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Endothelium-dependent vasodilation induced by *Hancornia speciosa* in rat superior mesenteric artery

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Abstract

The vasodilator effect of the ethanolic extract of leaves from *Hancornia speciosa* Gomes (HSE) was evaluated in superior mesenteric artery rings. HSE produced a concentration-dependent vasodilation ($IC_{50} = 10.8 \pm 4.0$ μg/mL) in arterial rings pre-contracted with phenylephrine, which was completely abolished in endothelium-denuded vessels. Endothelium-dependent vasodilation induced by HSE was strongly reduced by L-NAME (100 μM), a nitric oxide (NO) synthase inhibitor, but neither by atropine, a muscarinic receptor antagonist (1 μM), nor by indomethacin (10 μM), a cyclooxygenase inhibitor. In rings pre-contracted with 80 mM KCl, the vasodilator effect of HSE was shifted to the right and was completely abolished in the presence of L-NAME (100 μM). Similar effects were obtained in mesenteric rings pre-contracted with phenylephrine in the presence of KCl 25 mM alone or in addition to 100 μM L-NAME. In addition, $BaCl_2$ (1 mM) dramatically reduced the vasodilation induced by HSE. Together, these findings led us to conclude that HSE induces an endothelium-dependent vasodilation in rat mesenteric artery, by a mechanism dependent on NO, on the activation of potassium channels and endothelium-derived hyperpolarizing factor release. Rutin, identified as a major peak in the HPLC fingerprint obtained for HSE, might contribute for the observed vasodilator effect, since it was able to induce an endothelium-dependent vasodilation in rat superior mesenteric arteries.

Keywords: Resistance artery; Vasodilation; Endothelium; Nitric oxide; EDHF; *Hancornia speciosa*

Introduction

Hancornia speciosa Gomes (Apocynaceae), popularly named "mangaba", is a plant species found in *cerrado*, a savanna like vegetation occurring in Brazil. The bark of the species is popularly used to treat dermatosis, diabetes, hepatic diseases and as anti-inflammatory agent, whereas its roots and leaves are also employed as astringent, stomachic, to treat rheumatism and hypertension (Grandi et al., 1982; Britto and Britto, 1982; Hirschmann and Arias, 1990).

Recently, we have demonstrated that the ethanolic extract of leaves from *Hancornia speciosa* (HSE) inhibits angiotensin I-converting enzyme (ACE) (Serra et al.,

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2005). This report drove us to further investigate the cardiovascular effects induced by HSE, since other ACE inhibiting plants, such as *Ouratea semiserrata* and *Cecropia glaziovii*, also exhibited strong vasodilator activity (Braga et al., 2000; Lacaille-Dubois et al., 2001; Cortes et al., 2002). In the present work, we describe an endothelium-dependent vasodilator effect of HSE in superior mesenteric artery rings of rats, through a mechanism dependent on release of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). Besides, based on the HPLC fingerprint obtained for HSE, the compound that might contribute for the observed activity is also discussed.

Materials and methods

Plant material

The leaves of *Hancornia speciosa* Gomes (Apocynaceae) were collected in São Gonçalo do Rio Preto, Minas Gerais state, Brazil, in November 1999. The species was identified by Dr. J.A. Lombardi, from the Botanical Department, Instituto de Ciências Biológicas, UFMG, Belo Horizonte, Brazil and a voucher specimen (BHCB 3565) is deposited at the UFMG Herbarium.

Extract preparation

After drying at 40 °C, during 72 h, the plant material was powdered (10 g) and extracted with 96% EtOH (3 × 30 mL) under sonication (3 × 15 min). The solvent was vacuum removed in a rotavapor evaporator, at 70 °C, furnishing a dark residue (1180 mg).

HPLC characterization of HSE

Analyses were carried out on a Waters 2995 system (USA) composed of quaternary pump, auto sampler, photodiode array detector (model 2996) and Empower software for data processing. An ODS column (250 × 4.0 mm I.D., 5 μm) was employed (Merck, Germany) at a temperature of 40 °C and flow rate of 1.0 mL/min. UV-photodiode array detection (DAD) was performed at 254 nm. UV spectra from 210 to 400 nm were recorded on line for peak identification. A linear gradient of H_2O (A) and CH_3CN (B) was employed: 0 min 95% A, 5% B; 60 min 5% A, 95% B, followed by 5 min of isocratic elution. Solvents used were of HPLC grade (Merck, Germany) and were degassed by sonication before use. Samples were dissolved in MeOH to concentrations of 2 and 10 mg/mL, respectively, for rutin and HSE. After centrifugation at 8400*g* for 5 min, the sample solutions (10 μL) were automatically injected onto the apparatus.

