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Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats

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ABSTRACT

Objective: To investigate the toxic implications of ethanolic stem bark extract of *Azadirachta indica* (*A. indica*) at 50, 100, 200 and 300 mg/kg body weight in Wistar rats. **Methods:** Fifty male rats of Wistar strains were randomly grouped into five (A-E) of ten animals each. Animals in Group A (control) were orally administered 1 mL of distilled water on daily basis for 21 days while those in Groups B-E received same volume of the extract corresponding to 50, 100, 200 and 300 mg/kg body weight. **Results:** The extract did not significantly ($P>0.05$) alter the levels of albumin, total protein, red blood cells and factors relating to it whereas the white blood cell, platelets, serum triacylglycerol and high-density lipoprotein cholesterol decreased significantly ($P<0.05$). In contrast, the final body weights, absolute weights of the liver, kidney, lungs and heart as well as their organ-body weight ratios, serum globulins, total and conjugated bilirubin, serum cholesterol, low-density lipoprotein cholesterol and computed atherogenic index increased significantly. The spleen-body weight ratio, alkaline phosphatase, alanine and aspartate transaminases, sodium, potassium, calcium, feed and water intake were altered at specific doses. **Conclusions:** Overall, the alterations in the biochemical parameters of toxicity have consequential effects on the normal functioning of the organs of the animals. Therefore, the ethanolic extract of *A. indica* stem bark at the doses of 50, 100, 200 and 300 mg/kg body weight may not be completely safe as an oral remedy and should be taken with caution if absolutely necessary.

1. Introduction

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used with considerable importance in international trade[1,2]. In certain African countries, however, up to 90% of the population still relies exclusively on plants as a source of medicines[3]. Therefore, the continuous evaluation of these botanicals for safety/toxicity using different animal models since the responses by these animals to chemical agents varies widely.

Azadirachta indica (A. Juss), (family, Meliaceae) (*A. indica*), is popularly known as Neem (English) or "dongoyaro" (Yoruba-Western Nigeria) in Nigeria. The plant is perhaps one of the most studied and widely used medicinal plants of all ages[4]. The species is presently being cultivated worldwide because of its ability to adapt to different climatic conditions. Biological and pharmacological activities attributed to solvent extracts and products like oil from the different parts of the *A. indica* are as diverse as antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, larvicidal, fungicidal, antiulcer, spermicidal, anthelminthic, antidiabetic, anti-implantation, immunomodulating, molluscicidal, nematicidal, immunocontraceptive, insecticidal and antifeedant[5-13]. Furthermore, the bark of neem tree is known to possess tannins, phenolic compounds (salicylic acid and gallocatechin) which have been reported to be the anti-inflammatory principles[4]. Toxicological investigation of *A. indica* leaf extracts at 0.6 - 2.0 g/kg body weight in did not pose any lethal effects on haematology, enzyme levels and histopathological parameters of experimental animals whereas the leaf extract at 200 g/kg body weight reduced the body weight of the animals and were accompanied by weakness, anorexia and histopathological defects[15]. The

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ethanolic root extract has also been reported to exhibit a dose dependent hepatotoxicity whereas the aqueous extract was not injurious to the liver[16].

Although, Mbaya *et al*[13] had reported on the dose related clinical signs of toxicity (anorexia, dehydration, malaise, respiratory depression, coma and death) and histopathological changes in the trachea, bronchi, broncioles, lungs and kidney of both sexes of Swiss (Wistar) adult albino rats intraperitoneally administered with the doses of 100, 200, 400, 800, 1600 and 3200 mg/kg body weight of the crude ethanolic extract of *A. indica* stem bark for a period of 24 h, information is scanty in the open scientific literature that addressed the effect of the ethanolic extract of *A. indica* stem bark in the same manner as it is claimed to be used in the management of several diseases in the folklore medicine of Nigeria. In this study, we have evaluated the toxic implications of the extract at the doses of 50, 100, 200 and 300 mg/kg body weight orally administered to male rats on daily basis for 21 days. The toxicity was evaluated using parameters different from that of Mbaya *et al*[13] such as haematological profile, body weight ratio of selected organs of the animals, liver and kidney function indices, lipid profile, feed and water intake.

2. Materials and methods

2.1. Plant materials

The plant was collected in June, 2010 within the premises of the Lagos State University, Ojo Campus, Lagos, Nigeria. It was authenticated by Prof. OA Oke of the Botany Department of the same University. A voucher specimen (AshMed-01/2010/LASUHB) was deposited in the Herbarium of the University.

2.2. Assay kits

The assay kits for cholesterol, triacyglycerols, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alkaline phosphatase (ALP), Gamma glutamyl transerase (GGT), aspartate and alanine aminotransferases (AST and ALT) were products of Randox Laboratory Ltd, Co. Antrim, United Kingdom while those of sodium, potassium, calcium, albumin, globulin and bilirubin were products of Teco Diagnostics, Lakeview Avenue, Ahaheim, USA. All other reagents used were of analytical grade and were supplied by Sigma-Aldrich Inc., St. Louis, USA.

