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Toxicological Evaluation of *Phyllanthus pentandrus* Aqueous Extract on Hepatorenal Functions in Albino Rats

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ABSTRACT: Aqueous extract of *Phyllanthus pentandrus* Schum. & Thonn (Euphorbiaceae) shoot at 500, 1000, 2000 and 3000 mg/kg body weight was investigated for its toxic effect in Albino rats. Acute toxicity test at 3000 mg/kg produced no mortality after 48 hours of observation. Oral administration of low and moderate doses (500 to 2000 mg/kg) of the aqueous extract produced no obvious toxicity. However, at 3000 mg/kg weakness, reduced reaction to noise, slow movement and resting at the corner of cages were observed. Acute toxicity study did not produce any negative behavioural changes such restlessness, excitement, coma or convulsion in rats administered various doses. The lethal dose (LD_{50}) of the extract was greater than 3000 mg/kg body weight dose. There was no significant ($p > 0.05$) change in the weight of rats administered various doses of the extract. Liver function indices evaluated were not significantly affected at low doses (500 to 2000 mg/kg) except aspartate aminotransferase (AST) which was significantly affected ($p < 0.05$) at 3000 mg/kg when compared with the control. The concentrations of total proteins, bilirubin and albumin in the serum did not change significantly when compared with the control. The concentrations of sodium, magnesium, urea, and creatinine were significantly ($p < 0.05$) affected by the extract administration. The toxicological implication of this finding is discussed.

Key words: *Phyllanthus pentandrus,* Euphorbiaceae, Acute toxicity, Sub- acute toxicty, medicinal plants

INTRODUCTION

Toxicity in herbal therapy is an important challenge for traditional medicine. It should be emphasized that traditional use of any plant for medicinal purposes, by no means warrants the safety of such plant, particularly with regards to; mutagenicity, carcinogenicity, embryotoxicity, nephrotoxicity and hepatotoxicity, where the effects are rather complex and not easily recognized by the rural populations [1].

Phyllanthus pentandrus Schum. & Thonn. commonly called Bird's millet, belongs to the family Euphorbiaceae commonly found in sandy localities, amongst stones and rocks on sandy banks of rivers and dams, and also in dry river beds and lake side dunes [2]. Young shoot of the plant is a medicine for diarrhoea and dysentery [3]. In Nigeria, a decoction taken internally is believed to relieve ear ache, and the water extract of the plant ashes is also instilled in the ear. It is also used in the treatment of bacterial diseases in the mouth and sore throat, and can be chewed directly or grinded and mixed with milk or pap especially in case of infants in Sokoto state [4] and is often added to cereal in grooming process (Gumba) by the Hausa tribe in northern Nigeria, the seeds are eaten by birds [3]. Extract obtained from the shoot of *P. pentandrus* exhibited antimicrobial properties and contain a substantial amounts of nutrient which further confirm its nutritional properties [5].

Previous studies have indicated that some chromatographic fractions obtained from *P. amarus* indicated a potentially deleterious effect on the blood biochemistry [6] and its aqueous extract induced some pathological changes in rat necrotic foci with lymphocytes infiltration in the liver, seminiferous tubules devoid of spermatic cells following 30 days

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treatment [7]. Similarly, extracts of *P. amarus* could be considered to be safe in animals by oral route (LD 50 > 5 g/kg) even though it is slightly cytotoxic to the human adenocarcinoma cell line Caco-2[8]. Despite the multiple roles of *P. pentandrus* in food and medicine used for the treatment of different ailment, little or no information on its toxicological profile has been documented. There is the need for toxicological evaluation of *P. pentandrus* on the functioning of the liver and kidney to ascertain its toxicological status considering its wider application in herbal medicine. This research aimed at evaluating the toxicity of *P. pentandrus* on the various liver and kidney function indices in Albino rats which could provide information on the safety or otherwise on its utilization.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh shoot of *Phyllanthus pentandrus* was collected on the campus of the Usmanu Danfodiyo University, Sokoto permanent site. The plant was identified and voucher specimen was deposited at the Herbarium of the University. The shoots were shade dried and pulverized into coarse powder. The powdered (800g) plant material was extracted using water at room temperature by percolation method. The filtrate was concentrated to dryness at 40^0C using rotatory evaporator under reduced pressure. The residue was reconstituted in sterile distilled water at different concentrations for oral administration to albino rats.

