

Research Article

Evaluation of Acute and Sub-chronic Toxicities of Aqueous Ethanol Root Extract of *Raphia hookeri* (Palmaceae) on Swiss Albino Rats

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Abstract: This study evaluated the acute and sub-chronic toxicities of treatment with aqueous ethanol root extract of *Raphia hookeri* (Palmaceae) on rats. In acute toxicity study, the root extract in a graded doses of 125-2000 mg/kg bwt administered Intra-Peritoneal (IP) produced dose dependent mortality with median acute toxicity (LD₅₀) of approximately 562.3 mg/kg bwt. The animals fed with the extract by gavages tolerated up to 4000 mg/kg body weight (bwt) with no sign of physical/behavioural changes hence 1/20th of the dose (200 mg/kg) was used as the highest therapeutic dose. In sub-chronic toxicity study, significant increase (p<0.05) in the animals' body weight occurred at treatments with 50 and 100 mg/kg bwt doses whereas considerable decrease was observed at treatment with 200 mg/kg bwt extract dose. Significant increase (p<0.05) in aspartate aminotransferase (AST) level occurred compared to the control while alanine aminotransferase (ALT) showed decrease that was marked at the highest extract dose. Total protein was comparatively the same with the control while total bilirubin decreased markedly. In kidney function profile, creatinine level showed decrease while urea level exhibited fluctuation. There was an insignificant (p>0.05) decrease in Red Blood Cell (RBC) count and haemoglobin (Hb) level while White Blood Cell (WBC) showed increase. In tissue analysis, the extract caused marked deleterious effect on the testes leading to drastic reduction in sperm cells whereas tissues of liver, kidney and heart however showed normal appearance.

Keywords: Acute toxicity, *Raphia hookeri*, sub-acute toxicity, tissue histology

INTRODUCTION

The rising popularity of herbal medicines is based on their observed effectiveness in the treatment and prevention of diseases. This belief is attributed to the fact that herbal medicines are 'natural' and therefore safe. There is also the growing disillusionment with modern medicine due to lack of treatment success in many instances and unfavorable side effects. It has also been observed that public dissatisfaction with the cost of prescription medications combined with interest in returning to natural or organic remedies have led to an increase in herbal medicine use (Gbéassor *et al.*, 1989; Pierangeli and Windell, 2009). Furthermore, most countries do not impose prescription regulations upon herbal preparations, therefore, access to this kind of therapy is unrestricted and cheap (Rojas *et al.*, 2006). The lack of regulation in particular seemed to have greatly influenced herbal medicinal use appeal. The

major consequence however is a resultant indiscriminate use without appropriate dose thereby undermining the greater potential for adverse effect. Incidences of renal and hepatic toxicity resulting from the long time ingestion of medicinal herbs have been documented (Tédong *et al.*, 2007; Ogbonnia *et al.*, 2008). There is therefore apparent need for scientific documentation of information on the safety/toxic risk potentials of medicinal plants.

Raphia Hookeri (RH) is a member of the family Palmaceae. This plant is commonly found in West Africa and in abundance particularly in South Eastern Nigeria and usually grows up to 12 m high. It is one of the most important sources of forest food species in Southern Nigeria. The plant is found within the fresh water swamp forest and is adapted to life with its roots in water logged soil possessing breathing property (Akachukwu, 2001). The root of RH is used in traditional medicine in the treatment and prevention of

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various diseases (Akpan *et al.*, 1996). The cool root extract is normally given to infants with stomach pain (Akpan *et al.*, 1996). The effect of the root extract on the plasma level of ethanol has been observed in acute and chronic intoxication in humans where it was noted to be effective in the treatment of alcoholic intoxication (Akpan and Usoh, 2004). An investigation has also revealed the root extract to exhibit significant anti-diabetic property (Mbaka *et al.*, 2012a). Although the root of RH has many useful health benefits, to our knowledge no literature exists on its toxicity profile. This study was therefore undertaken to evaluate the acute and sub-chronic toxicities of the ethanolic extract of the root.

MATERIALS AND METHODS

The roots of *Raphia hookeri* were obtained in the month of November 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).

Preparation of the aqueous ethanol root extract of RH:

The roots were washed and dried before being subjected to size reduction to a coarse powder with electric grinder. The root powder, 810 g, was extracted with 95% aqueous alcohol in three cycles using Soxhlet extractor. The crude extract was filtered with filter paper (Whatman No. 4) and the filtrate was dried by rotary evaporator at 30°C to obtain 103 g dry residue (12.7% w/w) which was stored in an air tight bottle kept in a refrigerator at 4°C till used.