Superior mesenteric artery rings preparation and mounting

Animal experiments were performed according to the recommendations of the Brazilian Council for Animal Care and were approved by the Ethics Committee of the Universidade Federal de Minas Gerais. Male Wistar rats (200–250 g) were killed by decapitation. The viscera were exposed and a segment of the superior mesenteric artery excised, free of fat and connective tissue, cut into rings about 2–3 mm in length and set up in gassed (95% O_2 and 5% CO_2) Krebs-Henseleit solution of the following composition (mM): NaCl 110.8, KCl 5.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.07, $CaCl_2$ 2.49, NaH_2PO_4 2.33 and glucose 11.51. When necessary, the endothelium was removed by rubbing the intimal surface with a stainless steel stick. The tissues were maintained at 37 °C under a tension of 5.0 mN and equilibrated for a period of 1 h before initiating experimental protocols. During this period, the incubation media was changed every 15 min. After the equilibration period, two contractile responses were evoked by submaximal concentrations of phenylephrine (1 μM) to elicit reproducible responses. The presence of functional endothelium was assessed by the ability of acetylcholine (1 μM) to induce more than 70% relaxation of vessels pre-contracted with phenylephrine (1 μM). The absence of acetylcholine relaxant activity indicated the absence of functional endothelium. Mechanical activity recorded isometrically by a force transducer (World Precision Instruments, Inc., Sarasota, FL, USA) was fed to an amplifier-recorder (Model TBM-4; World Precision Instruments, Inc.) and to a personal computer equipped with an analogue-to-digital converter board (AD16JR; World Precision Instruments, Inc.), using CVMS data acquisition/recording software (World Precision Instruments, Inc., USA).

Vasorelaxant activity in pre-contracted superior mesenteric artery rings of rats

The determination of vasorelaxant activity was performed in superior mesenteric artery rings with or without functional endothelium pre-contracted to the same tension (approximately 8.0 mN of tension) with submaximal concentrations of phenylephrine (1.0 or 0.3 μM, respectively) or KCl (80 mM), as indicated. HSE was added cumulatively to increase the concentration once the response to phenylephrine or KCl had stabilized. In order to evaluate the participation of endothelium-derived products, experiments were carried out in the presence of L-NAME (100 μM), indomethacin (10 μM), in vessels pre-contracted with KCl (80 mM) or in vessels pre-contracted with phenylephrine (0.3 μM) in the presence of 25 mM KCl alone or in combination with L-NAME (100 μM). The activation of potassium

channels by HSE was investigated in vessels pre-treated with $BaCl_2$ (1 mM), a non-selective blocker of potassium channels, and pre-contracted with phenylephrine (0.3 μM). To evaluate the participation of muscarinic receptors in the relaxant effect of HSE, experiments were performed in the presence of atropine (1 μM). L-NAME, indomethacin and atropine were added to the bath 20 min prior to the addition of phenylephrine.

Drugs

Acetylcholine chloride, atropine sulphate, indomethacin, L-NAME and L-phenylephrine chloride were purchased from Sigma. Indomethacin was dissolved in 0.5% w/v sodium bicarbonate, whereas the other drugs were dissolved in distilled water to concentrations of 10 mM. All subsequent dilutions were made with Krebs–Henseleit solution immediately before use. Rutin, previously isolated and characterized by spectroscopic methods in our laboratory, was employed as reference compound for chromatographic analysis.

Statistical analysis

The experimental data are expressed as mean ± standard error mean (SEM) of at least five experiments. The statistical analyses were performed with two-way ANOVA plus Bonferroni post-test for the concentration-response curves and Student's *t*-test for the values of IC_{50}. The data were considered significantly different when $p < 0.05$.

