British Journal of Pharmaceutical Research
4(10): 1196-1208, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Acute and Sub-acute Toxicity Studies of Ethanol Seed Extract of *Raphia hookeri* on Swiss Albino Rats

G. O. Mbaka[1*], S. O. Ogbonnia[2] and F. O. Awoyemi[3]

[1]Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria.
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Idi- Araba, Lagos, Nigeria.
[3]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author GOM designed the experiment and the protocol for the study. He undertook the tissue processing and analysis and as well as partook in the write up and final editing of the manuscript. Author SOO partook in the experimental design and editing of the manuscript and also performed the statistical analysis. Author FOA conducted the laboratory work as well as managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 4th February 2014
Accepted 4th April 2014
Published 5th May 2014

ABSTRACT

Aim: To evaluate the acute and sub-acute toxicities of *Raphia hookeri* (Rh) seed hydroethanolic extract on experimental animals.
Materials and Methods: Acute toxicity study was evaluated on Swiss albino mice of both sexes. Administration of a single dose of 4000mg/kg of Rh seed extract by gavages to five mice showed no mortality, hence, its 1/20th dose was used as the highest therapeutic dose. The intra-peritoneal administration produced dose dependent mortality with median lethal dose (LD_{50}) of approximately 323.6mg/kg body weight (bwt). In sub-acute toxicity study, Wistar rats received daily administration of the extract in the dose range of 50 to 200mg/kgbwt for 30 days. The effects on biochemical, histological and haematological parameters were evaluated.
Results: The animals exhibited dose dependent body weight changes. There were some organs weight gains with the exception of the liver and testes which showed comparably

**Corresponding author: Email: mbaaka2gm@gmail.com;*

lower weight compared to the control. There was a significant (p<0.05) increase in total protein, aspartate aminotransferase (AST) and albumin levels compared to the control while bilirubin and alanine aminotransferase (ALT) levels decreased appreciably at the highest extract dose. The urea level decreased while the creatinine level increased in dose dependent manner. In lipid profile study, total cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-cholesterol) levels showed significant (p<0.05) decrease in value. There was significant (p<0.05) increase in high density lipoprotein cholesterol (HDL-cholesterol). Marked decrease in red blood cells, haemoglobin and haematocrit occurred. The white blood cells also decreased while neutrophil and lymphocytes increased appreciably. The extract caused marked deleterious effect on the testes leading to drastic reduction in sperm cells.
Conclusion: The extract caused undesirable effect on the male reproductive organ of the animals making it unsafe for consumption by males of reproductive age.

Keywords: Raphia hookeri; acute toxicity; sub-acute toxicity; tissue histology.

1. INTRODUCTION

Phyto-medicine from time immemorial has been the main stay of health care need for the treatment of various types of diseases. Despite improvement in science and advancement in medicine, greater number of the population of most developing countries still rely on herbal medicine to resolve their health problems [1]. Also, in most developed countries, there appears to be increased awareness of the usefulness of herbs in the management of various disease conditions. According to a WHO estimate, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [2].

It is generally presumed that herbal medicines are more effective and because of their natural source are free from undesirable side effect [3]. This belief has led to serious abuse such as prolonged administration without appropriate dose monitoring thereby undermining the greater potential for adverse effect. The danger associated with the potential toxicity of herbal therapies administered over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs [4,5].

Plants or herbal products used in the treatment of different ailments usually contain wide range of chemical compounds. Some of the chemical constituents could be of beneficial effects to the body system while others may possess toxic properties [6]. The incidence of adverse effects and sometimes life-threatening conditions allegedly emanating from these herbal medicines has been reported among various ethnic groups [7,8]. It is because some of these herbal therapies have narrow therapeutic index hence the need for scientific research to determine the appropriate dose for consumption. Unfortunately, of the vast number of medicinal plants available for use, only very few have been scientifically validated for their actual beneficial or toxic effects [9].

Raphia hookeri (Rh) commonly known as Raffia palm is a member of Palmaceae family. The plant is believed to have originated from the swamps of West Africa and is restricted to the tropical rainforest which is its ideal ecological condition [10]. It shows variation in character, particularly in size and the shape of their fruit. Rh appears more abundant in the eastern and western parts of Nigeria where they grow in fresh water swamps reaching a height of 9m

and possesses breathing roots thereby adapting it for life/support in water logged soils [11]. The fruit is large, cone-shaped with a single hard nut having an outer layer of rhomboid-triangular and overlapping reddish brown scales. Between this outer layer of scales and the very hard seed is a yellow, mealy, oil-bearing mesocarp or pulp. According to a report, Rh is probably the most diversely useful plant in Nigeria as all its parts have various economic values [12]. It was also noted to be an important source of forest food species in southern Nigeria. Rh has equally shown to have beneficial therapeutic property as it is used in herbal medicine in the treatment of various illnesses. Studies have shown the seed to possess significant antipyretic and analgesic activities [13,14]. Investigations have also revealed that the seed extract exhibited significant antioxidant [15], anti-inflammatory [16] and anti diabetic properties [11]. The aim of this study was to evaluate the safety of Rh seeds by carrying out the acute and sub-acute toxicity studies using animal model.

