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Full Length Research Paper

Vasorelaxant response induced by *Sida santaremnensis* H. Monteiro ethanol extract on rat superior mesenteric artery

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The aim of this work was to characterize the vasorelaxant effect produced by *Sida santaremnensis* ethanol extract (Ssan-EtOH) in rat superior mesenteric artery. Ssan-EtOH showed neither acute toxicity nor hemolytic activity. In the other hand, it induced a concentration-dependent vasorelaxant effect on phenylephrine (10 µmol/L) or KCl (80 mmol/L)-induced pre-contractions in endothelium-intact rat mesenteric artery rings, which attenuated after endothelium removal, without changes in maximum effect. N^G-nitro-L-arginine methyl ester (L-NAME) (100 µmol/L), indomethacin (10 µmol/L), atropine (1 nmol/L), KCl (20 mmol/L) or tetraethylammonium (3 mmol/L) pretreatment also induced a response attenuation. The endothelium-derived hyperpolarizing factor (EDHF) involvement in Ssan-EtOH-induced vasorelaxant response was verified after L-NAME (100 µmol/L) plus Indomethacin (10 µmol/L) plus tetraethylammonium (3 mmol/L) pretreatment and this vasorelaxation was decreased. In endothelium-denuded rings, Ssan-EtOH was able to inhibit phenylephrine-induced contractions (10^{-9} to 10^{-5} mol/L) in a concentration-dependent manner. In a nominally without Ca^{2+} depolarizing Tyrode solution, Ssan-EtOH inhibited $CaCl_2$ (10^{-6} – 3 x 10^{-2} mol/L)-induced contractions in a concentration-dependent manner. The endothelium-dependent Ssan-EtOH-induced vasorelaxant effect probably involves the participation of the nitric oxide synthase (NOS) and cyclooxygenases (COX) pathways, as well muscarinic receptors and EDHF and the endothelium-independent effect probably occurs by Ca^{2+} influx inhibition through voltage-operated calcium channels.

Key words: *Sida santaremnensis*, superior mesenteric artery, Malvaceae, vasorelaxation.

INTRODUCTION

The species *Sida santaremnensis* H. Monteiro (Malvaceae) is popularly known as “guanxuma” and occurs in the Americas, Africa and Asia. Species from the Sida genus have been used by traditional medicine to treat cough, fever (Hansen et al., 1995; Bork et al., 1997), vascular disorders and hypertension (Noumi et al., 1999). Other studies have shown that the *Sida cordifolia* induces hypotension and bradycardia in normotensive rats probably by decreasing the vascular resistance mediated by endothelium-derived relaxing factors (EDRFs) (Santos et al., 2006).

The smooth muscle is the main kind of muscle that

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controls and regulates the functioning of several organs and represents a useful tool for investigating biological activities of natural or synthetic substances (Watterson et al., 2005). The contraction of smooth muscle is dependent on intracellular Ca^{2+} mainly by extracellular Ca^{2+} influx increase and calcium release from intracellular stores (Somlyo and Somlyo, 1994).

The contractile activity of vascular smooth muscle cells from arteries and arterioles is a major determinant of resistance to blood flow through the circulation. Thus, vascular tone plays an important role in blood pressure regulation and blood flow distribution in several tissues and organs. The regulation of vascular tonus in the systemic circulation is dependent of the complex interplay of vasodilator and vasoconstrictor substances, stimuli from circulating hormones, neurotransmitters and endothelium-derived factors that regulate arterial blood pressure (Jackson, 2000).

Vessels such as superior mesenteric artery offers an increased resistance to blood flow and are more widely implicated in the regulation of capillary blood pressure, thus reflecting the evolution of the overall peripheral resistance (Mulvany and Aalkjaer, 1990; Folkow, 1979). Therefore, the purpose of this study was to evaluate the vasorelaxant effect and possibly involved mechanisms of ethanol extract obtained from *S. santaremnensis* aerial parts (Ssan-EtOH) in rat isolated superior mesenteric artery rings.

MATERIALS AND METHODS

Plant material and extraction

The aerial parts of *S. santaremnensis* H. Monteiro (Malvaceae) were collected from a local park in Teresina city, Piauí state, Brazil. The botanical identification was carried out at Graziela Barroso Herbarium of Federal University of Piauí, Brazil (voucher no. 21867). The aerial parts (2.5 kg) were dried at room temperature and then powdered. The powder was exhaustively extracted in 95% ethanol by maceration at room temperature.

The ethanol extract was evaporated to dryness under reduced pressure to yield 105 g (4.2%). Prior to the experiments, the ethanol extract was diluted in tween80/deionized water 0.1% (v/v) and diluted to the desired concentrations to give a water-soluble fraction (Ssan-EtOH). The final concentration of tween80 in the bath never exceeded 0.01%.

Animals

Male Wistar rats (270 ± 30 g) were used for all experiments. Animals were kept under conditions of controlled temperature (24±1°C) and 12-h light/dark cycle. They had free access to food (PURINA, Brazil) and tap water *ad libitum*. All experimental procedures were approved by the Animal Research Ethics Committee of Federal University of Piauí, Brazil (CEEAPI no. 04/2008). Procedures regarding euthanasia of animals were in accordance with the Resolution No. 714 (2002) of Federal Council of Veterinary Medicine, Brazil.

