

Evaluation of cardiovascular response induced by ethanolic extract of *Aspidosperma pyrifolium* wood on arterial pressure in spontaneously hypertensive rats

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Abstract

Aspidosperma pyrifolium (Apocynaceae) is a plant popularly known as “pereiro-de-sertão” and as another plants of the genus is known by the production of indole alkaloids. Until now there is no work regarding to any kind of cardiovascular activity. The present study evaluated the cardiovascular effects induced by the ethanolic extract of the wood of *A. pyrifolium* (EEAPW) on spontaneously hypertensive rats (SHR). The wood extract was obtained by maceration in 90%. To the measurement of arterial pressure (AP) and heart rate (HR), male SHR (250-300g) were anesthetized with thiopental (45 mg/Kg, i.p.) and polyethylene catheters were inserted in abdominal aorta and inferior vena cava for recording pressure and to drug administration, respectively. Experiments were performed 24 hours after surgical process. The results are presented as mean \pm standard error. The bolus intravenous administration of EEAPW (0.5, 1, 5, 10, 20 and 30 mg/Kg) produced a dose-dependent hypotension ($-7.8 \pm 1.5\%$; $-9.4 \pm 1\%$; $-10.9 \pm 1.2\%$; $-15.9 \pm 0.9\%$; $-21.2 \pm 1.3\%$ and $-30.2 \pm 0.9\%$, respectively) and bradycardia ($-11.20 \pm 1\%$; $-13.74 \pm 1.3\%$; $-12.8 \pm 1.5\%$; $-12.03 \pm 0.8\%$; $-17.37 \pm 1.2\%$ and $-13.90 \pm 1.4\%$), in non-anesthetized SHR (n=6). The hypotensive response produced by EEAPW remained unaltered after the administration of the nitric oxide synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 20 mg/Kg, i.v.). The hypotensive response was completely abolished after the administration of the cyclooxygenase (COX) inhibitor indomethacin, while the bradycardia was attenuated at the doses of 1, 5 and 10 mg/Kg. The obtained results shows that the hypotension produced by EEAPW depends on the endothelium-dependent relaxing factors, possibly prostacyclin and that the hypotension induced by EEAPW doesn't involves the activation of the nitrous oxide (NO) way.

Key words: Cardiovascular Effects, *Aspidosperma pyrifolium* Mart., Hypotension

Introduction

Medicinal plants and corresponding preparations have been used for a wide range of purposes and for many centuries people have been trying to treat diseases as well as alleviate symptoms by using different plant extracts and formulations [1]. Therefore, the research development and use of natural products as therapeutic agents, especially those derived from higher plants, have been increasing in recent years [2]. Plants such as *Evodia rutaecarpa* (Juss) Benth [3], *Bidens pilosa* L. [4], *Persea americana* Mill [5], *Erigeron breviscapus* Hand Mazz [6] have been investigated due to their cardiovascular properties, such as hypotensive effects, since hypertension is the most common cardiovascular disease and is a major public health issue [7], affecting more than 10% of the worldwide population [8].

The introduction, in the early 1950s, of the first truly effective antihypertensive drugs, dramatic advances have been made in the efficacy and tolerability of these agents. Nevertheless, it is acknowledged that less than 25% of treated individuals achieve target mean arterial pressure (MAP) [9] and trial outcomes have shown that each agent is associated with relative benefits and drawbacks, often within the context of various patient characteristics such as age, comorbidities and risk status [10]. The current trend towards lower the mean arterial pressure (MAP) goals suggests that more effective and better tolerated antihypertensive therapies will be needed, of which natural products can be considered as one of the potential sources [11].

Aspidosperma pyriforme Mart. (Apocynaceae) known as “pereiro” or “peroba-rosa”. The genus *Aspidosperma* just found in America [12] and 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* [13]. Antimalarial activity isolated from *A. nitidum* and *A. excelsum* and hypotensive effects of the *A. quebracho-blanco*

and *A. pyriforme* attributed to alkaloids from bark, stem bark and leaves, respectively [12].