2. MATERIALS AND METHODS

The seed of *Raphia hookeri* were obtained in the month of November 2010 from swampy farmland at Ikorodu, Lagos State, Nigeria. The plant sample was authenticated in the Forestry Research Institute of Nigeria (FRIN), Ibadan. The voucher specimen has been deposited in the herbarium (FHI/108941).

2.1 Preparation of the Aqueous Ethanol Seed Extract of RH

The fresh fruits of Rh obtained from swampy farm land were spread in the sun for a week to enable for the softening and easy removal of the mesocarp. The seeds obtained were dried before being subjected to size reduction to a coarse powder with electric grinder. The seed powder, 1140g, was extracted with 95% aqueous ethanol by maceration method with frequent stirring for up to two weeks. The crude extract was filtered with Whatman filter paper No. 4 and the filtrate concentrated in vacuum at 30°C to obtain 138g residue weight (12.1%w/w). The residue which was in form of paste was stored in an air tight bottle kept in a refrigerator at 4°C till used.

2.2 Animals

Wistar rats weighing between 140-150g of either sex obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria, were kept under standard environmental condition of 12/12h (light/dark cycle). A total of 20 rats were obtained and housed in polypropylene cages (5 animals per cage). They were maintained on mouse chow (Livestock Feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize for 7 days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals, in experimental studies [17].

2.3 Acute Toxicity Study

A total of 15 mice fasted for 14hrs were administered with Rh seed extract dispersed in acacia solution (2%w/v) intra zperitoneally in graded doses of 125, 250 and 500mg/kg of five mice per group until 100% mortality was recorded. The animals received the extract at the doses of 125, 250 and 500mg/kg body weight (bwt). The control group of five mice was given 0.3ml per kg body weight of acacia solution orally. LD_{50} was calculated using the method of Miller and Tanter [18]. Another group of five mice fasted for 14h were administered

a single dose of 4000mg/kg of Rh seed extract by gavages and then observed for seven days for mortality and physical/behavioural change. The animals did not show any mortality at the dose administered hence its 1/20th dose (200mg/kgbwt) was chosen as the highest dose with other graded doses of 100 and 50mg/kgbwt were used as the therapeutic doses [19] with modification.

2.4 Sub-acute Toxicity Study

A total of 20 male and female ten weeks old rats weighing 140-150g were randomly allotted five per group to the control and the extract treated groups. After fasting the animals overnight the control group received a dose of 0.6ml per kg body weight of acacia (2%w/v) solution and the treated received 50, 100 and 200mg/kg of the extract dispersed in acacia (2%w/v) solution. The doses were administered by gavages daily for a period of 30 days [20-22]. The animals were observed closely for any behavioural changes, body weight changes and mortality and were later sacrificed for haematological and biochemical investigations and organs histological changes.

2.5 Biochemical Parameters

Following the sacrifice under mild diethyl ether, blood was collected via cardiac puncture in two tubes. The EDTA tube was used to collect blood for the analysis of haematological parameters while the second with heparin to separate plasma for biochemical estimations. The collected blood was centrifuged within 20 min of collection at 4000rpm for 10min to obtain blood plasma, which was analyzed for total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-cholesterol) levels by modified enzymatic procedures from Sigma Diagnostics [23]. Low density lipoprotein cholesterol (LDL-cholesterol) level was calculated using Friedwald equation [24]. Plasma was analyzed for alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, and creatinine by standard enzymatic assay methods [25]. Urea was determined according to Urease-Berthelot method [26]. The protein content was determined using enzymatic spectroscopic methods [27]. Total bilirubin was estimated using Jandrassik and Grof technique [28]. Albumin was determined based on its reaction with bromocresol green (Binding method) [29].

2.6 Haematological Parameters

Haematocrit was estimated using the methods of Ekaidem et al. [30]. Haematocrit tubes were filled to mark with whole blood and the bottom of the tubes sealed with plasticide and centrifuged for 4-5min at 4000rpm using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a “critocap” chart until the meniscus of the plasma intersects the 100% line. Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method [30]. Haematocrit (HCT) was determined according to Ekaidem et al. [30] while white blood cells (WBC) and its differentials (neutrophil, eosinophil, basophil, lymphocyte and monocyte) were determined as described by Dacie and Lewis [31]. The blood samples were analyzed for red blood cells (RBC) by haemocytometic method [31].

