Cardiovascular Topics

Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals

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Summary

The cardiovascular effects of *Persea americana* Mill (Lauraceae) aqueous leaf extract (PAE) have been investigated in some experimental animal paradigms. The effects of PAE on myocardial contractile performance was evaluated on guinea pig isolated atrial muscle strips, while the vasodilatory effects of the plant extract were examined on isolated portal veins and thoracic aortic rings of healthy normal Wistar rats *in vitro*. The hypotensive (antihypertensive) effect of the plant extract was examined in healthy normotensive and hypertensive Dahl salt-sensitive rats *in vivo*.

***P americana* aqueous leaf extract (25–800 mg/ml) produced concentration-dependent, significant (p < 0.05–0.001), negative inotropic and negative chronotropic effects on guinea pig isolated electrically driven left and spontaneously beating right atrial muscle preparations, respectively. Moreover, PAE reduced or abolished, in a concentration-dependent manner, the positive inotropic and chronotropic responses of guinea pig isolated atrial muscle strips induced by noradrenaline (NA, 10^{-10}–10^{-5} M), and calcium (Ca^{2+}, 5–40 mM). PAE (50–800 mg/ml) also significantly reduced (p < 0.05–0.001) or abolished, in a concentration-dependent manner, the rhythmic, spontaneous, myogenic contractions of portal veins isolated from healthy normal Wistar rats. Like acetylcholine (ACh, 10^{-8}–10^{-5} M), the plant extract (25–800 mg/ml) produced concentration-related relaxations of isolated endothelium-containing thoracic aortic rings pre-contracted with noradrenaline. The vasorelaxant effects of PAE in the isolated, endothelium-intact aortic rings were markedly inhibited or annulled by N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-5} M), a nitric oxide synthase inhibitor. Furthermore, PAE (25–400 mg/kg iv) caused dose-related, transient but significant reductions (p < 0.05–0.001) in the systemic arterial blood pressure and heart rates of the anaesthetised normotensive and hypertensive rats used.**

The results of this laboratory animal study indicate that PAE caused bradycardia, vasorelaxation and hypotension in the mammalian experimental models used. The vasorelaxant action of PAE was endothelium dependent, and was, therefore, possibly dependent on the synthesis and release of nitric oxide (NO). The vasorelaxant effects of PAE appeared to contribute significantly to the hypotensive (antihypertensive) effects of the plant extract. However, the findings of this study tend to suggest that *P americana* leaf could be used as a natural supplementary remedy in essential hypertension and certain cases of cardiac dysfunctions in some rural Africa communities.

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In our current pharmaco-chemical exploration of African medicinal plants, we have examined in our laboratories some of the frequently used South African medicinal plants for their chemical constituents and pharmacological actions,[1-10] in an attempt to establish a scientific basis for their folkloric, ethnomedical uses. One of such commonly used African medicinal plants is *Persea americana* Mill (family: Lauraceae).

P americana, otherwise known as the avocado pear, Mexican avocado and so on, is a medium-sized, single-stemmed, terrestrial, erect, perennial, deciduous tree 15–20 m in height. Although a native of Central America (Mexico),

P americana is now found in most tropical and subtropical countries of the world. The branches are fissured and grey, but the twigs are green and smooth. The 15–25-cm long and 10–20-cm broad leaves with well-developed petioles are spirally arranged, often clustered near the branch ends, narrowly to broadly elliptical or obovate, and are usually pointed at the tip.[11]

The greenish-yellow flowers are borne on branched, compact panicles, which are shorter than the leaves. The often pear-shaped, one-seeded fruits are variable in size and shape according to the variety, up to 18 cm long and usually shiny and green, or brownish when ripe. The flesh is soft, oily, greenish or yellow surrounding one large, loose round seed.[11] The avocado is now cultivated commercially as a fruit crop in many countries of the world. In many parts of Africa, the fruits of the avocado are much sought after by humans and some animals as valuable foodstuff. Besides the oil, avocado fruit pulp contains carbohydrates and more protein than many other fruits, while its contents of vitamins A and B are high.[11,12]