However no reports studies with hypotensive effects of the ethanolic extract of the *Aspidosperma pyriforme* wood (EEAPW). Therefore, the aim of the present study was to investigate the acute cardiovascular effects of the ethanolic extract of the *Aspidosperma pyriforme* wood (EEAPW) in spontaneously hypertensive rats (SHR).

Materials and Methods

Plant collection and extraction

Aspidosperma pyriforme Mart., was collected in October 2001 in São José da Tapera, AL, Brazil, and identified by Dr J. E. de Paula (Universidade de Brasília, Brasília, DF, Brazil). A voucher specimen [number JEP3686 (UnB)] was deposited in the herbarium at the Universidade de Brasília. The dried wood of *A. pyriforme* was extracted exhaustively with ethanol in 3 times in cycles of 48 hours each, and the ethanolic solution was concentrated under reduced pressure to yield a brownish viscous liquid. When necessary, this extract was dissolved in distilled water, filtered, and known volumes were dried to determine its concentration, once only 70.5% of the crude ethanolic extract was soluble in water. This correction factor was utilized to calculate the desired final concentration of the samples.

Drugs

The following drugs were used: sodium thiopental, atropine sulphate, N^G-nitro-L-arginine-methyl ester (L-NAME), sodium nitroprussiate (NPS), indomethacin. Indomethacin was dissolved in sodium bicarbonate 5%. The other drugs were dissolved in distilled saline solution for experiments. All of the Sigma.

Animals

Male 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 arterial pressure, and pulse interval (referred to as heart rate, HR).

Hypotensive effects of EEAPW

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 EEAPW (0.5, 1, 5, 10, 20 and 30 mg/kg, i.v.) were administered randomly and the responses were recorded, MAP 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 EEAPW were administered after pretreatment with,

nitric oxide synthase inhibitor (NOS) (L-NAME, 20 mg/kg, i.v.), muscarinic antagonist (atropine, 2 mg/kg, i.v.) and inhibitor of cyclooxygenase (indomethacin, 3 mg/kg, i.v.). Doses of antagonists were chosen according to those recommended in the literature.

Statistical analysis

All values were expressed as mean \pm 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 $p < 0.05$. All analysis was performed using GraphPad™ Prism software, version 5.0

Results

Hypotensive and bradycardic effects of the EEAPW

In conscious, unrestrained rats, EEAPW (0.5, 1, 5, 10, 20 and 30 mg/kg, i.v., randomly) produced a significant ($p < 0.05$) and dependent-dose hypotension by: $-7.8 \pm 1.5\%$; $-9.4 \pm 1\%$; $-10.9 \pm 1.2\%$; $-15.9 \pm 0.9\%$; $-21.2 \pm 1.3\%$ and $-30.2 \pm 0.9\%$, respectively also induced decrease in heart rate in SHR: $-11.20 \pm 1\%$; $-13.74 \pm 1.3\%$; $-12.8 \pm 1.5\%$; $-12.03 \pm 0.8\%$; $-17.37 \pm 1.2\%$ and $-13.90 \pm 1.4\%$, respectively (**Figure 1**). There was no significant change in either MAP or HR after the i.v. administration of EEAPW vehicle.

Evaluation of endothelium-derived factors involvement in hypotensive effects

After pretreatment with L-NAME (20 mg/kg, i.v.), the hypotensive response induced by extract not was changed; however, the bradycardia was attenuate in lower doses 0.5; 1 and 10 mg/kg ($-4.49 \pm 1.1\%$; $-5.63 \pm 0.9\%$; $-6.62 \pm 1.7\%$ and $-6.40 \pm 0.5\%$, respectively) (**Figure 2**). After administration indomethacin (3 mg/kg, i.v.) the hypotensive effect was reversed in hypertension ($3.5 \pm 0.5\%$; $3.8 \pm 0.5\%$; $4.5 \pm 1.1\%$; $5.5 \pm 1.5\%$; $8.8 \pm 1.1\%$ and $7.2 \pm 1.1\%$, respectively) and as L-NAME the bradycardia was attenuate only

lower doses 0.5;1;5 and 10 mg/kg ($-5.14 \pm 0.7\%$; $-3.52 \pm 1.1\%$; $-5.85 \pm 0.6\%$ and $-3.95 \pm 0.6\%$, respectively) (Figure 3). After pretreatment with atropine the hypotension and bradycardic response induced by extract not was changed (Figure 4).

