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Effects of aqueous-alcoholic extract of *Persea Americana* (leaves) on isolated rabbit heart preparation and blood pressure of normotensive rats

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ABSTRACT: The aqueous-alcoholic extract of *Persea Americana* was investigated for its hypotensive activity on anaesthetized normotensive rats and effect on isolated rabbit heart preparations. It was found that the extract exhibited a dose-related hypotensive activity for all the dose levels (0.05 – 200 mg/kg) with a greater duration at higher dose level (200mg/kg). The extract was also found to reduce the force and the rate of contraction of isolated heart preparation, which was also dose-related. These results would justify the popular use of the plant to treat hyptertension.

Key Words: *Persea americana*; Hypotensive, Anaesthetized; Normotensive.

Introduction

Persea Americana – Lauraceae family called avocado, is a native to Central and South America. It is an evergreen tree, which grows up to 20m tall and can be equally wide. The fruit is pear-shaped with large, round to egg shaped central seed. The crop requires a minimum annual rainfall of 750mm (Susan and Anne, 1988).

Avocado is mainly grown for its food value. It has high fat content (25%), high protein, vitamins and minerals (Susan and Anne, 1988).

The fruit skin is antibiotic, and is employed as a vermifuge and remedy for dysentery. The leaves are chewed as a remedy for pyorrhoea, leaf poultices are applied on the forehead to relieve neuralgia. The aqueous extract of the leaves has an hypotensive effect (Perry, 1980). The leaf decoction is taken as a remedy for diarrhoea, sore throat and haemorrhage, it allegedly stimulates and regulates menstruation (Morton, 1987). The seeds yield milky fluid with the odour and taste of almond. The seed turns red on exposure providing an indelible red-brown or blackish ink, this ink is being used to mark cotton and linen textiles (Morton, 1987). The oil expressed from the flesh is rich in vitamins A, B, C and E with a digestibility coefficient of 93.890.

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Two resins derived from the skin of the unripe fruit are said to be toxic to guinea pigs by subcutaneous and peritoneal injection (Watt and breyer-Brandwijk, 1962). Large doses of *Persea Americana* leaves have been fatal in a pool to fish. The seeds, ground and mixed with cheese or cornmeal, have been used to poison rodents (Duke, 1979).

The present work was carried out to evaluate the phytochemical constituents of *Persea Americana*, and the effects of different concentrations of methanolic extract of the plant on the blood pressure of anaesthetized normotensive albino rats and the isolated heart preparations of rabbits.

Materials and Methods

Plant Material

Fresh leaves of *Persea Americana* were collected in October 2001 in Akure, Nigeria. A voucher sample which was authenticated by Mr. T. Arannilewa (Biology Department, federal University of Technology, Akure, Nigeria) was deposited in the herbarium of Biochemistry Department, Federal University of Technology, Akure, Nigeria.

Preparation of Extract

The leaves were air-dried for seven days at room temperature. The air-dried samples were ground to a mesh size of 1mm. A 350g sample of the powdered material was soaked in 100ml of a mixture of methanol and water (4:1) for 96 hours. This was filtered and concentrated to a small volume to remove the entire methanol using rotary evaporator. The small volume was later freeze-dried with the use of Freeze-drier and kept in the freezer at 4°C prior to analysis.

Phytochemical Screening

The methanolic extract was screened for the presence of the following: tannis, alkaloids, saponins, phlobatannins, anthraquionones and cardiac glycosides according to the method described by Sofowora (1993).

Animals

Wistar male albino rats (160 – 240g) and adult male rabbits were obtained from the Animal house of the Department of Physiology, Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. They were all clinically healthy and maintained in standard environmental conditions of temperature (27.0 ± 0.5%), relative humidity (73.0 ± 15%) and dark/light cycle. They were fed standard diet and water *ad lib*.

Hypotensive Activity

The hypotensive activity was investigated according to the method of Adeboye *et al* (1999). The Wistar albino rats were divided into 7 groups of 3 rats each. The rats were anaesthetized with urethane (1.75mg/kg). As soon as the animals reached the state of surgical anaesthesia, each of them was placed on its back and pinned on a rat dissecting board. The trachea of the rats was exposed by splitting the muscle in front of it using a forceps and scissors. The trachea cannula for rat was dissected pointing to the thorax and tied firmly in place using a soft thread. The femoral artery was located and exposed carefully and a bull dog clip was placed on the artery and it was then cannulated. The bull dog clip was removed and the cannula was pushed in further and then tied securely using a disposable syringe and the cannula was filled with heparinized saline to prevent blood clotting. The femoral vein was also located and exposed carefully and a bull dog clip was put on the vein and then cannulated at one end.

Heparinized saline was filled into a three-way tap and the cannular. The other end of the tap was connected to the transducer for blood pressure recording. After the stabilization period of 30 mins, the initial blood pressure was taken followed by the administration of extract in volume not exceeding 1ml/kg.

The extract was injected intravenously into the femoral artery and the blood pressure was recorded using a Glass polygraph Model 7D, calibration 1cm = 50mm Hg. The mean arterial blood pressure (MABP) was calculated using:

$$MABP = DP + (DP - SP)$$

where SP = systolic pressure; DP = diastolic pressure.

