Contractile effect of hydroalcoholic extract of *Sida cordifolia* L. leaves in rat aorta

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In the present work, the contractile effect induced by aqueous fraction of the hydroalcoholic extract of the leaves of *Sida cordifolia* L. (AFSC) was investigated in the rat aorta. In the presence of functional endothelium, AFSC produced contractions in a concentration-dependent manner (EC_{50} = 1.23 ± 0.09 mg/mL; n=6). In the absence of functional endothelium or in the presence of L-NAME (100 μM), the concentration-response curves to AFSC were significantly shifted to the left (EC_{50} = 0.84 ± 0.1 and 0.54 ± 0.15 mg/mL, respectively; n=6) with an increase of its maximal contractile response. In the presence of indomethacin (10 μM), the concentration-response curve produced by AFSC was not changed (EC_{50} = 1.28 ± 0.05 mg/mL; n=6). In addition, concentration-response curves to AFSC were significantly shifted to the right in the presence of 1 μM of prazosin (EC_{50} = 1.40 ± 0.01 mg/mL; n=7), but were not changed after 3 μM of yohimbine (EC_{50} = 1.22 ± 0.06 mg/mL; n=6). In conclusions, these results demonstrate that the contractions induced by AFSC in the rat aorta seem to be due to an activation of α_1-adrenergic receptors, and these seem to be negatively modulated by endothelium and release of NO.

Keywords: rat aorta, *Sida cordifolia*, α_1-adrenergic receptor

1. INTRODUCTION

Sida Cordifolia L. (MALVACEAE) is a native specie of the Brazilian Northeast and grows as a bush of up to 2 m. It is popularly known as "Malva Branca" and is used in the folk medicine for several purposes: antirheumatic, antipyretic [1], anti-inflammatory, analgesic [2], antiasthmatic, in the treatment of nasal congestion and as aphrodisiac [3]. A 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 (vasoconstrictors sympathomimetic amines) [4], vasicinone, and vasicinol (bronchodilators) and vasicine (bronchodilator and vasodilator) [5, 6].

A pharmacological study performed by Medeiros [7] demonstrated that the hydroalcoholic extract of *Sida cordifolia* leaves produced hypotension and bradycardia in rats, mainly due to a direct stimulation of the endothelial vascular muscarinic receptor and indirect cardiac muscarinic activation, respectively. Furthermore, previous studies have demonstrated that this plant present a vasodilator activity in rat superior mesenteric artery, which appears to be due to endothelial nitric oxide release [8].

In this context, the present study evaluated the response evoked by aqueous fraction of the hydroalcoholic extract of the leaves of *Sida cordifolia* L. (AFSC) in the rat aorta.

2. EXPERIMENTAL

2.1. Animals

Male *Wistar* normotensive rats (*Rattus norvegicus*) weighing 250-300 g were used in all experiments. Animals were housed under controlled conditions of temperature (25 ± 1ºC) and lighting (lights on: 6-18 h), and had free access to food and tap water *ad libtum*. All

procedures described in the present work are in agreement with the rules of the Animal Research Ethics Committee of Universidade Federal de Sergipe (UFS).

2.2. Preparation of the aqueous fraction of the hydroalcoholic extract of the leaves of Sida cordifolia

S. cordifolia leaves were collected from the herbal garden of the Federal University of Sergipe (Latitude: 10° 55' 23.32" S and Longitude: 37° 6' 10.40" w), Sergipe State, Brazil, deposited in the Department of Biology of the Federal University of Sergipe, Brazil (voucher specimen no. 30171) were dried at 40 ºC in an oven with air circulation and pulverized. The powder was extracted by repeated maceration with 70% ethanol in water at room temperature (25–30 ºC), for 72 h using a stainless steel percolator. The resulting extract was dried at 60 ºC under reduced pressure. A portion of this extract was dissolved in distilled water, filtered and known volumes were dried to determine the water-soluble fraction (72%). This factor was used to calculate the final concentration of the AFSC. When required, the extract was dissolved in a distilled water/cremophor EL solution and diluted to desired concentrations. The final concentration of cremophor EL in the bath never exceeded 0.01% and was without effect when tested in control preparations (data not shown).