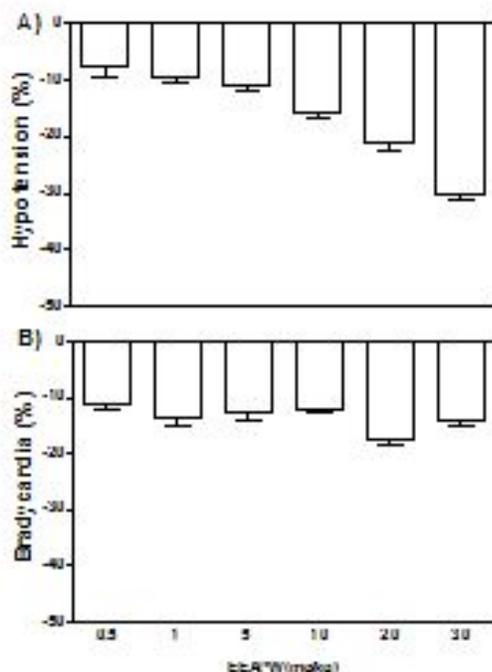


Figure 1: Effects on MAP and HR after acute administration of EEAPW (0.5; 1; 5; 10; 20 and 30 mg/kg, i.v.) in SHR. Values are mean \pm SEM of five experiments.

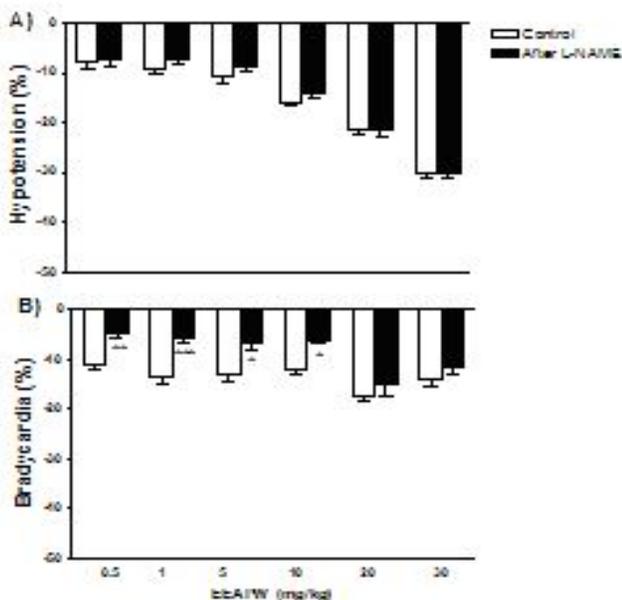
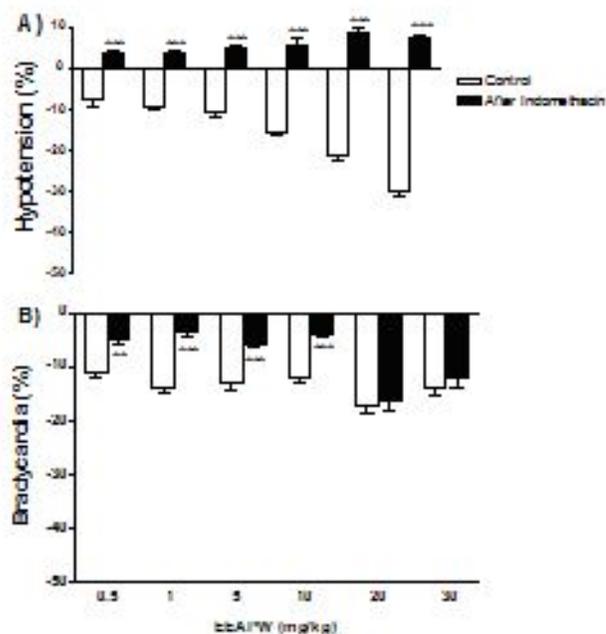
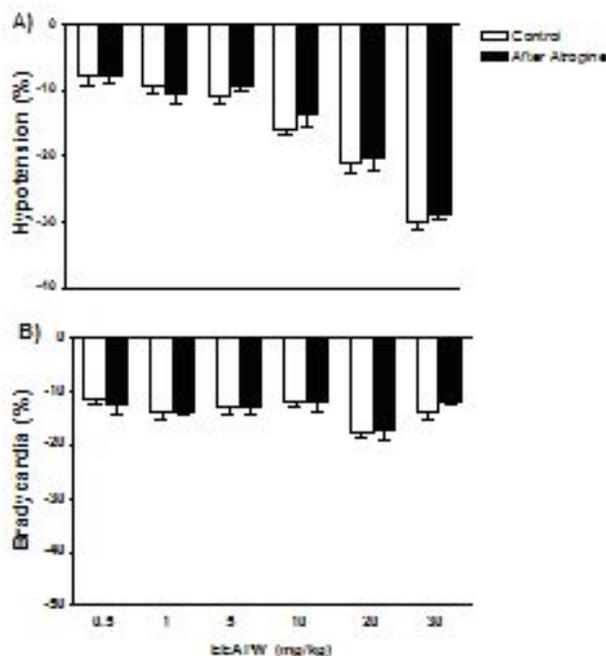


