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Toxic Effect of Aqueous Root-Bark Extract of *Vitex doniana* on Liver and Kidney Functions

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Abstract: This study investigated the sub-acute effects of aqueous root bark extract of *Vitex doniana* on some serum elemental components as well as serum biochemical parameters in rats. Serum sodium levels were significantly ($p<0.05$) increased following extract treatments while those of potassium were decreased. The extract decreased serum calcium concentration but no marked effect was observed on serum phosphorus concentration. The cholesterol level also decreased in the rats treated with higher concentration of extract (100 and 200 mg kg^{-1}), while the protein concentration in the serum of treated animals was comparable with that of control except at 200 mg kg^{-1} which showed significant ($p<0.05$) decrease in protein level at 21 days of extract treatment. Levels of liver marker enzymes and blood urea nitrogen in the extract treated rats increased in a dose-dependent manner and could be due to the pathological lesions in the liver and kidney which are the main excretory system. The aqueous extract of *Vitex doniana* though used for the management of some illness such as diarrhea, jaundice, anemia, mental illness, rheumatism and as tranquilizer in man, has been observed in rats under the conditions of this study to be toxic to both the liver and kidney.

Key words: Root bark extract, liver enzymes, serum electrolytes, pathology of kidney and liver

INTRODUCTION

Vitex doniana is a member of *Verbenaceae* family and has been described as woody or herbaceous often with quadrangles or branchlets. *Vitex* is a large genus and distributed throughout the tropics and sub-tropics. It has rare exceptional leaves which are composite and digitately compound and in Nigeria this combinations distinguishes it from all other genera (Hutchinson and Dalziel, 1963; Keay *et al*., 1964).

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 catalyst for exploring techniques of obtaining the required plants and probing their activities (Edeoga *et al*., 2005).

Vitex doniana has been used for both economic and medicinal purposes. The wood is for light construction work and the gum is considered an antidote against snakebites and arrow poison, the leaves and fruits in treatment of diarrhea, the aromatic bark serve as tonic (Sofowora, 1993).Other medicinal applications include treatment against jaundice, anemia, dysentery, mental illness, rheumatism, as anthelmintic and tranquilizer, gastrointestinal disturbances, urinary ailments etc. (http: //www. herbwen. com/herbage/A128htm: Igoli *et al*., 2005).

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Phytochemical analysis of the various parts of the plant extract revealed the presence of saponin, tannins, phenols, cardiac glycosides, flavonoids, sterols and triterpenes as well as high concentration of sodium, potassium, calcium, iron, phosphorus and sulphur (Abdulrahman and Onyeyili, 2006). Also Kilani (2006) have assessed the stem bark of *V. doniana* for antibacterial activity and establish its efficacy in the management of dysentery and gastroenteritis infections.

Despite the reported valuable therapeutic importance of medicinal plants, a large number of them have been implicated in causing toxicities (Frantisek, 1991; Anonymous, 2006).

The aim of this study therefore is to investigate the effect of aqueous root-bark extract of *Vitex doniana* on the pathology and physiology of liver and kidney.

MATERIALS AND METHODS

Plant Collection and Preparation

This study was carried out between the periods of April 2004 to June 2006. The root-bark of *Vitex doniana* was collected from the outskirts of Maiduguri, Borno State, Nigeria and identified by Dr. S.S. Sanusi a plant taxonomist in the Biological Sciences Department of University of Maiduguri, Nigeria. The air-dried root-bark was subsequently grounded into powder. Five hundred grams of the powdered root-bark was exhaustively soxhlet extracted with distilled water (Sofowora, 1993; WHO, 1992). The extract was concentrated *in vacuo* and stored at 4°C until required. A fresh solution was prepared from the residue on each day of extract administration.

Experimental Protocol

A total of 80 rats of both sexes weighing between 94.8-120 g were used for the experiments. They were obtained from a colony of rats maintained at the animal house of the Institute for Trypanosomiasis Research, Vom, Nigeria. The animals were housed in clean cages and had access to feed (ECWA Feeds Nigeria Ltd., Jos, Nigeria) and water *ad libitum*. They were allowed to acclimatize for two weeks in the Veterinary Physiology and Pharmacology Laboratory, University of Maiduguri, Nigeria, before the commencement of the studies. All the animals were handled according to the International Guiding Principles for Biomedical Research Involving Animal (CIOMS, 1985) and certified by the Animal Ethic Committee of the Faculty of Veterinary Medicine, University of Maiduguri.

