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Original Research Article

Evaluation of Cardiovascular Effects of Edible Fruits of *Syzygium cumini* Myrtaceae (L) Skeels in Rats

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Abstract

Purpose: *To evaluate the hypotensive, vasorelaxant and antihypertensive effects elicited by the hydroalcohol extract from the fruits of Syzygium cumini (EHSCF) in non-anesthetized rats.*
Methods: *The rats were anesthetized and polyethylene catheters were inserted into the lower abdominal aorta and into the inferior vena cava for blood pressure measurements and administration of drugs. After a recovery period of 24 h, EHSCF (0.5; 1; 5; 10; 20 and 30 mg/kg, i.v.) was administered in non-anesthetized rats. The mean arterial pressure and the heart rate were recorded. To investigate the effects of extract, doses EHSCF were administered after pretreatment with L-NAME, atropine, indomethacin, and hexamethonium. For measurement of isometric tension, a concentration-response curve was obtained after Phenylephrine and KCl (80 mM) pre-contractions. The bioactive extract was analyzed via mass spectrometry (MS) fingerprinting using direct electrospray ionization mass spectrometry (ESI-MS).*
Results: *EHSCF (0.5; 1; 5; 10; 20 and 30 mg/kg) induced hypotension (-15 ± 1, -14 ± 1, -15 ± 1, -13 ± 1, -11 ± 1 and -13 ± 2 %) and bradycardia (-6 ± 1, -5 ± 1, -6 ± 1, -14 ± 1, -8 ± 1 and -10 ± 2 %) in normotensive rats. These responses were attenuated by pre-treatment with L-NAME, indomethacin, hexamethonium or atropine. In phenylephrine, pre-contracted mesenteric rings, EHSCF-induced relaxation (E_{max} = 54.6 ± 4.5 % and pD2 = 2.7 ± 0.1) that were affected by endothelium removal. EHSCF caused relaxant effect of KCl (80 mM) pre-contracted rings (E_{max} = 100 ± 0.2 % and pD2 = 2.2 ± 0.1). This effect was not changed in denuded rings. A single oral administration of the extract reduced significant mean arterial pressure in spontaneously hypertensive rats. ESI-MS/MS analyses of EHSCF demonstrated that the major constituents of the analyzed samples coincided with the mass of the malic, gallic, caffeic and ferulic acids.*
Conclusion: *The results suggest that EHSCF induces hypotension probably due to a decrease in peripheral resistance, mediated by the endothelium. Bradycardia may be due to indirect cardiac muscarinic activation. The extract also causes an antihypertensive effect.*

Keywords: *Antihypertensive, Edible fruits, Hypotension, Syzygium cumini, Vasorelaxation*

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INTRODUCTION

The hypertension is the most common cardiovascular disease and is a major public health issue, affecting more than 10 % of the worldwide population [1]. The treatment of arterial hypertension with medicinal plants is well

reported in the literature [2-6].

Numerous species of Myrtaceae are used in folk medicine as diuretics and antihypertensives [3]. Biological assays have shown that many species of Myrtaceae exert hypotensive and antihypertensive action [4,5].

Syzygium cumini (L) Skeels (syn., *Eugenia jambolana* Lamk) is a plant belonging to Myrtaceae family. It is popularly known as "brinco-de-viúva" or "jamelão" in Brazil [6]. The fruits have been used as medicines in the traditional treatment of diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. Scientific reports have shown that the extracts of *S. cumini* possess cardioprotective, antioxidant, hypoglycemic, antidiabetic and antinociceptive effects [7,8].

In the literature there is report on the cardiovascular effects of *S. cumini*. For example: The aqueous extract of leaves produced hypotensive effects in anesthetized normotensive rats [6]. In anaesthetized dogs, the ethyl acetate fraction from leaves extract induced hypotension associated with bradycardia [9]. However, no study to date has investigated the cardiovascular effect of the edible fruit of *S. cumini*.

