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Endothelium-dependent vasodilator effect of *Euterpe oleracea* Mart. (Açaí) extracts in mesenteric vascular bed of the rat

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Abstract

Açai (Euterpe oleracea Mart.) a fruit from the Amazon region, largely consumed in Brazil is rich in polyphenols. Experiments were undertaken to determine whether hydro-alcoholic extract obtained from stone of açaí induces a vasodilator effect in the rat mesenteric vascular bed precontracted with norepinephrine (NE) and, if so, to elucidate the underlying mechanism. Açai stone extract (ASE, 0.3–100 μg) induced a long-lasting endothelium-dependent vasodilation that was significantly reduced by N^G-nitro-L-arginine methyl ester (L-NAME) and ^{1}H-[1,2,3] oxadiazolo [4,4-a] quinoxalin-L-one (ODQ) and abolished by KCl (45 mM) plus L-NAME. In vessels precontrated with NE and KCl (45 mM) or treated with K^{+2}_{Ca} channel blockers (charybdotoxin plus apamin), the effect of ASE was significantly reduced. However this effect is not affect by indomethacin, glybenclamide and 4-aminopiridine. Atropine, pyrilamine, yohimbine and HOE 140 significantly reduced the vasodilator effect of acetylcholine, histamine, clonidine and bradykinin, respectively, but did not change the vasodilator effect of ASE. In cultured endothelial cells ASE (100 μg/mL) induced the formation of NO that was reduced by N^G-nitro-L-arginine (L-NA, 100 μM). The present study demonstrates that the vasodilator effect of ASE is dependent on activation of NO-cGMP pathway and may also involve endothelium-derived hyperpolarizing factor (EDHF) release. The vasodilator effect suggest a possibility to use ASE as a medicinal plant, in the treatment of cardiovascular diseases.

Keywords: Açai; Euterpe oleracea Mart; Vasodilation; EDHF; NO

1. Introduction

The plant Euterpe oleracea Mart. also know by the popular name of açaí, is widely diffused in Amazon region especially in the Pará, Amazonas, Tocantins, Maranhão e Amapá states of Brazil. The skins of the fruits of açaí are commonly used to make juice, ice-cream, sweets, and is largely consumed in Brazil. Approximately 10.000 ton of frozen aqueous extract are consumed in Brazil and 1.000 ton are exported to many countries, as Japan, United States, Netherlands and Italy (Embrapa, 2004). Chemical studies have shown that açaí is rich in anthocyanic compounds (cyanidin 3-*O*-arabinoside, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside) and other polyphenols as epicatechine, catechine homoorientin, orientin, isovitexin and taxifolin deoxyhexose (Bobbio et al., 2000; Pozo-Isfran et al., 2004; Gallori et al., 2004). Many evidences suggest that diet rich in polyphenols might be involved in protection against cardiovascular risk (Stoclet et al., 2004). This beneficial effect of polyphenols may be due to many actions as antioxidant (Frankel et al., 1993) that increases bioavailability of nitric oxide (Fitzpatrick et al., 2000), vasodilation (Fitzpatrick et al., 2000) or antihypertensive (Soares de Moura et al., 2002a,b, 2004) properties. Therefore, experiments were undertaken to determine whether extracts obtained from fruits of açaí induce a

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vasodilator effect in the rat mesenteric vascular bed (MVB) and, if so, to elucidate the underlying mechanism.

2. Materials and methods

2.1. Preparation of extracts

Euterpe oleracea Mart. fruits were obtained from the Amazon Bay (Pará State, Brazil). Three extracts were studied. A frozen aqueous extract used to make juice, ice cream and other beverages was obtained from food stores. Shortly this extract (prepared in the North of Brazil) is obtained adding a certain amount of water to the fruits and then minced with rotatory spatula in order to separate the stones which are discharged. Once obtained, the concentrated aqueous extract of the skins is frozen until use for preparation of the beverages. Once in our laboratory, the aqueous extract was filtered through a Whatman n°. 1 filter paper, evaporated at low pressure until reduction of approximately 50% of the original volume and then lyophilized and frozen at −20 °C until use. Hydro-alcoholic extracts were obtained from decoction of skins or stones of the fruits. Fruits were washed in tap water and the skins were separated from the stones. Approximately 100 g of skins or 200 g of stones of açaí were boiled in 400 ml of water for 5 min, grinded for 2 min and then boiled again for another 5 min. The decoction was allowed to cool at room temperature, extracted with 400 of ethanol, shaken for 2 h and then kept in dark bottles inside a refrigerator (4 °C) for 10 days. After maceration period, the hydro-alcoholic extracts of açaí were filtered through Whatman n°. 1 filter paper and the ethanol was evaporated under low pressure at 55 °C. The extracts were then lyophilized and frozen at −20 °C until use. The concentration of polyphenols in açaí stone extract (ASE) and açaí skin extract, measured by analysing for total phenol by the Folin–Ciocalteau procedure (Singleton and Rossi, 1965) were 25 and 18%, respectively. Usually 100 g of stones or skins yields, respectively, 5 g and 3.2 g of lyophilized extract.