The rats were separated into four equal groups of twenty rats each. Animals in groups 1-3 were treated orally with 50, 100 and 200 mg kg^{-1} of the root-bark extract respectively for 3 weeks, while group 4 served as control and was given distilled water only by the same route. Serum electrolytes, serum enzymes and biochemical parameters were used to assess the effects of the various doses of the extract in the rats following sub-acute administration (Sandabe *et al*., 2005).

Sample Collection

Five rats from each group were humanely sacrificed every week according to the CIOMS (1985) guidelines and having been certified by the Animal Ethic Committee of the Faculty of Veterinary Medicine, University of Maiduguri. Blood samples were collected into plain sample bottles. Serum was allowed to separate at 4°C from blood samples and was used for the determination of Blood Urea Nitrogen (BUN), serum cholesterol and serum alkaline phosphatase (ALP), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total serum protein, sodium, potassium, calcium and phosphate using standard procedures. Tissue samples of the liver, kidney, lungs, stomach and intestines were harvested for histopathological examination.

Preparation of Standard and Test Solutions:

The test-samples were prepared by mixing 20 mL of distilled water with 0.1 mL of serum sample while the standard solution was obtained by mixing 20 mL of distilled water with 0.1 mL of the standard (using commercial kits).

Determination of the Serum Sodium (Na^+) and Potassium (K^+) by Flame Emission Spectroscopy

Three universal bottles labeled test standard and blank were used. With the help of a glass pipette 2 mm of de-ionized water was place into the bottle labeled blank while 2 mL of working standard was placed in the bottle labeled standard. 0.99 mL of de-ionized water and 0.1 mL of serum was placed in the bottle labeled test and mixed thoroughly.

Sodium light filter was inserted into the galvanometer and switched on. The gas supply was turned on and the flame ignited. Thereafter the air supply was turned on and the air pressure regulated to 1016 sq^{-1}. The gas supply was adjusted to obtain discrete cones of flame. The galvanometer scale was set to zero with the blank (de-ionized water), thereafter the galvanometer reading was set to 80. Using the working standard it was reset to zero. The test was read, checking the standard after test reading. The galvanometer gives reading in mEq L^{-1}. For a monovalent cation 1 mEq L^{-1} = mmol L^{-1} is equal to galvanometer reading×2.

For the determination of potassium, potassium light filter was used and the galvanometer was set with potassium working standard and continued as for sodium (Kotthoff and Elving, 1976).

Determination of Serum Calcium (Ca^{2+})

The procedure reported by Lorentz (1982) was use for the calcium ions determination. Commercial kits (Quimica Clinica Applicada, SA) were use for the study. Into appropriate tubes labeled test standard and blank, 2.5 mL each of O-cresolpthalein complexone CPC and dimethylamine buffer (DEA) buffer solution were added. Then 0.05 mL of serum, standard and distilled water were thereafter added, respectively into the 3 tubes. These were mixed well and allowed to stand for 5 min at room temperature. The optical density of the test, standard and blank were then read at wavelength of 575 nm using spectrometer (Boeringer, 4010, West Germany). The concentration of calcium ions in serum was obtained using the following formula.

$$\text{Calcium concentration in mmol L}^{-1} = \frac{\text{SA} \times \text{OD}}{\text{ST} \times \text{OD}} \times 10$$

SI unit: mg $dL^{-1} \times 0.2495$ = mmol L^{-1}
SA = Sample, ST = Standard, OD = Optical Density

Determination of Serum Inorganic Phosphorus

The method described by Henry (1964) was employed. The following mixtures were prepared as the test standard and blank solutions using commercial kits (Quimica Clinica Applicada, SA);

	Test (mL)	Standard (mL)	Blank (mL)
Sample/serum	0.05	-	-
Standard	-	0.1	-
Reagent	3.0	3.0	3.0

The materials in the various tubes were mixed thoroughly and allowed to stand for 10 min at room temperature. Thereafter, the optical density was read at wavelength of 650 nm using spectrophotometer (Boeringer, 4010, West Germany).

The concentration of phosphorus in serum sample was obtained from the formula

$$\text{Phosphorus concentration in mmol L}^{-1} = \frac{\text{SA} \times \text{OD}}{\text{ST} \times \text{OD}} \times 4$$

SA = Test, sample, ST = Standard, OD = Optical Density

Determination of Serum Urea

For analysis of serum urea, diacetylmonoxime colorimetric (non-UV) method was employed (Kaplan, 1965).