Solutions

The composition of the Tyrode´s solution used was (mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 10.0; and glucose, 5.6 mM (Tanaka et al., 1999). KCl 20, 60 and 80 mM Tyrode solution were prepared by an equimolar replacement of Na^+ for K^+. In a nominally, without Ca^{2+} solution, $CaCl_2$ was not added.

Drugs

The following drugs were used: L-phenylephrine hydrochloride (Phe), acetylcholine hydrochloride (ACh), atropine sulphate, tetraethylammonium (TEA), L-NAME (Sigma, St. Louis, MO, USA) and indomethacin (Calbiochem, San Diego, CA, USA). Indomethacin was dissolved in sodium bicarbonate 5%. The other drugs were dissolved in deionized water.

Preparation of rat superior mesenteric artery rings

The superior mesenteric arteries were removed and cleaned from the connective tissue and fat. Mesenteric rings (1 to 2 mm) were obtained and suspended by cotton threads in organ baths containing 10 ml of Tyrode's solution, at 37°C and gassed with carbogenic mixture (95% O_2 and 5% CO_2). Rings were stabilized with a resting tension of 0.75 gf for at least 1 h. During this time, the solution was changed every 15 min (Altura and Altura, 1970).

The isometric tension was recorded by a force transducer coupled to a data acquisition system (AECAD 1604, AQCAD 2.0.5., AVS Projects, São Paulo, SP, Brazil). When necessary, the endothelium was removed by gently rubbing the intimal layer with moistened cotton strings. The endothelium integrity was verified by relaxation to ACh (10 µmol/L) in rings pre-contracted by Phe (10 µmol/L). In endothelium-intact studies, preparations were discarded when ACh-induced relaxation was lower than 80%.

Determination of acute toxicity and hemolytic activity

The acute toxicity studies were performed according to Miller and Tainter (1944) with some modifications. Mice were divided into five groups (n = 10) and had free access to food (PURINA, Brazil) and tap water *ad libitum*. The animals were treated with saline (0.9% NaCl; control group) and Ssan-EtOH by oral route (500 or 2000 mg/kg) and the other groups were treated with saline and Ssan-EtOH by intraperitoneal route (500 or 1000 mg/kg). Animals were evaluated at 1, 2, 3, 4, 24, 48 and 72 h after the administration to verify the number of deaths.

The hemolytic activity was preformed according to methods described by Rangel et al. (1997) with some modifications. Male Wistar rats were anesthetized and euthanized and blood was immediately colleted in saline with EDTA. The erythrocytes were washed twice in saline solution (30 ml) with $CaCl_2$ (0.9% NaCl and 10 mmol/L $CaCl_2$) in constant agitation and centrifugation at 5000 rpm for 3 min. The Ssan-EtOH was evaluated at 3, 10, 30, 100, 300 and 1000 mg/L. The experiment was carried out in triplicate for concentrations at 4.5 ml with 0.5 ml of the suspension from erythrocytes. 100 µl of Triton X-100 1.0% (positive control) and saline(negative control) were used. The suspension was incubated for 1 h under constant agitation and centrifuged at 5000 rpm for 3 min. The cellular hemolysis was evaluated by absorbance of supernatant at 540 nm.

Investigation of Ssan-EtOH effect on L-phenylephrine hydrochloride (Phe) or KCl-induced pre-contractions

After 60 min stabilization period, Phe (10 µmol/L)-induced pre-contractions was elicited on endothelium-intact or endothelium-denuded rings to promote similar magnitude contractions. Thereby, Ssan-EtOH was added cumulatively (0.1 to 500 mg/L) after response to Phe had stabilized, approximately 30 min later. Then, a concentration-response curve was obtained. In other hand, Ssan-EtOH was also added cumulatively (0.1 to 500 mg/L) on KCl-induced tonic contractions (80 mmol/L) in endothelium-denuded rings (Oliveira et al., 2006).

Evaluation of endothelium-derived factors involvement in Ssan-EtOH-induced vasorelaxant response

In endothelium-intact preparations, some pharmacological tools were incubated 30 min before, in order to investigate the participation of endothelium-derived factors in Ssan-EtOH-induced vasorelaxant effect, as follows: L-NAME (100 µmol/L), a competitive inhibitor of NOS (Moncada and Higgs, 1993); Indomethacin (10 µmol/L) a COX inhibitor (Clark and Fuchs, 1997) and atropine (1 nmol/L) a muscarinic receptors non-selective antagonist (Sawyer et al., 1999). After stabilization of the tonic contraction induced by Phe (10 µmol/L), Ssan-EtOH cumulative concentrations (0.1 to 500 mg/L) were added to the organ bath.

Evaluation of potassium channels involvement in Ssan-EtOH-induced vasorelaxant response

In endothelium-intact preparations, non-selective potassium channel blockers were incubated 30 min before, in order to investigate their participation in Ssan-EtOH-induced vasorelaxant effect, as follows: KCl (20 mmol/L) or TEA (3 mmol/L) (Wang et al., 2008). After stabilization of the tonic contraction induced by Phe (10 µmol/L), Ssan-EtOH cumulative concentrations (0.1 to 500 mg/L) were added to the organ bath.

