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Acute and Sub-Chronic Toxicities and Antimicrobial Profiling of Hydro-Ethanol Extracts of *Moringa oleifera* (L) Seed in Swiss albino mice and Wistar rats

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ABSTRACT

Seeds and nuts have been employed since prehistoric era for their therapeutics and health benefits. *Moringa oleifera* (L.) is one of such seeds with poly-therapeutic activities and is consumed extensively in Nigeria for various health reasons. This study was carried out to evaluate the anti-microbial potency, acute and sub-chronic toxicities of *Moringa oleifera* seed extracts in rodents. The antibacterial activity of the hydro-alcohol extract of *Moringa oleifera* seeds were evaluated using standard methods. The acute toxicity of the extract was evaluated in Swiss albino mice by feeding the mice with the graded oral doses of the extract between 1.0 to 20.0 g/kg body weight while the graded oral doses of the extracts were administered to Wistar rats in sub-chronic toxicity evaluations. The extract revealed remarkable antimicrobial activities. In the acute toxicity, the animals fed with 15.0 and 20.0 g/kg body weight did not survive beyond 24 hours. The LD_{50} was 14.0 g/kg bodyweight. There was significant increase ($p < 0.05$) in the body weight, the serum electrolytes and the MCH values while a significant decrease in the MCV value was observed. The serum liver enzymes showed significant decrease while increase in the serum protein metabolites was observed. The high doses of the extract had some deleterious effects on the liver, kidney and testes.
Although the extracts showed a good safety margin with high LD_{50} value (14.0 g/kg), cautions should be exercised in the consumption since higher doses or prolonged consumption may exert deleterious effects on some organs.

Keywords: Acute toxicity, sub-chronic toxicity, lyophilize, *Moringa oleifera.*

Introduction

In developing countries, the major source of diseases is the poor quality of accessible drinking water, contaminated food, poor standard of personal hygiene and lack of appropriate sanitation.[1] The use of plant and plant extracts as therapeutic weapons against various human, animal and even plant diseases have been recognized since prehistoric era.[2] The discovery that such compounds could act as potential therapeutic weapons against various diseases, in addition to their food and nutritional values, has made plants invaluable and indispensable to human and animal lives.[3] Plants occupy a very important place in modern medicine as they are used as either raw materials for drugs or as a template for discovery and synthesis of drugs.[4] Herbal medicine either as an extract, pure compound, or as a derivative, offers unlimited opportunities for the discovery of new drugs. There is also growing disillusion with modern medicine coupled with the misconception that herbal products being natural may be devoid of adverse and toxic effects associated with convectional and allopathic medicines. Antimicrobials are being used as the mainstay for the treatment of infectious diseases worldwide.[5] Regardless of this fact, the problem of antimicrobial resistance has triggered interest in research for newer antimicrobial compounds of natural origin and less toxic to man.[6] According to the World Health Organization[5], medicinal plants can provide the best alternative source for obtaining a variety of drugs, since they possess a variety of bioactive principles known as phytochemicals which make them potential sources of antimicrobial agents.[7] Nevertheless, herbal preparations assumed to be safe could be contaminated with microbial and foreign materials such as heavy metals, pesticide residues or even aflatoxins due to the unhygienic way many are produced.[3] The renewed interest in the use of medicinal plants may be attributed to low cost, availability and accessibility by the local populace, high incidence of side effects of synthetic medicines and environmental friendliness of plant extracts. Apart from the problem of resistance, environmental degradation, cost and pollution associated with irrational use of orthodox medicines have necessitated renewed interest in the use of medicinal plants as sources of effective and safer alternatives in the management of human infections.[8]

Moringa oleifera popularly known as tree of life, mothers love etc is a tropical tree whose numerous economic applications and facility of propagation are arousing growing international interest.[9] Every part of this plant (leaves, roots, seed, bark, fruit, flowers and immature pods) has medicinal activity and are used for various health conditions like diabetes,[10] external sores and ulcers, anti-cancer, anti-diarrhea,[11] anti-inflammatory[12], antioxidant,[13] Hepatoprotective,[14] antihypertensive,[15] Antipyretic and wound healing,[16] atherosclerotic[17] and analgesic.[18] A good source of protein, vitamins, *B* carot-ene, amino acids and various phenolics.[19]

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The aim of this study was to evaluate the susceptibility of microorganisms to moringa seed extracts and determine the safety of the use of this extracts in rodents using haematology, serum chemistry and histopathological changes as indices of toxicities.

