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RESEARCH ARTICLE

Possible mechanisms of action of the hypotensive effect of *Annona muricata* (soursop) in normotensive Sprague–Dawley rats

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Abstract

Context: *Annona muricata* Linn (Annonaceae) (soursop) is a food plant reported to have antihypertensive properties.

Objective: We investigated the blood pressure reducing effect of its aqueous leaf extract and the possible mechanisms that may be responsible.

Methods: Intravenous administration of an aqueous leaf extract (9.17–48.5 mg/kg) of *A. muricata* on the mean arterial pressure and heart rate were recorded invasively on anaesthetized, normotensive Sprague–Dawley rats. Contractile responses of rat aortic rings to the extract (0.5–4.0 mg/mL) were studied using standard organ bath techniques.

Results: *A. muricata* (9.17–48.5 mg/kg) caused significant ($p < 0.05$) dose-dependent reduction in blood pressure without affecting the heart rates. The hypotensive effects were unaffected by atropine (2 mg/kg), mepyramine (5 mg/kg), propranolol (1 mg/kg) and L-NAME (5 mg/kg). *A. muricata* leaf aqueous extract significantly ($p < 0.05$) relaxed phenylephrine (10^{-9}–10^{-4} M) and 80 mM KCl induced contractions in endothelium intact and denuded aortic rings; and caused a significant ($p < 0.05$) rightward shift of the Ca^{2+} dose response curves in Ca^{2+}-free Kreb's solution containing 0.1 mM EGTA.

Conclusions: The hypotensive effects of *A. muricata* are not mediated through muscarinic, histaminergic, adrenergic and nitric oxide pathways, but through peripheral mechanisms involving antagonism of Ca^{2+}.

Keywords: Aorta, calcium antagonism, hypertension, rats, *in vitro*, *in vivo*

Introduction

Hypertension is a major risk factor for the development of cardiovascular disease, and medications aimed at reducing blood pressure levels have been shown to reduce the morbidity and mortality (Nwokocha et al., 2011a,b). However, many of these medications produce undesirable side effects. As a result, there is much interest in the use of alternative medicine in the treatment of hypertension and in the search for plants with hypotensive activity and less adverse side effects (Nwokocha et al., 2011c, 2012). Brussel (2004) reported that a decoction made from the fruits, leaves, and twigs of *Annona muricata* Linn (Annonaceae) is drunk to treat high blood pressure, and there have been reports that the plant extract has antihypertensive properties (Carbajal et al., 1991; Adeyemi et al., 2008).

A. muricata is found widely in the West Indies, South and Central America, tropical West Africa, and Asia. In the West Indies, various parts of the plant, including the leaves, bark and roots have been used to treat disease conditions such as diabetes (Adeyemi et al., 2008, 2010) and arthritis. Other reported medicinal uses of

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A. muricata include its anticancer (Oberlies et al., 1997; Liaw et al., 2002), antibacterial and antifungal (Takahashi et al., 2006) actions, as well as, its antinociceptive and anti-inflammatory effects (de Sousa et al., 2010).

Phytochemical screening of the leaves of *A. muricata* has shown it to consist of alkaloids such as reticuline, coreximine, coclarine and anomurine (Leboeuf et al., 1981, 1982), annomuricin E, annomuricin C, muricatocin C, gigantetronenin and muricapentocin with antioxidant and antitumor properties (Wu et al., 1995; Kim et al., 1998; Luna et al., 2006; Baskar et al., 2007), as well as, essential oils such as β-caryophyllene, δ-cadinene, epi-α-cadinol and α-cadinol (Pelissler et al., 1994; Kossouoh et al., 2007).

Although there are reports that the *A. muricata* plant may have antihypertensive properties, the mechanism of action has not been investigated and remains unknown. The present study therefore sought to investigate the possible mechanisms of action of the hypotensive effect of the aqueous leaf extract of *A. muricata* in normotensive Sprague–Dawley rats.

Materials and methods

Experimental animals

Twelve-week-old male Sprague–Dawley rats, weighing between 250 and 350 g, were used for the study. The animals were obtained from the Animal House of Basic Medical Sciences, University of the West Indies (UWI), Mona, Jamaica. They were housed in plastic cages under a 12 h light/dark cycle at 20–25°C and had free access to standard rat chow and tap water *ad libitum*. Ethical approval for the study was obtained from the FMS/UHWI/UWI Ethics Committee, Mona Campus Jamaica.