2.3. Animals

Male albino rats of Wistar strains (174.40±12.30) were bred within the animal house of the Department of Biochemistry, Lagos State University, Ojo Campus, Nigeria. The animals were kept in a clean metallic cages placed in a well ventilated house with optimum condition [temperature: (28±2) ℃; photoperiod: 12 h natural light and 12 h dark; humidity: 40%-45%]. They were allowed free access to commercial pelleted rat chow (Lagos State Agro-Products, Agric Farm, Ojo, Lagos, Nigeria) and water *ad libitum*. The floors of the cages were filled with saw dusts while the cleaning was done on daily basis. The study was carried out following the approval from the Ethical Committee on the Use and Care of Animals of the Lagos State University, Lagos, Nigeria.

2.4. Preparation of extract

The stem bark was cut into pieces, oven-dried at 40 oC to a constant weight before being pulverized. The powdered stem bark (40 g) was extracted with ethanol for 72 h with constant shaking. This was filtered with Whatman filter paper No 1 and the brownish coloured filtrate was concentrated at 40 ℃ using a rotary evaporator (Laborota 4000-Efficient, Heidolph, Germany) to give a yield of 6.52 g which was stored in a refrigerator maintained at 4 ℃. This was later reconstituted to give the required doses of 50, 100, 200 and 300 mg/kg body weight used in the present study. Ethanol was used in the present study instead of aqueous or hydro-alcohol because it was the most frequently mentioned vehicle for this particular plant during our ethnobotanical survey.

2.5. Animal grouping and extract administration

Fifty male rats of Wistar strains were randomly grouped into five (A-E) of ten animals each. Group A (control) were orally administered with 1 ml of distilled water on daily basis for 21 days. Animals in Groups B-E were treated like those of the control except they received same volume containing 50, 100, 200 and 300 mg/kg body weight of *A. indica* stem bark extract.

2.6. Determination of feed and water intake

The amount of feed and water intake were determined on daily basis. Briefly, the weight of daily feed supply and the left-over by the following day were recorded and the difference was taken as the daily feed intake. Similar procedure was adopted for the determination of the volume of water consumed. The average of the feed and water intake was computed for every three days of the experimental period.

2.7. Preparation of serum and isolation of organs

After 21 days of extract administration, the animals were humanely sacrificed by ether anaesthetization. The neck area was quickly cleared of fur to expose the jugular vein. The veins after being slightly displaced were sharply cut with sterile surgical blade and an aliquot of the blood

was collected into sample bottles containing EDTA for the haematological analysis. The remainder (5 mL) was collected into centrifuge tubes and spinned at 1 282 g × 5 min. The serum was carefully aspirated with Pasteur pipette into sample bottles for the various biochemical assays. The rats were further dissected and the liver, kidney, heart, lungs and spleen excised, freed of fat, blotted with clean tissue paper and then weighed. The organ-to-body weight ratios were determined according to the expression described by Yakubu *et al*[17].

2.8. Determination of biochemical parameters

The levels of cholesterol, triacyglycerol, HDL-C and LDL-C were determined in the serum of the animals using standard procedures[18-21]. The Automated Haematologic Analyzer (Sysmex, KX-21, Japan) was used to analyse the haematological parameters of red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell width coefficient of variation (RDW-CV), white blood cell (WBC), mean platelet volume (MPV), platelet distribution width (PDW) and platelets. The levels of other parameters were determined as described for bilirubin (total and conjugated)[22], total protein[23], albumin[24], globulin, sodium, calcium and potassium[25], whereas the activities of ALT and AST, GGT and ALP were determined using standard methods[26-28].

2.9. Statistical analysis

Data were expressed as means of ten replicates±SD and were subjected to one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test. Statistical significance was considered at $P<0.05$.

3. Results

The ethanolic extract of *A. indica* stem bark at all the doses investigated in the present study did not significantly ($P>0.05$) alter the red blood cells (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell width coefficient of variation (RDW-CV), red cell width

Table 1
Effect of administration of ethanolic extract of *A. indica* stem bark on some haematological parameters of male Wistar rats ($n=10$).

