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Research Paper

ACUTE AND SUB-ACUTE TOXICOLOGICAL ASSESSMENT OF THE AQUEOUS SEED EXTRACT OF *PERSEA AMERICANA* MILL (LAURACEAE) IN RATS

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Abstract

The aqueous seed extract of *Persea americana* Mill (Lauraceae) is used by herbalists in Nigeria for the management of hypertension. As part of our on-going scientific evaluation of the extract, we designed the present study to assess its acute and sub-acute toxicity profiles in rats. Experiments were conducted to determine the oral median lethal dose (LD_{50}) and other gross toxicological manifestations on acute basis. In the sub-acute experiments, the animals were administered 2.5 g/kg (p.o) per day of the extract for 28 consecutive days. Animal weight and fluid intake were recorded during the 28 days period. Terminally, kidneys, hearts, blood/sera were obtained for weight, haematological and biochemical markers of toxicity. Results show that the LD_{50} could not be determined after a maximum dose of 10 g/kg. Sub-acute treatment with the extract neither affected whole body weight nor organ-to-body weight ratios but significantly increased the fluid intake ($P < 0.0001$). Haematological parameters and the levels of ALT, AST, albumin and creatinine were not significantly altered. However, the concentration of total proteins was significantly increased in the treated group. In conclusion, the aqueous seed extract of *P. americana* is safe on sub-acute basis but extremely high doses may not be advisable.

Keywords: *Persea americana seed, herbal medicine, safety profile*

Introduction

The use of herbal medicines as complements or alternatives to orthodox medicines has been on the increase. The reasons, which have given rise to this trend, include the cheapness, availability and accessibility of these natural medicines. Besides, there has been the erroneous belief that these medicines are free from adverse effects (Larrey, 1994; Ernst, 2005). On the other hand they have been rejected because many of the acclaimed medicinal values have not been scientifically evaluated and their safety profiles uncertain (Ernst, 2005). It is, therefore, pertinent that safety assessments should be conducted on natural products for which certain medicinal uses have been scientifically validated.

The fruit of *Persea americana* Mill of family Lauraceae is eaten in many parts of the world. In recent years, research has focused on various parts of the plants. The fruit in particular has been shown to possess medicinal properties. The edible fruit pulp contains up to 33% oil rich in monounsaturated fatty acids (Ortiz et al., 2004) that are believed to modify the fatty acid contents in cardiac and renal membranes and enhance the absorption of α/β-carotene and lutein (Salazar et al., 2005). The carotenoid content has been reported to play significant role in cancer risk reduction (Lu et al., 2005). Other properties of the oil include wound healing (Nayak et al., 2008) and hepatoprotection (Kawagishi et al., 2001). Proximate analysis has been conducted on the seeds (Olaeta et al., 2007).

Other parts of the plant have been reported to possess medicinal properties. The aqueous leaf extract for example has analgesic and anti-inflammatory (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.

Although the pulp has been most widely consumed all over the world, in Nigeria, the powdered seed is often mixed with soups, pap and puddings in the belief that it is useful in the management of chronic hypertension. In recent times, we have been investigating the effects of the aqueous seed extract on vascular responses and blood

pressure in rats. Our data (not published) indicate that the aqueous seed extract possesses blood pressure lowering properties. Thus, suggesting possible scientific basis for the use of the seed extract for the treatment of hypertension.

Since hypertension for which the ground seed is being used locally is a chronic disease, safety assessment on a sub-acute basis is a near indication of what may be expected if the seed extract is used chronically. Having found no toxicological report on the aqueous seed extract of *P. americana* we designed experiments in order to evaluate its acute and sub-acute safety profiles in rats.

Materials and methods

Plant Material and Extract

The seeds of *P. americana* were obtained from a tree in Ora-ifite, Anambra State, Nigeria in the fruiting season of June. Experiments were performed with seeds obtained from one tree. It was identified and authenticated by Dr B. Ayinde of the Department of Pharmacognosy, University of Benin, Benin City, Nigeria, where a herbarium specimen exists. A hebarium specimen FHI – 108336 was deposited at the Forest Research Institute of Nigeria, Ibadan, 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 hr and filtered. The filtrate (aqueous extract) was concentrated in a rotary evaporator and was further dried in an oven set at 30^{o}C for 3 days (yield = 10.60% w/w). The extract was then packed into an amber-coloured bottle and stored at 4^{o}C until required for experiments.

Animals

Experiments were performed using adult male rats (244.0 ± 18.8 g) bred locally in the animal house of the Department of Pharmacology & Toxicology, University of Benin, Benin City, Nigeria. The rats were fed on rat pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria). Feeds and water were freely available to all animals all through the experiments. Animals were exposed to natural room temperature and 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).

