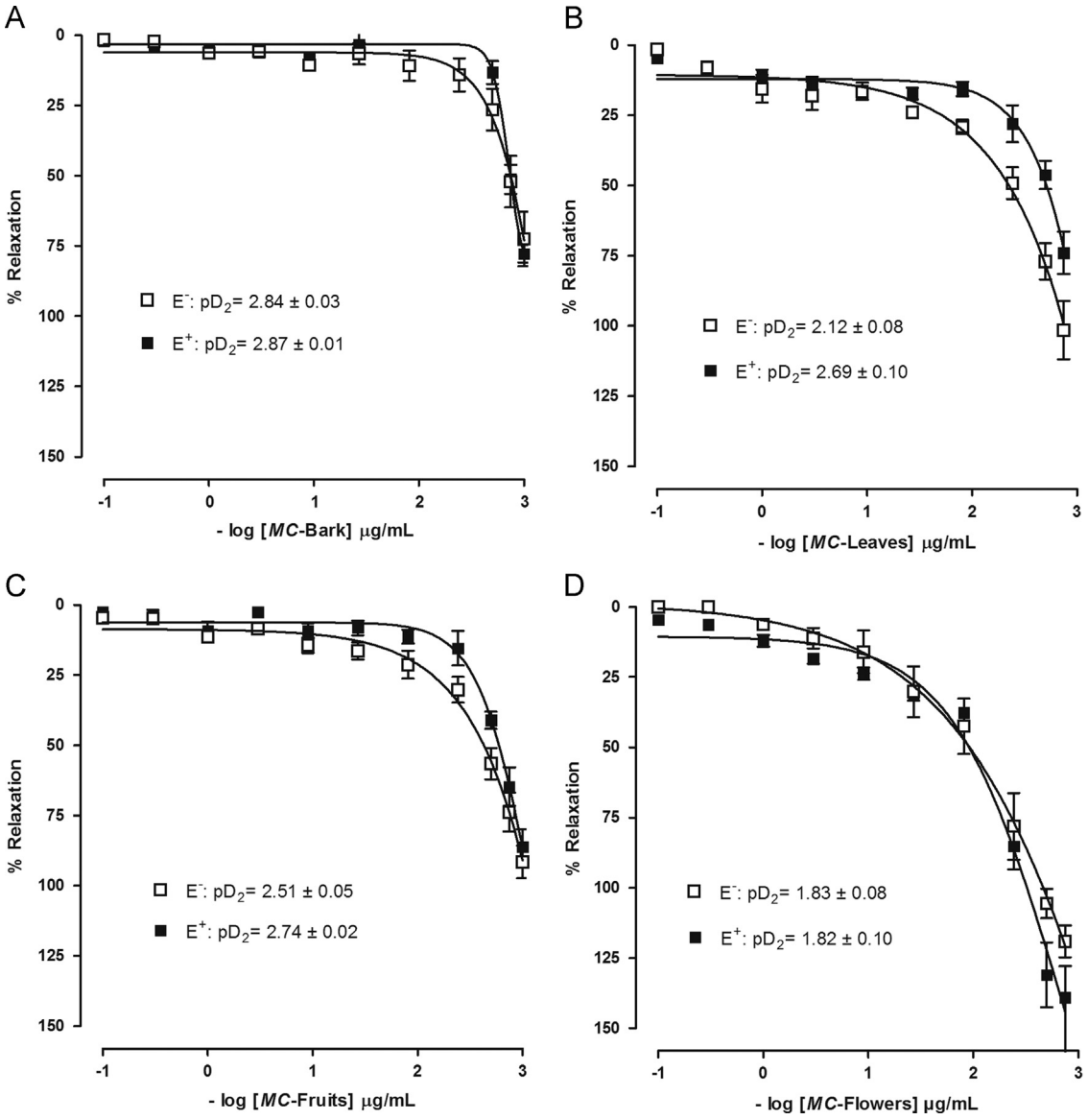

Fig. 3. Vasorelaxant effect from different ethanolic extracts of *Mimosa caesalpiniifolia* (MC-EtOH): stem bark (A), leaves (B), fruits (C) and flowers (D) (0.1–750 µg/mL) in rings of rat mesenteric artery: with (■), without (□) endothelium pre-contracted with Phe (10 µM). Values are mean ± S.E.M. $n=5$ experiments.