Investigation of Ssan-EtOH effect on Phe-induced concentration-response curves in endothelium-denuded preparations

After stabilization period, the effect of Ssan-EtOH on Phe-induced contractions in endothelium-denuded rings was assessed (Oliveira et al., 2006). Cumulative concentration-response curves for Phe (10^{-9} to 10^{-5} mol/L) were obtained before and after pre-incubation separately with Ssan-EtOH (9, 27, 81, 243 and 500 mg/L) for 30 min. The results were expressed as percentages of the maximal response for Phe-induced response and curves were statistically compared.

Investigation of Ssan-EtOH effect on $CaCl_2$-induced concentration-response curves in endothelium-denuded preparations

After stabilization period, the Ssan-EtOH effect on $CaCl_2$-induced contractions in endothelium-denuded rings was assessed (Oliveira et al., 2006). Cumulative concentration-response curves for $CaCl_2$ (10^{-6} to $3x10^{-2}$ mol/L) were obtained in endothelium-denuded rings exposed nominally without Ca^{2+} solution with KCl 60 mmol/L before and after pre-incubation separately with Ssan-EtOH (27, 81, 243 and 500 mg/L) for 30 min. The results were expressed as percentages of the maximal response for $CaCl_2$-induced response and curves were statistically compared.

Data analysis

All values were expressed as mean ± S.E.M. Experimental results were expressed as percentage decreases in Phe- or KCl-induced maximal contraction. The potency was expressed by EC_{50} (effective concentration that promotes 50% response). Student's t-test and ANOVA-one way Newman-Keuls post-test were used in the data analysis and results were considered significant when $p<0.05$. All analysis was performed using GraphPad™ Prism software, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Determination of toxicity for Ssan-EtOH

In acute toxicity studies, Ssan-EtOH did not induce death (500 to 2000 mg/kg) with a minuscule "k". Therefore, LD_{50} value was not determined. Likewise, Ssan-EtOH (1 to 1000 mg/L) did not elicit hemolytic activity in rat erythrocytes. Thereby, no toxicological effects were observed for Ssan-EtOH.

Effect of Ssan-EtOH on Phe- or KCl-induced pre-contractions

In rat endothelium-intact superior mesenteric artery rings, Ssan-EtOH induced a vasorelaxant response on Phe (10 µmol/L)-induced pre-contractions (Figure 1). The vasorelaxant response was markedly attenuated after endothelium removal. EC_{50} values for Ssan-EtOH in endothelium-intact or endothelium-denuded rings were significantly different (Figure 2). Ssan-EtOH-induced vasorelaxant response on KCl (80 mmol/L)-induced pre-contractions did not promote an attenuating response in rat endothelium-denuded superior mesenteric artery rings (Figure 3).

Effect of Ssan-EtOH on endothelium-derived factors

In rat endothelium-intact superior mesenteric artery rings, Ssan-EtOH-induced vasorelaxant effect was attenuated after L-NAME (100 µmol/L), Indomethacin (10 µmol/L) and atropine (1 nmol/L) (Figure 4). Likewise, a significant reduction in maximal effect value was observed only in the presence of Indomethacin (E_{max} = 87.41 ± 2.77 %*; *$p<0.05$ *vs*. control).

Effect of Ssan-EtOH on potassium channels

In endothelium-intact rat mesenteric rings after KCl (20

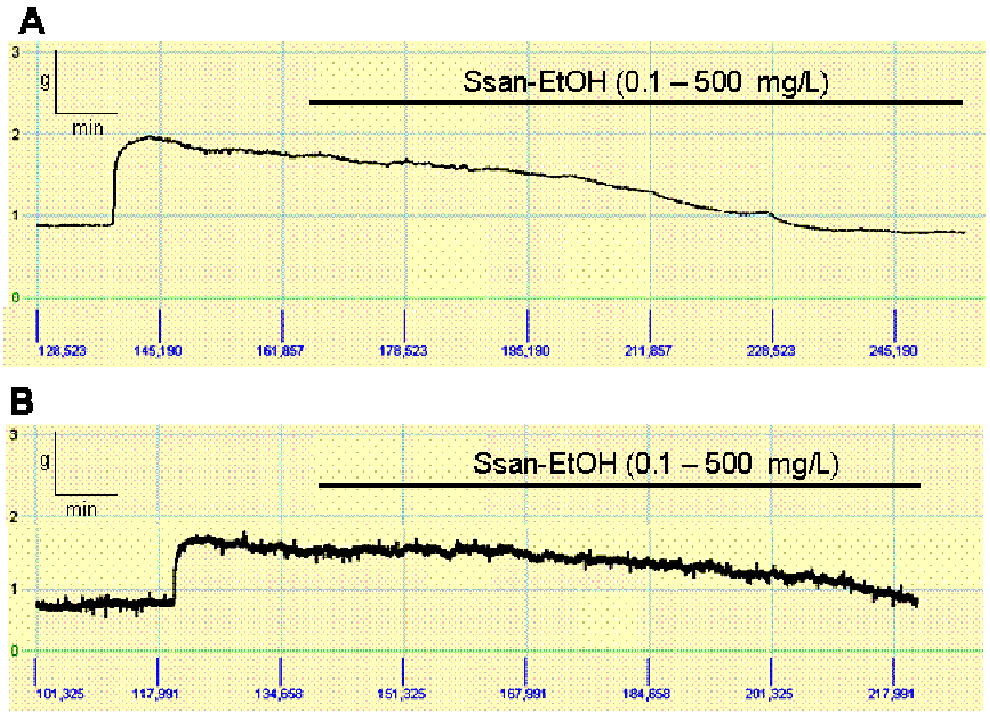

Figure 1. Original traces showing the vasorelaxant response of Ssan-EtOH (0.1 to 500 mg/L) in Phe 10 µmol/L pre-contracted mesenteric artery rings in the presence (A) or absence (B) of vascular endothelium.