Materials and Methods

Plant material and extract preparation

The fresh seeds and leaves (for identification) of *Moringa oleifera* were collected from Alor Anambra State, Nigeria in the month of June 2013. They were identified and authenticated by Mr Odewo at the Herbarium of the Department of Botany and Microbiology, University of Lagos where Herbarium specimen assigned with Voucher number LUTH 5905 was deposited in the Herbarium. The dried powder of *M. oleifera seeds* (620 g) was macerated in 4.5 L of 80% ethanol for 4 days with regular stirring. The extract was clarified by filtration with sterilized muslin cloth and dried in vacuum using rotary evaporator to yield 237.5 g powder (38.3% yields).

Microorganisms

Clinical isolates of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa* obtained from Lagos University Teaching Hospital (LUTH) were used for the research.

Laboratory Animals

Swiss albino mice (20.0 – 25.0 g) were used for the acute while adult Wistar rats (160.0 ± 20.0 g) were used for the sub-acute toxicity profiles. The animals were obtained from the Laboratory animal Centre, College of Medicine, University of Lagos, Idi-Araba and were kept under standard environmental conditions. They were kept in well spacious polypropylene cages (5 animals per cage) in full ventilated animal house with 12 hrs dark and light cycle and were fed on standard animal diet (Pfizer Feeds Ltd, Nigeria) and water ad libitum. They were acclimatized to the laboratory conditions for seven days prior to commencement of research. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animal Research (ILAR) guidelines on the use and care of animals, in experimental studies.[20] The animals were distributed randomLy into five groups of five animals each for acute and sub-chronic toxicity.[2]

Evaluation of antimicrobial activity of the extract

The antimicrobial activities of the hydro-ethanol extract of *Moringa oleifera* seeds on clinical isolates of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa* were evaluated using the agar diffusion method[21]. Different concentrations (50 µg, 100 µg and 200 µg) of the extract were used. Ciprofloxacin was used in different concentrations (2.5, 5.0, 10.0 and 20.0 µg/mL) as the positive control while sterile water was the negative control.

Acute Toxicity Study

Acute toxicity study was carried out using twenty-five (25) Swiss albino mice of both sexes weighing 20.0 – 25.0 g. The animals were randomLy distributed into one control group and four treated groups of five animals per group. After the overnight fasting, the control group received distilled water and 2% acacia orally. The test animals were fed orally with different doses (5.0, 10.0, 15.0 and 20.0 g/kg) of 80% (w/v) of the extract respectively. The 80% (w/v) solution of the extract was prepared by dispersing 16 g of the extract in 7 mL distilled water in a 100 mL beaker and transferred to a 20 mL volumetric flask. The beaker was thoroughly rinsed with distilled water; the content added to the volumetric flask and the volume made to mark. The animals were observed continuously for the first 4 hrs and every hour for the next 12 hrs, then 6 hourly for 56 h (72 h acute toxicity) to observe any death or changes in general behaviour and other physiological activities.[2, 22]

Sub-chronic Toxicity Study.

Male and female Wistar rats weighing (160.0 ± 20.0 g) acclimatized to the laboratory conditions for seven days and were maintained on standard animal feeds (Pfizer Feeds Ltd) with water ad libitum were used. The investigation was done in accordance with the Institute of Laboratory Animal Research guidelines[20] on the use and care of animals, in experimental studies. The weighed animals were divided into four groups of five animals per group. After overnight fasting, control group received a dose of 0.5 mL of distilled water and 2% acacia while the treated groups (1, 2 and 3) were fed with (100, 250 and 500 mg/kg body weight) of the extract formulated with distilled water and 2% acacia respectively. They were all fed orally once a day for 30 days.[3, 23-25] The gel suspension of the extract (12% w/v) was prepared by dispersing the gel (12 g) with 45 mL of sterile water in a beaker and transferred to a 100 mL volumetric flask. Then the beaker was rinsed with sterile water and the content transferred to the volumetric flask and volume made to mark with water. The animals were weighed every five days, from the start of the treatment to observe any change in weight. After 30 days, the animals were starved overnight and made unconscious on the 31st day by cervical dislodgement. The animals were bled through cardiac puncture and their blood collected in two different sterile tubes[3]: EDTA tube for analysis of haematological parameters and blood chemistry and heparin tube to separate plasma for biochemical estimations. The blood in heparin tube was centrifuged at 4000 revolutions per minute (rpm) for 10 minutes to obtain plasma, which was analysed for total cholesterol, total triglyceride, and HDL-cholesterol levels by the method of Ogbonnia *et al*.[3] Blood Plasma was analysed for Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine by standard enzymatic assay methods[26]. Protein contents were determined using enzymatic spectroscopic methods.[27] Haematocrit was estimated by the method.[28] Haematocrit tubes were filled with whole blood to the mark by capillary action and the bottom of the tubes sealed with plasticizer and centrifuged for 5 minutes using haematocrit centrifuge. The percentage cell volume and Haemoglobin contents were determined using the method of Ogbonnia *et al*.[3]

Histological evaluation

Different doses (of the lyophilized hydro-ethanol extracts of *Moringa oleifera* (L.) seeds were administered to Wistar rats for 30 days and the effects of the extracts on the visceral organs (liver, heart, kidney, and the testis) were histologically examined.