Three test tubes labeled blank, standard and test were used. Ten milliliters of distilled water was added to the three tubes. Thereafter, 0.01 mL each of standard (100 mmol L^{-1}) and test solution was, respectively placed in the tubes labeled standard and test. This was followed by addition of 2 mL of the color reagent (diacetylmonoxime, 5 g mL^{-1}) to each of the test tubes and was mixed thoroughly. Two milliliters of acid reagent (5 g of ferric chloride hexa-hydrate and 85% phosphoric acid in 25 mL of distilled H_2O) was then added to each of the three test tubes, mixed properly and incubated at 37°C for 15 min.

The contents of the tubes were then cooled and their absorbance's read using spectrometer (Boeringer, 4010, West Germany) at 560 nm. The concentration of urea was calculated using the formula.

$$\text{Urea concentratioin} = \frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

$$= \text{mmol L}^{-1}$$

Determination of Serum Total Protein

The total protein in the serum sample was estimated using direct Biuret method (Peters ***et al.***, 1982). Three test tubes labeled blank standard and test were used. Five milliliters of 3% NaOH solution was placed into the blank tube and 4.9 mL of the same solution was added to the tubes labeled standard and test. Thereafter 0.10 mL of protein standard was added into standard tube and 0.1 mL of serum sample added into the tube labeled test. One milliliter of Biuret solution (5% $CuSO_4$) was added into each of tubes. After 15 min, the absorbance of the standard and test were read against blank at 540 nm using spectrometer (Boeringer, 4010, West Germany). The protein concentration was calculated using the formula.

$$\text{Protien concentratioin} = \frac{\text{OA} \times \text{SA} \times \text{ST}}{\text{OD} \times \text{ST}} = \text{mmol L}^{-1}$$

SA = Sample; ST = Standard; OD = Optical Density

Determination of Serum Cholesterol

Cholesterol was assayed by Trindar's reaction (Evans and Steins, 1986). Commercial kits (Quinica Clinica Applicada, SA) were used for the assay. The following, mixtures were prepared for the test, standard and blank.

	Test (mL)	Standard (mL)	Blank (mL)
Serum	0.02	-	-
Standard	-	0.2	-
Reagent	2.0	2.0	2.0

The contents of the tubes were thoroughly mixed and allowed to stand for 5 min at room temperature after which the absorbance was read at the wavelength of 510 nm using spectrometer. The cholesterol concentration was calculated using the formula:

$$\text{Cholestrol concentratioin (mmol L}^{-1}) = \frac{\text{SA} \times \text{OD}}{\text{ST} \times \text{OD}} \times 200$$

SA = Sample; ST = Standard; OD = Optical Density.

Determination of Serum Aspartate Aminotransferase (AST) and Alanine Aminotranferase (ALT)

AST and ALT were measured colorimetrically at 505 nm using the procedure described by Moss *et al*. (1986). The colorimeter was zeroed with distilled water. The following components were pipetted into a 1.0 cm curvet that has 1.0 cm light path. Commercial kits (Quimica Clinica Applicada, SA) were used for the study.

	AST (mL)	ALT (mL)
AST substrate	0.1	-
ALT substrate	-	0.5

These were incubated for 5 min at 37°C

Serum sample	0.1	0.1
Incubated at 37°C	60 min	30 min
DNPH	0.50	0.50

Mixture allowed to stand for 20 min at room temperature

0.4 N NaOH	5.0	5.0

The mixtures in the various curvets were allowed to stand for 15 min at room temperature and the transmittance of the mixture read at wavelength of 505 nm. Calibration curves were prepared to determine the activity values of AST and ALT in which the transmittance obtained were, interpolated in the calibration curve.

In SI unit 1 $\mu L^{-1} = \mu\ mL^{-1} \times 0.483$

Determination of Serum Alkaline Phosphatase (ALP)

The procedure reported by Moss *et al*. (1986) was employed. Alkaline aminophosphatase levels were determined using commercial kits (Quimica Clinica Applicada, SA). Distilled water (1 mL) was pipetted into two curvets labeled test and standard. Thereafter one drop of substrate phenolphthalein monophosphate was added and the contents of the curvets incubated at 37°C for 5 min. The standard solution (0.1 mL) was added into the curvet labeled standard while 0.1 mL of serum was added into the curvet labeled test. Five milliliters of colors reagent was added to each of the tubes and the color reactions measured at wavelength of 550 nm using a spectrometer (Boeringer, 4010, West Germany). Alkaline phosphates level was calculated using the formula:

$$\text{Alkaline phosphatase concentratioin}(\mu L^{-1}) = \frac{SA \times OD}{ST \times OD} \times 30$$

SA = Sample; ST = Standard; OD = Optical Density.

