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## Antihypertensive Effect of *Bauhinia forficata* Aqueous Extract in Rats

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### ABSTRACT

*Bauhinia forficata*, known as “pata-de-vaca”, is used in folk medicine as antidiabetic. This study evaluated the phytochemical composition of the aqueous extract of *Bauhinia forficata* leaves (AEBF) and investigated its antihypertensive effect in conscious normotensive and Goldblatt hypertensive rats. Phytochemical screening of the aqueous extract was performed by colorimetric tests. The Mean Arterial Pressure (MAP) and Heart Rate (HR) were measured in normotensive and hypertensive rats through a catheter inserted in abdominal aorta via femoral artery. Aortic catheter was connected to a pressure transducer coupled to an amplifier-recorded apparatus. Phytochemical screening of the AEBF showed the presence of phenols, flobabenic tannins, flavonols, flavanones, alkaloids and saponins. In normotensive rats, it was observed that AEBF (5, 10, 20 and 40 mg kg<sup>-1</sup>, i.v.) induced hypotension and tachycardia that were not changed after indomethacin administration but were significantly (p<0.05) attenuated L-NAME. After treatment with atropine, only tachycardia was attenuated. In Goldblatt hypertensive rats, acute treatment with AEBF (400 mg kg<sup>-1</sup>; v.o.) was able to reduce 12% of the MAP (n = 6), while that HR was not affected. In summary, this study found possible benefits of this plant on the cardiovascular system. The results demonstrate that the Aqueous Extract of *Bauhinia forficata* (AEBF) present antihypertensive effect that seems to involve releasing of NO.

**Key words:** *Bauhinia forficata*, aqueous extract, phytochemical screening, antihypertensive effect

### INTRODUCTION

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide (Cirigliano and Sun, 1998; Sohail and Sohail, 2011; Karim *et al.*, 2011). In some regions, like South Asia, plants are the major source of drugs, while in other regions such as Latin and North America, the concurrent use of traditional forms of medical care and herbal remedies is very common (Eisenberg *et al.*, 1998).

Many herbal remedies have been popularly used for the treatment of diseases that produce harmful effects to the heart and the blood vessels such as hypertension, atherosclerosis and

diabetes mellitus. However, only some of these plants have their effectiveness, safety and mechanism of action confirmed (Vora and Mansoor, 2005). In this context, several research groups worldwide have studied medicinal plants with cardiovascular activity. Such studies have investigated possible beneficial and/or side effects of herbal products in order to develop a scientific basis for their therapeutic application (Menezes *et al.*, 2007).

*Bauhinia forficata* Link (Leguminosae) is a plant popularly known as “pata-de-vaca” in several regions of Brazil. It is an arboreal species originally from Asia, reaching 12 m in height (Pepato *et al.*, 2002). Its most important use in folk medicine is as antidiabetic, which is known as “natural insulin” (Vasconcelos *et al.*, 2004). Several studies have demonstrated its effects on diabetes (Pepato *et al.*, 2002; Silva *et al.*, 2002; Lino *et al.*, 2004) but any pharmacological study relating the activity of this plant on the cardiovascular system was not found in literature. Thus, this work aimed to evaluate the antihypertensive effects of Aqueous Extract of *Bauhinia forficata* (AEBF) on the arterial pressure and heart rate in normotensive and hypertensive rats.

## MATERIALS AND METHODS

**Drugs:** The following drugs were used: N $\beta$ -nitro-L-arginine Methyl Ester Hydrochloride (L-NAME), Atropine Sulphate, Indomethacin (INDO) (All from SIGMA), sodium thiopental (CRISTÁLIA), heparin sodium salt (ARISTON) and nifedipine (NIF) (from RBI).

**Botanical material and preparation of extract:** *Bauhinia forficata* was collected in the metropolitan region of Aracaju in the Brazilian state of Sergipe and identified by the Biology Department at the Universidade Federal de Sergipe. A voucher specimen was deposited in the Herbarium of the Biology Department at the Universidade Federal de Sergipe (No.: ASE9776). The Aqueous Extract of *Bauhinia forficata* (AEBF) was obtained from its dried leaves (80 g of powder) by an infusion with distilled water (1 L), at 100°C, followed by filtration. The filtrate was lyophilized and stored at 4°C. When required, the extract was dissolved in a saline solution at desired concentrations just before use.

