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 Intraperitoneal (IP) produced dose dependent mortality with median acute toxicity (LD$_{50}$) 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 whereas total bilirubin decreased markedly. In kidney function profile, creatinine level showed decrease while urea level exhibited fluctuation. There was no significant (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 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 (Mbak 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 (FHL/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 hr 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. LD<sub>50</sub> 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 (Mbak 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 sacrifice 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 Jandrassik 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

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

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Motility</th>
<th>% Motility</th>
<th>Log dose</th>
<th>Probit</th>
<th>Probit approximate</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>1/5</td>
<td>20</td>
<td>2.0969</td>
<td>4.1584</td>
<td>4.2</td>
</tr>
<tr>
<td>250</td>
<td>1/5</td>
<td>20</td>
<td>2.3979</td>
<td>4.1584</td>
<td>4.2</td>
</tr>
<tr>
<td>500</td>
<td>2/5</td>
<td>40</td>
<td>2.6989</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>3/5</td>
<td>60</td>
<td>3.0000</td>
<td>5.2533</td>
<td>5.3</td>
</tr>
<tr>
<td>2000</td>
<td>4/5</td>
<td>80</td>
<td>3.3010</td>
<td>5.8416</td>
<td>5.8</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50 mg/kg</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>65.9±1.9</td>
<td>66.1±0.5</td>
<td>66.5±2.1</td>
<td>65.2±2.2</td>
</tr>
<tr>
<td>Lung</td>
<td>36.0±2.2</td>
<td>37.9±1.3</td>
<td>33.5±3.9</td>
<td>39.1±3.6</td>
</tr>
<tr>
<td>Liver</td>
<td>12.2±1.1</td>
<td>14.1±4.2</td>
<td>151±3.1*</td>
<td>169.9±4.2*</td>
</tr>
<tr>
<td>Kidney</td>
<td>59.2±4.3</td>
<td>65.9±4.0</td>
<td>53.9±5.0</td>
<td>37.6±4.4*</td>
</tr>
<tr>
<td>Testes</td>
<td>9.3±1.6</td>
<td>9.7±1.5</td>
<td>7.8±0.2</td>
<td>9.4±0.6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>50 mg/kg</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/dL)</td>
<td>41.1±2.4</td>
<td>41.1±2.4</td>
<td>50.0±0.5*</td>
<td>43.8±3.8</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>9.3±1.6</td>
<td>9.7±1.5</td>
<td>8.7±0.2</td>
<td>9.4±0.6</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>64.2±0.4</td>
<td>62.7±0.1</td>
<td>50.6±1.2</td>
<td>50.2±0.2*</td>
</tr>
<tr>
<td>AST (μ/L)</td>
<td>9.3±1.6</td>
<td>9.7±1.5</td>
<td>7.8±0.2</td>
<td>9.4±0.6</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>41.1±2.4</td>
<td>41.1±2.4</td>
<td>50.0±0.5*</td>
<td>43.8±3.8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBC (x10²)</th>
<th>HB (g/dL)</th>
<th>HCT (%)</th>
<th>WBC (x10³)</th>
<th>MCV (fL)</th>
<th>MCH (%)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.1±2.4</td>
<td>9.3±1.6</td>
<td>41.1±2.4</td>
<td>50.0±0.5*</td>
<td>43.8±3.8</td>
<td>31.4±1.1</td>
<td>34.8±1.1</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>41.1±2.4</td>
<td>9.3±1.6</td>
<td>41.1±2.4</td>
<td>50.0±0.5*</td>
<td>43.8±3.8</td>
<td>31.4±1.1</td>
<td>34.8±1.1</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>41.1±2.4</td>
<td>9.3±1.6</td>
<td>41.1±2.4</td>
<td>50.0±0.5*</td>
<td>43.8±3.8</td>
<td>31.4±1.1</td>
<td>34.8±1.1</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>41.1±2.4</td>
<td>9.3±1.6</td>
<td>41.1±2.4</td>
<td>50.0±0.5*</td>
<td>43.8±3.8</td>
<td>31.4±1.1</td>
<td>34.8±1.1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neutrophil %</th>
<th>Lymphocyte %</th>
<th>Platelet %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3±0.1</td>
<td>62.6±0.5</td>
<td>70.0±1.6</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>1.0±0.4**</td>
<td>75.5±4.0</td>
<td>89.4±5.8*</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>1.7±0.6*</td>
<td>73.1±1.8*</td>
<td>89.4±5.8*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>1.4±0.3**</td>
<td>71.9±4.2*</td>
<td>76.7±4.2**</td>
</tr>
</tbody>
</table>

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

**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 cytoarchitecture of a cross section of normal testicular tissue.
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 hepatotoxic index. In toxic environment, the activity of the two enzymes in the bloodstream 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 indicative of hepatic cell injury. This could be due to the high level of AST in the extract treated.
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; Ogbonna 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


