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Full Length Research Paper

Effect of the aqueous seed extract of *Persea americana* mill (Lauraceae) on the blood pressure of sprague-dawley rats

Ogochukwu N. Anaka, Raymond I. Ozolua* and Stephen O. Okpo

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, P. M. B. 1154, Benin City 300001, Nigeria.

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The aqueous seed extract (AE) of *Persea americana* Mill (Lauraceae) is used by some herbal medicine practitioners in Nigeria for the treatment of hypertension. We investigated its effects on the mean arterial pressure (MAP) and Heart Rate (HR) of naïve and 260 mg/kg/day x 10 days pretreated rats. Naïve rats were given bolus injections of (a) - AE (240, 260, 280 mg/kg); (b) - 2 µg/kg of acetylcholine (ACh) + 240, 260, 280 mg/kg of AE; or bolus doses of ACh (1, 2, 4 µg/kg). Results show that 10-day pretreatment significantly reduced MAP (125.7 ± 11.2 vs 92.1 ± 8.5 mm Hg) and HR (274.6 ± 39.3 vs 161.6 ± 11.6 beats/min). Also, acute AE injections significantly decreased MAP from baseline values in naïve rats. The effects of AE on MAP were comparable with those of ACh. Combination of AE with 2 µg/kg of ACh only significantly potentiated the MAP reducing effect of 240 mg/kg of AE. It is concluded that the aqueous seed extract of *P. americana* reduces BP and HR in normotensive rats. This observation lends credence to its use by herbalists for the management of hypertension.

Key words: *Persia americana* seed, herbal medicine, blood pressure, heart rate.

INTRODUCTION

Herbal preparations are used in the treatment of hypertension and related cardiovascular diseases particularly in resource-poor countries (Ernst, 2005). The reasons for the increased popularity of these herbal medicines may include their relative cheapness compared to orthodox medicines, availability (since they are almost always derived from available plants in the locality), and time-trusted efficacy. Besides, orthodox medicines which are used in the treatment of hypertension have been known to present adverse effects which affect compliance to therapy and total quality of life of the patient (Fogari and Zoppi, 2004).

Persea americana Mill (Lauraceae) is one of the emerging plants of interest in the management of hypertension. It is commonly known as the avocado pear tree and is widely distributed in tropical countries. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids (Ortiz et al., 2004) which are believed to modify the fatty acid contents in cardiac and renal membranes (Salazar et al., 2005) and enhance the absorption of alpha/beta-carotene and lutein. The carotenoid content may play significant role in cancer risk reduction (Lu et al., 2005). Wound healing (Nayak et al., 2008) and hepatoprotective (Kawagishi et al., 2001) properties have been ascribed to the whole oil or its fatty acid constituents. Proximate analysis has been conducted on the seeds (Olaeta et al., 2007).

Other parts of the plant have been reported to have medicinal properties. The aqueous leaf extract for example has shown analgesic and antiinflammatory (Adeyemi et al., 2002), anticonvulsant (Ojewole and Amabeoku, 2006), hypoglycaemic and hypocholesterolaemic (Brai et al., 2007), vasorelaxant and blood pressure reducing (Owolabi et al., 2005; Ojewole et al., 2007) activities in animal studies. The leaf extract is used to treat hypertension and induce diuresis in Brazilian ethnomedicine (De A Ribeiro et al., 1986). The aqueous

*Corresponding author. E-mail: ozolua@uniben.edu or rozolua2000@yahoo.co.uk. Tel: +234 – 802 – 352 – 8166.

bark extract of the tree is used by traditional medicine practitioners in Nigeria for the treatment of parasitic skin diseases (Owolabi et al., 2005).

While studies on the antihypertensive properties of the plant have focused on the leaf extracts (Owolabi et al., 2005; Ojewole et al., 2007), we are not aware of any scientific report on the use of the aqueous seed extract for the management of hypertension. However, herbalists in Nigeria have through oral communication confirmed that the aqueous seed extract is equally effective in the treatment of hypertension. We therefore designed the present study in order to investigate the effect of the aqueous seed extract on the blood pressure of normotensive Sprague-Dawley rats.