2.2. Isolated mesenteric vascular bed

All experiments were reviewed and approved by the Ethics Committee of Animal Experiments of the State University of Rio de Janeiro. Male Wistar rats were killed with inhaled CO_2 and the superior mesenteric vascular bed (MVB) was cannulated (McGregor, 1965) and perfused at a flow rate of 4 ml min^{-1} with a physiological salt solution (PSS) by pulsatile pump (Lifecare Model 4, Abbott Shaw). The PSS had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, EDTA 0.02, and glucose 11. The PSS (37 °C) was bubbled with 95% O_2/5% CO_2. Perfusion pressure (PP) was measured with transducer connected to a preamplifier and chart recorded. Drugs were either dissolved in PSS and perfused at the desired concentration, or were administered as bolus injections directly into the perfusion stream (volume <300 µl).

The preparations were left to equilibrate for 30 min, and then injections of 120 µmol KCl, were administered every 10 min until consistent responses were obtained. The basal PP after the equilibration period was 24.8±1 mmHg (n=164). Different doses of extracts (0.1–3000 µg) were injected after the PP had been elevated (80–100 mmHg) with norepinephrine (NE; 6–30 µM) or phenylephrine (PE; 30 µM) added to the perfusion fluid. Acetylcoline (ACh; 10 pmol) and nitroglycerin (NG; 1 nmol) were also injected to test the endothelium-dependent and -independent responses before dose-responses curves to different extracts (açaí stone extract (ASE), açaí skin extract and aqueous extract of the hole fruit) were obtained. As ASE showed the most potent vasodilator effect comparing to açaí skin extract and aqueous extract, pharmacodynamic study were performed only with ASE.

The vasodilator effect of ASE, ACh and NG were studied after perfusion with deoxycholic acid (2.5 mM) dissolved in PSS for 3 min to chemically remove the endothelium or after perfusion with N^G-nitro-L-arginine methyl ester (L-NAME, 0.3 mM), an inhibitor of NO-synthase, 1H-[1,2,3] oxadiazolo [4,4-a] quinoxalin-L-one (ODQ; 10 µM), an inhibitor of guanylyl cyclase (GC), charybdotoxin (ChTx; 0,1 µM) plus apamin (0.1 µM), inhibitors of K^{+2}_{Ca} channel. In addition, the vasodilator effect of ASE, ACh

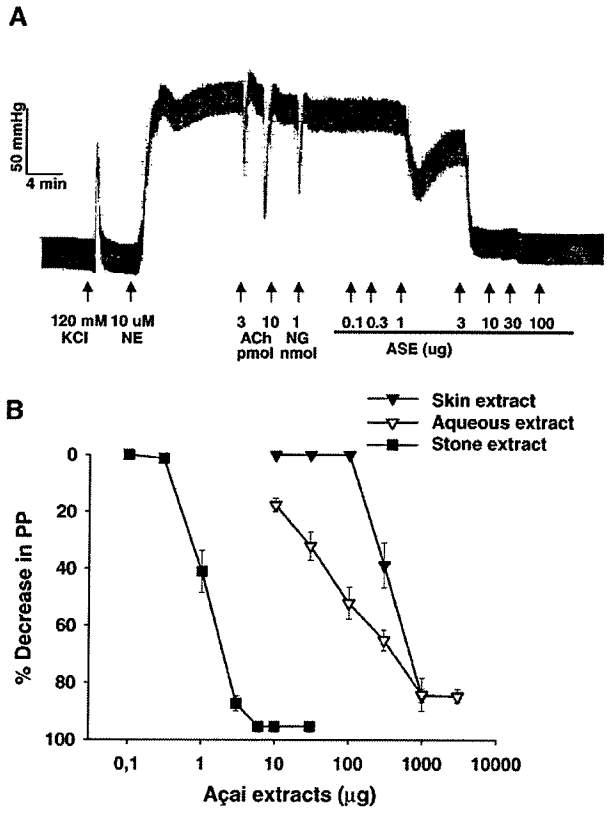

Fig. 1. Vasodilation effect of Euterpe oleracea Mart. extract in MVB of the rat. The MVB were pre-contract with NE (10 µM) added to the perfusion fluid. Acetylcoline (ACh; 10 pmol) and nitroglycerin (1 nmol) were also injected to test the endothelium-dependent and independent responses before dose-responses curves to extracts were obtained. A. Trace illustrates the response of ASE. Arrows indicate injections of ACh (3 and 10 pmol), NG (1 nmol) and ASE (0.1 to 100 µg). B. Vasodilation effect of extracts obtained from stones, skins and fruits in MVB. Different doses of the extract were injected in bolus: stones (0.1–100 µg), skins (10–1000 µg) and aqueous (10–3000 µg). Each line is presented as mean±SEM. n=6 rats/group.