Parameters	Extract (mg/kg body weight)				
	Control	50	100	200	300
White blood cell ($\times 10^9$/L)	30.40±13.07^a	11.45±2.28^b	6.35±1.79^c	9.60±2.69^d	9.03±0.92^d
Red blood cell ($\times 10^{12}$/L)	6.99±0.41^a	6.85±0.41^a	6.98±0.37^a	7.66±0.28^a	6.71±0.82^a
Packed cell volume	37.80±1.47^a	37.35±1.48^a	37.38±1.87^a	37.90±1.33^b	36.76±2.27^a
Haemoglobin (g/dL)	12.40±0.74^a	12.73±0.59^a	12.48±1.07^a	12.30±0.73^a	12.26±1.44^a
Mean corpuscular volume (fL)	54.20±1.65^a	54.60±1.78^a	55.53±0.36^a	54.68±1.27^a	54.77±1.81^a
Mean corpuscular haemoglobin (pg)	17.70±0.51^a	17.53±0.74^a	17.80±0.57^a	17.30±0.34^a	17.33±0.51^a
Mean corpuscular haemoglobin concentration (g/dL)	32.75±0.19^a	32.30±0.80^a	32.60±0.25^a	32.63±0.39^a	31.73±0.75^a
Red cell width coefficient of variation	14.90±0.54^a	15.18±0.29^a	15.35±0.21^a	15.40±0.35^a	15.60±0.17^a
Red cell width standard deviation	28.88±0.94^a	31.05±1.29^a	31.63±0.72^a	30.00±0.00^a	30.47±0.40^a
Platelets	858.80±80.52^a	673.00±97.68^b	427.25±36.79^c	641.25±99.50^d	622.67±85.16^e
Mean platelet volume	9.30±0.52^a	9.70±0.34^a	10.00±0.19^a	9.53±0.90^a	9.60±0.36^a
Platelet distribution width	16.08±0.39^a	16.15±0.21^a	16.20±0.18^a	16.13± 0.33^a	16.13±0.06^a

Mean±SD values carrying different superscripts from the control for each parameter are significantly different ($P<0.05$).

Table 2
Effect of administration of ethanolic extract of *A. indica* stem bark on the weights of some organs of male Wistar rats ($n=10$).

Parameters	Extract (mg/kg body weight)				
	Control	50	100	200	300
Initial body weight (g)	185.00±8.93^a	188.00±5.26^a	184.00±7.11^a	187.00±4.44^a	184.00±7.74^a
Final body weight (g)	205.00±10.00^b	218.00±9.15^b	214.00±7.54^b	207.00±8.09^b	210.00±9.00^b
Weight of liver (g)	5.56±0.58^a	7.05±0.93^b	8.86±0.55^c	7.27±0.28^d	8.58±0.76^c
Weight of kidney (g)	0.80±0.05^a	1.03±0.07^b	1.09±0.09^b	1.07±0.20^b	0.98±0.07^b
Weight of lungs (g)	1.16±0.05^a	1.59±0.11^b	1.95±0.81^c	2.03±0.28^c	1.49±0.66^b
Weight of spleen (g)	0.56±0.03^a	0.57±0.02^a	0.54±0.06^a	0.27±0.07^b	0.71±0.07^c
Weight of heart (g)	0.51±0.01^a	0.77±0.04^b	0.72±0.04^b	0.74±0.09^b	0.73±0.03^b
Liver-body weight (%)	2.71±0.02^a	3.23±0.05^b	4.14±0.03^c	3.51±0.01^d	4.08±0.05^c
Kidney-body weight (%)	0.39±0.01^a	0.47±0.08^b	0.51±0.02^b	0.52±0.01^b	0.47±0.03^b
Lung-body weight (%)	0.57±0.03^a	0.73±0.02^b	0.91±0.01^c	0.98±0.02^c	0.71±0.07^b
Spleen-body weight (%)	0.27±0.02^a	0.26±0.04^a	0.25±0.05^a	0.13±0.01^b	0.34±0.01^c
Heart-body weight (%)	0.25±0.01^a	0.35±0.01^b	0.34±0.03^b	0.36±0.02^b	0.35±0.01^b

Mean±SD values carrying different superscripts from the control for each parameter are significantly different ($P<0.05$).

Table 3
Liver and kidney function parameters of male Wistar rats administered with ethanolic extract of *A. indica* stem bark (n=10).

Parameters	Extract (mg/kg body weight)				
	Control	50	100	200	300
Total bilirubin (μmol/L)	1.47±0.05[a]	1.80±0.08[b]	2.64±0.03[c]	2.11±0.04[d]	2.96±0.06[c]
Conjugated bilirubin (μmol/L)	0.17±0.06[a]	0.22±0.03[b]	0.57±0.05[c]	0.41±0.04[d]	0.44±0.03[d]
Total protein (g/L)	8.85±0.02[a]	8.65±0.01[a]	8.85±0.06[b]	8.30±0.05[a]	8.73±0.04[a]
Albumin (g/L)	4.33±0.06[a]	3.82±0.03[a]	4.02±0.03 [a]	3.85±0.01[a]	4.04±0.02[a]
Globulin (g/L)	4.52±0.03[a]	6.83±0.06[b]	6.84±0.05[b]	6.46±0.04[b]	6.66±0.02[b]
Serum alkaline phosphatase (U/L)	58.28±0.04[a]	56.33±0.03[a]	99.23±0.02[b]	83.23±0.02[c]	103.47±0.04[d]
Serum gamma glutamyl transferase (U/L)	45.6±0.06[a]	23.75±0.03[b]	20.53±0.06[c]	42.05±0.06[d]	53.87±0.04[e]
Serum aspartate transaminase (U/L)	44.73±0.05[a]	41.25±0.02[a]	30.75±0.02[b]	25.75±0.03[c]	29.67±0.01[b]
Serum alanine trnasmainase (U/L)	17.00±0.01[a]	19.00±0.08[a]	12.25±0.04[b]	11.50±0.01[b]	11.67±0.03[b]
Sodium (mmol/L)	135.70±0.03[a]	132.50±0.04[a]	131.20±0.05[b]	143.00±0.06[c]	132.67±0.02[d]
Potassium (mmol/L)	2.03±0.07[a]	2.09±0.06[a]	3.70±0.03[b]	3.80±0.02[b]	3.63±0.03[b]
Calcium (mmol/L)	7.23±0.02[a]	7.73±0.01[a]	10.00±0.05[b]	12.93±0.03[c]	14.93±0.04[d]

