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Vasorelaxant action of aqueous extract of the leaves of *Persea americana* on isolated thoracic rat aorta

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Abstract

The present study investigated the vasorelaxant action of the aqueous leaves extract of *Persea americana* on isolated rat aorta. The results showed that the extract produced significant vasorelaxation and that the effect is dependent on the synthesis or release of endothelium-derived relaxing factors (EDRFs) as well as the release of prostanoid. The extract also reduced vasoconstriction probably by inhibiting Ca^{2+} influx through calcium channels.

Keywords: *Persea americana*; Vascular relaxation; Rat aorta; Nitric oxide

1. Introduction

Persea americana Mill (Lauraceae) is a deciduous plant, which is widely distributed throughout tropical and subtropical Africa. The fruit of the plant is commonly known as avocado pear. In Nigeria, the leaf is known in common names as Ewé pia (Yoruba), Ikבŋ eben mbakara (Efik), Akwukwo Ube oyibo (Igbo), and Ganyen piya (Hausa). The root, bark, fruit, and leaf are used extensively in traditional medicine for the treatment of various ailments. In Congo Brazzaville, a decoction of the stem bark is taken to relieve cough;

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while in Mexico, it is used as an aphrodisiac, emmenagogue, to prevent miscarriage, to speed up postpartum recovery, and in the treatment of haemorrhage between menstrual periods [1,2]. The leaves are used in Brazil and Jamaica for the treatment of high blood pressure [3,4]. In Nigeria, several ethnic groups use the leaves of *P. americana* in the treatment of hypertension. Adeboye et al. [5] have confirmed that the administration of the leaf extract of *P. americana* on anaesthetized normotensive male Sprague–Dawley rats produced a significant reduction in blood pressure.

However, the possible mechanisms by which *P. americana* lowers blood pressure have not been worked out. This study investigates the effects of the aqueous leaf extract of *P. americana* on endothelium-intact or -denuded aortic rings. In addition, the effects of L-NAME or methylene blue or indomethacin on *P. americana* extract activity were investigated. Finally, the effects of *P. americana* on aortic rings precontracted with noradrenaline or potassium chloride were investigated.

2. Experimental

2.1. Plant

P. americana leaves, collected in the University of Lagos Staff Quarters, Akoka, Lagos State, Nigeria, in June 1997 in the early hours of the morning, in accordance with the practice of traditional medicine practitioners, were authenticated by Dr. O. Ugboaja, Forestry Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen has been deposited in the FRIN Herbarium (no. FHI 106099).

2.2. Plant extract

P. americana leaves dried at 40 °C for 5 days were ground into fine powder and stored in an amber bottle. Fine powder material (840 g) was Soxhlet-extracted with distilled water and filtered. The solution (pH 5.4) was lyophilized, giving 143.7 g of extract (17.11% wt/wt). A new stock solution was prepared on each day of the experiment.

2.3. Animals

Sprague–Dawley rats of either sex weighing 250–300 g were used for the studies. The animals were obtained from the Laboratory Animal Center of the College of Medicine, University of Lagos, Lagos, Nigeria. They were kept in a well-ventilated animal house and received standard animal chow (Pfizer Feeds Nigeria, PLC) and water ad libitum. Prior to experimentation, they were fasted overnight with access to water ad libitum.

2.4. Drugs

Noradrenaline, acetylcholine hydrochloride, N^G-nitro-L-arginine methylester (L-NAME), indomethacin were from Sigma Chemical Company (St. Louis MO, USA). Methylene blue and potassium chloride were from British Drug Houses, UK.

2.5. Preparation of aortic rings

The rats were killed and the thoracic aorta were carefully excised and cleaned of connective tissue and adherent fat. The aortic lumen was carefully flushed with Krebs solution to free the lumen of its content, cut into 5-mm strips, and transferred into a Krebs solution [composition (mM): NaCl, 119; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; and glucose, 11.5; pH 7.4]. Each segment of the aortic ring was suspended in a 20-ml organ bath between two stainless-steel wire hooks. One hook was fitted to the ring at the bottom of the bath, while the second hook was attached to a Grass FT.03 force transducer connected to a Grass polygraph (Model 7D). Each aortic ring was placed under an initial tension of $2\times g$, which was kept constant throughout the experiments. The bath containing the Krebs solution was kept at 37 °C and bubbled with 95% O_2 and 5% CO_2. Responses to 1×10^{-7} M noradrenaline were obtained repeatedly until contractions were uniform during the initial stabilization period of 90 min. During this period, the bath solution was renewed every 30 min. Endothelium integrity was assessed by verifying that the contracted rings relaxed by at least 50% when stimulated with 1×10^{-6} M acetylcholine.