The animals were sacrificed by cervical dislocation and dissected to harvest the target organs, liver, kidney, heart, and testes. The harvested organs were fixed in 10% normal saline for seven days before embedding in paraffin wax. They were removed from the paraffin wax and dehydrated in increasing concentrations of alcohol; 70%, 80%, 90% and absolute alcohol (100%). The organs were treated with acetone and then cleared in xylene for 30 minutes to enhance the tissue transparency followed by impregnating and embedding in paraffin wax. Tissue from each of the organs was sectioned at 5µm and wax carefully removed for staining with haematoxylin and eosin.[29] The stained tissues were examined using light microscope at high magnification (x 400) to observe changes in organ structure while photomicrographs were taken.

Statistical analysis

Student's t-test was used to determine the statistical significant difference. The difference was regarded as significant when $P < 0.05$. Every data was expressed as mean ± standard error of the mean.

Results and Discussion

Seeds and nuts have served as important sources of drugs and their health benefits could be attributed to the presence of vitamins and some secondary metabolites especially polyphenols.[42] They contain various chemical compounds known as natural products or secondary products which are responsible for their physiological and pharmacological activities. Moringa oleifera seed is one of such seeds endowed with therapeutics potentials.

Weight variation

The effects of the *Moringa oleifera* seed extracts calculated based on the average of the body weight gain or loss of the treated animals compared with the control are shown in Figure 1. Observed was a significant ($p \leq 0.05$) gain in weight in all the groups of animals treated with the seed extract. Rapid increase in weight was observed to occur in the early days of the animals treated with the highest dose (500 mg/kg) of the extract, which declined sharply after some days of

exposure compared to the control. The least dose (100 mg/kg) exhibited sustained increase in weight followed by average dose (250 mg/kg). This is an indication that the extract has the tendency to cause increase in the body weight of the animals. The rapid increase in weight and sharp decline after some days of administration of the highest dose (500 mg/kg) suggests the dose might have triggered some deleterious effects and possible damages in the animal which culminated into stress and loss of weight. This is evident in the sustained weight increase exhibited by the least dose (100 mg/kg) which might have elicited little or no deleterious effect on the animal. The significant ($p \leq 0.05$) gain in weight of the animals is in consonant with the findings of Ufele et al[31] which clearly suggested increase in the appetite of the animals resulting from the increase in food intake.

Antimicrobial activity of the extract

The antimicrobial screening revealed that the extract in comparison with the standard ciprofloxacin had some antibacterial activities against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* and without activities on *Proteus mirabilis* and *Pseudomonas aeruginosa* as shown in table 1. Ciprofloxacin, the standard as active against all the organisms used. These findings are in contrast with the findings of Bukar *et al*[9] which reported that *Moringa oleifera* seed extracts were found active against *Pseudomonas aeruginosa*. It could also be that a different strain of *Pseudomonas aeruginosa* was used from that of Bukar *et al*.

Acute toxicity test

The acute toxicity study of the extract of *Moringa oleifera* seed revealed changes in behaviour and psychomotor reflexes of the animals fed with higher doses of 15.0 g/kg and 20.0 g/kg body weight but the LD_{50} was calculated to be 14.0 g/kg body weight. According to Ghosh,[43] Klaasen *et al*[44] and Ogbonnia *et al*.[3] the extract could be classified as being nontoxic, since the LD_{50} by oral route was found to be 14.0 g/kg body weight which was much higher than WHO toxicity index of 2.0 g/kg body weight. The LD_{50} of more than 14.0 g/kg body weight could be translated to 980 g dose in an average adult man of 70 kg. Despite the high safety margin, the behavioural changes and the psychomotor reflexes may occur in very high doses.

