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Hypotensive, vasorelaxant and antihypertensive activities of the hexane extract of *Anacardium occidentale* Linn

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Abstract: *Anacardium occidentale* is widely used in folk medicine in Brazil, India, and Africa to treat inflammation, diabetes and hypertension. The aim of the study was to assess the hypotensive, vasorelaxant and antihypertensive effects produced by the hexane extract from the leaves of *A. occidentale* (HEAO) in rats. The animals were anesthetized, and polyethylene catheters were embedded into the lower abdominal aorta and the inferior vena cava for blood pressure measurements and drug administration. The HEAO was injected and the mean arterial pressure and heart rate were measured. The effects of the HEAO on isolated rat mesenteric rings with and without endothelium were investigated. HEAO induced hypotension and bradycardia in normotensive rats. After pretreatment with L-NAME, indomethacin, atropine or hexamethonium, these effects were attenuated. In mesenteric rings, HEAO antagonized the contractile effects induced by phenylephrine or KCl. This effect was inhibited after removal of the vascular endothelium. Oral administration of the HEAO decreased significant mean arterial pressure in spontaneously hypertensive rats. Mass spectrometric analysis of HEAO identified malic acid and quercetin. Our findings demonstrate that HEAO produces hypotension probably due to a reduction in peripheral vascular resistance, mediated by the endothelium, in part, by a decrease in cardiac output. Bradycardia appears to be due to the indirect activation of cardiac muscarinic receptors via activation of the vagus nerve. The HEAO also induces an antihypertensive effect.

Key words: *Anacardium occidentale*; leaves, vasorelaxant activity; hypotension; antihypertensive effect

INTRODUCTION

Hypertension is an important public health problem. It is the primary common risk factor for the development of cardiovascular disease, heart attack, heart failure, stroke and kidney diseases [1]. Cardiac complications are responsible for high rates of morbidity and mortality, causing approximately 17 million deaths per year [2]. About three-quarters of hypertensive patients on antihypertensive treatment cannot reach target blood pressure [3]. One of the leading causes of this lack of control is the low rate of adherence to antihypertensive treatments [4]. It has been reported that 13.72-16.32% of hypertensive patients are resistant to antihypertensive treatment [5]. Some of the drugs used for the treatment of this disease are ineffective and produce side effects [6].

A significant number of individuals with cardiovascular diseases, including hypertension, make use of alternative therapies, such as medicinal plants, herbal medicines and supplements [7]. Nonetheless, many herbs have not been scientifically validated [8]. Evaluation of the cardiovascular actions of the extract and description of the pathways of action remain a logical research strategy in the search for new antihypertensive drugs.

Anacardium occidentale Linn is a plant belonging to the family Anacardiaceae. It is native to Brazil and is cultivated in South America, Africa and India. It is popularly known as cashew tree [9]. This plant has been widely used in folk medicine in Brazil, India and Africa to treat inflammation, diabetes, gastrointestinal diseases and hypertension [8,10,11].

Antimicrobial, antiinflammatory, antigenotoxic, antiulcerogenic, antioxidant, hypoglycemic and larvicidal properties, as well as acetylcholinesterase inhibition of extracts from *A. occidentale* have been reported [9,12-18]. There are three scientific studies on the cardiovascular actions of this plant: the stem bark extract of *A. occidentale* induced hypotensive and cardioinhibitory effects in rats [8], the extract of *A. occidentale* leaves produced vasorelaxant action in isolated rat aorta and in the mesenteric vascular bed [19], and one report describes the process for obtaining the antihypertensive principal from the bark of this plant [20].

Based on this information, the present study was designed to examine whether the hexane extract of the leaves of *A. occidentale* (HEAO) exerts effects on the blood pressure and heart rate in nonanesthetized normotensive rats, and on the contractile responses of isolated rat superior mesenteric artery preparations; we also examined its antihypertensive properties.

MATERIALS AND METHODS

Plant material

Leaves of *A. occidentale* L. (Anacardiaceae) were collected in Maceió (Alagoas, Brazil). Botanical identification was made in the Institute of Biological and Health Sciences of the Federal University of Alagoas by Dr. Flavia de B. P. Moura. The voucher specimen was deposited in the Herbarium of Prof. Honório Monteiro, at the Museum of Natural History under the number 4081.