**Phytochemical screening:** The aqueous extract was divided into different test tubes and various chemical constituents were qualitatively analyzed according to methods described by Matos (1994) and Harbone (1998). This analysis was conducted by observing colorimetric variation after the addition of specific reagents. The different chemical constituents and reagents tested included: phenolic compounds, pirogalic tannin, flobabenic tannins, anthocyanins, anthocyanidins, flavononols, leucoanthocyanidins, catechins, flavanones, alkaloids, steroids, triterpenoids and saponins.

**Animals:** Male Wistar normotensive rats (200-300 g) were used for all the experiments. Animals were housed under conditions of controlled temperature (25±1°C) and lighting (lights on: 06:00-18:00 h) and had free access to food and tap water. All procedures described in the present work were in compliance with the Animal Research Ethics Committee of the Universidade Federal de Sergipe, Brazil, approved under number 18/05, and Guide for the Care and Use of Laboratory Animals (NRC, 2011).

**Measurement of blood pressure and heart rate in conscious normotensive rats:** For measurement of the Mean Arterial Pressure (MAP) and Heart Rate (HR), rats were anaesthetized with sodium thiopental ( $45 \text{ mg kg}^{-1}$ ; i.p.). Polyethylene catheters were inserted into the abdominal aorta via left femoral artery for pressure recordings and into the lower vena cava via left femoral vein for the administration of drugs. Both catheters were filled with heparinized saline and led under skin to exit between the scapulae. After 24 h of the surgery, rats were placed in large individual cages and experiments were performed in conscious rats.

The arterial catheter was connected to a pre-calibrated pressure transducer (Edwards Lifescience, Irvine, CA, USA) and pressure outputs were recorded in an amplifier-recorder (BioData, Model BD-01, PB, Brazil) connected to a personal computer equipped with an analog-to-digital converter board (BioData, PB, Brazil). The arterial pressure waveform was presented as peak and valley (pulse) and for each cardiac cycle, the computer calculated the MAP and pulse interval (referred too as heart rate).

**Experimental protocols in normotensive rats:** After stabilization of the hemodynamic parameters, MAP and HR were recorded before (baseline values) and after administration of the randomized doses of AEBF (5, 10, 20 and  $40 \text{ mg kg}^{-1}$ ; i.v.). Dose-response curves were then obtained. Successive injections were separated by a time interval sufficient to allow full recovery of hemodynamic parameters. Similar records were obtained separately after administration of atropine, a muscarinic cholinergic antagonist ( $2 \text{ mg kg}^{-1}$ ; i.v.; 15 min), L-NAME, a Nitric Oxide (NO) synthase inhibitor ( $20 \text{ mg kg}^{-1}$ ; i.v.; 30 min) or INDO, a non-selective inhibitor of Cyclooxygenase (COX) ( $5 \text{ mg kg}^{-1}$ ; i.v.; 15 min).

**AEBF effect on hypertensive rats:** Two-kidney one-clip Goldblatt hypertension was induced in normotensive rats (weight between 160 and 180 g) by the method of Goldblatt *et al.* (1934). The animals were anaesthetized with sodium thiopental ( $45 \text{ mg kg}^{-1}$ ; i.p.) and submitted to median laparotomy, which exposed the renal pediculum. Afterwards, one silver clip ( $2 \times 5 \text{ mm}$  and  $0.2 \text{ mm ID}$ ) was placed around the left renal artery. After incision suture, oxytetracyclin chloride was administrated in the animals in a single dose ( $0.2 \text{ g kg}^{-1}$ ), by the intramuscular via.

Hypertension (MAP > 160 mm Hg) was evidenced by direct measurements of MAP (as described previously) 30 days after the surgical procedure. After this, the hypertensive animals were acutely treated by oral via with AEBF ( $200$  and  $400 \text{ mg kg}^{-1}$ ) or NIF ( $3 \text{ mg kg}^{-1}$ ). SHAM animals were only treated with vehicle. MAP and HR variations were observed at the times of 0.5; 1; 2; 3; 4; 8 and 24 h after administration.

**Statistical analysis:** Values are expressed as Mean  $\pm$  SEM. The significance of differences among means was evaluated by two-way ANOVA with Bonferroni post-test. All statistical analyses were done by using Graph Pad Prism™ version 3.02 software.