MATERIALS AND METHODS

Plant material and extract

The seeds of *P. americana* were obtained from a tree in Ifite-Oraifite, Anambra State, Nigeria, in the fruiting season of June. All experiments were done with seeds obtained from the particular tree. Although it is a commonly known tree, it was identified and authenticated by staff of the Department of Pharmacognosy, University of Benin where a herbarium specimen exists. Specimen with voucher number FHI - 108336 has been deposited with the Forest Research Institute of Nigeria. The seeds were chopped into small pieces and sundried for 5 days. The pieces were thereafter ground into powder with a mill and the powder was soaked in distilled water for 24 h and filtered. The filtrate (aqueous extract, AE) was concentrated in a rotary evaporator and was further dried in an oven set at 30°C for 3 days (yield = 10.60% w/w). The extract was then packed into an amber-coloured bottle and stored at 4°C until required for animal experiments.

Animals

Experiments were performed using adult male rats (235 – 285 g) bred locally in the animal house of the Department of Pharmacology and Toxicology, University of Benin. They were fed rat pellets (Bendel Feeds and Flour Mill Nigeria Ltd, Ewu, Nigeria). Feeds and water were freely available to all animals until they were used for experiments. Animals were exposed to natural lighting conditions and were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002). The study was approved by the institutional committee on the use of animals for experiments.

The animals were divided into two groups comprising of a naïve group which was given acute doses of the extract and a pretreatment group which was administered 260 mg/kg daily of the extract (*p.o.*) for 10 consecutive days.

Measurement of blood pressure and heart rate

Blood pressure was measured using an invasive technique earlier described (Omogbai et al., 2005). The rats were anaesthetized with urethane (1.75 g/kg, *i.p.*). The right jugular vein and the left common carotid artery were isolated and cannulated. While the jugular served as the route for the administration of drugs or extract, the carotid was connected through the cannula to a physiological pressure transducer (Bentley Trandec, USA), which was in turn connected to an Ugo Basile 2-channel "Gemini" recorder model 7070 (Ugo Basile, Italy). Prior to use, the instrument was switched on for 30 min, adjusted and then calibrated with a standard mercury sphygmomanometer (Aneroid, UK). In order to prevent clotting, the arterial (carotid) cannula was filled with heparinized saline. Animal body temperature was maintained at 37.0 ± 0.5°C by means of an overhead lamp to which a thermometer was fitted. Respiration was assisted by endotracheal intubation. BP measurement was begun after 10 min of surgery, with the pre- drug administration values taken as baseline. The volume of the solution of drug and or extract injected at any point in time was not in excess of 0.40 ml.

While the lower channel recorded the blood pressure, the upper channel recorded the heart rate. After baseline recording had been done for 5 min, drugs and/or extracts were administered according to a protocol such that reading returned to pre-injection level before another bolus injection. Depending on the protocol, doses of the extract (240, 260 and 280 mg/kg), and acetylcholine (1, 2 and 4 µg/kg) were administered alone or in combination (i.e., extract + acetylcholine). The doses of the extract for acute injections to naïve rats were decided from a pilot study which showed that only 240 mg/kg gave a consistent threshold response. The dose for pretreatment was taken as the average of the three doses used for acute injections to naïve rats. The recovery times (RT) after bolus injections were determined by measuring the distance from the point of recording pen deflection (upward or downward) to the point of return to baseline. The distances were converted to times using the set chart speed.

Drugs and chemicals

Acetylcholine (ACh) and urethane were all purchased from Sigma (UK). Heparin was manufactured by Biochemie (Austria) . Solutions of the drugs were prepared fresh and dilutions of ACh or extract were carried out in normal saline.

Statistics

Blood pressure readings are presented as mean arterial pressure (MAP) in millimeters of mercury and heart rates (HR) as beats per minute. The recovery times after bolus injection of ACh and or extract are in seconds. MAP indicates the percentage decrease occurring with the dose of ACh or extract. RT ACh_{Max} indicates recovery times occurring with the maximum dose of ACh (4 µg/kg). The data are presented as mean ± standard error of the mean (SEM) and *n* represents the number of rats used for each experiment. Comparisons were made using Student's *t*-test or Kruskal Wallis ANOVA with Dunn's post hoc test (GraphPad Prism Software, UK). Values were considered statistically significant at $p < 0.05$.