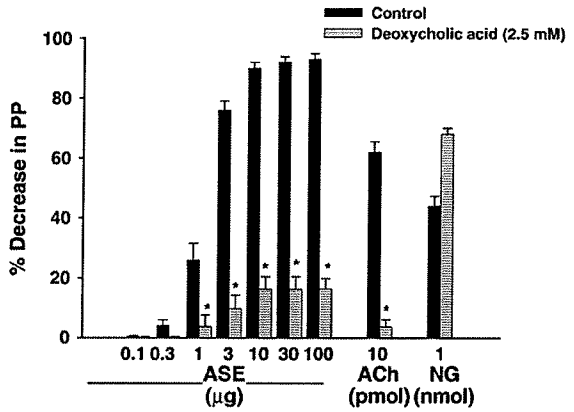

Fig. 2. Effect of endothelial removal by deoxycholic acid on the vasodilator effects induced by ASE, ACh, and NG in vessels precontracted with NE. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by NE. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. $n=6$ rats/group. *Significantly different from the corresponding control group ($p<0.05$).

and NG was studied in vessels perfused with high K^+ solution (45 mM) or in vessels perfused with L-NAME (0.3 mM) plus high K^+ solution (45 mM).

The vasodilator effects of ASE were also studied after perfusion with glibenclamide (1 μM), an inhibitor of the ATP-dependent K^+ (K_{ATP}) channel; indomethacin (0.1 μM), an inhibitor of cyclooxygenase; 4-aminopyridine (1 mM), an inhibitor of the voltage-dependent K^+ (Kv) channel; and pyrilamine (1 μM), atropine (0.03 μM); yohimbine (3 μM) and HOE-140 (0.01 μM) that significantly inhibited the vasodilator responses of histamine, ACh, clonidine and bradykinin (BK), respectively.

The concentrations of NE or PE were adjusted to maintain the same increase in PP in vessels pretreated with deoxycholic acid, L-NAME, indomethacin, or yohimbine, respectively.

As the ASE induced a long-lasting inhibitory effect on NE constrictor effect, only one dose-response curve were obtained in each vascular bed preparation. Control dose-response curves for ASE were obtained interspersed concurrently with drug-treated vessels, which prevented all the controls from being run on a single group of animals at one time during the course of the investigation.

2.3. Determination of NO formation in endothelial cells by electron spin resonance spectroscopy

Determination of NO formation was assessed by electron spin resonance spectroscopy (ESR) after formation of [Fe(II)NO

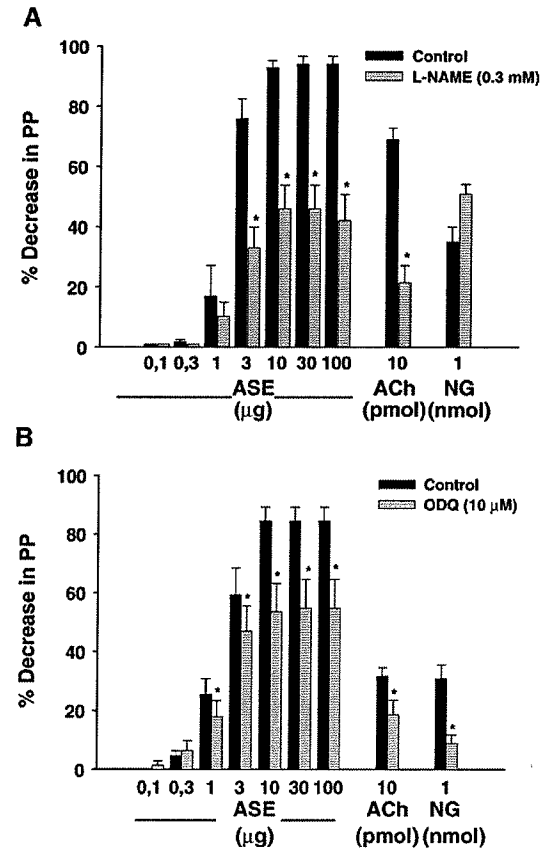

Fig. 3. Effects of l-NAME (A) and ODQ (B) on the vasodilator effects induced by ASE, ACh, and NG in the MVB precontracted with NE. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by NE. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. $n=6$–7 rats/group. *Significantly different from the corresponding control group ($p<0.05$).

