Toxicological studies of aqueous root extract of *Euphorbia lateriflora* (Schum and Thonn) in rats

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The effect of administration of graduated dose of aqueous root of *Euphorbia lateriflora* on ALT, AST, ALP, bilirubin, total protein, creatinine and urea in rats was determined to ascertain the toxic effects on the liver and kidney. There was a significantly higher (p<0.05) ALT activity and significantly lower urea level in group administered with 100 mg/kg of aqueous root extract compared to the control. There was a significantly higher (p<0.05) ALT, AST, and level of bilirubin in group administered with 250 mg/kg aqueous root extract compared to the control. The result suggest a possible hepatotoxic effect of root extract of *Euphorbia lateriflora* after administration of 100 mg/kg and 250 mg/kg, there was no possible kidney malfunction. The result of the oral acute toxicity study shown that *Euphorbia lateriflora* have oral LD₅₀ above 5000 mg/kg. Histopathological lesions were observed in liver and kidney after acute toxicity study.

**Keyword:** ALT: Alanine aminotransferase, AST: Aspartate aminotransferase and ALP: Alkaline phosphatase.

1. Introduction
Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [1, 2]. Many indigenous plants have been in the use of man since time immemorial for curing various ailments without the actual knowledge of their toxic potential [3]. Medicinal plants are now widely being used in many parts of the world for the remedy of many disease [4]. One of the problems of using plants as medicines is that in many cases no definite doses are prescribed, often resulting in overdose. Liver is the largest organ in the body of vertebrates. The liver is particularly susceptible to chemical injury because of its anatomical relationship to the most important portal of entry, the gastrointestinal tract; and the high concentration of xenobiotic – metabolizing enzymes [5].

The kidney performs the functions of getting rid of the body’s waste materials that are either ingested, produced by metabolism or as a result of detoxification of the liver. This, and other functions of the kidney, can be disrupted by accumulation of toxic metabolites or chemicals leading to renal diseases [6].

*Euphorbia lateriflora* (*Fidda sartse* in Hausa) is a shrub with smooth – graceful and erect branches. The leaves of this plant serve for the treatment of dermatoses [7], intestinal parasitosis and convulsive fever in children [8]. *E. Lateriflora* extract was shown to exhibit antiviral activity using the measles virus on human epidermoid carcinoma cell line [9].

In spite of its widespread use as medicine in some parts of Nigeria and other parts of West Africa, data on the toxicological analysis of *E. lateriflora* is scarce or non –existent.
2. Materials and Methods

2.1 Experimental Animals
Seventy six (76) albino rats of both sexes were obtained from National Veterinary Research Institute Vom, Jos. The animals were kept in cages in the animal house of the Department of Biological Sciences, Gombe State University and the animals had free access to normal diet (Growers marsh, vital feeds Ltd) and water.

2.2 Extracts Preparation
The roof of the plant was collected from around Galinja village in Madobi Local Government Area, Kano State. They were authenticated at the Botany unit of Biological Sciences Department, Bayero University Kano. The root of the plant was dried and ground to powder using pestle and mortar. For subacute toxicity studies 40 g of root was dissolved in 500 cm$^3$ of distilled water and filtered after 24 hours. The residues were dried. The difference between the original weight and final weight was found to be the concentration of the extract. The filtrates were concentrated to 300 cm$^3$ using oven drier. The concentration root was found to be 3.7 g/300 cm$^3$. For acute toxicity studies, the root was dissolved in water and filtered after 24 hours. The filtrate was evaporated to dryness in an oven. The dried extracts were weighed and dissolved in distilled water to a concentration of 4.5 g/40 cm$^3$.

2.3 Experimental Design

2.3.1 Subacute Toxicity
Twenty eight (12) rats were used. The animals were divided into three groups of 4 rats each. Groups I and II, were the study groups, while group III served as control group. The animals in groups I and II were administered orally with 100 mg/kg and 250 mg/kg of aqueous root extracts of *Euphorbia lateriflora* respectively. The animals in group III (control) were administered with distilled water. The administration of aqueous extracts was done once every twenty four (24) hours for the period of three weeks.

