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Antihypertensive Effects and Antioxidant Action of a Hydro-Alcoholic Extract Obtained from Fruits of *Euterpe oleracea* Mart. (Açaí)

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Abstract: Earlier, we have demonstrated that a hydro-alcoholic extract of the stone of *Euterpe oleracea* Mart., commonly known as Açaí, exerts a significant endothelium-dependent vasodilator action *in vitro*. The present study has evaluated potential antihypertensive properties of Açaí Stone Extract (ASE) in four established models of experimental hypertension in the rat; spontaneously hypertensive rats (adult and young 21 days of age SHR), Goldblatt (2 kidney, 1-clip; 2K-1C), L-NAME and DOCA-salt-induced hypertension. Blood pressure was measured non-invasively using the tail-cuff method. A significant antihypertensive effect of ASE (200 mg/kg/day) was observed in adult SHR, Goldblatt, L-NAME and DOCA-salt models of hypertension. Administration of ASE just after weaning prevented the development of hypertension in SHR. Interestingly, expression of eNOS (endothelial nitric oxide synthase), which was elevated in SHRs compared to normotensive rats, was reduced in SHRs by ASE treatment. In addition, a significant antioxidant action of ASE, evaluated by TBARS measured in the bronchoalveolar lavage of rats exposed to cigarette smoke, was observed. In conclusion, the present study has demonstrated an antihypertensive action of ASE that is probably mediated via its vasodilator and antioxidant actions and the current preclinical data suggest a potential therapeutic use of ASE in hypertensive patients.

Key words: *Euterpe oleracea* Mart., L-NAME, goldblatt 2K-1C, DOCA-salt, SHR

INTRODUCTION

Hypertension is the most common cardiovascular disease and constitutes a major risk factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease, myocardium infarct, heart failure, renal insufficiency, stroke and dissecting aneurysm of aorta (Oparil, 1999). Although various are drugs currently use for the treatment of hypertension, the last effective class of antihypertensive agents, AT1 antagonists, was introduced into therapeutics around twenty years ago. Thus, pharmacological studies designed to evaluated novel alternatives are important, which might both expand the clinician's arsenal and improve existing antihypertensive therapy. Natural polyphenols, obtained from many plants, have been shown to exert important actions on the cardiovascular system and may be a potential source of new compounds to treat cardiovascular diseases (Stoclet *et al.*, 2004).

Chemical studies have shown that fruits of *Euterpe oleracea* (Açaí) are rich in anthocyanic compounds (cyaniding 3-O-arabinoside, cyaniding 3-O-glucoside, cyaniding 3-O-rutinoside) and other

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polyphenols such as epicatechine, catechine homoorientin, orientin, isovitexin and taxifolin deoxyhexose (Bobbio *et al.*, 2000; Pozo-Isfran *et al.*, 2004; Gallori *et al.*, 2004). Antioxidant effects of Açaí fruit pulps have also been demonstrated (Lichtenthaler *et al.*, 2005). In addition, recent results from our laboratory demonstrated that both Açaí skin extract and Açaí Stone Extract (ASE) exert profound endothelium-dependent vasodilator effects, the latter being significantly more potent (Rocha *et al.*, 2007).

In the present study, we have evaluated potential antihypertensive effects of ASE in four classical models of experimental hypertension: hypertension induced by L-NAME (inhibition of oxide nitric synthase), renovascular hypertension (Goldblatt 2 kidney, 1-clip (2K-1C), renin-dependent), DOCA-salt hypertension (renin-independent) and genetically modulated hypertension (SHR). Furthermore, we have also investigated antioxidant properties of ASE as a potential mechanism of action. Our data show significant antihypertensive effects of ASE in all models of hypertension that may be mediated, at least in part, by antioxidant properties in addition to its established vasodilator action.

MATERIALS AND METHODS

All experiments done with rats were reviewed and approved by the Ethics Committee of Animal Experiments of the State University of Rio de Janeiro.

Preparation of Açaí Stone Extract (ASE)

Euterpe oleracea Mart. fruits were obtained from the Amazon Bay (Pará State, Brazil). Hydro-alcoholic extracts was obtained from decoction of stones of the fruits. Approximately 200 g of stones of Açaí were boiled in 400 mL of water for 5 min, ground for 2 min and then boiled again for another 5 min. The decoction was allowed to cool at room temperature, extracted with 400 mL of ethanol, shaken for 2 h and then kept in dark bottles inside a refrigerator (4°C) for 10 days. After the maceration period, the hydro-alcoholic extracts of Açaí were filtered through Whatman No. 1 filter paper and the ethanol evaporated under low pressure at 55°C. The extract was then lyophilized and frozen at -20°C until use. The concentration of polyphenols in ASE, measured by analyzing for total phenol by Folin-Ciocalteu procedure (Singleton and Rossi, 1965), was around 250 mg g⁻¹ of extract. Usually 100 g of stones yielded approximately 5 g of lyophilized extract.