Preparation of plant extract

The leaves were dried (450 g) at 37°C and powdered. The obtained powder was subjected to extraction by maceration with hexane for three cycles every 48 h. The extract was dried in a rotary evaporator under reduced pressure, yielding 4.5 g of the hexane extract.

Electrospray ionization mass spectrometry fingerprinting

ESI-MS analyses were conducted as described [21]. ESI-MS was performed by direct infusion using a syringe pump, with a flow rate of 10 μL min/mL. Crude extract was diluted in a solution containing 50% (v/v) chromatographic grade methanol and 50% (v/v) deionized water and 0.5% of ammonium hydroxide. Structural analysis of single ions in the mass spectra of the extract was performed by ESI-MS/MS. Identification of chemical constituents was accomplished by comparing the ESI-MS/MS fragmentation spectra with the fragmentation spectra of the pattern samples and with literature results.

Drugs and solutions

Phenylephrine hydrochloride, acetylcholine chloride, atropine sulfate, hexamethonium, indomethacin and N^{ω}-nitro-L-arginine-methyl ester (L-NAME) were purchased from Sigma (St Louis, MO, USA), heparin sodium salt was obtained from Roche Brazil (São Paulo, Brazil) and sodium thiopental was obtained from Cristalia (Butanta, Brazil). Indomethacin was dissolved in 5% sodium bicarbonate saline solution. The HEAO was dissolved in a mixture of distilled water and 3% cremophor. The other substances were dissolved in distilled water. The composition of Tyrode's solution was: 158.3 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1.05 mM $MgCl_2$, 0.42 mM NaH_2PO_4, 10 mM $NaHCO_3$ and 5.6 mM glucose. The K^+-depolarizing solution (80 mM KCl) was prepared by replacing 80 mM KCl in Tyrode's solution with equimolar NaCl.

Animals

Male Wistar rats (250-350 g) were used to determine the vasorelaxant and hypotensive effects of HEAO. Male spontaneously hypertensive rats (SHR) (250-350 g) were employed for evaluation of the antihypertensive activity. The animals were kept under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperature (22±2°C) with free access to food (Labina®, PURINA, Brazil) and tap wa-

ter. All experiments were conducted according to the Animal Research Ethical Committee of the Federal University of Alagoas, Maceió, AL, Brazil (Certificate no.: 0106/08).

Pharmacological analyses

Direct blood pressure and heart rate measurements in nonanesthetized normotensive rats

The rats were anesthetized with sodium thiopental (45 mg/kg, i.p.) and the catheter was embedded into the abdominal aorta via the left femoral artery (for the recording of arterial blood pressure). For substances' administration, another catheter was inserted into the inferior vena cava via the left femoral vein. Both catheters were filled with heparinized saline, tunneled subcutaneously, exteriorized, and sutured at the dorsal surface of the neck. Twenty-four hours after the surgical procedures, experiments were conducted in nonanesthetized rats. The arterial catheter was connected to a pre-calibrated pressure transducer (BLPR, AECAD, SP, Brazil). The transducer was connected to an amplifier-recorder (Model 04P, AECAD, SP, Brazil) and a computer implemented with an analog-to-digital converter board. The data were sampled every 500 Hz. The AQCAD software measured mean arterial pressure (MAP) and heart rate (HR).

Effect of the HEAO on blood pressure and heart rate in nonanesthetized normotensive rats

After the period of acclimatization and stabilization of hemodynamic parameters, saline (0.9% NaCl; 1 mL/kg; i.v.) or HEAO (0.5; 1; 5; 10; 20 and 30 mg/kg; i.v.) was injected randomly in the venous catheter. Mean arterial blood pressure (MAP) and heart rate (HR) were allowed to return to the resting level between injections. Changes in MAP and HR were recognized as the difference between the steady-state values before and the lowest readings after injection. To evaluate the mechanism of the hypotensive response of HEAO, a nitric oxide synthase inhibitor (L-NAME, 20 mg/kg), a muscarinic antagonist (atropine, 2 mg/kg), a ganglionic blocker (hexamethonium, 30 mg/kg) or an inhibitor of cyclooxygenase (indomethacin, 3 mg/kg) was administered intravenously before injection of random doses of the HEAO. The data were expressed as the percentage decrease or increase in MAP and HR. Doses of antagonists were determined according to those recommended in the literature.