Animals: Wistar rats weighing between 130-145 g of either sex obtained from the Animal House of the University of Ibadan, Oyo State, Nigeria, were kept under standard environmental condition of 12/12 hr (light/dark cycle). A total of 20 rats were obtained and housed in polypropylene cages (5 animals per cage) and were maintained on mouse chow (Livestock Feeds Nigeria Ltd), 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 (ILAR, 1996).

Acute toxicity study: A total of 15 mice fasted for 14 h were administered with Rh seed extract dispersed in acacia solution (2%w/v) intraperitoneally in graded doses of 125, 250 and 500 mg/kg of five mice per group until 100% mortality was recorded. The animals received the extract at the doses of 125, 250 and 500 mg/kg body weight (bwt). The control group of five mice was given 0.3 mL/kg body weight of acacia solution orally. LD₅₀ was calculated using the method of Miller and Tainter (1944). Another group of five mice fasted for 14 hrs were administered a single dose

of 4000 mg/kg of RH root extract orally and then observed for seven days for mortality and physical/behavioural changes. The animals did not show any mortality at the dose administered hence its 1/20th dose (200 mg/kg bwt) was taken as the highest extract dose together with lower graded doses of 100 and 50 mg/kg bwt were used as the therapeutic doses (Mbaka *et al.*, 2012b).

Sub-chronic toxicity study: A total of 20 male rats were randomly allotted to the control and the extract treated groups. The gel suspension (12%w/v) was prepared by dispersing the gel (12 g) with 45 mL of acacia (2%) solution in a beaker and transferred to a 100 mL volumetric flask. Then the beaker was rinsed with the solution and the content transferred to the volumetric flask and volume made to mark with the acacia solution. After fasting the animals overnight the control group received a dose of 0.6 mL acacia (2%w/v) solution and the treated received 50, 100 and 200 mg/kg of the extract dispersed in acacia (2%w/v) solution. The doses were administered orally daily for a period of 30 days (Pieme *et al.*, 2006; Joshi *et al.*, 2007; Mythilypriya *et al.*, 2007). 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.

Biochemical parameters: The animals were sacrificed under mild diethyl ether and thereafter 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 4000 rpm for 10 min to obtain plasma, which was analyzed for total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-cholesterol) levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan *et al.*, 2001). Plasma was analyzed for alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity and creatinine by standard enzymatic assay methods (Sushruta *et al.*, 2006). Urea was determined according to Urease-Berthelot method (Ekaidem *et al.*, 2006). The protein content was determined using enzymatic spectroscopic methods (Hussain and Eshrat, 2002). Total bilirubin was estimated using Jandrossik and Grof technique (Sherwin and Thompson, 2003). Albumin was determined based on its reaction with bromocresol green (Binding method) (Ekaidem *et al.*, 2006).

Haematological parameters: Haematocrit was estimated using the methods of Ekaidem *et al.* (2006). Haematocrit tubes were filled to mark with whole blood and the bottom of the tubes sealed with plasticide and centrifuged for 4-5 min 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. The blood samples were analyzed for Red Blood Cells (RBC) by haemocytometric method (Dacie and Lewis, 1995). Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem *et al.*, 2006). Haematocrit (HCT) was determined according to Ekaidem *et al.* (2006) while White Blood Cells (WBC) and its differentials (neutrophil, eosinophil, basophil, lymphocyte and monocyte) were determined as described by Dacie and Lewis (1995). The blood samples were analyzed for Red Blood Cells (RBC) by haemocytometric method (Dacie and Lewis, 1995).

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

Each organ tissue was sectioned at 5 µm and stained with Haematoxylin and Eosin (H and E) stain (Mbaka and Owolabi, 2011). The stained tissues were examined under PoTop© (Taiwan) light microscope at high power magnification X400 for changes in organ architecture and photomicrographs were taken.

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.