Figure 2: Effects of EEAPW on MAP and HR before (control) and after acute administration of L-NAME (20 mg/kg, i.v.) in SHR. Values are mean \pm SEM of five experiments. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs



0.001 vs control.

Figure 3: Effects of EEAPW on MAP and HR before (control) and after acute administration indomethacin (3 mg/kg, i.v.) SHR. Values are mean \pm SEM of five experiments. ** $p < 0.01$ and *** $p < 0.001$ vs



control.

Figure 4: Effects of EEAPW on MAP and HR before (control) and after acute administration of atropine (2 mg/kg, i.v.) in normotensive rats. Values are mean \pm SEM of five experiments.

Discussion

Vascular tone plays an important role in the physiological regulation of blood pressure. The development and maintenance of hypertension has been suggested to involve a reduced endothelium-dependent vasodilator influence on the vascular tissue [14].

The endothelium is an important regulator of vascular tone due to synthesis and release of vasodilatory substance such as NO and prostacyclin [15] in order to investigate the participation of NO and cyclooxygenase-derived products in the cardiovascular effects induced by EEAPW, was used L-NAME and indomethacin, respectively. In the presence of the L-NAME the hypotensive response not was reduced, however the EEAPW reduced bradycardic effect in lower doses. This is probably due to NOS inhibitors abolish of the negative inotropic effect of the NO produced by cardiac muscle [16], indicating that changes in heart rate do not play role in the hypotensive effect [17] and that the participation of the release of NO not appears to be involved in the hypotensive effect only bradycardic response of the EEAPW. After pretreatment with indomethacin the hypotension was abolished at all doses and as L-NAME bradycardia was attenuate in lower doses, these results strongly suggest the participation of cyclooxygenase-derived products in the hypotension caused by EEAPW.

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 by hypotension due of the cardiac output [18], beyond decrease of the total peripheral resistance through direct activation of endothelial muscarinic receptors in vessels [19]. It is well related that M₃ muscarinic receptors activation, located at endothelial cells, induces a release of endothelium-derived factors and consequently vasodilation and hypotension [19]. Whereas, stimulation of the M₂ subtype cardiac receptors, induce intense bradycardia followed by hypotension due to the decrease of the cardiac output [20]. Thus, in order to investigate

the role of muscarinic receptors in hypotensive and bradycardic effects induced by EEAPW, we performed experiments after atropine pretreatment, a muscarinic receptor blocker. In these conditions, both hypotensive and bradycardic responses not was reduced, these results suggest the non participation of the muscarinic receptors in effects.

In conclusion, the results obtained so far demonstrate that the extract of *Aspidosperma pyrifolium* wood induce hypotension and bradycardia, of the manner independent and which could be due to release of cyclooxygenase-derived products and NO, respectively and no participation of the muscarinic receptors in effects. However, further experiments are necessary to clearly elucidate the underlying mechanisms responsible for these responses.