Mean±SD values carrying superscripts different from the control for each parameter are significantly different (P<0.05).

Table 4
Serum lipid profile of male Wistar rats administered with ethanolic extract of *A. indica* stem bark (n=10).

Parameters	Extract (mg/kg body weight)				
	Control	50	100	200	300
Cholesterol (mmol/L)	311.25±0.03[a]	352.25±0.02[b]	335.25±0.01[c]	362.75±0.02[d]	374.00±0.05[e]
Triacylglycerol (mmol/L)	407.75±0.12[a]	235.50±0.40[b]	302.25±0.21[c]	257.25±0.11[b]	144.67±0.23[d]
High density lipoprotein-cholesterol (mmol/L)	157.25±0.02[a]	117.53±0.03[b]	126.43±0.07[c]	111.88±0.13[d]	138.07±0.09[e]
Low density lipoprotein-cholesterol (mmol/L)	90.15±0.06[a]	129.23±0.04[b]	151.28±0.05[c]	198.28±0.03[d]	147.10±0.02[e]
Atherogenic index (LDL-C/HDL-C)	0.57±0.03[a]	1.10±0.01[a]	1.19±0.03[b]	0.88±0.02[c]	1.06±0.01[b]

Mean±SD values carrying superscripts different from the control for each parameter are significantly different (P<0.05).

Table 5
Feed intake (g) of male Wistar rats administered with ethanolic extract of *A. indica* stem bark (n=10).

Animal grouping	Days						
	1-3	4-6	7-9	10-12	13-15	16-18	19-21
Control	44.59±2.75[a]	44.76±3.82[a]	44.24±3.29[a]	44.90±1.82[a]	45.03±1.47[a]	45.54±2.05[a]	44.48±3.89[a]
50 mg/kg body weight	48.18±3.71[b]	52.78±2.15[b]	51.34±3.35[b]	48.26±1.72[b]	48.27±1.09[b]	56.89±2.37[b]	57.42±1.59[b]
100 mg/kg body weight	47.98±3.98[b]	58.25±3.13[c]	47.45±2.33[c]	52.69±3.99[c]	52.20±1.69[c]	52.59±1.68[c]	59.48±3.97[b]
200 mg/kg body weight	48.21±3.02[b]	56.51±2.96[c]	51.13±2.94[b]	49.61±1.28[b]	57.36±2.52[d]	55.21±1.24[d]	58.78±1.31[b]
300 mg/kg body weight	29.82±4.09[c]	33.62±1.42[d]	30.10±1.15[d]	26.23±2.89[d]	33.23±1.54[e]	31.57±2.32[e]	34.59±2.75[c]

Mean±SD values carrying superscripts different from the control for each day interval are significantly different (P<0.05).

Table 6
Water intake (mL) of male Wistar rats administered with ethanolic extract of *A. indica* stem bark (n=10).

Animal grouping	Days						
	1-3	4-6	7-9	10-12	13-15	16-18	19-21
Control	48.85±3.88[a]	49.09±1.89[a]	47.15±1.35[a]	46.37±3.30[a]	46.75±5.88[a]	47.48±1.89[a]	48.48±1.89[a]
50 mg/kg body weight	75.69±2.16[b]	50.66±4.18[b]	56.75±4.92[b]	53.67±2.04[b]	52.95±0.99[b]	57.42±1.51[b]	55.39±1.54[b]
100 mg/kg body weight	87.36±1.89[c]	55.17±2.82[b]	71.22±4.41[c]	67.19±3.60[c]	77.07±4.87[c]	60.56±2.55[b]	61.73±2.38[c]
200 mg/kg body weight	80.32±8.18[c]	54.03±1.01[b]	73.04±4.22[c]	80.42±3.29[d]	96.08±4.57[d]	87.69±3.86[c]	90.20±5.10[d]
300 mg/kg body weight	30.40±1.17[d]	32.03±3.21[c]	34.11±1.17[d]	34.88±1.27[e]	35.80±1.81[e]	34.57±0.97[d]	34.62±1.90[e]

Mean±SD values carrying superscripts different from the control for each day interval are significantly different (P<0.05).

standard deviation (RDW-SD), mean platelet volume (MPV) and platelet distribution width (PDW) (Table 1). In contrast however, the white blood cell (WBC) and platelets decreased significantly (P<0.05) following the administration of the extract (Table 1).