## RESULTS

**Phytochemical screening:** Phytochemical screening of the AEBF showed the presence of phenols, flobabenic tannins, flavonols, flavanones, alkaloids and saponins (Table 1). Phytochemical screening of the AEBF showed the presence of several groups of the substances, mainly of phenols, flavonols, flavanones and alkaloids. In accordance to literature, intake of these compounds can promote some benefits on the cardiovascular system.

Table 1: Phytochemical screening of aqueous extract of *Bauhinia forficata*

Parameters	Extract	Parameters	Extract
Phenols	+	Catechins	-
Pirogalic tannin	-	Flavanones	+
Flobabenic tannins	+	Alkaloids	+
Anthocyanins	-	Steroids	-
Anthocyanidins	-	Triterpenoids	-
Flavonols	+	Saponins	+
Leucoanthocyanidins	-		

+: Presence and -: Absence of chemical constituents

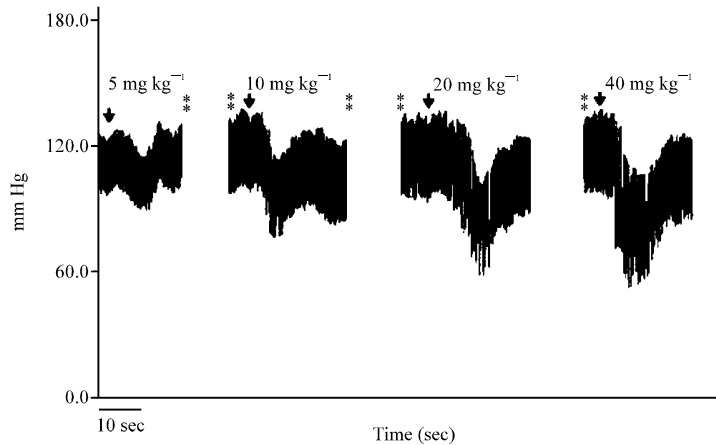


Fig. 1: Original traces showing the variations of arterial pressure (mm Hg) and heart rate (X axis) induced by acute administration of AEBF (5, 10, 20 and 40 mg kg<sup>-1</sup>, i.v.) in a conscious normotensive rat in function of the time (Y axis). The arterial pressure waveform is presented as peak and valley (pulse) and for each cardiac cycle, the computer calculated the MAP and pulse interval (referred too as heart rate). The indicates time scale in sec (s), \*\*Indicates an interruption of the trace, and arrows indicate the moment of AEBF application

Several studies have supported the view that the consumption of foods containing phenols and flavonoids, in particular flavonols and flavanones, exert cardioprotective effects (Peters *et al.*, 2001; Mennen *et al.*, 2004; Graf *et al.*, 2005; Manach *et al.*, 2005). Sagara *et al.* (2004) demonstrated that these benefits included reduced baseline measures of systolic and diastolic blood pressures. Furthermore, a recent study has suggested that the intake of certain subclasses of flavonoids may be associated with lower mortality by cardiovascular diseases (Mink *et al.*, 2007). In the present study, AEBF was able to induce hypotension associated to tachycardia in the normotensive animals.

**AEBF effect on MAP and HR in conscious normotensive rats:** In conscious normotensive rats, baseline MAP and HR values were 115±6 mm Hg and 342±6 bpm, respectively. In these animals, the intravenous in bolus injections of AEBF (5, 10, 20 and 40 mg kg<sup>-1</sup>) induced a dose-dependent, intense and transitory hypotension (-12±1; -17±2; -18±2 and -21±2%; n = 9) associated with tachycardia (8±2; 10±2; 10±2 and 12±2%; n = 9) (Fig. 1 and 2). The duration of AEBF effect on MAP and HR was approximately of 25 sec.

