ABSTRACT
This study evaluated the hepato-renal toxicological indices following 21 days administration of n-hexane leaf extract of *Vitex simplicifolia* in Wistar rats. Acute toxicity studies with very high concentrations of the crude extract was carried out followed by sub chronic toxicities studies involving administration of 250mg/kg, 500mg/kg and 1000mg/kg body weight of the ethyl acetate extract to the experimental animals for 21 days. Liver and Kidney toxicological indices were evaluated from the serum as well as the tissues of the experimental animals after the 21 days period of administration. The result of acute toxicity studies indicate that this extract is well tolerated at doses as high as 5000mg/kg body weight. The results of sub-chronic toxicity studies indicated significant increase in the activities of ALT and AST while ALP activities was lower compared to the control. Unconjugated bilirubin levels were also significantly (P<0.05) higher in the test groups compared to the control. Similarly, the result of kidney toxicological indices showed the levels of urea, HCO$_3$- and creatine were significantly higher in the test animals compared to the control. While Na$^+$, K$^+$ and Cl$^-$ levels showed no significant change in the extract administered groups compared to the control. Histopathology examination of the liver and kidneys exhibited mild hepatic damage at the highest dose (1000mg/kg body weight). These observations suggest that n-hexane extract of *Vitex simplicifolia* as a phytoremedy against any ailments as high concentrations of the extract may induce hepato-renal damage.

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INTRODUCTION
Plants are known to be efficacious and most often could contain compounds that are potential drugs which would require further examinations. Interest in and the search for medicines from natural sources has served as a catalyst for exploring techniques of obtaining the required plants and probing their activities (8,9). A large proportion of such medicinal compounds have been discovered with the aid of ethnobotanical knowledge of their traditional uses (15). At least 12,000 such compounds with diverse pharmacological functions (17,28 *Vitex simplicifolia* (Verbenaceae) is used in aqueous medium for the treatment of diseases in (21), there is paucity of information on the toxicity of the lipophilic medium especially as it used in the treatment of skin diseases in Africa (19). Similarly, it will be interesting to know if such extracts will have a better cictrization activity in wound healing (12,22). Many aromatic plants are known to contain volatile constituents such as monoterpenoids and sesquiterpenoids known to exert defined physiological action on the human system (6), hence such examination may assist in understanding the overall role of volatile...
compounds in the entire extract cocktail in the management of disease. Furthermore, since it is used as Vitamin A supplement (7,27), its toxic evaluation may be desirable to ensure that correct dosage is evaluated. The plant is known in Hausa as dinyar biri, Ucha koro in Igbo and Oori nla in Yoruba (7).

The aim of this study therefore is to investigate the effect of n-hexane extract of *Vitex simplicifolia* leaf on physiology and pathology of the liver and kidney.

**Material and Methods**

**Plant and animal**
The leaves of *Vitex simplicifolia* were collected from Bayero University Kano and were dried in shade at room temperature and grounded into powder. Wistar rats were obtained from the department of physiology animal house, Bayero University Kano; they were housed in colony cages at an ambient temperature and relative humidity. The animals had free access to standard palletized grower feed and drinking water.

**Extract preparation**
The powdered plant was dissolved in n-hexane overnight. It was filtered and the residue was discarded. The filtrate was evaporated to dryness using Vacuum evaporator. The dried plant residue was used to prepare different concentrations.

**Experimental Design**
A total of 33 Wister rats were used for the study. 13 rats were used for the acute toxicity study while 20 rats were used for the sub-chronic toxicity study. For the sub-chronic toxicity study, the 20 animals were divided into four groups of five rats each. Group 1 was the control fed only feed and water throughout the period of the experiment while groups 2, 3, and 4 were administered 250, 500 and 1000 mg/kg body of the n-hexane extract respectively for 21 days. After the 21 days of administration, the animals were sacrificed, blood samples were collected in heparin bottles and the liver and kidney of the animals were removed and preserved in 9% formalin until histopathological analysis.

**Determination of LD50**
The lethal dose (LD50) was determined by the method of Lorke (17). In the first phase, Nine (9) Wister rats were used. The nine animals were divided into three groups of three animals each. Each group were administered 10,100 and 1000mg/kg body weight of the extracts and then observed for 24 hours to monitor their behaviour and mortality. In the second phase two of the experiment, three animals were used; the animals were divided into three groups of one animal each. They were administered higher doses (1600, 2900 and 5000 mg/kg body weight) of the extracts and observed for behaviour as well as mortality. (18). LD50 was calculated by the formula: \( LD_{50} = \sqrt{D_0 \times D_{100}} \) where:

\[
D_0 = \text{Highest dose that gave no mortality},
\]

\[
D_{100} = \text{Lowest dose that produce mortality}.
\]