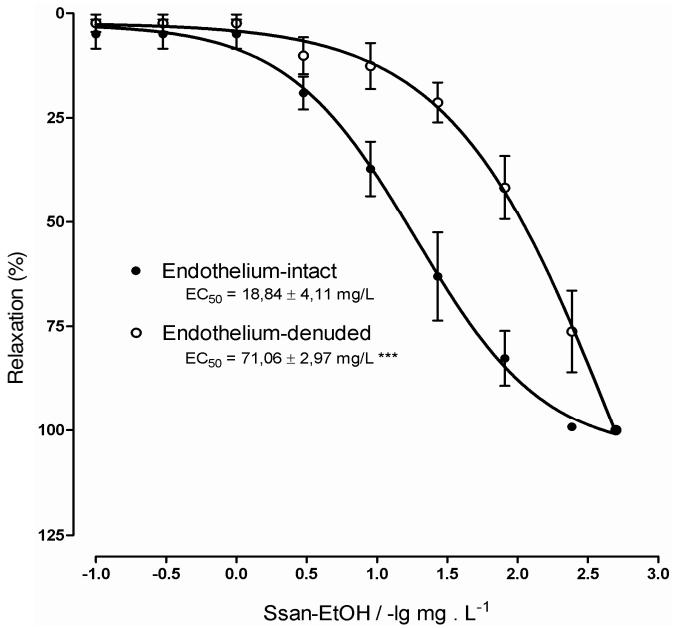

Figure 2. Vasorelaxant effect induced by Ssan-EtOH on Phe 10 µmol/L pre-contracted endothelium-intact (●) (n=6) or endothelium-denuded (○) (n=6) rat mesenteric rings. Values are each mean ± S.E.M.; ***p<0.001 vs. endothelium-intact.

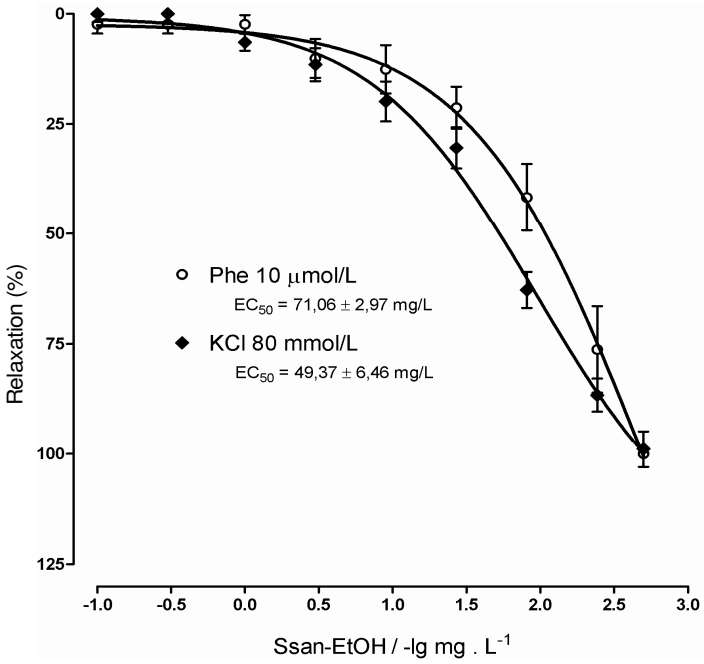

Figure 3. Vasorelaxant effect induced by Ssan-EtOH on Phe 10 µmol/L (○) or KCl 80 mmol/L (◆) pre-contracted endothelium-denuded rat mesenteric rings. Values are mean ± S.E.M.

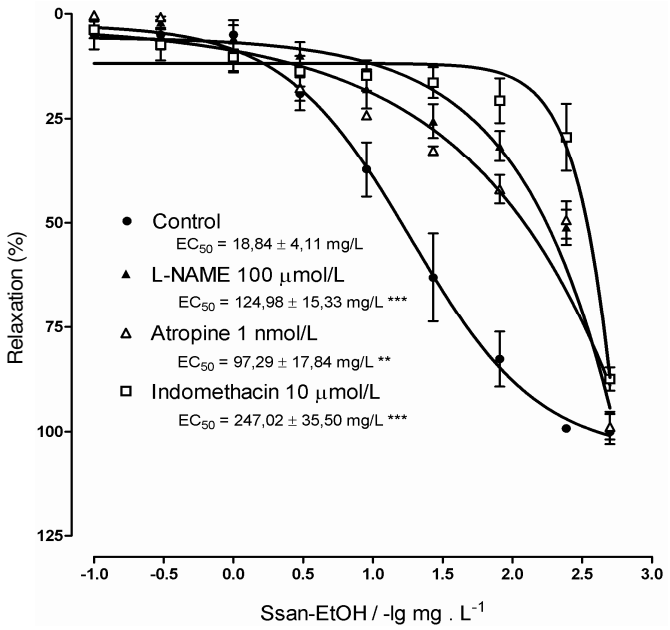

Figure 4. Concentration-response curves induced by Ssan-EtOH on endothelium-intact rat mesenteric rings pre-contracted with phenylephrine in the presence of L-NAME 100 µmol/L (▲) (n=9), Indomethacin 10 µmol/L (□) (n=7) or atropine 1 nmol/L (△) (n=5). The values are mean ± S.E.M., ***p<0.01; ***p<0.001 vs. control (●).