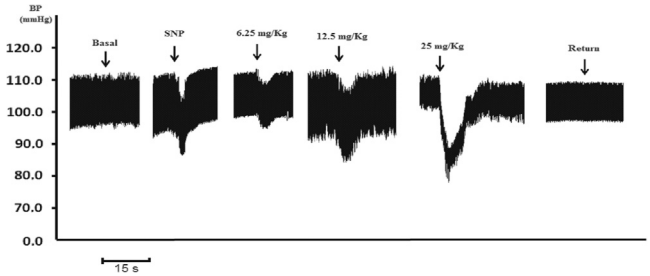

Fig. 4. Original traces of the MC-EtOH/flowers-induced hypotensive effect on MAP in non-anesthetized normotensive rats.

orally through gavage; after 30 min, the arterial pressure values were registered. The MC-EtOH/flowers decreased arterial pressure from 90 min after the administration on (Fig. 8A). Significant alteration in the heart rate was not observed when compared to the control (Fig. 8B).

4.4. Effect of the MC-EtOH/flowers on the cumulative addition of phenylephrine or $CaCl_2$ on mesenteric artery rings isolated from rats

The cumulative concentration–response curves for phenylephrine were dislocated to the right with reduction of the maximal effect in the presence of different concentrations of MC-EtOH/flowers (81, 243 and 500 µg/mL; $E_{max}=77.78\pm4.00\%$*; $57.69\pm3.57\%$*, $28.18\pm2.07\%$*, control, $E_{max}=100\pm2.15\%$), (*$p<0.05$ vs control, Fig. 9) in superior mesenteric artery rings isolated from rats. In a depolarizing medium nominally without calcium, the MC-EtOH/flowers (81, 243, 500 and 750 µg/mL) inhibited the contractions induced by $CaCl_2$ in a concentration-dependent way ($E_{max}=93.50\pm5.40\%$, $81.77\pm9.91\%$*, $45.31\pm3.75\%$*, $24.08\pm7.10\%$*, Control, $E_{max}=99.60\pm5.86\%$) with reduction of the maximal effect (*$p<0.05$ vs control, Fig. 10).

4.5. Effect of the MC-EtOH/flowers on superior mesenteric artery rings isolated from rats pre-contracted with S(-)-BayK8644 and KCl 80 mM

The MC-EtOH/flowers inhibited the chronic contractions induced by S(-)-Bay K 8644, ($pD_2=2.06\pm0.12$ µg/mL*, $n=4$) in a significant