**Liver and Kidney function test**
Four enzymes indices of liver damage were assayed to determined liver toxicity. Aspartate Aminotrasferase (AST) activity was determined by the method described by Karmen, (13), Alkaline Phosphatase (ALP) and Alanine Aminotransferase (ALT) activities were determined by the methods of Reitman and Frankel (25) respectively while bilirubin levels was determined by the method of Sherloch (26). Kidney function was evaluated by determining the levels of kidney function indices; urea, creatinine, sodium ion, potassium ion, chloride ion and bicarbonate ion using the methods described by Annino and Giese (3) and Henry et al. (10)

**Histopathological studies** (4,20)
The liver biopsies were fixed with 10% formal saline and then transferred to a
cassette, a container designed to allow reagents to freely act on the tissue inside. This cassette was immersed in multiple baths of progressively more concentrated ethanol (to dehydrate the tissue with ascending grade of alcohol), cleared with toluene, infiltrated with molten paraffin wax. During this 12 to 16 hour process, paraffin will replace the water in the tissue, turning soft, moist tissues into a sample miscible with paraffin, a type of wax. This process is known as tissue processing. The processed tissue was then taken out of the cassette and set in a mould. Additional paraffin was added to create a paraffin block which is attached to the outside of the cassette. The process of embedding allows the sectioning of tissues into very thin (2 - 7 micrometer) sections using a microtome. The slices are thinner than the average cell, and are layered on a glass slide for staining. Tissue was dewax and hydrated, stained in Erich’s haematoxylin for 15mins, rinsed in water, differentiated in 1% HCl and 70% alcohol for 1min, rinsed in water, counterstained with 1% eosin for 1min, rinsed in water again and finally dehydrated, cleared and mounted on microscope for examination.

**Statistical analyses**
Data were presented as mean ± SD and analysed using SPSS® software, version 14.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Differences in biochemical values between control and treated groups were assessed by independent Student’s t-test. A P-value <0.05 was considered statistically significant.

**Results**
The result of phase I and II acute toxicity studies is presented in Table 1&2 below. In both phases no signs of toxicity or mortality were recorded after 24 hours of the administration.

**Table 1: Phase I LD_{50}, of the n-hexane leaf extract of Vitex simplicifolia**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Doses (mg/Kg )</th>
<th>No. of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2: Phase II LD_{50} of the n-hexane leaf extract of Vitex simplicifolia**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Doses (mg/Kg )</th>
<th>No. of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1600</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2900</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5000</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3: Effect of oral administration of n- hexane of leaf extract of *Vitex simplicifolia* on liver enzymes and unconjugated billirubin in Wister rats.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Dose mg/Kg</th>
<th>Body weight</th>
<th>ALP/ (U/l)</th>
<th>AST/ (U/l)</th>
<th>ALT/ (U/l)</th>
<th>U.Bil (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td></td>
<td>137.00±10.00</td>
<td>24.00±2.00</td>
<td>21.00±1.00</td>
<td>0.28±0.115</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>200.33±7.84a</td>
<td>84.667±4.70a</td>
<td>27.67±0.57a</td>
<td>0.89±0.79a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>537.00±10.00a</td>
<td>104.00±2.00a</td>
<td>30.00±3.05a</td>
<td>1.148±0.325a</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>786.50±237.50b</td>
<td>107.00±13.00a</td>
<td>39.00±13.005a</td>
<td>2.28±0.550a</td>
<td></td>
</tr>
</tbody>
</table>

Result expressed as mean±sem, sample size=5

*p<0.05 significantly greater than control

The result in Table 3 shows the activities of AST, ALT, ALP and Unconjugated bilirubin in rats administered with extracts. ALP, AST, ALT and unconjugated bilirubin were significantly (P<0.05) increased in all the test groups compared to the control. The result of kidney toxicological indices following administration of n- hexane leaf extract of *Vitex simplicifolia* is presented in table 4. Urea, creatinine and carbonate levels of the test animals showed significant (P<0.05) increase compared to that of the control. However, the concentration of sodium, potassium and chloride did not differ significantly (P>0.05) between the test and the control groups.

