Nephrotoxicity and Hepatotoxicity Evaluation in Wistar Albino Rats Exposed to Nauclea latifolia Leaf Extracts

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ABSTRACT
Consumption of the aqueous leaf extract of Nauclea latifolia as anti-malaria concoction without any recourse or regard for its safety is a common practice in the Northern Nigeria. The aim of this study was to evaluate the safety efficacies of the ingestion of the methanolic leaf extract of this plant on the liver and kidney functions in wistar albino rats. Acute toxicity tests were carried out to determine LD₅₀, while sub-chronic toxicity study was carried out by oral administration of graded doses (200, 400, 800, 1600 and 3200mg/ Kg) of the extract to different groups of rats for 30 days. Both the liver and kidney functions assessed biochemically using standard methods revealed the LD₅₀ of N. latifolia at 3200mg/Kg body weight as being non-lethal. Meanwhile, biochemical and histological results obtained for the liver and kidney function parameters indicated that ingestion of N. latifolia leaf extract has no observable toxic effects on these organs at the tested doses. It was therefore suggested that these results could form the basis for clinical trial in human.

Keywords: Hepatotoxicity, Nauclea latifolia Nephrotoxicity, wistar albino rats

INTRODUCTION
Medicinal plants have been known to be useful in the treatment of various diseases all over the world since the time immemorial.
the documented severe toxic reactions arising from the use of herbs, general public and professional traditional medical practitioners/healers sometimes mistakenly think of herbs as natural alternative to drugs, failing to recognize/realize that herbs contain bioactive chemicals, some of which may be toxic (Tyler, 1994). However, many patients are under false assumption that naturally derived herbal medicines are safer with fewer side effects but this is not totally true (Gamaniel, 2000).

Nauclea latifolia (Rubiaceae) is a tree species grown in the northern parts of Nigeria, commonly known as “Tuwonbiri” or “Tafashiya” in Hausa, “Ubulumu” in Igbo and “Opepe” in Yoruba, has been claimed to be valuable in a wide spectrum of ailments (Onyeyili et al. 2001; Ajagbonna et al., 2002). Nworgu et al. (2008) reported blood pressure lowering effect of N. latifolia in rats, while potential anti-diabetic properties of the plant were recorded by Gidado et al. (2005). Many people in Northern Nigeria treat malaria by drinking aqueous leaf extracts of N. latifolia; however, the responses of various organs, especially the liver and kidney (sites of biotransformation) in humans to ingestion of this extract, remain scientifically unknown. More so, there was little or dearth of information on the effects of the extract of this plant at the cellular level. Thus, this study was undertaken to examine to what extent the liver and kidney would be affected in rats exposed to N. latifolia leaf extract.

MATERIALS AND METHODS

Plant Materials and Preparation of Plant Extracts

The leaves of N. latifolia were collected within Sokoto metropolis and authenticated at the Biological Sciences Department, University of Agriculture, Abeokuta by Dr. Aworinde D.O. (Plant Taxonomist/Anatomist). The leaves were washed with tap water, air-dried and pulverized using a grinding machine. Three hundred grams (300g) of the ground sample was immersed in absolute methanol (1000ml) for 72 hours, under rigorous shaking/mixing to ensure maximum extraction. The extract was filtered through Whatman filter paper No 1, and the decoction was concentrated to dryness in rotary evaporator to obtain the crude methanolic extract, which was stored in a refrigerator until used. The extract yield was 9.8% of the starting materials.

Phytochemical Screening of the Aqueous and Methanolic Extracts of Nauclea latifolia

Phytochemical screening was carried out according to the methods proposed by Trease and Evans (1978), as described by Edeoga et al. (2005).

Test for tannins: The dried powdered leaf (0.5g) was boiled in 20ml of water in a test tube and then filtered. Two drops of 0.1% (w/v) ferric chloride reagent were added and observed for brownish-green or brownish-green to indicate the presence of tannins.
Test for saponins: Two (2) grams of powdered leaf was boiled in 20ml of distilled water in water bath and filtered. 10ml of filtrate was mixed with 5 ml of distilled water and shaken vigorously for stable persistence froth. The frothing was mixed with 3 drops of olive oil, before it was shaken vigorously and observed for the formation of emulsion.

Test for flavonoids: Powdered leaf (5mg) was heated in 10ml of ethylacetate over a steam bath for 3 min. The mixture was filtered and 4ml of filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed, indicating a positive test for flavonoid.

Test for steroid: Acetic anhydride (0.2ml) was added to 0.5g methanolic extract of each sample with 2ml H$_2$SO$_4$. The colour was expected to change from violet to blue or green.

Test for terpenoids (Salkowski test): The extract (0.5g) was mixed with 2ml chloroform and concentrated H$_2$SO$_4$ (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoids.

Test for cardiac glycosides (Keller Killani test): Methanolic extract (0.5g) was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of conc. H$_2$SO$_4$. A brown ring of the interface indicates a deoxy sugar that characterized a cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring was gradually formed throughout thin layer.