2.7 Tissue Histology

The organs were fixed in 10% formal saline for ten days before embedding in paraffin wax.

Each organ tissue was sectioned at 5µm and stained with Haematoxylin and Eosin (H and E) stain [11]. The slide specimens were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.8 Statistical Analysis

Significant differences were determined using a Student's t-test. Differences were considered significant if $p<0.05$ and $p<0.01$. All data were expressed as mean±standard error of the mean.

3. RESULTS

The intra-peritoneal (IP) administrations of *R. hookeri* seed extract produced dose dependent mortality with median lethal dose (LD_{50}) of approximately 323.6mg/kgbwt Table 1. The rats dosed with the extract by gavages tolerated up to 4000 mg/kgbwt with no sign of physical/behavioural changes hence 200mg/kgbwt (1/20th of the dose) was used as the highest therapeutic dose with other graded doses of 100 and 50mg/kgbwt respectively.

Table 1. Determination of the acute toxicity of aqueous ethanol extract of *Raphia hookeri* seed on mice (Intra-peritoneal route)

Dose	Motility	% Motility	Log dose	Probit	Probit approximate
125	1/5	20	2.0969	4.1584	4.2
250	2/5	40	2.3979	4.7467	4.7
500	3/5	60	2.6989	5.2533	5.3

The LD_{50} of the extract intra-peritoneal is 323.6mg/kg

The percentage body weight changes compared to the control is shown in Fig. 1. There was an insignificant ($p\geq0.05$) initial body weight which became marked during the course of the extract administration that progressed over time but decreased at the last week of the experiment. The weight change showed dose-effect relationship.

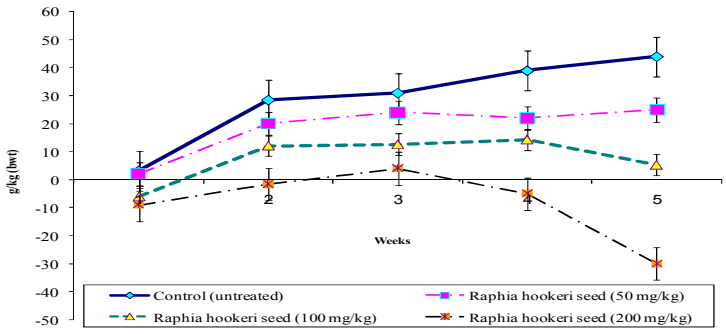

Fig. 1. Weight differential in control and treated rats after sub-acute exposure to *Raphi hookeri* seed extract

The result of the effect of the extract on the harvested organs weight is presented in Table 2 which showed slight weight gain in all the organs compared to the control with the exception of the weight of liver and testes that were comparably lower than the control.

Table 2. Data on the organ weight (100g body weight) of rats after sub-acute treatment with aqueous ethanol extract of *Raphia hookeri* seed

Treatment	Mean organ weight per 100g body weight				
	Heart	Lung	Liver	Kidney	Testes
Control	0.3±0.2	1.9±0.5	5.8±0.3	1.0±0.3	3.0±0.4
50mg/kg	0.4±0.1	2.1±0.2	4.6±0.3	1.3±0.3	3.0±0.2
100mg/kg	0.4±0.1	2.1±0.2	4.0±0.2	1.3±0.2	2.8±0.0
200mg/kg	0.4±1.1	2.2 ±0.1	3.5±0.5	1.2±0.4	2.5±0.3

Table 3 showed the effect of the extract on biochemical parameters. There was increase in total protein and albumin with significant (p<0.05) increase occurring in albumin level particularly at the higher doses. The total bilirubin level however decreased markedly (p<0.05). The AST increased (p<0.05) with increase in dose while ALT showed increase at the lower doses but decreased at the highest extract dose compared to the control. The urea level decreased insignificant (p≥0.05) while the creatinine level increased significantly (p<0.05) in dose manner compared to the control. In lipid profile study, total cholesterol, triglycerides and LDL-cholesterol levels showed significant (p<0.05) decrease in value. There was however significant increase (p<0.05) in the HDL-cholesterol level. The alkaline phosphatase level decreased significant (p<0.05) with dose compared to the control.