2.3. Isolated rat aortic rings preparations

Aortic rings (2-4 mm) were obtained free from connective tissue and fat. Then, they were suspended by platinum hooks in a organ bath containing 10 mL Krebs Henseleit solution (composition in mM: NaCl 118.0, KCl 4.6, CaCl2.2H2O 2.5, NaHCO3 25.0, glucose 11.0, MgCl2.7H2O 5.7 and KH2PO4; pH 7.4) maintained at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2, for isometric tension recordings. The rings were allowed to equilibrate for 1h under a resting tension of 1g. During this time, the solution was changed every 15 min to protect against interfering metabolites [9]. The presence of functional endothelium was assessed by the ability of acetylcholine (1 μM) to induce more than 60 % relaxation of vessels pre-contracted with phenylephrine (1 μM). In some experiments, the endothelium was removed by rubbing the intimal surface of the vessels with a cotton swab. Removal of functional endothelium was verified by the lack of any relaxation to acetylcholine (1 μM) in rings pre-contracted with phenylephrine (1 μM).

2.4. Drugs

The drugs used were: cremophor EL, N^w-nitro-*L-arginine* metyl ester (L-NAME), acetylcholine chloride, indomethacin, prazosin, yohimbine (all purchased from SIGMA, St. Louis, MO, USA). Indomethacin was dissolved in 0.5 % w/v sodium bicarbonate. The other compounds were freely dissolved in distilled water.

2.5. Experimental protocols

After stabilization period of 60 min., AFSC (0.01, 0.03, 0.1, 0.3, 1 and 3 mg/mL) was cumulatively added to the bath. The AFSC-induced response was evaluated in rat aortic rings with and without endothelium, and after incubation separately with L-NAME (100 μM), an inhibitor of the NO-synthase [10], indomethacin (10μM), an inhibitor of the cyclooxygenase (COX) [11], prazosin (1 μM), a selective α_1-adrenergic receptor antagonist [12] and yohimbine (3 μM), a selective α_2-adrenergic receptor antagonist [13]. These inhibitors were added in the bath 30 min. before the application of AFSC. The contractions were measured by percentage of maximal response (E_{max}) and the values of concentration required to produce 50% of maximal response (EC_{50}) were calculated as subsequently described.

2.6. Statistics

Values are expressed as mean ± SEM. When appropriate, *student´s t-test* was done to evaluate the significance of the differences between means. EC_{50} values were calculated by non-linear regression of individual concentration-response curves using Graph Pad Prism, version 3.0.2 software (San Diego, CA, USA).

3. RESULTS

3.1. Effect of AFSC in rat aortic rings with and without endothelium

AFSC (0.01 – 3 mg/mL) induced concentration-dependent contractions in aortic rings with and without functional endothelium, however, in the absence of the endothelium, the concentration-response curves to AFSC were significantly shifted to the left with increase of E_{max} values (Figure 1; Table 1).