Experimental Animals

Thirty six (36) Wistar albino rats (weighing 180-240g) of both sexes, obtained from the Department of Veterinary Anatomy, University of Ibadan, were used for the study. They were housed in well-ventilated rat cages, kept at 27-30°C, with 12 hour natural light and 12 hour darkness, and allowed free access to tap-water and dried rat pellets (Ladokun & Sons Feeds, Ltd). They were also allowed to acclimatize for a week before the commencement of the experiment.

LD$_{50}$ Determination/Acute Oral Toxicity Study

This was carried out according to the procedure described by Oduola et al. (2010). Briefly, graded doses of the extract were administered orally to six (6) groups of rats consisting of six (6) rats per group. Thus, Group 1 served as a control and received normal saline, while Groups 2, 3, 4, 5, and 6 received 200, 400, 800, 1600 and 3200 mg/Kg body weight respectively, with the aid of canula attached to a graduated syringe. All the rats were placed under observation for 24 hours, after which the number of dead rats was recorded and LD$_{50}$ was calculated using the formula described by Aliu and Nwude (1982).

Sub-chronic Toxicity Study

After 72 hours, none of the rats in oral toxicity study died. Thus, the extract was administered to the animals for 30 more days, at the end of which, the rats were weighed, and their blood samples were
collected through cardiac puncture under chloroform anesthesia into lithium-heparin specimen bottles for biochemical assays. The rats were then sacrificed by cervical dislocation, while liver and kidney collected for function tests and histopathological examinations were carried out using the standard techniques.

All the biochemical parameters were determined using the Chromatest reagents diagnostic kits, except for Glutathione-S-transferase (GST) whose activity was determined using the method of Habig and Jakoby (1980).

**RESULTS**

The present study attempted to evaluate the effects of ingestion of the methanolic leaf extract of the plant *Nauclea latifolia* on the liver and kidney functions in wistar albino rats. The results of the phytochemical screening of the aqueous and methanolic extracts of *N. latifolia* are presented in Table 1.

**Histopathological Studies**

The histological examinations of the liver section of the representative samples of these groups of rats were carried out following standard procedures.

**Statistical Analysis**

The mean, standard deviation and level of significance for the difference between the means of the data generated were computed using student test SPSS 6.

**TABLE 1**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Methanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac glycosides</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++ Highly present  
+ Present  
Absent.

The results of the acute oral toxicity study revealed that there was no record of death even at the highest dose of 3200mg/kg.
Kg b.wt, as shown in Table 2. This indicated that the LD$_{50}$ of the plant was higher than 3200mg/Kg. In fact, all the animals appeared healthy and active throughout the experiment.

Table 3 shows the values of some electrolytes (Na$^+$, K$^+$ and HCO$_3^-$), urea and creatinine levels between the control and the studied groups, as well as between the groups. Table 4 presents the values of total protein and albumin, ALT, AST, ALP and GST obtained for the control and studied groups (B, C, D, E and F). The plant extract over the range of tested doses showed very insignificant changes rather than producing toxicity as compared to normal.

**DISCUSSION**

Liver and the kidney play important roles in the biotransformation of the ingested. In particular, the liver is much more prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation and its anatomic-physiologic structure. The kidney, on the other hand, is highly susceptible to toxicants because of a high volume of blood flows through it and it also filters large number of toxins which can concentrate in the tubules. The leaf extract *N. latifolia* was found to contain high level of cardiac glycoside, moderate levels of flavonoids, saponins, terpenoid and tannins. However, steroids were not present in the tested doses. Judging by the current means of estimating the current LD$_{50}$ values, based on acute oral toxicity recommended by the Global Harmonised Systems of classification and labelling of chemicals on toxicants (Link/URL, 2010), the LD$_{50}$ for this particular plant extract would be greater than 3200mg; this suggests or implies that the extract is non-lethal at 3200mg, and it is therefore assumed to be safe for consumption.