Arterial Pressure Measurement

Adult male SHR and Wistar rats (250-350 g) were housed in plastic cages (3 rats per cage). Cardiovascular parameters, systolic, mean and diastolic blood pressures were measured by the tail-cuff method using a Letica 5000 device. During the period of adaptation, rats were trained for at least 2 weeks to stay under a piece of cloth until the arterial pressure was steadily recorded with minimal restraint and stress during arterial pressure measurements. The first measurement of cardiovascular parameters was discarded and a mean of 2 or 3 subsequent measurement was recorded on each particular day.

Experimental Models of Hypertension

Goldblatt (2K-1C) Hypertension

To induce 2K-1C renovascular hypertension, after the period of adaptation, sixteen adult Wistar rats were anesthetized with pentobarbital (40 mg kg⁻¹) plus xylazine (20 mg kg⁻¹) i.p. The left renal arterial was occluded by a silver clip (0.2 mm in diameter). The right kidney was not touched. After this surgical procedure the animals were separated into two groups. The control group (n = 8) did not receive any treatment and the ASE group (n = 9) was treated orally with ASE (200 mg/kg/day, dissolved in drinking water), after the surgical procedure.

Desoxycorticosterone Acetate (DOCA)-Salt Hypertension

Fifteen adult Wistar rats were uninephrectomized under anesthesia with pentobarbital (40 mg kg^{-1}) plus xylazine (20 mg kg^{-1}) ip. After recovery from surgery, the rats were trained for measurements of systolic, mean and diastolic blood pressure and separated into two groups, a control and an ASE group. All the animals were treated subcutaneously with desoxycorticosterone acetate (15 mg/kg/week) and were given a 0.9% NaCl / 0.2% KCl drinking solution *ad libitum*. The animals of the control group were treated with DOCA and 0.9% NaCl / 0.2% KCl drinking solution ($n = 7$) until the end of the experimental period. The animals of ASE group were treated orally with 100 mg/kg/day of ASE from the first day of treatment with DOCA and 0.9% NaCl / 0.2% KCl drinking solution ($n = 8$). After 23 days the treatment was interrupted.

L-NAME Hypertension

After the period of adaptation, the animals were treated orally with N^G -nitro-L-arginine methyl ester (L-NAME; 50 mg/kg/day in the drinking water, L-NAME group, $n = 7$) or with 50 mg kg^{-1} of L-NAME (50 mg/kg/day) plus ASE (200 mg/kg/day , L-NAME+ASE group, $n = 7$) dissolved in drinking water, daily for 21 days. The possibility of a chemical reaction between ASE and L-NAME leading to chemical changes on L-NAME was discharged since HPLC estimations showed that the concentration of L-NAME in the drinking water, did not change after three days (Soares de Moura, personal observation).

Spontaneously Hypertensive Rats

After period of adaptation, adults SHR ($n = 8$) were treated orally with ASE (200 mg/kg/day) dissolved in drinking water for 33 days. After 33 days of treatment, ASE treatment was interrupted. Cardiovascular parameters were evaluated at least two days/week.

In another experimental protocol, ASE (200 mg/kg/day) was administered orally (drinking water) in young rats ($n = 8$), from day 21 (after weaning) until day 95. Cardiovascular parameters of SHR treated and untreated (control animals, $n = 8$) were evaluated from day 75 to day 95 of age.

Expression of Endothelial Nitric Oxide Synthase (eNOS)

The expression of eNOS was evaluated in SHR and Wistar rats treated and untreated with ASE (200 mg/kg/day) for 20 days. The mean arterial pressure of untreated ($n = 5$) and treated ($n = 5$) SHR groups were recorded before and after treatment with ASE. Mesenteric vascular beds of SHR and Wistar rats of 16 weeks-old, treated orally with ASE and untreated ($n = 4$ per group), were pulverized in ice using a stainless steel pestle and resuspended in homogenization buffer (phosphate buffer solution-PBS-with Triton X-100 1%, NaF 1M, NaPPI 100 mM, Na_3VO_4 1 M) and protease inhibitor mixture (pepstatine $10 \mu\text{g mL}^{-1}$, leupeptin $10 \mu\text{g L}^{-1}$, aprotinin $10 \mu\text{g mL}^{-1}$). Samples were centrifuged at -4°C , on 13000 rpm for 20 min and the supernatant was collected and assayed for total protein concentrations using the Lawry method (Bio-rad). Total protein ($40 \mu\text{g}$) was subject to SDS-PAGE (12%) and transferred onto a PVDF membrane for 2 h at 328 mAmp at 4°C . (Amersham Bioscience). The blots were blocked with PBS plus tween 20 (0.1%) and albumin (3%) and incubated overnight with rabbit polyclonal IgG raised against eNOS or β -tubuline antibody (1:200, Santa Cruz Biotechnology). The blots were washed with PBS plus tween (0.1%) and exposed to horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1: 5000 and 1:7000, respectively) dissolved in 5% (w/v) nonfat milk in buffer and immunoreactivity was visualized using an ECL detection system and autoradiographic film.