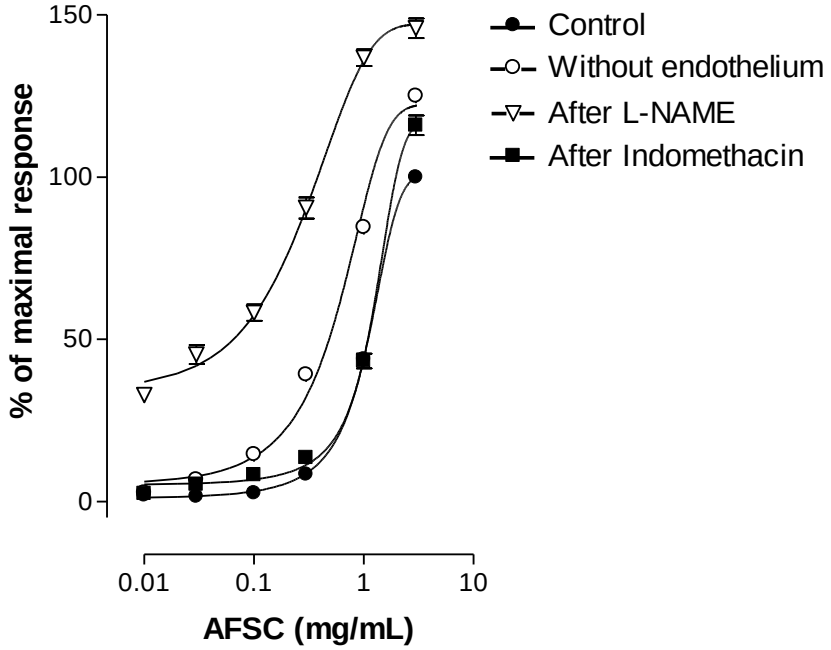

Figure 1: Concentration-response curves to AFSC in rat aorta with (●) and without functional endothelium (○), and in the presence of L-NAME (▽) or indomethacin (■). Values are mean ± SEM of

3.2. Effect of L-NAME or indomethacin on AFSC-induced contractile effect in intact rat aorta

In the presence of L-NAME (100 μM), the concentration-response curves to AFSC were significantly shifted to the left. When the preparations were incubated with indomethacin (10 μM), these concentration-response curves were not changed. E_{max} values were increased in both experimental conditions (Figure 1; Table 1). Interestingly, EC_{50} and E_{max} values in the presence of L-NAME were greater than those obtained in aortic rings without endothelium (Table 1).

Table 1 – EC_{50} and E_{max} values for the contractile effect of AFSC in rat aortic rings, in different experimental conditions.

Experimental conditions	EC_{50} (mg/mL)	E_{max} (%)
Control (with endothelium)	1.23 ± 0.09	100 ± 5.2
Without endothelium	0.84 ± 0.1 **	125 ± 4.5 **
L-NAME (100 µM)	0.54 ± 0.15 ** #	146 ± 6.8 ** #
Indomethacin (10 µM)	1.28 ± 0.05	116 ± 8.0 *
Prazosin (1 µM)	1.40 ± 0.01 *	22 ± 3.9 **
Yohimbine (3 µM)	1.22 ± 0.06	71 ± 6.5 **

*Values are means ± SEM of six experiments. * p>0.05, ** p>0.01 vs. control. # p>0.05 vs. without endothelium.*

3.3. Effect of prazosin or yohimbine on AFSC-induced contractile effect in intact rat aorta

The incubation of the preparations with prazosin (10 µM) significantly shifted to the right the concentration-response curves to AFSC, with change of E_{max} values. On the other hand, after incubation with yohimbine (3 µM), any shift was observed, but the E_{max} values were decreased (Figure 2; Table 1).

