Cardiovascular effects of *Hyptis fruticosa* essential oil in rats

M.R.V. Santos a,⁎, A.A. Carvalho a, I.A. Medeiros b, P.B. Alves c, M. Marchioro a, A.R. Antoniollia

a Laboratório de Farmacologia Cardiovascular, Universidade Federal de Sergipe, Av. Marechal Rondon, S/N, Rosa Elze, CEP: 49100-000, São Cristóvão, SE, Brazil
b Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, João Pessoa, PB, Brazil
c Departamento de Química, Universidade Federal de Sergipe, São Cristóvão, SE, Brazil

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Abstract

In non-anesthetized normotensive rats, *Hyptis fruticosa* essential oil (HFEO, 5, 10, 20 and 40 mg/kg; i.v.) induced hypotension associated with tachycardia. In intact and isolated rings of rat superior mesenteric artery (control), HFEO (1–1000 μg/ml, n = 6, cumulatively) induced concentration-dependent relaxations of tonus induced by 10 μM phenylephrine (Phe) (pD2 = 2.6 ± 0.27; Emax = 64 ± 8.3%). In denuded endothelium pre-contracted rings with Phe or K+-depolarizing solution (80 mM), the concentration–response curves to HFEO were not shifted (pD2 = 2.3 ± 0.25 and 2.3 ± 0.28, respectively), but their maximal responses were significantly (P < 0.05 vs control) increased (Emax = 122.3 ± 18.2% and 92 ± 3.6%, respectively). HFEO was also capable of antagonizing the concentration–response curves to CaCl2 (3 μM–30 mM) in a dose-dependent manner.

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1. Introduction

The use of medicinal plants for the treatment of human diseases has increased considerably worldwide. Evaluation of the effects of these plants on organs and systems contributes to the development of the scientific basis for their therapeutic application, and also enriches considerably the therapeutic arsenal for the treatment of a number of diseases [1].

*Hyptis* has ca. 400 species distributed mainly at the central states of Brazil [2]. Various species are used in folk medicine as antiinflammatory, antinociceptive, anticonvulsant and antiulcerogenic [3–6].

*Hyptis fruticosa* popularly known as “alecrim do campo” or “alecrim do vaqueiro” is an aromatic sub-bush plant which grows up to 1.5 m found on the Brazilian northeast coast. Previous studies have demonstrated that the aqueous extract, the ethanolic extract, and the essential oil from *H. fruticosa* presented analgesic and anticonvulsive activities.

Considering that there are no pharmacological studies relating the effects of *H. fruticosa* to the cardiovascular system, this work aimed to evaluate the cardiovascular effects of HFEO in rats using in vivo and in vitro studies.
2. Experimental

2.1. General

The drugs used were: sodium thiopental (CRISTÁLIA), heparin sodium salt (ARISTON), acetylcholine chloride (Ach), l-phenylephrine chloride (Phe) and cremophor (a derivative of castor oil and ethylene oxide used to emulsify water-insoluble substances) (SIGMA). All compounds were freely dissolved in distilled water (for in vitro experiments) or saline (for in vivo experiments).

2.2. Plant

_H. fruticosa_ Salzm., ex Benth (Lamiaceae), collected near São Cristóvão, Brazilian State of Sergipe was identified by Prof. Dr. A. S. Ribeiro, Botanist in the Biology Department, Universidade Federal de Sergipe. A voucher specimen was deposited in the Herbarium of the Biology Department, Universidade Federal de Sergipe. Code 007912.

2.3. Extraction

The HFEO was obtained from fresh leaves by steam distillation and stored at 4 °C. Oil was dissolved in a saline/cremophor (0.1% v/v) solution for in vivo experiments and in water distilled/cremophor (0.1% v/v) solution for in vitro experiments. Cremophor had no effect when tested in control condition (data not shown).

2.4. Animals

Male Wistar rats (200–350 g) were used for all experiments. Animals were housed under standard environmental conditions, fed with rodent diet and tap water ad libitum.

2.5. Solutions

The composition of the normal Tyrode’s solution used was: NaCl 158.3, KCl 4.0, CaCl$_2$·2H$_2$O 2.0, NaHCO$_3$ 10.0, C$_6$H$_{12}$O$_6$ 5.6, MgCl$_2$·6H$_2$O 1.05 and NaH$_2$PO$_4$·H$_2$O 0.42 mM. K+-depolarizing solutions (KCl 60 mM and KCl 80 mM) were done by replacing 60 or 80 mM KCl in the Tyrode’s solution with equimolar NaCl, respectively. In the nominally without Ca$^{2+}$ solution, CaCl$_2$ was omitted.