Acute toxicological experiments

Experiments to determine the oral median lethal dose (LD_{50}) of the aqueous seed extract of *P. americana* were carried out by using the method described by Miller and Tainter (1944). Six groups of male rats comprising of 4 rats per group were given the vehicle for extract reconstitution (control), 2, 4, 6, 8, 10 g/kg of the extract using an orogastric tube (CU.FNC-16-3). Mortality and other signs of acute toxicity were monitored for 72 hr in the first instance and 2 weeks after.

Sub-acute toxicological experiments

Rats of either sex were allotted to two groups of control and treated, such that the number of either sex was the same in both groups but rats of opposite sex were not placed in the same cage and pregnant rats were excluded from the study. The treated group was given daily doses of 2.5 g/kg of extract in 0.4 ml of distilled water by orogastric tube for 28 days. The dose of extract was taken as a quarter of the maximum dose (10 mg/kg) used for the acute toxicological experiments. Each rat in the control group was sham-treated with 0.4 ml of distilled water (*p.o*) daily for 28 days. At the end of the treatment period of 28 days, animals were anaesthetized with diethyl ether vapour in a chamber and blood samples were obtained via the abdominal aortae for biochemical and haematological assays. The hearts and left kidneys were isolated, cleaned of the adherent tissues and kept on absorbent papers for 10 mins before they were weighed.

Biochemical assays

Serum was obtained by allowing blood in specimen bottles to clot and retract. The serum samples were centrifuged at 3000 rpm and then separated by use of Pasteur pipettes into clean bottles. The serum samples were stored at -20^{o}C until analysis using Chemwell Chemistry autoanalyzer (Awareness Technology, USA, model 2910). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method described by Schumann et al. (2002). Serum creatinine was quantified by kinetic method of Larsen (1972). Total serum protein

was assayed by the Biuret method (Doumas et al., 1981), serum albumin was quantified by the method of Doumas et al. (1971).

Haematological assays

White blood cell (WBC), platelet count (PC), packed cell volume (PCV), haemoglobin concentration (HB), and the WBC cell differentials (lymphocytes (LYM) and neutrophils (NEU)) were all analyzed by use of an automated blood analyzer (QBC Autoread Plus, UK). The blood samples were first pipetted into QBC capillary tubes and spurn in a parafuge centrifuge (Becton Dickson, UK) for 5 mins and read by means of an autoread analyzer.

Measurement of body weight and daily fluid intake

Fluid intake was recorded daily within the 28-day period. Animals drank from graduated water bottles and at the same time each day, the decrement in the amount of fluid consumed over 24 hr was measured by subtracting the day's reading from that of the previous day. End-of-treatment weights were used for weight analysis.

Statistics

Data are presented as mean ± SEM (standard error of the mean) and *n* represents the number of rats used for a particular experiment. Comparisons were made between treated and control groups by use of Student's *t*-test. All data were analyzed using GraphPad Prism software (UK). $P < 0.05$ indicates statistically significant difference.

Results

The oral LD_{50} was indeterminable being in excess of 10 g/kg (not presented). The animals for the oral LD_{50} determination did not exhibit any toxicological signs such as depression, writhing, diarrhoea, hypermotility and aggression compared to the control.

Weight gain by the control animals was minimal (Table 1) while the treated had a slight reduction in weight although there was no statistical difference in the percentage change in weight between both groups. Similarly, there were no statistically significant changes in the heart-to-body weight and kidney-to-body weight ratios in both groups. Fluid intake was significantly higher in the treated groups compared to the controls ($P < 0.0001$). Mean fluid intake was 21.5% higher in the treated group.

In Table 2 some haematological parameters following the sub-acute treatment of rats with the extract are shown. The parameters measured: white blood cell count and differentials (lymphocytes and neutrophils), packed cell volume, haemoglobin concentration and platelet count were all not significantly different between the groups.
Also, there were no significant changes in ALT and AST levels after 28 days of treatment with the extract (Table 3). However, the value of total proteins was significantly higher in the treated group than the control but albumin concentrations were comparable. Similarly, creatinine concentrations were not significantly altered by the treatment.
In general, the animals did not exhibit any obvious external symptoms of toxicity before they were sacrificed for the various sub-acute experiments.

Table 1. Weight indices and fluid intake following oral treatment of rats with 2.5 g/kg/day (x 28) aqueous *P. americana* seed extract.

	Weight Change (%)	Daily fluid intake (mL)	H:BW (x 10^{-4})	K:BW (x 10^{-4})
Control	4.7 ± 4.6	196.8 ±10.6	29.0 ± 1.5	29.4 ± 1.3
Treated	-1.9 ± 9.9	251.6 ± 8.1*	30.0 ± 3.1	32.0 ± 3.3

*$P < 0.0001$, $n = 4 – 8$. H:BW, heart-to-body weight ratio; K:BW, kidney-to-body weight ratio.