Sub-chronic toxicity test

Effects of the ethanol extract of moringa oleifera seed on serum electrolytes

The observed effects of the extract on serum electrolytes were summarized in Figure 2. The extract exhibited significant ($p \leq 0.05$) reduction of sodium and chlorine ions levels. The reductions of Na^+ and Cl^- ions were observed to progress with increased dose of the extract. Also observed was significant ($p \leq 0.05$) increase of serum bicarbonate and slightly ($p \leq 0.01$) elevation of the serum calcium level. The observed reduction of sodium (Na^+) and chlorine (Cl^-) levels coupled with significant ($p \geq 0.5$) increase in serum bicarbonate with no significant ($p \leq 0.05$) increase in calcium (Ca^{2+}) level could be an indication of electrolyte imbalance and could also be a serious sign of compromised kidney which could be traced to consumption of the seed extract.

Effects of the ethanol extract of moringa oleifera seed on blood parameters

Table 3 reveals the effects of the seed extracts on blood parameters. There was observed significant increase ($p < 0.05$) in the platelets (PLT) which was more with the smallest dose (100 mg) than the larger doses. The red blood cells (RBC) and the Haematocrit (HCT) were observed to be significantly ($p \leq 0.05$) increased which was also dose dependent. Haemoglobin (HB) and mean corpuscular haemoglobin concentration (MCHC) levels increased significantly ($p \leq 0.05$) with the dose of 250 mg/kg body weight of the extract recording the highest increment. The white blood cells (WBC) and the mean corpuscular volume (MCV) were significantly ($p \leq 0.05$) reduced. The production of red blood cells occurs in the bone marrow and one of the important enzymes regulating this process is called erythropoietin (Epo). The majority of Epo is produced and released by the kidneys, and a smaller portion is released by the liver. The polythemia, an increase in red blood cells (RBC), haematocrit (HCT), haemoglobin (HB) and mean corpuscular haemoglobin concentration (MCHC) levels observed in the test animals when compared with the normal is an indication that *Moringa oleifera* seed extract had the potential to boost production of blood components especially in the conditions of blood loss (hypovolemia) or anaemia, however, in normal haematological conditions or excessive use, the functions of the bone marrow, liver and the kidney could be compromised.[32,33] The WBC is known to rise as body defence in response to toxic environment.[34,40] A higher white blood cell count could usually be attributed to viral or other infections that temporarily disrupt the work of bone marrow. Some disorders could also diminish bone marrow function. Significant ($p \leq 0.05$) reduction of the WBC and the MCV associated with the *Moringa* seed extract could be as a result of deleterious effects of the extract on the bone marrow.

Effects of the ethanol extract of moringa oleifera seed on serum proteins and metabolites

The effects of *Moringa oleifera* seed extract on serum proteins and metabolites are summarised on Table 4. The higher doses of the extract were observed to significantly ($p \leq 0.05$) increase the total protein and urea while the smallest doses caused significant ($p \leq 0.05$) decrease in total protein and urea. Also observed is significant ($p \leq 0.05$) dose dependent increase in total bilirubin. Observed was significant ($p \leq 0.05$) increase in creatinine level with the least dose causing the highest increase. Some proteins in the blood may be elevated as the body fights infections and inflammation as people with certain bone marrow diseases, such as multiple myeloma, may have high blood protein levels before manifestations of any other symptoms.[35, 36] High bilirubin levels in adults usually indicate that there may be an underlying problem involving the red blood cells, liver, or gallbladder; however, other problems also may be involved.[37] Increased serum creatinine levels have adverse downstream effects on the heart, lungs and other organs[38]. This study, revealed that the seed extract had a significant ($p \leq 0.5$) increasing effects on the total protein, urea, bilirubin and creatinine levels indicating deleterious effects of the seed extract on the vital organs such as liver and kidney.

Effects of the ethanol extract of moringa oleifera seed on liver enzymes

The effects of *Moringa oleifera* seed extract on liver enzymes are shown in Figure 3. The alanine amino transferase (ALT) was observed to show significant increase ($p \leq 0.05$) at low (100 mg) and average (250 mg) doses of the extract but showed significant ($p \leq 0.05$) decrease at the highest dose (500 mg/kg body weight). There was significant decrease ($p \leq 0.05$) in aspartate amino transferase (AST) and alkaline phosphate (ALP) values in the doses administered. Elevated concentrations of liver enzymes particularly ALT and AST in the blood plasma has been found to be indicators of inflamed or injured liver.[39, 41] *Moringa oleifera* seed extract was observed to have caused an increase in the alanine amino transferase (ALT) level, which implied that the extract had some deleterious effects in the liver. The extract did show a decrease in aspartate amino transferase (AST), alkaline phosphate (ALP) levels which suggested the extract might have had some dangerous or toxic effected on the heart functions. It can be deduced that noticed increase in ALT values could not be completely attributed to the extract but to some other unidentified hidden factors.