In the present study, we examined the effects of the hydroalcoholic extract of the fruits of *S. cumini* (EHSCF) on blood pressure and vascular tone in non-anesthetized rats, using *in vitro* and *in vivo* approaches.

EXPERIMENTAL

Acquisition and extraction of plant materials

The edible fruits of the plant were collected at the specimen was collected at 09:00 am, 01.05.2011, at the Campus of the Universidade Federal de Alagoas and identified by Flavia de B. P. Moura, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas. Voucher samples (no. MUFAL 4078) were deposited in the Herbarium Prof. Honório Monteiro (MUFAL), at the Museu de História Natural, Universidade Federal de Alagoas. The seeds were separated from the fruits, which were dried in an oven at 45 ºC and powdered. Due to the high content of polyphenols, namely anthocyanins in *S. cumini* fruits [10], the powder (950 g) was extracted with ethanol 90 % at room temperature in order to avoid degradation of these thermolabile compounds. The solvent was filtered and evaporated under reduced pressure and temperature (below 40 ºC), affording 38.13 g (4 % yield) of EHSCF.

Animals

Male spontaneously hypertensive rats (SHR) and Wistar rats (250 – 350 g) were used in the experiments. They were housed in conditions of controlled temperature (21 ± 1 °C) and exposed to a 12 h/12 h light/dark cycle with free access to food (Labina®, PURINA, Brazil) and tap water. All experimental procedures were carried out in accordance with the internationally accepted principles for laboratory animal use and care as contained in European Community guidelines (EEC Directive of 1986; 86/609/EEC), and approved by the Animal Ethics Committee of the Universidade Federal de Alagoas (certification no. 009715/2008-34).

Electrospray ionization mass spectrometry fingerprinting

The crude EHSCF (10.0 µg/mL) was diluted in a solution containing 50 % (v/v) chromatographic grade methanol, 50 % (v/v) deionized water, and 0.5 % of ammonium hydroxide (Merck, Darmstadt, Germany). The fingerprinting using electrospray ionization mass spectrometry analyses (ESI-MS) were performed according to Salvador [8,11]. The solutions were injected by direct infusion in the mass spectrometer using UPLC-MS equipment, model ACQUITY TQD (Waters Corporation, Milford, MA). ESI-MS and ESI-MS/MS spectra were acquired in the negative ion mode. The operating conditions were as follows: capillary voltage of 3.0 kV; source temperature of 100 °C; desolvation temperature of 100 °C, and cone voltage of 30 V. The total time for acquisition was set at 1 minute. Samples were infused by an automatic injection pump (Harvard Apparatus) with a continuous flow of 10 µL/min. The full scan spectra were acquired in the range of m/z 100 - 1000 and ESI-MS/MS spectra were acquired at collision energies of 10 - 30 eV from m/z 50 up to the m/z of the ion under study. Structural analysis of single ions in the mass spectra from the extract was performed by ESI-MS/MS. The ion with the m/z of interest was selected and submitted to 15 – 45 eV collisions with argon in the collision quadrupole. The collision gás pressure was optimized to produce extensive fragmentation of the ion under investigation. The compounds were identified by comparing their ESI-MS/MS fragmentation spectra to fragmentation spectra of authentic standard samples and literature data [8,11,12].

In vivo experiments

Normotensive rats were anaesthetized with sodium thiopental (45 mg/kg, i.p.), the lower abdominal aorta and inferior vena cava were cannulated via left femoral artery and vein using polyethylene catheters. Both catheters were filled with heparinized saline and led to exit between the scapulae. Arterial pressure was measured 24 h after surgery by connecting the arterial catheter to a pre-calibrated pressure transducer (BLPR, AECAD, SP, Brazil) and connected to a personal computer equipped with an analog to digital converter board. Using AQCAD software (AVS Projects, SP, Brazil), data were sampled every 500 Hz. The computer calculated mean arterial pressure (MAP) and heart rate (HR). The venous catheter was used for drug administration. EHSCF (0.5, 1, 5, 10, 20 and 30 mg/kg, i.v.) was randomly administered in non-anesthetized rats. To characterize pharmacologically the response of EHSCF on MAP, bolus injections of atropine (2 mg/kg, i.v.), hexamethonium (30 mg/kg, i.v.), L-NAME (20 mg/kg, i.v.) or indomethacin (3 mg/kg, i.v.) were first given followed by EHSCF.