In addition to the nutritional value of its fruit, the leaves and other morphological parts of *P americana* possess medicinal properties and are widely used in traditional medicines of many African countries. For example, the fruit pulp is eaten as an aphrodisiac and as an emmenagogue in South Africa,[12] while a hot-water extract of the leaves is taken orally as a diuretic and for hypertension in many West African countries.[11] In some other parts of the world, various morphological parts of *P americana* have been employed for a wide range of human ailments. Products of the plant have been effectively used for the management, control and/or treatment of amenorrhoea, anaemia, insomnia, hyperlipidaemia, hypertension, diabetes mellitus, diarrhoea, dysentery, gastritis, peptic ulcers, bronchitis, cough, hepatitis, and so forth.[11,12]

Previous studies on the avocado have shown that leaf extracts of *P americana* possess a catalogue of pharmacological activities, including analgesic, anti-inflammatory, antidiabetic, hypoglycaemic, hypotensive and antihypertensive properties.[13-16] The present study was prompted by the claim of some traditional health practitioners in KwaZulu-Natal that decoctions and infusions of avocado leaves are effective remedies for the management and/or control of hypertension and certain cardiac disorders.

The aim of the present study was, therefore, to investigate the cardiac, vascular and antihypertensive (hypotensive) effects of *P americana* aqueous leaf extract in experimental animal paradigms, with a view to providing a pharmacological justification (or otherwise) for the ethnomedical uses of the plant leaf in the management, control and/or treatment of essential hypertension and certain cardiac dysfunctions in some rural African communities.

Materials and methods

The experimental protocol used in this study was approved by the ethics committee of the University of Durban-Westville and conforms to the *Guide to the Care and Use of Animals in Research and Teaching*.[17]

Plant material and preparation

Fresh leaves of *P americana* were collected from a playground behind Willowpark Centre along Umbilo Road in Durban, between January and June 2003. The leaves were identified by Prof H Baijnath, the former chief taxonomist/curator of the Department of Botany, University of Durban-Westville, as those of *P americana* Mill (family: Lauraceae). A voucher specimen of the plant has been deposited in the Botany Departmental Herbarium.

Room air-dried leaves (1 kg) of *P americana* were milled in a Waring commercial blender. The powdered leaf was macerated in distilled water and extracted twice, on each occasion with 2.5 l of distilled water at room temperature for 48 hours, with occasional shaking. The combined distilled water extracts were concentrated to dryness at 60 ± 1°C in a rotary evaporator. Freeze drying and solvent elimination under reduced pressure finally gave 21.50 g (2.15% yield) of a light-brown, powdery aqueous leaf extract. This crude extract was used in our study without further purification. Aliquot portions of the residue from the aqueous extract were weighed and dissolved in distilled water for use on each day of our experiments.

Animal material

Healthy male Dunkin-Hartley guinea pigs (*Cavia porcellus*) weighing 300–450 g, and healthy young adult male Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept under laboratory conditions of temperature, humidity and light and were allowed free access to food (standard pellet diet) and water *ad libitum*. All the animals were fasted for 16 hours, but allowed free access to water before the commencement of our experiments. Guinea pig isolated atrial muscles were used for the *in vitro* evaluation of the effects of the aqueous extract on myocardial contractility, whereas rat isolated portal veins and thoracic aortic rings were used to examine the vasorelaxant effects of the extract. Normotensive (normal) Wistar, and hypertensive Dahl salt-sensitive rats were used for the *in vivo* investigation of the hypotensive (antihypertensive) effect of the aqueous extract.

Isolated muscle experiments

Guinea pig muscle strips

The guinea pigs were sacrificed by stunning and exsanguination. The left and right atrial muscles of the animals were isolated and mounted as previously described by Ojewole.[18]

The isolated left atrium of each guinea pig was impaled on a thin platinum wire electrode and suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution (composition in mmol/l, pH adjusted to 7.4: NaCl, 118; KCl, 4.7; NaH_2PO_4, 1.28; $NaHCO_3$, 25.0; $MgCl_2$, 1.2; $CaCl_2$, 2.52; glucose, 5.55) maintained at 34 ± 1°C and continuously aerated with carbogen (95% O_2 + 5% CO_2 gas mixture). Each left atrial muscle preparation was electrically driven with square wave pulses of 5-ms duration at a frequency of 3 Hz and a supramaximal voltage of 5–10 V, delivered by

an SRI stimulator. The spontaneously beating right atrium of the animal was also set up under the same physiological experimental conditions and allowed to beat spontaneously. Two isolated electrically driven left atrial muscle strips and two isolated spontaneously beating right atrial muscle preparations were always set up at a time (one as the test, and the other as the control) to allow for changes in the atrial muscle sensitivity.