2.3.2 Acute Toxicity: Sixteen (17) rats were used for acute toxicity studies. Four groups of rats (4 per group) were administered orally with different doses (2000, 3000, 4000 and 5000 mg/kg) of the extracts. The number of deaths in each group within 24 hours was recorded.

2.4 Collection and Preparation of Blood Samples
At the end of third week of oral administration of aqueous extracts (leaf, root and stem), the rats were sacrificed by decapitation. Blood samples were collected into centrifuge tubes. The blood was allowed to clot at room temperature for 5 minutes, after which an applicator stick was used to carefully loosen the blood. The blood was then centrifuged at 2500 rpm for 10 minutes. A clean Pasteur pipette was used to carefully collect the serum and dispensed into a clean labeled specimen bottle.
Sera samples collected were analyzed for activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and levels of bilirubin, total protein, creatinine and urea.

3. Results
The result of the oral acute toxicity indicates that two deaths were recorded, one in group administered with 3000 mg/kg and other one in group administered with 5000 mg/kg. According to the toxicity scale of Hodge and Sterner, any compound with an oral LD$_{50}$ above 5000 mg/kg should be considered practically nontoxic. The rats that died during acute toxicity test were subjected to postmortem examination. The Histopathological lesions observed in some organs of the rats that died as a result of root extract administration are shown in Table 2.

4. Discussion
From table 1, the serum activity of ALT was found to be significantly higher (P<0.05) than the control in both groups administered with 100 mg/kg and 250 mg/kg aqueous root extract of *Euphorbia lateriflora*. This signifies that oral
administration of aqueous root extract of *Euphorbia lateriflora* could have possible hepatotoxic effect. This can be linked to the fact that, ALT is released from the liver when parenchymal cells become necrotic; the increased in their activity in the plasma is related to the extent of cell breakdown\[17\]. When the liver is damaged, levels of enzymes in the blood rise above normal ranges. The enzymes usually affected include ALT among others. Liver toxicity is ascertained if there is an increase in ALT three times its level at the start of study\[18\]. However, the ALT activities in groups administered with 100 mg/kg and 250 mg/kg aqueous root extract of *Euphorbia lateriflora* were not up to three times higher than in control group.

**Table 1:** Effects of the aqueous root extract of *Euphorbia lateriflora* on serum ALT, AST, ALP, bilirubin, total protein creatinine and urea

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/dm³)</th>
<th>AST (U/dm³)</th>
<th>ALP (U/dm³)</th>
<th>Bilirubin (µmol/dm³)</th>
<th>Total protein (g/dm³)</th>
<th>Creatinine (µmol/dm³)</th>
<th>Urea (mmol/dm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/kg</td>
<td>11.68±0.23a</td>
<td>21.75±1.31</td>
<td>84.15±13.87</td>
<td>9.25±1.51</td>
<td>73.80±7.68</td>
<td>59.60±0.34</td>
<td>8.93±0.68a</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>11.24±1.18a</td>
<td>27.879±1.59a</td>
<td>80.03±6.80</td>
<td>13.41±2.33a</td>
<td>66.00±4.16</td>
<td>59.65 ±0.44</td>
<td>10.09±0.92</td>
</tr>
<tr>
<td>Control</td>
<td>9.40±0.68</td>
<td>21.38±0.41</td>
<td>80.85±11.27</td>
<td>8.33±1.07</td>
<td>73.80±5.32</td>
<td>59.75±0.27</td>
<td>11.10±1.58</td>
</tr>
</tbody>
</table>

Values with superscript a are significantly different from control values at p<0.05  
Values represent mean ± SD (n = 4)

**Table 2:** Histopathological lesions observed in kidney and liver of the rats that died after acute toxicity test using aqueous root extract of *Euphorbia lateriflora*.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>Section of kidney shown infarction with loses of tubular epithelium and occasional necrosis (i.e. kidney infarcted).</td>
<td>Liver congestion</td>
</tr>
<tr>
<td>5000</td>
<td>Section of kidney shown infarction and necrosis of tubular epithelium.</td>
<td>Section of liver shown marked congestion</td>
</tr>
</tbody>
</table>

