

## Cardiovascular effects of *Sida cordifolia* leaves extract in rats

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### Abstract

The cardiovascular activity of the aqueous fraction of the hydroalcoholic extract of *Sida cordifolia* leaves (AFSC) was evaluated. In normotensive non-anaesthetized rats was observed that AFSC (5, 10, 20, 30 and 40 mg/kg, i.v.) induced hypotension ( $6 \pm 2\%$ ;  $8 \pm 2\%$ ;  $11 \pm 2\%$ ;  $19 \pm 3\%$  and  $33 \pm 3\%$ , respectively) and bradycardia ( $0.3 \pm 3\%$ ;  $13 \pm 4\%$ ;  $38 \pm 6\%$ ;  $64 \pm 7\%$  and  $80 \pm 5\%$ , respectively). Hypotensive response was completely abolished after atropine (2 mg/kg; i.v.) but potentialized after hexamethonium (20 mg/kg; i.v.) ( $12 \pm 2\%$ ;  $21 \pm 5\%$ ;  $28 \pm 3\%$ ;  $32 \pm 2\%$  and  $32 \pm 3\%$ , respectively), while bradycardic response was completely abolished after atropine (2 mg/kg; i.v.) and attenuated with hexamethonium (20 mg/kg; i.v.) ( $1 \pm 0.3\%$ ;  $5 \pm 1\%$ ;  $7 \pm 1\%$ ;  $7 \pm 1\%$  and  $10 \pm 1\%$ , respectively). In hexamethonium treated rats, L-NAME significantly attenuated the hypotensive response ( $9 \pm 2\%$ ;  $14 \pm 1\%$ ;  $16 \pm 1\%$ ;  $16 \pm 2\%$  and  $22 \pm 3\%$ , respectively). In normotensive anaesthetized and vagotomized rats, hypotensive and bradycardic responses were significantly attenuated ( $0.5 \pm 0.2\%$ ;  $1 \pm 0.4\%$ ;  $3 \pm 0.6\%$ ;  $4 \pm 0.8\%$  and  $6 \pm 1\%$ , respectively,  $n=6$ , and  $7 \pm 2\%$ ;  $12 \pm 5\%$ ;  $15 \pm 2\%$ ,  $17 \pm 2\%$  and  $25 \pm 3\%$ , respectively). The anaesthesia with sodium thiopental did not affect the AFSC-induced responses when compared with those induced in non-anaesthetized rats (data not showed). In conclusion, the results obtained so far show that AFSC produce hypotension and bradycardia, mainly due to a direct stimulation of the endothelial vascular muscarinic receptor and indirect cardiac muscarinic activation, respectively.

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## 1. Introduction

*Sida cordifolia*, a native specie of the Brazilian Northeast, popularly known as “Malva Branca”, grows as a bush of up to 2 m. It is used in the folk medicine for several purposes: antirheumatic, antipyretic [1], laxative, diuretic, antiinflammatory, analgesic [2,3], hypoglycaemic [2], antiasthmatic, in the treatment of nasal congestion and as aphrodisiac [4,5]. Further studies showed antiviral [6], antimicrobial [7] and antifungal [8] activities.

A preliminary phytochemical screening of the hydroalcoholic extract of the leaves of *S. cordifolia* demonstrated the presence of alkaloids, steroids, flavonoids and saponins. Chemical studies of the leaves of this plant revealed the presence of ephedrine, pseudoephedrine (vasoconstrictor), vasicinone [9], vasicine and vasicinol (bronchodilators) [10]. Its toxicity in mice is very low, ca. 3 g/kg, p.o. [3].

Till now, no pharmacological study relating the activity of this plant on the cardiovascular system has been reported.

In present study we evaluate the cardiovascular activity of AFSC in rats, using direct blood pressure measurements in non-anaesthetized and anaesthetized rats.

