ANTIMICROBIAL AND TOXICOLOGICAL STUDIES OF RICINODENDRON HEUDELOTII (BAILL.)

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INTRODUCTION

Herbal plants, known to contain countless biologically active compounds [1], have been utilized for the management and cure of various ailments throughout human history. Above 50% of all contemporary drugs are from natural products, which play a significant role in drug evolution programs [2]. Nature has been the significant role in drug evolution programs [2]. Nature has been the origin of healing agents over the years. Medicinal plants, for many centuries and today, have been used for curing different types of diseases in virtually all cultures [3]. Ricinodendron heudelotii (Ball.) is a member of the Euphorbiaceae family. It is a broad-growing secondary forest tree native to West and Central Africa [4]. It is traditionally called “Njansang” in Cameroon and “Okwe” in South East Nigeria [5]. Ricinodendron heudelotii has been used in folk tradition for treating cough, yellow fever, anemia, malaria, stomach pain, and intestinal disease and used as a poison neutralizer [6,7]. A blend of the bark of R. heudelotii has been reported to stimulate sexual desires and increase passing out of urine in some parts of Cameroon [6]. Bark extracts are also used to prevent abortion [8,9]. Its leaves are used to treat looseness of the bowels while the fruits are used as seasoning [7]. It is cultivated by farmers for topsoil fertility advancement, light wood work, shades, and pasturage [10]. The seed is often used as a thickener in soup making [5]. The seeds have been reported to contain hydrogen cyanide, tannin, alkaloid, phenol, saponin, and flavonoid [5]. The effectiveness of existing antibiotics has been challenged by the advent of drug-resistant pathogens, thus making antibiotic resistance to be a global concern [11]. Thus, in view of the fact that adequate scientific information regarding the use of the plant is lacking, this makes preclinical toxicological and therapeutic studies important. This study is therefore focused on exploring the biochemical, antimicrobial, histological, and hematological effects of R. heudelotii in rats.

METHODS

Plant material

The leaves were collected on October 2015 in Covenant University Ota, Ogun, Nigeria, and certified by Dr. J.O. Popeolu of the Department of Biological Sciences, Covenant University, Ota, Nigeria. Sample of the leaf was deposited herbarium section of the Forest Research Institute of Nigeria with voucher no FHI 110573. Leaves were dried at room temperature (25°C) and grinded using an electric blender into coarse powder. These powdered samples were sealed in plastic bags until needed for the study.

Microorganisms

The selected bacterial strains for the current study were Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella sp., Escherichia coli (Gram-negatives), Staphylococcus aureus, Bacillus sp., Micrococcus sp., Streptococcus faecalis, and Salmonella sp. (Gram-positive) while the fungal strain was Candida albicans. They were all obtained from culture collection center, Department of Biological Sciences, Covenant University, Ota. All organisms were sustained on nutrient broth (NB) at 37°C and fungus on potato dextrose agar (PDA) at a temperature of 28°C.

Experimental animals and housing

In this study, 35 male adult albino Wistar rats having weight between 170 and 200 g were purchased from Lagos University Teaching Hospital,
Lagos, Nigeria, and kept at a maintained temperature and fed with rat
chow (Graceline Feeds Ota, Ogun State) along with water. The rats were
permitted to adapt to the environment for 2 weeks before the inception
of the experiment. This study was approved by the Biological Sciences
Research Ethics Committee, Covenant University. All experimental rats
were managed in adherence to the rules of the National Institute of
Health for the use and care of laboratory animals [12].

Extraction and phytochemical screening of the plant
The powdered leaf samples (580 g) were extracted by maceration with
3 L of 95% ethanol (Sigma-Aldrich) and kept at room temperature for
3 days. The solution was stirred daily for thorough mixing and sieved
thereafter to obtain the filtrate which was further condensed at reduced
temperature of 40°C and pressure through a rotary evaporator [13].
The yield of the plant extract obtained was 1.38%.

The ethanolic extract was exposed to preliminary phytochemical
tests to ascertain the specific phytoconstituents contained in the leaf
such as tannins, alkaloids, glycosides, steroids, flavonoids, terpenoids,
saponins, anthocyanin, and phenols. Such tests were determined
by the typical color change following guidelines as described by
Harborn [14] and Sofowora [15].