Results and discussion

In superior mesenteric artery rings pre-contracted with phenylephrine, HSE elicited a concentration-dependent relaxant effect in vessels containing a functional endothelium, with an $IC_{50} = 10.8 \pm 4.0$ μg/mL (Fig. 1A). This effect was abolished in the absence of a functional endothelium (Fig. 1A), indicating that the vasodilator effect of HSE was dependent on endothelium-derived relaxing factors. To evaluate the participation of NO in the vasodilator effect induced by HSE, the superior mesenteric artery rings were treated with L-NAME, a classical NO synthase inhibitor. In this experimental condition, the vasodilation induced by HSE was strongly inhibited (Fig. 1B), but a small relaxation can still be observed with higher amounts of HSE. These results suggest that NO is the main factor involved in HSE vasodilator activity. Moreover, they also suggest the participation of other endothelium-derived relaxant factor in the vasodilator effect induced by HSE. Therefore, we used indomethacin, a cyclooxygenase inhibitor, to evaluate the participation of cyclooxygenase derivatives in the HSE vasodilator effect. As shown in Fig. 1B, indomethacin at a concentration able to inhibit contractions induced by arachidonic acid (data not shown) did not alter the effect of HSE ($IC_{50} = 19 \pm 6.0$ μg/mL), ruling out the participation of prostanoids in the vasodilation induced by HSE. Atropine, a selective muscarinic antagonist, was also unable to inhibit the vasodilator effect of HSE ($IC_{50} = 12.5 \pm 2.8$ μg/mL; Fig. 1B), although it strongly inhibited the vasodilations induced by 1 μM ACh (data not shown). These findings show that HSE effect is not due to activation of muscarinic receptors, which could be one of the mechanisms involved in the endothelium-dependent vasodilation.

Endothelium-derived NO plays an important role in the control of vascular homeostasis. NO controls vascular tone, modulates the growth of vascular smooth muscle cells and decreases platelet adhesion and aggregation, as well as the adherence of other blood components (Scott-Burden and Vanhoutte, 1994; Moncada et al., 1991). A decrease in NO production by

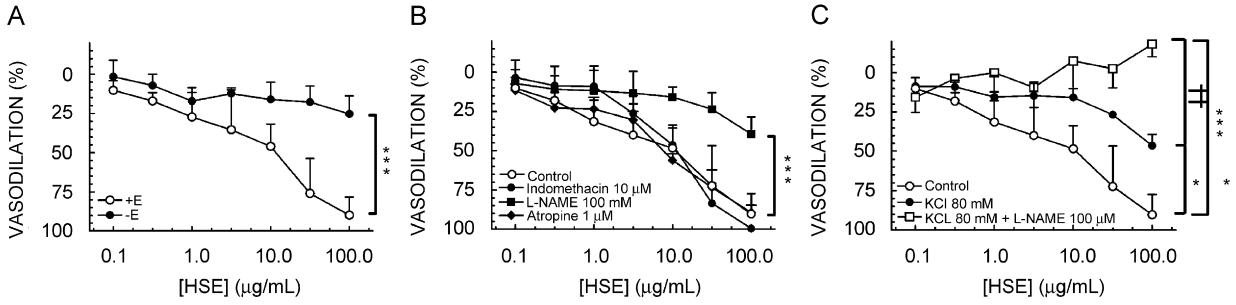

Fig. 1. Concentration-response curve of HSE on phenylephrine-pre-contracted rat superior mesenteric artery rings (A) in the presence (+E) or absence (−E) of a functional endothelium, (B) in the absence (control) or in the presence of L-NAME (100 μM), indomethacin (10 μM) or atropine (10 μM), and (C) in arteries pre-contracted with 80 mM KCl in the absence or in the presence of L-NAME (100 μM). The results are expressed as mean ± SEM of at least five experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ against control curve; ††< 0.01 against 80 mM KCl alone.

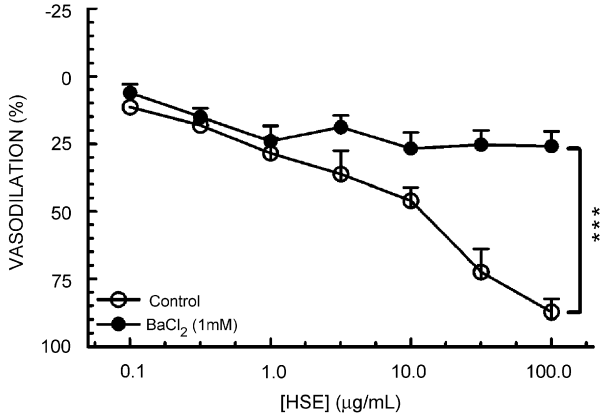

Fig. 2. Effect of the inhibition of potassium channels by $BaCl_2$ (1 mM) on the vasodilation induced by HSE in rat superior mesenteric artery. The results are expressed as mean ± SEM of at least five experiments. ***$p < 0.001$ against control curve.