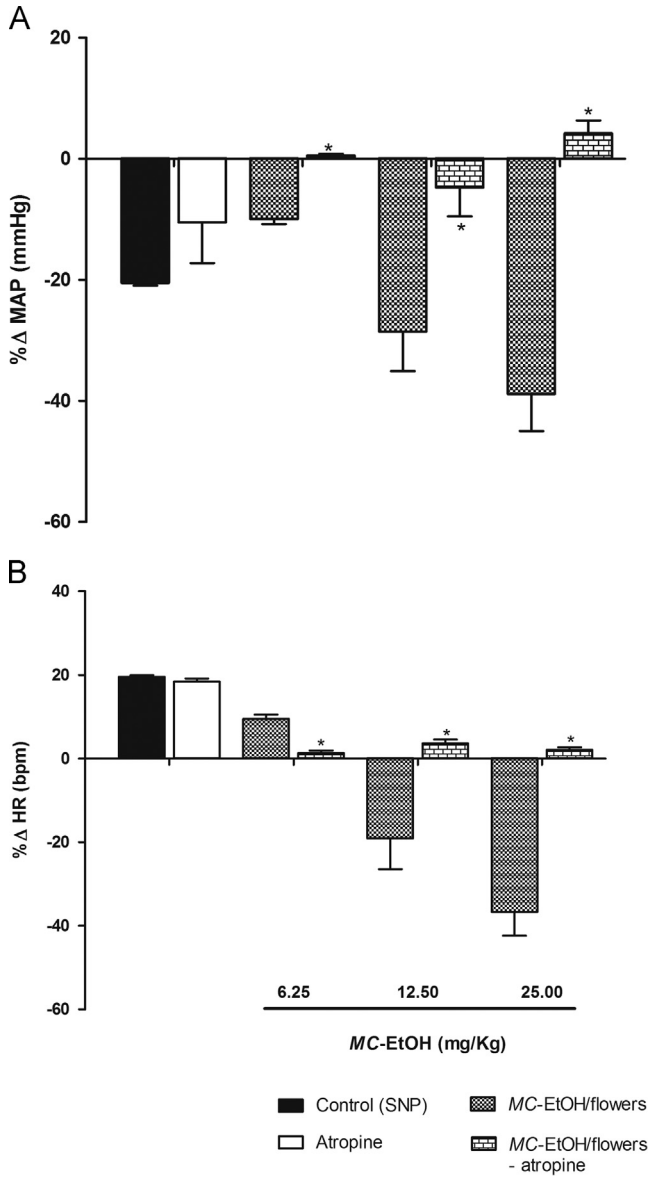

Fig. 5. Effect of MC-EtOH/flowers on mean arterial pressure (A) and heart rate (B) in non-anesthetized rats in presence and absence of atropine (2 mg/kg, i.v.). Values are mean ± SEM of five experiments. *$p < 0.05$ vs MC-EtOH/flowers; Test *t*-Student.