Antimicrobial studies of plant extract
The antimicrobial effect of ethanolic extract of R. heudelotii was
carried out using agar diffusion method described by Benkeblia [16]
with slight modifications. Bacterial cell suspensions of about 18–24 h
were adjusted to a 0.5 McFarland standard. Mueller-Hinton agar plates
were seeded with 100 μL of each of the bacterial cell suspensions, and
wells were bored using 0.8 mm diameter cork borer. The extract was
dissolved in dimethyl sulfoxide and aseptically introduced into the
bored wells, allowed to spread out, and incubated at 37°C for 18–24 h
for bacteria, and thereafter, the diameter of the inhibition zones were
measured. The same method was employed for fungal isolate which
was initially cultured on PDA and incubated at room temperature for 48 h. For standard, standard antibiotic discs of 6 mm diameter (Hi-Media)
for gentamicin were used.

Minimum inhibitory concentration (MIC) was determined by preparing
different concentrations of the extracts using sterile distilled water.
Standardized bacterial suspension (0.1 mL) was introduced into test
tubes containing nutrient broth, and different concentrations of the
extract were introduced to them after which they were aerobically
incubated at 37°C for 18–24 h. The highest dilution where there was no
bacterial (or fungal) growth was recorded as the MIC.

Acute toxicity
Lethal dose (LD₅₀) was determined using the method as described
by Lorke [17]. This involves two stages. At the initial stage, rats were
grouped into 3, each containing 3 rats and received the ethanolic extract
of R. heudelotii at an oral dose of 10, 100, and 1000 mg/kg body weight
(b.w.), respectively. For the second stage, different doses of 2900, 3600,
and 5000 mg/kg b.w. were administered to another set of 3 groups of
three rats. The rats were monitored over 72 h and 2 weeks period for
morbidity or mortality; changes in behavior were recorded. However, there were behavioral changes
in the group administered 3600 and 5000 mg/kg b.w. of the extract; decreased locomotive activity, food intake, weight loss, and weakness
were observed in these groups. No mortality of rats was experienced all through the experiment.

Experimental design
A total of 35 rats were purchased and equally distributed across five
experimental groups. Group I rats was administered 1ml of distilled
water while Groups II-IV were given orally 250, 500, 1000, and
2000 mg/kg b.w. of the extract, respectively, for 28 days. At the end of
the 28 days treatment, food was withdrawn from the animals overnight,
and the following morning they were anesthetized in diethyl ether.
They were afterward dissected from the abdomen; blood samples were
obtained through the cardiac puncture into heparinized tubes. Plasma
was obtained by centrifuging at 3000 rpm for 15 min [18] and kept
at −20°C inside Eppendorf tubes until needed for biochemical assays.
The organs (liver and kidney) obtained were used for histological
examination.

Biochemical assay
The commercial test kits for liver function test were purchased from
Randox Laboratory, United Kingdom. Standard procedures were used
to evaluate the protein concentration [19], aspartate aminotransferase
(AST) [20], alkaline phosphatase (ALP) [21], alanine aminotransferase
(ALT) [22], cholesterol [23], total albumin [24], total bilirubin [25],
urea [26], and creatinine [27].

Hematological assays
Hematological parameters were estimated with the aid of an
automated hematology system analyzer, mean cell volume, white
blood cell count, hemoglobin, red blood cell count, hematocrit (HCT),
platelet count (PLT), mean cell hemoglobin (MCH), mean corpuscular
hemoglobin concentration, percentage lymphocyte, and percentage
granulocyte [28].

Histopathological studies
The method according to Aliyu et al. [29] was adopted. The organ tissues
were fixed in normal saline for 72 h and cut into thin slices 2.1 mm
thick. The tissues were dehumidified using liquor. They were thereafter
processed with paraffin wax and cast into blocks; tissue sections were then
slit into 5μm using microtome and allowed to dry on a slide. The slides
were afterward soiled with hematoxylin-eosin stain, analyzed using a
light microscope, and photomicrographs recorded [29,30].

Statistical analysis
Data were analyzed through one-way analysis of variance (ANOVA) and
Tukey’s test using the statistical package for the social sciences (SPSS),
version 21.0 (SPSS Inc., Chigaco, IL, USA). Probability of *p<0.05 was
considered to be statistically significant. All data were represented as mean±standard error mean for 7 animals graphically using Graph pad
prism, version 5.0.

RESULTS

Phytochemical analysis
The qualitative phytochemical analysis revealed the existence of
tannins, flavonoids, alkaloids, cardiac glycosides, saponins, steroids,
and terpenoids in the leaf extract (Table 1).

Acute toxicity
Administration of varying concentrations of ethanolic extract of
R. heudelotii at doses 10, 100, 1000, and 2900 mg/kg did not cause
any changes in behavior. However, there were behavioral changes
in the group administered 3600 and 5000 mg/kg b.w. of the extract; decreased locomotive activity, food intake, weight loss, and weakness
were observed in these groups. No mortality of rats was experienced all
through the experiment.

