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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 billirubin, 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 (Edeoga *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 aproximatively 8 m and is widely distributed from Egypt to Guinea. In BurkinaFaso, 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 cicatrisation 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) (Burkill, 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.

The plant extracts have been used as medication for infertility, liver disease, anodyne, stiffness, hypertension, cancer, febrifuge, as tonic galactagogue to aid milk production in lactating mothers, sedative, digestive regulator and treatment of eye, kidney and as supplement for lack of vitamin A and B (Sofowora, 1993; Burkill, 2000). Despite the extensive use of different parts of this plant for managing various ailments, there has not been to our knowledge, an extensive review of its possible toxicity against any organ or the whole system. This study therefore aim to bridge this gap by evaluating the toxicity indices of the liver following sub-chronic administration of fractionated leaf extracts of this plant.

MATERIAL AND METHODS

Plant and animals

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 weighing between 160 – 240g were obtained from the department of Physiology animal house, Bayero University Kano and 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.

Extraction and fractionation

Powdered materials of the plant were extracted with 95% ethanol at room temperature for seven days. The filtrates from this extract were concentrated under vacuum to yield crude ethanol extract. The latter was further fractionated using solvents of varying polarities (H_2O, CH_3OH, Ethyl acetate and hexane) to generate respective fractions (Prassanna *et al*, 2012)

Experimental Design

A total of sixty five (65) Wistar albino rats weighing between 160 – 240g were used for the study. The rats were divided into thirteen (13) groups of five (5) each. Extracts were administered orally using 1ml syringe.

Group 1 - Normal untreated rats

Groups 2, 3 and 4 – Rats administered 250, 500 and 1000 mg/kg body weight of aqueous fraction of the leaf extract of *Vitex simplicifolia* respectively.

Groups 5, 6 and 7 – Rats administered 250, 500 and 1000 mg/kg body weight of methanolic fraction of the leaf extract of *Vitex simplicifolia* respectively.

Groups 8, 9 and 10 – Rats administered 250, 500 and 1000 mg/kg body weight of ethyl acetate fraction of the extract of *Vitex simplicifolia* respectively.

Groups11, 12 and 13 –Rats administered 250, 500 and 1000 mg/kg body weight of n- hexane fraction of the leaf extract of *Vitex simplicifolia* respectively.

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 LD_{50}

The lethal dose (LD_{50}) 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 1000mg/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). LD_{50} was calculated by the formula: $LD_{50} = \sqrt{(D_0 x D_{100})}$ 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

Group	No. of Animals	Doses (g/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 2: Phase 2 LD_{50}, (Oral) of the aqueous fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	1	1600	0
2	1	2900	0
3	1	5000	0

Table 3: Phase I LD_{50}, (Oral) of the methanolic fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (g/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 4: Phase II LD_{50} (Oral) of the methanolic fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	1	1600	0
2	1	2900	0
3	1	5000	0

Table 5: Phase I LD_{50}, (Oral) of the ethyl acetate fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (g/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 6: Phase II LD_{50} (Oral) of the ethyl acetate fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	1	1600	0
2	1	2900	0
3	1	5000	0

Table 7: Phase I LD_{50}, (Oral) of the n-hexane fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (g/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 8: Phase II LD_{50} (Oral) of the n-hexane fraction of *Vitex simplicifolia* leaf extract

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	1	1600	0
2	1	2900	0
3	1	5000	0

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.