Plant material and extraction

Mature leaves of *A. muricata* were harvested in January 2010, and the species authenticated by Mr. Patrick Lewis the resident botanist at the herbarium of The University of the West Indies. A voucher specimen number (35448) was deposited in the herbarium. The powdered leaves were macerated in distilled water and left overnight. The mixture was then filtered and the filtrate was evaporated using a rotary evaporator (Buchi, Switzerland), and further evaporated to dryness by freeze-drying (Millrock Technologies, USA). The dark brown solid residue of 9.10 g was stored in a capped container and refrigerated at –4°C until ready for use.

Measurement of blood pressure and heart rate

The rats were anesthetized with 15% urethane (8 mL/kg) given intraperitoneally, and the trachea exposed and cannulated to facilitate respiration. A polyethylene catheter (PE 50) was then inserted into the right jugular vein (for infusion of drugs and extracts) and another catheter was inserted into the left carotid artery and connected to a pressure transducer (Statham P23XL, USA) and a Grass Polygraph (Model 7D, Quincy, MA, USA) for blood pressure and heart rate (HR) measurements. Soon after the cannulation, 500 IU/kg of heparin (Upjohn, USA) was injected to prevent intravascular blood clotting. The animals were allowed to stabilize for at least 30 min before measurements were taken or any test substance administered.

Effects of *A. muricata* extract on blood pressure and heart rate

After equilibration of the animals for 30 min, the systolic and diastolic blood pressure and heart rate were recorded. Graded doses of the *A. muricata* aqueous leaf extract (9.17–48.5 mg/kg) were then administered 10 min apart. The blood pressure and heart rate were again recorded. Mean arterial pressure (MAP) was calculated as the sum of the diastolic pressure and one-third pulse pressure (Nwokocha et al., 2012).

Effect of *A. muricata* on atropine, propranolol, histamine and eNOS blockade

The effect of the aqueous leaf extract was examined after administration of the muscarinic receptor antagonist, atropine (2 mg/kg), the beta-adrenoceptor antagonist, propranolol (1 mg/kg), the histamine H_1 receptor agonist, mepyramine (5 mg/kg), or the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME, 5 mg/kg) (Weldon et al., 1995; de Moura et al., 2005; Ghayur et al., 2005; Lessa et al., 2008; Diaz-Juarez et al., 2009). Each drug was given intravenously and allowed to incubate for 5 min before a bolus injection of 19.41 mg/kg (ED_{50}) *A. muricata* extract was infused. The corresponding blood pressure and heart rate changes were then recorded.

Effects of *A. muricata* extract on the aortic rings in isolated organ bath studies

Thoracic aortae were isolated from the male rats and placed in a cold (4°C) physiological Kreb's solution (PSS) with the following composition (mM): NaCl, 112; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1, $NaHCO_3$, 25; KH_2PO_4, 0.5; NaH_2PO_4, 0.5; glucose, 10; pH 7.4. All chemicals were purchased from Sigma-Aldrich, St Louis, MO, USA. Each aorta was cleaned of connective tissues under the dissecting microscope and cut into ring segments (~3 mm long). The segments were mounted in thermostated 10 ml organ baths (37°C) containing physiological Kreb's solution through which a gas mixture of 95% O_2/5% CO_2 was passed. The rings were connected to an isometric force transducer (SS12LA, Biopac Systems Inc., Goleta, CA, USA), connected to a data acquisition unit (Biopac Student Lab MP36 systems) and each isometric contraction was recorded using a Biopac BSL PRO 7 computer software. A passive tension of 1 g was applied to the tissue using a movable device. The rings were equilibrated for 90 min while being rinsed with PSS every 10 min (Nwokocha et al., 2011). During the equilibration period, the rings were challenged with 10^{-6}

M phenylephrine and the aorta was relaxed with 10×10^{-6} M acetylcholine to test the endothelial integrity.