## 2. Experimental

### 2.1. Drugs

Heparin sodium salt (ROCHE), sodium thiopental (CRISTÁLIA), cremophor (a derivative of castor oil and ethylene oxide used to emulsify water-insoluble substances), sodium nitroprusside, atropine sulfate, hexamethonium bromide, N<sup>w</sup>-nitro-L-arginine methyl ester (L-NAME) (SIGMA). The drugs were all dissolved in saline solution.

### 2.2. Plant

*S. cordifolia* L. (Malvaceae) leaves. A voucher specimen (no. 30171) was deposited in the Department of Biology of the University of Sergipe, Brazil.

### 2.3. Extraction

Leaves dried at 40 °C and pulverized were extracted with 70% EtOH at r.t. for 72 h and dried at 60 °C to give a residue. A portion of the residue was dissolved in distilled water, filtered and dried to determine the amount of the water-soluble fraction in the residue. Prior to the experiments the residue was dissolved in a saline/cremophor (0.025% v/v) solution and diluted to desired concentrations to give a water-soluble fraction (AFSC).

### 2.4. Animals

Male Wistar rats weighing 200–300 g were used in all experiments. Animals were housed under controlled conditions of temperature (25 ± 1 °C) and lighting (lights on: 6–18 h), and had free access to food and tap water ad libitum.

### 2.5. Direct blood pressure measurements in non-anaesthetized rats

Intra-aortic blood pressure was recorded according to Oliveira et al. [11]. Under sodium thiopental anaesthesia (45 mg/kg, i.v.), the lower abdominal aorta and inferior vena cava were cannulated via left femoral artery and vein using a polyethylene catheter. Thereafter, catheters were filled with heparinized saline solution and led under the skin to emerge between the scapulae. Arterial pressure was measured after 24 h by connecting the arterial catheter to a pre-calibrated pressure transducer (Statham P23 ID; Gould, Cleveland, OH, USA) coupled to an amplifier-recorder (Model TBM-4M, WPI, Sarasota, FL, USA) and connected to a computer equipped with an analog–digital convert board (CIO-DAS16/JR, Computer Boards, Inc., Mansfield, MA, USA) and CVMS software (WPI, Sarasota, FL, USA). The data were sampled at a frequency of 500 Hz. For each cardiac cycle, the computer calculated mean arterial pressure (MAP) and heart rate (HR) (pulse interval).

After cardiovascular parameters had stabilized, the MAP and HR were recorded before (baseline values) and after administration of randomized doses of AFSC (5, 10, 20, 30 and 40 mg/kg). For the construction of a dose-response curve, the difference between baseline and after administration values for each dose was expressed as percentage of baseline value. Successive injections were separated by a time interval sufficient to allow full recovery of haemodynamic parameters. Similar records were obtained separately after administration of atropine (2 mg/kg; i.v.; 15 min), a non-selective antagonist of muscarinic receptor; hexamethonium (20 mg/kg; i.v.; 30 min), a ganglionic blocker and L-NAME (20 mg/kg, i.v. 30 min), a competitive inhibitor of NO-synthase.

### 2.6. Direct blood pressure measurements in anaesthetized and vagotomized rats

The animals were cannulated as previously described, separated in two groups (SHAM and vagotomized) and were maintained under anaesthesia with sodium thiopental (45 mg/kg; i.v.) and controlled conditions of body temperature through a heater blanket ( $35 \pm 1$  °C). An intra-tracheal probe coupled to an artificial ventilator (Rodent Ventilator, UGO BASILE) was placed. The first group was sham-operated (SHAM), while in the second group was performed a cervical bilateral vagotomy.

When the cardiovascular parameters had stabilized, MAP and HR were recorded before (baseline values) and after administration of AFSC (5, 10, 20, 30 and 40 mg/kg, injected randomly). Dose-response curves were obtained as previously described.