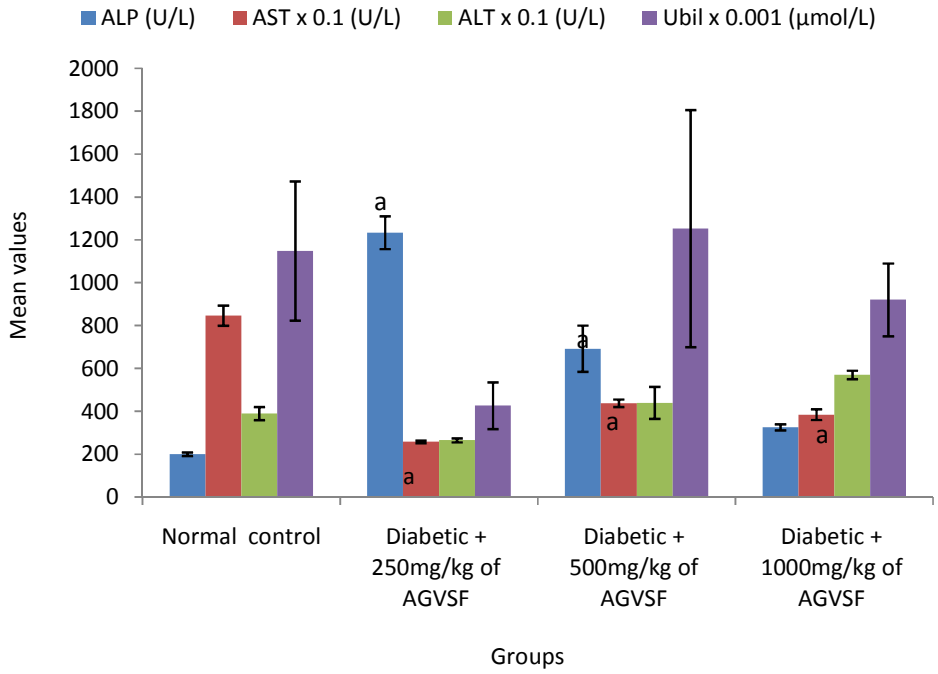

Figure 1: Shows the effect of aqueous leaf extract of *Vitex Simplicifolia* on ALP, AST, ALT and unconjugated billirubin in alloxan- induced diabetic rats.
[a] = significant compared to control.
AGVSF-Aqueous fraction of *Vitex simplicifolia* leaf

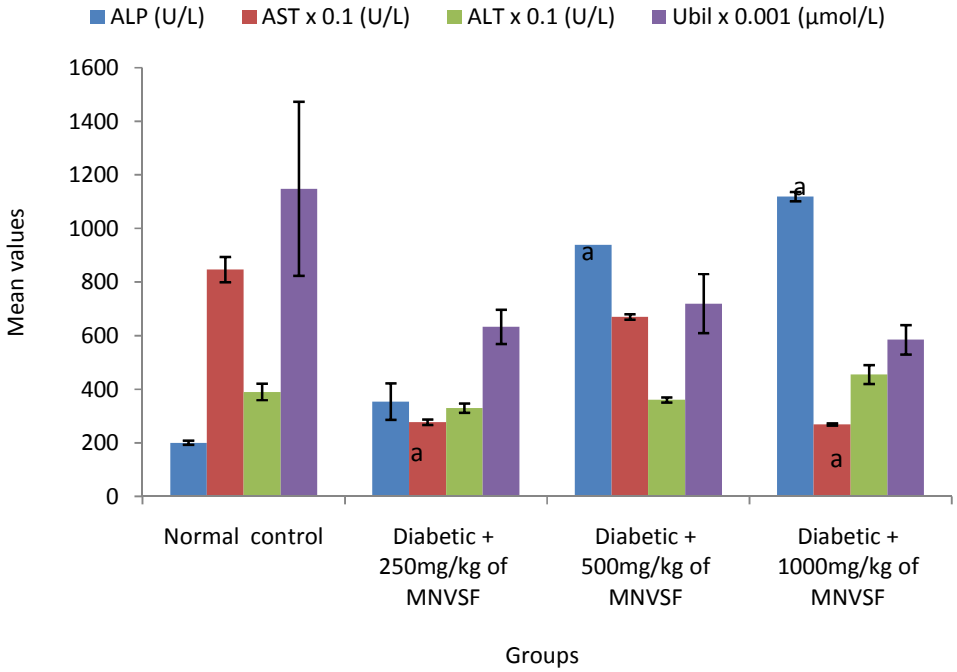

Figure 2: Shows the effect of methanolic leaf extract of *Vitex Simplicifolia* on ALP, AST, ALT and unconjugated billirubin in alloxan- induced diabetic rats.
[a] = significant compared to control.
MNVSF-Methanolic fraction of *Vitex simplicifolia* leaf