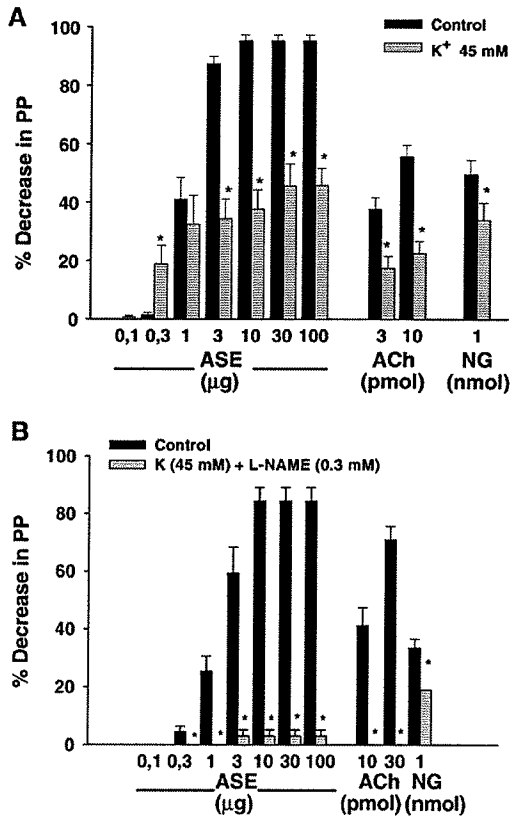

Fig. 4. Effects of high K^+ solution (A) and high K^+ plus L-NAME (B) on the vasodilator effects induced by ASE, ACh, and NG in the MVB precontracted with NE. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by NE. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. $n=6$–7 rats/group. *Significantly different from the corresponding control group ($p<0.05$).

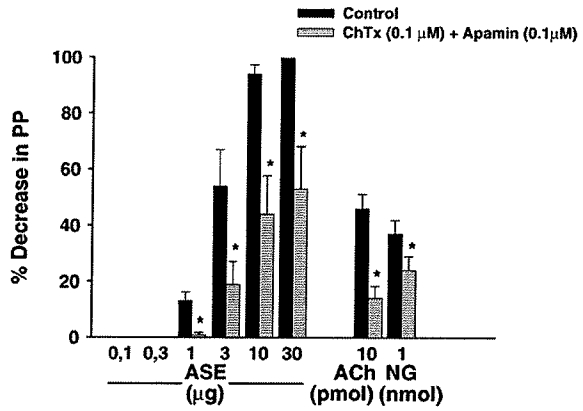

Fig. 5. Effects of ChTx plus apamin on the vasodilator effects induced by ASE, ACh, and NG in the MVB precontracted with NE. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by NE. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. $n=6$ rats/group. *Significantly different from the corresponding control group ($p<0.05$).

$(DETC)_2$], a paramagnetic diethyldithiocarbamate iron complex with NO, in cultured human umbilical vein endothelial cells (HUVECs). The ESR methodology was used as reported previously with minor modifications (Vanin, 1999; Kleschyov and Munzel, 2002). Confluent cultures of HUVECs (first passage) were washed twice with Hanks balanced salt solution (HBSS) buffered with 10 mmol/L HEPES, and then incubated in a HBSS-HEPES solution in the presence of bovin serum albumin (20.5 mg/mL), 1.5 mmol/L $CaCl_2$, 0.3 mmol/L L-arginine for 15 min at 37 °C. In some experiments HUVECs were treated with N^G-nitro-L-arginine (L-NA, 100 μM) for 30 min before addition of spin trap chemicals $FeSO_4$ (0.8 mmol/L) and DETC (1.6 mmol/L) at a final concentration of 0.2 mmol/L. After 5 min, the endothelial formation of NO was induced by addition of ASE (0.1–100 μg/mL) for 30 min. Thereafter, dishes were placed on ice, and the incubation medium was removed before addition of 0.3 mL of the HBSS-HEPES buffer. Cells were then scraped and the cell suspension was collected in a calibrated tube. Tubes were rapidly frozen at 77 K for ESR measurements. ESR measurements were performed on an MS100 spectrometer (Magnettech Ltd., Berlin, Germany) under the following conditions: temperature 77 K, microwave frequency 9.34 GHz, microwave power 20 mW, modulation frequency 100 kHz, modulation amplitude 1 mT. The third component of the ESR signal was used for relative comparison of the concentration of NO trapped in each sample.

2.4. Statistical analysis

The vasodilator effect of all the drugs was expressed as the percentage decrease on the increase in PP induced by NE or PE. The dose of the extracts producing a half maximal relaxation amplitude (ED_{50}) was determined after log transformation of the normalized dose-response curves and is reported as the mean with 95% confidence interval by the use of the Prism GraphPad 4.04 software (San Diego, CA). All results are presented as mean± SEM for the numbers of rats. The Student unpaired t test was used for statistical analysis. Values of $p<0.05$ were considered statistically significant.

2.5. Drugs

The following compounds were used: NE, PE, ACh, histamine, BK, L-NA, L-NAME, HOE-140, yohimbine, atropine, ChTx, apamin, indomethacin, deoxycholic acid, and glibenclamide were purchased from Sigma (St. Louis, MO; USA). ODQ