After a 90 min equilibration period, dose response to PE was done to determine the concentration that gave a sub-maximal response and EC_{70} calculated. Graded doses of the *A. muricata* extract were added to the rings with or without endothelium pre-contracted with 10^{-6} M phenylephrine (PE). The endothelium was removed mechanically by gently rubbing the intimal surface of the aortic rings and removal was confirmed by the absence of relaxation to 10×10^{-6} M acetylcholine (Nwokocha et al., 2011a). In another set of experiments, a concentration versus response relationship to phenylephrine was done in the presence of EC_{50} of the extract (1.32 mg/mL) determined in a pilot study.

To further elucidate the mechanism of action, the calcium channel blocking effect was assessed by testing on high K^+ (80 mM)-induced contractions. The aortic ring was allowed to stabilize in normal Kreb's solution, which was then replaced with a Ca^{2+}-free Kreb's solution (mM): KCl, 50; NaCl, 91.04; $MgSO_4$, 1.05; $NaHCO_3$, 11.90; glucose, 5.55 and EGTA, 0.1. for 30 min in order to remove calcium from the tissues. This solution was replaced with a K^+-rich and Ca^{2+}-free Kreb's solution (mM): KCl, 80; NaCl, 43.7; $MgSO_4$, 1.05; $NaHCO_3$, 11.90; glucose, 5.55. Following an incubation period of 30 min, control dose-response curves of Ca^{2+} were obtained and then re-determined after pre-treating the aortic rings for 60 min with the aqueous extract (Ghayur et al., 2005).

Drugs and chemicals

All drugs were purchased from Sigma-Aldrich. Drugs and chemicals used were prepared fresh in distilled water, except for EGTA, which was prepared in normal saline.

Statistical analysis

The results are presented as mean ± standard error of mean (SEM). The data were analysed using GraphPad Prism software version 5 (GraphPad Software, San Diego, CA, USA). Student's *t*-test was used to compare the means. A *p* value of 0.05 was considered statistically significant.

Results

Effect of graded doses of *A. muricata* on blood pressure and heart rate

Intravenous administration of *A. muricata* aqueous leaf extract caused a dose-dependent reduction in systolic blood pressure (SBP), diastolic blood pressure (DBP) and MAP. Heart rate was not significantly affected by increasing doses of the *A. muricata* extract (Table 1).

Effect of *A. muricata* on mepyramine, propranolol, L-NAME and atropine blockade

The administration of mepyramine (5 mg/kg), propranolol (1 mg/kg), L-NAME (5 mg/kg) and atropine (2 mg/kg) did not significantly attenuate the reduction of blood pressure induced by the *A. muricata* leaf extract (Figure 1). However, in the presence of propranolol, there was significant ($p < 0.05$) further reduction of MAP compared with the effect of the extract alone.

Table 1. Effect of graded doses of *Annona muricata* on blood pressure and heart rate.

Parameter	Control	9.17 mg/kg	19.41 mg/kg	48.53 mg/kg
SBP (mm Hg)	134 ± 10	100 ± 7* (25.4)	84 ± 6* (37.3)	73 ± 8* (45.5)
DBP (mm Hg)	102 ± 8	90 ± 16 (11.8)	64 ± 4* (37.3)	50 ± 8* (51.0)
MAP (mm Hg)	144 ± 11	93.3 ± 9* (35.2)	70.7 ± 5* (50.9)	57.7 ± 5* (60)
HR (beats/min)	420 ± 10	420 ± 15 (0)	400 ± 6 (4.76)	400 ± 10 (4.76)

Results are expressed as mean ± SEM. Values in parenthesis denotes percentage reduction in each parameter *$p < 0.05$ compared with control. $n = 5$.

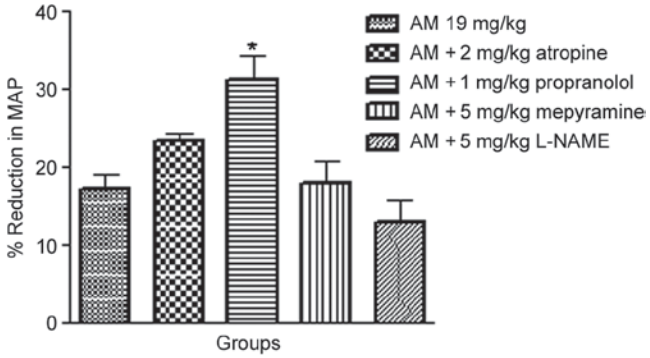

Figure 1. The maximal immediate changes in mean arterial pressure (MAP) in anaesthetized rats A. muricata aqueous leaf extract (19.17 mg/kg) in the presence or absence of blockers. Each point represents the mean ± SEM of five rats *$p < 0.05$ vs. the value without antagonisms.