**TABLE 3**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (control)</th>
<th>Group 2 (200mg/Kg)</th>
<th>Group 3 (400mg/Kg)</th>
<th>Group 4 (800mg/Kg)</th>
<th>Group 5 (1600mg/Kg)</th>
<th>Group 6 (3200mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/l)</td>
<td>125 ± 3.75</td>
<td>124 ± 4.32</td>
<td>127 ± 5.24</td>
<td>126 ± 3.45</td>
<td>125 ± 5.10</td>
<td>122 ± 4.13</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.58 ± 0.88</td>
<td>4.42 ± 0.76</td>
<td>4.38 ± 0.93</td>
<td>5.43 ± 0.47</td>
<td>5.23 ± 0.81</td>
<td>4.34 ± 0.62</td>
</tr>
<tr>
<td>Bicarbonate (mmol/l)</td>
<td>26.17 ± 2.1</td>
<td>24.66 ± 2.81</td>
<td>25.17 ± 1.31</td>
<td>27.17 ± 1.8</td>
<td>25.50 ± 1.10</td>
<td>22.71 ± 3.40</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>111.83±9.4</td>
<td>109.28 ± 5.68</td>
<td>108.19±9.30</td>
<td>103.50 ± 5.4</td>
<td>105.25 ± 5.40</td>
<td>107.33 ± 6.21</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>6.71 ± 1.50</td>
<td>6.30 ± 1.81</td>
<td>6.32 ± 1.64</td>
<td>6.52 ± 0.99</td>
<td>6.04 ± 0.98</td>
<td>6.21 ± 0.65</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>89.97±5.71</td>
<td>90.19 ± 14.33</td>
<td>93.11±10.36</td>
<td>90.34±12.61</td>
<td>92.11 ± 10.30</td>
<td>90.38 ± 8.21</td>
</tr>
</tbody>
</table>
The observed non-toxic effect or the absence of hepatocellular or nephrotoxical damage at these investigated concentrations could be buttressed by the non-significant differences in the liver and the kidney function parameters, which revealed that the conjugating ability of the liver was not compromised, especially from the total and conjugating bilirubin levels obtained. Meanwhile, non-hepatocellular damage as revealed by the ALT and AST values which were further buffered by the histological revelation. The liver sections of the control and the tested groups showed no gross lesion, except for mild hepatic vacuolation which had been observed at 3200mg.

Meanwhile, the histopathological examinations of the kidney in the control and treated rats showed no visible lesion or necrotic sign. The results of this study suggest that ingestion of *N. latifolia* (at the tested concentration) has no adverse effect on the liver and kidney functions in rats. Therefore, the present study has established that ingestion of *N. latifolia* extract has no observable adverse effect(s) on the liver and kidney of rats and this could form a basis for

**TABLE 4**
Effect of intake of *Nauclea latifolia* methanolic extract on liver function profiles

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Group 1 (control)</th>
<th>Group 2 (200mg/Kg)</th>
<th>Group 3 (400mg/kg)</th>
<th>Group 4 (800mg/kg)</th>
<th>Group 5 (1600mg/kg)</th>
<th>Group 6 (3200mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Proteins (mg/L)</td>
<td>3.42±0.31</td>
<td>3.85±0.32</td>
<td>3.91±0.29</td>
<td>3.66±0.33</td>
<td>3.67±0.31</td>
<td>3.33±0.40</td>
<td></td>
</tr>
<tr>
<td>Albumin (mg/L)</td>
<td>1.30±0.07</td>
<td>1.43±0.07</td>
<td>1.45±0.11</td>
<td>1.34±0.08</td>
<td>1.42±0.07</td>
<td>1.36±0.08</td>
<td></td>
</tr>
<tr>
<td>Total Bilirubin (µmol/L)</td>
<td>10.5±2.10</td>
<td>7.65±2.02</td>
<td>8.76±2.03</td>
<td>8.33±2.01</td>
<td>8.45±2.11</td>
<td>8.14±2.30</td>
<td></td>
</tr>
<tr>
<td>Conj. Bilirubin (µmol/L)</td>
<td>2.77±0.16</td>
<td>2.63±0.36</td>
<td>2.54±0.16</td>
<td>2.67±0.75</td>
<td>2.70±0.12</td>
<td>2.40±0.34</td>
<td></td>
</tr>
<tr>
<td>ALT (1 U/L)</td>
<td>25.32±1.30</td>
<td>26.38±1.28</td>
<td>26.67±1.40</td>
<td>27.14±1.61</td>
<td>27.83±2.01</td>
<td>25.19±2.34</td>
<td></td>
</tr>
<tr>
<td>AST (1 U/L)</td>
<td>20.17±1.29</td>
<td>21.88±1.26</td>
<td>21.64±1.70</td>
<td>21.28±2.00</td>
<td>20.71±2.11</td>
<td>20.86±1.44</td>
<td></td>
</tr>
<tr>
<td>ALP (1 U/L)</td>
<td>50.34±5.00</td>
<td>51.07±5.10</td>
<td>52.22±4.20</td>
<td>51.98±4.20</td>
<td>53.06±4.46</td>
<td>53.18±4.31</td>
<td></td>
</tr>
<tr>
<td>GST(units/mg)</td>
<td>0.96±0.04</td>
<td>0.99±0.03</td>
<td>0.94±0.02</td>
<td>0.90±0.04</td>
<td>0.82±0.03</td>
<td>1.12±0.41</td>
<td></td>
</tr>
</tbody>
</table>
Nephrotoxicity and Hepatotoxicity Evaluation in Wistar Albino Rats Exposed to *Nauclea latifolia* Leaf Extracts

Fig. 1: Section of the liver tissue showing normal hepatocytes (control, X400 magnification)

Fig. 2: Liver section showing no visible lesion (Group 2, X400 magnification)

Fig. 3: Liver section showing hepatic regeneration (HR) without visible pathology (Group 3, X100 magnification)
In conclusion, it can be concluded that *N. latifolia* has potential to be used in the management of hepatic and nephritic damages.

**REFERENCES**