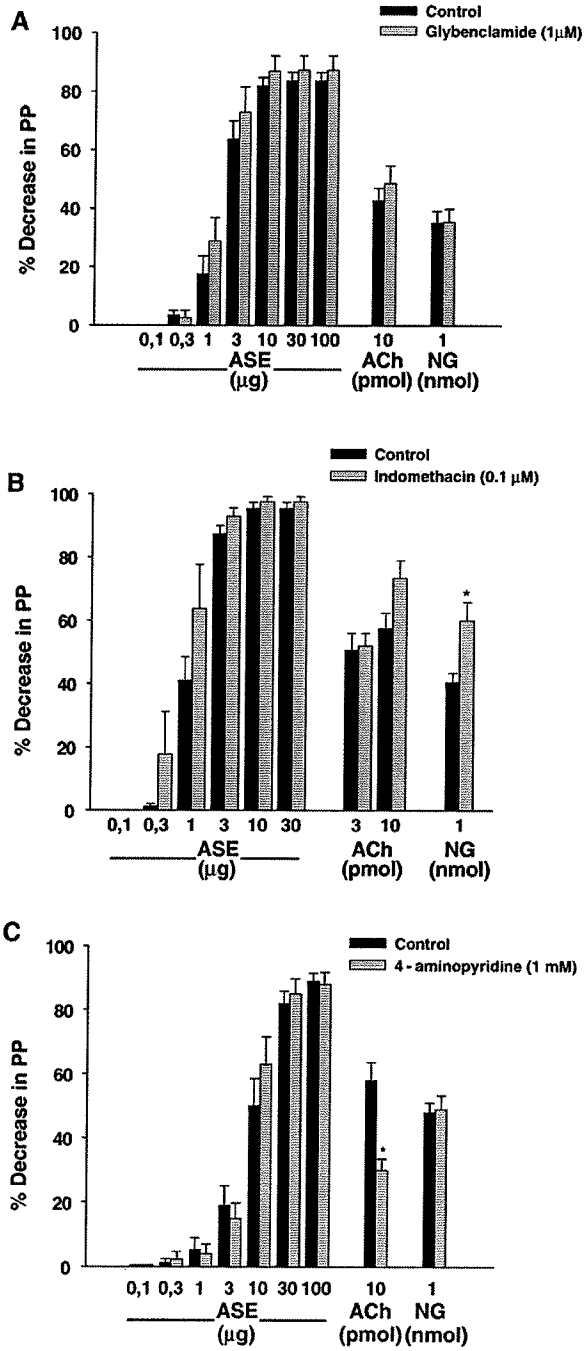

Fig. 6. Effects of glybenclamide (A), indomethacin (B) and 4-aminopyridine (C) on the vasodilator effects induced by ASE, ACh, and NG in the MVB precontracted with NE. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by NE. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. $n=6$ rats/group. *Significantly different from the corresponding control group ($p<0.05$).

was purchased from Tocris (Ellisville, MO). NG and clonidine were a gift from Innovatec-Divisão Cristalia Produtos Químicos Farmacêuticos Ltda, Brazil and Boehring Ingelhein Química Farmacêutica Ltda, São Paulo, Brazil, respectively. All drug solutions were freshly prepared before each experiment.

3. Results

3.1. Effects of açaí stones extract (ASE), açaí skin extract and aqueous extract in the mesenteric vascular bed of the rat

In vessels precontracted with NE, bolus injection of ACh (30 pmol) and NG (1 nmol) induced rapid and transient decrease of PP. All three extracts of açaí (0.1–3000 μg) induced a dose-dependent, sustained and long-lasting (more than 1 h) vasodilator effect (Fig. 1). The maximal response was similar between the three extracts. However, the vasodilator response of ASE was more potent (ED_{50}=1.11 μg; CI=0.99 to 1.26) than those obtained by the skin extracts (ED_{50}=317.8 μg; CI=246.2 to 410.2) and aqueous extract (ED_{50}=77.6 μg; CI=15.36 to 392.1) in the MVB of rat (p<0,05). Therefore the underline mechanism of vasodilator effect of ASE was studied.

3.2. Action of deoxycholic acid on the vasodilator effect of ASE in the mesenteric vascular bed of the rat

In vessels precontracted with NE (control group, n=6), bolus injections of ACh (10 pmol) and NG (1 nmol) induced rapid and transient decrease in PP and ASE induced a dose-dependent, sustained and long lasting (more than 1 h) vasodilator effect (Fig. 1). In separated experiment after vasodilator effect of ACh and NG were demonstrated, the vessels were treated with deoxycholic acid (n=6) and bolus injection of ACh, NG and ASE were performed. Under those conditions, the vasodilator effect of ACh and ASE (30 μg: 90±2% (control) vs 16.3±4% (deoxycholic acid), p<0.05) were significantly inhibited, while the effect of NG was not reduced (Fig. 2).

3.3. Actions of l-NAME, ODQ, high K^+, ChTx plus Apamin and KCl (45 mM) plus L-NAME on the vasodilator effect of ASE in the mesenteric vascular bed of the rat

In vessels precontracted with NE (control group), the vasodilator effect of ACh, NG and ASE were studied. In MVB precontracted with NE and perfused with L-NAME (0.3 mM, n=6), the vasodilator effects ACh (10 pmol) and ASE (30 μg, 94±3 (control) vs 46±8%, p<0.05) were significantly reduced, while the vasodilator response to NG (1 nmol) was not modified (Fig. 3A). In separate experiments and after the control responses to ACh, NG and ASE, pre-treatment of the vessels with ODQ (10 μM, n=6) significantly reduced the vasodilator effects of ASE (30 μg: 84±5 (control) vs 54.7±9% (ODQ), p<0.05), ACh and NG (Fig. 3B).