Antihypertensive activity

Measurements of the MAP and HR were performed in SHR rats. The rats were randomly selected and separated into three groups of five rats each. The control rats (saline group) received saline solution, and the treated groups received an intragastric dose of 100 (treated group 1) or 300 mg (treated group 2) of HEAO/kg body weight. MAP and HR were measured according to the protocol described above and were registered before and after the treatment with extract at 0, 1, 2, 4 and 6 h. The percent decreases in MAP and HR were also calculated.

Preparation of isolated normotensive rat superior mesenteric artery rings

The rats were anesthetized and killed by aortic exsanguination. The superior mesenteric artery was isolated, and the connective tissue and fat were removed. The isolated mesenteric artery was cut into rings approximately 4 mm long, placed between two stainless steel stirrups and connected to an isometric force transducer. The vasorelaxant and vasoconstrictive responses were recorded using a computerized system (AECAD 1604, DATAQ, AVS Projects and SP). The mesenteric rings were placed in isolated organ baths containing 5 mL of Tyrode's solution. The solution was maintained at pH 7.4 and gassed with 95% O_2 and 5% CO_2 at 37°C. The mesenteric rings were stabilized with a resting tension of 0.5 g for at least 60 min. In some rings, the endothelium was mechanically removed, and the removal effectiveness was demonstrated by the absence of relaxation to acetylcholine (10 μM ACh) in rings precontracted with phenylephrine (10 μM Phe). For studies in rings with endothelium, the rings were discarded when the relaxation to ACh was less than 80%.

Effect of HEAO on mesenteric rings precontracted with Phe or KCl

The ability of HEAO to cause vascular relaxation was studied in both endothelium-denuded and endothelium-intact mesenteric rings previously contracted by Phe (10 μM) or KCl (80 mM). Under the sustained contraction elicited by Phe or KCl, the vessels were exposed to increasing concentrations of HEAO (1-1000 μg/mL). The extent of relaxation was expressed as the percentage of Phe- or KCl-induced contraction.

Data analysis and statistics

The data are presented as the mean±SEM. The maximum effect (E_{max}) was considered as the maximal amplitude response reached in the concentration-effect curves. The obtained results were statistically analyzed using one-way ANOVA followed by Bonferroni's post hoc test or unpaired Student's t-test, when appropriate, and values of $P<0.05$ were considered significant. Generation of graphs and statistical analysis was performed using GraphPad Prism ver. 3.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Analysis of the chemical constituents by electrospray ionization mass spectrometry fingerprinting

The ESI-MS fingerprints technique with direct infusion was used to characterize the presence of compounds in the extract. The extract was analyzed by direct insertion in the negative ion mode. The constituents of the extract were recognized by comparison of their ESI-MS/MS fragmentation spectra with fragmentation spectra of the pattern samples and with literature results. Malic acid and quercetin were identified in the HEAO (Table 1).

Table 1. Compounds identified in the HEAO using ESI-MS/MS analyses.

	ESI-MS ions (*m/z*)	
Compound	**Deprotonated ions [M-H] *m/z***	**MS/MS ions *m/z***
Malic acid	133	15 eV: 133 →115
Quercetin	301	25 eV: 301→179, 151,121, 107
Myricetin	317	25 eV: 317
Quercetin 3-*O*-β-D-glucopyranoside	433	25 eV: 433→301, 121, 107
Quercetin 3-*O*-β-D-rhamnopyranoside	448	25 eV: 448→303, 301, 151
Myricetin 3-*O*-β-D-rhamnopyranoside	463	25 eV: 463→435, 349, 319, 317
Anacardic acid	342	25 eV: 342→314, 343, 374

Effects of HEAO on mean arterial pressure and heart in nonanesthetized normotensive rats

As shown in Fig. 1, HEAO (0.5, 1, 5, 10, 20, and 30 mg/kg i.v., randomly injected) produced significant decreases in MAP (-13±1, -11±1, -15±2, -20±4, -21±3 and -17.7±1%) and HR (-7±1, -4±1, -9±2, -17±1, -31±2 and -20±1%) in nonanesthetized rats. The administration of vehicle did not cause any significant changes to baseline MAP or HR.

