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Cardiovascular effects of the *Aspidosperma macrocarpum* leaves ethanol extract in rats

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Abstract

The cardiovascular activity of *Aspidosperma macrocarpum* leaves ethanol extract (AMLEE) was evaluated. In non-anesthetized normotensive rats showed hypotensive response without significant alterations in heart rate. AMLEE (1, 5, 10 and 20 mg/kg, iv) induced hypotension that was completely abolished after atropine (2 mg / kg, iv). The extract also produced in vitro relaxant effect on isolated mesenteric artery endothelium-intact rings. EEAM (200mg/kg, v.o.) promoted an antihypertensive activity. In conclusion, our results suggest that cardiovascular responses could be mediated by the endothelial muscarinic receptors.

Key words: Cardiovascular Effects, Aspidosperma macrocarpum Mart., Hypotension.

Introduction

Hypertension is one important public health problems affecting more than 10% of the worldwide population (1). It is the most common risk factor for the development of cardiovascular disease, heart attack, heart failure, stroke, and kidney diseases (2). Non-adherence to medication for systemic high blood pressure (HBP) is one of the leading current causes of the lack of control of hypertension and has implications on morbidity and mortality (3).

The use of alternative therapies, herbs, and supplements occurs at a very high rate among patients with cardiovascular disease including hypertension (4). The World Health Organisation have significant interest in complimentary health systems, perhaps due to the integral part played by ethnomedicinal plants in folkloric healthcare (5). A considerable part of the population in developing countries, use folk medicines for their daily healthcare. Some of the traditional medicine involves the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects. In contrast to most drugs, there is a paucity of information on the mechanism of action by which most natural products exert a biological effect. It is also an impediment to sound clinical practice (6).

In this context, *Aspidosperma* is a genus distributed almost exclusively in the Americas. In Brazil it is represented by about 52 species (7). These genus is characterized by the occurrence of indole alkaloids. Taking into account the various biological activities attributed to these alkaloids. As the antimicrobial and anti-leishmanial activity of alkaloids from stem bark and bark of *A. ramiflorum* (8). Antimalarial activity isolated from *A. nitidum and A. excelsum* and hypotensive effects of the *A. quebracho-blanco* and *A.pyrifolium* attributed to alkaloids from bark, stem bark and leaves, respectively (9).

However no reports studies with hypotensive effects of *Aspidosperma macrocarpum* leaves ethanol extract (AMLEE). Therefore, the aim of the present study was to investigate the acute cardiovascular effects of ethanolic extract of the *Aspidosperma macrocarpum* leaves ethanol extract in rats.

Materials and Methods

Plant collection and extraction

The plant was collected in Planaltina (GO), Brazil, in November 2007 and identified by Prof. José Elias de Paula, University of Brasilia (UnB). Voucher samples (N° JEP 3767) are deposited in the Herbarium of University of Brasilia (UnB). The dried and powdered stem bark (4 Kg), twigs (1.2 Kg), stem (3.5 Kg) and leaves (1.6 Kg) were extracted with ethanol 95% (3 x 6 L each) at room temperature (27 ± 1 °C). The ethanolic solutions were filtered and evaporated under reduced pressure and temperature (below 40 °C), affording 507g, 187.1g, 560g and 436g, respectively, of crude ethanolic extract.

Drugs

The following drugs were used: sodium thiopental, atropine sulphate, N^G-nitro-L-arginine-methyl ester (L-NAME), sodium nitropussiate (NPS), phenylephrine hydrochloride, acetylcholine chloride. All of the Sigma Chemical Co. The other drugs were dissolved in distilled water for *in vitro* experiments and saline solution for *in vivo* experiments.

Animals

Male Wistar and SHR weighing 250 to 350 g were obtained from our local colonies maintained at the Experimental Sciences Nucleus, Federal University of Alagoas (UFAL), Maceió, Brazil. They were kept under conditions of constant temperature (21 ± 2 °C) with a 12-h light–dark cycle and free access to food and water. The experiments performed to the Guide for care and Use of Laboratory Animals published by the UK Animals (Scientific Procedures) Act 1986 and to the directives 609/86 EEC.

Measurement of mean arterial pressure and heart rate

Rats were anesthetized intraperitoneally (i.p.) using sodium thiopental (45 mg/kg) and catheters (PE-10 fused to PE-50) was inserted into the abdominal aorta (for the recording of arterial blood pressure) and in the inferior vena cava (for drug administration) via the left femoral artery and vein, respectively. Both catheters were filled with heparinized saline (1: 10 v/v) and led under the skin to exit between the scapulae. After catheterization, rats were placed individually in plastic cages and 24 hours later, the arterial catheter was connected to a pre-calibrated pressure transducer (BLPR, AECAD, SP, Brazil). The transducer was fed to an amplifier-recorder (Model 04P, AECAD, SP, Brazil) and 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. For each cardiac cycle, the computer calculated mean blood pressure (MBP) and pulse interval (referred to as heart rate, HR).

