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CARDIOVASCULAR EFFECTS AND ACUTE TOXICITY OF THE AQUEOUS EXTRACT OF *COSTUS SPICATUS* LEAVES (ZINGIBERACEAE)

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ABSTRACT

Cardiovascular effects and acute toxicity of the aqueous extract of *Costus spicatus* leaves (AECS) were studied in rats and mice. In non anaesthetized normotensive rats (n = 6), AECS (0.5, 1.0, 5.0 and 10.0 mg/kg; i.v., randomly) induced hypotension associated with tachycardia. In intact or endothelium-denuded rings of rat superior mesenteric artery, AECS (0.1 - 100.0 µg/mL, n = 4) provoked relaxation of the tonus induced by 10 µM phenylephrine. The extract showed low acute toxicity in mice.

Keywords: *Costus spicatus*, aqueous extract, cardiovascular effects, acute toxicity.

RESUMO

Os efeitos cardiovasculares e a toxicidade aguda do extrato aquoso das folhas de *Costus spicatus* (AECS) foram estudados em ratos e camundongos. Em ratos normotensos não-anestesiados, (n = 6), AECS (0.5,1.0, 5 e 10.0 mg/kg; i.v., aleatoriamente) induziu hipotensão associado com taquicardia. Em anéis intactos ou sem endotélio da artéria mesentérica superior de rato, AECS (0.1 - 100.0 µg/mL, n = 4) provocou relaxamento do tônus induzido por 10 µM de fenilefrina. O extrato demonstrou baixa toxicidade aguda em camundongos.

Palavras-chave: *Costus spicatus*, extrato aquoso, efeitos cardiovasculares, toxicidade aguda.

INTRODUCTION

The plant *Costus spicatus* Swartz (Zingiberaceae) is a bush that grows up to 2 meters and can be found in the coastal brasilian forest. Popularly known as "cana-do-brejo" or "cana-de-macaco", this plant is used in folk medicine as diuretic (Martinez, 1984), for treatment of colds, sore throats, dysentery, diarrhea (Cruz, 1965), bladder, urethra and kidney complaints (Manfred, 1947).

Phytochemical analysis of *C. spicatus* rhizomes and leaves showed the presence of flavonoids (Williams & Harbone, 1977), flavonol glicosides (Silva *et al*, 2000), saponins and sapogenins (Silva *et al*., 1999a, 1999b). Other studies on this plant revealed antimicrobial (Misas *et al.*, 1979), haemolytic (Silva *et al*., 1999a, 1999b) and diuretic activities (Caceres *et al*., 1987; Souza *et al*, 2004). The objective of this study

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is to evaluate the cardiovascular effects of AECS in rats, through the combined use of the *in vivo* and *in vitro* approaches.

MATERIAL AND METHODS

Plant: The plant *Costus spicatus* was collected around the city of Aracaju (10º54'S, 37º04'W). The voucher specimen was identified and deposited in the Herbarium of the Biology Departament at the Universidade Federal de Sergipe.

Aqueous extract: The AECS was obtained from dried leaves (40g of powder) by an infusion with distilled water (1 L/100° C) followed by filtration. The filtrate was lyophilized (aqueous extract) and stored at 4º C. When required, extract was dissolved in a saline solution, for in vivo experiments, or nutritive solution, for in vitro experiments, at the desired concentrations.

Animals: Male Wistar rats (200 - 300 g) were used for the cardiovascular experiments; male Swiss mice (20-35g) were used in the toxicity experiments. Animals were housed under conditions of controlled temperature and lighting (lights on: 06:00 - 18:00 hours) and had free access to food and tap water.

Drugs: The drugs used were: acetylcholine chloride (Ach), L-phenylephrine chloride (Phe), (all from SIGMA), sodium nitroprusside (SNP), heparin sodium salt (ARISTON) and sodium thiopental (CRISTÁLIA). All compounds were freely dissolved in distilled water (for in vitro experiments) or saline (for in vivo experiments).

Acute toxicity: Experimental groups of 6 mice received orally doses of 1, 3 and 5 g/kg of AECS, while the control group received only vehicle (distilled water). All groups were observed for 48 hours; at the end of this period the mortality was recorded for each group (Dietrich, 1983).