In MVB precontracted with NE and perfused with high K^+ (45 mM, n=6), the vasodilator effects of ACh (10 pmol), NG (1 nmol) and ASE (30 μg: 95.4±2% (control) vs 45.7±7% (K^+) p<0.05), were significantly reduced (Fig. 4A). The addition of L-NAME to the PSS containing high K^+ (45 mM) almost abolished the response to ASE (30 μg: 84.6±4% (control) vs 3.1±2% (L-NAME+K^+) p<0.05, Fig. 4B). In vessels precontracted with NE (n=6), the vasodilator effect of ASE (30 μg: 100±0% (control) was significantly reduced by ChTx (0.1 μM) plus apamin (0.1 μM) (53±15%), p<0.05) as well as the responses to ACh and NG (Fig. 5).

3.4. Actions of glybenclamide, indomethacin and 4-aminopyridine on the vasodilator effect of ASE in the mesenteric vascular bed of the rat

In vessels precontracted with NE the vasodilator effect of ACh, NG and ASE were evaluated (control group). In separated experiments and after the control responses to ACh and NG were demonstrated the vessels were treated with glibenclamide (1 μM, n=6), indomethacin (0,1 μM, n=6) or 4-aminopyridine (4-AP, 1 mM, n=6), and the vasodilator effects of ACh, NG and ASE were studied. Under this condition the vasodilator effect of ASE (30 μg) was not significantly reduced by glybenclamide (83.7±3% (control) vs 87±5%), indomethacin (95.4±2% (control) vs 94±2%) or 4-aminopyridine (82±4% (control) vs 85±4% (Fig. 6).

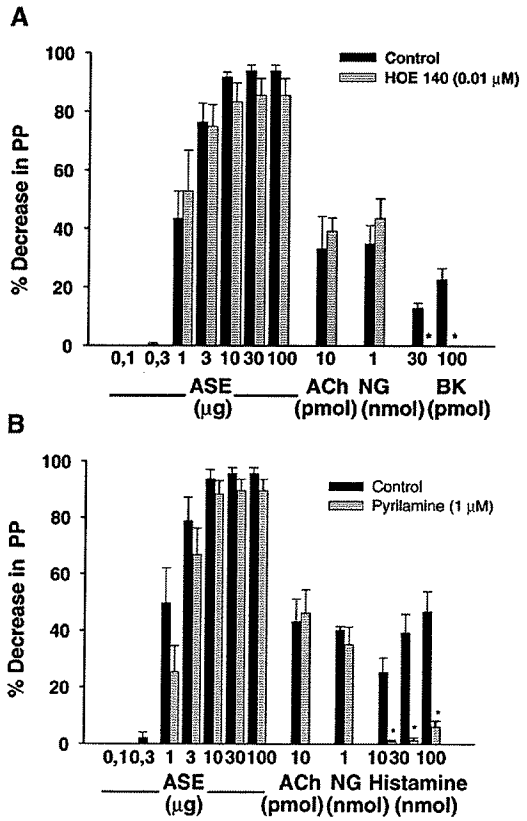

Fig. 7. Effects of HOE 140 (A) and pyrilamide (B) on the vasodilator effects induced by ASE, ACh, and NG in the MVB precontracted with NE. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by norepynephrine. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. n=6 rats/group. *Significantly different from the corresponding control group (p<0.05).

3.5. Actions of HOE-140, pyrilamine, atropine and yohimbine on the vasodilator effects of ASE in mesenteric vascular bed of the rat

In vessels precontracted with NE, the vasodilator effects of ACh, bradykinin, histamine, NG and ASE were studied (control group). In separated experiments the vessels were treated with atropine (0.03 μM, $n=6$), HOE-140 (0.01 μM, $n=6$) or pyrilamine (1 μM, $n=6$) and the vasodilator effects of ASE, ACh, BK or histamine were studied. Under these conditions the vasodilator effects of NG and ASE (30 μg: 93.9±2 vs 85.7±5% with and without HOE140, respectively; 95.6±2 vs 89.6±4% with or without pyrilamine, respectively; 95.4±2 vs 92.6±4% with or without atropine, respectively) were not significantly reduced but the vasodilator effects of ACh, BK or histamine, were significantly inhibited by atropine, HOE-140 or pyrilamine, respectively. In vessels precontracted with PE (30 μM), the vasodilator effects of ACh, NG, clonidine and ASE were studied (control group). In other group of experiments the vessels were treated with yohimbine (3 μM, $n=6$) and the vasodilator effects of ACh, NG, clonidine, and ASE were evaluated. Under this condition the effects of ACh, NG and ASE (30 μg: 95.4±2% (control) vs 95±2% (yohimbine) were not significantly reduced but the vasodilator effect of clonidine was significantly inhibited by yohimbine (Figs. 7 and 8).