Antioxidant Activity

Cigarette Smoke Exposure

The antioxidant effects of ASE were studied in adult male rats ($n = 10$) exposed to Cigarette Smoke (CS) from 2 commercial filtered cigarettes, 3 times per day, for 5 consecutive days) using a

smoking chamber as described previously (Silva Bezerra *et al.*, 2006). CS rats were divided into two groups of five animals: (a) exposed rats untreated, control animals, (b) exposed rats treated orally with ASE 100 mg/kg/day in the drinking water, for 5 days. Rats (n = 10) exposed only to ambient air were divided in two groups of five animals: (a) untreated rats, control animals and (b) rats treated with ASE 100 mg kg⁻¹ in the drinking water, during 5 days. ASE was prepared daily and dissolved in tap water. Each cigarette smoked produced 300 mg m⁻³ of total particulate matter in the chamber (Silva Bezerra *et al.*, 2006).

Determination of Oxidative Damage (TBARS)

Lipid peroxidation was estimated in the bronchoalveolar lavage (BAL) of all rats killed at the end of the experimental period, by formation of thiobarbituric acid reaction substances (TBARS) during an acid-heating reaction as previously described by Draper *et al.* (1993). Briefly, the samples from BAL were mixed with 1 mL of trichloroacetic acid, 10% and 1 mL of thiobarbituric acid, 0.67%, subsequently they were heated in a boiling water bath for 30 min. TBARS were determined by the absorbance at 532 nm and were expressed as malondialdehyde equivalents (nmol mg⁻¹ protein).

Bronchoalveolar Lavage Fluid

Airspaces from rats (n = 4) were washed with buffered saline solution (500 µL) three consecutive times in the right lungs (final volume 1.2-1.5 mL). The fluid was withdrawn and stored on ice. Total mononuclear and polymorphonuclear cell numbers were determined in a Zi Coulter counter (Beckman Coulter, Carlsbad, CA, USA). Differential cell counts were performed on cytopsin preparations (Shandon, Waltham, MA, USA) stained with Diff-Quik. At least 200 cells per Bronchoalveolar Lavage (BAL) fluid sample were counted using standard morphologic criteria.

Statistical Analysis

All results are presented as Mean±SEM for number of rats. One-way Analysis of Variance (ANOVA) plus Bonferroni or unpaired Student's t-test were used for statistical analysis. Values of p<0.05 were considered statistically significant.

Drugs

L-NAME and desoxycorticosterone acetate and all chemical reagents used were purchased from Sigma (St. Louis, MO, USA). All drug solutions were freshly prepared before each experiment.

RESULTS

Effect of ASE in Goldblatt Hypertension

Baseline systolic, mean and diastolic arterial pressure before the surgical procedure in control group (106.3±2, 84.7±3, 74.4±3, respectively) and ASE treated group (113.9±4, 91.0±3, 79.5±3, respectively), were not significantly different. After 21 days the mean and diastolic arterial pressures were significantly higher in the control group (199.4±6, 192.4±6, respectively) compared to the ASE group (156.6±10, 147.4±11, respectively) (Fig. 1).

Effect of ASE in DOCA-Salt Hypertensive Rats

The baseline systolic, mean and diastolic arterial pressure in the control group (123.2±6, 78.2±5, 58.1±6, respectively) and ASE group (114.6±6, 87.1±5, 74.5±6, respectively), at the beginning of treatment (day zero) were not significantly different. After twenty three days the systolic, mean and diastolic arterial pressures were significantly higher in the control group (180.5±7, 163.5±7, 155.5±8, respectively) compared to the ASE group (114.6±6, 87.0 5, 74.5±6, respectively). When ASE treatment was interrupted from day 23 until day 37 the blood pressure showed a significant rise (Fig. 2).

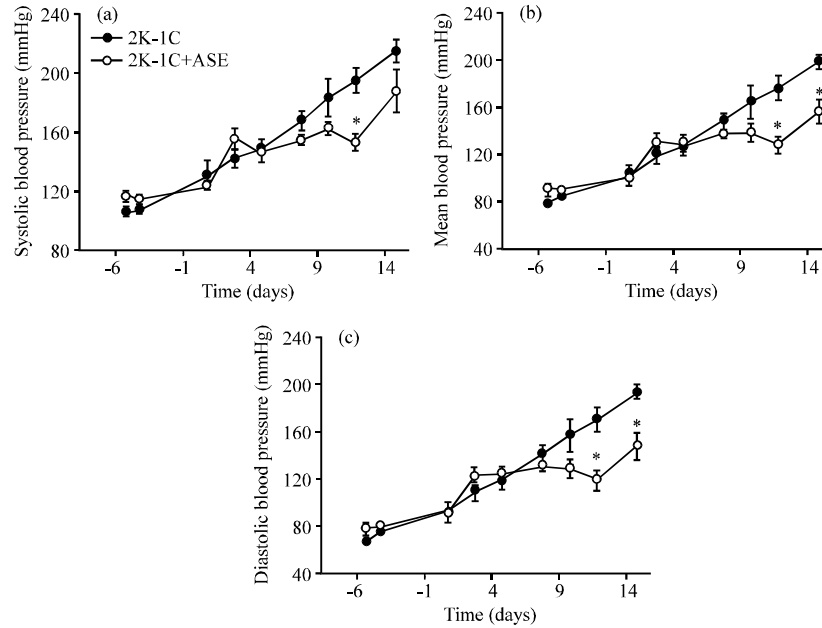


Fig. 1: Effect of ASE (200 mg/kg/day) on systolic (a), mean (b) and diastolic (c) blood pressure in 2K-1C rats. *Significantly different ($p < 0.05$; Student's t-test) between 2K-1C and 2K-1C plus ASE