Effects of L-NAME and indomethacin on HEAO-induced responses in nonanesthetized normotensive rats

The pretreatment with L-NAME (20 mg/kg, i.v.), a nitric oxide synthase inhibitor, significantly attenuated the hypotensive effect induced by HEAO (-4.4±1.1, -2.8±0.2, -3.3±0.4, -4.8±0.9, -6.4±1.2 and -4.6±1.3%). The bradycardic response evoked by HEAO was completely blocked or reversed (-0.7±0.2, 0.0±0.5, 0.1±0.9, 1.6±1.6, 0.5±0.5 and 1.4±0.4%) (Fig. 1). A similar effect was produced by the inhibitor of cyclooxygenase (indomethacin, 3 mg/kg, i.v.) on MAP (-4.0±0.3, -6.6±1.3, -7.9±0.7, -7.3±0.6, -13.8±1.0 and -13.2±0.4%) and HR (2.0±0.2, 0.7±0.6, 3.0±1.0, -1.6±0.5, -0.2±0.9 and -1.0±0.6%) (Fig. 1).

Effects of atropine and hexamethonium on HEAO-induced responses in nonanesthetized normotensive rats

The bradycardic effect of HEAO was completely inhibited in animals previously treated with hexamethonium (a ganglionic blocker; 30 mg/kg, i.v) (-0.7±0.2, 0.7±0.1, 0.0±0.6, 0.3±0.3, -1.0±0.5 and -0.5±0.3%), whereas the hypotensive action was significantly reduced by doses of 0.5, 1.0 and 10 mg/kg (-4.5±1.1, -5.6±2.4 and -12.4±0.6%, respectively) (Fig. 1). The

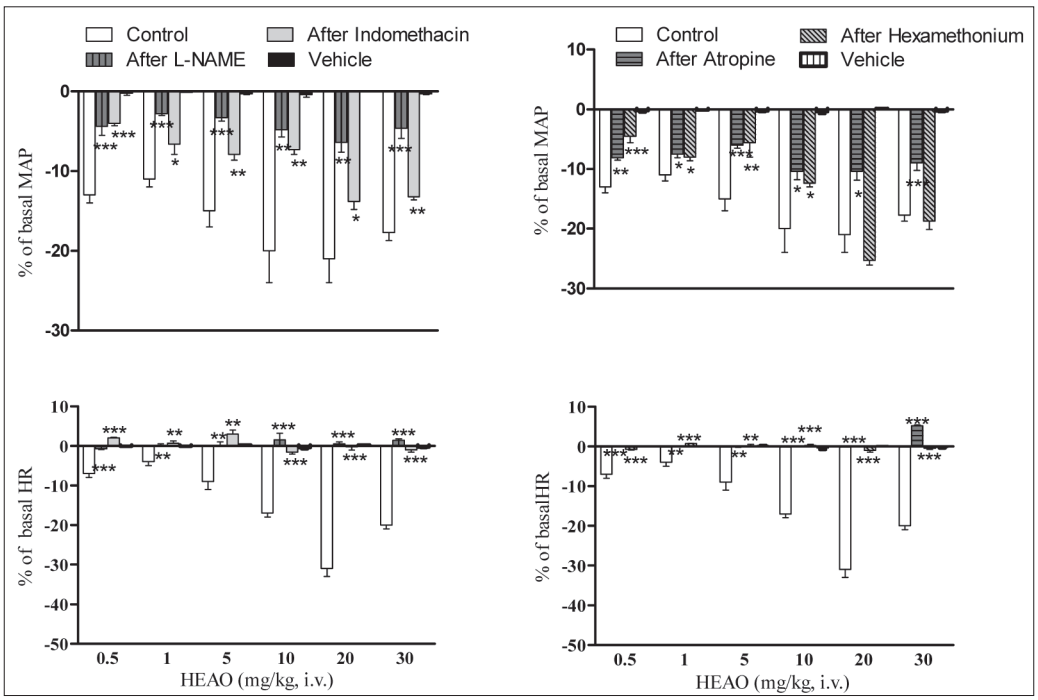

Fig. 1. Effect of HEAO on MAP and HR before administration of HEAO and after acute administration of L-NAME (20 mg/kg, i.v.), indomethacin (3 mg/kg, i.v.), atropine (2 mg/kg, i.v.) or hexamethonium (30 mg/kg, i.v.) in normotensive rats; *p<0.05, **p<0.01 and ***p<0.001 vs. control. Control – effect of the extract without the blockers; vehicle – mixture of distilled water and 3% cremophor.