2.6. Measurement of mean arterial pressure and heart rate in non-anesthetized normotensive rats

For measurement of mean arterial pressure (MAP) and heart rate (HR), rats were anesthetized with sodium thiopental (45 mg/kg, i.p.). A polyethylene catheter was inserted into the abdominal aorta via the left femoral artery for pressure recordings. Another catheter was inserted into the lower vena cava via the left femoral vein for the administration of drugs. Both catheters were filled with heparinized saline and led under the skin to exit between the scapulae. After 24 h of surgery, rats were placed in large individual cages and experiments were performed in non-anesthetized 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). For each cardiac cycle, the computer calculated the MAP, and pulse interval (referred to as heart rate).

After cardiovascular parameters had stabilized, MAP and HR were recorded before (baseline values) and after i.v. administration of randomized doses of HFEO (5, 10, 20 and 40 mg/kg). Successive injections were separated by a time interval sufficient to allow full recovery of cardiovascular parameters.

2.7. Preparation of isolated 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 in organ baths containing 10 ml of
Tyrode’s solution, gassed with a mixture of 95% O₂ and 5% CO₂ and maintained at 37 °C. Isometric tension was recorded under a resting tension of 0.75 g. During the stabilization period the solution was changed every 15 min [7]. 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.

2.7.1. HFEO effect on phenylephrine (10 μM) induced tonus in isolated rat superior mesenteric artery rings with or without endothelium

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 HFEO (1, 3, 10, 30, 100, 300 and 1000 μg/ml) were added cumulatively to the organ bath. The relaxations were measured by comparing the developed tension before and after the addition of HFEO and expressed as percentage of relaxation from induced tonus.

2.7.2. Effect of HFEO on contraction induced by KCl in rings without endothelium

After the stabilization period, rings without endothelium were pre-contracted with K⁺-depolarizing solutions (KCl 80 mM) and, on the tonic phase, different concentrations of HFEO (1, 3, 10, 30, 100, 300 and 1000 μg/ml) were added cumulatively to the organ bath. The relaxations were measured as previously described.

2.7.3. Effect of HFEO on concentration–response curves to CaCl₂ in rings without endothelium

After the stabilization period, the rings without endothelium were contracted with K⁺-depolarizing solution (KCl 60 mM) and washed with normal Tyrode’s solution until full recovery of initial tension. After this, they were incubated with nominally without Ca²⁺ solution for 15 min and afterwards exposed to nominally without Ca²⁺ solution with KCl of 60 mM for another 15 min [8]. Then, a first cumulative concentration–response curve to CaCl₂ (3×10⁻⁶, 10⁻⁵, 3×10⁻⁵, 10⁻⁴, 3×10⁻⁴, 10⁻³, 3×10⁻³, 10⁻² and 3×10⁻² M) was obtained. In these same preparations, HFEO (0.3, 3,
30 and 300 μg/ml) was individually pre-incubated for 15 min and a second cumulative concentration–response curve

to CaCl₂ was obtained. This curve was compared with that obtained in the absence of HFEO and the results were

expressed as percentages of the maximal response to CaCl₂ alone.

2.8. Statistical analysis

Values were expressed as the mean±SEM. When appropriate, Student’s t-test or one-way ANOVA was done to

evaluate the significance of the differences between means. The EC₅₀ values were obtained by non-linear regressions of

concentration–response curves and pD₂ values were calculated through the negative logarithm of the EC₅₀. All these

procedures were done by using Graph Pad Prism™ version 3.02 software.

3. Results

In non-anaesthetized normotensive rats, baseline MAP and HR values were 110±2 mm Hg and 329±6 bpm,

respectively. In these animals, the intravenous bolus injections of HFEO (5, 10, 20 and 40 mg/kg) induced a transitory
reduction of blood pressure from 6 to 19% associated with an increase in heart rate from 2 to 16% (Fig. 1). The effects to each dose were fully recovered after 30 s (data not shown).

In intact isolated superior mesenteric artery rings of rats, HFEO (1, 3, 10, 30, 100, 300 and 1000 μg/ml, cumulatively) induced concentration-dependent relaxations of tonus induced by 10 μM phenylephrine (pD2=2.6±0.27; Emax=64±8.3%). In rings without endothelium pre-contracted with Phe or K+-depolarizing solution (KCl 80 mM), the concentration–response curves to HFEO were not significantly shifted (pD2=2.3±0.25 and 2.3±0.28, respectively), but their maximal responses were significantly increased (Emax=122.3±18.2% and 92±3.6%, respectively, P<0.01) (Fig. 2).