Effect of *A. muricata* on phenylephrine-induced contraction

The effect of *A. muricata* on phenylephrine-induced contraction is presented in Figure 2. The aqueous leaf extract did not have any vasoconstrictor effect on the aortic rings after incubation. However, the extract caused a significant ($p < 0.05$) reduction in phenylephrine-induced contraction of the aortic rings with a maximum contraction of 60.2 ± 5.1% and a shift of the dose-response curve to the right. The sensitivity (pD_2) to phenylephrine in the presence of *A. muricata* was 5.23 which was significantly ($p < 0.05$) reduced when compared with the pD_2 of the control (6.63).

Effect of *A. muricata* on relaxation of the aorta

The aqueous leaf extract of *A. muricata* caused a dose-dependent relaxation of aortic rings precontracted with phenylephrine with increasing doses (Figure 3). The maximum relaxation to phenylephrine-induced contraction was 45.3 ± 4.4% in aorta with intact endothelium. In endothelium-denuded aortic rings, the extract also

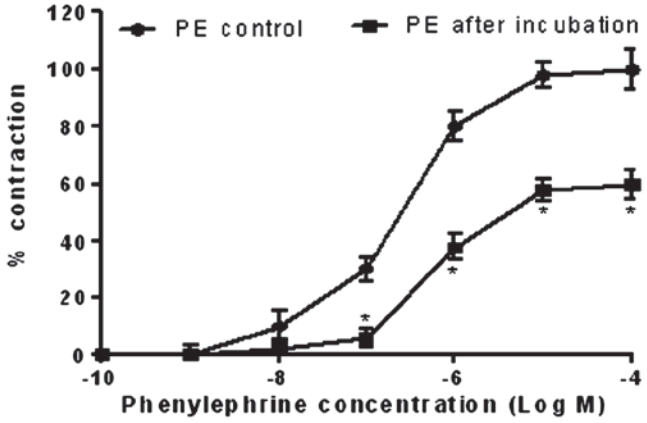

Figure 2. Effect of aqueous leaf extract of *A. muricata* (1.32 mg/mL) on phenylephrine-induced vasoconstriction of aortic rings. Each data point represents the mean ± SEM *$p < 0.05$ vs. Control. $N = 5$.

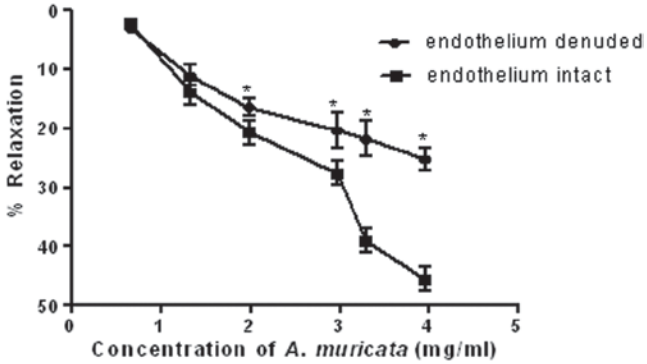

Figure 3. Concentration-response curves for the relaxation induced by the *A. muricata* extract on PE pre-contracted rat aortic rings, in endothelium-denuded and intact rings. The responses are expressed as % of maximum PE-induced contraction. Each data point represents the mean ± SEM *$p < 0.05$ vs. Control. $N = 5$.

caused vasorelaxation in a dose-dependent manner with a maximum relaxation of 27.4 ± 5.3%. However, relaxation of the aortic rings with intact endothelium was significantly ($p < 0.05$) higher when compared with aortic rings without endothelium.

Effect of *A. muricata* on calcium-induced contraction

The effect of *A. muricata* on Ca^{2+} dose-response curve constructed in a Ca^{2+}-free medium on rat aortic rings is presented in Figure 4. The extract shifted the Ca^{2+} dose-response curves to the right with suppression of the maximum effect. In the Ca^{2+}-induced contraction of the aortic rings, the extract reduced the maximum contraction to 27.4%, which was significantly ($p < 0.05$) lower than that of the control.