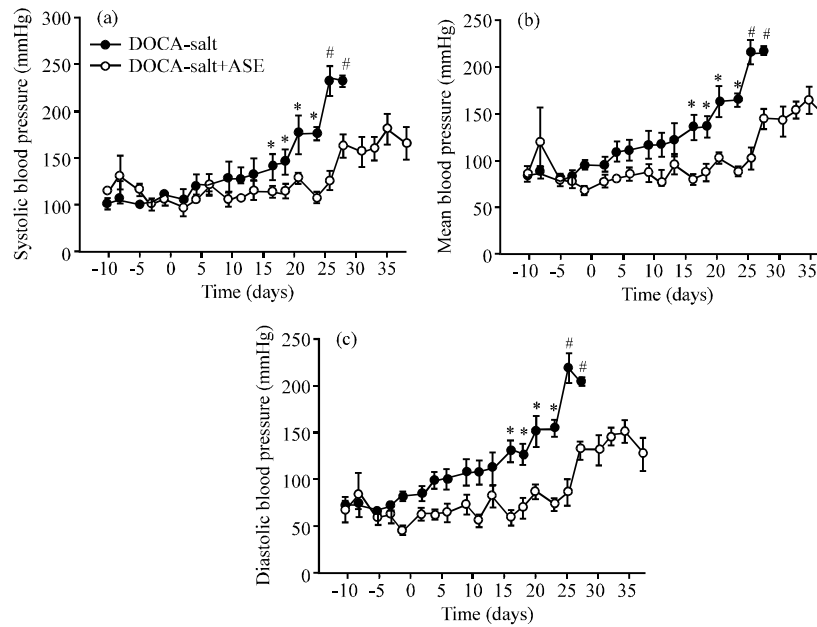


Fig. 2: Effect of ASE (100 mg/kg/day) on systolic (a), mean (b) and diastolic (c) blood pressure in uninephrectomized rats treated with deoxycorticosterone acetate (DOCA) and salt solution. Arrows up represent the beginning of treatment with ASE and arrows down represent the interruption of treatment. *Significantly different ($p < 0.05$ student t-test) between control and ASE. #Significantly different ($p < 0.05$ Student's t-test) between day 25 and 21

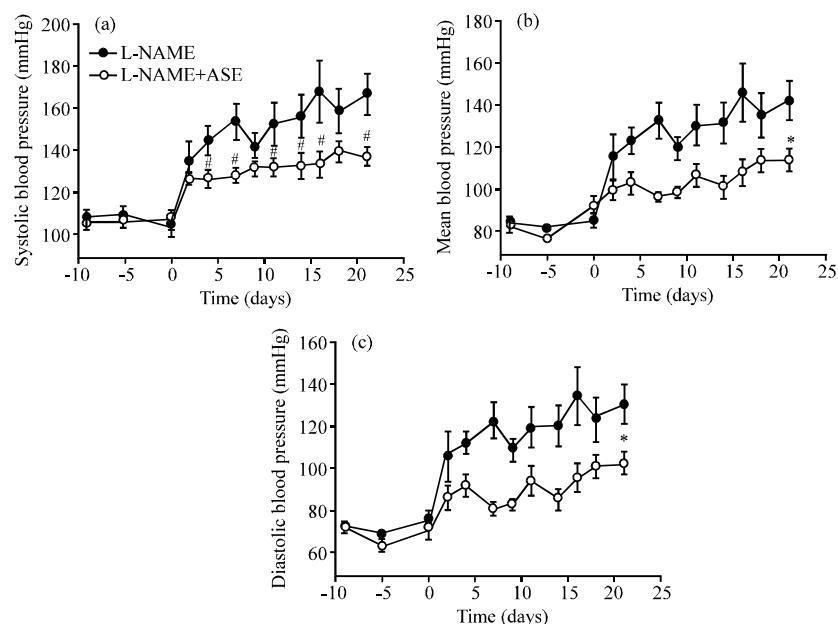


Fig. 3: Effect of ASE (200 mg/kg/day) on systolic (a), mean (b) and diastolic (c) blood pressure in L-NAME rats. Significantly different ($p < 0.05$; *ANOVA; #Student's t-test) between L-NAME and L-NAME+ASE groups

Effect of ASE in L-NAME Hypertensive Rats

The baseline systolic, mean and diastolic arterial pressures before the beginning of L-NAME treatment in the control group (105 ± 5 , 86.2 ± 3 , 76.6 ± 4 , respectively) and ASE group (107.8 ± 4 , 93.2 ± 4 , 71.3 ± 4 , respectively), were not significantly different. However systolic, mean and diastolic arterial pressures 21 days after L-NAME (50 mg/kg/day) administration in the control group (167.5 ± 9.9 , 142.5 ± 9.6 , 130.7 ± 9.6 , respectively) were significantly greater than in the ASE treated group (138.0 ± 4 , 114.3 ± 5.2 , 103.1 ± 6 , respectively) (Fig. 3).