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References

1. Höfling JF, Anibal PC, Obando-Pereda GA, et al. Antimicrobial potential of some plant extracts against *Candida* species. *Braz J Biol* 2010; 70:1065-1068.
2. Hipólito UV, Rodrigues JG, Lunardi CN, et al. Mechanisms underlying the vasorelaxant action of the pimarane ent-(14),15-pimaradien-3 α -ol in the isolated rat aorta. *Eur J Pharmacol* 2009; 616:183-191.
3. Wang GJ, Wu XC, Chen CF, et al. Vasorelaxing action of rutaecarpine: effects of rutaecarpine on calcium channel activities in vascular endothelial and smooth muscle cells. *J Pharmacol Exp Ther* 1999; 289:1237-1244.
4. Dimo T, Rakotonirina S, Kamgang R, et al. Effects of leaf aqueous extract of *Bidens pilosa* (Asteraceae) on KCl- and norepinephrine-induced contractions of rat aorta. *J Ethnopharmacol* 1998; 60:179-182.
5. Owolabi MA, Jaja SI, Coker HA. Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta. *Fitoterapia* 2005; 76:567-573.
6. Pan Z, Feng T, Shan L, et al. Scutellarin-induced endothelium-independent relaxation in rat aorta. *Phytother Res* 1998; 22:1428-1433.

7. Nikam AP, Bodhankar SL, Piplani P, Bansal J, Thakurdesai PA. β -adrenoreceptor blocking and antihypertensive activity of PP-24, a newly synthesized aryloxypropranolamine derivative. *J Pharm and Pharmacol* 2008; 60:1501-1506.
8. Chen S, Lv G, Zhang X, et al. Anti-hypertensive effects of laiju extract in two different rat models. *Asia Pac J Clin Nutr*; 16:309-312.
9. Evans RG, Kett MM, Shweta A, et al. New physiological target within the kidney for antihypertensive therapy. *Drug Des Rev* 2005; 2:167-178.
10. Germino FW. The management and treatment of hypertension. *Clin Cornerstone* 2009; 9:S27-S33.
11. Ayele Y, Urga K, Ephrem E. Evaluation of in vivo antihypertensive and in vitro vasodepressor activities of the leaf extract of *Syzygium guineense* (Willd) D.C. *Phytother Res* 2010; 24:1457-1462.
12. Pereira MM, Jácome RL, Alcântara AFC, et al. Alcalóides indólicos isolados de espécies do gênero *Aspidosperma* (apocynaceae). *Quim. Nova* 2007; 30: 970-983.
13. Ferreira ICP, Lonardoní MVC, Machado GMC, et al. Anti-leishmanial Activity of Alkaloidal Extract from *Aspidosperma ramiflorum*. *Mem Inst Oswaldo Cruz* 2004; 99(3):325-327.
14. Ojewole JA. Vasorelaxant and Hypotensive Effects of *Sclerocarya Birrea* (A Rich) Hochst (Anacardiaceae) Stem Bark Aqueous Extract in Rats. *Cardiovasc J South Afr* 2006; 17:117-123.
15. Stankevicius E, Kevelaitis E, Vainorius E, Simonsen U. Role of nitric oxide and other endothelium-derived factors. *Medicina* 2003; 39:333-339.
16. Balligand J, Cannon PJ. 1997. Nitric oxide synthases and cardiac muscle. *Atheroscler Thromb Vasc Biol* 17: 1846-1858.
17. Côrtes SF, Rezende BA, Corriu C et al. Pharmacological evidence for the activation of potassium channels as the mechanism involved in the hypotensive and vasorelaxant effect of dioclein in rat small resistance arteries. *Br J Pharmacol* 2001; 133:849-858.
18. Peterson GL, Herron GS, Yamaki M, Fullerton DS, Schimerlik MI. Purification of the muscarinic acetylcholine receptor from porcine atria. *Proc Natl Acad Sci USA* 2001; 81: 4993-4997.
19. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; 43:109-142.
20. Medeiros IA, Santos MRV, Nascimento, NMS, Duarte JC. Cardiovascular effects of *Sida cordifolia* leaves extract in rats. *Fitoterapia* 2006; 77:19- 27.