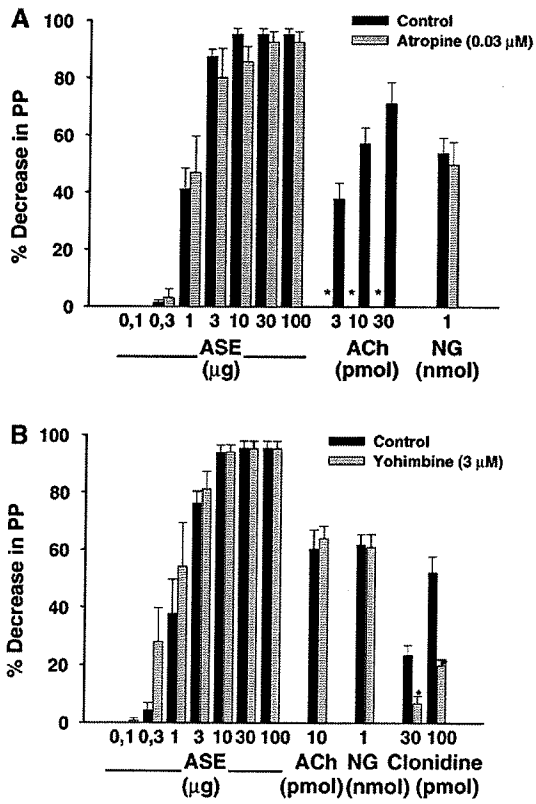

Fig. 8. Effects of atropine (A) and yohimbine (B) on the vasodilator effects induced by ASE, ACh, and NG in the MVB precontracted with NE or PE, respectively. Ordinate: vasodilation (%), expressed as a percentage decrease of the vasoconstriction induced by norepynephrine or phenylephrine. Abcissa: doses of ASE, ACh, or NG. Each bar is presented as mean±SEM. $n=6$ rats/group. *Significantly different from the corresponding control group ($p<0.05$).

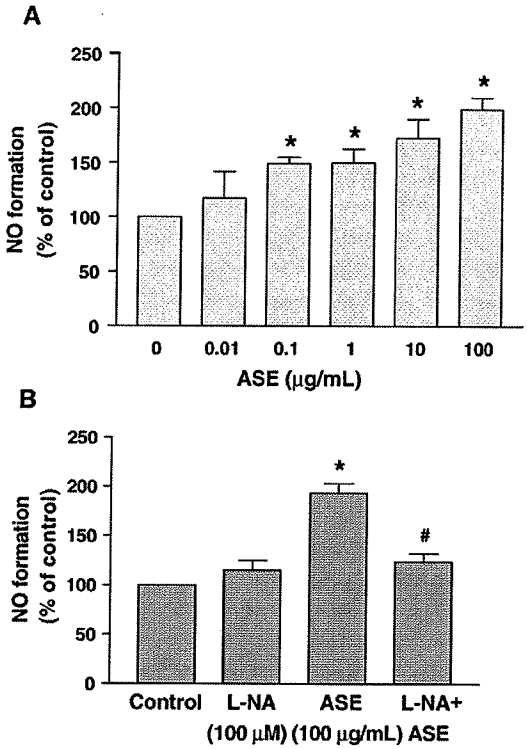

Fig. 9. Formation of NO induced by ASE (0.01–100 μg/mL) in HUVECs (A) and the effect of L-NA (100 μM) on NO formation induced by ASE (100 μg/mL). Each bar is presented as mean±SEM. $n=5$ experiments. *Significantly different from the corresponding control ($p<0.05$).

3.6. Effect of ASE on the endothelial formation of NO

In HUVECs, ASE induced the formation of NO in a dose-dependent manner (Fig. 9A). Treatment of endothelial cells with L-NA (100 μM) significantly ($n=5$, $p<0.05$) reduced the formation of NO induced by ASE (100 μg/mL), while L-NA alone did not modify the basal formation of NO (Fig. 9).

4. Discussion

The present study has demonstrated for the first time that the three extracts of açaí exerted dose-dependent sustained vasodilator effects on the MVB of the rat. The magnitude of the vasodilator effects induced by aqueous and hydro-alcoholic extracts of fruits of açaí, demonstrated in the present study were similar to that of ACh and NG, delineating the importance of the present findings. Differently from ACh and NG, that induce a reversible vasodilatation, the vasodilator effect of açaí extracts is long lasting suggesting a pharmacodynamic difference among those vasodilators. The amplitude of vasodilator effects of the three extracts were similar, however, ED_{50} of ASE was significantly smaller than the other two extracts suggesting that compounds that induce vasodilation are more concentrated in the stone of açaí. Therefore, ethanol plus water seems to be more

effective than water in the extraction of the active vasodilator principles occurring in the fruits of açaí. It is important to recall that açaí fruits are rich in polyphenols, compounds that have a significant vasodilator effect in vitro (Stoclet et al., 2004). The difference in vasodilator activity observed in this work might be due to different concentration of polyphenols in the extracts since the present study demonstrated that the concentration of total polyphenols in ASE is higher than in extract obtained from the skins. As ASE was the most potent of the three extracts, all the protocols to study the mechanism of the vasodilator effect were performed with ASE.