RESULTS

The *R. hookeri* aqueous ethanolic root extract following Intra-Peritoneal (IP) administration produced

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

Dose	Motility	% Motility	Log dose	Probit	Probit approximate
125	1/5	20	2.0969	4.1584	4.2
250	1/5	20	2.3979	4.1584	4.2
500	2/5	40	2.6989	4.7467	4.7
1000	3/5	60	3.0000	5.2533	5.3
2000	4/5	80	3.3010	5.8416	5.8

Table 2: Data on the organ weight (100 g body weight) of rats after sub-chronic treatment with aqueous ethanol extract of *Hookeri* root

Treatment	Mean organ weight per 100 g body weight				
	Heart	Lung	Liver	Kidney	Testes
Control	0.3±0.2	2.1±0.5	5.8±0.3	1.4±0.3	2.7±0.4
50 mg/kg	0.5±0.5	2.1±0.2	4.4±0.7	1.5±0.1	3.7±0.3
100 mg/kg	0.4±0.1	2.1±0.2	3.7±0.2	1.4±0.6	3.2±0.2
200 mg/kg	0.4±0.4	2.2 ±0.1	3.4±0.2	1.4±0.1	3.3±0.5

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

Table 3: Blood chemistry of rats after sub-chronic treatment with aqueous ethanol extract of *R. hookeri* root

Parameter	Control	50	100	200
Total protein (mg/dL)	65.9±1.9	66.1±0.5	66.5±2.1	65.2±2.2
Albumin (mg/dL)	36.0±2.2	37.9±1.3	33.5±3.9	39.1±3.6
Total bilirubin (mg/dL)	1.2±1.1	0.6±0.0**	0.6±0.0**	0.6±0.0**
AST (iµ/L)	135.4±4.2	141.1±2.9**	151±3.1*	169.9±4.2*
ALT (iµ/L)	59.2±4.3	59.9±4.0	53.9±5.0	37.6±4.4*
Alkaline phosphatase (iµ/L)	161.5±4.4	164.6±5.9	161.4±4.0	146.3±8.5*
Urea (mg/dL)	9.3±1.6	9.7 ±1.5	7.8±0.2	9.4±0.6
Creatinine (mg/dL)	41.1±4.2	41.1±4.2	50.0±0.5**	43.8±3.8
Total cholesterol (mg/dL)	90.0 ±0.2	61.5±0.0 *	53.8±0.1*	51.4±0.1*
Triglycerides (mg/dL)	64.2±0.4	62.7±0.1	50.6±1.2*	50.2±0.2*
HDL-cholesterol (mg/dL)	41.1±0.1	44.4± 0.0	49.1±0.1**	51.0±1.1*

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

Table 4: Haematological values of rats after sub-chronic treatment with aqueous ethanol extract of *R. hookeri* root

Treatment	RBC (x10 ⁶)	Hb (g/dL)	HCT (%)	WBC (x10 ³)	MCV (fl)	MCH (%)	MCHC (%)
Control	7.0±0.9	11.5±1.0	39.9±0.9	4.6±0.2	56.2±1.2	16.1±2.1	28.3±1.1
50 mg/kg	6.1±0.8	11.1±0.3	36.8±1.0**	5.2±0.2	60.1±2.1	16.1±2.1	31.4±1.1
100 mg/kg	6.3±0.4	11.1±1.5	36.6±1.8**	7.7±1.4**	57.7±3.1	17.5±0.6	30.2±1.0
200 mg/kg	5.7±0.8	10.0±2.5	44.5±2.5	5.9±0.5	59.0±7.3**	18.4±2.1**	29.9±0.9

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

Table 5: Quantitative data on WBC differentials of rats after sub-chronic treatment with aqueous ethanol extract of *R. hookeri* root

Treatment	Neutrophil %	Lymphocyte %	Platelet %
Control	0.3±0.1	62.6±0.5	70.0±1.6
50 mg/kg	1.0±0.4**	75.5±4.0*	89.4±5.8*
100 mg/kg	1.7±0.6*	73.1±1.8*	89.4±7.6*
200 mg/kg	1.4±0.3**	71.9±4.2*	76.7±4.2**

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

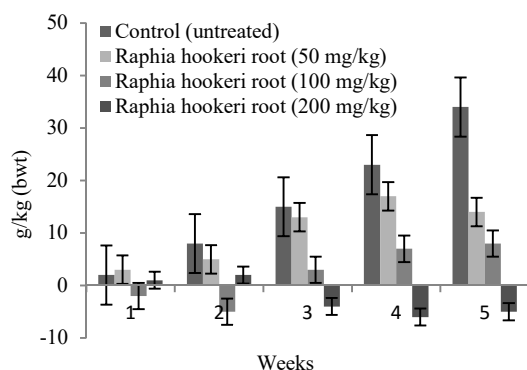


Fig. 1: Weight differential in control and treated animals

dose dependent mortality with median lethal dose (LD_{50}) of approximately 562.3 mg/kg bwt (Table 1). The rats fed with the extract by gavages tolerated up to 4000 mg/kg bwt with no sign of physical/behavioural changes hence 200 mg/kg bwt (1/20th of the dose) was used as the highest therapeutic dose and two lower graded doses of 100 and 50 mg/kg bwt, respectively.