Tissue histology/histopathology

The effects of *Moringa oleifera* seed extract on the target organs are represented in Figures. 4-7. Figures 4a - 4d represent the hepatic tissues of the control and treated animal showing the hepatocytes (blue arrow), the portal tract (yellow arrow) and the sinusoids (red arrow). The histology of the liver revealed that the seed extract caused oedematous hepatic tissues particularly at the low and median concentrations of the extract but at a highest dose, no histological abnormalities were observed. It could be inferred that abnormalities observed at lower doses could not entirely be attributed to the extract but might have resulted from some unidentified factors.

Figures 5a – 5d show the photomicrograph of normal (control) and treated renal tissue indicating the convoluted tubules and the renal corpuscles separated from the surrounding structures by Bowman's space. Figure 5c reveals diffused renal corpuscles with stretched convoluted tubules indicating kidney degeneration.

The photomicrograph of normal cardiac tissue (Figure 6a) shows the muscle fibres, the shielded myocytes interspaced by the interstitium.

The cardiac tissues across all extract concentrations administered (Figures 6b-d) showed normal appearance.

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Table 1: Effect of various concentrations of *Moringa oleifera* seed extract, distilled water and ciprofloxacin on the microorganisms.

	ORGANISM/ ZONES OF INHIBITION (mm)				
Extract conc.(µg/mL)	***B. subtilis***	***E. coli***	***S. aureus***	***P. mirabilis***	***P. aeruginosa***
Distilled water	-	-	-	-	-
50	20.0	-	-	-	-
100	23.0	20.0	15.0	-	-
200	30.0	30.0	20.0	-	-
Ciprofloxacin (µg/mL)			ZONES OF INHIBITION		
2.5	7.0	13.0	6.0	7.0	10.0
5.0	24.0	23.0	10.0	12.0	9.0
10.0	24.0	25.0	17.0	18.0	19.0
20.0	26.0	26.0	23.0	24.0	21.0

- : No zone of inhibition.

Table 2: Acute toxicity of *Moringa oleifera* seed extract in Swiss albino mice.

Dose of extract (g/kg)	**Number of animals**	**Number of dead animals**	**% death/**	**% cumulative death**
Control	5	0	0	0
5	5	0	0	0
10	5	1	20	9.1
15	5	5	100	54.5
20	5	5	100	100

Control group received distilled water and 2% acacia orally.

Table 3: Effect of *Moringa oleifera* seed extract on blood parameters.

Blood parameters	**Control**	**100 mg**	**250 mg**	**500 mg**
RBC	6.54 ± 0.81	8.09 ± 0.68 *	7.92 ± 0.71	7.74 ± 0.68
HB	12.3 ± 1.32	13.8 ± 1.06	14.2 ± 0.99	13.5 ± 0.93
HCT	45.9 ± 3.44	49.1 ± 2.56	48.7 ± 2.13	48.6 ± 3.01
WBC	9.8 ± 1.29	6.5 ± 0.34 *	6.6 ± 1.03 *	8.9 ± 1.09
PLT	500 ± 28.00	837 ± 40.03 *	707 ± 43.00 *	724 ± 29.09 *
MCV	70.3 ± 5.30	60.8 ± 3.55 *	61.5 ± 3.47 *	62.9 ± 4.07 *
MCH	18.8 ± 1.96	17.0 ± 2.10	17.9 ± 2.19	17.4 ± 1.87
MCHC	26.7 ± 2.21	28.1 ± 2.05	29.1 ± 1.97	27.7 ± 3.01

* = $P < 0.05$ when compared with control group.

Table 4: Effect of *Moringa oleifera* seed extract on serum proteins and metabolites.

Serum protein and metabolites	**Control**	**100 mg**	**250 mg**	**500 mg**
Total protein	26.6 ± 1.26	16.9 ± 2.01	20.1 ± 2.11	27.9 ± 3.03
Total Bilirubin	0.3 ± 0.02	0.4 ± 0.01	0.4 ± 0.03	0.5 ± 0.03
Creatine	0.31 ± 0.01	5.84 ± 0.13 *	5.55 ± 0.22 *	4.16 ± 0.34 *
Urea	1.9 ± 0.06	1.6 ± 0.07	1.6 ± 0.04	2.4 ± 0.09

* = $P < 0.05$ when compared with control group.