### 2.7. Electrocardiogram records (ECG) and simultaneous direct blood pressure measurements in anaesthetized rats

The animals were cannulated as previously described and maintained under anaesthesia with sodium thiopental (45 mg/kg; i.v.) and conditions of controlled body temperature through a heater blanket ( $35 \pm 1$  °C). An intra-tracheal probe coupled to an artificial ventilator (Rodent Ventilator, UGO BASILE) was placed. To evaluate electrical cardiac activity changes induced by AFSC, the ECG was recorded using DII derivation, through subcutaneous electrodes implanted in the superior and inferior members of the animals. Immediately after surgical procedure and cardiovascular parameters had stabilized, a

single dose of AFSC (40 mg/kg) was administrated and MAP and HR were recorded. A similar record was obtained after treatment of the rats with atropine (2 mg/kg; i.v.; 15 min).

### 2.8. Statistic analysis

Values are expressed as mean  $\pm$  SEM. When appropriate, Student's *t*-test was done to evaluate the significance of the differences between means.

## 3. Results

### 3.1. Effect of AFSC on mean arterial pressure and heart rate in non-anaesthetized rats

The Fig. 1 show a typical record of the effect induced by AFSC (30 mg/kg, i.v.) on MAP and HR in non-anaesthetized rat. AFSC (5, 10, 20, 30 and 40 mg/kg; i.v.) induced hypotension ( $6 \pm 2\%$ ;  $8 \pm 2\%$ ;  $11 \pm 2\%$ ;  $19 \pm 3\%$  and  $33 \pm 3\%$ , respectively) and bradycardia ( $0.3 \pm 3\%$ ;  $13 \pm 4\%$ ;  $38 \pm 6\%$ ;  $64 \pm 7\%$  and  $80 \pm 5\%$ , respectively) (Fig. 2).

### 3.2. Effect of atropine, hexamethonium and L-NAME on AFSC-induced responses in non-anaesthetized rats

As expected in control animals, atropine and hexamethonium significantly reduced MAP from  $110 \pm 4$  to  $97 \pm 2$  and  $89 \pm 2$ , respectively. On the contrary, HR was increased

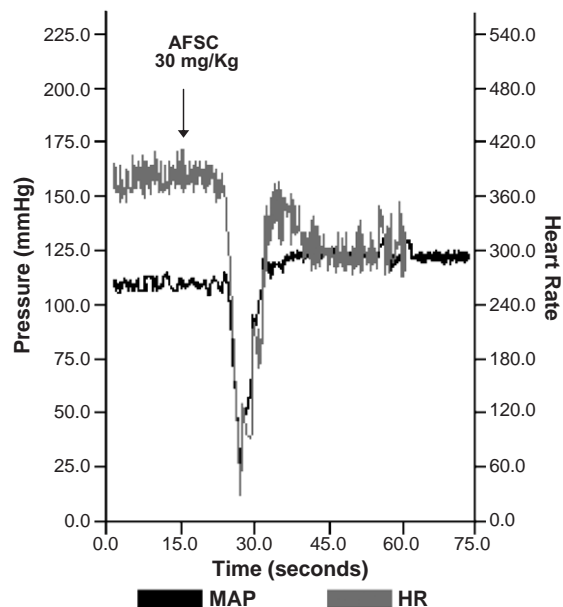


Fig. 1. Effect of 30 mg/kg i.v. of AFSC on MAP and HR in one non-anaesthetized rat.