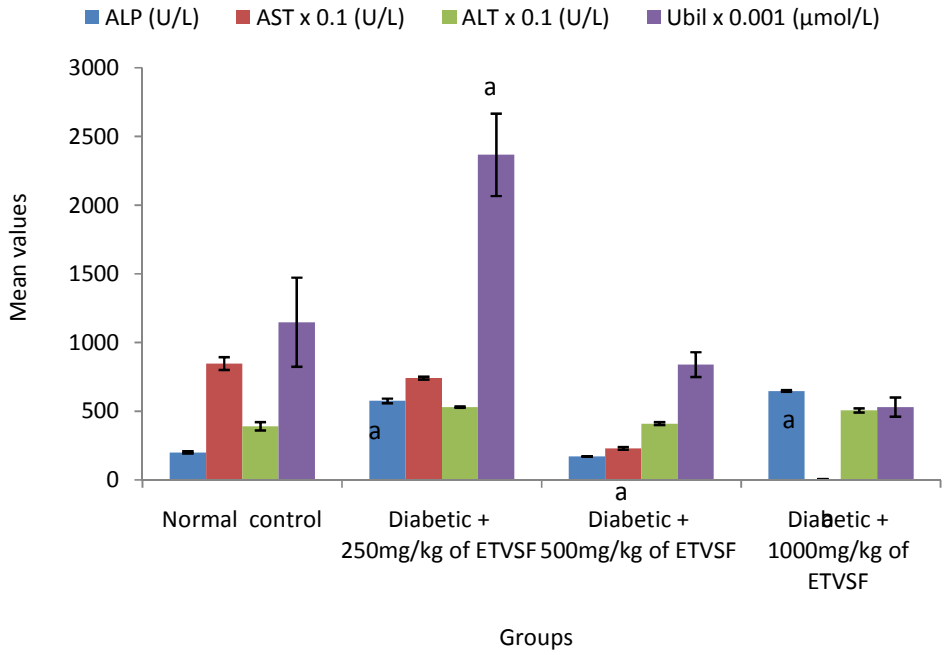

Figure 3: Shows the effect of ethyl acetate leaf extract of *Vitex Simplicifolia* on ALP,AST,ALT and unconjugated billirubin on alloxan- induced diabetic rats.
[a] = significant compared to control
ETVSF-Ethyl acetate fraction of *Vitex simplicifolia* leaf