The weight gain compared to the initial body weight of the animal is shown in Fig. 1. Increase in the animals' body weight occurred in the two lower doses whereas considerable decrease was observed in the highest extract dose. Table 2 showed the organ weights of the treated and the control group with no colour changes compared to the control. Marginal weight increase occurred on the heart and the testes while decrease was observed on the liver. In Table 3 are the results of the biochemical studies. In liver function profile, there was significant increase ($p < 0.05$) in AST level compared to the control whereas ALT showed decrease which was marked at the highest extract dose. Total protein was comparatively the same while total bilirubin decreased markedly compared to the control. There was fluctuation in the albumin level which showed marked decrease ($p < 0.01$) at 100 mg/kg bwt compared to the control. In kidney function profile, creatinine level showed decrease that was appreciable at the lowest and medium extract doses compared to the control whereas urea level exhibited fluctuation. In lipid profile study, total cholesterol and triglycerides levels decreased with increase in extract doses while HDL cholesterol level increased with increase in extract dose. Alkaline phosphatase exhibited fluctuation in level.

The summary of the effect of the extract on the haematological parameters of the animals is shown in Table 4. There was insignificant ($p > 0.05$) decrease in RBC count while Hb and HCT levels exhibited fluctuation compared to the control. On the other hand, the MCV, MCHC and MCH levels showed marginal increase compared to the control. Also, WBC count increased marginally compared to the control. Table 5 showed the effect of the extract on the WBC differentials. There was significant ($p < 0.05$) increase in the levels of neutrophil, lymphocytes, mixed differential and platelets compared to the control.



Fig. 2a: Photomicrograph of a cross section of hepatic tissue of the control showing portal tract (red arrowed) and normal hepatocytes separated by ill-defined sinusoids. (H and E stain) Mag. X100



Fig. 2b: Photomicrograph of a cross section of hepatic tissue administered with 200 mg/kg bwt of the extract showing no pathological changes. Mag. X100



Fig. 3a: Photomicrograph of treatment with 200 mg/kg bwt extract dose showing dense spermatogonia cell layer at the basement (green arrowed). The central portion contains scanty spermatozoa (red arrowed). (H&E stain) Mag. X100



Fig. 3b: 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 (green arrowed) while the spermatozoa clustered in the centre (red arrowed) (H&E stain) Mag. X100

Tissue histology: The photomicrograph of normal hepatic tissue (Fig. 2a) 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 indistinct hepatic sinusoids. In Fig. 2b, no abnormal change was observed. Figure 3a showed the cyto-architecture of a cross section of normal testicular tissue

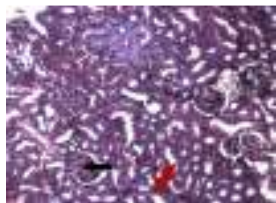


Fig. 4a: Photomicrograph of a cross section of the cortical region of renal tissue (untreated) indicating renal corpuscles (black arrowed) and convoluted tubules (red arrowed). (H&E stain) Mag. X100

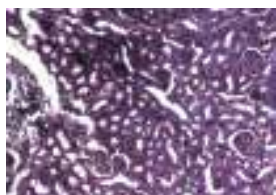


Fig. 4b: Photomicrograph of a cross section of cortical region of the renal tissue administered with 200 mg/kg bwt of the extract showing no pathological changes. Mag. X100



Fig. 5a: Photomicrograph of a cross section of myocardium (heart) showing darkly spotted myocytes (thin arrowed) separated by an unremarkable interstitium (H and E stain) Mag. X100