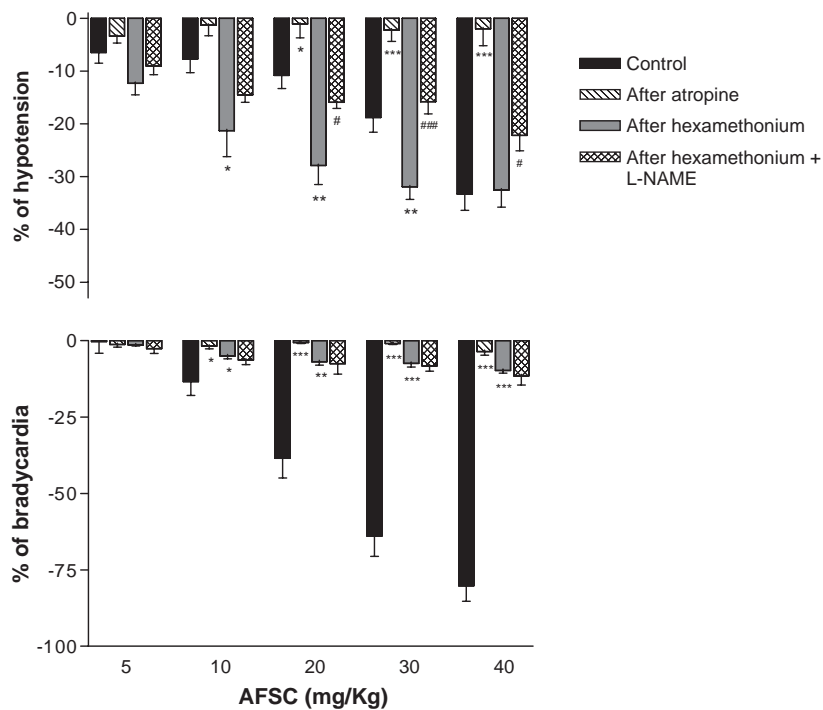


Fig. 2. Effect of AFSC on MAP and HR in non-anaesthetized rats before (control) and after acute administration of atropine (2 mg/kg, i.v.), hexamethonium (20 mg/kg, i.v.) and hexamethonium (20 mg/kg, i.v.)+L-NAME (20 mg/kg, i.v.). Values are mean  $\pm$  SEM of six experiments. \*  $P < 0,05$ , \*\*  $P < 0,01$  and \*\*\*  $P < 0,001$  vs. control and #  $P < 0,05$ , ###  $P < 0,001$  vs. hexamethonium given alone.

from  $361 \pm 8$  to  $442 \pm 10$  and  $443 \pm 14$ , respectively. The injection of hexamethonium + L-NAME increase MAP to  $134 \pm 4$  and decrease HR to  $328 \pm 5$ .

As shown in Fig. 2, administration of atropine completely abolish the AFSC-induced hypotensive and bradycardic responses. Administration of hexamethonium potentiate significantly the hypotensive response ( $12 \pm 2\%$ ;  $21 \pm 5\%$ ;  $28 \pm 3\%$ ;  $32 \pm 2\%$  and  $32 \pm 3\%$ , respectively) and significantly attenuate bradycardic response ( $1 \pm 0,3\%$ ;  $5 \pm 1\%$ ;  $7 \pm 1\%$ ;  $7 \pm 1\%$  and  $10 \pm 1\%$ , respectively).

The administration of hexamethonium+L-NAME significantly attenuate the AFSC-induced hypotensive response ( $9 \pm 2\%$ ;  $14 \pm 1\%$ ;  $16 \pm 1\%$ ;  $16 \pm 2\%$  and  $22 \pm 3\%$ , respectively), but did not affect the bradycardic response.

### 3.3. Effect of a bilateral cervical vagotomy on AFSC-induced responses in anaesthetized rats

The baseline values of MAP was not affected in vagotomized rats ( $117 \pm 4$  vs.  $121 \pm 4$ ). On the contrary, HR resulted increased significantly ( $502 \pm 15$  vs.  $424 \pm 14$  SHAM).

The anaesthesia with sodium thiopental did not affect the AFSC-induced responses when compared with the those induced in non-anaesthetized rats (data not showed).

Bilateral cervical vagotomy was capable of slightly attenuating the hypotensive response ( $0.5 \pm 0.2\%$ ;  $1 \pm 0.4\%$ ;  $3 \pm 0.6\%$ ;  $4 \pm 0.8\%$  and  $6 \pm 1\%$ , respectively), while bradycardic response was strongly reduced ( $7 \pm 2\%$ ;  $12 \pm 5\%$ ;  $15 \pm 2\%$ ,  $17 \pm 2\%$  and  $25 \pm 3\%$ , respectively) (Fig. 3).