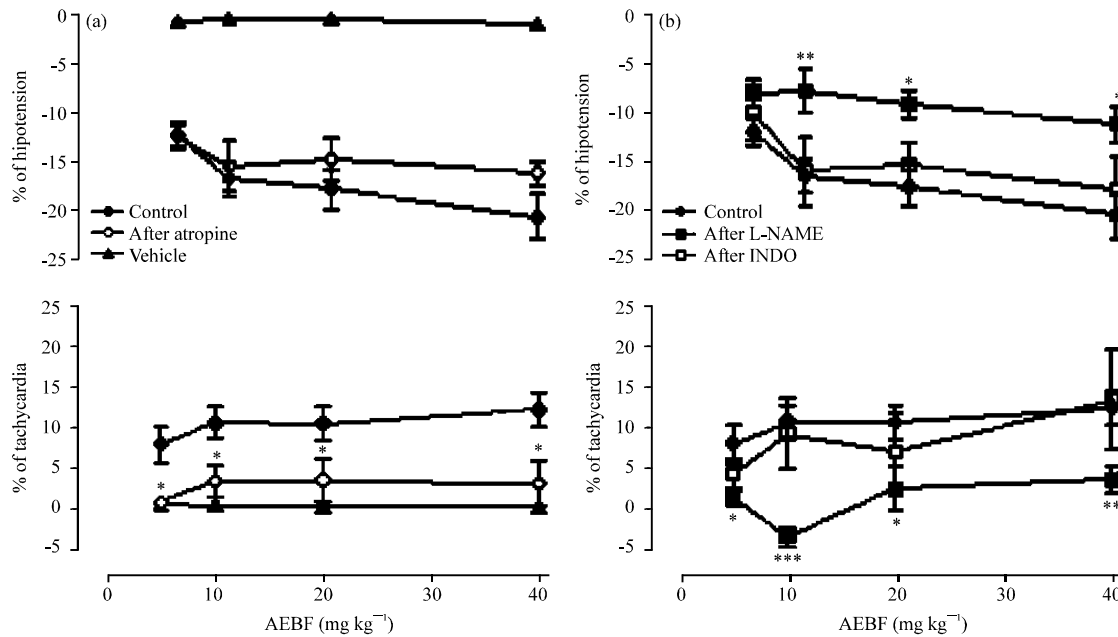


Fig. 2(a-b): Effect of vehicle and AEBF (5, 10, 20 and 40 mg kg<sup>-1</sup>, i.v.) on MAP and HR of conscious normotensive rats (a) Before (control) and after pre-treatment with atropine (2 mg kg<sup>-1</sup>) and (b) Before (control) and after pre-treatment with L-NAME (20 mg kg<sup>-1</sup>) or indomethacin (INDO: 5 mg kg<sup>-1</sup>). Value are expressed as Mean±SEM of six experiments. The data were analyzed with repeated measures two-way ANOVA followed by Bonferroni post-test. \*p<0.05; \*\*p<0.01 and \*\*\*p<0.001 vs. control

**Effect of atropine, L-NAME or indomethacin on AEBF-induced responses in conscious rats:** The baseline values of MAP (115±6 mm Hg; n = 9) were not affected after administration of atropine (121±5 mm Hg) or indomethacin (118±6 mm Hg) but were significantly increased after L-NAME (153±2 mm Hg; p<0.001). On the other hand, baseline values of HR (342±6 mm Hg; n = 9) were significantly increased after atropine (423±12 mm Hg; p<0.001).

As showed in the Fig. 2a, the hypotensive effect induced by AEBF was not changed in rats pre-treated with atropine but tachycardic response was significantly reduced in all doses. On the other hand, in rats pre-treated with L-NAME, the hypotensive effect induced by AEBF was reduced in the doses of 10, 20 and 40 mg kg<sup>-1</sup>, while tachycardic response was significantly reduced in all doses (Fig. 2b). In rats pre-treated with INDO, neither hypotension nor tachycardic effects induced by AEBF were modified (Fig. 2b).

**Antihypertensive effect of AEBF:** In all Goldblatt hypertensive rats, the baseline values of arterial pressure and heart rate were of 185±6 mm Hg e395±11 bpm, respectively. In these animals, the acute oral administration of the AEBF (400 mg kg<sup>-1</sup>) or NIF (3 mg kg<sup>-1</sup>) reduced significantly the MAP and did not change the HR. No significant effect was observed for EABF in the dose of 200 mg kg<sup>-1</sup> (Fig. 3).