The mechanism of the vasodilator effect of ASE is not known, but the possibility of this effect being modulated by endothelial cells has to be considered, since chemical elimination of endothelial cells with deoxycholic acid significantly reduced its vasodilator effect. The use of deoxycholic acid to remove the endothelial cell layer suggested that the vasodilator effect of hydro-alcoholic extract of açaí stone is endothelium-dependent. The vasodilator effect of ACh was also significantly inhibited after deoxycholic acid treatment. This endothelium-dependency is consistent with previous studies using ACh in this preparation (Moore et al., 1990; Parsons et al., 1994). Importantly, within this experimental protocol there appeared to be little or no disruption of the smooth muscle of the vascular bed by a detergent action of deoxycholic acid, since the vasodilator response to NG, which occurs via an endothelium-independent elevation of intracellular cGMP in the mesenteric vasculature (Shibata et al., 1986; Khan et al., 1992), remained unaltered. Thus damage of the vascular preparation as a possible explanation for the reduction of the ASE vasodilator effect after deoxycholic acid treatment appeared unlikely from the present findings.

The vasodilator effect of ASE in the rat MVB might be due to the release of vasodilator autacoids by the endothelial cells, such as prostacyclin, since its stable synthetic analogue iloprost has been shown to elicit vasodilation of the rat mesenteric vascular bed (Yamawaki et al., 2000) and NE has been reported to release prostacyclin in this preparation (Peredo and Adler-Graschinsky, 2000). However in the present study we found that the cyclooxygenase blocker indomethacin did not alter the vasodilator effect of ASE and thus the current data appeared to exclude the involvement of prostaglandins in its mechanism of action.

EDRF can be released from endothelial cells by activation of many receptors (Vanhoutte, 1999). The endothelium-dependent vasodilator effect of ASE is independent on stimulation of muscarinic, histaminergic, alpha$_2$ adrenoceptors or bradykinin receptors at the level of endothelial cells since treatment with atropine, pyrilamine, yohimbine or HOE 140, compounds that inhibit the EDRF release from the endothelium cells induced by ACh, histamine, epinephrine or bradykinin, did not reduce the vasodilator effect of ASE.

The vasodilator effects of ASE and ACh but not NG an endothelium-independent vasodilator, were significantly reduced by L-NAME, an inhibitor of NO-synthase (Rees et al., 1989) suggesting that NO, a modulator of vascular function (Vanhoutte and Mombouli, 1996) plays an important role in the vasodilator effect of ASE in the MVB of the rat. This hypothesis is corroborated by the present findings that indicating that ASE stimulated the formation of NO in endothelial cells, an effect which is abolished by inhibition of NO synthase by L-NA. The vasorelaxation induced by NO is dependent on a reduction of the intracellular calcium due not only to activation of soluble guanylate cyclase (Waldman and Murad, 1987) but also to hyperpolarization induced by opening >of K^+ channels (Tare et al., 1990). Activation of soluble guanylate cyclase seems to play a significant role on the endothelium-dependent vasodilator effect of ASE since ODQ, an inhibitor of soluble guanylate cyclase, significantly reduced its effect.

Data from the literature suggest that the remaining portion of ACh-induced vasodilation that is resistant to NO synthase inhibition is likely to be mediated via release of an as yet unidentified endothelium-derived hyperpolarizing factor (EDHF) that acts ultimately by opening of plasmalemmal potassium channels (Chen et al., 1988; Feletou and Vanhoutte, 1988). As in our study the endothelium-dependent vasodilator effects of ACh and ASE were not completely inhibited by L-NAME, we can speculate that EDHF might play a role in the vasodilator effect of ASE. Indeed in our study we demonstrated that the vasodilator effect of ASE is inhibited in vessels pretreated with high potassium solution and also by charybdotoxin plus apamin, compounds that inhibit the hyperpolarization induced by opening of calcium-dependent K^+ channels. In addition the remained portion of ASE-induced vasodilation resistant to L-NAME or high potassium solution is almost completely abolished by combination of L-NAME plus high potassium solution. The participation of K_{ATP} and K_V channels on the vasodilator effect of ASE seems improbable since its vasodilator effect was not changed by glybenclamide or 4-aminopyridine, inhibitors of K_{ATP} and K_V channels, respectively (Choquet and Korn, 1992; Aguilar-Bryan et al., 1995, Philipson and Steiner, 1995).

5. Conclusion

In the present study, we demonstrate that ASE induces an endothelium-dependent vasodilator effect that does not involve prostanoids release, receptors activated by ACh, histamine, adrenaline, bradykinin and opening of K_{ATP} or K_V channels. Probably the vasodilator effect of ASE is dependent on the activation of NO-GMPc pathway and also may involve EDHF release. Finally, the vasodilator effect of ASE demonstrated in the present study provides experimental support for the possibility to use ASE, as a medicinal plant, in the treatment of cardiovascular diseases.

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