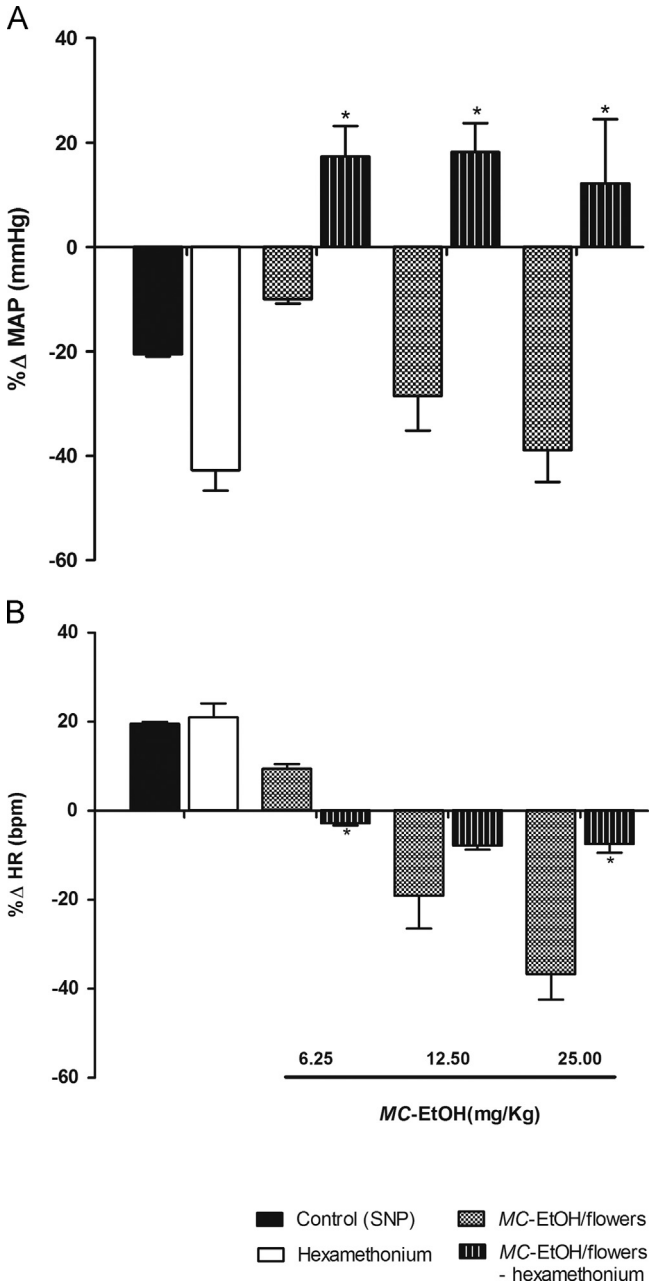

Fig. 6. Effect of MC-EtOH/flowers on mean arterial pressure (A) and heart rate (B) in non-anesthetized rats in presence and absence of hexamethonium (20 mg/kg, i.v.). Values are mean ± SEM of five experiments. *$p < 0.05$ vs MC-EtOH/flowers; Test *t*-Student.

way when compared to the vasorelaxant effect in mesenteric artery rings pre-contracted with KCl 80 mM ($pD_2 = 2.62 \pm 0.04$ μg/mL, $n = 7$, *$p < 0.05$ Fig. 11).

4.6. *Vasorelaxant effect of MC-EtOH/flowers on superior mesenteric artery rings isolated from rats in presence of the TEA 3 mM*

The vasorelaxant effect induced by the MC-EtOH/flowers in mesenteric artery rings pre-contracted with phenylephrine ($pD_2 = 1.83 \pm 0.08$ μg/mL) did not show statistical difference in relation to the rings ($pD_2 = 2.12 \pm 0.06$ μg/mL) after the pre-treatment for 30 min with TEA 3 mM, a non-selective blocker of the channels for potassium (Rocha and Bendhack, 2009) (Fig. 12).

5. Discussion

The main finding of this study was that the ethanolic extract obtained from the inflorescences of *M. caesalpiniifolia* was able to relax the superior mesenteric artery rings of normotensive rats, thus showing for the first time its vasorelaxant effect. The fingerprint thrush HPLC showed a simple profile identified flavonoids, rutin, quercetin and others (Fig. 2) might contribute for the observed vasodilator effect. The MC-EtOH/flowers administered through venous pathway induced a hypotensive effect in te three doses used (6.25, 12.5 and 25 mg/kg) followed by a bradycardia effect in the two higher doses. Aiming at assessing the participation of the muscarinic receptors in this response, atropine, a non-selective antagonist of these receptors, was used (2 mg/kg, i.v.) (Mitchelson, 1984). In such conditions, the hypotension and bradycardia were practically abolished in all doses (Fig. 5B), suggesting that the effect of the MC-EtOH/flowers on the arterial pressure of rats involves either the direct activation of the muscarinic receptors or the indirect activation by vagal activation, leading to the release