vascular endothelial cells is closely associated with the endothelial dysfunction or injury, which is suggested to be an important factor in pathologies such as atherosclerosis, restenosis and hypertension (Lüscher, 1994; Busse and Fleming, 1996). In addition to NO, the endothelium is also able to release other relaxant factors, such as prostaglandin I_2 (PGI_2) and EDHF (Mombouli and Vanhoutte, 1997). In rat mesenteric arteries EDHF, but not PGI_2, is the additional relaxant factor released in association with NO (Andriantsitohaina et al., 1996). The relaxant effect of EDHF is inhibited by high concentrations of potassium at the extracellular medium (Andriantsitohaina et al., 1996). In our experimental conditions, we observed that the vasodilator effect of HSE is significantly shifted to the right in rings pre-contracted with 80 mM KCl (Fig. 1C). We observed that in the presence of L-NAME (100 µM),

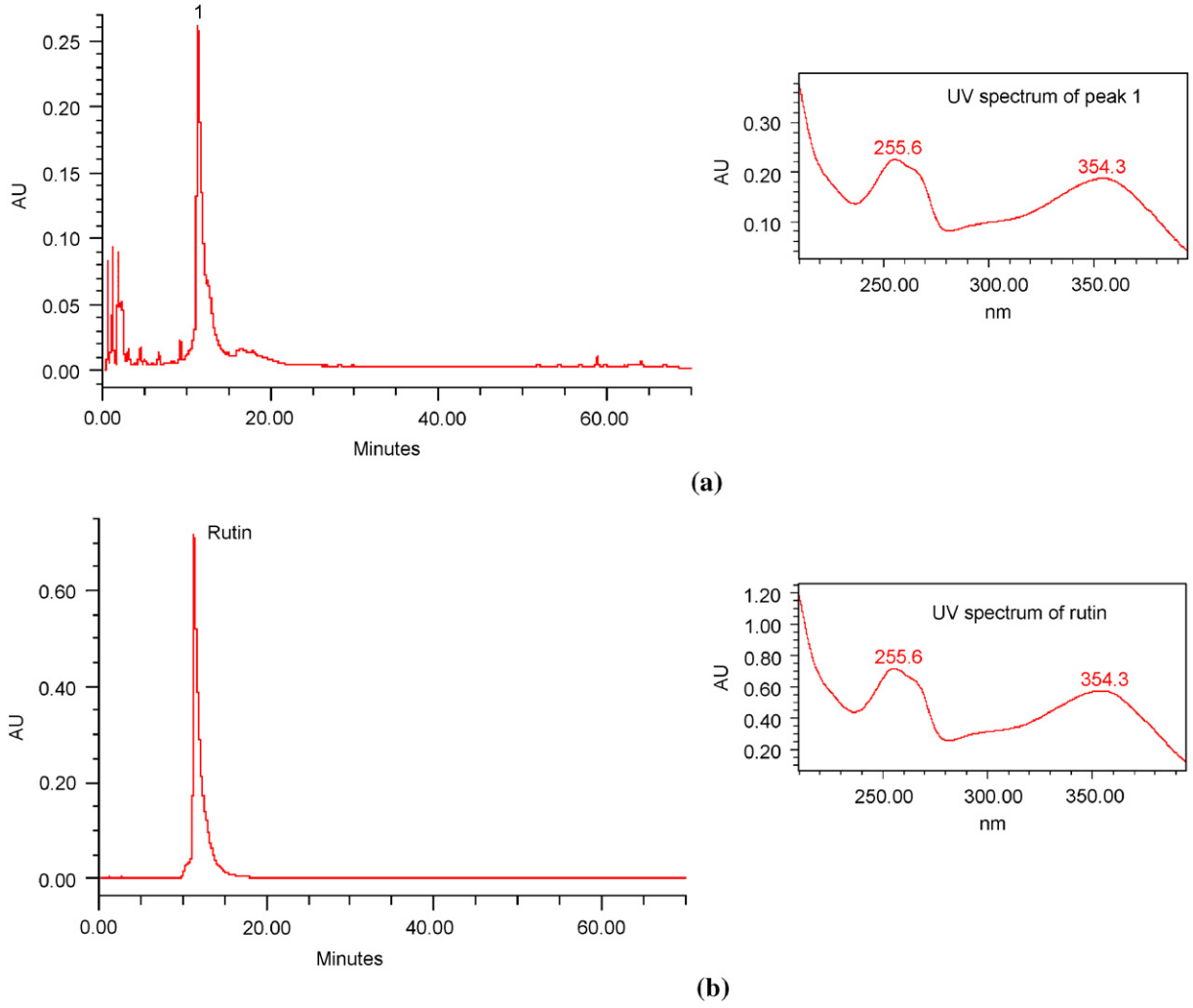

Fig. 3. RP-HPLC chromatograms obtained (a) for the ethanol extract (HSE) of *Hancornia speciosa* leaves (b) and for a sample of rutin (retention time of 11.35 min), along with the respective UV spectra recorded on line by the DAD detector. Chromatographic conditions: see experimental.