Hypotensive effects of AMLEE

Before each experiment, cardiovascular parameters had stabilized and NPS (10 mg/kg) was injected to check the efficacy the insert venous catheter. Different doses of AMLEE (1, 5, 10 nad 20 mg/kg, i.v.) were administered randomly and the responses were recorded, MBP and heart rate (HR) were first allowed to return to their baseline levels, obtained before the first injection of the extract. In order to investigate the effects of extract, doses AMLEE were administered after pretreatment with muscarinic antagonist (atropine, 2 mg/kg, i.v.).

Antihypertensive activity

Antihypertensive activity study of AMLEE was conducted according to (11) in SHR and rats. Animals were allotted into three groups (five animals each). Negative control (Saline group), treated SHR with single dose of AMLEE (200 mg/kg, v.o.) (Treated group) and a group treated SHR with single dose of nifedipine (3 mg/kg, v.o.) (Positive control group). Measurements (blood pressure and heart rate) were recorded before and after the treatment of test compound at 0, 1, 2, 4 and 6 h by a tail cuffmethod using a LE 5007 automatic blood pressure computer (Letica, PanLab, Barcelona, Spain). Percent decrease in HR and MBP was calculated.

Preparation of rat superior mesenteric artery rings

Male Wistar rats were anaesthetized and killed by aortic exsanguination, the superior mesenteric arteries were quickly removed, cleaned of adherent connective tissues and cut into rings (2–4 mm length) and suspended by cotton threads in organ baths containing 5 ml of Tyrode's solution. Rings were stabilized with a resting tension of 0.5 g for at least 60 minutes. During this time, the solution was changed (every 15 min) to prevent the accumulation of metabolites that could otherwise lead to misinterpretation of results. The isometric tension was recorded by a force transducer coupled to a data acquisition system (AECAD 1604, DATAQ, AVS Projects and SP). When necessary, the endothelium was removed by mechanically by gently rolling the lumen of the vessel on a thin wire. The endothelium integrity was verified by relaxation to ACh (100 ìmol/L) in rings precontracted by Phe (100 ìmol/L). In endothelium-intact studies, preparations were discarded when ACh-induced relaxation was lower than 70% .

Vasorelaxant activity of the AMLEE

After 60 min stabilization period, Phe (100 ìmol/L)-induced pre-contractions was elicited on endothelium-intact rings to promote similar magnitude contractions. Thereby, AMLEE was added cumulatively (0.1 to 300 mg/L) after response to Phe had stabilized, approximately 40 min later, in on endothelium-intact rings.

Statistical analysis

All values were expressed as mean ± S.E.M. Student's t-test and ANOVA-one way Bonferroni post-test were used in the data analysis and results were considered significant when $p < 0.05$. In vitro experimental results were expressed as percentage decreases in Phe-induced maximal contraction. All analysis was performed using GraphPad™ Prism software, version 5.0.

Results

Hypotensive effects of the AMLEE

In conscious, unrestrained rats, AMLEE (1, 5, 10 and 20 mg/kg, i.v., randomly) produced a significant ($p < 0.05$) and non dependent-dose hypotension (-12.6 ± 4.1%; -10.4 ± 2.3%; -12.2 ± 1.3% and -16.6 ± 3% respectively. The AMLEE not was effect in the heart rate. (**Figure 1**). There was no significant change in either MBP or HR after the i.v. administration of vehicle.