Effect of ASE in Spontaneously Hypertensive Rats

In adult SHR, ASE significantly reduced significantly systolic, mean and diastolic arterial pressures. Before treatment the baseline systolic, mean and diastolic arterial pressures were 216.6 ± 5 , 193.1 ± 2 , 181.9 ± 3 , respectively, whereas after 33 days of treatment with ASE, diastolic, mean and systolic blood pressure were 193.0 ± 7 , 167.4 ± 5 , 157.1 ± 6 , respectively. The blood pressure increased after the ASE was removed (Fig. 4).

In SHR treated with ASE from 21 days of age (after weaning) the systolic, mean and diastolic blood pressures in the ASE group measured at day 95 were 135 ± 3.3 , 80.0 ± 2.1 , 55.5 ± 3.4 , respectively, which significantly reduced ($p < 0.05$) compared with the control group (221.9 ± 5.1 , 211.17 ± 5.6 , 201.3 ± 4.5 , respectively) (Fig. 5).

Western Blot Analysis of Vascular eNOS

Adult SHR showed significantly higher eNOS expression compared with SHR treated with ASE ($p < 0.05$). eNOS protein expression in vessels from Wistar rats was significantly reduced compared with control SHR and SHR treated with ASE ($p < 0.05$). However, the eNOS expression was also significantly increased ($p < 0.05$) in Wistar rats treated with ASE compared with control Wistar

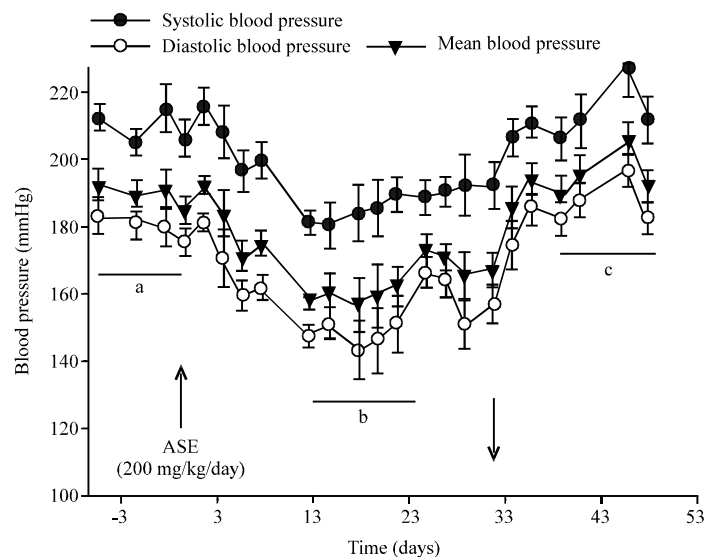


Fig. 4: Effect of ASE (200 mg/kg/day) on systolic, mean and diastolic blood pressure in adults SHR. Arrows up represent the beginning of treatment with ASE and arrows down represent the interruption of the treatment. Significantly different ($p < 0.05$ ANOVA) between untreated period (a) and treated with ASE period (b). Significantly different ($p < 0.05$ ANOVA) between treated with ASE period (b) and after interruption of the treatment (c)

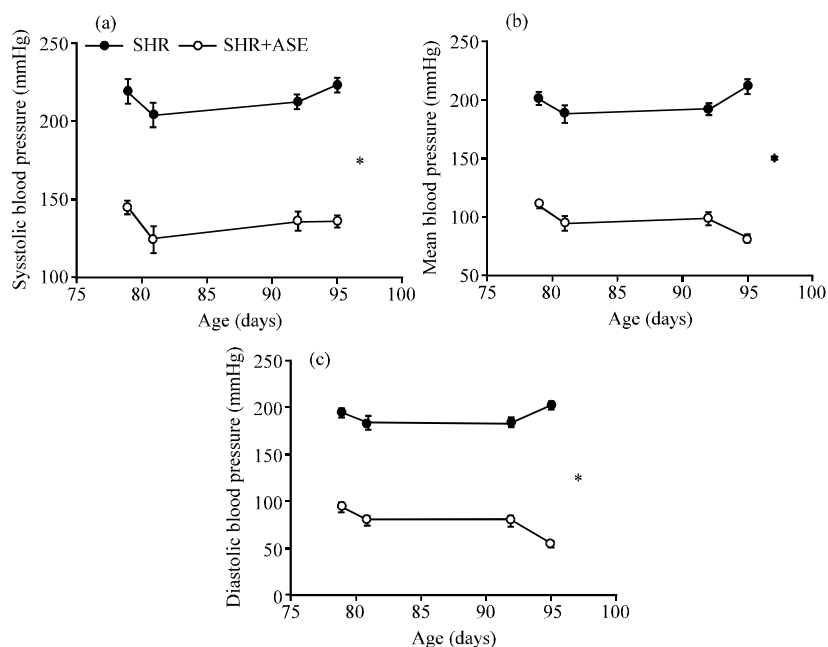


Fig. 5: Effect of ASE (200 mg/kg/day, treated from day 21) on systolic (a), mean (b) and diastolic (c) blood pressure in young SHR. *Significantly different ($p < 0.05$ ANOVA) between SHR and SHR+ASE groups