Compared with their respective initial body weights of the animals, their final body weights increased (P<0.05) throughout the exposure period. The extract also increased the absolute weight of the liver, kidney, lungs and heart of the animals (Table 2). The 50 and 100 mg/kg body weight of the extract did not significantly alter the absolute weight of the spleen whereas the 200 mg/kg body weight decreased it. In contrast, the highest dose (300 mg/kg body weight) increased the weight of the pancreas. Similarly, all the doses of the extract increased the liver-, kidney-, lung- and heart-body weight ratios. The spleen body weight ratio was not significantly different from the control at 50 and 100 mg/kg body weight. In addition, the 200 mg/kg body weight of the extract decreased the spleen-body weight ratio whereas the 300 mg/kg body weight increased it (Table 2).

The ethanolic extract of *A. indica* stem bark significantly increased (P<0.05) the serum globulin as well as total and

conjugated bilirubin (Table 3). In contrast, the levels of albumin and total protein were not significantly altered in the serum of the animals. Apart from the 50 mg/kg body weight which produced values of alkaline phosphatase, alanine transaminase, aspartate transaminase, sodium, potassium and calcium ions that compared well with the control, other dose levels significantly altered the levels of the liver and kidney function parameters. Specifically, the higher doses increased the levels of alkaline phosphatase, potassium and calcium. The levels of aspartate and alanine transaminases and sodium decreased significantly at the doses of 100, 200 and 300 mg/kg body weight. The serum gamma glutamyl transferase activity decreased at all the doses of the extract (Table 3).

The ethanolic extract of *A. indica* stem bark significantly decreased the serum concentrations of triacylglycerol and HDL-C. In contrast, all the doses of the extract increased the serum concentrations of cholesterol, LDL-C and the computed atherogenic index (Table 4).

Compared with the distilled water treated control, the feed consumed by the animals administered with 50, 100 and 200 mg/kg body weight increased significantly throughout the experimental period (Table 5). In contrast however, administration of the extract at the dose of 300 mg/kg body weight reduced the amount of feed consumed right from the beginning of the experiment and was sustained throughout the remaining period of the exposure to the extract (Table 5).

The extract at 50, 100 and 200 mg/kg body weight significantly increased the water intake of the animals (Table 6). The increase by the 200 mg/kg body weight between the 7-21 d was higher than in the 50 and 100 mg/kg body weight treated animals. Furthermore, the 300 mg/kg body weight of the extract significantly reduced the volume of water consumed by the animals (Table 6).

4. Discussion

Administration of herbal preparations without any standard dosage coupled with non- availability of adequate scientific studies on their safety has raised concerns on their toxicity[29]. Toxicity studies in animals are commonly used to assess potential health risk in humans caused by intrinsic adverse effects of chemical compounds/plant extracts[30-32]. These adverse effects may manifest significant alterations in the levels of biomolecules such as enzymes and metabolic products, normal functioning and histomorphology of the organs[33].

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Such toxicity testing is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when data are translated from animal studies[34]. It can also be used to explain blood relating functions of chemical compounds/plant extract[35]. The reductions in only the WBC and platelets out of all the haematological parameters investigated in the present study could imply selective systemic toxicity effect by the extract. Therefore, the reduction in the WBC at all the doses of the extract investigated could possibly imply that the rate of entrance of the blood parameter from the bone marrow did not commensurate with the rate of its removal from the circulation or may also be due to underproduction of haematopoietic regulatory elements by the stroma cells and macrophages in the bone marrow at those doses[36]. The present study has revealed that it was only the platelet count that was affected whereas there was no effect on the volume and distribution width of the platelets. Furthermore, the reduction in platelets may hamper the process of blood clotting. Hb, RBC and PCV are associated with total population of red blood cells while MCV, MCH, MCHC, RDW-CV, RDW-SD relates to individual red blood cells. Lack of an effect on these parameters indicates that both the individual and total population of the red blood cells was not affected.

Organ body weight ratio is a useful index of swelling, atrophy or hypertrophy[37]. The increases in the absolute and the computed organ-body weight ratios for liver, kidney, lungs and heart by the ethanolic extract of *A. indica* stem bark may suggest hypertrophy while the reduction in same parameters for the spleen could be an indication of atrophy. These speculations will await histopathological examinations.