RESULTS

A representative tracing of the effect of AE on the blood pressure of normotensive naïve rats is shown in Figure 1. Each dose of the extract caused a decrease in both systolic and diastolic pressure and heart rate. The data obtained from the tracings were pooled and analyzed.

In Figure 2 the effects of doses of AE alone and in combination with 2 µg/kg of ACh on MAP of naïve rats are shown. Basal MAP value was 125.7 ± 11.2 mm Hg but AE given alone at the three doses (240, 260 and 280

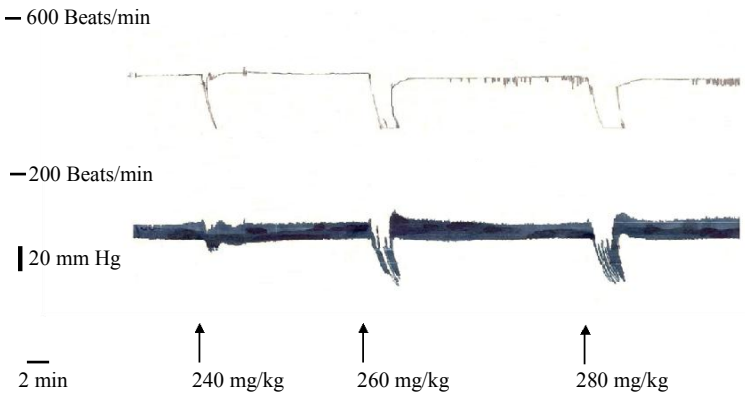

Figure 1. Representative tracing of the effect of aqueous extract (AE) of *P. americana* on heart rate (upper panel) and blood pressure (lower panel) of an anaesthetized naïve rat.

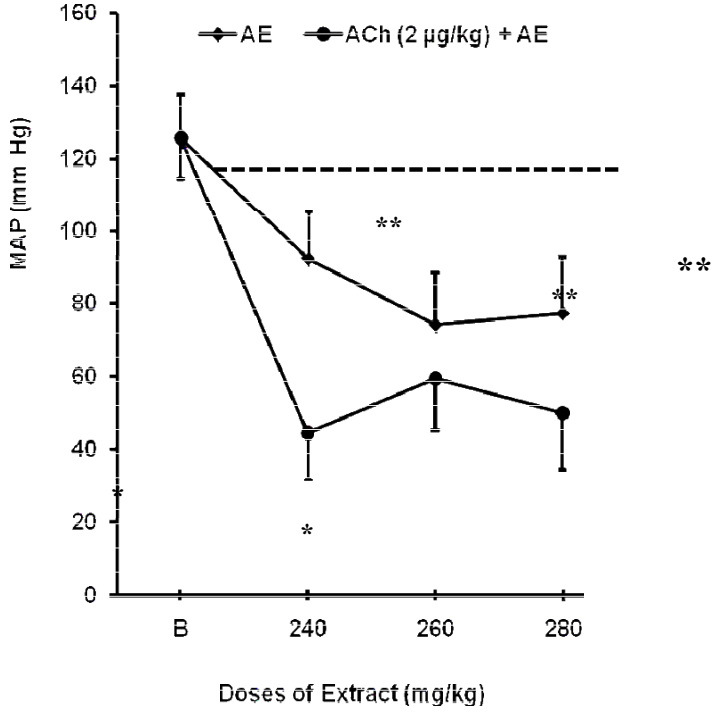

Figure 2. The effect of aqueous seed extract of *P. americana* (AE) alone and in combination with 2 µg/kg ACh on blood pressure of naïve rats. ***p* < 0.005 compared to corresponding baseline values (B). **p* < 0.05 compared to AE alone, n = 5 per group.

mg/kg) significantly ($p < 0.005$) reduced this value in a dose-dependent manner. The corresponding MAP values for each dose of the extract were: 92.2 ± 12.0, 74.0 ± 12.9 and 77.18 ± 15.6 mm Hg. The concurrent administration of 2 µg/kg of ACh with 240 mg/kg of AE was significantly ($p < 0.05$) more effective than the extract alone in MAP reduction in naïve rats. This was not seen with the higher doses of the extract (Figure 2).