Fig. 5b: Photomicrograph of a cross section of myocardium of treatment with 200 mg/kg bwt showing no lesion. (H and E stain) Mag. X100

indicating the seminiferous tubules in transverse plane with distinct boundary. Close to the basement of the epithelium were primitive spermatogenic cell series compactly arranged while the tails of matured sperm cells show wavy appearance towards the lumina. The extract administration at 200 mg/kg bwt (Fig. 3b) caused marked deleterious effect on the testes resulting to testicular cell mass depletion. The basement cell layers (primitive sperm cell) showed intact formation. However, the matured sperm cells were largely scanty

at the luminal. Figure 4a showed the normal photomicrograph of renal tissue indicating the cortical area containing the renal corpuscles that appeared as a dense rounded mass. It is separated from the surrounding structures by Bowman's space. The photomicrograph of the extract treated (Fig. 4b) showed normal appearance. Figure 5a showed the photomicrograph of normal cardiac tissue in which the muscle fibres were branched to give the appearance of three dimensional networks. Also indicated were the nuclei deeply stained. The photomicrograph of the extract treated (Fig. 5b) showed normal appearance.

DISCUSSION

Plants have varied secondary products acting as active principles; the type and the concentration found in the diet and the metabolic clearance rate in the body are factors that may likely influence the toxicity of the plant. The root extract of RH is shown to be rich in tannins, flavonoids, triterpenes, saponins, polyphenols, alkaloids and cardiac glycosides (Akpan and Usoh, 2004). The acute toxicity study suggests that the root extract has high safety margin since the animals tolerated up to 4000 mg/kg bwt by gavages translating to 280 g equivalence dose in human adult (Ogbonnia *et al.*, 2013). According to World Health Organization (WHO) toxicity index of 2 g/kg bwt (WHO, 1966), the extract could be considered to be relatively safe for consumption.

In sub-chronic toxicity study, there was a significant increase in the animals' body weight at the lower extract doses while decrease occurred at the highest extract dose compared to their initial body weight. The internal organs showed weight variation compared to the control. The heart and the testes of the extract treated exhibited increase in weight while the liver showed decrease in weight compared to the control. The gross anatomy of the organs however revealed no detectable colour changes. There are reports that reduction in body and organ weights are sensitive indices of toxicity after exposure to toxic substance (Raza *et al.*, 2002; Teo *et al.*, 2002; Thanabhorn *et al.*, 2006). Significant increase occurred in AST at all doses while ALT showed appreciable decrease in level which was more marked at the highest extract dose. These two enzymes are vital in establishing hepato-toxic index. In toxic environment, the activity of the two enzymes in the blood stream is known to increase significantly (Crook, 2006). Although AST level increased markedly, ALT which is more specific to the liver decreased appreciably thus suggesting that the high level of AST in the extract treated may not have resulted from liver damage, more so, hepatic tissue morphology showed no detectable inflammatory changes. Total protein and the albumin showed increase in level with albumin indicating more marked increase, the increase in albumin point to the fact that the extract helped to prevent oxidative damage to the liver. Increase in albumin and total protein is

reported to have hepato-protective effect (Oyagbemi *et al.*, 2008). Creatinine and the urea which are end-products of protein metabolism showed insignificant increase indicating that the risk of potential inflammatory challenge was minimal. The renal tissue histology also showed normal appearance invariably buttressing that the kidney did not compromise its function.

In lipid profile study, the decrease in total cholesterol and triglycerides demonstrated the presence of hypolipidaemic agent in the extract. The extract equally exhibited marked increase in HDL-cholesterol an indication that it has the tendency to minimize cardiovascular risk factor a major contributor of death in diabetes (Barnett and O'Gara, 2003; Ogbonnia *et al.*, 2014). The beneficial effect of the extract on lipid profile accounts for its use in the treatment diabetes and diabetic complications (Mbaka *et al.*, 2012a, b).

It appeared the extract interfered with the integrity of the testes because there was a considerable quantitative decrease in sperm cells in the seminiferous tubules particularly within the lumina where more remarkable decrease was observed. Despite the reduction of sperm cells, the testes exhibited weight increase which suggested other inflammatory changes. It is therefore most likely that the use of the extract as therapeutic agent might pose a threat to male fertility.