Experimental Animals

Albino rats (Wister strains) of either sex weighing (101-216g) were purchased from the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. The animals were kept in wire mesh cages at animal house of the same department, and divided in 5 groups of 5 rats each. They were maintained on rat pellets and clean tap water, before and after daily administration of the extract. The study was conducted with strict adherence to the ethical procedure of the Usmanu Danfodiyo University, Sokoto on the use of animals for experiment.

Acute Toxicity Studies (LD_{50} determination)

Aqueous extract of *P. pentandrus* (3000 mg/kg body weight) was administered to 5 groups of 1 rat each (one after the other) at a grace observation period of 48 hours in a single oral dose by using a feeding needle. The control group received distilled water only. Observation of toxic symptoms were made and recorded systematically after administration. The toxicological effect was assessed on the basis of mortality which was expressed as LD_{50}.

Sub Acute Toxicity Studies

A total of 25 albino rats were divided into 5 groups of 5 rats each. Animals in group 2, 3, 4, and 5 were orally administered 1ml of graded doses of plant extract (500, 1000, 2000 and 3000 mg/kg body weight) once daily for 28 days respectively. Animals in group 1 served as the control group and received distilled water. The parameters analyzed in the rats during pre-treatment and post-treatment phases include body weights of all the animals before and within 28 days (weekly) of administration.

Animals were sacrificed 24 hours after the last treatment, blood samples were collected, allowed to clot and centrifuged to obtain sera. Serum Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined using Randox assay kit, based on standard methods of Reitman and Frankel [9]. Alkaline phosphatase activity was estimated by the Randox kit (colorimetric) of Rec GSCC [10]. Total bilirubin was assayed by the method of Malloy and Evelyn as reported by Varley *et al.* [11]. Albumin (Bromocresol green) was by the method of Cheesbrough [12]. Total protein was determined by the biuret method as described by Gornall *et al.* [13]. Urea (Diacetyl monoxime) was by the method of Wybenga *et al.* [14] and uric acid by Collins and Diehl [15] and Morin and Prox [16]. Electrolytes and bicarbonate were estimated by the methods of Uriyo and Singh [17] and creatinine by the modified method of Jaffe [18].

Data Analysis

Results are presented as mean ± S.D. (Standard Deviation). Student's *t*-test was used for comparison between the untreated and treated groups. $P<0.05$ was considered to be statistically significant. Organ to body weight ratio was computed using the expression; Weight of organ/total weight of the animals x 100.

RESULTS AND DISCUSSION

Acute toxicity test of *P. pentandrus* at 3000 mg/kg produced no mortality after 48 hours of observation. The mean lethal dosage (LD_{50}) of the aqueous shoot extract of *P. pentandrus* was greater than the 3000 mg/kg body weight dose. Oral administration of low and moderate doses (500 to 2000 mg/kg) produced no obvious sign of toxicity. However, higher dose of 3000 mg/kg caused weakness, reduced reaction to noise, slow movement and resting at the corner of cages. Acute toxicity did not produce any negative behavioural changes such restlessness, excitement, coma or convulsion in rats administered various doses. There was no significant ($p > 0.05$) change in the weight of rats administered various doses of *P. pentandrus* shoot extract (Table 1). Liver function indices were not significantly affected at low doses (500 to 2000 mg/kg) except aspartate aminotransferase (AST) which was significantly different ($p < 0.05$) at 3000 mg/kg when compared with the control (Table 2).