#### 3.4. Effect of AFSC on the ECG records

AFSC (40 mg/kg; i.v.) was capable to induce sinoatrial blockade (Fig. 4b), which was completely abolished by pre-treatment with atropine (Fig. 4c).

## 4. Discussion

The major finding of this work is that AFSC administration in non-anaesthetized rats induced a marked hypotension and an intense bradycardia.

It is well-established that the primary autonomic regulation of the sinoatrial node function is by vagal action via stimulation of cardiac muscarinic receptors [12]. The stimulation of these receptors induce intense bradycardia followed by hypotension due to the decrease of the cardiac output. These receptors are predominantly  $M_2$  subtype [13,14], as confirmed by the localization of  $M_2$  mRNA in the rat heart by in situ hybridization [15].

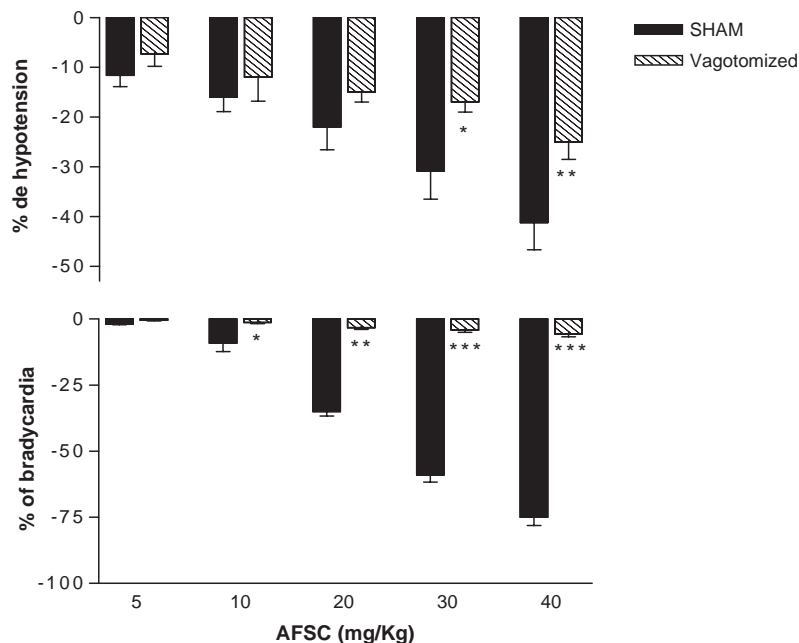


Fig. 3. Effect of AFSC MAP and HR in SHAM and vagotomized anaesthetized rats. Values are mean  $\pm$  SEM of six experiments. \*  $P < 0.05$ , \*\*  $P < 0.01$  and \*\*\*  $P < 0.001$  vs. SHAM.