Antihypertensive activity study of EHSCF was conducted in non-anesthetized SHR rats. The rats were randomly assigned to four groups of five rats each. Control rats (Saline group), Treated group 1: received a single oroagastric dose of 100 mg of EHSCF/kg body weight, Treated group 2: dose of 200 mg/kg and Treated group 3: dose of EHSCF 500 mg/kg. The MAP was measured according to a protocol previously described above. The MAP was recorded before and after the treatment with extract at 0, 1, 2, 4 and 6 h. Percent decrease in MAP was calculated.

In vitro experiments

Normotensive rats were killed by stunning and exsanguination in anaesthesia. The superior mesenteric artery was removed, cleaned of from connective tissue and fat. Rings (1-2 mm) were obtained and placed in physiological Tyrode's solution, maintained at 37 °C, gassed with carbogenic mixture, and maintained at pH 7.4. The stabilization period was of 1 h under a resting tension of 0.75 g. The isometric tension was recorded by a force transducer coupled to a data acquisition system (AECAD 1604, DATAQ, AVS Projects and SP). Endothelium was removed by gently rubbing the intimal surface of the vessels. The presence of functional endothelium was assessed by the ability of acetylcholine (ACh, 10 µmol/L) to induce more than 80 % relaxation of pre-contracted vessels with phenylephrine (Phe, 10 µmol/L). The absence of the relaxation to ACh was taken as evidence that the vessel segments were functionally denuded of endothelium. We studied the concentration-dependent relaxant effect of EHSCF on endothelium-intact and endothelium-denuded mesenteric rings that were pre-contracted with Phe (10 µmol/L) or 80 mM KCl. During the tonic phase of the contraction, EHSCF (1 - 1000 mg/L, cumulatively) was added to the organ bath. The relaxant effect was expressed as the percentage of Phe- or KCl-induced contraction.

Statistical analysis

All values were expressed as mean ± SEM. The results were analysed with Student's t-test, one-way ANOVA, and Bonferroni post-test. $P < 0.05$ was considered as significant. In vitro experimental results were expressed as percentage decreases in Phe-or KCl (80 mM) induced maximal contraction. Non-linear regression was used to derive the potency expressed by pD_2 (-Log EC_{50}, effective concentration that promotes 50 % response). All analysis was performed using GraphPad™ Prism 3.0 version software.

RESULTS

Electrospray ionization mass spectrometry fingerprinting

The ESI-MS fingerprints technique was used to characterize the presence of bioactive compounds in theses edible fruits. The EHSCF extract was analysed by direct insertion in the negative ion mode and this method is a sensitive and selective for the identification of polar organic compounds with acidic sites, such as the organic acids [8,11,13].

Deprotonated forms of the compounds of interest were then selected and dissociated and their ESI-MS/MS were compared to those of standards. The compounds were identified by comparison of their ESI-MS/MS fragmentation spectra with fragmentation spectra of the authentic standard samples. These analyses showed that the detected constituents in EHSCF were coincided with the mass of the phenolic acids such as: malic, gallic, caffeic and ferulic acids (Table 1, Figure 1). The investigation by direct infusion electrospray ionization mass spectrometry (ESI-MS) analyses provided important information about bioactive components present in the extract.