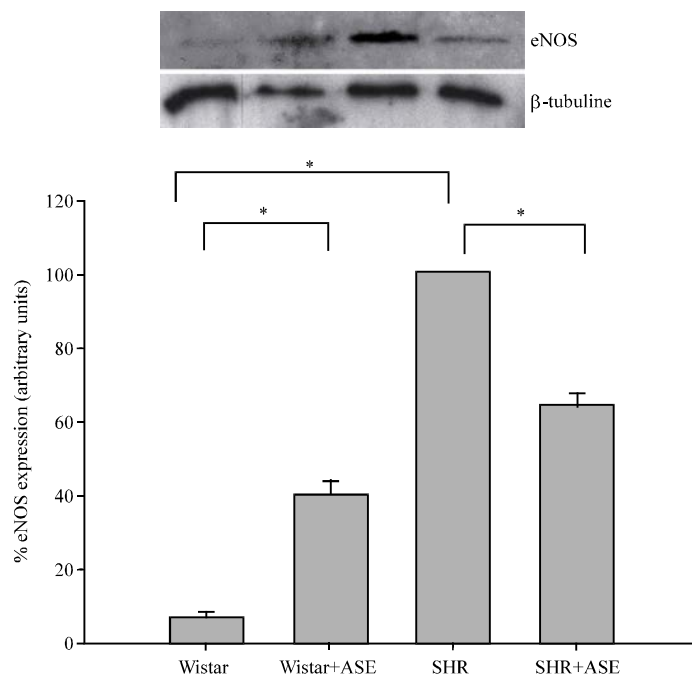


Fig. 6: Western blot analysis comparing expression of eNOS (40 μ g protein per lane) in mesenteric arteries from Wistar, Wistar+ASE, SHR and SHR+ASE. Data from three separate experiments are expressed as mean $\pm$ SEM, *Significantly different from the corresponding control groups ($p < 0.05$ Student's t-test)

rats (Fig. 6). The mean blood pressures of SHRs before and after treatment were 181.3 ± 5.4 and 157.8 ± 5.8 mmHg, respectively. Mean blood pressures of untreated SHR at the beginning and at the end of 20 days were, respectively 177.4 ± 4.0 and 195.2 ± 7.7 mmHg.

Antioxidant Effect of ASE on Rats Exposed to Cigarette Smoke

Oxidative damage expressed by TBARS in the Bronchoalveolar Lavage (BAL) of adult Wistar rats exposed to Cigarette Smoke (CS), was significantly higher ($p < 0.05$) than in rats exposed only to ambient air. Rats exposed to CS and treated with ASE showed significantly lower levels of TBARS as compared to rats exposed to CS alone ($p < 0.05$). Rats treated with ASE and exposed to ambient air showed no significant difference in TBARS from control animals exposed to ambient air (Fig. 7).

ASE Modulated Inflammatory Cell Influx

The alveolar macrophages number in BAL fluid (cells $\times 10^3$ mL $^{-1}$) was 126.3 ± 7.1 in control group, 139.5 ± 20.5 in ASE group, 220.8 ± 24.9 in CS group and 159.0 ± 16.4 in CS+ASE group. The neutrophils number in BAL fluid (cells $\times 10^3$ mL $^{-1}$) was 15.0 ± 0.6 in control group, 15.25 ± 1.9 in ASE group, 51.7 ± 9.2 in CS group 17.0 ± 2.5 in CS+ASE.

The inflammatory cell influx (macrophages and neutrophils) into the BAL of adult Wistar rats exposed to CS was significantly higher ($p < 0.05$ and 0.001 , respectively) than in rats exposed only to ambient air. Rats exposed to CS and treated with ASE presented a reduction in alveolar neutrophils as compared to rats exposed to CS alone ($p < 0.01$). Rats treated with ASE and exposed to ambient air showed no significant difference in BAL cells from control animals exposed to ambient air (Fig. 8).