Effects of R. heudelotii extract on biochemical parameters
The ethanolic extract showed a significant increase (p<0.05) in the
activity of ALT and AST (Fig. 1). Similarly, the levels of urea were
significantly (p<0.05) elevated (Fig. 2) in Groups II, III, and IV. The
activity of ALP significantly increased (p<0.05) in the group treated with
2000 mg/kg b.w. of leaf extract. Total protein levels were significantly
decreased (Fig. 1) while creatinine levels were unaltered (Fig. 2) across all the treated groups.

Table 1: Qualitative phytochemical screening of R. heudelotii

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>Absent</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Present</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>Absent</td>
</tr>
</tbody>
</table>
Histopathological studies
Compared with the control groups, no remarkable alterations were noticed in the morphology of the organ tissues of rats administered 250 mg/kg b.w. However, noticeable cellular alterations such as severe portal and central venous congestion were observed in the liver organ of rats administered 500 mg/kg b.w. of the extract including severe periportal cellular infiltration and vacuolar degeneration of hepatocytes of those given 1000 and 2000 mg/kg b.w. (Fig. 3). The kidney of rats treated with 1000 mg/kg b.w. of extract showed a mild tubular degeneration (long arrow Fig. 4) with interstitial hemorrhage (short arrows Fig. 4) at the renal cortex, while the 2000 mg/kg b.w. group showed mild-to-moderate interstitial hemorrhage (Fig. 4).

DISCUSSION
Toxicological evaluations are essential in determining the safety limit of plant extracts and herbs in animals. These are usually conducted to determine the safety of the plant extract in humans. In this study, no death occurred all through the treatment period. There were neither occurrences of diarrhea nor changes in locomotor activity observed.

From the preliminary qualitative phytochemical test carried out on the ethanolic leaf extract, tannins, steroids, terpenoids, flavonoids, saponin, cardiac glycosides, and alkaloids were discovered present (Table 1). These secondary metabolites contribute significantly to biological activities of medicinal plants as some of them have shown excellent antimicrobial, antioxidant, and antimalarial properties. Tannins have...
amazing stringent properties. They are known to hasten the healing of wounds [31], treat bacterial infections, and dysentery [32]. Flavonoids have also been reported to be efficient reactive oxygen species scavengers as their anticancer property ascertained on their chelating and antioxidant attributes [33].

The antimicrobial potential of the leaf extract may be hard to correlate to a specific compound as a result of their complexity [34]; however, tannins have been reported by Hatano et al. [35] to strongly inhibit microorganisms, especially effect on S. aureus. This is in line with this present study where the leaf extract showed a zone of inhibition of
Mean LD$_{50}$ value is often used as the basis for assessing acute toxicity [19]. Our study has shown that the ethanolic leaf extract of Ricinodendron heudelotii could produce signs of toxicity at very high concentrations (3600 and 5000 mg/kg b.w.) but not death. This shows that the extract is relatively safe or may be slightly toxic just like any xenobiotics since it falls within the range of 1000-5000 mg/kg b.w. [37]. No significant difference (p<0.05) in the weight of the animals was observed. However, the organ weights in the 2000 mg/kg b.w. group showed a significant difference (Table 4), this implies that small doses can be effective against microorganisms.

Hematological parameters are useful markers used to ascertain the adverse effect of plant extracts or even drugs on blood constituents [39]. In this study, treatment with the plant extract led to a significant decrease (p<0.05) in PLTs in rats in the 2000 mg/kg b.w. group (Table 5). According to McLellan et al. [40], reduction in PLT in experimental rats indicates detrimental action on the blood’s oxygen transporting ability as well as thrombopoietin. The observed reduction in the PLTs in this study indicates that RH extract may cause disorder in the blood oxygen transporting ability. Reductions in red blood cell (RBC) and HCT were also observed (Table 5). Reduction in RBC was statistically significant in the group treated with 2000 mg/kg b.w. of R. heudelotii extract while the reduction in HCT was dose dependent but not significant. This could be as a result of osmoregulatory system disturbance of the blood cells or impairment of the cell membrane. The observed reduction in hematological indices could indicate erythrocite destruction [41]. Therefore, the reduction observed in RBC count and HCT may be linked to delayed hemopoiesis, shrinkage, and destruction of RBC. Likewise, the oxygen-transporting ability of the blood and the oxygen supplied to the tissues may be disrupted following administration of the extract.

Biochemical analysis is useful for predicting the toxicological effect of the leaf extract in animals and the safety of plant products for human use [42]. ALP, AST, and ALT are distinct markers of hepatic injury [43].