Discussion

The aqueous leaf extract of *A. muricata* produced a dose-dependent decrease in systolic blood pressure, diastolic blood pressure and MAP in normotensive rats without a significant effect on heart rate. These findings support the traditional use of the plant leaves for the treatment

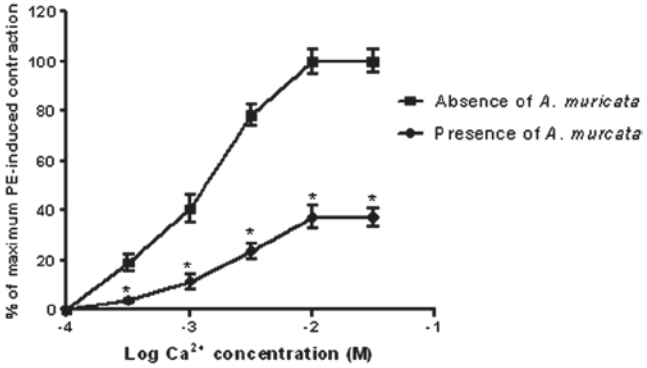

Figure 4. The effect of increasing concentrations of Ca^{2+} in the presence or absence aqueous leaf extract of *A. muricata* in isolated rat aortic rings in Ca^{2+} free medium. Values shown are mean ± SEM, $n = 4$.

of hypertension. The results of the *in vivo* study also suggest that the muscarinic, endothelial, histaminergic and adrenergic mechanisms are unlikely to be involved in the observed cardiovascular effects of the aqueous leaf extract of *Annona muricata*. Additionally, the data show that *A. muricata* did not have any significant negative chronotropic effect on the heart suggesting that the active substances may be acting on the periphery of the cardiovascular system and not directly on the heart.

To further characterize the mechanism of action of the aqueous extract of this plant we performed experiments *in vitro* using isolated thoracic aortic rings. Our observations were that the aqueous extract decreased the phenylephrine-induced contractions in both endothelial intact and denuded aortic rings. This finding further suggests that the hypotensive effects of the extract did not involve the endothelial or nitric oxide-dependent pathways.

The hypotensive effect of *A. muricata* may instead be attributed to the alkaloid compounds present in the leaves of the plant. The alkaloids, isoquinoline, corexi-mine, and anomurine, have been documented to have a transient depressive effect on the blood pressure (Oliver-Bever, 1986). Essential oils such as β-caryophyllene, δ-cadinene, epi-α-cadinol and α-cadinol have also been isolated from the *A. muricata* plant (Pelissler et al., 1994; Kossouoh et al., 2007). Some essential oil like beta-caryophyllene present in other plants have been reported to exhibit hypotensive and vasodilator activities (Damiani et al., 2004; Sundufu & Shoushan, 2004; Morteza-Semnani, 2006; Kamboj & Saluja, 2008) and the presence of such compounds in *A. muricata* might possibly contribute to the cardiovascular effects observed with the aqueous plant extract (Abdalla et al., 1995).

The present data indicate that the extract may exert its blood pressure lowering effect through the blockade of calcium ion channels; and this Ca^{2+} antagonism is further demonstrated by its ability to relax high K^+-induced contractions (Ghayur et al., 2005). Thus, the hypotensive properties of the aqueous extract of *A. muricata* may be through its calcium antagonism. Similar results have

been reported for the plant extract of *Acorus calamus* L (Acoraceae), which has been found to have hypotensive and vasorelaxant effects mediated through calcium ion antagonism (Shah & Gilani, 2009).

Dias et al. (2004) have reported that reticuline caused hypotension through voltage-dependent Ca^{2+} channel blockade and/or inhibition of Ca^{2+} release from norepinephrine-sensitive intracellular stores. Reticuline, an alkaloid, is one of the components of *A. muricata* leaves (Leboeuf et al., 1981, 1982) and may contribute to the observed results.

In conclusion, these results suggest that the aqueous leaf extract of *A. muricata* lowers blood pressure by a mechanism that does not involve cholinergic, histaminergic or endothelial-dependent pathways, and that the mechanism of action more likely involves voltage-dependent Ca^{2+} channel blockade and/or inhibition of Ca^{2+} release from intracellular stores of the blood vessels.

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Declaration of interest

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