

Effect of administration of aqueous leaf extract of *Vitex simplicifolia* on hepato-renal toxicological indices in white albino rats.Salim M A¹ Imam A A^{2*} Bala M² Khalifa M H² Tanko Y³ Mohammed A³¹Department of Human Physiology, Bayero University Kano-Nigeria²Department of Biochemistry, Bayero University Kano-Nigeria³Department of Human Physiology, Ahamadu Bello University, Zaria-Nigeria

ABSTRACT

This study evaluated the hepato-renal toxicological indices following 21 days administration of aqueous leaf extract of *Vitex simplicifolia* in white albino 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 aqueous 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 indicate that there was a significant increase in the activities of ALT and ALP while AST activities was lower compared to the control. Total and direct 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 that the levels of urea, creatinine and Cl^- were significantly higher in the test animals compared to the control while Na^+ , K^+ , and HCO_3^{2-} levels were significantly lower in the extract administered groups compared to the control. Histopathology examination of the liver and kidneys showed mild hepatic and renal damage at the highest dose (1000mg/kg body weight). These observations shows that care should be taken when using the aqueous extract of *Vitex simplicifolia* as a phytoremedy against any ailments as high concentrations of the extract may induce low to marginal injury to the liver and kidney.

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Introduction

Phytochemicals as medicinal agents over years have been used as lead compounds of modern drugs (4). "Return to nature" is a phrase used by advocates of natural products over the use of synthetic drugs (1). Such advocacy has led to patronage of natural products for the prevention and management of ailments (22). Challenges for the survival of early man against diseases, physical and mental discomfort led to the exploration of the natural surrounding for solution and thus, resulted in the discovery of many plant, animal products and minerals as agents with therapeutic benefits (16). The reservoir of

basic information derived from rich cultures of India and China in the use of traditional medicine has led to the keen interest of pharmaceutical companies to use ethnobotanical knowledge to develop drugs with potent therapeutic effects as well as greater acceptance by such communities as the first line of treatment and management of a variety of diseases (10). Natural products have not only provided chemical compounds with important biological and therapeutic functions, but are also used for defence against predators such as herbivorous animals, insects and fungi. It is estimated that about 12,000 bioactive compounds

with variety of pharmacological, physiological and biochemical functions have so far been identified (11,24). As majority of synthetic compounds are derived from plants, with increase of potency achieved through chemical manipulation, it is thought possible that the both positive and adverse effects of the two sets of compounds are mediated through identical mechanism in human (11,24). *Vitex simplicifolia* (Verbenaceae) is a perennial shrub, widely distributed from Egypt to Guinea with diverse pharmacological functions against skin diseases, dermatitis, bilharzia, migraines, fever, aches, amoebiasis, sore teeth, colic, infant tetanus (15). Strong advocacy for the use of tradition medicine coupled with the high cost of synthetic drugs has led to the use of this plant (30%) in the treatment of skin diseases over synthetic drugs (1-3%) in Burkino Faso (13). The healing process associated with immune system is in three stages. The stages are (i) vascular and inflammatory (ii) tissue repair and (iii) maturation. It has been established that chemicals with both antioxidant and antimicrobial activities have high therapeutic impact in term of accelerating cicatrisation and wound healing (7,17). Many aromatic plants are identified to have the dual activities, hence considered as alternative therapeutic agents to modern medicine. Such therapeutic values are identified within their volatile constituents like monoterpenoids, sesquiterpenoids and phenolic compounds (2). It is locally called vitex (English), dinya birri (Hausa), Ucha koro (Igbo) and Oori-nla (Yoruba) (3). Several previous studies have established different parts of *vitex simplicifolia* as a remedy against many ailments. The stem decoction is taken orally for the management of diabetes mellitus and other conditions in Nigeria. The plant extracts have been used as medication for infertility, liver disease, hypertension, cancer, and as tonic galactagogue to aid milk production in lactating mothers. It is also used as sedative, digestive regulator

and treatment of eye, kidney disease and as supplement for lack of vitamin A and B (3,23). Despite the extensive use of different parts of this plant for management of 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 is aimed to bridge this gap by evaluating the toxicity indices of the liver and kidney following sub-chronic administration of aqueous extract of this plant.

Material and Methods

Plant and animal

The leaves of *vitex simplicifolia* were collected from Bayero University Kano, the leaves were dried in shade at room temperature and grounded into powder. Albino 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 distilled water overnight. It was filtered and the residue was discarded. The filtrate was evaporated to dryness using Freeze dryer. The dried plant residue was used to prepare different concentrations.

Experimental Design

A total of 33 white albino 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 1000mg/kg body of the aqueous 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 (12). In the first phase,

nine (9) wistar rats were used. The nine animals were divided into three groups of three animals each. Each groups 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(12). LD₅₀ was calculated by the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$ where:

D₀ = Highest dose that gave no mortality,

D₁₀₀ = Lowest dose that produce mortality.