Table 1: Compounds identified in hydroalcohol extract of the fruits of *Syzygium cumini* using negative ion mode ESI-MS/MS

Compound	ESI-MS ions (*m/z*)		
	***Syzygium cumini* fruit hydroalcoholic extract (EHFCS)**	***Deprotonated ions [M-H]⁻ (m/z)***	***MS/MS ions (m/z)***
Malic acid	+	133	25 eV: 133→115
Gallic acid	+	169	25 eV: 169→125, 79
Caffeic acid	+	179	25 eV: 179→135
Ferulic acid	+	195	25 eV: 193→178, 149, 134

+ = detected compound

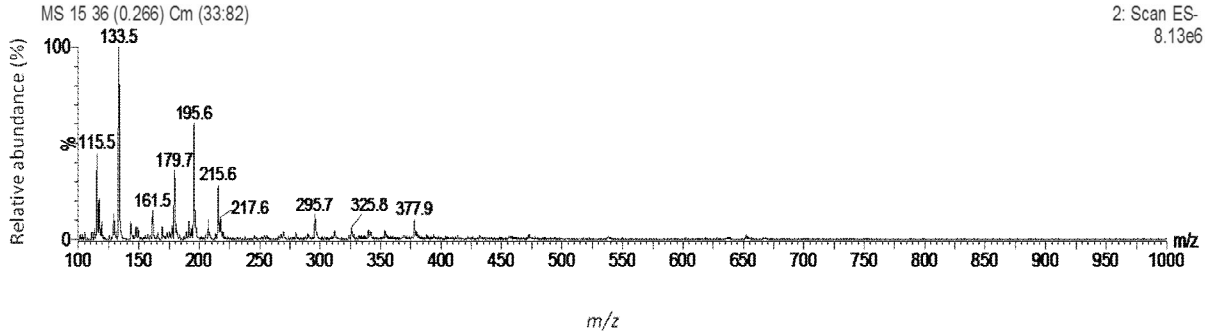

Figure 1: ESI-MS fingerprints of hydroalcoholic extract of *Syzygium cumini* fruits

Effect of EHSCF on MAP and HR in non-anesthetized normotensive rats

In these animals, intravenous EHSCF injections induced hypotension (-15.1 ± 1.4 %; -13.8 ± 1.1; -14.9 ± 1.5, -13 ± 0.9, -11.2 ± 1.5 and -13 ± 1.7 %, respectively) which were associated with bradycardia (-5.7 ± 1, -5.3 ± 1.3, -5.6 ± 0.5, -14 ± 1.5, -8.3 ± 1 and -9.6 ± 1.7%, respectively) that was not dose-dependent. The administration of vehicle produced no significant change in MAP or HR (Figure 2).

Effect of L-NAME and indomethacin on EHSCF-induced responses in non-anesthetized normotensive rats

Hypotension induced by EHSCF was attenuated significantly in 4 doses (0.5, 1, 5 and 10 mg/kg, i.v.) while bradycardic effect was reversed in tachycardia (2.3 ± 0.5, 1.3 ± 0.9, 2.4 ± 1.3, 2.5 ± 1.2, 0.1 ± 1.1 and 5 ± 0.6 %) in rats pretreated with L-NAME. In the presence of indomethacin, hypotension (-4.8 ± 0.9, -3.8 ± 0.4, -8.6 ± 0.9, -6.1 ± 0.9, -3.8 ± 0.5 and -4.6 ± 1.1 %) and bradycardia (1.6 ± 1.2, 2.1 ± 0.4, -0.9 ± 0.5, 0.3 ± 0.7, -2.0 ± 1.6 and -1.7 ± 0.8 %) induced by EHSCF were significantly attenuated (Figure 2).

Effect of atropine and hexamethonium on EHSCF-induced responses in non-anesthetized normotensive rats

The pre-treatment with atropine caused significant changes in the hypotensive and bradicardic responses in 4 doses (5, 10, 20 and 30 mg/kg, i.v.). The bradycardia was significantly reduced in rats previously treated with hexamethonium (-0.1 ± 0.5, -1.5 ± 0.9, -2,4 ± 0.9, -2.3 ± 0.7, 1.5 ± 1.5 and 3.7 ± 0.8 %, respectively), but caused significant hypotension ($p < 0.05$) at some dose levels (0.5, 5 and 10 mg/kg, i.v.) (Figure 2).

