TOXICITY STUDY OF FOUR PARTIALLY PURIFIED LEAF EXTRACT OF Vitex simplicifolia ON LIVER FUNCTION IN WISTAR RATS


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ABSTRACT
This study evaluated the liver toxicological indices, following 21 days administration of fractionated leaf extracts of Vitex simplicifolia in Wistar rats. Acute toxicity studies with very high concentrations of the four fractionated extracts were carried out followed by sub chronic toxicities studies involving administration of 250mg/kg, 500mg/kg and 1000mg/kg body weight of the four fractionated leaf extracts to the experimental animals for 21 days. Aqueous, methanolic and ethyl acetate fractions significantly increase Alanine transaminase ALP (P < 0.05) and significantly decrease (P < 0.05) Aspartate transaminase (AST). However, the n-hexane fraction showed significant increase in ALP, AST and unconjugated bilirubin. The histopathological studies of the liver showed mild to moderate sinusoidal lymphocytosis with prominent kupfer cells and focal portal inflammation in the test groups. These observations shows that care should be taken when using Vitex simplicifolia as a phytoremedy against any ailments as sub chronic administration at high concentrations of the extract may induce injury to the liver.

Keywords: Vitex simplicifolia, Unconjugated bilirubin, Alanine aminotransferase, Aspartate transaminase, Aspartate transaminase

INTRODUCTION
Plants are known to be efficacious and most often could contain compounds that are potential drugs which would require further examinations. Interest in the search for medicines from natural sources has served as a catalysts for exploring techniques of obtaining the required plants and probing their activities (Edogho et al., 2005). A large proportion of such medicinal compounds have been discovered with the aid of ethnobotanical knowledge of their traditional uses. Chemical compounds in plants mediate their effects on the human system through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they function. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Lai and Roy, 2004, Tapsell, et al., 2006).

Vitex simplicifolia (Verbenaceae) is a perennial shrub or small tree which grows to a height of approximately 8 m and is widely distributed from Egypt to Guinea. In Burkina Faso, the plant is used to treat various internal or external diseases like skin diseases, dermatitis, bilharzia, migraines, fever, aches, amoebiasis, sore teeth, colic, infant tetanus (Nacoulma,1996). Investigations have also revealed that this plant is also used in the treatment of skin infections and wounds healing. In Burkina Faso, infectious diseases are the leading cause of infant (2.37%) and maternal (14.6%) mortality; therefore they constitute public health problems. The treatment of skin diseases dates back to ancient times, and many of their treatments were using medicinal plants. About 30% of traditional remedies are used to treat wounds and skin lesions, compared to only 1-3% of modern drugs (Mantle et al., 2001). The healing process is an immune response that begins after injury and takes place in three stages: vascular and inflammatory stage, phase of tissue repair and phase of maturation. A drug having simultaneously the potential antioxidant and antimicrobial activities may be a good therapeutic agent to accelerate cicatrization and wound healing (Phillips et al., 1991; Heike et al., 1999). Aromatherapy is now considered to be another alternative way in healing people, and therapeutic values of aromatic plants lie in their volatile constituents such as monoterpenoids, sesquiterpenoids and phenolic compounds that produce a definite physiological action on the human system (Bruneton, 1993). It is locally called Vitex (English), Dinya birri (Hausa), Ucha koro (Igbo) and Oori-nla (Yoruba) (Burkii, 2000). Several previous studies have established different parts of Vitex simplicifolia as a remedy against many ailments. In Nigeria, information available from the indigenous traditional healers indicates that, a decoction of the chopped stem barks and leaf of Vitex Simplicifolia is prepared and taken orally for treatment of diabetes and other disease conditions.
Animals were treated for a period of three (3) weeks. After the 21 days of administration, the animals were sacrificed, blood samples were collected in heparin bottles and the liver 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 (1983). In the first phase, nine (9) Wistar rats were used. The nine animals were divided into three groups of three animals each. Each group were administered 10,100 and 1000 mg/kg body weight of the extracts and then observed for 24 hours to monitor their behaviour and mortality. In the second phase 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. (Lorke, 1983). LD₅₀ was calculated by the formula: \( LD_{50} = \sqrt{D_0 \times D_{100}} \) where:

- \( D_0 \) = Highest dose that gave no mortality,
- \( D_{100} \) = Lowest dose that produce mortality.