Statistical Analysis

The result is presented as mean±standard deviation. Significant ($p<0.05$) differences between means were compared using ANOVA (graph pad instat package).

RESULTS

Effect of Aqueous Extract of *Vitex doniana* Root-Bark on Some Serum Electrolytes

The effects of *Vitex doniana* root-bark extract (50, 100 and 200 mg kg^{-1}) on some elemental and biochemical components of blood in rats are shown in Table 1. The concentration of sodium ion (Na^+) in the serum samples at the various dose levels of the extract showed an increase in Na^+ ion concentration up-to twenty one days of extract treatment. The increases in Na^+ ion concentration appear to be time and dose-dependent. There was no appreciable decrease in concentration of sodium ion 14 days post extract administration.

The potassium ion (K^+) concentration decreased in all the treated groups compared with the control, however, following the termination of treatments, the K^+ ion concentration increased. Calcium (Ca^{2+}) concentration also decreased following treatment with the extract at 100 and 200 mg kg^{-1} doses. The animals treated with 50 mg kg^{-1} dose showed increased Ca^{2+} ion concentration at day 14 of treatment when compared with the control and those treated with higher doses of the extract. There was no marked effect of the extract on serum phosphorus concentration.

The effect of the various doses of the extract on serum urea levels showed that at higher doses of extract (100 and 200 mg kg^{-1}) there was an increase in serum urea concentrations compared with the control (Table 2). However, the urea concentration decreased 14 days post extract administration. The protein concentration in the blood of treated animals appear to be comparable with that of the control except at 21 days of extract treatment with 200 mg kg^{-1} dose which recorded significantly decreased protein levels. Cholesterol levels following treatment with 100 and 200 mg kg^{-1} doses decreased significantly ($p<0.05$) but subsequently rose following termination of treatment (Table 2).

Effect of Aqueous Extract of *Vitex doniana* Root-Bark on Some Serum Enzymes

The serum AST increased with extract treatment with the differences being significant ($p<0.05$) when compared with the control especially at the doses of 100 and 200 mg kg^{-1} of extract.

Serum ALT was also observed to increase with extract treatment. Treatment with 200 mg kg^{-1} of the extract resulted in an extremely significant ($p<0.05$) increase of ALT from day seven and the value did not return to the control level 14 days post treatment. Alkaline phosphatase levels following treatment with the various doses of the extract increased from the first week of treatment with the levels decreasing after termination of treatment (Table 3).

Table 1: Effect of aqueous extract of *Vitex doniana* root-bark on some serum electrolyte components of blood in rats

Parameters (mmol L^{-1})	Extract dose (mg kg^{-1})	Days of extract treatment 7	14	21***	28	35
Sodium	Control	128.00±0.28	127.00±2.30	128.00±3.30	129.00±3.20	128.00±2.50
	50.0	128.00±2.38	130.00±2.40	136.00±2.40*	139.00±2.80*	133.00±2.60*
	100.0	123.00±2.95	132.00±2.80*	140.00±1.90*	138.00±2.70*	139.00±2.80*
	200.0	157.00±3.53	158.00±2.60*	158.00±1.80*	146.00±3.30*	142.00±3.70*
Potassium	Control	12.60±1.31	12.90±0.22	12.95±0.25	11.90±0.12	11.90±0.12
	50.0	10.70±2.65**	7.38±0.18	5.60±0.38**	11.80±0.03	11.60±0.87
	100.0	10.80±2.88**	8.76±0.42**	6.60±0.28**	12.30±0.52	10.00±1.50
	200.0	10.60±2.84**	6.86±0.22**	7.10±0.48**	15.20±0.43*	13.60±0.35*
Calcium	Control	2.20±0.99	2.25±0.33	2.32±0.23	2.35±0.05	2.30±0.35
	50	3.30±0.78	5.53±0.12*	2.74±0.05	2.08±0.12	2.25±0.15
	100.0	2.10±0.29	1.88±0.28**	1.22±0.30**	1.85±0.15**	1.95±0.30
	200.0	2.80±0.71	2.12±0.25**	1.80±0.28**	1.60±0.01**	2.20±0.60
Phosphorus	Control	2.70±1.33	2.88±0.38	2.78±0.80	2.95±0.65	3.10±0.65
	50.0	2.20±1.16	2.55±0.27	2.56±0.60	2.53±0.17	2.80±0.42
	100.0	2.65±1.27	2.54±0.44	2.21±0.20	2.93±0.07	2.50±0.60
	200.0	2.95±0.98	2.90±0.76	2.65±0.17	3.13±0.17	3.18±0.40