From Table 1, there was a significantly higher level (p<0.05) in the activity of AST in group administered with 250 mg/kg of root extract of *Euphorbia lateriflora* compared to the control. There was no significant difference (p>0.05) in the activity of AST in group administered 100 mg/kg of root extract compared to the control. The increase in the activity of AST could be linked to the fact that, AST increase in the serum in a wide variety of conditions particularly, cardiac, liver and muscles disease due to cellular necrosis allowing enzyme leakage into the circulation\[19\]. However, concurrent increase in ALT and AST is a suggestive of mild liver necrosis. This could be linked to the fact that liver toxicity is ascertained if there is an increase in ALT three times its level at the start of study as reported by Hosein\[18\]. From Table 1, it has been found that, there was no significant difference (p>0.05) in the activity of ALP compared to the control in group administered orally with 100 mg/kg and 250 mg/kg root extract of *Euphorbia lateriflora*. Liver ALP is a non-plasma specific enzyme that is released from the sinusoidal surface of the liver cell and thus is present in the serum at low levels in the absence of liver damage\[17\]. From Table 1, it was observed that the serum level of bilirubin was significantly higher in group administered with 250 mg/kg root extract
of *Euphorbia lateriflora* compared to the control. The increase could be linked to the fact that hyperbilirubinemia is caused by such factors as decreased uptake into liver cells [20]. The plasma bilirubin concentration reaches about twice the upper reference limit when the normal load of bilirubin cannot be conjugated and/or extracted by damage liver cells [21]. However, increased plasma levels in this study was not up to twice the control value as shown in Table 1, in group administered with 250 mg/kg root extract of *Euphorbia lateriflora*. The liver damage is not severe because, there was no significant difference (p>0.05) in the activity of ALP and level of Total protein in group administered with 250 mg/kg aqueous root extract of *Euphorbia lateriflora*.

From Table 1, there was no significant difference (P>0.05) in groups administered with 100 mg/kg and 250 mg/kg root aqueous extract of *Euphorbia lateriflora* compared to the control. From Table 1, there was no significant (P>0.05) difference in the levels of creatinine compared to the control in groups administered with 100 mg/kg and 250 mg/kg root extract of *Euphorbia lateriflora*. This shows that, there was no possible kidney malfunction. Creatinine is removed from the plasma by glomerular filtration and is then excreted in the urine without being reabsorbed by the tubules to any significant extent. In addition when plasma level increase above the normal the kidney can also excrete creatinine through the tubules. Consequently, serum creatinine levels in renal disease generally do not increase until the renal function is substantially impaired [22]. High serum creatinine may also be indicative of renal failure [5]. A normal renal function depends on a normal filtration rate. A high glomerular filtration rate leads to excretion of creatinine. In addition, the plasma creatinine concentration may not exceed the upper limit of the reference range until the glomerular filtration therefore the creatinine clearance, has been reduced by approximately 60 percent [23].

From Table 1, it was observed that serum urea level was significantly lower than the control in group administered with 100 mg/kg and with no significant difference (P>0.05) in group administered with 250 mg/kg aqueous root extract of *Euphorbia lateriflora*. This shows that, there was no possible kidney malfunction. The significantly lower in serum urea seen is contrary to the fact that urea concentration is raised in renal disease [19]. In addition, the significantly lower serum urea could be linked to the fact that, rats administered extract for 3 weeks showed less appetite than the control group and they were all fed with a feed rich in protein. Hotellier and Delaveau reported that manifestation of inappetence observed in the rats may however be linked to the present of some chemicals such as tannins in the extract [24]. It has been reported that *Euphorbia lateriflora* contain tannins [25]. From table Table 1, it was found that there was a significantly lower (p>0.05) urea level in group administered with 100 mg/kg root extract of *Euphorbia lateriflora* compared to control. There was no significant difference (p>0.05) in creatinine levels in groups administered with 100 mg/kg and 250 mg/kg root aqueous extract of *Euphorbia lateriflora* compared to the control. It has been reported that change in blood urea are normally parallel by changes in blood creatinine [5]. The decrease in the urea level could be due to the fact that urea concentration is influenced by diet [26].

5. Conclusion
The results have shown that oral administration of 100 mg/kg and 250 mg/kg root extract could possess possible hepatotoxic. Results of the levels of urea and creatinine revealed that, there was no possible kidney malfunction.

6. References