Antihypertensive effect of EHSCF in SHR rats

In the set of experiments, baseline MAP values were recorded at 0 h and considered with 100 % of activity. EHSCF produced no significant change on MAP (Group 1). After 4 and 6 h of extract administration, the MAP was significantly decreased (Treated group 2) when compared to saline group (saline group) and the Treated group 3 showed hypertensive effects (Figure 3).

Effect of EHSCF on mesenteric rings pre-contracted with Phe

Figure 4 shows that EHSCF in a concentration-dependent manner relaxed the Phe induced contraction in artery segments with intact

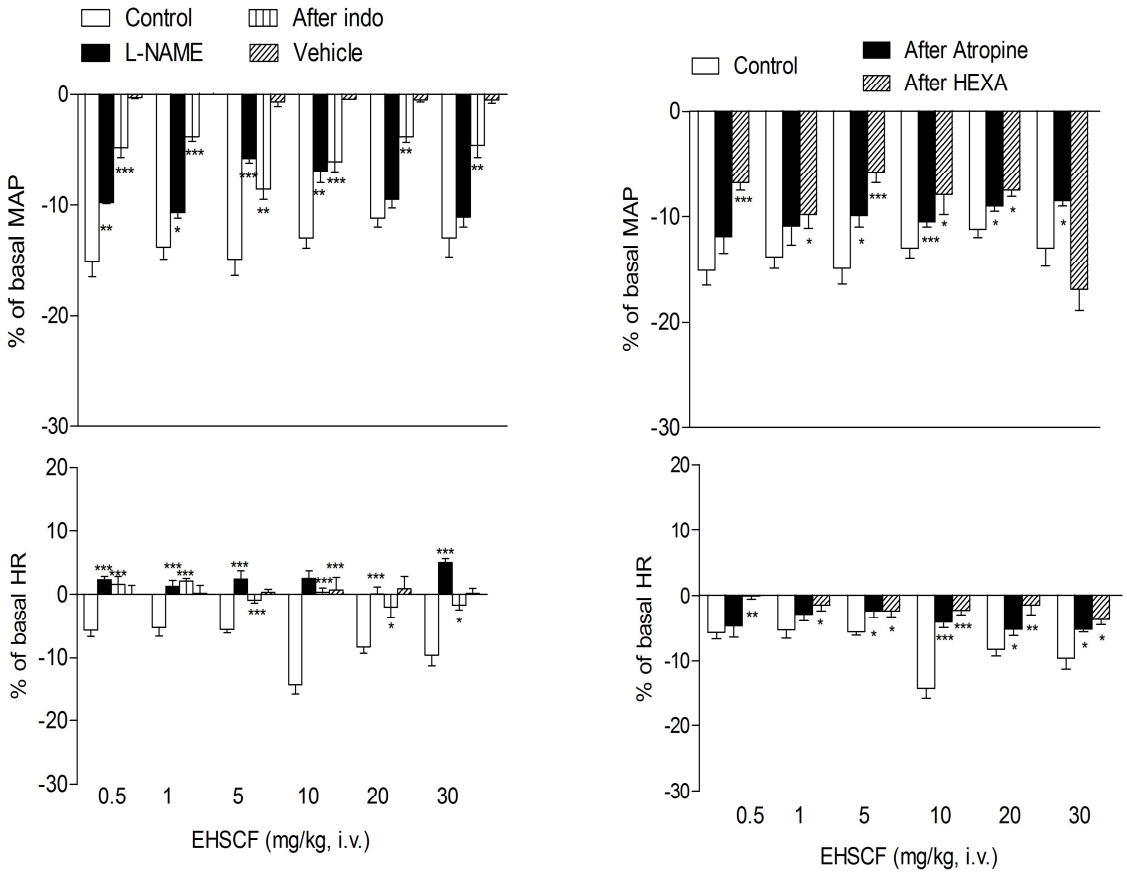

Figure 2 Effect of EHSCF on MAP and HR before administration of EHSCF, and after acute administration of L-NAME (20 mg/kg, i.v.), indomethacin (3 mg/kg, i.v.), atropine (2 mg/kg, i.v.) and hexamethonium (30 mg/kg, i.v.) in normotensive rats; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ *vs* control