As showed in Fig. 3, CaCl2 (3×10−6, 10−5, 3×10−5, 10−4, 3×10−4, 10−3, 3×10−3, 10−2 and 3×10−2 M) induced contractions in denuded rat mesenteric artery rings in a dose-dependent manner that were strongly inhibited after incubation with HFEO in doses of 3, 30 and 300 μg/ml (Emax=12±2; 41±2.4 and 81±2%, respectively).

4. Discussion

In this work, we chose to evaluate the effects of HFEO on the blood pressure and heart rate in non-anesthetized rats in order to avoid anesthesia and surgical stress influences [9,10]. Baseline MAP and HR values were in agreement to those previously reported in other studies [11–14]. In these animals, acute intravenous administration of HFEO induced hypotension associated with tachycardia.

The control of blood pressure is mainly maintained by peripheral vascular resistance and the major contributor is the vascular tone of several arterial beds [15], so is the mesenteric bed [16]. In order to verify if the hypotensive response could be induced by the decrease in the peripheral vascular resistance due to a possible vasorelaxation, we performed in vitro experiments using rings from the isolated rat superior mesenteric artery. In these preparations, HFEO induced vasorelaxation in a concentration-dependent manner of Phe-induced tonus, suggesting that the hypotensive response induced by HFEO may be due to a direct action on the peripheral vascular resistance. These initial results are in agreement with those of other studies that had demonstrated that several essential oils present a potent hypotensive effect through a direct vasorelaxant effect [13,14,17–19]. Furthermore, HFEO contains α-pinene and caryophyllene, substances with potent smooth muscle relaxant activity [20,21], and 1,8 cineole, with hypotensive activity [11]. Then, it is possible to hypothesize that the effects induced by HFEO could be due to the presence of these compounds. However, further experiments are necessary to clearly elucidate this assumption.

It is well known that the endothelium is an important regulator of the vascular tone by releasing endothelium-derived relaxing factors [22], mainly NO and COX-derived products, such as PGI2 [22,23]. In order to investigate the participation of the endothelium in the vasorelaxant response induced by HFEO, we performed experiments in the absence of functional endothelium. In these conditions, the vasorelaxant response induced by HFEO was increased. This suggests that the presence of endothelium is not essential for relaxant response expressions and that an endothelium-independent pathway is probably implicated in this effect.

Calcium is the primary regulator of tension in vascular smooth muscle [24]. It is well known that the maintenance of smooth muscle contraction depends on Ca2+ influx from extracellular space through voltage and/or receptor operated calcium channels (VOCCs and/or ROCCs, respectively) [25]. It is well reported that the increase in external K+ concentration (KCl 80 mM) induces smooth muscle contraction through VOCCs activation and subsequent calcium release from the sarcoplasmic reticulum [25]. The high K+-induced contraction is inhibited by Ca2+ channel blockers or by removal of external Ca2+ and is, therefore, entirely dependent on Ca2+ influx [25]. Thus, we evaluated the HFEO effect on intact rings pre-contracted with K+-depolarizing solutions (KCl 80 mM). This set of experiments revealed that HFEO-induced vasorelaxations, which were more efficacious in this experimental condition than in those in rings pre-contracted with Phe, suggest that HFEO appears to inhibit Ca2+ influx through VOCCs.

In order to check the hypothesis above, we constructed a concentration–response curve to CaCl2 in high K+ solution before and after incubation with HFEO. In these conditions, HFEO was capable of antagonizing the CaCl2-induced contractions in a concentration-dependent manner. As reported by Chan et al. in Ref. [26] nifedipine, a L-type voltage-operated Ca2+ channel blocker, also inhibited the concentration–response curve to CaCl2, suggesting strongly that HFEO could be acting possibly as a calcium channel blocker.

In conclusion, the results obtained in this work showed that HFEO induces hypotensive effect with a contemporaneous increase in heart rate probably of reflex origin. This hypotensive effect may probably be due to a...
direct vasodilatation and a consequent decrease in the peripheral vascular resistance. This vasodilatation seems to be due to an inhibition of the Ca\(^{2+}\) influx through voltage-operated Ca\(^{2+}\) channels.

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