Table 4: Effect of oral administration of n- hexane leaf extract of *Vitex simplicifolia* on urea, creatinine and electrolytes in Wister rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (µmol/l)</th>
<th>Na⁺ (mEq/L)</th>
<th>K⁺ (mEq/L)</th>
<th>Cl⁻ (mEq/L)</th>
<th>HCO₃⁻ (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.72±0.83</td>
<td>40.33±3.18</td>
<td>143.48±2.31</td>
<td>5.86±0.80</td>
<td>104.71±1.04</td>
<td>23.33±1.45</td>
</tr>
<tr>
<td>2</td>
<td>56.19±0.74a</td>
<td>50.00±2.301a</td>
<td>142.34±2.89</td>
<td>5.00±0.75</td>
<td>105.91±1.07</td>
<td>22.00±1.15</td>
</tr>
<tr>
<td>3</td>
<td>70.00±0.85a</td>
<td>60.00±1.00a</td>
<td>139.00±1.00</td>
<td>5.00±0.85</td>
<td>105.00±1.07</td>
<td>24.00±1.00</td>
</tr>
<tr>
<td>4</td>
<td>91.50±0.50a</td>
<td>70.50±63.50a</td>
<td>140.50±15.50</td>
<td>5.00±95</td>
<td>102.50±12.5</td>
<td>23.50±1.50</td>
</tr>
</tbody>
</table>

Result expressed as mean±sem, sample size=5

*p<0.05 significantly greater than control
Though Electrolytes were not significantly affected by the administration of extract, levels of urea and creatinine were profoundly affected (Table 4) by the treatment at all levels. Figure 1 below shows the result of liver histopathological examination of the control and group 1 administered 1000 mg/kg body weight of the n-hexane extract of the plant. The liver architecture of the control group showed no pathological changes, while that of the test group show mild increase in kupfer cells, focal portal inflammation.

Figure 1: Photomicrograph of a section from a liver of the wistar rats administered 1000 mg/kg of N-hexane fraction of Vitex simplicifolia leaf extract using H and E Stain and a magnification of x250 (A) control (B) Treated with 1000 mg/Kg Body weight Extract

Figure 2 below is the photomicrograph of kidney histopathological examination of the control (Group 1) and test group 4 administered 1000mg/kg body weight of the methanolic extract of the plant. The kidney architecture when compared to the group I control showed multifocal expansion of the interstitium by aggregates of lymphocytes and histiocytes.

Figure 2: Figure 1 Photomicrograph of a section from a Kidney of the wistar rats administered 1000 mg/kg of N-hexane fraction of Vitex simplicifolia leaf extract using H and E Stain and a magnification of x250 (A) control (B) Treated with 1000 mg/Kg Body weight Extract
Discussion

The lipophilic extract of Vitex rotundifolia has been demonstrated to exhibit inhibit phosphorylation activity of MAP kinase and cancer chemotherapeutic potential (16), it is thought possible that Vitex simplicifolia belonging to approximately 270 of Vivex species (16) may have the same potential therapeutic benefit. Hence the need to conduct toxicological study on two key critical organs involved in drug detoxicification and excretion.

The lipophilic extract of The administration of n-hexane leaf extracts of Vitex simplicifolia at 250, 500 and 1000 mg/kg doses for 21 days orally was observed to significantly increase all the biochemical indices under investigation. The elevation of levels of Alkaline Phosphatase (ALP) as observed in the present study may be an indication of either liver problem or bone disease, since the two main sources of ALP are liver and bone. ALT is a cystosolic enzyme more specific to the liver, so a rise only occurs with liver diseases (14). A high level of serum bilirubin is used as indices of liver function and bile excretion status (11). The levels of these marker enzymes is a strong index of liver toxicity as has been substantiated by histological analysis which indicated gross tissue abberation. There is paucity of data on the toxic effect of Vitex simplicifolia; however aqueous extract of Vitex donniana is reported to be toxic to the liver at the dose given in this studyto the liver and kidney (1).

The administrations of n-hexane extract of Vitex simplicifolia to Wister rats showed no significant change in sodium, and chloride but a significant increase was observed in urea and cretinine. This corroborates with the reported study on vitex doniana by Ahmed et al. (2) The elevation of serum urea and creatinine observed in this study may have resulted from kidney damage from exposure to the extract. It is an established fact that a wide variety of renal diseases with different permutation of glomerular, tubular, interstitial or vascular damage can cause an increase in serum urea and creatinine concentration (5,25). Histopathology result of the kidney (Fig 2) with multifocal expansion of the interstitium by aggregates of lymphocytes and histiocytes substantiates this observation. Urea is a by product of protein metabolism that is excreted through the urine (1). Previous studies (1,24) on Vitex donniana; a related specie of Vitex simplicifolia reported similar observations which indicate that the Vitex family may contain some phytochemicals that could induce damage to the kidney.

Conclusion

This study evaluated hepato-renal toxicological indices following oral administration of n-hexane leaf extract of Vitex simplicifolia to experimental animals. The recorded observations suggest that the plant is well tolerated up to a dose of 5000mg/kg body weight at acute level but produced moderate sub-chronic hepato-renal injury to the liver and kidney. Thus care should be exercised when using this plant as a phytoremedy against ailments especially with respect to the duration of administration.

References

1. doniana Root-Bark on the Peripheral and Central Nervous System of Laboratory Animals J Applied Sci 7(10):1397-1403

http://dx.doi.org/10.4314/ajtcam.v12i1.14