Table 3. Blood chemistry values of rats after sub-acute treatment with aqueous ethanol extract of *Raphia hookeri* seed

Parameter	Control	50mg/kg	100mg/kg	200mg/kg
Total protein(mg/dL)	65.9±1.9	68.6±2.0	67.3±3.3	70.1±1.2**
Albumin(mg/dL)	41.0±2.2	50.0±3.3**	73.9±3.9*	54.0±2.0*
Total bilirubin(mg/dL)	1.2±1.1	0.5±0.0*	0.6±0.0*	0.5±0.1*
AST(iμ/L)	139.4±4.2	161.4±9.2*	188±0.1*	170.4±1.3*
ALT(iμ/L)	59.2±4.3	59.5±2.3	63.8±3.0	51.3±1.4*
Alkaline(iμ/L) phosphatase	161.5±4.4	150±3.5*	141.4±4.0*	130.8±8.0*
Urea(mg/dL)	9.3±1.6	9.2 ±1.5	7.3±3.2	8.8±0.2
Creatinine(mg/dL)	45.2±5.6	48.0±5.2	53.7±2.5**	56.3±3.3*
Total cholesterol(mg/dL)	90.3±0.2	73.0 ±0.1*	65.3±1.1*	50.3±1.0*
Triglycerides(mg/dL)	64.9±0.4	64.8±0.0	62.6±1.0	58.7±0.4**
LDL-cholesterol(mg/dL)	95.2±1.1	80.5± 2.0*	73.3±1.7*	72.7±0.9*
HDL-cholesterol(mg/dL)	41.2±0.1	50.3± 0.2*	61.3±0.0*	67.4±1.0*

*Mean ± SEM, (n=5) *p<0.05; **p<0.01 vs. control group*

The summary of the effect of the extract on the haematological parameters of the animals is shown in Table 4. Administration of the extract led to decrease in RBC, Hb and HCT. The decrease in PCV was more marked compared to the control. The WBC count equally recorded significant (p<0.05) decrease compared to the control. The MCV, MCH and MCHC levels fluctuated with no marked variations except in MCH and MCHC which showed significant (p<0.01) decrease at the highest extract dose compared to the control.

In Table 5 is shown the effect of the extract on WBC differentials. There was a significant increase (p<0.01) in neutrophil, lymphocytes and in plate late (increase (p<0.05) levels in all the extract dose treatment compared to the control.

Table 4. Haematological values of rats after sub-acute treatment with aqueous ethanol extract of *Raphia hookeri* seed

Treatment	RBC ($x10^6/mm^3$)	Hb (g/dL)	HCT (%)	WBC ($10^3/mm^3$)	MCV (fl)	MCH (Pg)	MCHC (g/dL)
Control	7.0±0.9	10.5±.00	39.9±0.9	4.6±0.2	56.2±1.2	16.1±2.1	28.3±1.1
50mg/kg	5.0±0.5	9.1±1.0	29.7±3.1*	2.6±0.7**	56.3±0.1	17.3±0.1	29.9±0.8
100mg/kg	4.6±0.9**	8.8±1.2	26.7±1.0*	3.9±0.4	58.3±3.7	18.5±1.0	31.7±0.3
200mg/kg	6.3±0.2	8.7±1.3	30.0±1.0*	2.2±0.5*	54.9±1.5	14.5±1.0**	25.6±2.0**

*Mean±SEM, (n=5) *p<0.05; **p<0.01 vs. control group*

Table 5. Quantitative data on WBC differentials of rats after sub-acute treatment with aqueous ethanol extract of *Raphia hookeri* seed

Treatment	Neutrophil %	Lymphocyte	Platelets %
Control	0.3±0.1	62.6±0.5	70.0±1.6
50mg/kg	0.7±0.1**	65.7±2.0	98.7±9.8 *
100mg/kg	0.5±0.3	86.7±4.7*	112.5±6.9*
200mg/kg	0.5±0.1	66.5±2.7**	121.1±7.5*

*Mean± SEM, (n=5) *p<0.05; **p<0.01 vs. control group*

3.1 Tissue Histology

Fig. 2a showed the cyto-architecture of a cross section of normal testicular tissue indicating the seminiferous tubules in transverse plane with remarkable boundary. The epithelium towards the basement showed primitive spermatogenic cells compactly arranged while the tails of matured sperm cells show wavy appearance towards the lumina. The extract administration at the highest dose Fig. 2b caused marked deleterious effect on the testes resulting in drastic reduction of the number of sperm cells. At the basement, the spermatogonia formed a scanty thin layer while the remaining large central portion was devoid of sperm cells. Fig. 3a indicated the photomicrograph of normal cardiac tissue in which the muscle fibres showed branched networks. Also indicated were the deeply stained nuclei nuclei. The photomicrograph of the extract treated Fig. 3b showed normal appearance. The photomicrograph of normal hepatic tissue Fig. 4a showed the portal tracts at the periphery of indistinct hepatic lobule. The hepatocytes radially arranged continued from the lobular margins towards the centre vein with each column interspaced by sinusoids. In Fig. 4b no abnormal change was observed. Fig. 5a showed the normal photomicrograph of renal tissue indicating the cortical area containing the renal corpuscles that appeared as a dense rounded mass. The photomicrograph of the extract treated Fig. 5b showed normal appearance.