**Liver function test**

Four enzymes indices of liver damage were assayed to determined liver toxicity. AST activity was determined by the method described by Karmen, (1955), ALP and ALT activities were determined by the methods of Reitman and Frankel (1957), while bilirubin levels were determined by the method of Sherloch (1951).

**Histopathological studies** (Avwioro, 2010; Mitchell et al, 2011)

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 mold. 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.

**RESULTS**

The results of phase I and II acute toxicity studies are presented in Table 1- 8 below. In both phases no signs of toxicity or mortality were recorded after 24 hours of the administration.
Table 1: Phase I LD$_{50}$ (Oral) of the aqueous fraction of *Vitex simplicifolia* leaf extract

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Doses (g/Kg)</th>
<th>No. of Death</th>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
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<td>3</td>
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Table 2: Phase 2 LD$_{50}$ (Oral) of the aqueous fraction of *Vitex simplicifolia* leaf extract

<table>
<thead>
<tr>
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<tr>
<td>1</td>
<td>1</td>
<td>1600</td>
<td>0</td>
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<tr>
<td>2</td>
<td>1</td>
<td>2900</td>
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<td>3</td>
<td>1</td>
<td>5000</td>
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Table 3: Phase I LD$_{50}$ (Oral) of the methanolic fraction of *Vitex simplicifolia* leaf extract

<table>
<thead>
<tr>
<th>Group</th>
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Table 4: Phase II LD$_{50}$ (Oral) of the methanolic fraction of *Vitex simplicifolia* leaf extract

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<tr>
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<tr>
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<td>3</td>
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Table 5: Phase I LD$_{50}$ (Oral) of the ethyl acetate fraction of *Vitex simplicifolia* leaf extract

<table>
<thead>
<tr>
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Table 6: Phase II LD$_{50}$ (Oral) of the ethyl acetate fraction of *Vitex simplicifolia* leaf extract

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<tbody>
<tr>
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<tr>
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<td>1</td>
<td>2900</td>
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<tr>
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Table 7: Phase I LD$_{50}$ (Oral) of the n-hexane fraction of *Vitex simplicifolia* leaf extract

<table>
<thead>
<tr>
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<td>3</td>
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Table 8: Phase II LD$_{50}$ (Oral) of the n-hexane fraction of *Vitex simplicifolia* leaf extract

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The lethal dose (LD$_{50}$) determination was conducted using the method of Lorke (1983) through oral route in rats in two different phases. In both phases no signs of toxicity or mortality were observed after 24 hours of the administration.
Figure 1: Shows the effect of aqueous leaf extract of *Vitex Simplicifolia* on ALP, AST, ALT and unconjugated bilirubin in alloxan-induced diabetic rats.

AGVSF - Aqueous fraction of *Vitex simplicifolia* leaf

Figure 2: Shows the effect of methanolic leaf extract of *Vitex Simplicifolia* on ALP, AST, ALT and unconjugated bilirubin in alloxan-induced diabetic rats.

MNVSF - Methanolic fraction of *Vitex simplicifolia* leaf
Figure 3: Shows the effect of ethyl acetate leaf extract of *Vitex Simplicifolia* on ALP, AST, ALT and unconjugated bilirubin on alloxan-induced diabetic rats.

\( ^a \) = significant compared to control.

ETVSF = Ethyl acetate fraction of *Vitex simplicifolia* leaf

Figure 4: Shows the effect of N hexane leaf extract of *Vitex Simplicifolia* on ALP, AST, ALT and unconjugated bilirubin on alloxan-induced diabetic rats.