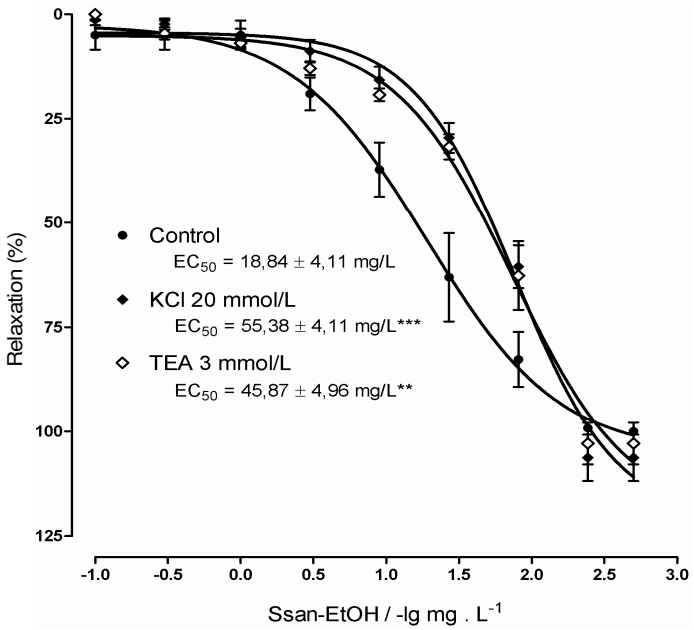

Figure 5. Concentration-response curves induced by Ssan-EtOH on Phe 10 µmol/L pre-contracted intact rat mesenteric rings in the presence of KCl 20 mmol/L (◆) (n=7) or TEA 3 mmol/L (◇) (n=6). The values are mean ± S.E.M., **p<0.01; ***p<0.001 vs. control (●).

mmol/L) pretreatment, Ssan-EtOH-induced vasorelaxant response was markedly decreased. A similar response was observed in the preparations after TEA (3 mmol/L)pretreatment (Figure 5), but not in endothelium-denuded preparations (Figure 6). In both cases, the E_{max} attenuation was not observed.

Effect of Ssan-EtOH on endothelium-intact rings after L-NAME plus Indomethacin plus TEA association

In endothelium-intact preparations, pretreatment with L-NAME (100 µmol/L) plus Indomethacin (10 µmol/L) did not induce attenuation of vasorelaxant response differently as observed in preparations with these pharmacological tools separately. However, L-NAME (100 µmol/L) plus Indomethacin (10 µmol/L) plus TEA (3 mmol/L) pretreatment showed response attenuation and E_{max} decrease (E_{max} = 88.6 ± 9.6 %*) (Figure 7).

Effect of Ssan-EtOH on Phe- or $CaCl_2$-induced contractile response

After Ssan-EtOH pretreatment, Phe-induced vasoconstriction was markedly inhibited in endothelium-denuded rings by a concentration-dependent manner (E_{max} = 98.0 ± 1.6 %; E_{max} = 91.1 ± 5.4%; E_{max}= 45.9 ± 1.9%***; E_{max}=11.2 ± 2.5 %***, E_{max}=5.6 ± 1.1%***, respectively) (Figure 8). Likewise, $CaCl_2$-induced vasoconstriction was attenuated nominally, without Ca^{2+} depolarizing Tyrode solution (E_{max} = 97.6 ± 3.8 %; E_{max} = 52.9 ± 4.8 %***; E_{max} = 43.5 ± 5.4 %***, E_{max} = 26.7 ± 3.8 %***, respectively) (Figure 9).

DISCUSSION

The major finding of this study was that the ethanol extract from *S. santeremnensis* induced an endothelium-dependent and –independent vasorelaxant effect. The vascular endothelium plays an important role in homeostasis by modulating the vascular smooth muscle tone and acts as a main target in hypertension and atherosclerosis treatments. Endothelial cells produce various vasorelaxant substances such as EDRFs: NO (Moncada et al., 1991), EDHF (Félétou and Vanhoutte, 1999) and vasorelaxant prostaglandins. These EDRFs diffuse to adjacent smooth muscle cells and promote vasorelaxation (Vanhoutte and Boulanger, 1995). In order to investigate the role of endothelium-derived vasorelaxant factors, experiments using L-NAME, an inhibitor of the NOS (Bartunek et al., 2000) were realized. L-NAME pretreatment inhibited the Ssan-EtOH-induced