the vasodilator effect of HSE was completely abolished in mesenteric rings pre-contracted with 80 mM KCl (Fig. 1C), suggesting that the vasodilator effect of HSE was not due to inhibition of voltage-operated calcium channels. Moreover, similar effects were obtained in mesenteric rings pre-contracted with phenylephrine in the presence of KCl 25 mM alone or in addition to 100 μM L-NAME (not shown). Finally, $BaCl_2$ (1 mM), a non-selective inhibitor of potassium channels, dramatically reduced the effect of HSE (Fig. 2). These results demonstrate the activation of potassium channels during the vasodilator effect of HSE in rat superior mesenteric arteries and suggest that the release of an EDHF may be involved, as the effect of HSE was endothelium-dependent.

The HPLC fingerprint of HSE showed a simple profile, with predominance of peaks of polar compounds (Fig. 3a). The UV spectra registered on line for the major peak, eluted at 11.34 min, revealed a flavonoid. A standard solution of rutin, injected in the same chromatographic conditions, produced a peak with identical retention time and UV spectrum (Fig. 3b). The presence of rutin in HSE was further indicated by comparison with authentic sample in TLC analysis (data not shown). As far as we know, the chemical composition of *Hancornia* species remains to be investigated and the chromatographic profiles registered for HSE points out rutin as a constituent of *H. speciosa*.

Rutin induced a concentration-dependent vasodilator effect in mesenteric artery rings with a functional endothelium (Fig. 4). However, its effect was absent in mesenteric artery rings where the endothelium was removed (Fig. 4). These results demonstrate for the first time that rutin induces an endothelium-dependent vasodilation in rat superior mesenteric arteries, which is compatible with the effect observed with HSE. On the other hand, the maximal vasodilation observed with rutin was only 55.2 ± 8.9%, which is significantly lower than observed with HSE (90.3 ± 5.8%; $p < 0.01$). Consequently, it is feasible to suppose the participation of other compounds in the vasodilator effect induced by HSE.

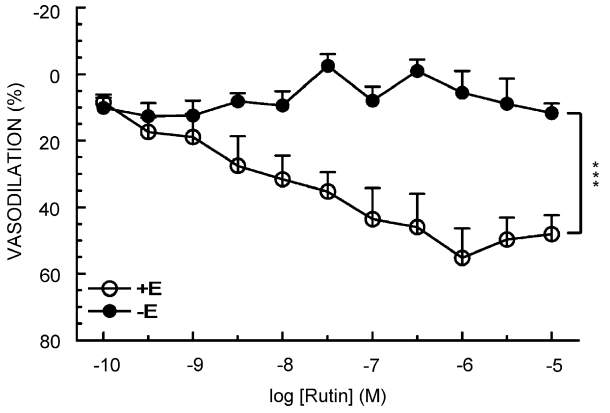

Fig. 4. Concentration-dependent vasodilation induced by rutin in rat superior mesenteric artery in the presence (+E) or in the absence (−E) of a functional endothelium. The results are expressed as mean ± SEM of five experiments in arteries +E and of four experiments in arteries −E. ***$p < 0.001$ against +E curve.

The endothelium-dependent, NO-mediated vasodilator effect of flavonoids and other polyphenols has been previously demonstrated (Fitzpatrick et al., 1995; Rice-Evans et al., 1996; Lemos et al., 1999; Huang et al., 1999). Although several reports on plant extracts and constituents that affect the NO pathways and modulate the vascular contractility have been published (reviewed in Achike and Kwan, 2003), only a number of works signalizes a mechanism based on the release of EDHF (Schuldt et al., 2005; Kwan et al., 2004a, b; Villar et al., 2004).

In conclusion, in the present study we demonstrated that HSE induces a potent vasodilation in the rat superior mesenteric artery through a mechanism dependent on the production of NO, on activation of potassium channels and likely in the release of an EDHF. In addition, our results also demonstrate that the flavonoid rutin contributes for the vasodilator effect of HSE and points out the participation of other compounds in this effect. Since smaller muscular arteries play a significant role in the regulation of blood pressure and organ perfusion, the vasodilator effect of HSE corroborates the traditional use of *H. speciosa* for treating hypertension.

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