pretreatment with atropine (2 mg/kg, i.v., a muscarinic antagonist) significantly attenuated the hypotensive effect induced by HEAO (-8.1±0.4, -7.5±0.6, -6.0±0.5, -10.4±1.4, -10.4±1.5 and -9.0±1.2%). Similarly, the bradycardic response was completely blocked or reversed (0.0±0.1, 0.0±0.1, -0.1±0.1, 0.0±0.1, -0.1±0.0 and 5.2±0.2%) (Fig. 1).

Antihypertensive effect of HEAO in SHR rats

The values of MAP and HR before administration of the extract were taken as 100% (0 h) of activity. After 2 h of oral administration of HEAO, the MAP was significantly decreased when compared with saline control extract administration (Fig. 2). There were no significant changes in HR in either group analyzed in SHR.

Effect of HEAO on the contraction induced by phenylephrine or KCl

As shown in Fig. 3, HEAO relaxed Phe (10 μM)- and KCl (80 mM)-precontracted mesenteric rings with endothelium, in a dose-dependent manner (E_{max}=55.1±3.1% and E_{max}=37.9±2.3%, respectively). The E_{max} values for the relaxant effect of HEAO in endothelium-intact and endothelium-denuded rings that were precontracted with Phe were significantly different (55.1±3.1% and 13.7±5.3%, respectively). In rings precontracted with KCl (80 mM), the absence of the endothelium abolished HEAO-induced vasorelaxation. The E_{max} values established for HEAO in endothelium-intact rings precontracted with KCl were significantly different from those observed in Phe-precontracted rings.

DISCUSSION

We investigated the possible cardiovascular effects produced by HEAO using *in vivo* and *in vitro* approaches. Previous research has shown that anesthesia modifies the principal systems of regulation of blood pressure, such as the sympathetic nervous and baroreflex [22-24]. Anesthesia also induces synaptic depression in the central nervous system and changes the autonomic responses [25,26]. Thus, all *in vivo* experiments were conducted in conscious and freely moving animals to

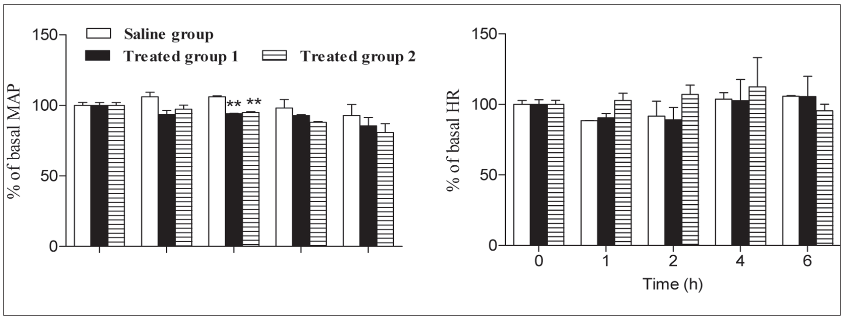

Fig. 2. Effect of HEAO on MAP and HR after administration of saline (Saline group) and HEAO (100 and 300 mg/kg, orogastric administration) (Treated group) in SHR. Values are mean±SEM (n=5); $^{**}p<0.01$ vs. the Saline group.

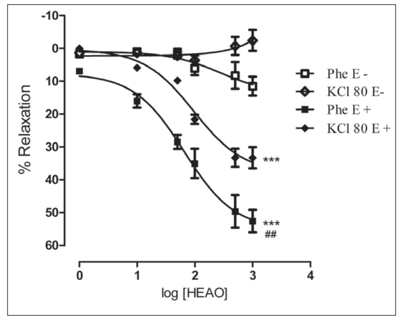

Fig. 3. Vasorelaxant effect of HEAO in superior mesenteric artery rings. Values are expressed as the mean±SEM of five experiments. $^{***}p<0.001$ vs. Phe or KCl E-; ##$p<0.01$ vs. contracted by KCl (80 mM) E+. E- – endothelium denuded; E+– endothelium intact.

avoid a direct impact of anesthesia and surgical stress on the analyzed cardiovascular parameters. Our results demonstrated that in nonanesthetized normotensive rats, the intravenous administration of HEAO induced hypotension and bradycardia.