Enzymes such as phosphatases, dehydrogenases and transferases are often found in appreciable quantities in the serum but are not of the extracellular fluid origin. These occur as results of tissue damage or disrupted cell membranes that lead to the leakage of such enzymes from the tissue and become elevated in the serum[38]. Therefore, serum enzyme measurement provides a valuable tool in clinical diagnosis because it provides information on the effect and nature of pathological damage to the tissues[39]. The reduction in the activities of serum ALP, AST, ALT and GGT at varying doses could suggest inhibition or inactivation of the enzyme molecules[40]. Similarly, the elevated levels of ALP could constitute threat to the cells since the cells might be deprived of the much needed energy as a result of indiscriminate hydrolysis of the phosphate ester. These alterations will have their consequential effects on the normal functioning of the enzymes in the animals. The elevation in the levels of K, PO4 and Ca at 300 mg/kg body weight of the extract as well as decrease in the levels of Na at 100, 200 and 300 mg/kg body weight of the extract suggest interference in the normal homeostasis of these ions. However, the lack of an effect on these ions at the 50 mg/kg body weight suggests that the dose may be relatively safe for consumption on daily basis for 21 d.

Evaluation of serum proteins such as albumin and globulin are good criteria for assessing the secretory ability/functional capacity of the liver[41]. The non significant effect of the extract on the albumin and total protein in the serum of animals at all the doses investigated could imply that the synthetic and secretory functions of the liver with respect to these proteins were not affected. Furthermore, the increase in serum total and conjugated bilirubin could indicate

mild haemolysis and obstruction in the normal excretion of bile[41-48]. The elevated level of globulin suggests increase in functional activity of the liver with respect to the plasma protein.

Changes in the levels of major lipids such as LDL-C, HDL-C, cholesterol and triacylglycerol could provide useful information on the predisposition of the heart of animals to atherosclerosis and its associated coronary heart disease[49]. The significant reduction in triacylglycerol may be associated with impaired lipolysis while reduction in HDL-C at all doses investigated may not be clinically beneficial to the animals since the rate at which plasma cholesterol are carried to the liver will be also decreased. Furthermore, the enhanced level of cholesterol and LDL-C may suggest cardiovascular risk in the animals. This is supported in the present study by the increase in the computed atherogenic index, a useful indicator of cardiovascular diseases[50].

Water is an essential nutrient to every life since it is the most important nutrient for growth and development [51]. Any factor influencing water intake will also affect feed consumption[52]. Therefore, it is not surprising the existence of same pattern of feed and water intake in the present study. Thus, the increase in water intake by the animals administered with 50, 100 and 200 mg/kg body weight of the extract implied that water was readily consumed by the animals. Similarly, the increased feed consumption by these animals, may also suggests that the doses of the extract enhanced the sense of taste and appetite of the animals after their consumption[53]. In contrast, the decreased water and feed intake by animals treated with 300 mg/kg body weight probably decreased the appetite of the animals and will have consequential effects on their performances.

In conclusion, the alterations in biochemical parameters by the ethanolic extract of *A. indica* stem bark are indications of adverse effects on the various organs of the animals. These will have consequential effects on the normal functioning of these organs. The ethanolic extract of *A. indica* stem barks may not be safe as an oral remedy most especially at 100, 200 and 300 mg/kg body weight. The dose of 50 mg/kg body weight appeared to be relatively safe.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Ahmad I, Agil F, Owais M. Modern phytomedicine: turning medicinal plants into drugs. West-Sussex England: John Wiley and Sons; 2006, p. 2-24.

[2] Ebong PK, Atangwho IJ, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (*A. Juss*) (Neem) and Vernonia amygdalina (Del.) (African bitter leaf). *Am J Biochem Biotechnol* 2008; **4**: 239-244.

[3] Isaac AB, George IN, Oladimeji TA, James DH. A bioactive flavonoid from Pavetta crassipes K. Schum. *Org Med Chem Lett* 2011; **1**: 14.

[4] Nwachukwu N, Iweala EJ. Influence of extraction methods on the hepatotoxicity of *Azadirachta indica* bark extract on albino rats. *Global J Pure Appl Sci* 2009; **15**: 369-372.

[5] Devmurari VP, Jivani NP. Hepatoprotective activity of methanolic and aqueous extracts of Azadirchata Indica leaves. *Int J Pharm Tech Res* 2010; **2**: 1037-1040.

[6] Mahabub-Uz-Zaman M, Ahmed NU, Akter R, Ahmed K, Aziz MSI, Ahmed MS. Studies on anti-inflammatory, antinociceptive and antipyretic activities of ethanol extract of *Azadirachta indica* leaves. *Bangladesh J Sci Ind Res* 2009; **44**: 199-206.

[7] Prakash AO, Tiwari RK, Mathur R. Non-hormonal postcoital contraceptive action of neem oil in rats. *J Ethnopharmacol* 1988; **23**: 53-59.

[8] Patil P, Gaikwad RD, Sawane MV, Waghmare VS. Effect of neem oil on sperm mitochondrial activity. *Online J Health Allied Sci* 2009; **8**: 12.