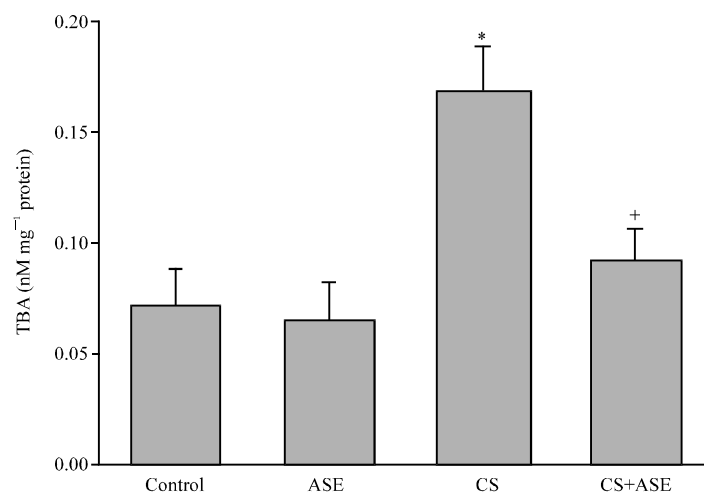


Fig. 7: Effect of ASE on lipid peroxidation estimated as TBA in bronchoalveolar lavage of adult rats.
 *Significantly different ($p < 0.05$; Student's t-test) between CS and control and CS and ASE.
 *Significantly different ($p < 0.05$; Student's t-test) between CS+ASE and CS

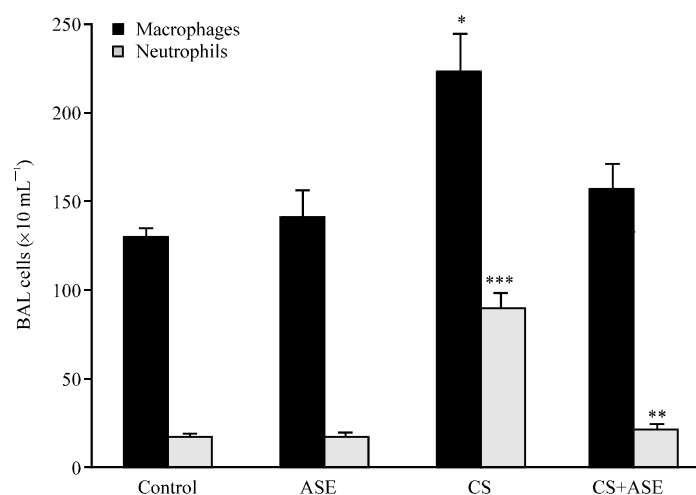


Fig. 8: Effect of ASE on cell influx (macrophages and neutrophils) into the BAL from exposed mice to Cigarette Smoke (CS). *Significantly different ($p < 0.05$; Student's t-test) between CS and control and CS and ASE; ***Significantly different ($p < 0.001$; Student's t-test) between CS and control and CS and ASE; **Significantly different ($p < 0.01$; Student's t-test) between CS+ASE and CS

DISCUSSION

The present results demonstrate, for the first time, a significant antihypertensive effect of an extract obtained from the seed of Açai, a polyphenol-rich plant. The antihypertensive effect of ASE was demonstrated in all experimental models (DOCA-Salt, Goldblatt, L-NAME and SHR) of hypertension in rats.

Goldblatt renovascular hypertension (2K-1C) is dependent on activation of the renin-angiotensin system (Lerman *et al.*, 2005). The increase in blood pressure observed in 2K-1C hypertension is probably dependent on an increase in arterial vascular resistance induced by angiotensin II due to its direct activation of AT₁ receptors and also, to an indirect vasoconstrictor action of angiotensin II through activation of the sympathetic nervous system (Faber and Brody, 1984). Since we have demonstrated that ASE induces an endothelial-dependent vasodilator effect (Rocha *et al.*, 2007) the anti-hypertensive action of ASE in 2K-1C hypertension may be due to a decrease in vascular resistance due to inhibition of vasoconstriction induced by angiotensin II and norepinephrine. An increase in oxidative stress occurs in 2K-1C hypertension (Welch *et al.*, 2003) probably due to activation of NAD(P)H oxidase induced by angiotensin II and norepinephrine (Griendling *et al.*, 1994; Paravicini *et al.*, 2006). The anti-oxidant action of ASE demonstrated in the present study may also participate in the anti-hypertensive action of ASE in 2K-1C since tempol, a superoxide dismutase mimetic compound not only inhibited the oxidative stress but also the increase in blood pressure observed in 2K-1C model of hypertension (Welch *et al.*, 2003).

The antihypertensive action of ASE seems also to be independent of the renin-angiotensin system since it occurs also in a model of low renin hypertension that is DOCA-salt hypertensive rats. In this model of hypertension, angiotensin II seems not to participate in the mechanism of the elevated blood pressure since compounds that inhibit angiotensin converting enzyme, do not induce reduction of the high levels of blood pressure observed in DOCA-salt hypertensive rats (Wada *et al.*, 1995; Wong and Zimmerman, 1982; Li *et al.*, 1996). The present results have demonstrated that in nephrectomized rats treated with DOCA-salt the increase in blood pressure is significantly reduced by ASE. Probably the anti-hypertensive effect of ASE in DOCA-salt hypertension is due to its vasodilator effect (Rocha *et al.*, 2007) since hydralazine, a potent vasodilator compound, reduces hypertension in DOCA-Salt hypertensive rats (Cuman *et al.*, 1994). The antioxidant effect of ASE may also be involved on the antihypertensive effect since increased production of superoxide by NADPH oxidase has also been reported in DOCA-salt hypertensive rats (Callera *et al.*, 2003). Additionally, involvement of NO production may participate in the antihypertensive effect of ASE in DOCA-salt hypertensive rats since reduction of NO formation and NO synthase activity and expression has been found to occur in this hypertension model (Takanohashi *et al.*, 1996; Hara *et al.*, 2001). Since we have previously demonstrated that ASE increased nitric oxide formation in human umbilical vein endothelial cells (Rocha *et al.*, 2007) and now also eNOS expression in vessels of normotensive rats (present results), the antihypertensive effect of ASE in DOCA-salt hypertension may be also dependent on the activation of eNOS/NO system.