Oral pretreatment of rats with 260 mg/kg of AE daily for 10 consecutive days significantly ($p < 0.05$) reduced MAP compared to their naïve counterparts (Table 1). Basal heart rates and recovery times after bolus maximum dose of acetylcholine (ACh_{Max}) were also significantly reduced in the pretreated group (Table 1).

As shown in Figure 3, the depressor responses by naïve rats to doses of ACh were similar to the responses by pretreated rats to the drug. The figure shows that the depressor responses following each dose of the extract

Table 1. Basal mean arterial pressure and heart rate of naïve and *P. americana* pretreated rats, and effects of maximum doses of acetylcholine (ACh) on heart rate and recovery time in the rats.

	Parameters			
	Basal MAP (mm Hg)	**Basal HR (Beats/min)**	**HR ACh_{Max} (Beats/min)**	**RT ACh_{Max} (S)**
Naïve	125.7 ± 11.2	274.6 ± 39.3	98.8 ± 18.0	170.4 ± 43.9
Pretreated	92.1 ± 8.5*	161.6 ± 11.6*	90.8 ± 11.7	31.5 ± 6.2*

*p < 0.05 compared to corresponding value in column (Student's *t*-test). MAP, mean arterial pressure; HR, heart rate; and RT, recovery time. The pretreated group was given 260 mg/kg/day (*p.o.*) of extract for 10 consecutive days. n = 4 - 5.

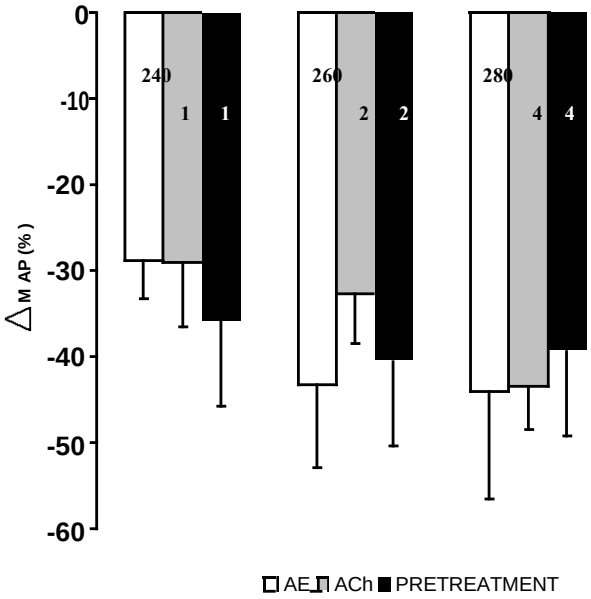

Figure 3. Comparative effects of the aqueous seed extract (AE) of *P. americana* on change from baseline mean arterial pressure following bolus doses of acetylcholine (ACh) on naïve and AE-pretreated rats. Values are not significantly different. Figures in bars represent doses. *n* = 4 – 5.

were not significantly different from those produced by ACh. That is, responses by naïve and pretreated animals to 1, 2 and 4 µg/kg of ACh were not significantly different from those by naïve rats to doses of 240, 260 and 280 mg/kg of the extract. For example, while 4 µg/kg of ACh gave MAP values of -43.5 ± 12.6% (naïve) and -39.3 ± 10.0% (pretreated), 280 mg/kg of AE gave value of -44.0 ± 7.5%.

The acute changes in heart rate occurring after bolus doses of ACh are shown in Figure 4. Although both AE and ACh caused negative changes in heart rate in the naïve rats, doses of 2 and 4 µg/kg of ACh were more effective than 260 and 280 mg/kg of AE respectively. Values for 2 and 4 µg/kg of ACh were - 48.3 ± 6.2% and - 47.4 ± 4.8% respectively. The values for 260 and 280 mg/kg of AE were -9.4 ± 5.3 and 19.8 ± 8.4% respectively.