The endothelium plays a significant role in modulating vascular smooth muscle (VSM) tone and regulating blood pressure through the release of a variety of vasoactive factors, including nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) and cyclooxygenase metabolites of arachidonic acid, such as prostacyclin (PGI_2) and prostaglandins (PGs) [27].

To investigate the participation of NO in hypotensive and bradycardic effects induced by HEAO, we conducted experiments in animals pretreated with L-NAME, an inhibitor of NO synthase. Thus, in the presence of L-NAME, the hypotension produced by HEAO was attenuated, and the bradycardia was blocked or reversed, implying that NO seems to participate in these effects. Previous studies showed that NO can reduce sympathetic tone, producing hypotension and bradycardia [28]. NO also promotes negative chronotropic and inotropic effects in the rat [29]. We suggest that NO is involved in these responses by decreasing blood pressure and heart rate or by acting directly on the heart. Nonetheless, further experiments are needed to clarify the mechanisms involved.

PGI_2 is as an endothelium-derived vasodilator that, by stimulating the PGI_2 receptors and activating adenylate cyclase, induces an increase in intracellular cyclic-AMP concentration, producing smooth muscle relaxation [30]. Indomethacin, a nonselective cyclooxygenase inhibitor, was used to evaluate the involvement of vasodilator prostanoids in HEAO-induced responses. This inhibitor significantly reduced HEAO-elicited hypotension and abolished or reversed the bradycardic response. These results suggest that cyclooxygenase metabolites are involved in the effect elicited by the extract (probably PGI_2).

It has been reported in the literature that PGE_2 plays a dynamic role in the regulation of blood pressure homeostasis, exerting a pressor response when EP1 and EP3 receptors are activated, while EP2 and EP4 receptors mediate the depressor response [31, 32]. Thus, the development of new pharmacologic agents targeting specific PGE_2 receptors could reduce blood pressure and provide end-organ protection for the treatment of hypertension.

Preclinical trials have reported promising effects of *A. occidentale* in modulating the inflammatory response, probably by reduction in PGE_2 levels [33]. Thus, we also suggest that the prostaglandin pathway is involved in the hypotensive response induced by the extract, probably by the reduction in the PGE_2 levels and by decreasing the pressor response. However, more pharmacological studies will be needed to clarify the mechanism(s) responsible for the hypotensive and antihypertensive action.

The primary autonomic regulation of the sinoatrial node function is through the vagal response via stimulation of cardiac muscarinic receptors. These activated receptors produce excessive bradycardia, a decrease in the cardiac output, followed by hypotension [34]. Therefore, to evaluate the role of muscarinic receptors in hypotensive and bradycardic responses induced by HEAO, we performed experiments in animals pretreated with atropine, a nonselective antagonist of these receptors. Under this treatment, the hypotensive response was significantly changed. The depressor effects of the extract on heart rates were abolished or reversed, suggesting that cardiac muscarinic receptors participate in this effect. Accordingly, we suggest that HEAO acts either directly on these receptors or indirectly via vagal activation.

This hypothesis was investigated using a ganglionic blockade with hexamethonium. Under this treatment, the bradycardic effect was abolished, and the hypotensive response was attenuated, suggesting that most of the HEAO-induced bradycardic effect was due to the indirect activation of muscarinic cardiac receptors, and that hypotension is partly due to a fall in cardiac output as a result of severe bradycardia.

Several physiological mechanisms are involved in short-term blood pressure control, such as neural and hormonal regulatory mechanisms. These regulatory mechanisms significantly influence the blood vessels and heart. Consequently, a drug that intervenes with the function of arteries, veins or the heart will immediately modify blood pressure [35]. The superior mesenteric artery is a small-caliber artery. It plays a significant role in the determination of peripheral resistance and regulation of blood pressure [36]. To better understand and confirm the hypotensive effect induced in *in vivo* experiments, we used an *in vitro* approach with isolated superior mesenteric arteries. In mesenteric rings precontracted with Phe, the HEAO produced a relaxant effect on endothelium-intact rings in a concentration-dependent manner.