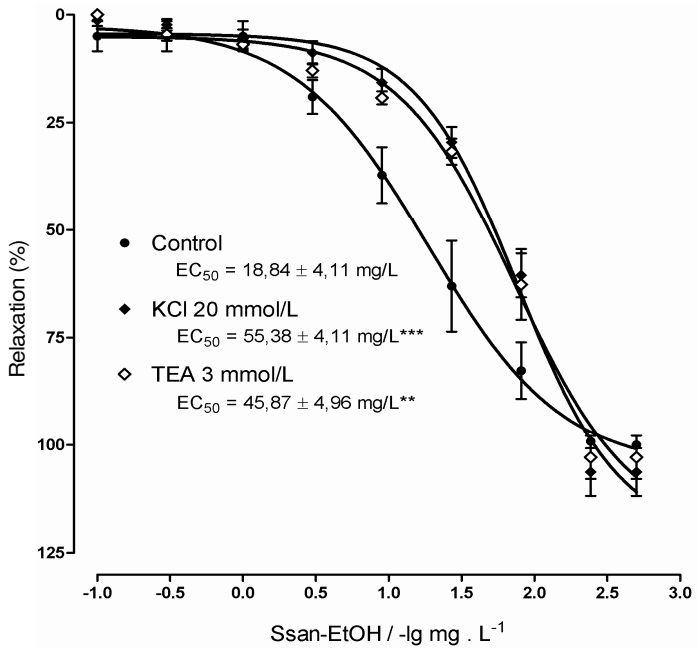

Figure 5. Concentration-response curves induced by Ssan-EtOH on Phe 10 µmol/L pre-contracted intact rat mesenteric rings in the presence of KCl 20 mmol/L (◆) (n=7) or TEA 3 mmol/L (◇) (n=6). The values are mean ± S.E.M., **p<0.01; ***p<0.001 vs. control (●).

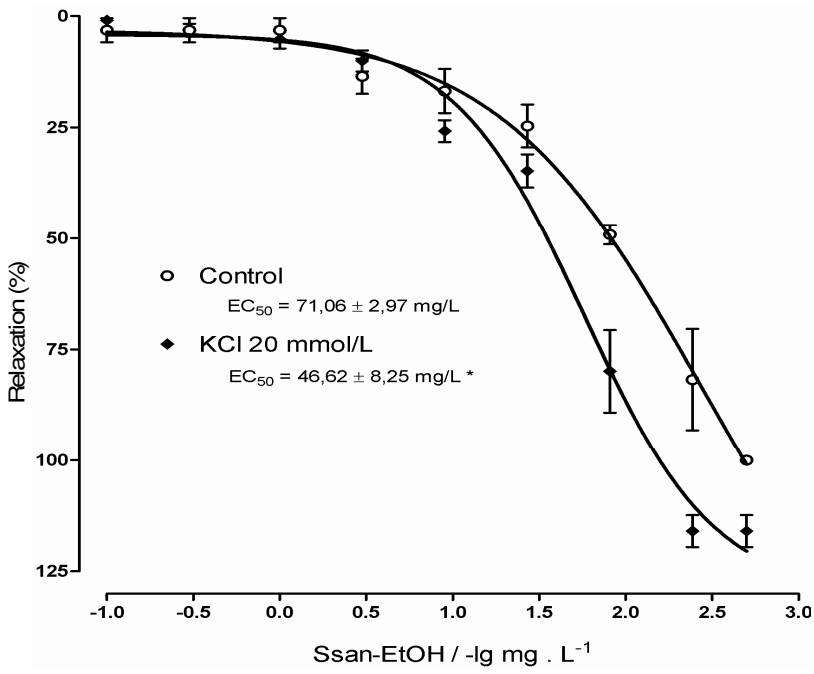

Figure 6. Concentration-response curve induced by Ssan-EtOH on Phe 10 µmol/L pre-contracted endothelium-denuded rat mesenteric rings in the presence of KCl 20 mmol/L (◆) (n=7). The values are mean ± S.E.M.; *p<0.05 vs. control (○).

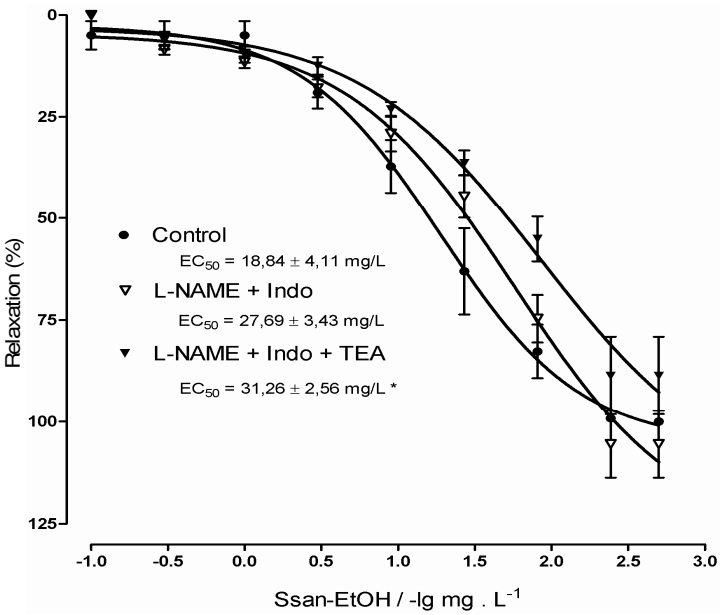

Figure 7. Concentration-response curves induced by Ssan-EtOH on endothelium-intact rat mesenteric rings in the presence (n= 6) of L-NAME 100 µmol/L plus indomethacin (Indo) 10 µmol/L (▽) (n=5) and L-NAME 100 µmol/L plus Indo 10 µmol/L plus TEA 3 mmol/L (▼) (n=5). The values are a mean ± S.E.M.; *p<0.05 vs. control (●).