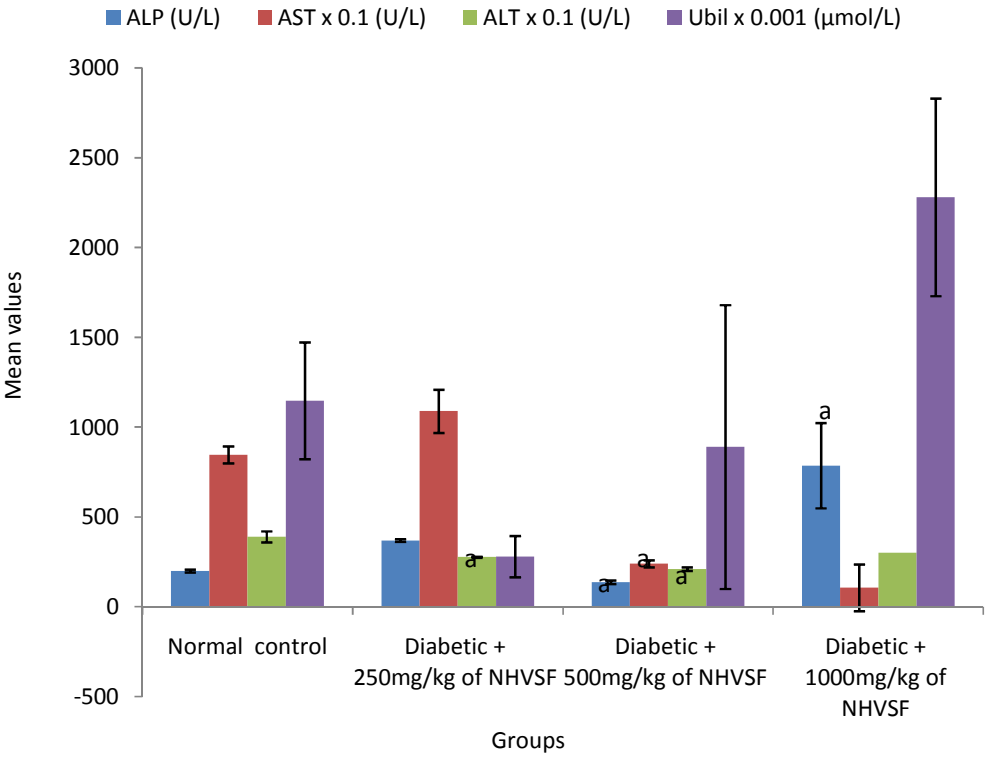

Figure 4: Shows the effect of N hexane leaf extract of *Vitex Simplicifolia* on ALP,AST,ALT and unconjugated billirubin on alloxan- induced diabetic rats.
[a] = significant compared to control.
NHVSF-N-hexane fraction of *Vitex simplicifolia* leaf

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 sinoidal 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, ×250).

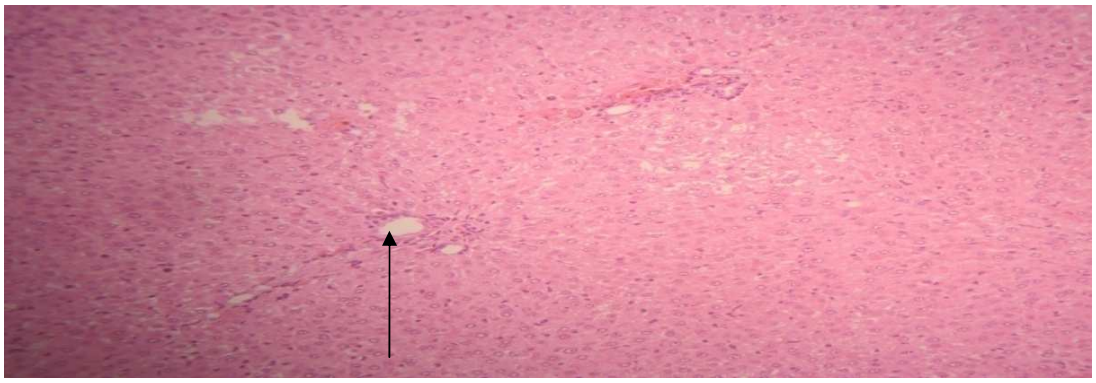

Plate III: Photomicrograph of section of liver of alloxan induced wistar rats that were administered 1000 mg/kg of aqueous fraction of *Vitex simplicifolia* leaf extract for twenty one days showing portal tract area(black arrows), with no pathological changes (H and E stain x250).

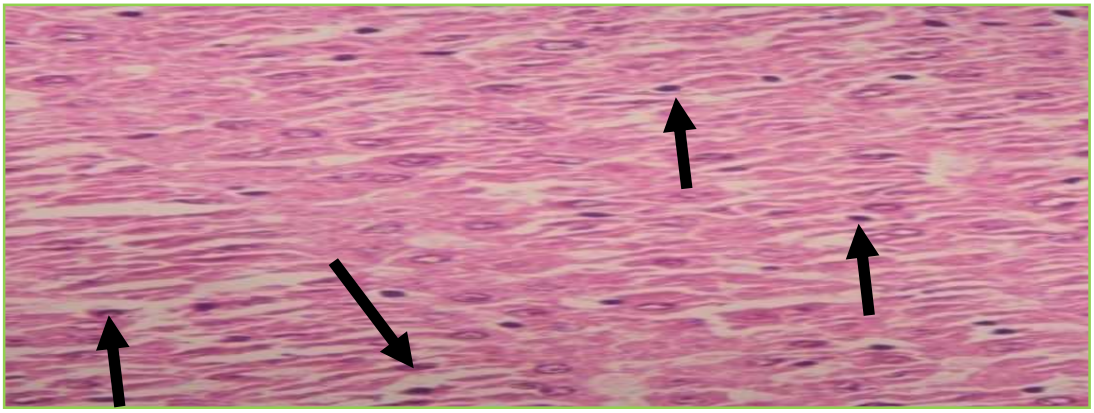

Plate IV: III:Photomicrograph of a section of liver from rats that were administered 1000 mg/kg methanolic fraction of *Vitex simplicifolia* leaf extract showing the portal tract area (black arrows), with mild sinusoidal lymphocytosis and mild expansion (H and E stain x250).