The atrial muscle preparations were left to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with PAE or any of the reference drugs used. The test atrial muscle preparations were treated with sequentially applied graded concentrations of PAE and/or reference agonist drugs used, whereas the control atrial muscle strips were treated with volumes of distilled water (0.1–0.6 ml) equivalent to the volumes of bath-applied PAE solution used. The electrically provoked and spontaneous contractions of the atrial muscles, as well as the PAE- and reference agonist drug-induced responses of the atrial muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat portal veins

The rats were sacrificed by stunning and exsanguination. The abdomen of each rat was quickly opened by midline incision, and the intestines were pulled aside. The portal vein of each rat, with an *in situ* length of approximately 2 cm, was cleaned of extraneous connective and fatty tissues and then removed from the animal. Each isolated portal vein was suspended under an applied resting tension of 0.5 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiological solution. Two isolated venous tissue preparations (one control and the other PAE- or reference drug-treated test) were always set up in order to make allowances for changes in the venous tissue sensitivity. Control venous muscle strips were treated with distilled water only (the vehicle in which PAE and reference drugs were dissolved). The venous tissue preparations were allowed to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with PAE or any of the reference drugs used. The plant extract- (25–800 mg/ml) and reference drug-induced responses of the venous smooth muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat thoracic aorta rings

The rats were sacrificed by decapitation. The descending thoracic aorta of each normotensive rat was quickly and carefully excised and placed in a Petri dish filled with ice-cold Krebs-Henseleit physiological solution. The aorta was cleaned of extraneous fat and connective tissues and cut into rings approximately 3–4 mm in width. All dissecting procedures were carefully done to protect the functional endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface three times with distilled water-moistened cotton wool, followed by six times with a small, plastic tubing. A pair of rat isolated aortic rings, one with intact functional endothelium, and the other one with endothelium denuded, were always set up in parallel for appropriate comparison.

Each of the isolated endothelium-containing and endothelium-denuded aortic rings was suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution maintained at 36 ± 1°C and continuously aerated with carbogen (95% O_2 + 5% CO_2). The aortic tissue preparations were left to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with graded concentrations of PAE or any of the reference drugs used. At the end of the equilibration period, the aortic ring preparations were initially contracted with bath-applied noradrenaline (10^{-5} M).

Endothelial integrity and successful removal of the functional endothelium was assessed by the presence or absence, respectively, of relaxant response to acetylcholine (10^{-5} M). ACh-induced relaxation ≤ 5% was taken as satisfactory removal of the functional endothelial layer. Such endothelium-denuded aortic muscle preparations were used in this study. After the subsequent wash-out and equilibration period of 30 min, cumulative dose-response curves were obtained with noradrenaline in aortic rings with and without endothelium.

Subsequently, 20-min pretreatment of the aortic muscle preparations with graded concentrations of the plant extract (25–800 mg/ml) was carried out before the next cumulative additions of noradrenaline (10^{-10}–10^{-5} M) to the bath fluid. After the addition of each NA concentration, a plateau response was obtained before the addition of the next higher dose in all cases of cumulatively applied noradrenaline concentrations. Consecutive dose-response curves were taken at 30-min intervals, during which time the physiological bath solution was changed three to five times until the tension developed returned to basal level.

Following 20-min incubation of the aortic ring preparations with the plant extract (25–800 mg/ml), the arterial relaxant effect of PAE was examined on endothelium-containing and endothelium-denuded aortic ring preparations pre-contracted with sequentially applied or cumulatively administered noradrenaline (10^{-10}–10^{-5} M). The effect of the vehicle in which PAE and the reference drugs used were dissolved (distilled water), was also tested. After each challenge, the aortic rings were washed three to five times with fresh physiological solution and allowed to equilibrate for 30 min before they were challenged again with any of the reference drugs or PAE. The contractile and/or relaxant effects of all the reference drugs, as well as PAE-induced relaxations of the isolated aortic ring preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Whole-animal experiments

Normotensive Wistar and hypertensive Dahl salt-sensitive rats weighing 250–300 g were used. Before the commence-

ment of our experiments, the salt-sensitive rats were placed on 4% saline water and normal food (standard pellet diet) for six to eight weeks (during which time the arterial blood pressure of the animals rose to between 170/130 and 190/140 mmHg). Salt-sensitive rats with arterial blood pressure ≥ 170/120 mmHg were considered to be hypertensive and used in this study.