Blood pressure measurements: Under sodium thiopental anesthesia (45 mg/kg, i.v.), the lower abdominal aorta and inferior vena cava were canulated via the left femoral artery and vein using polyethylene catheters. The catheters were then filled with heparinized saline solution and slid 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 (Edwards Lifescience, Irvine, CA, USA) and pressure outputs were recorded by an amplifier-recorder (BioData, Model BD-01, PB, Brasil) connected to a personal computer equipped with an analog-to-digital converter board (BioData, PB, Brasil). Data were collected at a frequency of 200 Hz. For each cardiac cycle, the computer calculated mean arterial pressure (MAP) and pulse interval, referred to here as the heart rate (HR). The venous catheter was inserted for drug administration. Sodium nitroprusside (10 µg/kg) was injected to check the efficacy of catheter insertion.

Rat superior mesenteric artery rings: Rats were killed by stunning and exsanguination. The superior mesenteric artery was removed, cleaned from connective tissue and fat, and sectioned in rings (1 - 2 mm), which were suspended by cotton threads in organ baths containing 10 ml of Tyrode´s solution (Composition mM: NaC 158.3, KCl 4.0, $CaCl_2 2H_2O$ 2.0, $NaHCO_3$ 10.0, $C_6H_{12}O_6$ 5.6, $MgCl_2.6H_2O$ 1.05 and $NaH_2PO_4H_2O$ 0.42), gassed with carbogenic mixture (95 % O_2 and 5 % CO_2) and maintained at 37º C for isometric tension recordings. The stabilization period was of 1 h under a resting tension of 0.75 g. During this time the solution was changed every 15 min, to prevent the accumulation of metabolites. The isometric tension was recorded through a force transducer (Gould, Model GM2, USA) coupled to an amplifier-recorder (Gould, USA). Endothelium was removed by gently rubbing the intimal surface of the vessels. The

presence of functional endothelium was assessed by the ability of acetylcholine (ACh) (10 µM) to induce more than 70 % relaxation of pre-contracted vessels with phenylephrine (10 µM). The absence of the relaxation to ACh was taken as evidence that the vessel segments were functionally denuded of endothelium.

In vivo experiments: After cardiovascular parameters had been stabilized, MAP and HR were recorded before (baseline values) and after i.v. administration of randomized doses of AECS (0.5, 1.0, 5.0 and 10.0 mg/ kg). Dose-response curves were then obtained. These administrations were separated by a time interval sufficient to allow full recovery of cardiovascular parameters. A control dose-response curve was then obtained.

In vitro experiments: After the stabilization period, two successive contractions of similar magnitude were induced with 10 µM Phe in rings with or without endothelium. During the tonic phase of the third contraction, different concentrations of AECS (0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0 µg/ml) were added cumulatively to the organ bath. The relaxations were measured by comparing the developed tension before and after the addition of AECS and expressed as percentage of relaxation from induced tonus.

Statistics: Values are expressed as means ± SEM. When appropriate, Student´s t test was conducted in order to evaluate the significance of the differences between two means. All procedures were carried out on Graph Pad Prism™ version 3.02 software.

RESULTS

In non-anaesthetized normotensive rats, baseline MAP and HR values were 117 ± 1 mmHg and 369 ± 10 bpm, respectively. In these animals, the intravenous *in bolus* injections of AECS (0.5, 1, 5 and 10 mg/kg) induced a light and transitory hypotension (-7.4 ± 0.5; -14.9 ± 0.6; -11.5 ± 0.4 and –10.7 ± 0.4 %) associated with taquycardia (2.2 ± 0.3; 8.9 ± 0.4; 5.7 ± 0.3 and 9.4 ± 0.4 %). The effects to each dose were fully recovered after 30 seconds (Figure 1). In rat isolated rings of the superior mesenteric artery with intact endothelium, AECS (0.1, 0.3, 1.0, 3.0, 10.0, 30.0 and 100.0 µg/mL, n = 4, cumulatively) induced relaxations of tonus induced by 10 µM phenylephrine, which was not significantly affected after the endothelium remotion (Figure 2). The AECS did not present toxicity up to the oral dose of 5g/kg, so the LD50 could not be determined.

DISCUSSION

We chose to evaluate the effects of AECS on the cardiovascular parameters in non-anaesthetized rats, in order to avoid anesthesia and surgical stress influences (Smith & Hutchins, 1980; Fluckiger *et al.*, 1985). Baseline MAP and HR values were analogous to those previously reported in other studies (Lahlou *et al.*, 2002; Cunha *et al.*, 2004). In these animals, acute administration of AECS induced light hypotension associated with tachycardia.