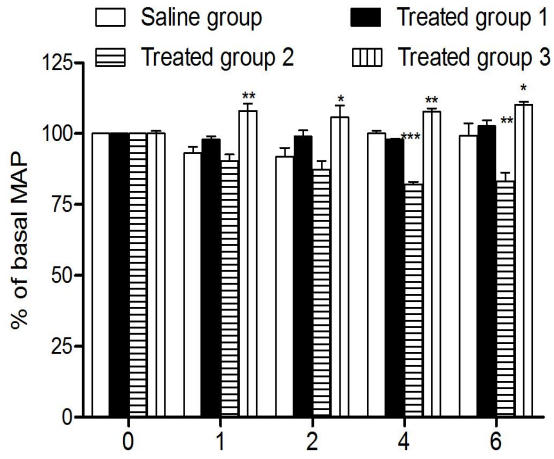

Figure 3: Effect of EHSCF on MAP after of administration of saline (Saline group) and EHSCF (100, 200 and 500 mg/kg, oroagastric administration) (Treated group) in SHR. Values are mean ± SEM (n = 5); * $p < 0.05$, and ***$p < 0.001$ *vs* saline group

endothelium {pD_2 = 2.7 ± 0.1 and maximal effect (E_{max}) = 54.6 ± 4.5 %}. In endothelium-denuded mesenteric rings, the vasorelaxant response was significantly decreased (E_{max} = 6.4 ± 0.7 %).

Effect of EHSCF on contraction induced by depolarization with high K+ concentration

In endothelium-intact rings, EHSCF inhibited the sustained tonic contraction induced by 80 mM KCl in a concentration-dependent manner (pD_2 = 2.2 ± 0.1 and E_{max} = 100 ± 0.2 %). However, the extract could not induce a vascular relaxation in endothelium-denuded rings (Figure 4). The E_{max} values found for EHSCF in endothelium-intact rings pre-contracted with KCl were significantly different from those found in phenylephrine-pre-contracted rings (E_{max} = 100.0 ± 0.2 % and E_{max} = 54.6 ± 4.5 %, respectively).

DISCUSSION

In this study, we investigated the possible cardiovascular effects produced by EHSCF, using *in vivo* and *in vitro* approaches. The anesthesia modifies the blood pressure regulation systems, generating depression of the central nervous system synapses and changing the autonomic responses [14]. To avoid anesthetic effect on hemodynamics parameters, the experiments were performed in non-

anesthetized rats. The results show that, in non-anesthetized normotensive rats, the intravenous administration of EHSCF induced hypotension associated with bradycardia.

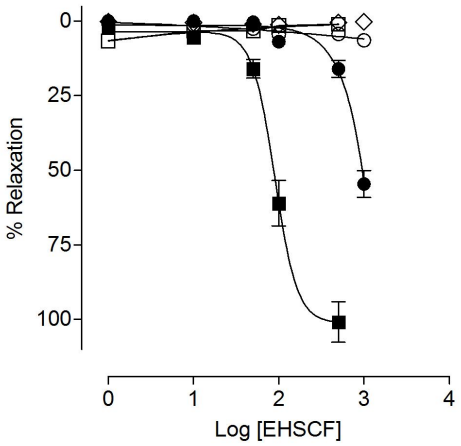

Figure 4: Effect of increasing concentrations of EHSCF (from 1 to 1000 mg/L) on phenylephrine (10 μM) and KCl (80 mM) induced contractions in rings of rat mesenteric artery; ○ = Phe without endothelium, ● = Phe with endothelium, ◊ = vehicle (distilled water), □ KCl 80 without endothelium and ■ = KCl 80 with endothelium; values are expressed as mean ± SEM

The vascular endothelium plays an important role in homeostasis by modulating vascular smooth muscle tone and regulating blood pressure. The regulation of vasodilatation by the endothelium is due to the synthesis and release of vasodilatory substances such as NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) [15].