Chronic inhibition of NOS by L-NAME induces arterial hypertension in rodents (Ribeiro *et al.*, 1992; Baylis *et al.*, 1992). The pathophysiology of L-NAME hypertension is at the moment, not completely elucidated, but probably depends on reduction of NO synthesis (De Genaro *et al.*, 2005) activation of sympathetic drive (Sakuma *et al.*, 1992) and an increase in the renin-angiotensin system (Zanchi *et al.*, 1995) leading therefore to general vasoconstriction that induces an increase in vascular resistance. An increase in oxidative stress due to activation of NADPH may also participate in the mechanism of L-NAME hypertension (Li *et al.*, 2002; Toba *et al.*, 2005; Kitamoto *et al.*, 2000). Present results demonstrated a significant inhibitory effect of ASE on the arterial hypertension induced by L-NAME. Probably this effect is dependent on the vasodilator (Rocha *et al.*, 2007) and anti-oxidant effects of ASE (present results) since antioxidants such as tempol (Kopkan and Majid, 2005) and vasodilators as hydralazine (Okazaki *et al.*, 2006) also inhibit the hypertension induced by L-NAME. In the present study we demonstrated a significant effect of ASE on the development of hypertension in SHR. Thus, while control SHR showed high levels of blood pressure from day 75 until day 95 of age, ASE-treated animals (from 21 of age until day 95) showed no rise in blood pressure, demonstrating a preventative action on the development of hypertension in this model of genetic hypertension. At

the moment the mechanism(s) involved in this important action of ASE is not completely elucidated. An antioxidant effect of extracts obtained from *Euterpe oleracea* Mart. has been shown to occur *in vitro* (Lichtenthaler *et al.*, 2005). In the present study, we demonstrated that an antioxidant action of ASE also occur *in vivo*, since the increase in lipid peroxidation induced by cigarette smoke in rats was significantly reduced by ASE. Present results also showed that the increase in neutrophils influx into the BAL induced by cigarette smoke, was significantly inhibited by ASE. This effect could be due to its antioxidant action. Several studies have shown increased reactive oxygen species in SHR (Suzuki *et al.*, 1995; Grundfeld *et al.*, 1995) and a reduction in blood pressure in SHRs with antioxidant treatment (Schnackenberg *et al.*, 1998; Varizi *et al.*, 2000). In this study, we also demonstrated a reduction in the high levels of blood pressure of adult SHRs treated with ASE. Probably this antihypertensive effect may be due, in part, to the antioxidant action of ASE. The present study demonstrated an increase in eNOS expression in adult SHRs compared to normotensive rats. Interestingly, we found that ASE treatment reduced not only the high levels of blood pressure but also reduced the high expression of eNOS observed in adult SHRs. Increases in eNOS have been also demonstrated in SHRs by other authors (Varizi *et al.*, 1998, 2000; Li *et al.*, 2006).

Present results are in accordance with data from the literature demonstrating a reduction of blood pressure and concomitant decrease in the high level of eNOS induced by antihypertensive treatment with AT-1 receptor blockade and calcium channel blockade in spontaneously hypertensive rats (Varizi *et al.*, 2002). The *in vitro* increase in NO formation by ASE observed by our group in isolated human umbilical cells (Rocha *et al.*, 2007), suggesting an increase in eNOS activity, has now also been demonstrated *in vivo* since we showed an increase in eNOS in adult normotensive rats treated with ASE. In the present study we demonstrated an increase in eNOS expression in SHRs that was reduced by ASE. It has been suggested that in spontaneously hypertensive rats there is an increased inactivation of NO by ROS generated at the expense of a larger NO production, a condition referred as eNOS uncoupling (Li *et al.*, 2002). Thus an increase in ROS might have contributed to upregulation of eNOS by limiting the availability of biologically active NO that exerts a negative feedback action on eNOS expression (Varizi *et al.*, 2002). Therefore, the anti-oxidant effect of ASE and the reduction of blood pressure, that decreases the expression of eNOS induced by high shear stress, probably play an important role on the reduction of eNOS expression induced by ASE in spontaneously hypertensive rats.

In conclusion, the present study has demonstrated an anti-hypertensive effect of ASE that is probably due to its combined vasodilator and antioxidant actions. The present preclinical results support a potential therapeutic indication of ASE in hypertensive patients.

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