*: Significant increase at $p<0.05$; **: Significant decrease at $p<0.05$; ***: Last day of extract treatment

Table 2: Effect of aqueous extract of *Vitex doniana* on some biochemical parameters in rats

Parameters (mmol L^{-1})	Extract dose (mg kg^{-1})	Days of extract treatment 7	14	21*	28	35
Urea	Control	3.60±1.04	3.60±0.32	3.60±0.37	3.58±0.42	3.53±0.24
	50.0	3.80±1.39	3.90±0.26	3.50±0.08	3.53±2.80	3.53±0.17
	100.0	4.40±1.23	4.90±0.29**	5.90±0.78**	3.93±0.39	2.55±0.27
	200.0	4.80±1.37	5.30±0.18**	6.30±0.18**	3.13±0.17	3.18±0.08
Protein	Control	54.00±3.00	54.00±2.50	54.20±3.20	54.30±2.50	54.50±2.60
	50.0	53.50±4.50	49.40±2.60	52.20±2.70	52.20±2.70	53.20±2.70
	100.0	52.80±2.50	52.00±2.30	51.30±3.80	53.50±3.50	53.50±3.50
	200.0	54.30±1.80	53.50±16.0	46.50±2.50***	54.00±2.50	53.80±3.20
Cholesterol	Control	2.29±1.40	2.29±0.09	2.30±0.30	2.35±0.55	2.29±0.30
	50	2.20±0.70	2.30±0.21	2.34±0.36	2.60±0.56	2.17±0.35
	100.0	2.48±0.56	1.68±0.06***	1.85±0.25***	2.70±0.53	2.86±0.68
	200.0	2.30±0.28	1.60±0.02***	1.40±0.60***	1.80±0.20***	2.50±0.20

*: Last day of extract treatment; **: Significant increase at (p<0.05); ***: Significant decrease at (p<0.05)

Table 3: Effect of aqueous extract of Vitex doniana root-bark on some serum enzymes in rats

Parameters (µL^{-1})	Extract dose (mg kg^{-1})	Days of extract treatment 7	14	21**	28	35
AST	Control	82.0±3.70	81.5±2.75	81.8±2.50	82.0±3.50	82.0±2.25
	50.0	84.0±1.26	83.0±3.25	83.0±3.50	83.0±2.61	82.0±3.45
	100.0	85.0±2.16*	84.0±2.35*	84.0±2.75*	84.0±2.30	83.0±1.36
	200.0	85.0±1.26*	85.0±3.25*	85.0±2.25*	85.0±2.53*	84.0±3.54
ALT	Control	54.0±3.30	55.0±3.75	54.2±1.25	54.3±3.52	54.5±2.50
	50.0	56.5±3.25	56.4±3.10	56.0±2.00	54.2±1.25	54.2±2.50
	100.0	58.0±3.05	58.1±2.10*	57.1±0.58*	56.3±3.20	55.3±3.60
	200.0	59.4±2.40*	59.3±2.60*	58.4±3.20*	57.4±1.90	56.3±1.80
ALP	Control	151.0±2.70	150.0±1.65	151.0±3.40	151.0±2.65	151.0±2.75
	50	157.0±3.60*	155.0±1.80*	157.0±3.50*	154.0±1.90	152.0±2.35
	100.0	153.0±2.60	158.0±2.36*	159.0±2.80*	152.0±2.80	151.0±2.68
	200.0	155.0±4.30*	158.0±2.50*	161.0±2.10*	154.0±2.30	153.0±2.30

*: Significant increase at (p<0.05); **: Last day of extract treatment

Histopathological Studies of the Effects of Aqueous Extract of *Vitex doniana* Root-Bark

Two weeks following extract treatment the kidneys of the animals that were treated with 50 mg kg^{-1} of extract showed mild congestion and a few lymphocytic infiltration by lymphocytic cells. Also some of the vascular walls were disrupted.