Each of the normotensive and hypertensive rats was anaesthetised with intraperitoneal injection of 0.11 g/kg of Trapanal® [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate]. The right femoral vein was cannulated with a small polythene cannula for the administration of the plant extract and reference drugs. In order to minimise blood coagulation, heparin (500 units/kg) was intravenously administered to the animal, and flushed in with 0.2 ml of 0.9% w/v sodium chloride solution. The left carotid artery of each rat was also cannulated and connected to a four-channel Grass polygraph for systemic arterial blood pressure recording. The trachea of each rat was cannulated for artificial respiration, but the animal was allowed to breathe spontaneously. The rat's body temperature was maintained at 36 ± 1°C with an incandescent lamp placed over the abdomen.

After 20 min stabilisation period, systemic arterial blood pressure (systolic, diastolic and mean arterial pressures) and heart rate of each rat were measured and recorded. The effects of PAE and the reference drugs [acetylcholine (0.5–4.0 µg/kg iv) and noradrenaline (0.5–4.0 µg/kg iv)] on systemic arterial blood pressure and heart rates (calculated from the ECG limb lead II recording at a fast paper speed of 25 mm/sec) were recorded by means of a four-channel Grass polygraph recorder (model 79D). In some of the rats, the hypotensive (depressor) effect of PAE (25–400 mg/kg iv) was examined after atropinisation [pretreatment of the rats with atropine sulphate (1.5 mg/kg ip) 18–24 hours before use]. Because PAE and other drugs used in this study were dissolved in distilled water, rats treated with distilled water (2 ml/kg iv) alone were used as control animals under the same experimental conditions.

Compounds and drugs used

The following compounds and drugs were used: *P americana* aqueous leaf extract, acetylcholine chloride (Sigma, England); (-)-noradrenaline hydrochloride (Sigma, England); atropine sulphate (Sigma, England); N^G-nitro-L-arginine methyl ester (L-NAME) (Sigma, England); Trapanal® [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate] (Byk Gulden, Konstanz, Germany); (±)-propranolol hydrochloride (Sigma, England); calcium chloride and potassium chloride (Sigma, England). The drugs were dissolved in distilled water each day at the beginning of our experiments. Drug concentrations and doses quoted in the text refer to the salts, except PAE, and denote final organ bath concentrations in the *in vitro* experiments.

Data analysis

Data obtained from test guinea pig isolated atria, rat isolated portal vein, aortic ring strips, and anaesthetised normotensive and hypertensive rats treated with PAE alone, as well as those obtained from distilled water-treated control isolated atria, portal veins, aortic rings and anaesthetised rats, were pooled and expressed as means (± SEM). Statistical comparison of the differences between PAE- and reference drug-treated test means, and distilled water-treated control means, was performed with GraphPad InStat Software (version 3.00, GraphPad Software, San Diego, California, USA) using one-way analysis of variance (ANOVA; 95% confidence interval), followed by Tukey-Kramer multiple-comparison tests. Values of $p \leq 0.05$ were taken to imply statistical significance.

Results

Isolated muscle experiments

Guinea pig muscle preparations

Sequential administrations to the bath fluid of relatively low to high concentrations of PAE (25–800 mg/ml) significantly reduced ($p < 0.05$–0.001) or abolished the force of contractions of guinea pig isolated electrically driven left atrial muscle preparations in a concentration-related manner (Fig. 1). The negative inotropic effect of PAE on these muscle strips was not affected by prior exogenous administration of atropine to the bath fluid.

At the same concentration range, the plant extract also significantly reduced ($p < 0.05$–0.001) or abolished the rate of contractions of guinea pig isolated spontaneously beating right atrial muscle preparations in a concentration-dependent manner (Fig. 2). However, the negative chronotropic effect of PAE on these muscle strips was not antagonised by atropine which reduced or abolished the negative chronotropic effect of acetylcholine on six other spontaneously beating right