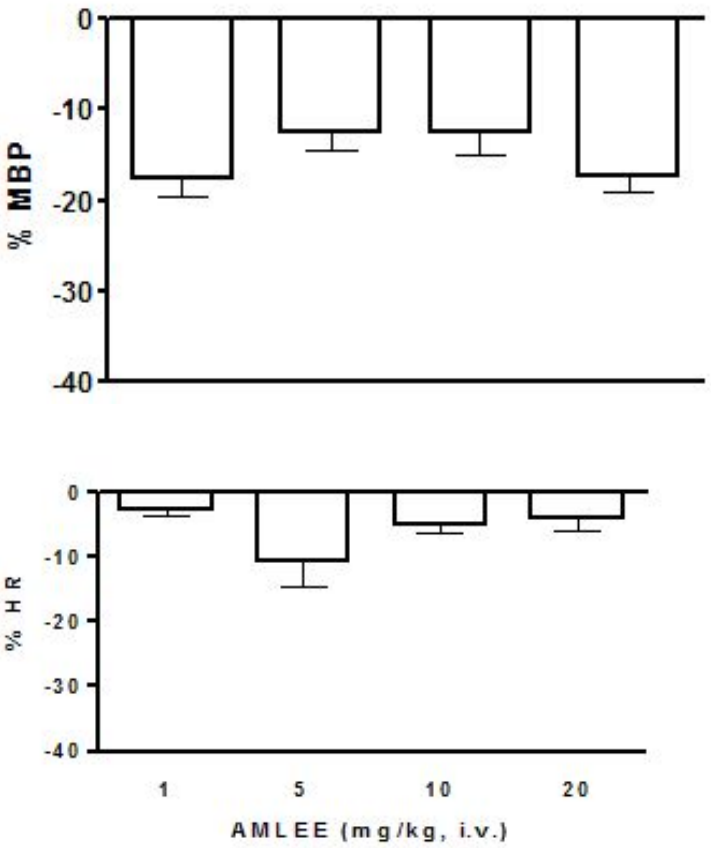


Figure 1: Effects on MBP and HR after acute administration of MALEE (0.5; 1; 5; 10 and 20 mk/kg, i.v.) in wistar. Values are mean ± SEM of five experiments.

Effect of atropine in the hypotensive effect

After pretreatment with atropine the hypotension was completely reversed (16.7 ± 7.7%; 20.3 ± 5.6%; 19.5 ± 3.8% and 35 ± 0.8%, respectively). (**Figure 2**).

Effect of AMLLE on Phe-induced pre-contractions

In rat endothelium-intact superior mesenteric artery rings, the cumulative AMLEE (0.1 to 300 µg/mL) additions induced a vasorelaxant response on Phe induced pre-contractions in a concentration-dependent manner and in a similar way inducing a maximal relaxant effect of 59.1 5.16 (Figure 3).

Antihypertensive effects of AMLEE

Baseline HR and mean blood pressure before administration were recorded at 0 h and were considered with 100% of activity. After 2h of AMLEE administration in SHR we observed a significant decrease in MBP, without changed HR compared saline group. Nevertheless, after this time elapsed the antihypertensive effect of extract was modified and returned to basal (**Figure 4**).

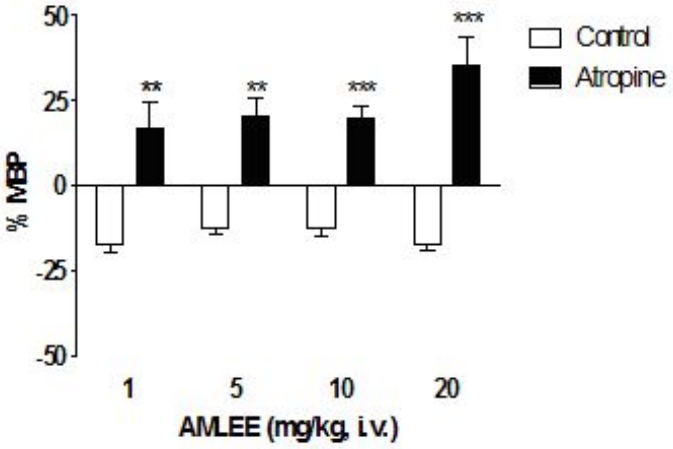


Figure 2: Effects of AMLEE on MBP and HR before (control) and after acute administration of Atropine (2 mg/kg, i.v.) in SHR. Values are mean ± SEM of five experiments. ** p < 0.01 and *** p < 0.001 vs control.

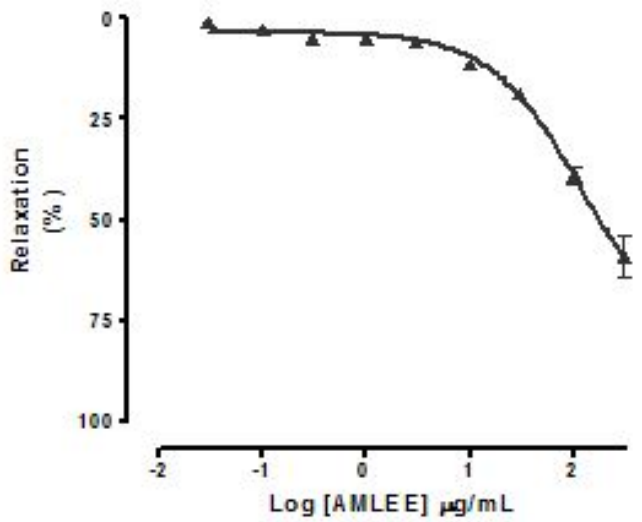
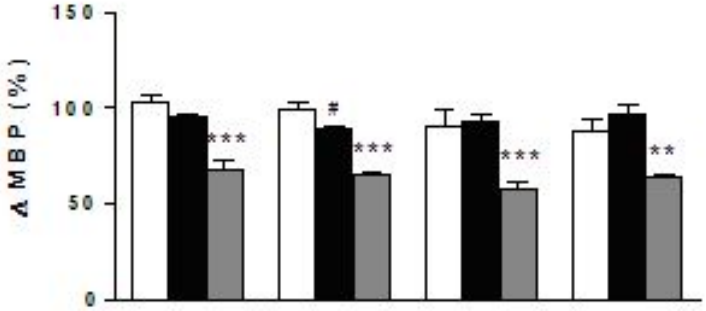