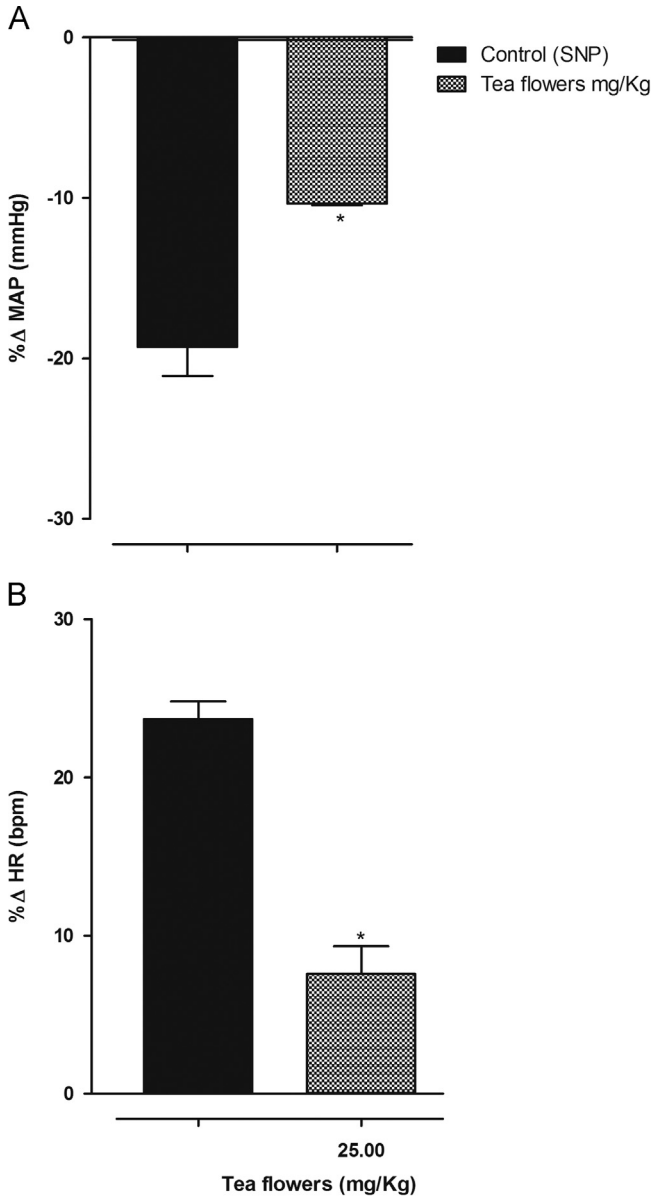

Fig. 7. Effect of (25 mg/kg) dose of tea/flowers on mean arterial pressure (A) and heart rate (B) in non-anesthetized rats. All results are expressed as mean ± SEM of at least five experiments. $^{*}p < 0.05$ vs control, Test-*t*-Student.

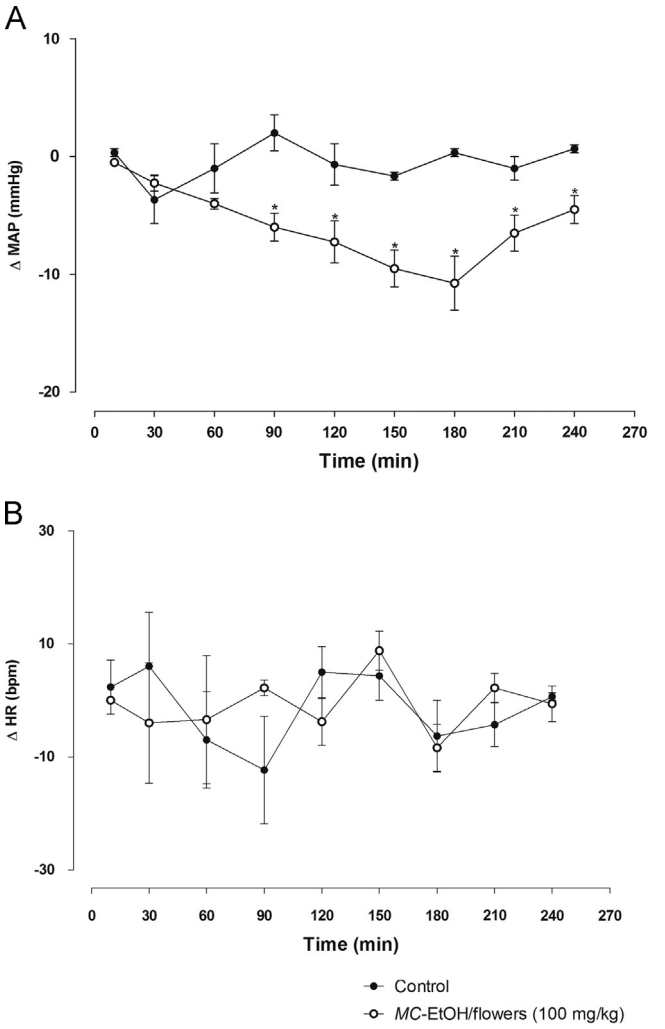

Fig. 8. Effect of (100 mg/kg) dose of MC-EtOH/flowers, orally administered on the mean arterial pressure (A) and heart rate (B) in rats. All results are expressed as mean ± SEM of at least five experiments. $^{*}p < 0.05$ vs saline, Test-*t*-Student.