In another preparation, the vascular endothelium was denuded by gently rubbing the aortic lumen with an 18-gauge hypodermic needle. Removal of the endothelium was confirmed by a relaxant response to acetylcholine (1×10^{-6} M) of less than 10% [6]. The integrity of the vascular smooth muscle function was assured by contraction response to 6×10^{-2} M potassium chloride and 10^{-7} M noradrenaline [7].

2.6. Relaxation experiments

Following the equilibration period, the tissues were precontracted by addition of noradrenaline 1×10^{-7} M. Once the tonic responses became stable, at the peak of each contraction, the cumulative concentration–response curves to the aqueous extract of *P. americana* (0.01–12.8 mg/ml) were obtained, in preparations with intact or denuded endothelium and in the absence or in the presence of N^G-nitro-L-arginine methylester (L-NAME, 10^{-4} M, a nitric oxide synthetase inhibitor) or methylene blue (10^{-6} M, soluble guanylate cyclase inhibitor) or indomethacin (10^{-5} M, cyclooxygenase inhibitor). All antagonists were incubated with the tissue 30 min before extract addition. Each preparation was exposed to only one antagonist.

In another experiment, cumulative concentration–response curves to noradrenaline (1×10^{-9} to 1×10^{-5} M) and potassium chloride (1×10^{-2} to 8×10^{-2} M) were obtained in preparations of rat aortic rings with intact endothelium before or after addition of the aqueous extract at a final bath concentration of 1 mg/ml or 5 mg/ml. The extract was kept in contact with the preparation for 30 min and throughout the construction of the second concentration–response curve. In all cases, responses to each concentration of agonist was expressed as percent of the maximum contraction obtained in the initial concentration–response curve.

2.7. Statistical analysis

Data are presented as mean ± S.E.M. The EC_{50} values (i.e., the concentration of the agonist or extract that produced 50% reduction of maximal relaxant responses) were

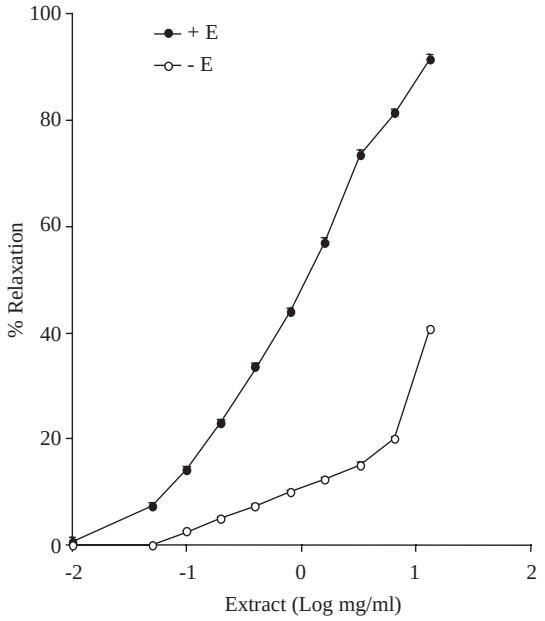

Fig. 1. Effect of leaves aqueous extract of *P. americana* on rat aortic rings with intact endothelium (+E) and denuded endothelium (−E) precontracted with 10^{-7} M noradrenaline. Values are mean+S.E.M. ($n=6$).

determined from the concentration–response curves by linear regression analysis. Statistical significance of the data for control and treated groups was assessed by Student's *t*-test for paired and unpaired samples. Statistical significance was accepted when $P<0.001$.

3. Results and discussion

The cumulative addition of the aqueous extract of *P. americana* (0.01–12.8 mg/ml) produced a concentration-related vasorelaxation response in rings of rat aorta with

Table 1
Effect of the inhibitors of endothelium-dependent relaxing factors (EDRFs) on the vasorelaxant action of the leaves' aqueous extract of *P. americana* in rat aortic rings with intact endothelium precontracted with 10^{-7}M noradrenaline

EDRF inhibitors	Concentration (M)	EC_{50} (mg/ml)
Control	–	0.88 ± 0.03
Indomethacin	10^{-5}	15.9 ± 1.2
Methylene blue	10^{-6}	89.6 ± 10.9
L-NAME	10^{-4}	127.8 ± 6.3
Endothelium-denuded	–	2001.1 ± 252.2

$n=6$ in each group.
Values are mean ± S.E.M.
$P<0.001$ compared to control.