Liver and Kidney function test

Four enzymes indices of liver damage were assayed to determined liver toxicity. AST activity was determined by the method described by Karmen, et al. (8), ALP activities were determined by the methods of Reitman and Frankel (21) while bilirubin levels was determined by the modified method of Jendrassik and Gróf (6). 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 (6,25).

Histopathological studies (1,14)

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 result of acute toxicity study is presented in Table 1. There were no signs of toxicity or mortality after 24 hours of the administration of the various dosages of the plant extract

Table 1: Phase LD₅₀, of the crude aqueous leaf extract of *Vitex simplicifolia*

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0
4	3	1600	0
5	3	2900	0
6	3	5000	0

Table 2 below shows the activities of AST, ALT, ALP total and direct bilirubin in extract administered and control rats. ALP was significantly ($P < 0.05$) increased in the test groups (Groups II, III and IV) compared to the control. However, a decrease in ALP was observed with increase dosage of the extract. There was no significant change in the activity of

AST between test and control groups. Moderate but insignificant increase in ALT activity was also observed in the test groups (II and III). There was a significant decrease in ALT activity in test group II compared with control ($p > 0.05$). Similarly, the levels of direct bilirubin and total bilirubin were not significantly different from the control.

Table 2. Effect of oral administration of aqueous leave extract of *vitex simplicifolia* on liver toxicological indices in white albino rats.

GROUP	AST/ (U/l)	ALT/ (U/l)	ALP/ (U/l)	DB/ ($\mu\text{mol/l}$)	TB/ ($\mu\text{mol/l}$)
I. Control	84.67 \pm 4.70 ^a	39.00 \pm 3.06	200.33 \pm 7.4	1.482 \pm 0.5	1.15 \pm 0.33
II 250mg	25.82 \pm 0.61 ^a	26.50 \pm 0.96 ^a	1233.5 \pm 76.30 ^a	1.48 \pm 0.13	0.43 \pm 0.11
III 500mg	43.81 \pm 1.74 ^a	44.00 \pm 7.50 ^b	692.33 \pm 108.2 ^a	1.93 \pm 0.12	1.25 \pm 0.55
IV 1000mg	38.50 \pm 2.50 ^a	57.00 \pm 2.00 ^b	326.00 \pm 14.00 ^a	0.88 \pm 0.24	0.92 \pm 0.17

Values are presented as mean \pm standard error of mean (SEM) for five determinations.

^a $p >$ significantly lower than control.

^b $p <$ significantly higher than control

The result of kidney toxicological indices following administration of aqueous leaf extract of *Vitex simplicifolia*. is presented in table 3. Urea and creatinine levels of the test animals showed significant ($P<0.05$) increase compared with control, while

bicarbonate levels decreased significantly ($P<0.05$). However, the concentration sodium, potassium and chloride ions did not differ significantly between the test and the control groups.

Table 3 . Effect of oral administration of aqueous leave extract of *vitex simplicifolia* on

Group	UREA/(mg/dl)	CREAT/(μ mol/l)	Na ⁺ /(mEq/L)	K ⁺ /(mEq/L)	Cl ⁻ /(mEq/L)	HCO ₃ ⁻ /(mEq/L)
Control	25.72± 0.83	186.33±3.18	219.48±2.31	5.86 ±0.80	75.71±1.04	44.33±1.45 ^{ab}
Group I	100.3± 1.38 ^b	249.25±2.93 ^b	207.39±9.89	5.29 ±0.05	89.09±0.92	25.25±1.11 ^a
Group II	142.53±2.03 ^b	278.00±17.32 ^b	240.39±8.15 ^b	5.59±0.1	88.1±4.23	38.33±2.03
Group III	99.52 ± 7.10 ^b	113.50±4.50 ^a	191.08±8.00 ^a	4.60±0.91	84.5±7.97	30.00±2.00 ^b

kidney toxicological indices in white albino rats.

Values are presented as mean ± standard error of mean (SEM). Number of animals per group was 5; ^ap>significantly lower than control; ^bp< significantly higher than control.

Figure1 shows the result of liver histopathological examination of the control and group administered with

1000mg/kg body weight of the aqueous extract of the plant. The liver architecture of the two groups showed no pathological changes.