There was decrease in RBC, Hb and HCT value suggesting decrease in erythropoiesis and the oxygen carrying capacity of blood and the amount of the oxygen delivered to the tissues (De Gruchy, 1976). The decrease in Hb level also suggested the likelihood of decrease in iron absorption. There was however an increase in RBC indices, MCV, MCH and MCHC noted to be of unique importance in anaemia diagnosis in most animals (Coles, 1986). Increase in these parameters showed that macrocytic anaemia occurred which is believed to be linked to iron deficiency anaemia (Agbor *et al.*, 1999). The decrease in RBC indices is further confirmatory that the extract has the tendency of potentiating iron induced anaemia. The level of WBC also increased appreciably. In toxic environment WBC is said to increase significantly to boost the body immune system (Teguia *et al.*, 2007). There was also marked increase in lymphocyte the main effectors cell of the immune system (Teguia *et al.*, 2007). The stimulatory increase in lymphocyte may have been triggered by the extract toxic challenge on the internal organs particularly in the testis which in this case was most vulnerable. In a similar manner, there was a percentage increase in neutrophil indicating that the WBC differential was active as phagocytic agent against foreign compounds.

CONCLUSION

RH root extract exhibited high safety margin which is an indication that it could be safe for consumption. The extract showed beneficial effect on the lipid profile which may account for its use in the management of

diabetes and its complications. However it appears the use of the extract as a therapeutic agent may pose a threat to male fertility.

REFERENCES

- Agbor, G.A., J.E. Oben, D.C. McKnight, R.G. Mills, J.J. Bray and P.A. Crag, 1999. Human Physiology. 4th Edn., Churchill Livingstone, USA, pp: 290-294.
- Akachukwu, C.O., 2001. 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.
- Akpan, E.J. and I.F. Usuh, 2004. Phytochemical screening and effect of aqueous root extract of *Raphia hookeri* (raffia palm) on metabolic clearance rate of ethanol in rabbits. Biokemistri, 16(1): 37-42.
- Akpan, E.J., E.O. Akpanyung and D.O. Edem, 1996. Antimicrobial properties of the mesocarp of the seed of *Raphia hookeri*. Nig. J. Biochem. Mol. Biol., 11: 89-93.
- Barnett, H.A. and G. O'Gara, 2003. Diabetes and the Heart. Clinical Practice Series. Churchill Livingstone, Edinburgh, UK.
- Coles, E.H., 1986. Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, USA, pp: 10-42.
- Crook, M.A., 2006. Clinical Chemistry and Metabolic Medicine. 7th Edn., Hodder Arnold, London, pp: 426.
- Dacie, J.C. and S.M. Lewis, 1995. Practical Haematology. 7th Edn., Churchill Livingstone, Edinburgh, pp: 12-17.
- De Gruchy, G.C., 1976. Clinical Haematology in Medical Practice. Blackwell Scientific Publication, Oxford, London, pp: 33-57.
- Ekaidem, I.S., M.I. Akpanabiatu, F.E. Uboh and O.U. Eka, 2006. Vitamin B12 supplementation: Effects on some biochemical and haematological indices of rats on phenytoin administration. Biokemistri, 18(1): 31-37.
- Gbéassor, M., Y. Kossou, K. Amegbo, K. Koumaglo and A. Denke, 1989. Antimalarial effects of eight African medicinal plants. J. Ethnopharmacol., 25: 115-118.
- Hussain, A. and H.M. Eshrat, 2002. 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., 17(2): 33-43.
- ILAR, (Institute of Laboratory Animal Research), 1996. Commission on Life Science. National Research Council, Retrieved from: www.edu/openbook.php?record_id=5140.
- Joshi, C.S., E.S. Priya and S. Venkataraman, 2007. Acute and sub acute studies on the polyherbal antidiabetic formulation Diakyur in experimental animal model. J. Health Sci., 53(2): 245-249.