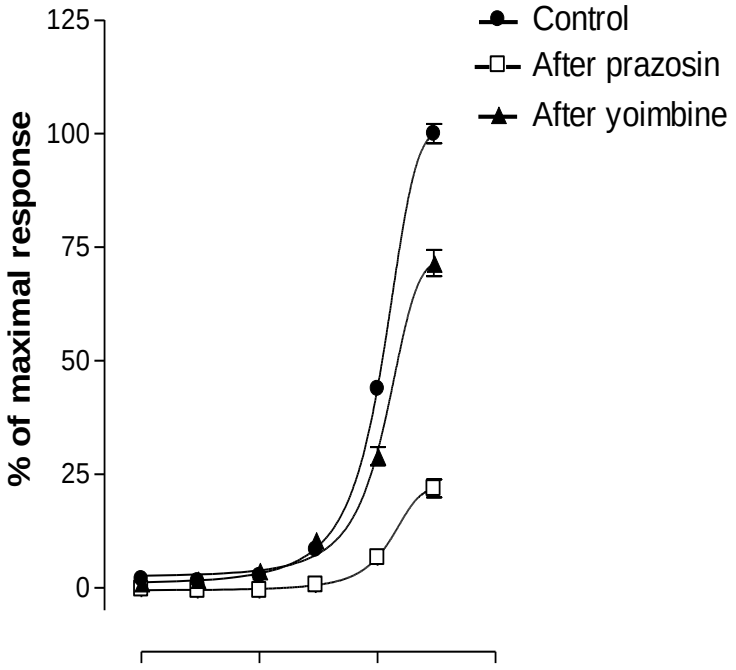

Figure 2: Concentration-response curves to AFSC in rat aorta with functional endothelium (●) and in the presence of prazosin (□) or yohimbine (▲). Values are mean ± SEM of six

4. DISCUSSION

The major finding of this study is that AFSC induced concentration-dependent contractions in rat aortic rings by activation of α_1-adrenergic receptor.

In the endothelium denuded aorta rings, AFSC-induced concentration-response curves were significantly shifted to the left, indicating that contractile effect induced by AFSC was negatively modulated by the endothelium.

The endothelium plays an important role in the control of the vascular tone through the release of endothelium derived relaxant factors (EDRFs), including NO and COX-derived relaxant products [10]. In order to investigate the participation of these EDRFs in the effect induced by AFSC, the rings were incubated with L-NAME or indomethacin. In these conditions, only the L-NAME was able of shifting the concentration-response curves to the left, suggesting that the NO, but not COX-derived relaxant products, exerts a negative modulation of this response.

Interestingly, the shifts observed in the rings incubated with L-NAME were significantly greater than those observed in the rings without endothelium, suggesting that other sources of NO, that not endothelial, also could be involved in the modulation of the contractile effect induce by AFSC. It is well reported in the literature that NO-containing nerve fibers, called nitrergic innervations, are present in the wall of several blood vessels and may participate in the modulation of vasomotor response [14]. Furthermore, the expression of neuronal NOS has been demonstrated in rat vascular smooth muscle [15]. Thus, we hypothesized that the non-endothelial NO involved in the modulation of the contractile effect induced by AFSC may possibly be linked to this neuronal source. However, further experiments are necessary to clearly elucidate this point.

Several studies have demonstrated that the activation of the α_1- and α_2-adrenergic receptors in the vascular smooth muscle induces contraction [16]. Furthermore, in the chemical study has been demonstrated the presence of ephedrine and pseudoephedrine, amines sympathomimetic with potent vasoconstrictor action, in the leaves of *S. cordifolia* [4]. Thus, we hypothesized that this effect could be due to an activation of these receptors. In order to investigate a role of the α_1- and α_2-adrenergic receptors in the contractile effect evoked by AFSC, we performed experiments in the presence of prazosin, a selective α_1-adrenergic receptor antagonist [12], or yohimbine, a selective α_2-adrenergic receptor antagonist [13]. In these conditions, only prazosin was able to significantly shift to right the concentration-response curves to AFSC, suggesting that the activation of α_1-adrenergic receptor is very important to expression of this effect.

Contradictory findings were obtained by Santos [8], which demonstrated a vasodilator activity of this extract in rat superior mesenteric artery. This can be explained because the experiments were performed in mesenteric artery and previous studies have demosntrated differential responses of rat aorta and mesenteric artery to norepinephrine, a α_1-adrenergic agosnist [17].

It is popularly known that the tea of *S. cordifolia* leaves is very used for treatment of nasal congestion. Similarly to this, drugs with α_1-adrenergic agonist activity, such as naphazolline, phenylpropanolamine, phenylephrine, including the ephedrine and pseudoephedrine [18], which are founded in the leaves of *S. cordifolia* [4], also are used for treatment of this symptom. Then, our findings can provide scientific basis to the use this plant as a decongestant in according to popular use.

In conclusions, these results show that AFSC induces contractions in the rat aorta that seem to be due to an activation of α1-adrenergic receptors. Furthermore, this effect seems to be negatively modulated by the endothelium and by the release of NO.

5. ACKNOWLEDGEMENTS

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