Table 2
Effect of the inhibitors of endothelium-dependent relaxing factors (EDRFs) on the vasorelaxant action induced by acetylcholine in rat aortic rings

EDRF inhibitors	Concentration (M)	EC_{50} (M)	E_{max} (%)
Control	–	82.7 ± 0.11	94.6 ± 0.15
Indomethacin	10^{-5}	71.3 ± 1.12	87.1 ± 1.02 (NS)
Methylene blue	10^{-6}	n.d. (18.8%)	18.8 ± 0.07***
L-NAME	10^{-4}	n.d. (7.2%)	7.2 ± 0.02***
Denuded endothelium	–	n.d. (9.8%)	9.8 ± 0.11***

$N=6$ in each group.
Values are mean ± S.E.M.
n.d. = not detectable; percentages of maximum response are presented in parentheses.
*** $P<0.001$ compared to control.

intact endothelium precontracted with noradrenaline (1×10^{-7} M), with an EC_{50} of 0.88 ± 0.03 mg/ml. In the endothelium-denuded rings, the vasorelaxant action of the aqueous extract of *P. americana* was significantly attenuated (EC_{50} 2001.14 ± 252.18 mg/ml; Fig. 1). The vasorelaxant effect of the aqueous extract of *P. americana* was also significantly attenuated by L-NAME (10^{-4} M), methylene blue (10^{-6} M), or indomethacin (10^{-5} M) (Table 1). Cumulative addition of acetylcholine (1.1×10^{-8} to 1.4×10^{-5} M) produced relaxation of endothelium-intact rat aortic rings precontracted with noradrenaline (1×10^{-7} M). The vasorelaxant effect was significantly reduced by

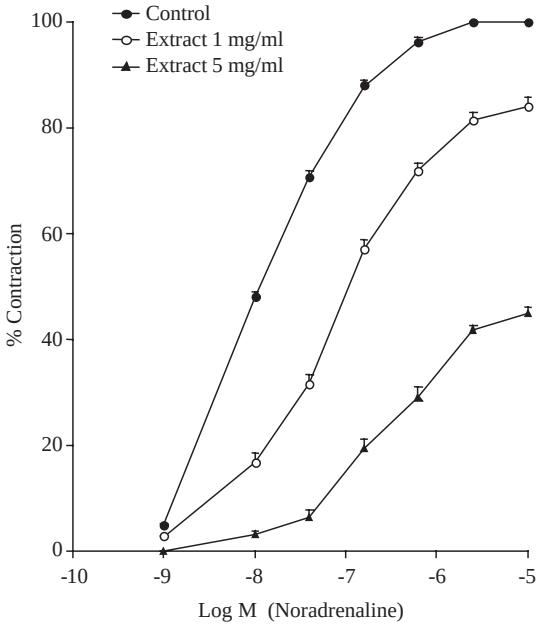

Fig. 2. Effect of leaves aqueous extract of *P. americana* on rat aortic rings with intact endothelium on contraction induced by noradrenaline. Values are mean + S.E.M. ($n=6$).

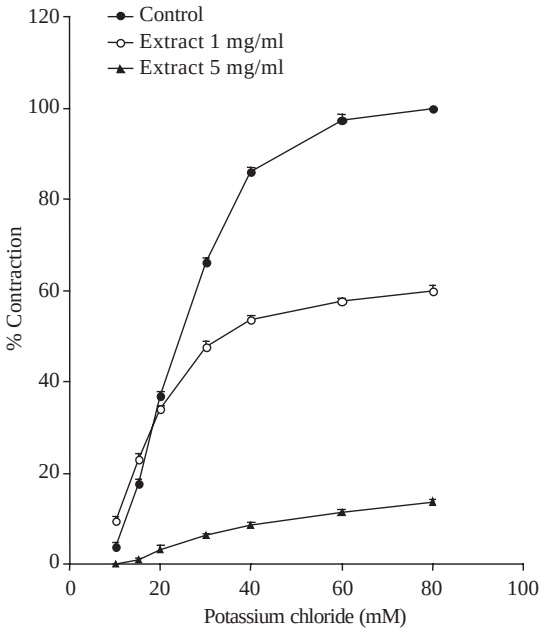

Fig. 3. Effect of leaves aqueous extract of *P. americana* on rat aortic rings with intact endothelium on contraction induced by potassium chloride. Values are mean + S.E.M. ($n = 6$).

L-NAME (10^{-4} M) and methylene blue (10^{-6} M), but not affected by indomethacin (10^{-5} M) (Table 2).

The aqueous extract of *P. americana* (1 mg/ml or 5 mg/ml) produced a rightward shift of the concentration–response curves to noradrenaline (1×10^{-9} to 1×10^{-5} M) and potassium chloride (10–80 mM) (Figs. 2 and 3).

Taken together, the above results showed that the vasorelaxant effect of the aqueous leaves extract of *P. americana* is endothelium-dependent. In fact, this activity was blocked by L-NAME or methylene blue, suggesting that the vasorelaxation is dependent on the synthesis and release of endothelium-derived relaxing factors (EDRFs). The blockade by indomethacin suggests that *P. americana* may act also by activating PGI_2 and PGE_2 receptors. The vasorelaxant effect may also be produced by the inhibition of Ca^{2+} mobilization through voltage-dependent channels and, to a lesser extent, receptor-operated channels.

These vascular effects provide an explanation of its hypotensive action and a basis for the use of the extract in the management of high blood pressure in folkloric medicine.

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