- Mbaka, G.O. and M.A. Owolabi, 2011. Evaluation of haematinic activity and subchronic toxicity of *Sphenocentrum jollyanum* (Menispermaceae) seed oil. Eur. J. Med. Plant, 1(4): 140-152.
- Mbaka, G.O., S.O. Ogbonnia and A.E. Banjo, 2012a. Activity of *Raphia hookeri* root extract on blood glucose, lipid profile and glycosylated haemoglobin on alloxan induced diabetic rats. J. Morphol. Sci., 29(3): 1-9.
- Mbaka, G.O., S.O. Ogbonnia, K.J. Oyeniran and P.I. Awopetu, 2012b. Effect of *Raphia hookeri* seed extract on blood glucose, glycosylated haemoglobin and lipid profile of alloxan induced diabetic rats. Br. J. Med. Med. Res., 2(4): 621-635.
- Miller, L.C. and M.L. Tainter, 1944. Estimation of the LD₅₀ and its error by means of logarithmic probit graph paper. P. Soc. Exp. Biol. Med., 24: 839-840.
- Mythilypriya, R., P. Shanthi and P. Sachdanandam, 2007. Oral acute and sub acute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. J. Health Sci., 53(4): 351-358.
- Ogbonnia, S.O., J.I. Odimegwu and V.N. Enwuru, 2008. Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treulia africana* Decne and *Bryophyllum pinnatum* Lam. And their mixture on streptozotocin (STZ)-induced diabetic rats. Afr. J. Biotechnol., 7(15): 2535-2539.
- Ogbonnia, S.O., G.O. Mbaka, A.M. Nwozor, H.N. Igbokwe, A. Usman and P.A. Odusanya, 2013. Evaluation of microbial purity and acute and subacute toxicities of a Nigerian commercial polyherbal formulation used in the treatment of diabetes mellitus. Brit. J. Pharm. Res., 3(4): 948-962.
- Ogbonnia, S.O., G.O. Mbaka, F.E. Nkemehule, J.E. Emordi, N.C. Okpagu and D.A. Ota, 2014. Acute and subchronic evaluation of aqueous extracts of *Newbouldia laevis* (Bignoniaceae) and *Nauclea latifolia* (Rubiaceae) roots used singly or in combination in Nigerian traditional medicines. Br. J. Pharmacol. Toxicol., 5(1): 55-62.
- Oyagbemi, A.A., A.B. Saba and R.O.A. Arowolo, 2008. Safety evaluation of prolonged administration of stresroak in grower cockerels. Int. J. Poult. Sci., 7(6): 1-5.
- Pieme, C.A., V.N. Penlap, B. Nkegoum, C.L. Taziebou, E.M. Tekwe, F.X. Etoa and J. Ngongang, 2006. Evaluation of acute and subacute toxicities of aqueous ethanol extract of leaves of *Senna alata* (L.) Roxb (*Ceasalpinaceae*). Afr. J. Biotechnol., 5(3): 283-289.
- Pierangeli, G.V. and L.R. Windell, 2009. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f). King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts. J. Med. Plants Res., 3(7): 511-518.
- Raza, M., O.A. Al-Shabanah, T.M. El-Hadiyah and A.A. Al-Majed, 2002. Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Sci. Pharm., 70: 135-145.
- Rojas, J.J., V.J. Ochoa, S.A. Ocampo and J.F. Munoz, 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine. A possible alternative in the treatment of nonnosocomial infections. BMC Complem. Altern. M., 6: 2-8.
- Sherwin, J.E. and C. Thompson, 2003. Liver Function. In: Kaplan, L.A., A.J. Pesce and S.C. Kazmierczak (Eds.), Clinical Chemistry: Theory, Analysis, Correlation. 4th Edn., Mosby Inc., EDS St Louis, USA, pp: 493.
- Sushruta, K., Satyanarayana, N. Srinivas and R.J. Sekhar, 2006. Evaluation of the blood-glucose reducing effects of aqueous extracts of the selected umbelliferous fruits used in culinary practice. Trop. J. Pharm. Res., 5(2): 613-617.
- Tédong, L., P.D.D. Dzeufiet, T. Dimo, E.A. Asongalem, S.N. Sokeng, J.F. Flejou, P. Callard and P. Kamtchouing, 2007. Acute and Subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. Afr. J. Tradit. Complem., 4(2): 140-147.
- Teguia, A., P.B. Telefo and R.G. Fotso, 2007. Growth performances, organ development and blood parameters of rats fed graded levels of steeped and cooked taro tuber (*Esculenta*) meal. Livest. Res. Rural Dev., 19(6): 1-8.
- Teo, S., D. Stirling, S. Thomas, A. Hoberman, A. Kiores and V. Khetani, 2002. A 90-day oral gavage toxicity study of D-methylphenidate and D.L-Thethylphenidate in sprague-dawley rats. Toxicology, 179: 183-196.
- Thanabhorn, S., K. Jenjoy, S. Thamaree, K. Ingkaninan and A. Panthong, 2006. Acute and subacute toxicity study of the ethanol extract from *Lonicera japonica* Thunb. J. Ethnopharmacol., 107: 370-373.
- Wasan, K.M., S. Najafi, J. Wong and M. Kwong, 2001. 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., 4(3): 228-233.
- WHO, 1966. Specifications for identity and purity and toxicological evaluation of food colours. WHO/Food Add/66.25 Geneva.