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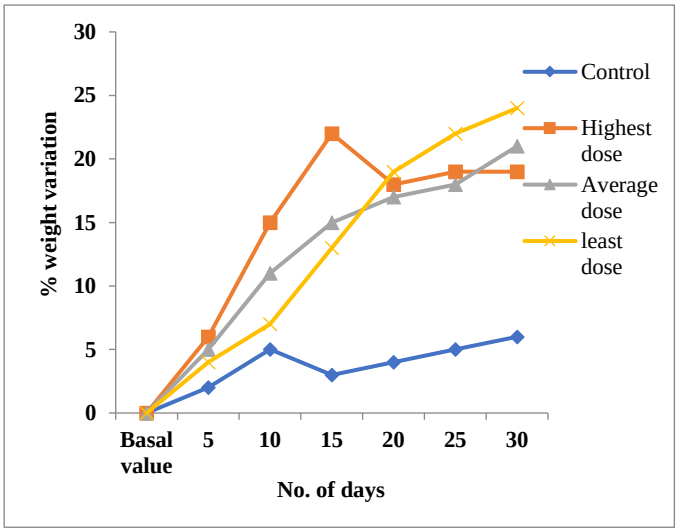

Figure 1: Percentage weight control of the treated and control animals versus number of days of exposure. Highest dose: 500 mg/Kg, Average dose: 250 mg/Kg, Least dose: 100mg/kg.

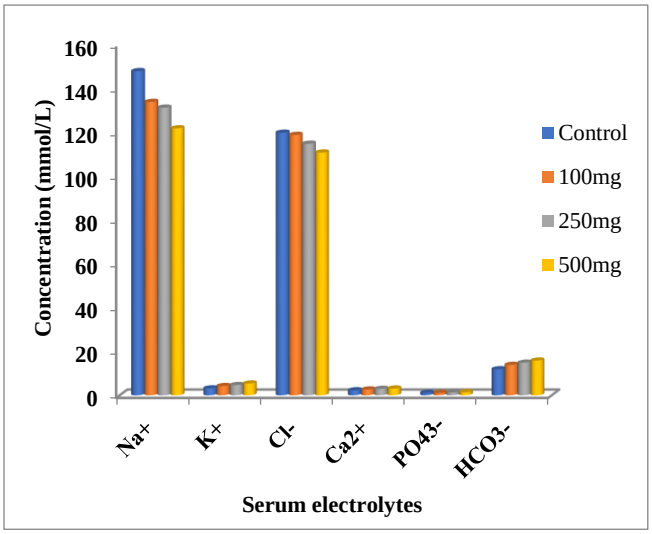

Figure 2: Effect of *Moringa oleifera* seed extract on serum electrolytes.

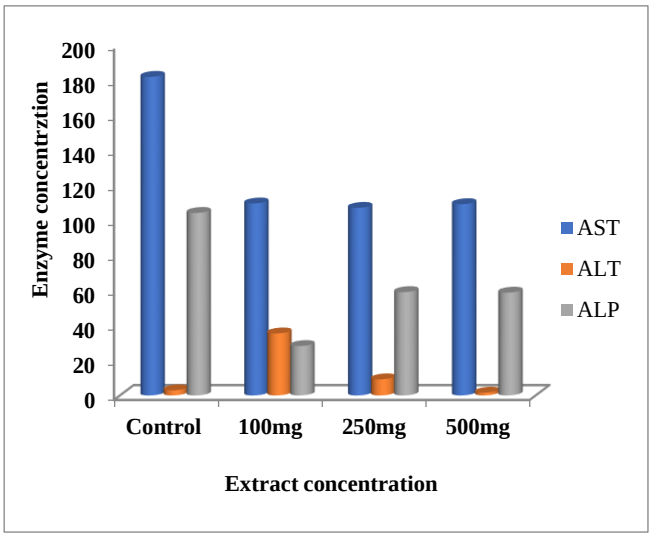

Figure 3: Effect of *Moringa oleifera* seed extract on liver enzymes.

The photomicrograph of the testicular tissue of the control group (Figure 7a) shows clusters of primitive spermatogenic cells (Myocyte) separated by the interstitium. The spermatids and spermatozoa formed clustered at the centre. Figure 7c which represents the group treated with 250 mg extract shows slightly loosened myocyte, maintaining normal spermatids and spermatozoa. The groups administered with 100 mg and 500 mg/kg body weight revealed loosened myocyte with empty centres indicating spermatids and spermatozoa clearance which could also be attributed to some unidentified factors.
There were no abnormalities observed at the cardiac tissues at all extract doses administered (Figures 6b - d) compared to the control.