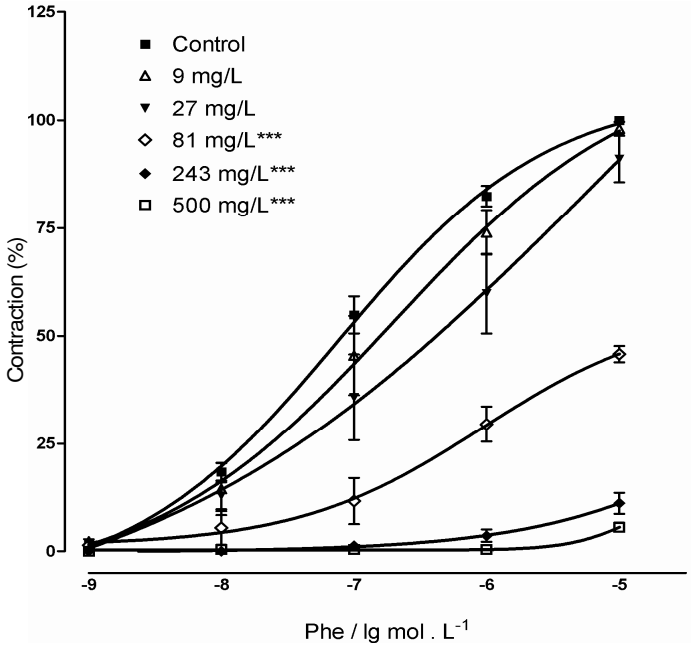

Figure 8. Concentration-response curves induced by Phe (10^{-9} – 10^{-5} mol/L) on endothelium-denuded rat mesenteric rings (n=7) in the presence of Ssan-EtOH (9, 27, 81, 243 or 500 mg/L) for 30 min. The values are ± S.E.M.; ***p<0.001 vs. E_{max} control.

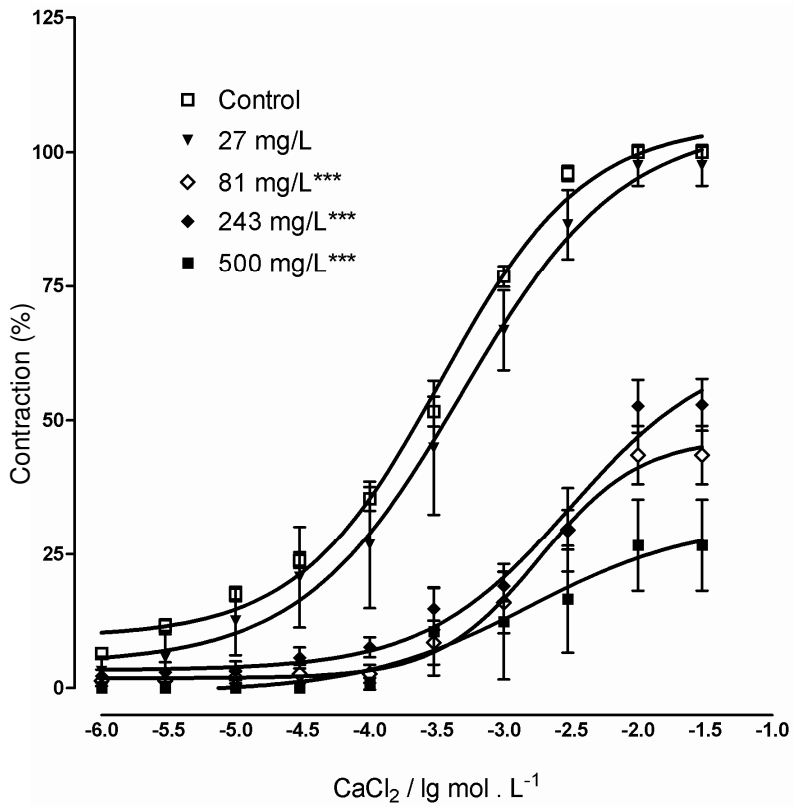

Figure 9. Concentration-response curves induced by $CaCl_2$ (10^{-6} – $3x10^{-2}$ mol/L) on endothelium-denuded rat mesenteric rings (n=6) in the presence of Ssan-EtOH (27, 81, 243 or 500 mg/L) for 30 min. The values are mean ± S.E.M.; ***p<0.001 vs. E_{max} control.

vasorelaxation, suggesting the participation of the NO-Synthase in this response.