Figure 3: Concentration-response curves to AMLEE (0,1 - 300 ìg/ml) in rat endothelium-intact mesenteric rings rat pre-contracted with 100 ìM Phe (ï'5e). Values are expressed as mean ±SEM, n=6.



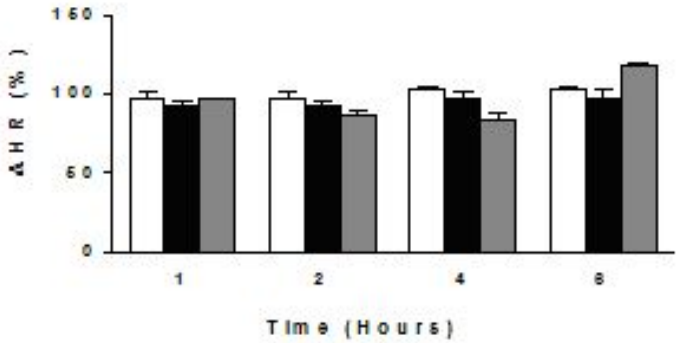
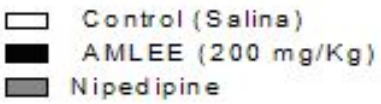


Figure 4: Effect of oral administration of AMLEE 200 mg/kg, v.o. and nifedipine (3mg/kg, v.o.) on MBP and HRin SHRs non-anesthetized at 1, 2, 4 and 6 h after treatment. Values are expressed as mean ± e.p.m. # p< 0.05 vs control. ** p < 0.01 and *** P <0.001 vs. control.

Discussion

The findings of this study indicate that acute intravenous administration of AMLEE in normotensive rats Wistar possesses hypotensive properties. The in vivo reduction in blood pressure by the extract occurred without significant alterations in heart rate, possibly suggesting that the in vitro vasorelaxation effect of extract significantly contributed to its hypotensive effects.

As established in the literature, the vascular tone of the arterial bed underlies the maintenance of peripheral resistance in the circulation and it is the major contributor to the control of blood pressure (11). In most vascular beds the stimulation of muscarinic receptors M_3in the endothelial cells induces vasorelaxation by the release of endothelium-derived vascular relaxant factors (EDRFs), including nitric oxide, cyclooxygenase metabolites and endothelium-derived hyperpolarizant factors, and consequently vasodilation and hypotension (12).Therefore, to evaluate the role of muscarinic receptors in hypotensive responses induced by AMLEE, we performed experiments in animals pre-treated with



atropine, a non-selective antagonist of these receptors. Under this condition, the hypotensive responses were significantly change, the depressor effects of extract on blood pressure were completely reversed, suggesting that AMLEE act via muscarinic receptor activation.

The present study also demonstrated that the extract had in vitro relaxant effect on isolated mesenteric artery endothelium-intact rings in a concentration-dependent manner of phenylephrine-induced tonus. It could be suggested that the BP lowering effect of the plant is owed to relaxation of the vascular system.

SHRs are widely used as an animal model for the study of essential hypertension in human, to measure an antihypertensive effect SHR rats have been used. The administration of a single intragastric dose of 200 mg/kg AMLEE in conscious and freely moving SHR rats, signi□'3fcantly decreased MBP (only, 2 h) compared with that of vehicle-treated SHRs. The HR was not signi□'3fcantly different compared with vehicle-treated group. It could be suggested that antihypertensive effect of the plant is owed to relaxation of the vascular system (13). Nifedipine, a voltage operated calcium channel (VOCC) L-type (dihydropyridine sensitive) blocker, was used as a positive control.

In conclusion, the results obtained so far show that the AMLEE induces hypotensive effect, which may be due to stimulation of endothelial muscarinic receptors, as well as, induced in vitro vasorelaxation effect. Moreover, the extract produced antihypertensive effect SHR rats probably owed to relaxation of the vascular system. However, further experiments are clearly needed to elucidate the underlying mechanisms responsible for these responses.

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