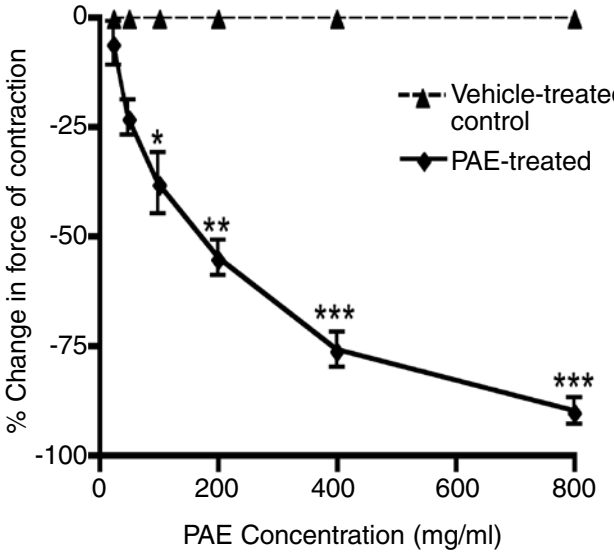

Fig. 1. Effects of graded concentrations of PAE (25–800 mg/ml) on guinea pig isolated electrically driven left atrial muscle strips. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

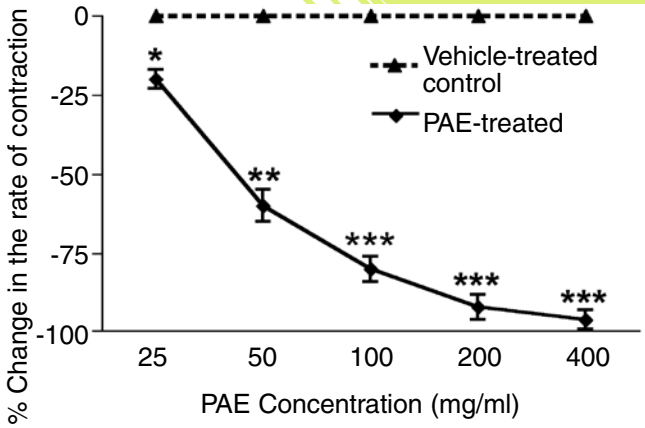

Fig. 2. Effects of graded concentrations of PAE (25–400 mg/ml) on guinea pig isolated spontaneously beating right atrial muscle strips. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

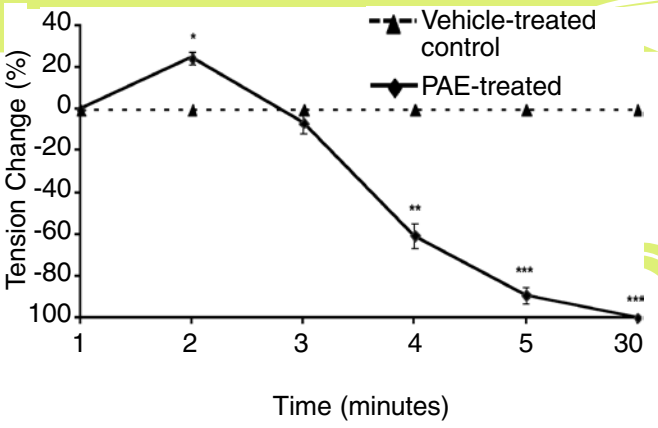

Fig. 3. Effects of PAE (800 mg/ml) on rhythmic myogenic spontaneous contractions of rat isolated portal veins. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight to 10 preparations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

atrial muscle preparations examined. PAE significantly reduced ($p < 0.05$–0.001) or abolished, like propranolol, the positive inotropic and chronotropic effects of noradrenaline on all eight other isolated atrial muscle strips tested. The plant extract also significantly ($p < 0.05$–0.001) inhibited or abolished calcium-induced positive inotropic and chronotropic responses on all other nine atrial muscle strips examined.

Rat portal veins

Sequential administrations to the bath fluid of relatively low to high concentrations of PAE always induced concentration-dependent, biphasic effects on the amplitude and frequency of the rhythmic myogenic contractions of the rat isolated portal veins. The biphasic effects produced by PAE always consisted of an initial slight but significant ($p < 0.05$) contraction (stimulation) of short duration, followed by a secondary, longer-lasting and significant ($p < 0.05$–0.001) relaxation (inhibition) of the venous muscle preparations (Fig. 3). At the same concentration range, the plant extract also inhibited or abolished in a concentration-dependent manner, contractions of the venous muscle preparations induced by noradrenaline or potassium.

Rat aortic ring strips

Cumulative additions of graded concentrations of noradrenaline to the bath fluid provoked concentration-dependent contractions of both endothelium-containing and endothelium-denuded normotensive rat isolated aortic ring strips, with a maximum of 3.76 ± 0.30 g tension developed. Acetylcholine provoked concentration-related, significant relaxations ($p < 0.05$–0.001) of endothelium-containing aortic ring preparations pre-contracted with bath-applied noradrenaline, but did not significantly relax ($p > 0.05$) endothelium-denuded aortic ring preparations pre-contracted with bath-applied noradrenaline.