[9] Dehghan MH, Daryani A, Robabeh D. Histological evidenceof male potent reproductive sites by Iranian botanical *Azadirachta indica* (Neem) seed extract. *Int J Mol Med Adv Sci* 2006; **2**: 7-15.

[10] Sithisarn P, Roongtawan S, Gritsanapan W. Antioxidant activity of Siamese neem tree (VP1209). *J Ethnopharmacol* 2005; **99**: 109-112.

[11] Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC. Protective role of extracts of neem seeds in diabetes caused by steptozotocin in rats. *J Ethnopharmacol* 2004; **90**: 185-189.

[12] Vinothini G, Manikandan P, Anandan R, Nagini S. Chemoprevention of rat mammary carcinogenesis by *Azadirachta indica* leaf fractions: Modulation of hormonestatus, xenobiotic-metabilizing enzymes, oxidative stress, cell proliferation and apoptosis. *Food Chem Toxicol* 2009; **47**: 1852-1863.

[13] Mbaya AW, Ibrahim UI, Thank God O, Ladi S. Toxicity and potential anti-trypanosomal activity of ethanolic extract of *Azadirachta indica* (Maliacae) stem bark: An in vivo and in vitro approach using Trypanosoma brucei. *J Ethnophamarcol* 2010; **128**: 495-500.

[14] Lewis KC. Uses of medicinal plants. *Am J Trop Med* 2001; **140**: 261-268.

[15] Ghimeray AK, Jin C, Ghimire BK, Cho DH. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta Indica A. Juss* grown in foothills of Nepal. *Afri J Biol* 2009; **8**: 3084-3091.

[16] Nwachukwu N, Igwenyi I. Influence of extraction methods on the hepatotoxicity of *A. indica* root. *J Res Biosci* 2006; **2**: 10-23.

[17] Yakubu MT, Akanji MA, Oladiji AT, Adesokan AA. Androgenic potentials of aqueous extract of Massularia acuminata (G. Don) Bullock ex Hoyl. Stem in male Wistar rats. *J Ethnopharmacol* 2008; **118**: 508-513.

[18] Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins-An integrated approach to mechanisms and disorders. *Nutri Rev* 2009; **45**: 271-273.

[19] Faizal P, S Suresh S, Kumar RS, Augusti KT. A study on the hypoglycemic and hypolipidemic effects of an Ayurvedic drug Rajanyamalakadi in diabetic patients. *Indian J Clin Biochem* 2009; **24**: 82-87.

[20] Albers JJ, Warnick GR, Chenng MC. Quantitation of high density lipoproteins. *Lipids* 1978; **13**: 926-932.

[21] Agrawal M, Spencer HJ, Faas FH. Method of LDL cholesterol measurement influences classification of LDL cholesterol treatment goals: clinical research study. *J Invest Med* 2010; **58**: 945-949.

[22] Ekanem JT, Yusuf OK. Some liver function indices and blood parameters in T. brucei-infected rats treated with honey. *Biokemistri* 2007; **19**: 81-86

[23] Gornal AC, Bardawill CJ, David MM. Determination of serum protein by means of biuret reaction. *J Biol Chem* 1949; **177**: 751-766.

[24] Doumas BT, Watson WA, Biggs HG. Albumin standards and measurement of serum-albumin with bromocresol green. *Clin Chim Acta* 1971; **31**: 87-96.

[25] Tietz NW. Clinical guide to laboratory tests. 3rd edn. Philadelphia: W.B. Saunders; 1995.

[26] Yakubu MT, Adebayo OJ, Egwim EC, Owoyele VB. Increased liver alkaline phosphatase and aminotransferase activities following administration of ethanolic extract of Khaya senegalensis stem bark to rats. *Biokemistri* 2005; **17**: 27-32.

[27] Solomon BA, Adebayo AO, Olugbenga BE. Effect of Cassava based diet on hepatic proteins in albino rats fed with crude oil contaminated diet. *J Appl Sci Environ Manage* 2011; **15**: 223-229.

[28] Wright PJ, Leathwood PD, Plummer DT. Enzymes in rat urine. Alkaline phosphatase. *Enzymologia* 1972; **42**: 317-327.

[29] Saad B, Azaizeh H, Abu-Hijleh G, Said S. Safety of traditional Arab herbal medicine. *Evid Compl Altern Med* 2006; **3**: 433-439.

[30] Klaassen CD, Eaton DL. Principles of toxicology. In: Klaassen CD. Ed. Casarett and Doull's toxicology: the basic science of poison. New York: Pergamon Press; 1991, p. 32.

[31] Afolayan AJ, Yakubu MT. Effect of Bulbine natalensis Baker stem extract on the functional indices and histology of the liver and kidney of male Wistar rats. *J Med Food* 2009; **12**: 814-820.