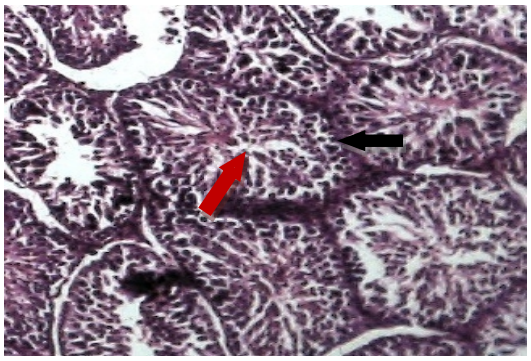

Fig. 2a. Photomicrograph of untreated testicular tissue indicating cross section of the seminiferous tubules separated by the interstitium. Close to the basement are compact primitive cells, the spermatogonia (black arrowed) while the spermatozoa clustered in the centre (red arrowed) (H&E stain) Mag.X100

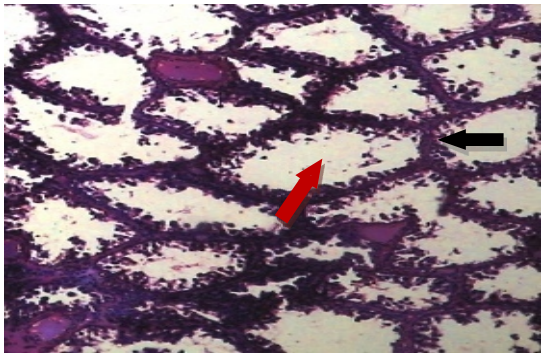

Fig. 2b. Photomicrograph of treatment with 200mg/kgbwt extract dose showing scanty spermatogonia cell layer at the basement (black arrowed). The central portion is devoid of sperm cells (red arrowed). (H&E stain) Mag.X100

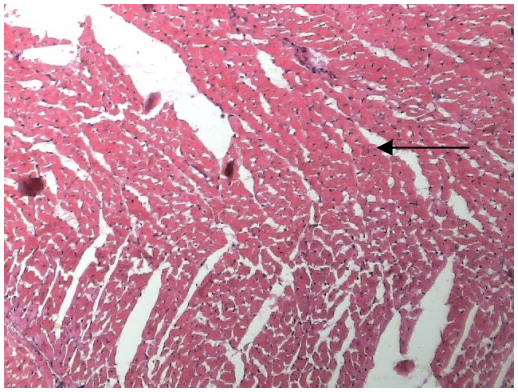

Fig. 3a. Photomicrograph of a cross section of myocardium (heart) showing myocytes (thin arrowed) separated by an unremarkable interstitium (H&E stain) Mag.X100

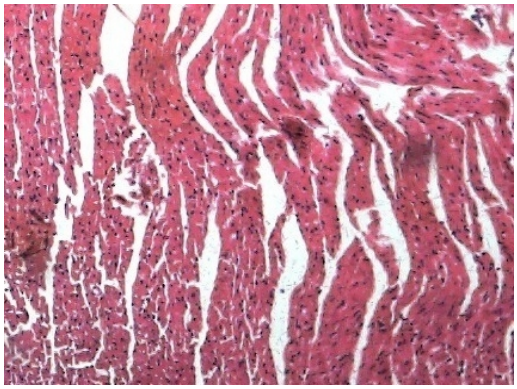

Fig. 3b. Photomicrograph of a cross section of myocardium showing no lesion. (H&E stain) Mag.X100

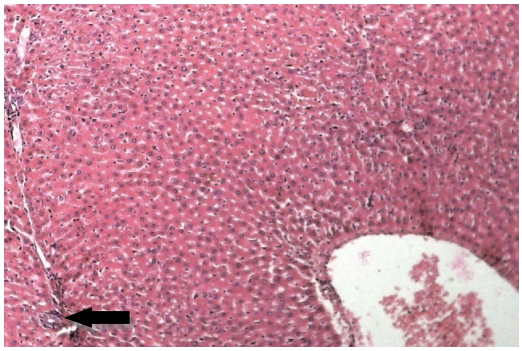

Fig. 4a. Photomicrograph of a cross section of hepatic tissue of the control showing portal tract (thick arrowed) and normal hepatocytes separated by ill-defined sinusoids. (H&E stain) Mag.X100