Effect on isolated rabbit hearts preparations

The rabbit was killed by a blow on the head and immediately the throat was cut to expel the blood. The cheat was cut open and the heart exposed and the aorta was freed from its attachment to the pulmonary artery. It was then quickly transferred into a basin containing Ringer Locke solution and squeezed gently to remove as much blood as possible from the aorta (Burande *et al*., 1983). The heart was then tied to the Langendorff's set up with the aorta tied to the cannula connected to the tube leading from the reservoir of the Ringer Locke solution (temperature 37°C, flow rate of 7-8 ml/min with a pH of 7.4). The Ringer Locke solution was continuously bubbled with oxygen. After the stabilization period of 5 minutes, extract was administered at concentration 10mg/ml (stock) through the side arm of the cannula at various volumes of 0.1, 0.2 and 0.3ml. The contractility of the heart was being recorded on the Glass polygraph model 7D by means of a palmar clip placed at the apex of the heart connected through a lever to the transducer for recording (calibration, 2.0cm = 1.0g).

Toxicity

Twenty-four rats (160 – 200g), divided into six equal groups, were used. Single doses of the extract (250 mg/kg) were given daily for a period ranging from 1 – 15 days. The control group received saline.

Statistical Analysis

This includes mean ± SD, analysis of variance (ANOVA) and Duncan multiple range test.

Results and Discussion

The results of the phytochemical screening showed the presence of alkaloids, saponins, tannins, flavonoids and cardiac glycosides (Table 1). Tannins are used pharmacologically in the treatment of bruises and superficial wound (Trease and Evans, 1985). Saponins bind to cholesterol to form insoluble complexes; dietary saponins in the guts of monogastrics combine with endogenous cholesterol excreted via the bile. This prevents cholesterol re-absorption and results in a reduction of serum cholesterol (Cheeke, 1971). Saponins have been found to be potentially useful for the treatment of hypercholesterolemia, which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol (Malinow *et al*., 1977a; 1977b). Cardiac glycosides are cardioactive compounds whose pharmacological effectiveness is dependent on both the aglycones and sugar attachment; the inherent activity resides in the aglycones. The clinical effect in cases of congestive heart failure is to increase the force of myocardiac contraction. The heart arresting properties of these glycosides also render them most effective arrow poisons (Brian *et al*., 1985).

Results of hypotensive activity of *Persea Americana* in Table 2 revealed that at low concentrations (0.05 – 0.5mg/kg), there was no significant ($P>0.05$) difference in the meanm arterial blood pressure. However, there was significant ($P<0.05$) decrease in the mean arterial blood pressure of the treated rats at 50 – 200 mg/kg) compared to the control group. The hypotensive effect was found to be dose dependent. This dose-related hypotensive activity showed by the aqueous – methanolic extract of *Persea Americana* could be due to the presence of one or more metabolites (saponins, alkaloids, tannins and/or cardiac glycosides) present in them. The duration (the time between initial fall in blood pressure and the recovery of the pre-treatment value) was shorter for smaller concentrations (generally less than 60 seconds). But this was, however, greater (128 seconds) with higher concentrations (200 mg) (Table 2).

The hypotensive effect of this plant is in support of the earlier finding of Perry (1980) that the aqueous leaf extract had a hypotensive activity. The short duration of the hypotensive effect of the plant at low concentrations and longer duration at high concentrations indicated that the extract may be a cardio-depressant at low concentration and a ganglion-blocker at high concentration (Sofowora, 1984).

For the isolated rabbit heart preparation, the extract of *Persea Americana* had effect on the isolated heart by lowering the force of contraction of the cardiac muscle.(Table 3). Cholinergic drugs (e.g. acetylcholine, carbarchol) that are associated with the lowering of the force of the cardiac muscle are said to stimulate the parasympathetic system, which causes the heart to beat more slowly, and weakly, which may then lower the pumping action of the cardiac muscle (Alfred *et al*., 1984). hence the above result would suggest that *Persea Americana* extract may possess parasympatholytic activity.

Toxicity results showed that there was no death recorded in all the rats used. However, there were temporary behavioural changes during the administration of first dosage. The changes, which lasted 7mins, include stretching, prostration, sluggishness and slow response to external stimuli.

The results of the present study would lend support to the popular use of *Persea Americana* as an antihypertensive preparation by the Yorubas in South Western Nigeria. Further work, however, is in progress to purify and characterize the active principles in this plant.

Table 1: Preliminary phytochemical analysis of methanol extract of *Persea americana* leaves[a]

Constituents	**Extract**
Alkaloids	+
Anthraquinones	-
Saponins	+
Tannins	+
Phlobatannins	-
Cardiac glycosides	
Cardinolides	+
Steroidal nucleus	+
Deoxy sugar	+
Flavonoids	+

+, Present; -, Absent.

Table 2: Effect of extract of *Persea Americana* leaves on blood pressure of anaesthetized normotensive rats.

Dosage (mg/kg)	**MABP**	**% Change in MABP**	**Duration (sec.)**
Control (saline, 0.02)	$93.24^{c} \pm 2.04$	0	30
0.05	$85.35^{c} \pm 5.98$	-11.64	23
0.5	$76.96^{c} \pm 4.70$	-6.59	20
5.0	$38.48^{b} \pm 6.78$	-8.33	15
50.0	$35.52^{b} \pm 1.30$	-23.36	24
100.0	$29.24^{a} \pm 1.20$	-27.15	34
200.0	$20.72^{a} \pm 2.10$	-28.82	128

Each value of MABP is a Mean ± SD of triplicates.

Table 3: Effect of *Persea Americana* on the isolated rabbit heart preparation

Dose (mg)	Response (g)	Heart Rate (beats/min)
0.0	8.0	708
1.0	2.5	240
2.0	2.0	180
3.0	1.5	0

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