Another very important vasorelaxation-involved pathway and subsequent regulation of vascular tone is related to production and release of COX metabolites. COXs are a group of enzymes involved in arachidonic acid metabolism and biosynthesis of some derivatives substances. Prostacyclin is a potent endothelium-derived vasodilator biosynthesized from arachidonic acid, important for the regulation of muscle tone (Schulz and Triggle, 1994; Moncada and Vane, 1978). The influence of COX in Ssan-EtOH-induced vasorelaxant response was carried out after Indomethacin pretreatment, a non-selective inhibitor of this enzyme (Moncada and Higgs, 1993). The vasorelaxant effect of Ssan-EtOH was markedly attenuated, suggesting the involvement of metabolites from arachidonic acid in this response.

It is well related that M_3 muscarinic receptors activation, located at endothelial cells, induces a release of endothelium-derived factors, mainly NO and consequently vasodilation and hypotension (Moncada et al., 1991; Furchgott and Zawadzki, 1980). Thus, in order to investigate the role of muscarinic receptors in vasorelaxant effect induced by Ssan-EtOH, we performed experiments after atropine pretreatment, a muscarinic receptor blocker (Sawyer et al., 1999) and we observed that the concentration curve to Ssan-EtOH was dislocated toward the right. Thus, Ssan-EtOH-induced vasorelaxant effect probably involves the activation of muscarinic receptors.

Moreover, potassium channels have an important role in the regulation of vascular tone. It is well recognized that potassium channels activation relaxes blood vessels by inducing a cell membrane hyperpolarization, which promotes a decrease in the open-state probability of voltage-dependent calcium channels and thus decreases the level of intracellular Ca^{2+} (Wellman and Nelson, 2003; Tanaka et al., 1999).

The involvement of potassium channels in Ssan-EtOH-induced endothelium-dependent vasorelaxant effect was evaluated in KCl (20 mmol/L)-induced depolarizing Tyrode

solution preparations. This procedure partially inhibits the potassium channels opening-mediated vasodilation in cell membrane, resulting in a decrease of K^+ efflux (Campbell and Harder, 1996). This series of experiments were performed in endothelium-intact and removed, and it was observed that Ssan-EtOH-induced vasorelaxant response was attenuated only in endothelium-intact preparations, suggesting an involvement of potassium channels in the endothelium-dependent vasorelaxant response of Ssan-EtOH.

In order to confirm the possible role of potassium channels in the vasorelaxant effect induced by Ssan-EtOH, TEA (3 mmol/L), a non-selective blocker of potassium channels, was used. In this condition, the vasorelaxant response of Ssan-EtOH was significantly attenuated. These results indicate that Ssan-EtOH may activate vascular TEA-sensitive potassium channels and would inhibit the Ca^{2+} influx via voltage-operated calcium channels by the subsequent membrane hyperpolarization (Wang et al., 2008). In rat mesenteric arteries, K^+ channels contribute substantially to the regulation of vascular resistance and systemic circulation (Christensen and Mulvany, 1993) and the endothelium-dependent dilation in these arteries is mediated predominantly by EDHF and NO (McCulloch and Randall et al., 1998), although EDHF plays the key role in the response (Hwa et al., 1994).

Classically, the remnant dilation to endothelium-dependent vasodilators in the presence of L-NAME plus indomethacin has been attributed to EDHF (Chauhan et al., 2003). The vasorelaxant response of the Ssan-EtOH was markedly attenuated after L-NAME plus Indomethacin pretreatment, suggesting EDHF participation. The EDHF induced hyperpolarization of vascular smooth muscle cells, thereby promoting vasodilation by inhibition of voltage-gated calcium channels (Nagao and Vanhoutte, 1991).

Ssan-EtOH was able to inhibit contractions induced by the cumulative addition of phenylephrine in endothelium-denuded preparations, suggesting that Ssan-EtOH could probably act in some stage of the vascular smooth muscle contractile machinery, since α_1-adrenergic receptors activation until the increase of extracellular Ca^{2+} influx through receptor-operated Ca^{2+} channels - ROCCs (Karaki and Weiss, 1998). Besides, it also produced a dilation of isolated arteries pre-contracted by depolarization with KCl (80 mmol/L), which induces contraction by allowing the influx of extracellular Ca^{2+} through voltage-dependent (L- and T-type) Ca^{2+} channels, called electromechanical coupling (Gurney, 1994; Yoshihima et al., 1992; Vogalis et al., 1991). Since the resting membrane potential is inverted by a high K^+ Tyrode solution, the vasorelaxation in endothelium-denuded preparations can be produced by a voltage-gated calcium channels blockade, but not by mediators that promote hyperpolarization. The vasorelaxation induced by Ssan-EtOH on KCl (80 mmol/L) depolarization-induced pre-contraction therefore suggest the probably Ca^{2+} influx blockade through voltage-gated calcium channels. This is further supported by the findings that Ssan-EtOH inhibited $CaCl_2$-induced contractions in endothelium-denuded preparations in a nominal without Ca^{2+} depolarizing Tyrode solution.

Likewise, this work demonstrate that Ssan-EtOH-induced vasorelaxant effect is probably mediated by both endothelium-dependent manner, mainly involving NO, prostacyclin and EDHF pathways and by a calcium channel blockade in endothelium-denuded preparations. Further experiments are necessary to identify other possible mechanisms involved and what steps in cell signaling pathways are responsible for these effects.

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Abbreviations

Ssan-EtOH, ethanol extract from aerial parts of *Sida santaremnensis* H. Monteiro; **LD_{50},** lethal dose that induced 50% from death.

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