Like acetylcholine, PAE produced concentration-dependent, significant relaxations ($p < 0.05$–0.001) of the endothelium-containing aortic ring preparations pre-contracted with noradrenaline (Fig. 4), but did not relax endothelium-denuded aortic ring preparations pre-contracted with bath-applied noradrenaline. Moreover, the plant extract shifted cumulatively administered noradrenaline concentration-response curves to the right in a non-parallel and non-competitive fashion, and suppressed NA-induced maximal contractions of endothelium-containing aortic ring muscle preparations. Ten minutes' prior incubation of the endothelium-intact aortic ring tissues with L-NAME, a nitric oxide synthase inhibitor, inhibited or abolished PAE- or ACh-induced

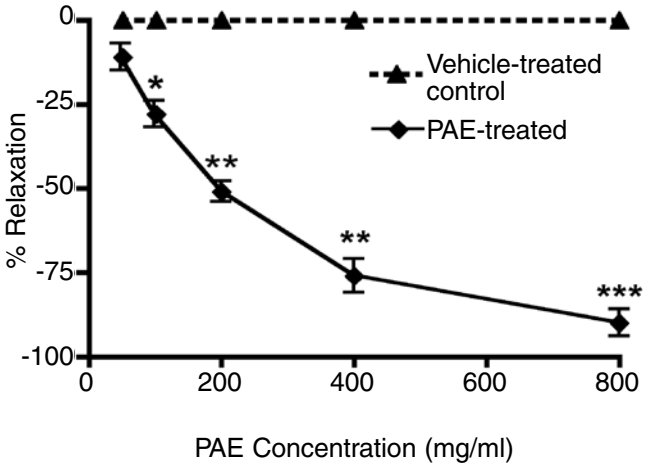

Fig. 4. Arterial relaxant effects of graded concentrations of PAE (50–800 mg/ml) on noradrenaline (10^{-5} M)-induced contractile responses of endothelium-intact aortic rings from normal rats. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$ vs vehicle-treated control.

TABLE 1. EFFECTS OF PAE ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF NORMOTENSIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	*Before treatment: control values*	*After treatment: PAE (25–400 mg/kg iv)*				
		25	*50*	*100*	*200*	*400*
Systolic BP (mm Hg)	124.5 ± 4.6	112.5 ± 4.4	91.6 ± 4.6*	73.4 ± 4.1**	58.5 ± 4.0***	42.6 ± 3.4***
Mean BP (mm Hg)	111.4 ± 4.1	98.8 ± 4.7	84.3 ± 4.8*	66.5 ± 4.0**	51.4 ± 4.1***	38.4 ± 3.1***
Diastolic (mm Hg)	94.3 ± 4.0	80.4 ± 4.2	68.5 ± 4.0*	56.4 ± 4.3**	44.3 ± 4.3***	31.4 ± 3.0***
Heart rate (beats/min)	396.6 ± 18.6	364.8 ± 18.2	332.3 ± 15.4*	302.8 ± 14.4**	283.5 ± 12.3***	242.7 ± 11.5***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control.

TABLE 2. EFFECTS OF PAE ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF HYPERTENSIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	*Before treatment: control values*	*After treatment: PAE (25–400 mg/kg iv)*				
		25	*50*	*100*	*200*	*400*
Systolic BP (mmHg)	188.2 ± 6.4	173.6 ± 6.6	156.4 ± 6.3*	140.6 ± 6.0**	124.4 ± 4.8***	101.3 ± 4.8***
Mean BP (mmHg)	146.8 ± 6.1	132.4 ± 6.4	120.6 ± 5.8*	106.4 ± 5.2**	92.5 ± 4.0***	76.7 ± 4.4***
Diastolic (mmHg)	120.4 ± 6.3	106.4 ± 4.0	91.3 ± 5.1*	78.5 ± 4.4**	64.3 ± 4.2***	50.5 ± 4.0***
Heart rate (beats/min)	424.6 ± 20.4	391.4 ± 18.6	356.5 ± 16.0*	318.2 ± 15.2**	286.4 ± 14.6***	236.4 ± 12.5***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control.

relaxations of the endothelium-containing aortic rings pre-contracted with noradrenaline. Ten minutes' prior incubation of the aortic ring tissues with atropine sulphate also inhibited or abolished acetylcholine-induced relaxations of the endothelium-containing aortic ring preparations pre-contracted with noradrenaline.