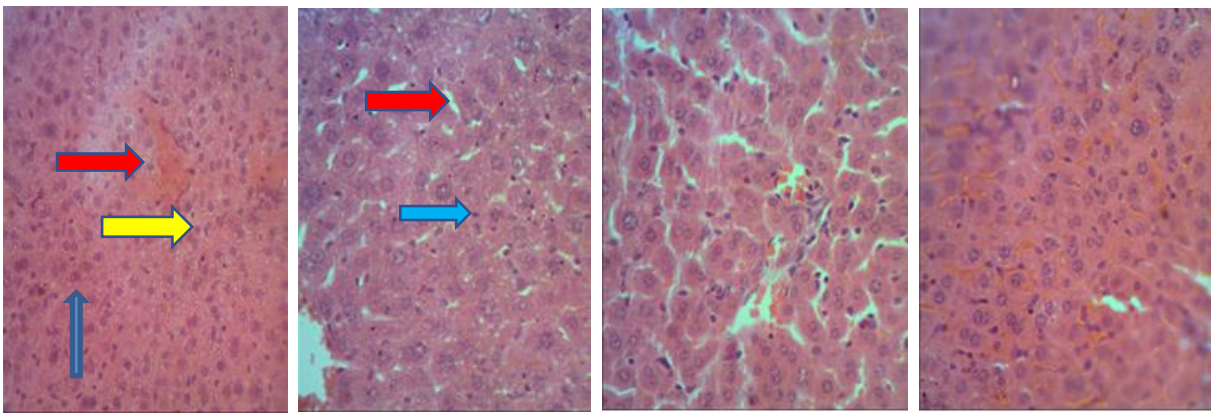

Figure 4a: Photomicrograph of control hepatic tissue.

Figure 4b: Photomicrograph of hepatic tissue treated with 100 mg extract indicating signs of degeneration.

Figure 4c: Photomicrograph of hepatic tissue treated with 250 mg extract showing clear degeneration.

Figure 4d: Photomicrograph of hepatic tissue treated with 500 mg extract.

Yellow arrow: Portal tract, blue arrow: hepatocyte, red arrow: sinusoid. Mag. X 400.

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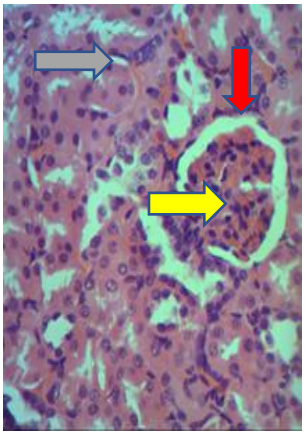
Figure 5a: Photomicrograph of the control renal tissue.

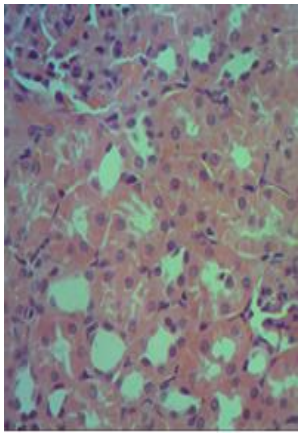
Figure 5b: Photomicrograph of renal tissue treated with 100 mg extract.

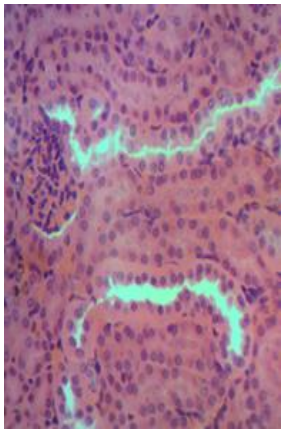
Figure 5c: Photomicrograph of renal tissue treated with 250 mg extract.

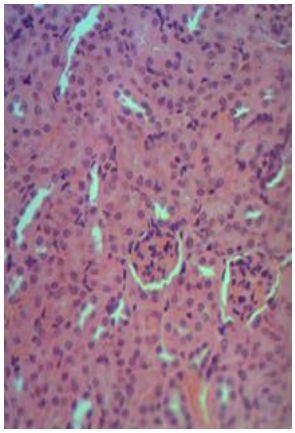
Figure 5d: Photomicrograph of renal tissue treated with 500 mg extract.

Yellow arrow: Renal corpuscles, green arrow: Bowman's space , red arrow: Convoluted tubule.

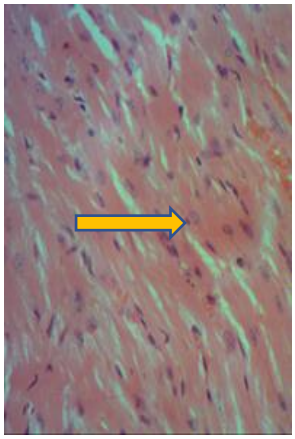
Figure 6a: Photomicrograph of the control myocardium.