Table 1: Weight of rats as affected by the oral administration of *P. pentandrus* extract.

Dose (mg/kg)	Weight/Weeks				
	Initial	1	2	3	4
Control	177.50 ± 0.06	180.07 ± 0.25	176.47 ± 4.73	190.35 ± 0.15	**202.66 ± 0.17**
500	101.20 ± 0.21	117.76 ± 0.47	124.26 ± 0.82	131.38 ± 0.36	**145.58 ± 0.24**
1000	117.14 ± 0.14	121.00 ± 0.49	129.50 ± 0.09	137.71 ± 0.26	**133.07 ± 0.08**
2000	152.82 ± 0.41	157.05 ± 0.57	151.48 ± 0.35	171.24 ± 0.17	**185.73 ± 0.15**
3000	**216.28 ± 0.25**	**202.85 ± 0.15**	**212.73 ± 0.20**	**218.56 ± 0.12**	**221.24 ± 0.24**

Values are means ± S.D. of three replicates.

Table 2: Effect of *P. pentandrus* shoot extracts on some liver function indices after four weeks of administration to rats.

Parameter	Extract (mg/kg body weight)				
	Control	500	1000	2000	3000
Total protein (g/l)	4.65 ± 0.30	4.66 ± 0.06	6.11 ± 0.02	6.53 ± 0.04	**7.08 ± 0.05**
Albumin (g/l)	2.02 ± 0.01	2.23 ± 0.01	2.36 ± 0.02	2.44 ± 0.02	**2.46 ± 0.01**
Aspartate transferase(U/l)	9.48 ± 0.13	10.70 ± 0.20	11.76 ± 0.30	13.33 ± 0.18	**4.67* ± 1.49**
Alanine transferase (U/l)	4.65 ± 0.03	4.66 ± 0.06	6.11 ± 0.02	6.52 ± 0.04	**7.08 ± 0.05**
Alkaline transferase (U/l)	85.37 ± 1.69	80.40 ± 1.94	89.57 ± 1.34	106.53 ±1.46	**127.88 ± 2.09**
Total bilirubin (mg/dl)	4.79 ± 0.13	4.76 ± 0.14	5.20 ± 0.02	5.97 ± 0.12	**13.29 ± 0.11**
Direct bilirubin (mg/dl)	**6.08 ± 0.12**	**11.05 ± 0.25**	**10.97 ± 0.13**	**19.00 ± 0.19**	**21.63 ± 0.19**

Values are means ± S.D. of three replicates. * =significant different from the control ($p<0.05$).

The concentrations of total proteins, bilirubin and albumin in the serum did not show any significant change compared with the control. The non significant ($p > 0.05$) change in liver function indices at low and moderate doses (500 to 2000 mg/kg) suggests that the shoot extract of *P. pentandrus* is non-toxic at these doses, but showed effect on the aspartate aminotransferase level at 3000 mg/kg body weight. The concentrations of total proteins, bilirubin and albumin in the serum may indicate the state of the liver and the type of damage. The fact that the total bilirubin, albumin and total protein levels were not altered by the extract suggest that the secretory function of the liver was not impaired. The increase in serum aspartate aminotransferase activity at 3000 mg/kg body weight may indicate cellular damage to the plasma membrane of the rats under study [19]. Bilirubin is formed by the breakdown of haemoglobin in the spleen, liver and bone marrow. In the liver, bilirubin is conjugated with glucuronic acid to form soluble compound. The conjugated bilirubin passes down the bile duct and is excreted into the gastrointestinal tract. An increase in bilirubin concentration in the serum is called jaundice which occurs in toxic or infectious liver diseases [20]. The non-significant change in the levels of total and direct bilirubin when compared with the control suggests that the aqueous extract of *P. pentandrus* has no obvious effect on the liver at all doses. Albumin is the most abundant serum protein representing 55-65% of the

total protein. It is synthesized in the liver and its main biological functions are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands such as fatty acids, calcium, bilirubin and thyroxine. Hypoalbuminaemia is associated with low albumin secretion, liver disease, malnutrition or malabsorption and also indicates dehydration. The absence of both in rats administered the extract of *P. pentandrus* indicate no significant (p> 0.05) effect on albumin levels when compared with control at all doses.