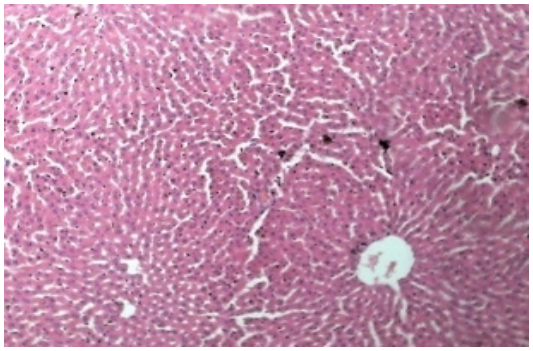

Fig. 4b. Photomicrograph of a cross section of hepatic tissue administered with 200mg/kgbwt of the extract showing no pathological changes. Mag.X100

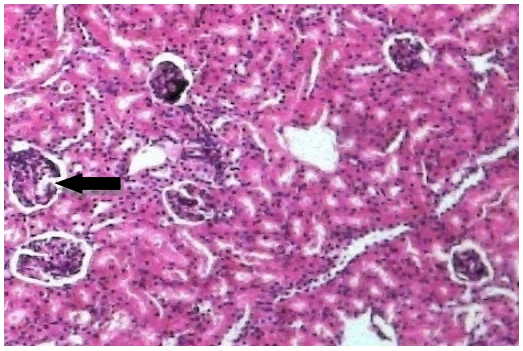

Fig. 5a. Photomicrograph of a cross section of the cortical region of renal tissue (untreated) indicating renal corpuscles (thick arrowed) and convoluted tubules (thin arrowed). (H&E stain) Mag.X100

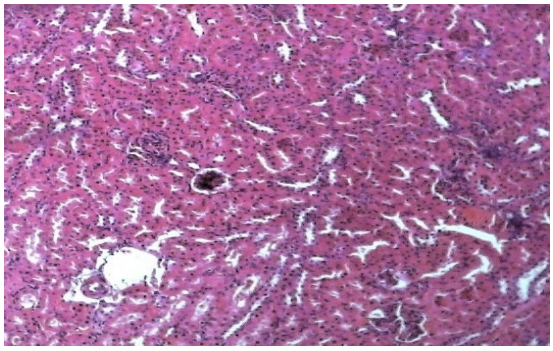

Fig. 5b. Photomicrograph of a cross section of cortical region of the renal tissue administered with 200mg/kgbwt of the extract showing no pathological changes. Mag.X100

4. DISCUSSION

In rural communities, the exclusive use of herbal drugs prepared and dispensed by herbalists without formal training in the drug formulation and preparation for disease treatment is still very common requiring that experimental screening method be established to ascertain the safety and efficacy of these herbal products [5]. In acute toxicity study, animals administered with the extract by gavages at 4000mg/kgbwt showed no visible sign of toxicity nor was there any mortality. According to World Health Organization (WHO) toxicity index of 2g/kgbwt the extract could be classified as being non toxic, since the LD_{50} was found to be 4.0g/kgbwt translating to 280g equivalence dose in human adult [32]. This is a very high toxicity index value thereby making the preparation to be relatively safe for use. In sub-acute toxicity study, the animals exhibited body weight increase which progressed but

at the last week of the study decreased. Also, the liver and testes showed decrease in weight compared to the normal. Reduction in the body and organ weights is a sensitive index of toxicity after exposure to toxic substance [33,34].

In sub-acute toxicity study, AST showed significant (p<0.05) increase with dose while ALT decreased appreciably compared to normal. These are two major marker enzymes in liver whose activities are known to increase significantly (p<0.05) in toxic conditions [24]. The marked increase observed only in AST might be linked to unknown factors since the hepatic tissue histology did not indicate any pathological changes. More so, the extract caused marked increase in the levels of albumin and total protein indicated to have hepato-protective effect [35]. The slight decrease in urea level coupled with normal appearance of renal tissue suggested that there was no considerable kidney compromise. There was however, significant (p<0.05) increase in plasma creatinine level, and since creatinine is an end product of protein metabolism its increase might be attributed to a rise in protein synthesis. The decrease in weight of testis after treatment with the extract was complemented with severe histopathological changes in the seminiferous tubules showing very scanty spermatogenic cell content. It is therefore obvious that the use of Rh seed extract as therapeutic agent poses a serious threat to male fertility. The extract at the administered dose had beneficial effect on the plasma lipids. The total cholesterol, triglycerides and LDL-cholesterol levels decreased markedly whereas HDL-cholesterol level showed significant (p<0.05) increase compared to the control.