The vascular endothelium performs a crucial role in the control and maintenance of cardiovascular homeostasis, which is achieved by production of biochemical mediators [30]. To verify whether the vasorelaxant effect could involve the participation of the vascular endothelium, we conducted experiments with mesenteric rings without the endothelium. In this state, the relaxant effect induced by HEAO was altered, suggesting that the presence of the endothelium is essential for the vasodilator effect. Results from these *in vitro* experiments support the effects observed *in vivo*, as the hypotensive and bradycardic effects induced by the extract involved the possible participation of the endothelium. It is important to mention that our findings corroborate evidence from previous studies demonstrating that the extract of *A. occidentale* leaves produced a vasorelaxant effect in aortic rings [19].

Elevation of K^+ concentration in smooth muscle cells promotes membrane depolarization by voltage-gated Ca^{2+} channels (Cav) opening and a consequent increase in Ca^{2+} influx in the intracellular medium, causing a sustained contraction [37]. The present study demonstrated that the HEAO was capable of antagonizing, in a concentration-dependent manner, KCl-induced contractions in intact mesenteric rings. However, in denuded mesenteric rings, the vasorelaxant effect of the HEAO was wholly abolished, suggesting that the presence of the endothelium is crucial for relaxant response expression.

The Phe-induced vasoconstriction is mediated by the stimulation of G-protein linked to adrenoceptors, while the KCl-induced vasoconstriction is mediated by activation of voltage-dependent calcium channels and following the release of Ca from the sarcoplasmic reticulum [38]. In intact mesenteric rings, we determined that HEAO was capable of antagonizing, in a concentration-dependent manner, KCl- and Phe-induced contractions with lower effectiveness for rings precontracted with KCl. Since HEAO relaxed mesenteric rings that were precontracted with both agonists, we suggest that the HEAO blocks Ca^{2+} influx by interfering with both voltage- and receptor-operated channels.

The SHR model is similar to hypertension in humans [39]. This model is widely used for the screening of new antihypertensive drugs. It provides a similar pattern of blood pressure decrease as in hypertensive humans [40]. Thus, we reasoned that the SHR was a relevant model to investigate the effect of HEAO on hypertension. The intragastric administration of HEAO in nonanesthetized SHR elicited a significant reduction in MAP (2 h after administration) and did not change the HR. The antihypertensive effect of this

extract could be assigned to both its vasorelaxant and hypotensive effects.

The compounds identified in the HEAO were malic acid and quercetin. Preliminary qualitative chemical tests show the presence of alkaloids, saponins and polyphenols [41]. Our results are in agreement with other published studies that demonstrated the presence of a mixture of quercetin in the hexane extract of the leaves of the plant [42]. Previous research has shown that quercetin is capable of exerting endothelium-dependent vasodilation by increased NO and by decreasing blood pressure [43,44]. The literature reports that quercetin shows its antihypertensive effects by modification of multiple factors controlling blood pressure [45]. It was reported that malic acid caused hypotensive activity in normotensive Sprague-Dawley rats [46]. Based on this, we propose that these compounds probably contributed to the cardiovascular effects induced by the hexane extract.

CONCLUSION

HEAO induces hypotensive and bradycardic effects in normotensive nonanesthetized rats. The hypotensive effect is probably due to a reduction in peripheral vascular resistance, mediated by the endothelium, and in part by a decrease in cardiac output. The bradycardic effect appears to be due to an indirect cardiac muscarinic activation through the vagus nerve. HEAO also exhibits an antihypertensive effect. Further experiments are necessary to elucidate the underlying mechanisms responsible for these responses.

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Author contributions: Êurica Ribeiro and Cintia da Costa conceived and designed the study. Cintia da Costa, Edla Herculano, Jessyka Silva, Emanuel Paulino and Alessandro Bernardino performed the *in vitro* and *in vivo* experiments. João Araújo-Júnior, Antônio Sant'Ana and Marcos J. Salvador performed the chemical experiments. Êurica Ribeiro and Cintia da Costa wrote the paper. All authors read and approved the final manuscript.

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