\( ^a \) = significant compared to control.

NHVSF = N-hexane fraction of *Vitex simplicifolia* leaf
Histopathology Result

Histopathological examination of the rats liver from normal group 1 (Plate 1) showed normal hepatocytes arrange as radiating cord forming hexagonal units containing a central venules and that of the aqueous fraction (Plate 2) where as that of the methanol fraction of the extract (Plate 3) shows mild sinusoidal lymphocytosis and mild expansion. The liver section of the ethyl acetate fraction (Plate 4) and that of the n- hexane fraction (Plate 5) showed moderate sinusoidal lymphocytosis, prominent kupfer cells and focal portal inflammation indicating liver damage, but the section of the liver of the positive control (Plate 6) is normal after 3 weeks of administering glibenclamide.

Plate 11: Photomicrograph of a section of liver from untreated Wistar rat showing the portal tract area (black arrows), with no pathological changes (H and E stain, x250).

Plate III: Photomicrograph of section of liver of alloxan induced wistar rats that were administered 1000 mg/kg of aqueous fraction of *Vitex simplicifolia* leaf extract for twenty one days showing portal tract area(black arrows), with no pathological changes (H and E stain x250).

Plate IV: III:Photomicrograph of a section of liver from rats that were administered 1000 mg/kg methanolic fraction of *Vitex simplicifolia* leaf extract showing the portal tract area (black arrows), with mild sinusoidal lymphocytosis and mild expansion (H and E stain x250).
DISCUSSION
The result of acute toxicity study indicated that the 
LD$_{50}$ of the leaf extract of *Vitex simplicifolia* is greater 
than 5000mg/kg body weight (Tables 1 – 8). Thus, 
the non-lethal effects produced with the high dose of 
this extract are an indication that the leaf extracts of 
*Vitex simplicifolia* is relatively safe on acute oral 
exposure. It can therefore be established that *Vitex 
simplicifolia* leaf extract is non-toxic, which is in 
agreement the reported study by Abdelmajid (2014) 
on essential oil of the leaves of *Vitex simplicifolia* and 
with Bruce (1987), American Society for Testing and 
Materials (1987), Aditya and Ravi (2014), Kingsley et 
al (2014) and Ravichandra et al (2014), that any 
chemical substance with LD$_{50}$ estimate greater than 
3000-5000mg/kg (oral route) could be considered of 
low toxicity and safe on acute exposure.
The administration of aqueous, methanolic and ethyl 
acetate fractionated extracts of *Vitex simplicifolia* at 
250, 500 and 1000 mg/kg doses for 21 days orally 
was observed to significantly increase ALP (P>0.05) 
and decrease significantly (P>0.05) AST and had no 
significant change in ALT and Unconjugated billirubin 
(Figures 1, 2 and 3). The elevation of levels of 
Alkaline Phosphatase (ALP) as observed in the present 
study may be an indication of either liver or bone 
disease, since the two main sources of ALP are liver 
and bone. However the experimental animals treated 
with n- hexane fraction of *Vitex simplicifolia* showed 
significant increase in ALP, AST and Unconjugated 
billirubin (Figure 4). The indicators of liver function 
were all increased indicating possible liver damage. 
ALT is a cystosolic enzyme more specific to the liver, 
so a rise only occurs with liver diseases (Almdal et al., 
2008). The organ morphological changes in the liver 
are mild characterised by preservation of the native 
arhitecture but mild expansion of the sinusoids with 
lymphocytosis. This is not unexpected because, liver 
cells being stable cells heal by regeneration and mild 
sub lethal injury to the liver cells may heal within a 
short time.
Conclusion
This study evaluated liver toxicological indices following oral administration of fractionated leaf extracts 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 with tendency of liver toxicity as the dose and duration increases. Care should be exercised when using this plant as a phytoremedi against ailments especially with respect to the dose and duration of administration.

REFERENCE