In order to investigate the participation of the NO in hypotensive and bradycardic effects induced by EHSCF, we performed experiments in animals pre-treated with L-NAME, an inhibitor of NO synthase. Under this condition, the hypotensive response significantly changed, suggesting that NO appears to be participating in this effect. However, bradycardic response was completely reversed in tachycardia. Previous studies have shown that NO can act on brain areas involved in cardiovascular control, reducing sympathetic tone to the vessels and heart, causing hypotension and bradycardia [16]. It is well documented that NO produced negative chronotropic and inotropic effects in rat or mouse cardiac myocytes [17]. Based on this premise, we can suggest that these effects were possibly due to the action of extract-derived NO on the cardiovascular control, decreasing the blood pressure and heart rate or acting directly on the heart. Nevertheless further experiments are needed to clarify the mechanisms involved.

Prostacyclin (PGI2) is as an endothelium-derived vasodilator that, by stimulating the PGI2 receptors and activating adenylate cyclase, induces an increase in intracellular cyclic-AMP concentration thus producing smooth muscle relaxation [18]. To determine whether the hypotensive effect could involve the PGI2, we performed experiments with indomethacin, a potent nonselective COX inhibitor. In the presence of indomethacin, hypotension and bradycardia induced by extract were significantly attenuated when compared to that induced by the extract given alone, suggesting that PGI2 are involved in these effects elicited by extract.

It is well established that the primary autonomic regulations of the sinoatrial node function is by vagal action via stimulation of cardiac muscarinic receptors, since these receptors induced intense bradycardia followed by hypotension due to the decrease of the cardiac output [19], beyond decrease of the total peripheral resistance through direct activation of endothelial muscarinic receptors in vessels [15].

To evaluate the role of these receptors in the EHSCF-induced response, we performed experiments in the presence of atropine, a non-selective antagonist of muscarinic receptor. In these conditions, the hypotensive and bradycardic action of EHSCF were affected in animals pretreated with atropine. Thus, we can suggest that EHSCF would be acting, either directly in these receptors or indirectly via vagal activation, decreasing heart rate, cardiac output and consequently arterial pressure. To evaluate this hypothesis, experiments were performed with hexamethonium, a ganglionic blocker, which was able to significantly attenuate bradycardic response. The result suggests that EHSCF induced hypotension and bradycardia could be mediated by a direct via activation of cardiac muscarinic receptors and indirectly, via vagal stimulation.

Short-term systemic blood pressure control in humans and other mammals is regulated by a sophisticated multi-input and multi-output, multi-feedback system involving hormonal and neural regulations that strongly influence blood vessel and heart function. Thus, a substance that interferes with the function of arteries, veins or the heart will quickly alter blood pressure [20]. It is also important to mention that small arteries, as the superior mesenteric artery, play an important role in the determination of the peripheral resistance and in the regulation of blood pressure [21]. Based on this premise, we suggest the hypothesis that hypotensive response could be due to a decrease in

peripheral vascular resistance caused by a possible vasorelaxation.

In order to investigate this hypothesis, we performed experiments in rat-isolated superior mesenteric arteries. In mesenteric artery intact rings, EHSCF induced vasorelaxation in a concentration-dependent manner of phenylephrine-induced tonus. These results suggest that hypotensive response is due to vasodilation induced by extract. Regulation of vasodilatation by the endothelium is determined by three main components: NO, prostacyclin and endothelium derived hyperpolarizing factor (EDHF). These endothelium-derived relaxing factors diffuse to adjacent smooth muscle cells and cause them relaxation [15,17,18]. To determine whether the vasodilatory effect could involve the participation of endothelium, we performed experiments with mesenteric artery denuded rings. In this condition, the relaxant effect induced by EHSCF was changed, suggesting that the presence of the endothelium is important for vasodilator effect.