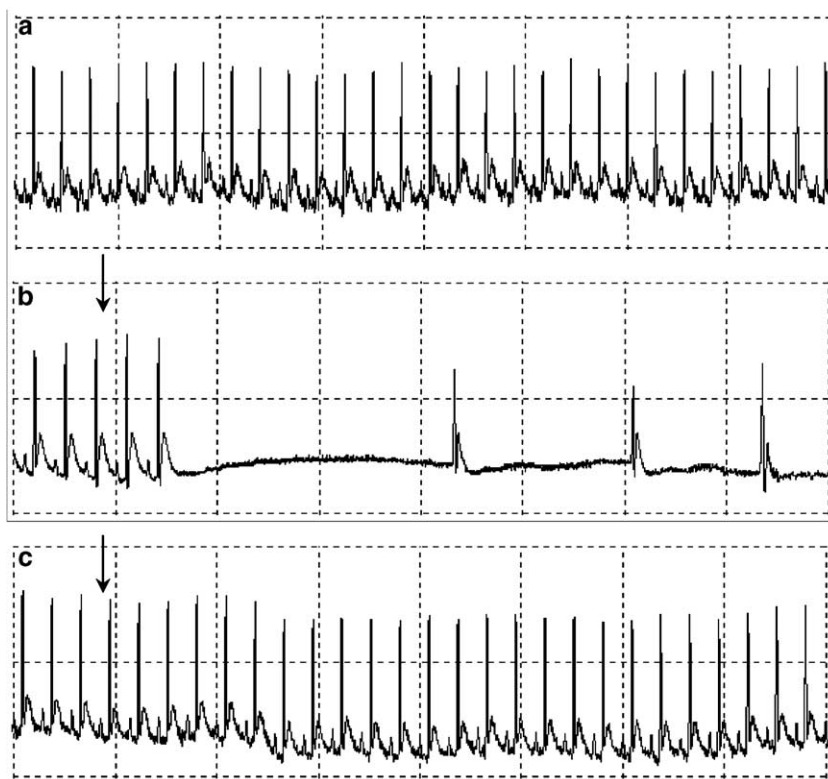


Fig. 4. Original traces showing a typical record of the ECG in control anesthetized rats (a), after AFSC administration (40 mg/kg, i.v.) (b) and after AFSC (40 mg/kg, i.v.)+atropine (2 mg/kg, i.v.) (c). The arrows indicate administration point.

In order to evaluate the role of these receptors in the AFSC-induced responses, we performed experiments in the presence of atropine, a non-selective antagonist of muscarinic receptor [16]. In these conditions, both hypotensive and bradycardic responses were completely abolished. Thus, we could suggest that AFSC would be acting, either directly in these receptors or indirectly *via* vagal activation. This was investigated by using a ganglionic blockade, hexamethonium (20 mg/kg, i.v.), which was capable to significantly attenuate the bradycardia and to potentiate the hypotensive response, suggesting that most of the AFSC-induced bradycardic effect appears to be really due to an indirect activation of muscarinic cardiac receptors. To confirm the participation of the vagus nerve pathway in this effect, we used anaesthetized cervical bilateral vagotomized rats. In these animals, the responses to AFSC were similar to that observed in animals after acute treatment with hexamethonium, suggesting that AFSC probably induced a vagal stimulation. In addition, in anaesthetized rats we found that AFSC induced sinoatrial blockade which was completely abolished by atropine. These results suggest that AFSC-induced bradycardic effect is indirect, possibly by changing the sinoatrial conduction in consequence of a vagal stimulation.

However, hypotensive response appears not to be exclusively due to a decrease of the cardiac output, since this response, differently of the bradycardia, which was potentiated by hexamethonium treatment and unaffected after vagotomy. The potentiation of the hypotensive response induced by AFSC after hexamethonium, when compared to that observed after vagotomy, can be explained by the fact that the ganglionic blockade affects both cholinergic and adrenergic pathways, the later being involved in the control of the vascular tonus. Since the hypotensive response induced by AFSC was completely abolished by atropine, we could hypothesize that this effect could be due to a decrease of the total peripheral resistance through direct activation of endothelial muscarinic receptors in vessels. It is well related in the literature that muscarinic activation of  $M_3$  receptors, located on the endothelial cells of the vessels, induce release of endothelium-derived relaxing factors [17], mainly NO [18], and consequently vasorelaxation and hypotension. AFSC could be activating this via and promoting decrease of the total peripheral resistance and hypotension. To evaluate the involvement of NO in this effect we administrated AFSC in normotensive and non-anaesthetized rats pre-treated with hexamethonium plus L-NAME, a competitive inhibitor of NO-synthase [19]. We found that L-NAME was able to significantly attenuate the hypotensive response induced by AFSC, suggesting that NO appears to be involved in this effect.

In conclusion, the results obtained so far demonstrate that the aqueous fraction of the hydroalcoholic extract of the leaves of *Sida cordifolia* induce hypotension and bradycardia, which could be due to both indirect cardiac muscarinic activation through the vagus nerve, and direct activation of endothelial vascular muscarinic receptors and consequent release of NO. However, further experiments are necessary to clearly elucidate the underlying mechanisms responsible for these responses.

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