Peripheral vascular resistance mainly maintains the blood control pressure and the major contributor is the vascular tone of several arterial beds (White *et al.*, 1996), as the mesenteric one (Mulvany & Aalkjaer, 1990). In order to verify if hypotensive response could be induced by the decrease of the peripheral vascular resistance due to a possible vasorelaxation, we performed experiments using rings from the rat superior mesenteric artery, an *in vitro* approach. In these preparations, AECS induced vasorelaxation suggesting that the hypotensive response appears to be due to a direct action on the peripheral vascular resistance.

It is well known that the endothelium is an important regulator of the vascular tone by releasing

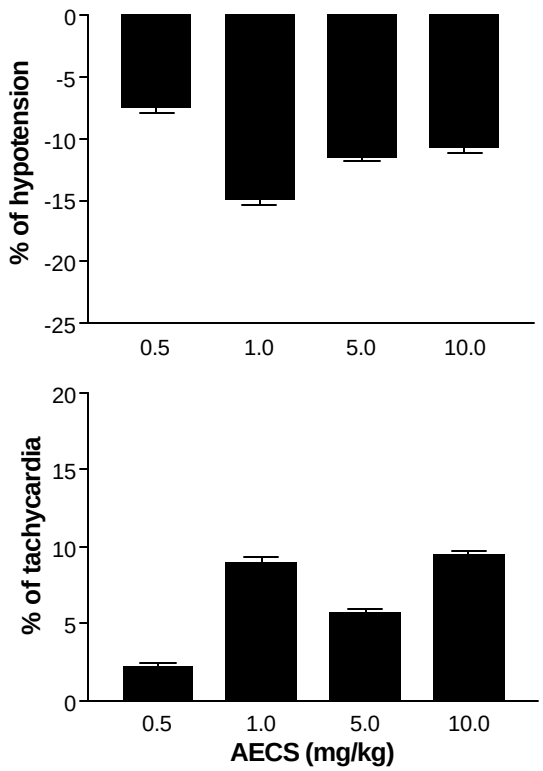

Figure 1. Hypotensive and tachycardic response induced by AECS (0.5, 1.0, 5.0, 10.0 mg/Kg; i.v.) in non-anaesthetized normotensive rats. Values are expressed as mean ± SEM.

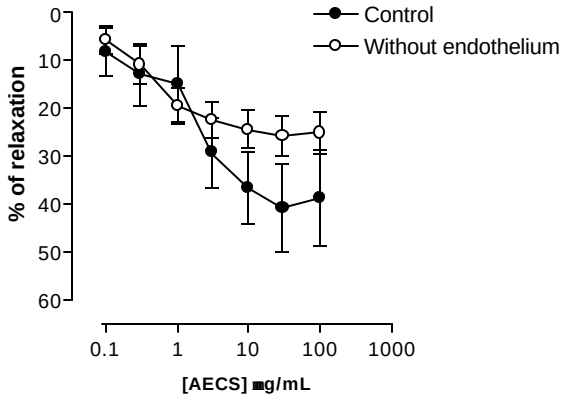

Figure 2 – Concentration-response curves to AECS (0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0 µg/ml) in rat superior mesenteric artery rings pre-contracted with 10.0 µM. Values are expressed as mean ± SEM. * $p < 0.05$ vs Control.

endothelium-derived relaxing factors (Moncada *et al*., 1991), mainly NO and COX-derived products, such as PGI_2 (Furchgott & Zawadzki, 1980; Moncada *et al.*, 1991). In order to investigate the participation of the endothelium in the vasorelaxant response induced by AECS, we performed experiments in the absence of functional endothelium. In these conditions, the vasorelaxant response induced by AECS was not significantly changed. This suggests that the presence of endothelium is not essential for relaxant responses expressions and that an endothelium-independent pathway is probably implicated in this effect.

The cardiovascular effects of AECS may be due to the presence of flavonoids (Williams & Harbone, 1977), such as, quercetin, kaempferide, tamarixetin and isorhamnetin 3-O-neohesperidoside (Silva *et al*, 2000), which have vasodilator properties (Perez-Viscaino *et al*, 2002) possibly involving opening potassium channels (Calderone *et al*, 2004). These cardiovascular effects associated with the recent discovery of *Costus spicatus* diuretic effect (Souza *et at*, 2004) showed that this medicinal plant has a great therapeutic potential, because the association between diuretic and vasorelaxant effects are frequently used in treatment of hypertension and other cardiovascular diseases (World Health Organization, 1999; Boydak *et al.*, 2004). However, further pharmacological studies are necessary to confirm this assumption.

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