Table 2: MIC values of extract of Ricinodendron heudelotii leaf against test organisms

<table>
<thead>
<tr>
<th>Plant/microorganism</th>
<th>R. heudelotii MIC (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>-</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>62.5</td>
</tr>
<tr>
<td>E. coli</td>
<td>31.25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>62.5</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>62.5</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>62.5</td>
</tr>
<tr>
<td>S. faecalis</td>
<td>31.25</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans</td>
<td>-</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of seven replicates. *p<0.05 compared to control. R. heudelotii: Ricinodendron heudelotii; MIC: Minimum inhibitory concentration

Table 3: Effects of Ricinodendron heudelotii extract on organ weight after 28 days treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.25±0.26</td>
<td>0.97±0.02</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>1.03±0.04</td>
<td>5.63±0.19</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>5.97±0.39</td>
<td>1.15±0.06</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>5.74±0.22</td>
<td>1.17±0.05</td>
</tr>
<tr>
<td>2000 mg/kg</td>
<td>6.8±0.30</td>
<td>1.20±0.37</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of seven replicates. *p<0.05 compared to control. R. heudelotii: Ricinodendron heudelotii; SEM: Standard error mean

Table 4: Zone of inhibition of Ricinodendron heudelotii against bacteria and fungus isolate

<table>
<thead>
<tr>
<th>Organism</th>
<th>R. heudelotii gentamicin Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>27</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>28</td>
</tr>
<tr>
<td>E. coli</td>
<td>25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>-</td>
</tr>
<tr>
<td>Micrococcus sp.</td>
<td>-</td>
</tr>
<tr>
<td>S. faecalis</td>
<td>15</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans</td>
<td>-</td>
</tr>
</tbody>
</table>

Values represent mean±SEM of seven replicates. *p<0.05 compared to control. R. heudelotii: Ricinodendron heudelotii; SEM: Standard error mean

ALT is an enzyme found in highest amount in the liver [31]. Thus, an increase of the enzyme in the blood (Fig. 1) indicates hepatic injury.
This is in alignment with work done by Oyono et al. [34] when acute toxicity was conducted on methanolic bark extract of *R. heudelotii*. AST which is also a significant biomarker for liver disease [44] which showed a significant increase (Fig. 1). Since the liver is the major organ of detoxification and mostly exposed to different levels of ingested xenobiotics which can adversely affect hepatic cells [45]. This result suggests that the extract is likely to exert damage to liver cells at higher concentrations. The liver also helps to synthesize proteins that maintain the electrolyte balance in plasma. In line with that, *R. heudelotii* leaf extract reduced the total protein content in the groups administered 500, 100, and 2000 mg/kg b.w. of the extract. Albumin is also an important marker for liver diseases [46]. Our study showed a significant elevation of albumin in the 2000 mg/kg b.w. group.

ALP, a biomarker of liver disease and obstructive jaundice, was found to be significantly increased (p<0.05) (Fig. 1) in the 2000 mg/kg b.w. group; this suggests that the observed liver injury might be linked to biliary obstruction of the liver. This is also in line with research done by Oyono et al. [34]. This is further substantiated by a significant and steady rise in the total bilirubin (Fig. 1) concentration in the plasma caused by an obstruction in the bile duct causing an accumulation of bilirubin in the liver. Plasma urea concentration significantly increased (p<0.05) at high extract doses, and this may be due to nephrotoxicity. An elevated plasma urea concentration has been associated with diseases related to nephrotoxicity since the kidney is known to dispose of waste products of metabolism [47]. During renal breakdown, there is a rise in nitrogenous substances such as urea and uric acid. However, plasma creatinine concentration was found to be insignificant.

Toxicological studies also show an increase in cholesterol concentration. High blood cholesterol concentration is one of the important possibilities during cardiovascular disease [13]. Thus, the rise in plasma total cholesterol concentration (Fig. 5) effect by the extract is harmful and may increase the risk of cardiovascular disease. This is, however, combated by a very rapid increase in the HDL-cholesterol concentration (Fig. 5) to mop up cholesterol from the blood vessels. This also shows that, at the right dose, *R. heudelotii* can be used in the treatment of coronary heart disease and cardiovascular diseases [48]. The toxicity exhibited by the ethanolic extract of *R. heudelotii* was reaffirmed through the histological sectioning which revealed sinusoidal congestion and severe vascular degeneration of hepatocytes in all treated groups. Vascular degeneration detected in the present study may be due to mitochondrial intermembrane space expansion and extension of the outer mitochondrial membrane [49].

**CONCLUSION**

The ethanol leaf extract of *R. heudelotii* may not induce significant toxic effects when administered in rats below 3600 mg/kg b.w. and thus may be safe for use as a potential candidate for the enhancement of new antimicrobial formulations as it demonstrated high antimicrobial activities. However, one should apply caution on the dosage to be administered as higher concentration could induce liver cell injury.

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**REFERENCES**