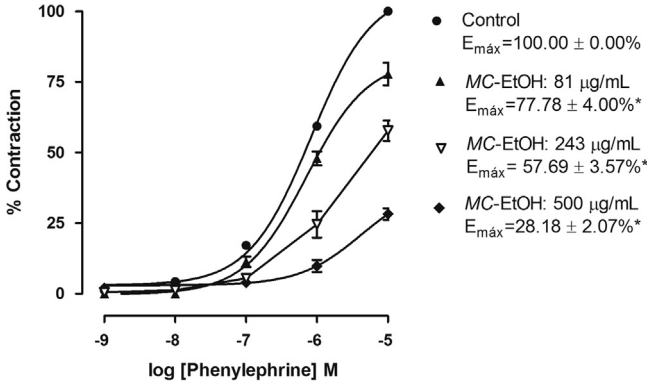

Fig. 9. Inhibitory effect of MC-EtOH/flowers (81, 243 or 500 μg/ml) on the cumulative-contraction curve induced by phenylephrine (10^{-9}–10^{-5} M) in (E-) mesenteric artery rings. Data are reported as mean ± SEM ($n=5$). $^{*}p < 0.05$ vs control, Test-*t*-Student.

of acetylcholine in the sinoatrial node and a consequent muscarinic activation (Bernardes et al., 2013).

Aiming at assessing whether the action of the MC-EtOH/flowers could be via cholinergic neuronal activation, hexamethonium, a ganglionic blocker (Borodinova et al., 2013), was used (20 mg/kg i.v.), thus reducing both the cholinergic and the adrenergic transmissions (Medeiros et al., 2006). The hypotensive effect of the MC-EtOH was completely reversed after the ganglionic blockade with hexamethonium in the three doses used with attenuation of bradycardia (Fig. 6B). According to Huangfu et al. (1992), the nucleus of the solitary tract (NTS) plays a key role in the modulation of the autonomic activity efferent to the cardiovascular system. They emit myelinated nervous fibers (C Fibers), which project to the heart, more precisely in the atrium (Cheng and Powley, 2000). The activation of the C Fibers results in bradycardia through the cholinergic stimulation and release of the P substance in the cardiac nervous terminations, and that effect can be abolished by atropine, but not by hexamethonium (Haxhiu-Poskurica et al., 1992; Jones et al., 1995), similar to what has been found in the results with the MC-EtOH/flowers. It is then suggested that a possible bradycardia effect induced by the MC-EtOH/flowers is due to an indirect activation of the cardiac muscarinic receptors. The venous administration of the tea/flowers at the same dose as the MC-EtOH/flowers promoted a decrease in the pressure. However, it induced tachycardia, highlighting the fact that there can be some substance in the tea with inotropic

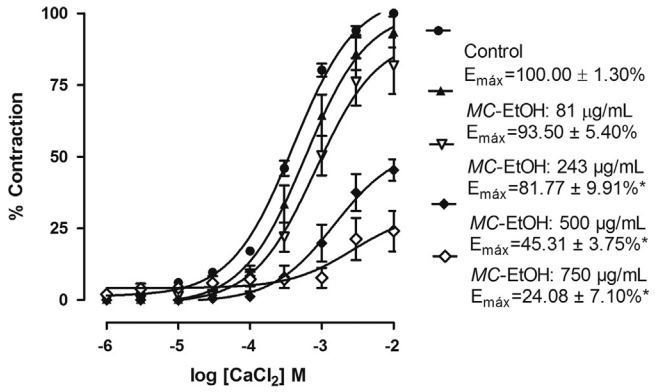

Fig. 10. Inhibitory effect of MC-EtOH/flowers (81, 243, 500 and 750 µg/mL) on $CaCl_2$-induced contractions (10^{-6}–3×10^{-2} M) in endothelium-denuded rat mesenteric artery ring in nominally without Ca^{2+} depolarizing Tyrode solution. Values are mean ± SEM from 5 experiments, $^*p < 0.05$ vs control, Test-*t*-Student.