It is well known that the contractions to α1-adrenoceptor agonists, such as phenylephrine, are initiated by Ca^{2+} release from intracellular stores, which is followed by activation of Ca^{2+}-activated channels causing depolarization of the vascular smooth muscle cell membranes and activation of voltage-gated Ca^{2+} channels [22]. Whereas that high-K^+-induced contraction in smooth muscle is mediated by cell membrane depolarization and an increase in calcium influx through VOCC [23]. In intact mesenteric rings, we found that extract was able to antagonize, in a concentration-dependent manner, KCl-induced contractions. Nevertheless, in denuded mesenteric rings, the vasorelaxant effect of EHSCF was completely abolished, suggesting that the presence of the endothelium is essential for relaxant response expression. Scientific reports have shown that amplitude of the endothelium-dependent hyperpolarization of smooth muscle is related nonlinearly to extracellular K^+ ion concentration and K^+ overload has been reported to abolish the endothelium-dependent hyperpolarization [23]. The vasorelaxant effect of extract was not inhibited by 80 mM K^+ containing Tyrode solution. Thus, we can suggest that EHSCF-induced vasorelaxation could not be caused by hyperpolarization of the smooth muscle cells. Our results suggest that EDHF not participate in the vasorelaxant response induced by the extract. Based on this assumption, we can propose that prostacyclin or NO could be involved in the vasorelaxant effect induced by the extract. Nevertheless further experiments are needed to clear up the mechanisms involved.

Studies indicate that essential hypertension is genetically determined. The rats SHR are an animal model of genetically determined, thus are appropriate for verifying the antihypertensive activity of the extract. The intragastric administration of EHSCF in conscious SHR rats evoked reduction significant in MAP (four and six hours after administration) and did not alter the heart rate. It is important to mention also that EHSCF induced hypotension activity in animals with essential hypertension (SHR rats) (unpublished data). The anti- hypertensive effect of this extract could be attributed to both its vasorelaxant and hypotensive effects.

Mass spectrometry (MS) is currently the gold standard technique for the analysis of complex chemical mixtures mainly due to its unmatched ability to detect, count and characterize atoms and molecules of many types, compositions and sizes. Although previous separation is needed particularly for precise quantitation, direct MS analysis using electrospray ionization mass spectrometry (ESI-MS) of complex mixtures has been shown to provide fast and reliable characterization of complex mixtures via distinctive chemical profiles The MS fingerprinting approach is used to characterize numerous samples and shown it to provide reliable qualitative distinction [11-13, 24-26].

Phenolic acids, such as malic, gallic, caffeic and ferulic acids were detected in EHSCF using ESI-MS analysis. These results are in agreement with other reports that have demonstrated that the major acid present in the fruit of *S. cumini* is malic acid [8]. It is reported in literature that ferulic acid possess the hypotensive effect in SHR due to NO-mediated vasodilation [27]. The caffeic acid decreases the HR and blood pressure in rat [28] and promotes inhibition of contractile responses of thoracic aortic rings to phenylephrine or to KCl [29]. Based on these premises, we can suggest that the phenolic acids possess in EHSCF are possibly responsible for the hypotensive, antihypertensive and vasorelaxant effects of edible fruits of *S. cumini*. Taken together, our data show that the edible fruits of *S. cumini* could be used as a potential agent for antihypertensive treatment. Therefore, the anti-diabetic and antihypertensive activities of *S. cumini* have the most promising nutraceutical value.

CONCLUSION

The present study demonstrates that the edible fruit extract of *S. cumini* contains phenolic acids and promotes hypotensive and bradycardic effects in normotensive non-anaesthetized rats. The hypotension is due to a decrease in peripheral resistance, mediated by endothelium, and that bradycardia may be due to both indirect and direct cardiac muscarinic activation. EHSCF also shows an antihypertensive effect.

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