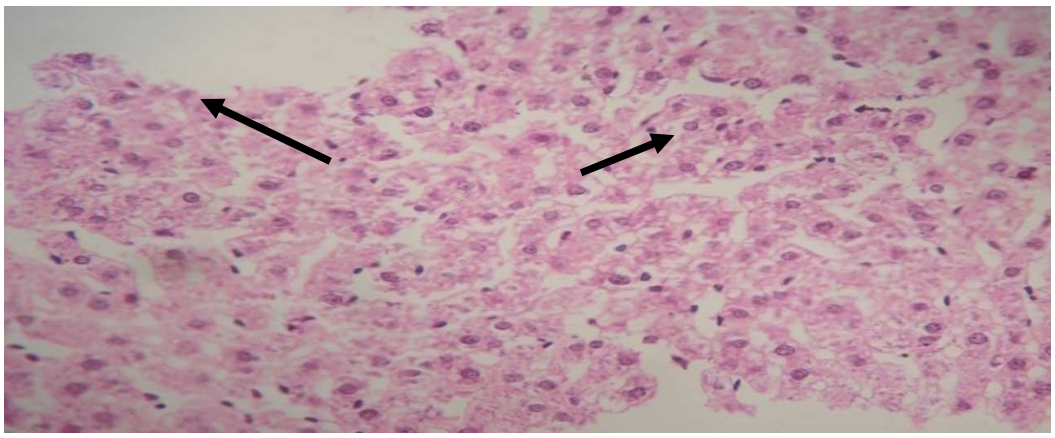

Plate V: Photomicrograph of a section of liver from wistar rats administered ethyl acetate fraction of *Vitex simplicifolia* leaf extract for twenty one days, showing the portal tract area(black arrows), with mild fatty change, moderate sinusoidal lymphocytosis, with prominent kupfer cells (H and E stain x250).

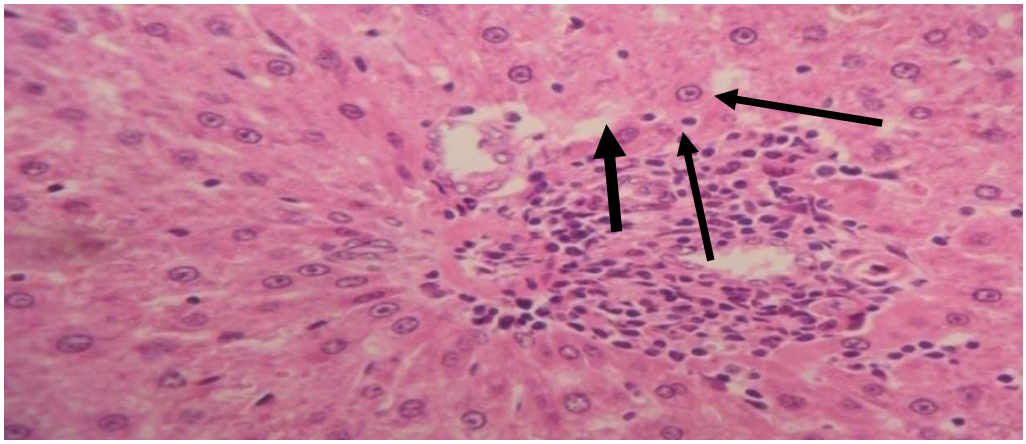

Plate VI: Photomicrograph of a section from a liver of the wistar rats administered 1000 mg/kg of N- hexane fraction of *Vitex simplicifolia* leaf extract,showing the portal tract area (black arrows), with mild increase in kupfer cells, focal portal inflammation (H and E stain x250).

DISCUSSION

The result of acute toxicity study indicated that the LD_{50} of the leaf extract of *Vitex simplicifolia* is greater that 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 architecture but mild expansion of the sinusoids with lymphocytosis.This is not unexpected becouse, 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 phytoremedy against ailments especially with respect to the dose and duration of administration.

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