Whole animal experiments

Acute intravenous administrations of PAE into anaesthetised normotensive and hypertensive rats produced transient, dose-related, significant reductions ($p < 0.05$–0.001) in the systemic arterial blood pressure and heart rates of the rats (Tables 1, 2). The transient hypotensive (antihypertensive) effect of the plant extract persisted for 12–85 min, depending on the PAE dose administered. Furthermore, the plant extract dose-dependently inhibited or abolished the pressor effects of noradrenaline on systemic arterial blood pressure and heart rates of the animals. Pre-treatment of the normotensive and hypertensive rats with atropine sulphate abolished or markedly reduced the depressor effects of acetylcholine on systemic arterial blood pressure and heart rates of the animals. However, the depressor effects of PAE on blood pressure and heart rates were not affected by pre-treatment with atropine sulphate.

Discussion

The results of this study indicate that the aqueous leaf extract of *P americana* possessed cardiodepressant, vasorelaxant and hypotensive (antihypertensive) effects in the experimental animal paradigms used. These observations are in agreement with the findings of some of the earlier investigators who have reported vasorelaxant[16] and hypotensive[15] effects of the leaf extract in experimental animal models.

Furchgott and Zawadzki[19] first described the involvement of the endothelium-derived relaxing factor (EDRF), which was subsequently determined to be nitric oxide or NO derivatives synthesised from guanidine groups of L-arginine.[20,21] Endothelium-dependent relaxation, which has been demonstrated in many vascular preparations, including some veins, arteries and microvascular vessels, occurs in response to stimulation by a variety of substances, such as acetylcholine, adenine nucleotides,[22] thrombin, substance P,[23] calcium ionophore A23187, bradykinin and histamine.[24] The vasodilatation effects of endothelium-dependent substances can be inhibited by several L-arginine analogues, such as N-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME).[20,25-27]

Endothelial nitric oxide plays a vital role in the control of vasomotor tone and structure.[22,28] On the other hand, vascular tone plays an important role in the regulation of arterial blood pressure. The development and maintenance of hypertension has been suggested to involve a reduced endothelium-dependent vasodilator influence on the vascular tissue.[28] Impairment of endothelium-dependent vascular relaxation in human and experimental hypertension has been observed by Luscher and Vanhoutte,[22] and the ability of nitric oxide to maintain vascular tone has been shown to be deficient in this condition.[28,29] Because NO is a potent vasodilator, a deficient production and/or release of endothelium-derived NO will result in diminished vasodilator tone, thus allowing vascular resistance to rise, and this, in turn, will lead to elevated blood pressure.[22,28]

Relaxation of vascular smooth muscle by NO involves a series of steps. Nitric oxide is formed in functional endothelium by the activation of nitric oxide synthase (NOS), which uses L-arginine as a substrate. Once formed, NO diffuses out of the endothelium, with some entering the underlying vascular smooth muscle where it binds to and activates soluble guanylate cyclase.[28] This enzyme catalyses the conver-

sion of guanidine triphosphate (GTP) to cyclic guanidine monphosphate (cGMP), which in turn, causes relaxation of the vascular smooth muscle cells.[20,28,30,31]

In pathological conditions of the cardiovascular system, there is a dysfunction in the integrity of the vascular endothelium with a subsequent reduction in the release, bioavailability and/or action of nitric oxide.[28] NO release and function have been shown to decrease in cardiovascular diseases, such as hypertension,[22] atherosclerosis[32] and congestive heart failure.[33] Therefore, the development of vasodilators which can restore the level and integrity of NO in the vascular system would potentially contribute to the treatment of these cardiovascular diseases.[28]

In the present study, the plant extract, like acetylcholine, caused concentration-dependent relaxation of the normotensive rat isolated endothelium-containing aortic ring preparations pre-contracted with noradrenaline. This vasorelaxant property would appear to have contributed, at least in part, to the antihypertensive (hypotensive) effect of the plant extract. The arterial muscle relaxant effect of the extract disappeared by removal of the functional endothelium.