Table 2. Haematological indices following 28-day daily treatment of rats with 2.5 g/kg (p.o.) aqueous *P. americana* seed extract.

	WBC (x 1000/µL)	LYM (%)	NEU (%)	PCV (%)	HB (g/dL)	PC (x 1000/µL)
Control	10.1 ± 0.7	67.5 ± 3.7	25.8 ± 2.4	37.3 ± 0.5	11.5 ± 0.6	878.6 ± 46.7
Treated	11.1 ± 2.9	60.4 ± 9.9	33.7 ± 6.4	35.6 ± 1.8	11.4 ± 0.5	778.4 ± 193.5

Values are not significantly different. WBC, white blood cell count; LYM, lymphocytes; NEU, neutrophils; PCV, packed cell volume; HB, haemoglobin; PC, platelet count. $n = 5$

Table 3. Effect of 28-day daily treatment of rats with 2.5 g/kg (p.o.) aqueous seed extract of *P. americana* on some biochemical parameters.

	CREAT. (mg/dl)	T. PROT mg/dl)	ALB (mg/dl)	ALT (IU/L)	AST (IU/L)
Control	0.46 ± 0.02	5.3 ± 0.3	2.7 ± 0.3	53.2 ± 10.7	180.8 ± 53.9
Treated	0.45 ± 0.02	7.4 ± 0.09*	2.8 ± 0.2	54.2 ± 10.7	163.8 ± 36.0

* $P < 0.05$, $n = 5$. T. BIL, total bilirubin; C.BIL, conjugated bilirubin; T. PROT, total protein; ALB, albumin.

Discussion

Acute treatment of rats with doses in excess of 10 g/kg did not cause death or obvious toxicological manifestations in the rats. Our laboratory results (not published) show that acute doses of 260 mg/kg per day given for 10 consecutive days to rats resulted in lower mean arterial blood pressure values compared to controls. Locally, the quantity of the powdered seed often added to food ranges from 1.0 to 2.0 g per adult per day. Estimating with the yield of 10.6% w/w of the aqueous extract, this is a maximum of 212.0 mg of the aqueous extract per day for an adult or about 3.0 mg/kg/day. Therefore, the maximum dose used in the LD_{50} determination (10 g/kg) in this study is more than 3000 times the recommended daily dose. Use of much higher doses in toxicological assessment gives an idea of the safety margin of the extract over the period.

The extract is being used for the treatment of hypertension which is a chronic disease. Therefore sub-acute toxicological assessment is an indication of its safety profile. Daily dosing with 2.5 g/kg per day for 28 days significantly increased the fluid intake by rats. Diseases associated with the kidney often manifest as water imbalance with alteration in fluid intake following polyuria or oliguria (Alcázar, 2008). Since urine output was not measured within the period, it is not immediately known if the alteration in fluid intake is as a result of increased urination. Taken together with comparable serum creatinine concentrations between the two groups, the significantly increased fluid intake in the treated group would be suggestive of alteration in body water regulatory mechanisms rather than renal injury.

Organ-to-body weight ratios are indices which are often used in toxicological evaluations (Michael et al., 2007). These ratios may be altered by the presence of tumours and hyperplasias although definite genotoxicity such as by the use of *Salmonella typhimurium* is often necessary to evaluate carcinogenic potentials of substances as established by the National Toxicology Program (1993). The results in this study show that these indices were not significantly altered by sub-acute treatment. This lends credence to the absence of injuries to the heart and kidney.

This study has also shown that sub-acute treatment with the extract did not cause any change in haematological parameters. Haematological changes such as anaemia are often accompaniments of bone marrow toxicity (Flanagan and Dunk, 2008) among other causes. The lack-of-effect on neutrophil levels indicates that the extract may not have induced any inflammatory process since these cells are usually elevated in the course of inflammations (Formela et al., 1995). Additional proof that organ injuries might not have occurred is seen in ALT and AST levels which were not significantly different between the treated and the control groups. Elevated levels of AST and ALT are often diagnostic of underlying cellular injuries (Karthikeyan et al., 2006; Wittekind, 1995). The reason for the increased total protein levels in the extract-treated group is not known with certainty but may be due to increase in hepatic protein synthesis. The seeds have low protein content (Olaeta et al., 2007).

In conclusion, using doses much higher than what is ordinarily taken ethnomedicinally, the seed extract appears safe at least on acute and sub-acute basis. While the medicinal importance of the extract is increasingly becoming of scientific interest, the consumption of high quantities may not be advised.

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