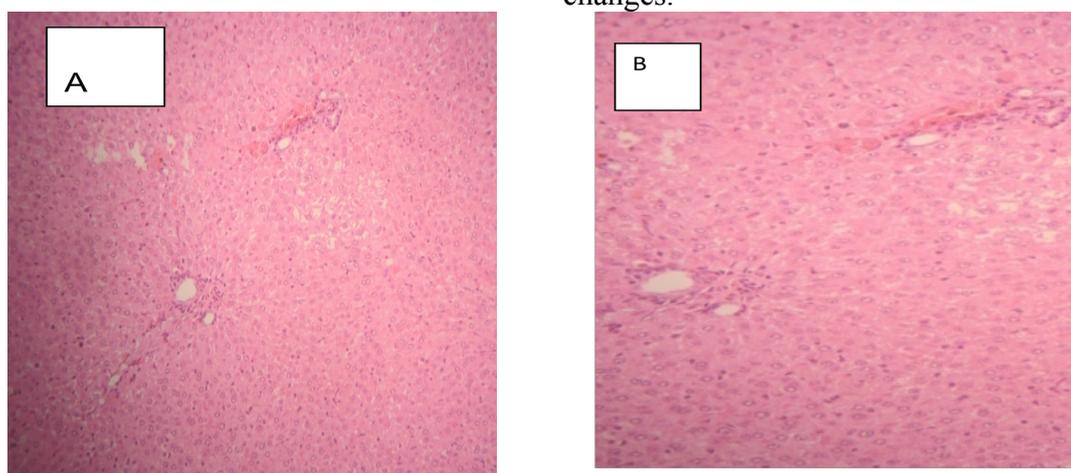
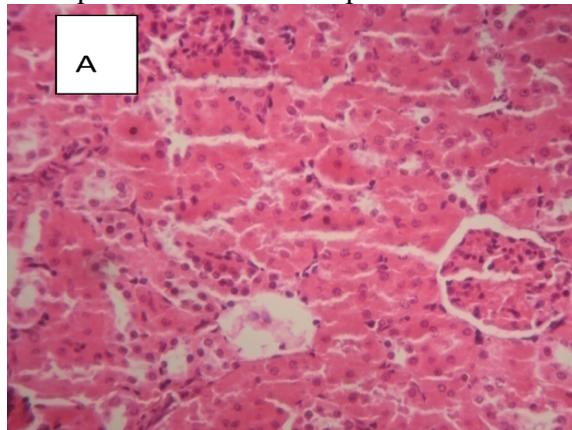


Fig.1 (A) Stained cross section of liver of group I (control) albino rats administered no extract showing the portal tract area, with no pathological changes (normal). (Hand E Stain (×100) (B) Stained cross section of liver of rats that were administered 1000mg/kg body weight aqueous extract of *vitex simplicifolia* leaf showing the portal tract area, with no pathological changes (normal) (Hand E Stain (×100)

While figure 2 shows the result of kidney histopathological examination of the control Group and test group administered 1000mg/kg body weight of the aqueous extract of the plant. There was



a mild damage to the kidney architecture as demonstrated by the mild damage to distal tubules at dosage of 1000mg/kg body weight extract.

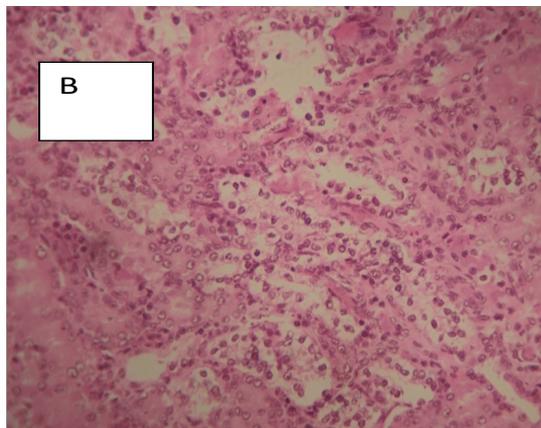


Fig. 2 Fig 2. (A) : Stained cross section of kidney of group I (control) albino rats administered no extract showing the portal tract area, with no pathological changes (normal). (Hand E Stain ($\times 100$)) (B) : Stained cross section of Kidney of rats that were administered 1000mg/kg body weight aqueous extract of *vitex simplicifolia* leaf showing the portal tract area, with mild damage to the distal tubules (Hand E Stain ($\times 100$))

Discussion

The administration of aqueous leaf extracts of *vitex simplicifolia* at 250, 500 and 1000 mg/kg⁻¹ doses for 21 days orally was observed to significantly increase ALP and decrease AST, but had no significant change on the level of unconjugated bilirubin. 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 cytosolic enzyme more specific to the liver, so a rise only occurs with liver disease (9). Although high level of serum bilirubin is used as indices of liver function and bile excretion status (18), this study did not record appreciable differences in bilirubin levels between the test and the control groups.

The administrations of aqueous extract of *vitex simplicifolia* to white albino rats showed no significant change in sodium, potassium, chloride but a significant

increase in urea and creatinine levels and decrease in carbonate levels was recorded. 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 (20). Histopathology result of the kidney with mild distal tubular damage substantiate this observation. Urea is a by product of protein metabolism that is excreted through the urine. Previous studies (19) 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 mild damage to the kidney.

Conclusion

This study evaluated hepato-renal toxicological indices following oral administration of aqueous 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 a low to 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.

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