DISCUSSION

Pretreatment of rats with the aqueous seed extract of *P. americana* over a period of 10 days resulted in the reduced mean arterial pressure in the rats. Acute administration of the extract to naïve rats also resulted in blood pressure reduction. These findings lend some credence to the use of the extract by herbalists for the treatment of hypertension. The blood pressure reducing effect of bolus doses of the extract was not increased at the higher doses by the concurrent administration of ACh, a blood pressure reducing agent which releases nitric oxide from the endothelium and causes reduced heart rate (Brown and Taylor, 2006). This may be due to homeostatic mechanisms aimed at limiting the fall in blood pressure. The doses of ACh used in the study produced similar levels of blood pressure and heart

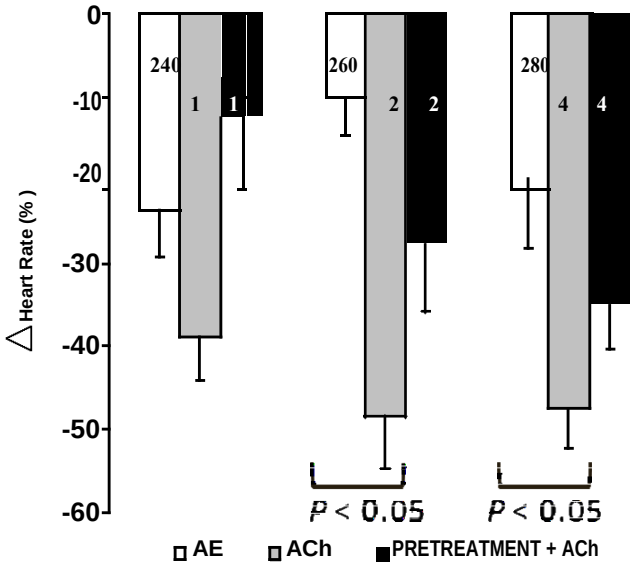

Figure 4. Effects of acute doses of acetylcholine (ACh) on change from baseline heart rates of naïve and aqueous seed extract (AE) of *P. americana* pretreated rats in comparison with acute doses of the extract on naïve rats. Figures in bars represent doses. n = 4 – 5.

reduction as acute doses of the extract, thereby enabling comparison of relative potency.

The mechanism involved in the blood pressure lowering effect of the extract appears to be cardio-dependent as heart rate was significantly decreased following pretreatment with the extract or its acute administration to naïve rats. Reduction in heart rate is the main mechanism by which -adrenoceptor blockers such as propranolol reduce blood pressure in hypertensive animals although these drugs do not reduce blood pressure in normotensive humans (Westfall and Westfall, 2006).

Other mechanisms may also contribute to the blood pressure reduction. For example our unpublished data indicate that 1 mg/ml of the extract resulted in attenuated aortic ring responses to cumulative concentrations of noradrenaline in an organ bath, irrespective of whether the rings were obtained from normotensive or hypertensive rats. In addition, the leaves of the plant have been used as a diuretic in Brazilian ethnomedicine (De A Ribeiro et al., 1986). However, the possible diuretic properties of the seed extract were not evaluated in the present study.

Our phytochemical screening of the extract has identified the presence of tannins. Tannins are polyphenolic compounds that possess vasorelaxant effects (Stoclet et al., 2004), most of which like ACh are endothelium-dependent (Stoclet et al., 2004; Diebolt et al., 2001) . Mechanisms attributed for the vasorelaxant actions of polyphenols include inhibition of protein kinase C; inhibition of cyclic nucleotide phosphodiesterases; and/or decreased Ca^{2+} uptake (Herrera et al., 1996). The polyphenol content of the extract may be responsible for the reduction in blood pressure. However, some polyphenols have been reported to possess contractile properties in vascular smooth muscles (Sanae et al., 2003).

The recovery time may give an indication of the duration of action of acutely administered bolus doses of drugs. The present study has shown that extract-pretreated animals recovered faster from bolus doses of ACh than their naïve counterparts. Although the mechanistic basis for this observation is not known, it may be protective against profound fall in blood pressure.

The literature currently contains a number of scientifically confirmed uses of the parts of *P. americana*. For example its aqueous leaf extract possesses antihypertensive properties possibly by causing relaxation of blood vessels (Owolabi et al., 2005; Ojewole et al., 2007). We have in a previous study reported the acute and sub-acute safety profiles of the seed extract in rats (Ozolua et al., 2009). The current study therefore adds to the economic and medicinal values of the plant.

Conclusion

The results from this study indicate that the aqueous seed extract of *P. americana* possesses blood pressure lowering properties in normotensive Sprague-Dawley rats. The effect of the extract may be due to reduction in heart rate. The results from this study justify the use of

the extract for the management of hypertension by herbalists in Nigeria.

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