The liver of the animals in this group showed mild lymphocytic cellular infiltration in the lumen of the blood vessels. There was slight congestion, thickening of the vascular walls and focal areas of necrosis of some of the hepatocytes. The observed lesion appear to be severe by the third week of extract treatment (Fig. 1a and b).

The kidneys of the animals that received 100 mg kg^{-1} of aqueous extract, by the first week of treatment showed slight to severe congestion of the blood vessels due to cellular infiltration by inflammatory cells. The Bowman's capsule and vascular walls were thickened. The liver of the animals in this group had mild vascular congestion and cellular infiltration with lymphocytes and a few neutrophils and monocytes.

At three weeks of extract treatment some of the glomeruli were sclerosed. There were also diffused lymphocytic and neutrophilic cellular infiltration into the vascular lumen and interstitium. Mild vascular wall disruption and diffused areas of mild to moderate vascular congestions were also observed.

Fig. 1a: Liver of rats exposed to 50 mg kg $^{-1}$ of extract for 14 days showing focal area of Necrosis with mononuclear cell infiltration (lymphocytes and macrophages) (H and E: ×400)

Fig. 1b: Liver of normal rats treated with distilled water showing intact structures (H and E: ×400)

Fig 2a: Kidney of rats treated with aqueous extract at dose of 200 mg kg $^{-1}$ for 21 days showing haemorrhage in the cortex adjacent to a Bowman's capsule (H and E: x 400)

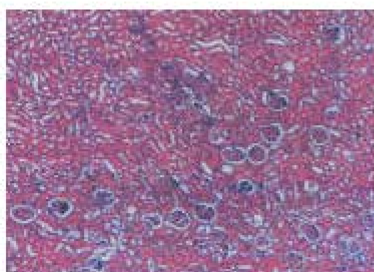

Fig 2b: Kidney of normal rats treated with distilled water showing intact structures (H and E: x400)

Fig 3a: Liver of rats treated with extract at dose of 200 mg kg^{-1} for 21 days showing Non-supportive hepatesis with periportal necrosis of hepatocytes; (b) Mononuclear cell infiltration (H and E: ×400)

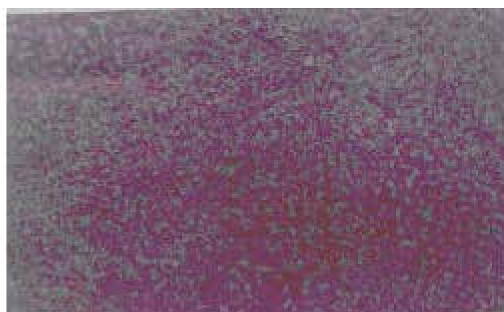

Fig 3b: Liver of normal rats treated with distilled water showing intact structures (H and E: ×400)

In the animals that received 200 mg kg^{-1} of the extract, the kidney showed tubular edema, there was cellular infiltration by some neutrophils and thickening of Bowman's capsule within two weeks of treatment. Within this period, the liver showed cellular infiltration into the lumen of the blood vessel, with thickening of the vascular walls and focal hemorrhages. At three weeks of extract treatment, there were also cellular infiltration in the interstitium by lymphocytic and neutrophilic cells; the vascular walls were thickened and some of the glomeruli were sclerosed (Fig. 2a and b).

In the liver, some of the vessels showed moderate to severe areas of hemorrhages and cellular infiltration especially by lymphocytes and a few neutrophils in the sinusoids of the lumen of the vessels. Also some of the hepatocytes showed coagulative necrosis (Fig. 3a and b). In the group of rats that received 50,100 and 200 mg kg^{-1} of extract for three weeks, no lesion was observed in the intestine, stomach, brain and spleen. Tissue preparations of organs in the control groups showed no observable lesions.

DISCUSSION

The administration of *Vitex doniana* root-bark extract to rats resulted in significantly ($p<0.05$) increased sodium ion and decreased potassium ion concentrations in the blood. The increased blood sodium ion concentration could be an indication of decreased sodium excretion or dehydration

(Odutula, 1992). However, in this study the animals were not diarrheic, hence dehydration may not explain the presence of high sodium level in the blood (Abdulrahman and Onyeyili, 2006). *Vitex doniana* has been observed to induce central sedative or depressant effect, hence could have affected glomerula filtrations and thereby decreasing the excretion of substances in the urine including sodium ion. Furthermore, high level of sodium ions observed to be present in the plant sample from elemental analysis could have contributed to the increased sodium load in the treated animals (Abdulrahman and Onyeyili, 2006).