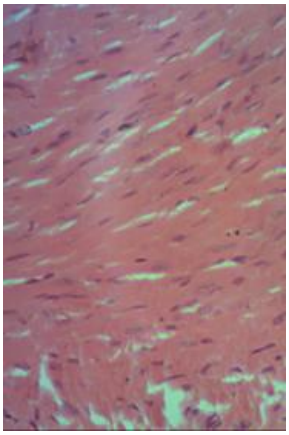
Figure 6b: Photomicrograph of myocardium treated with 100 mg extract.

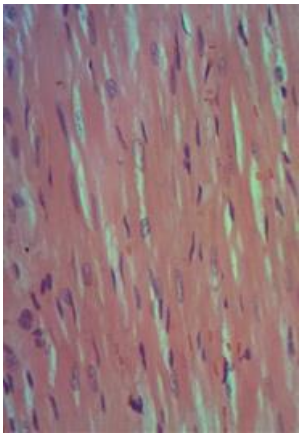
Figure 6c: Photomicrograph of myocardium treated with 250 mg extract showing signs of oedema.

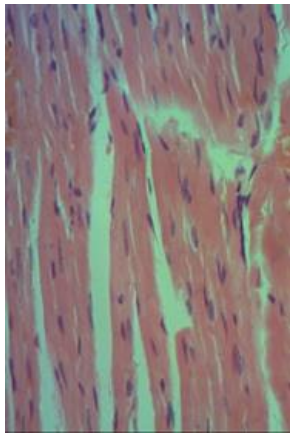
Figure 6d: Photomicrograph of myocardium treated with 500 mg extract.

Yellow arrow: myocyte.

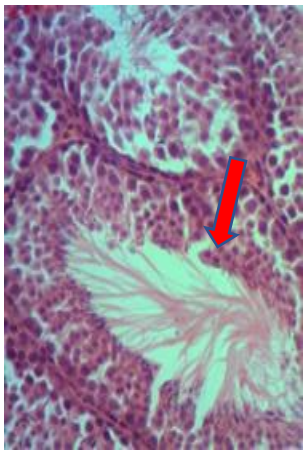
Figure 7a: Photomicrograph of the control testicular tissue showing densely packed spermatogenic cells.

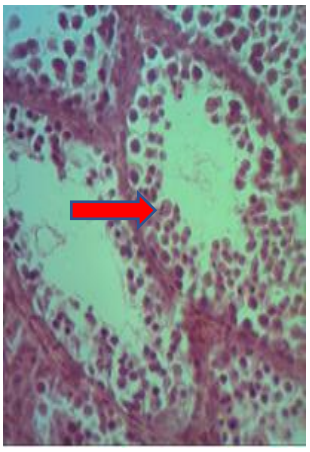
Figure 7b: Photomicrograph of testicular tissue treated with 100mg extract.

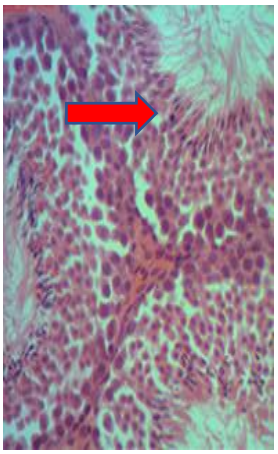
Figure 7c: Photomicrograph of testicular tissue treated with 250 mg extract.

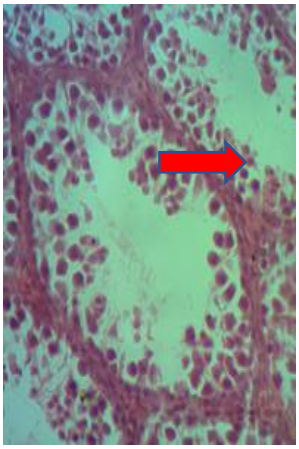
Figure 7d: Photomicrograph of testicular tissue treated with 500 mg extract.

Red arrow: myocyte spermatogenic cells.

Conclusion

Moringa oleifera seed extract exhibited high safety margin with LD_{50} value of 14.0 g/kg, an indication that the extract might be safe for consumption. The extract demonstrated the potential to stimulate appetite leading to increase in food consumption and weight gain of the animals. It could also be used in treatment of anaemia. Despite the enormous benefits of the extract, caution should be exercised in its use since high dose or prolonged use of it could cause electrolyte imbalance, deterioration of the bone marrow, liver and particularly the kidney.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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