[32] Oyedemi SO, Yakubu MT, Afolayan AJ. Effect of aqueous extract of Leonotis leonorus (L.) R. Br. Leaves in male Wistar rats. *Hum Exp Toxicol* 2010; **29**: 377-384.

[33] Yakubu MT, Bukoye BB, Oladiji AT, Akanji MA. Toxicological implications of aqueous extract of Bambusa vulgaris leaves in pregnant Dutch rabbits. *Hum Exp Toxicol* 2009; **28**: 591-598.

[34] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of toxicity of pharmaceuticals in humans and in animals. *Reg Toxicol Pharmacol* 2000; **32**: 56-67.

[35] Yakubu MT, Akanji MA, Oladiji AT. Haematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogia argrestis stem. *Pharmacog Mag* 2007; **3**: 34-38.

[36] Adebayo JO, Adesokan AA, Olatunji LA, Buoro DO, Soladoye AO. Effect of ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats. *Biokemistri* 2005; **17**: 45-50.

[37] Amresh GR, Singh PN, Rao CV. Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. *J Ethnopharmacol* 2008; **116**: 454-460.

[38] Morrone FB, Spiller F, Edelweiss MIA, Meurer L, Engroff P, Barrios CH, et al. Effect of temozolomide treatment on the adenine nucleotide hydrolysis in blood serum of rats with implanted gliomas. *Appl Cancer Res* 2009; **29**: 118-124.

[39] Ashafa AOT, Sunmonu TO, Afolayan AJ. Toxicological evaluation of aqueous leafand berry extracts of Phytolacca dioica L. in male Wistar rats. *Food Chem Toxicol* 2010; **48**: 1886-1889.

[40] Akanji MA, Nafiu MO, Yakubu MT. Enzyme activities and histopathology of selected tissues in rats treated with potassium bromate. *Afr J Biomed Res* 2008; **11**: 87-95.

[41] Naganna B. Plasma proteins. In: Tawlar GP, Srivastava LM, Moudgils KD. (Eds). Textbook of Biochemistry and Human Biology. 2nd end. India: Prentice-Hall of India Private Ltd; 1989, p. 172.

[42] TC Okoye, PA Akah, AC Ezike, MO Okoye, CA Onyeto, F Ndukwu, et al. Evaluation of the acute and sub acute toxicity of *Annona senegalensis* root bark extracts. *Asian Pac J Trop Med* 2012; **5**(4): 277-282.

[43] Rathee P, Rathee D, Rathee D, Rathee S. *In-vitro* cytotoxic activity of β-Sitosterol triacontenate isolated from *Capparis decidua* (Forsk.) Edgew. *Asian Pac J Trop Med* 2012; **5**(3): 225-230.

[44] Vital PG, Rivera WL. Antimicrobial activity, cytotoxicity, and phytochemical screening of *Voacanga globosa* (Blanco) Merr. leaf extract (Apocynaceae). *Asian Pac J Trop Med* 2011; **4**(10): 824-828.

[45] Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. *Asian Pac J Trop Med* 2010; **3**(10): 769-774.

[46] Hussain T, Fareed S, Siddiqui HH, Vijaykumar M, Rao CV. Acute and subacute oral toxicity evaluation of *Tephrosia purpurea* extract in rodents. *Asian Pac J Trop Dis* 2012; **2**(2): 129-132.

[47] Kumbhare MR, Guleha V, Sivakumar T. Estimation of total phenolic content, cytotoxicity and *in-vitro* antioxidant activity of stem bark of *Moringa oleifera*. *Asian Pac J Trop Dis* 2012; **2**(2): 144-150.

[48] Chairman K, Ranjit Singh AJA, Alagumuthu G. Cytotoxic and antioxidant activity of selected marine sponges. *Asian Pac J Trop Dis* 2012; **2**(2): 234-238.

[49] Yakubu MT, Akanji MA, Oladiji AT. Alterations in serum lipid profile of male rats by oral administration of aqueous extract of Fadogia agrestis stem. *Res J Med Plant* 2008; **2**: 66-73.

[50] Panagiotakos B, Pitsavos C, Skoumas J, Chrysohoou C, Toutouza M, Stefanadis CI, et al. Importance of LDL/HDL ratio as a predicator for coronary heart disease events in patients with heterozygous familial hypercholesterolemia: A 15 year follow-up (1987-2002). *Curr Med Res Opin* 2003; **19**: 89-94.

[51] Counotte G. Avicultural professional: conocer la calidad del aqua de bebida. Doetinchem: Reed Business Information; 2003, p. 20-22.

[52] Macari M. Metabolismo hídrico da poedeira comercial. In: V Simpósio Técnico deProdução de Ovos. *Jaboticabal Resumos. Jaboticabal:* APA; 1995, p. 109-131.

[53] Marai IFM, Askar AA, Bahgat LB. Tolerance of New Zealand white and Californian doe rabbits at first parity to the sub-tropical environment of Egypt. *Livestock Sci* 2006; **104:** 165-172.