Potassium ion is the major cation of the intracellular fluid with only ten percent of the total body potassium found extracellular. Therefore, serum potassium concentration is not a good measure of total body potassium because the bulk of potassium resides within the cells. Although the intracellular potassium cannot be easily measured, potassium deficiency correlates well with increased sodium concentration (Odutula, 1992).

The decreased serum potassium concentration observed in this study may have resulted from the increased sodium load in the tubular urine which probably enhances the excretion of potassium ions. Disturbances of potassium homeostasis could have serious health consequences (Tietz *et al*., 1986), however it should be noted that because extracellular potassium concentrations are maintained at the expense of intracellular supply, functions of body systems could be normal even with substantial total body loss of potassium, hence the absence of the clinical manifestations of potassium loss in the treated animals in this study.

The decreased serum calcium concentration of rats treated with extract doses of 100 and 200 mg kg^{-1} may be due to various factors including problem of either the production or response to Vitamin D. The abnormality in Vitamin D system could be due to decreased absorption of Vitamin D or increased metabolism of 25-hydroxylcholecalciferol because of stimulation of enzymes by the extract. Drugs such as Phenobarbital, diphenylhydantoin and rifampin which induce microsomal enzymes are known to increase 25-hydroxylcholecalciferol metabolism (Odutula, 1992).

Hypocalcaemia causes prolonged coagulation times. In addition calcium is also important in cell membrane and capillary permeability and a host of others (Schalm *et al*., 1975)

The administration of *Vitex doniana* extract to rats at 100 and 200 mg kg^{-1} doses for 21 days orally was observed to induce increased serum urea levels. The elevated serum urea level observed in this study may have resulted from kidney damage from exposure to the extract. It is a known 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 concentration (Roct *et al*., 1998), urea being the product of protein metabolism that should be excreted through urine. Rabo (1998) in his study with the extract of *Butyrospermum paradoxum* also observed increased serum urea levels in treated rats.

The total protein was unaffected except with 200 mg kg^{-1} dose of extract at 21 day of treatment. The difference observed was significant ($p<0.05$) and might be due to protein sparing action at this dose level. The decreased cholesterol level observed with the administration of 100 and 200 mg kg^{-1} doses of *Vitex doniana* to rats in the present study indicates a tendency of the extract to lower the level of cholesterol in the peripheral tissues of the treated animals. In an earlier report by Odutula (1992), it was shown that the administration of some drugs or agents could result in hypocholesterolaemia. The reduction in serum cholesterol was observed to be dose-dependent. The reduced serum cholesterol may be due to increased metabolism of cholesterol by the liver, liver injury or decreased cholesterol absorption from gastrointestinal tract.

The result obtained in this study appear to be in conformity with the hypocholesterolaemic effect of other plant extracts. *Amaranthus cadatus* and *Amaranthus spinosa* are known to induce hypocholesterolaemia in treated animals (Arowolo *et al*., 1989; Akinloye and Olerede, 2001).

Indicators of liver function-AST and ALT were all increased by the extract administration. The increase in ALT appear to be extremely significant ($p<0.05$) especially at 200 mg kg^{-1} body weight indicating possible liver damage. An elevated ALT is a sensitive clinical indicator of hepatocellular

injury (Tietz, 1994). 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. Sub-acute oral administration of the extract to rats at various doses appears to have adversely affected the liver and kidneys. The pathological changes observed in these two organs also appear to be dose and time dependent. The presence of pathological lesions in the liver and kidney may not be surprising since the kidney is the primary organ of excretion while the liver is the main organ of biotransformation in the body, hence the two organs may have been exposed to the toxic/active principle(s) present in the extract. The above appear to be in agreement with the observations of Jovanoic *et al*. (1991) who noted tubular necrosis with the presence of cast in the renal tubules of animals fed with feeds containing anti-nutritive factors such as saponins and tannins. Above findings also agree with the theory of target organ toxicity by Heywood (1981), since metabolism of environmental chemicals take place mostly in the liver, while excretion occur through kidneys (Clarke and Clarke, 1977; Parke, 1982).

In this study the extract was observed to be toxic to liver and kidney, it is therefore suggested that it should be used with caution.

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