The RBC count decreased significantly (p<0.05) indicating that the extract may have adversely affected haematopoietic system. There was also corresponding decrease in Hb content and HCT value in directly proportional manner to RBC count. The decrease in Hb level was suggestive of decreased iron absorption. In toxic environment conditions, total RBC count is known to decrease while total WBC count increases [36]. But in this case, there was also a decrease in the WBC count compared to the control. The factor responsible for this is unclear but might not be unconnected with the suppression on body defense system. The RBC indices MCHC, MCH and MCV decreased respectively compared to the control. The decrease in these parameters has particular importance in anaemia diagnosis in most animals [37]. Low MCHC is noted to be associated with iron deficiency anaemia where microcytic hypochromic RBC are produced as a result of lack of iron to support haemoglobin synthesis [38]. This shows that the extract may have affected the average size of RBC (microcytes) as well as haemoglobin weight per RBC. Hence the extract possesses the potential to induce microcytic anaemia.

There was significant increase in lymphocytes, which are the main effectors cells of the immune system [36]. The rise was therefore, indicative of the implicit harmful effect of the extract which caused stimulatory effect on the body immune system. In like manner the neutrophil might be due to increased need for phargocytosis of damaged tissues. The increased platelet count could be due to stimulatory effect on thrombopoietin [39].

5. CONCLUSION

The administration of Rh seed extract at the doses investigated showed obvious deleterious effect on some of the internal organs of the treated animals particularly on the testes as was evident in the testicular tissue histology. Therefore, the therapeutic agent might pose a serious threat to male fertility hence the need for caution in the medicinal use of the plant's seeds in males.

CONSENT

This was not applicable since the study was on animals and not on humans.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the appropriate ethics committee of our Institution". The authors hereby declare that all experiments were examined and approved by the appropriate ethics committee and was therefore, performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Res. 2005;19:633-642.
2. Pierangeli GV, Windell LR. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (.F). King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. Extracts. J Med Plants Res. 2009;3(7):511-518.
3. Mbaka GO, Owolabi MA. Evaluation of haematinic activity and subchronic toxicity of *Sphenocentrum jollyanum* (Menispermaceae) seed oil. Euro J Med Plant. 2011; 1(4):140-152.
4. Tédong L, Dzeufiet PDD, Dimo T, Asongalem EA, Sokeng SN, Flejou JF, Callard P, Kamtchouing P. Acute and Subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr J Trad Alter Med. 2007;4(2):140-147.
5. Ogbonnia SO, Odimegwu JI, Enwuru VN. Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. And their mixture on streptozotocin (STZ)-induced diabetic rats. Afr J Biotechnol. 2008;7(15):2535-2539.
6. Ahsan MR, Islam KM, Haque ME, Mossaddik MA. *In vitro* antibacterial screening and toxicity study of some different medicinal plants. World J Agric Sci. 2009;5(5):617-621.
7. Elvin-Lewis M. Should we be concerned about herbal remedies? J Ethnopharmacol. 2001;75:141-164.
8. Chan K. Some aspect of toxic contaminants in herbal remedies. A review. Chemosphere. 2003;52:1361-1371.
9. Al-Kubaisy K, Al-Essa LY, Al-Jamain H, Shawagfeh MT. Hematological and biochemical evaluation of *Teucrium polium* in albino rats. Br J Pharm Toxicol. 2012;3(3):123-126.
10. Ndon BA. Some morphological and chemical characteristics of developing fruits of *Raphia hookeri*. J Exp Botany. 1985;36:817-830.
11. Mbaka GO, Ogbonnia SO, Oyeniran KJ, Awopetu PI. Effect of *Raphia hookeri* seed extract on blood glucose, glycosylated haemoglobin and lipid profile of alloxan induced diabetic rats. Br J Med Med Res. 2012;2(4):621-635.