Furthermore, pre-treatment of the endothelium-containing aortic ring preparations with L-NAME, a nitric oxide synthase inhibitor, inhibited or abolished the vasorelaxant effect of PAE. Taken together, these observations would appear to suggest that the vasorelaxant effect of the extract, like that of acetylcholine, was dependent on the formation and/or synthesis and release of endothelium-derived nitric oxide, since removal of the functional endothelial cells led to the absence of relaxant response to PAE in the endothelium-denuded aortic ring preparations. These observations are in agreement with the findings of Martin *et al*.,[34] Ignarro *et al*.,[35] Kang *et al*.,[36-38] Baisch *et al*.[20] and Yin *et al*.[28]

The present study also suggests that the endothelium-dependent vasorelaxant effect of PAE could be mediated via endothelial NO signaling in the aortic tissue preparations. However, the release of endothelial NO and the opening of potassium channels have also been implicated in the vasorelaxant effects of extracts from some other medicinal plants.[39-41]

Noradrenaline-induced contractions of blood vessels have been shown to be partly due to calcium release from intracellular storage sites and partly due to the influx of extracellular calcium into the cell via receptor-gated channels following alpha$_1$ (α_1)-adrenoceptor activation.[42] In the present study, endothelium-containing aortic rings pre-contracted with NA in Krebs-Henseleit solution with and without normal calcium concentrations were relaxed by exogenous additions of PAE or acetylcholine. Moreover, the non-parallel shift of the noradrenaline concentration-response curves to the right by the plant extract seems to suggest a mechanism of non-competitive α_1-adrenoceptor blockade. This hypothesis is in consonance with the work of Abreu *et al*.[43] on ethanolic extract of *Jatropha gossypiifolia* Linn in rats.

The findings of the present study indicated that PAE induced vasorelaxation in normotensive rat isolated portal veins and endothelium-containing aortic rings, and caused hypotension in anaesthetised, normotensive and hypertensive rats. Although α_1-adrenoceptor blockade may have partially contributed to the hypotensive effect of the plant extract, the experimental evidence obtained in the present study tends to suggest that vasorelaxation might largely have been responsible for the hypotensive action of the plant extract. This vasorelaxant effect of the extract was probably mediated through endothelium-dependent NO production and cGMP release, and not related to activation of vascular endothelial muscarinic receptors.

Although the precise mechanism of the hypotensive action of PAE could not be established in the present study, we excluded involvement of cholinergic mechanisms. However, a complicating factor in the interpretation of the data obtained in the hypotensive experiments was the bradycardia associated with the reduction in systemic arterial blood pressure of the rats. Firstly, the reduction in heart rate could, on its own, have been the cause of the hypotension. However, based on the results obtained from the rat isolated aortic rings, it would seem unlikely that the fall in arterial blood pressure produced by PAE was solely dependent on reduction in heart rate. Secondly, the observed transient, secondary reflex tachycardia accompanying the fall in arterial blood pressure would probably suggest that the plant extract did not affect central cardiovascular centres and/or brain cardiovascular receptors. The plant extract may, therefore, also have had a direct effect on the sinus node of the heart, or on the central nervous system control machinery of arterial blood pressure.

P americana has been reported to contain many bioactive chemical compounds, including polyphenolics, tannins, coumarins, flavonoids, triterpenoids, phytosterols (especially β-sitosterol), biotin, α-tocopherol, carotene, ascorbic acid, scopoletin, quercetin, oils, organic acids and inorganic substances such as calcium, magnesium, zinc and phosphorus.[11,12] However, our present state of knowledge of the chemical constituents of the leaf extract is limited. It is therefore impossible for us at this stage to identify with certainty the vasorelaxant and antihypertensive constituent/s of PAE. Although we speculate that one or more of the major chemical constituents of the plant [namely flavonoids, polyphenols, tannins, coumarins (especially scopoletin and other coumarins), triterpenoids and phytosterols] may possibly have accounted for the observed cardiodepressant, vasorelaxant and antihypertensive properties of the plant extract, there are no sufficient scientific data at present to justify this speculation. However, the experimental evidence obtained in the present study showed that *P americana* aqueous leaf extract produced significant cardiodepressant, vasorelaxant and hypotensive (antihypertensive) effects in the laboratory animal paradigms used.

In conclusion, the findings of the present laboratory animal study lend pharmacological support to the suggested anecdotal ethnomedical uses of *P americana* aqueous leaf extract as a natural supplementary remedy in the management, control and/or treatment of hypertension and certain cardiac disorders in some rural Africa communities.

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