The concentration of Na, Mg, Urea and creatinine were significantly different at some doses when compared with the control (Table 3). Renal function tests are required either to demonstrate the presence or absence of active lesion in the kidney, or to assess the normal functioning capacity of different parts of the nephron [19].

Table 3: Effect of *P. pentandrus* shoot extracts on kidney function indices after four weeks of administration to rats.

Parameter	Extract (mg/kg body weight)				
	Control	500	1000	2000	**3000**
Creatinine (mg/dl)	45.57 ± 0.43	66.82 ± 0.77	82.15 ± 1.35	88.23 ± 11.19*	**117.85 ±1.55**
Urea (mg/dl)	103.41 ± 0.24	89.09 ± 0.18*	113.0 ± 0.49	113.91 ± 0.30	**100.06 ± 0.31**
Sodium (mmol/l)	2.86 ± 0.02	3.42 ± 0.02	3.36 ± 0.04	3.48 ± 0.03	**3.66 ± 0.02**
Potassium (mmol/l)	3.06 ± 0.02	4.13 ± 0.01	1.42 ± 0.02	1.15 ± 0.01	**1.59 ± 0.03**
Magnesium (mmol/l)	0.043 ± 0.00	0.04 ± 0.02	0.02 ± 0.00	0.04 ± 0.00	**0.05 ± 0.00***
Calcium (mmol/l)	0.05 ± 0.00	0.07 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	**0.06 ± 0.01***
Uric acid (µmol/l)	**2.84 ± 0.08**	**3.44 ± 0.06**	**5.19 ± 0.05**	**2.56 ± 0.09**	**9.89 ± 0.03**

Values are means ± S.D. of three replicates. * =significantly different from the control (p<0.05).

The significant (p> 0.05) effect observed in some renal function parameters indicates that the shoot extract of *P. pentandrus* may have toxic effect. Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. Due to their ability to dissociate readily into their constituent ions or radicals, they constitute the single most important factor in the transfer and movement of water and electrolytes between three divisions of the extracellular and intracellular compartments. The non significant (p> 0.05) effect observed in some of the electrolytes evaluated, suggests that the extract of *P. pentandrus* is non toxic. Table 4 shows a significant effect (p< 0.05) on the organ to body weight ratio of rats after four weeks of extract administration. The extract caused a decrease in the weight of the liver which is significantly different from the control.

Table 4: Effect of *P. pentandrus* shoot extracts on the organ to body weight ratio of rats after four weeks of administration.

Dose (mg/kg)	Weight (%)	
	Liver	**Kidney**
Control	4.61^{b}	**0.77^{b}**
500	3.80^{d}	**0.74^{b}**
1000	4.69^{a}	**1.02^{a}**
2000	4.31^{c}	**0.87^{b}**
3000	3.99^{d}	**0.69^{b}**
LSD (0.05)	**0.24**	**0.56**

Values represent mean of organ weight ratio of three replicates. Mean in a column with the same superscript are not significantly different at (P< 0.05).

From the results obtained in this study, it was observed that aqueous shoot extract of *P. pentandrus* is non toxic at low and moderate doses. The LD_{50} value is higher than 3000 mg/kg body weight and it could be used safely and effectively if taken in small and medium doses. Since, the liver and kidney indices are affected at high dose; the aqueous shoot extract of *P. pentandrus* could be used at lower concentrations to treat infectious diseases with a certain level of safety. The toxic effect observed may suggest that the normal functioning of the liver and kidney were affected. The histopathological changes and the mechanisms of toxicity of the extract at higher doses are being investigated.

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