12. Akachukwu CO. Production and utilization of wine palm (*Raphia hookeri*) Mann and Wendland). An important Weland Species Occasionally visited by honey bees. Proc Aquatic Sc. 2001:282-297.
13. Dalziel JM. In: The useful plants of West Africa. Crown agents for overseas governments and administration, London. 1955;15.
14. Muko KN, Ohiri PC, Ezegwu CO. Antipyretic and analgesic activities of *Sphenocentrum jollyanum*. Nig J Nat Prod and Medicine. 1998;2:52-53.
15. Nia R, Paper DH, Essien EE, Iyadi KC, Bassey AIL, Antai AB, Franz G. Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum jollyanum* Pierre. African J Biomed Res. 2004;7:129-132.
16. Moody JO, Roberts VA, Connolly JD, Houghton PJ. Anti- inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmacol. 2006;104:87-91.
17. ILAR, (Institute of Laboratory Animal Research). Commission on life science. National research council. www.edu/openbook. 1996; php?record_id=5140.
18. Miller LC, Tainter ML. Estimation of the LD_{50} and its error by means of logarithmic probit graph paper. Proc Soc Exp Biol Med. 1944;24:839-840.
19. Yemitan OK, Adeyemi OO. Toxicity study of the aqueous root extract of Lecaniodiscus cupanioides. Nig J Health Bio Sci. 2004;3:20-23.
20. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwe EM, Etoa FX, Ngongang. Evaluation of acute and subacute toxicities of aqueous ethanol extract of leaves of *Senna alata* (L.) Roxb (*Ceasal piniaceae*). Afr J Biotech. 2006;5(3):283-289.
21. Joshi CS, Priya ES, Venkataraman S. Acute and sub acute studies on the poly herbal anti diabetic formulation Diakyur in experimental animal model. J Health Sci. 2007;53(2):245-249.
22. Mythilypriya R, Shanthi P, Sachdanandam P. Oral acute and sub acute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats. J Health Sci. 2007;53(4):351-358.
23. Wasan KM, Najafi S, Wong J, Kwong M. Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound FMVP4, to gerbils. J Pharm Sci. 2001;4(3):228-233. www.ualberta.ca/~csps.
24. Crook MA. Clinical Chemistry and Metabolic Medicine. 7th edn. Hodder Arnold, London. 2006;426.
25. Sushruta K, Satyanarayana S, Srinivas N, Sekhar RJ. Evaluation of the blood–glucose reducing effects of aqueous extracts of the selected umbellifereous fruits used in culinary practice. Trop J Pharm Res. 2006;5(2):613-617.
26. Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Annal Chem. 1967;39:971-974.
27. Hussain A, Eshrat HM. Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa*, Linn and partially purified product from *Abroma augusta*, Linn. in streptozotocin induced diabetes. Indian J Clin Biochem. 2002;17(2):33- 43.
28. Koch TR, Doumas BT. Bilirubin, total and conjugated, modified Jendrassik Grot method. In selected methods of clinical chemistry vol. 9, edited by W Faulkner and S Meites Washington DC. Am Ass Clin Chem. 1982;113.
29. Spencer K, Price CP. Determination of serum albumin using Bromoscresol techniques. Annals Clin Biochem. 1971;14:105-115.
30. Ekaidem IS, Akpanabiatu MI, Uboh FE, Eka OU. Vitamin B12 supplementation: Effects on some biochemical and haematological indices of rats on phenytoin administration. Biokemistri. 2006;18(1):31-37.

31. Dacie JC, Lewis SM. Practical haematology 5th Edn, Churchill Livingstone, London; 1984.
32. Ogbonnia SO, Mbaka GO, Nwozor AM, Igbokwe HN, Usman A, Odusanya PA. Evaluation of microbial purity and acute and sub-acute toxicities of a Nigerian commercial polyherbal formulation used in the treatment of diabetes mellitus. British J Pharm Res. 2013;3(4):948-962.
33. Raza M, Al-Shabanah OA, El-Hadiyah TM, Al-Majed AA. Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Sci Pharm. 2002;70:135-145.
34. Teo S, Stirling D, Thomas S, Hoberman A, Kiores A, Khetani V. A 90-day oral gavage toxicity study of D-methylphenidate and D. L-Thethylphenidate in Sprague-Dawley rats. Toxicol. 2002;179:183-196.
35. Oyagbemi AA, Saba AB, Arowolo ROA. Safety evaluation of prolonged administration of stresroak in grower cockerels. International J Poultry Sci. 2008;7(6):1-5.
36. Teguia A, Telefo PB, Fotso RG. Growth performances, organ development and blood parameters of rats fed graded levels of steeped and cooked taro tuber (*Colocasia esculenta var esculenta*) meal. Livestock Res Rural Dev. 2007;19(6):1-7.
37. Coles EH. Veterinary clinical pathology. WB Saunders, Philadelphia, USA, 1986:10-42.
38. Ogbonnia SO, Mbaka GO, Igbokwe NH, Anyika EN, Alli P, Nwakakwa N. Antimicrobial evaluation, acute and subchronic toxicity studies of Leone Bitters, a Nigerian polyherbal formulation, inrodents. Agric Biol J N Am. 2010;1(3):366-376. Science Huβ. Available: http://www.scihub.org/ABJNA.
39. Li J, Xia Y, Kuter DJ. Interaction of thrombopoiethin with the platelet complements receptor in plasma: Binding, internalization, stability and pharmacokinetics. Brit J Haematol. 1999;106:345.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=508&id=14&aid=4449