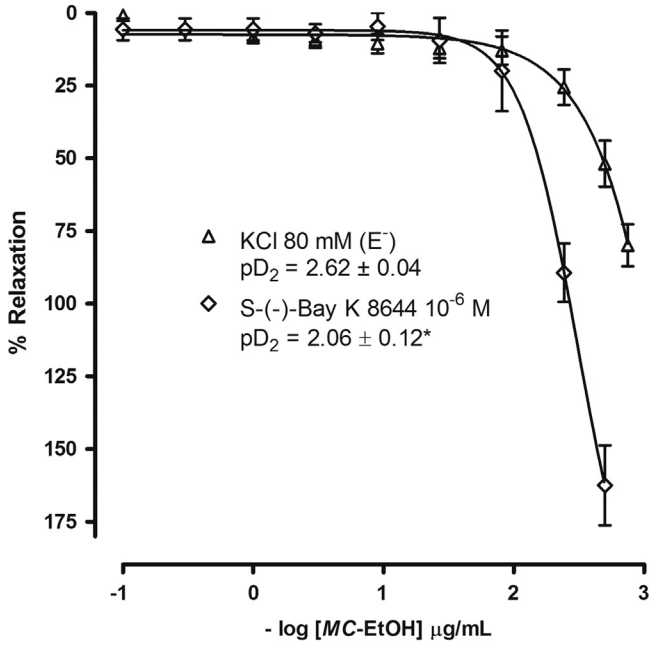

Fig. 11. Vasorelaxant effect of MC-EtOH/flowers on isolated mesenteric arteries pre-contracted of S(-)-Bay K 8644 0.1 mM (◊) or (E-) pre-contracted with KCl 80 mM (Δ). Values are mean ± SEM ($n=5$). $^*p < 0.05$ vs KCl 80 mM.

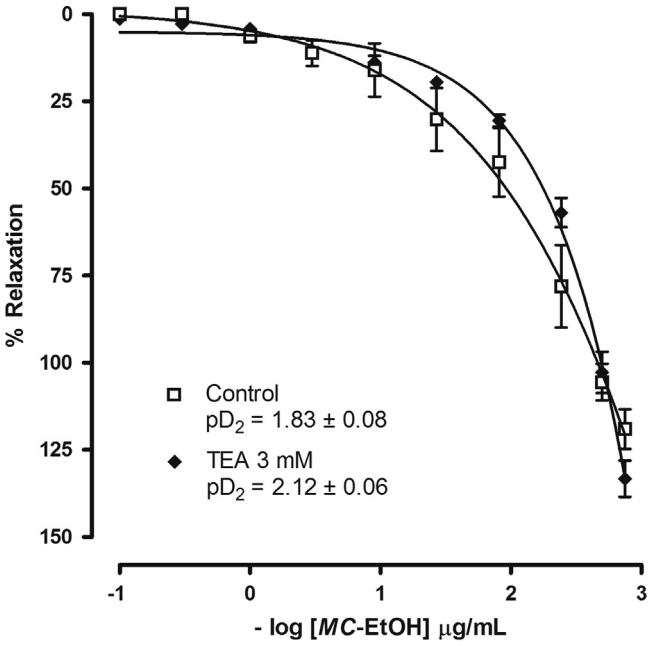

Fig. 12. Concentration–response curves for the vasorelaxant effect of MC-EtOH/flowers (0.1–750 µg/mL) on isolated mesenteric arteries (E-) pre-contracted with phenylephrine 10–5 M (□) and in the presence of TEA 3 mM (♦). Values are mean ± SEM ($n=5$).