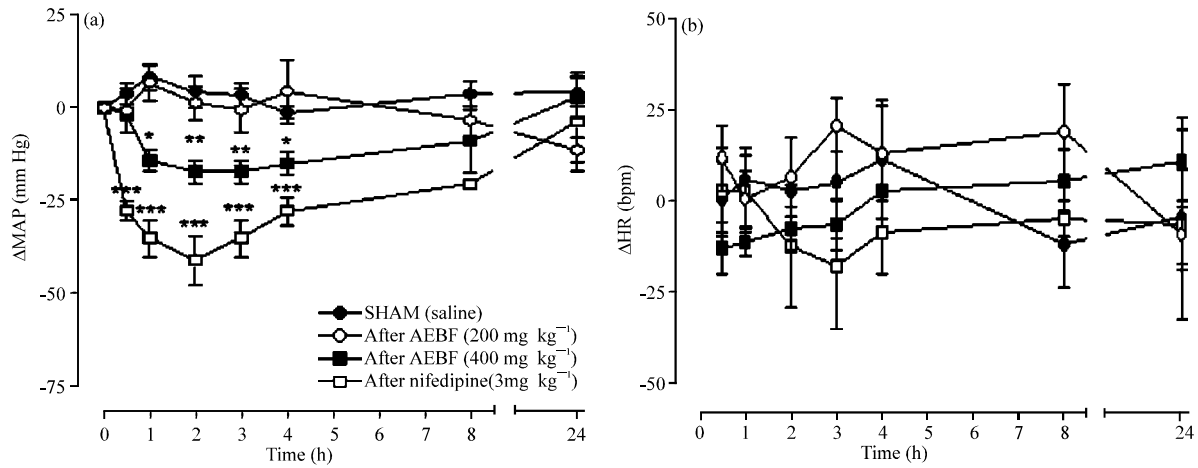


Fig. 3(a-b): Effect of AEBF (200 and 400 mg kg<sup>-1</sup>) and nifedipine on (a) MAP and (b) HR variations ( $\Delta$ ) of conscious hypertensive rats at the times of 0.5; 1; 2; 4; 8 and 24 h after oral administration. Value are expressed as Mean $\pm$ SEM of six experiments. The data were analyzed with repeated measures two-way ANOVA followed by Bonferroni post-test. \*p<0.05; \*\*p<0.01 and \*\*\*p<0.001 vs. control

## DISCUSSION

It was known that hypotensive response can be caused by an intense vasodilatation due to a stimulation of endothelial muscarinic receptors, in spite of apparent absence of cholinergic enervation in the most blood vessels (Bruning *et al.*, 1994). This stimulation induces an increase in intracellular Ca<sup>2+</sup> in the endothelial cells, promoting the production and releasing of the Endothelium-derived Relaxing Factors (EDRFs) (Furchgott and Zawadzki, 1980), mainly NO (Moncada *et al.*, 1991) and prostacyclin (PGI<sub>2</sub>), both potent vasodilators (Schulz and Triggle, 1994). Thus, to verify the participation of muscarinic receptors in these responses, the animals were pre-treated with atropine (2 mg kg<sup>-1</sup>, i.v.), a non-selective antagonist of these receptors.

In the six studied animals, the pre-treatment with atropine produced a significant and sustained increase in baseline values of HR but it did not changed MAP. In these animals, the hypotension induced by AEBF was not altered by atropine. On the other hand, tachycardia was attenuated significantly in all doses. This result suggests that muscarinic receptors do not seem to be important to expression of hypotensive response. However, the attenuation of the tachycardic response seems to be due to the intense positive inotropic response caused by the atropine. Since the heart rate was strongly rose, the tachycardic response induced by the indirect action of AEBF, possibly caused by baroreflex response, was attenuated.

In order to evaluate a possible participation of NO and PGI<sub>2</sub> in hypotensive and tachycardic responses induced by AEBF, experiments were performed in animals pre-treated with L-NAME, a NO synthase inhibitor, or INDO, a COX inhibitor. In these animals, MAP baseline values were significant increased only by L-NAME, while HR baseline values were not changed. In the animals treated with L-NAME, hypotensive and tachycardic responses induced by AEBF was attenuated significantly, while in the animals treated with INDO, no change was observed. These results suggest that NO but not PGI<sub>2</sub>, is relevant to expression of response induced by AEBF. The attenuation of AEBF-induced tachycardia caused by L-NAME pre-treatment appears to be associated to reduction of the hypotension and consequently to the baroreflex activation.

In order to evaluate the possible antihypertensive effects of AEBF by oral via, experiments were performed by using Goldblatt hypertensive rats. In these animals, the oral acute treatment with AEBF in dose of 400 mg kg<sup>-1</sup> was able to induce decrease of hypertension without modifying HR.

In Brazil, many diabetic patients with associated cardiovascular diseases, like hypertension, drink daily tea of *Bauhinia forficata* leaves. In summary, this study demonstrated possible benefits of the use of this medicinal plant on the cardiovascular system. These results demonstrated that *B. forficata* aqueous extract appears to low blood pressure by a pathway involving nitric oxide.

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