action (Fig. 7A and B). Based on such facts and also on the popular use that these flowers have in the treatment of hypertension (Albuquerque et al., 2007), we sought to assess the effect of the oral administration in normotensive rats. This way, it was observed that the MC-EtOH/flowers promoted a reduction in the arterial pressure in the dose of 100 mg/kg after 90 min of the administration, persisting for two hours and a half, and did not cause a significant alteration in the heart rate (Fig. 8A and B, respectively). Those data are relevant because they show that the bradycardia effect caused by the MC-EtOH/flowers through venous pathway is not perceived when administered orally, suggesting then that the substance (or substances) that causes (or that cause) bradycardia present in the MC-EtOH/flowers is (or are) deactivated by the action of the first-pass metabolism, corroborating the findings in folk medicine for the treatment of hypertension. Aiming at highlighting the vasorelaxant mechanism of action that can contribute to the hypotensive response obtained, the mesenteric artery was used due to its substantial contribution for the regulation of the systemic flow, what reflects the variation of the vascular resistance (Mulvany and Aalkjaer, 1990). The MC-EtOH/flowers promoted a vasorelaxant effect dependent on the concentration and independent on the vascular endothelium in mesenteric artery rings pre-contracted with phenylephrine, an agonist of the α1-adrenergic receptors, which is bound to the $G_{q/11}$ protein. By being activated, it induces the formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) through the hydrolysis of the phosphatidylinositol 4,5-biphosphate (PIP_2) of the plasmatic membrane. The IP_3 binds to its receptor in the sarcoplasmic reticulum (RIP_3), which induces the release of calcium, thus generating a process of vascular smooth muscle contraction (Zhang et al., 2010). The contraction induced by phenylephrine is mediated by an increase in the Ca^{2+} influx through the receptor-operated calcium channels and also sensitive towards the voltage (Lee et al., 2001), whereas the contraction induced by the depolarization (KCl 80 mM) in the smooth muscle is mediated by the depolarization of the plasmatic membrane with an increase in the Ca^{2+} influx through the voltage-operated channels (Cav) (Cunha et al., 2013). In this study, the MC-EtOH/flowers inhibited the contractions induced by the cumulative addition of phenylephrine in endothelium-denuded preparations (Fig. 9), thus suggesting that the MC-EtOH/flowers probably acts on the vascular smooth muscle blocking the phenylephrine-sensitive calcium channels. The voltage-dependent calcium channels (Cav) are transmembrane proteins that supply calcium influx to a variety of intracellular activities in excitable cells. In blood vessels, this calcium input produces vasoconstriction and the Cav-blocking substances have been used in the treatment of cardiovascular disturbances (Catterall et al., 2005). The MC-EtOH/flowers inhibited the contractions induced by the cumulative addition of $CaCl_2$ in a depolarizing medium nominally without calcium (Fig. 10). Corroborating these results, the MC-EtOH/flowers promoted vasorelaxation in preparations pre-contracted with S(-)-Bay K 8644, an L-type activator of Ca^{2+} channel (Cav-L). These results suggest that the vasorelaxation induced by MC-EtOH/flowers involves the blockade of the calcium influx through the plasmatic membrane (Fig. 11) and the vasorelaxant effect observed with MC-EtOH does not seem to involve the opening of the K^+ channels, because there was no alteration in the response in the presence of TEA 3 mM, a pharmacological tool that is a nonspecific blocker of the K^+ channels in this concentration (Bernardes et al., 2013) Fig. 12. Hence, it is concluded that the inflorescences of *M. caesalpiniifolia* present hypotensive and

vasorelaxant effects involving the muscarinic pathway and blockade of the CavL, respectively, corroborating this way with the popular use of the flowers tea in the treatment of hypertension.

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