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Chronic Toxicity Studies of Aqueous Leaf Extract of *Voacanga africana* in Wistar Rats

***[1]IGHODARO, IGBE; SILVANUS, INNIH[2]; STEPHEN, VINCENT OGEDENGBE[1]; LUCY, AMAMINA [2]**

Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. [2]Department of Anatomy, School of Basic Medical Sciences, University of Benin
igbe.ighodaro@uniben.edu, silvanusinnih@yahoo.co.uk, vinstephen36@gmail.com, amaminalucy@gmail.com
***Corresponding author:** Ighodaro Igbe*
Department of Pharmacology & Toxicology, Faculty of Pharmacy,
University of Benin, Benin City, Nigeria. PMB1154
igbe.ighodaro@uniben.edu. +2348166058559

ABSTRACT: *Voacanga Africana stapf* (Apocynaceae) leaves is being used in herbal medicine to treat leprosy, diarrhoea, generalized oedema and convulsion in children as an infant tonic and the present study was aimed at its toxicological evaluation in wistar rats. The sub-acute toxicity was evaluated after administering daily oral doses of *Voacanga Africana stapf* (100, 400 and 800 mg/kg) for 28 days after which the effect on anthropometric, haematological and histopathological parameters were assessed. There was a significant reduction ($p<0.05$) in the pattern of weight gain in the female albino rats and alkaline phosphatase (ALP) but no significant difference in the organ weight index in all selected organs. There were no gross abnormalities or histopathological changes observed among any the groups treated. The results suggest that the aqueous leaf extract of *Voacanga africana* can be considered relatively safe on chronic administration to rats and may cause reduction in weight gain in female rats probably due to changes in female hormones.
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***KEYWORDS*;** *Voacanga africana*, toxicity, weight gain, haematology

INTRODUCTION

As the use of medicinal plants increases, experimental screening of their toxicity is crucial to guarantee the safety of users (Asgarpanah and Ramezanloo, 2012). Toxicity is an expression of being poisonous, indicating the state of adverse effects caused by the interaction between toxicants and cells (Syahmi et al, 2010). Natural products are the cornerstone of health care delivery especially in resource poor settings. Present estimates indicate that about eighty percent of the world's population relies on traditional medicine for health care delivery (Peter, 1995). So much has been done in screening medicinal plants for efficacy based on traditional claims while less emphasis is placed on the issue of safety, as reports of efficacy far outnumber those of toxicity, probably as a result of the greater demands for resources and time such exercise warrant. Pharmacological and toxicological evaluations of medicinal plants are essential for drug development (Sofowora, 1993)

Voacanga africana is a small tropical tree, distributed mainly in West Africa. It is locally known by different names such as; in Igbo (petepete), in Yoruba (Ako-Dodo) and in Hausa (Kokiyar.) It is a perennial plant grows to the height of 6m. The flower is bisexual, the leaves are broad and oval and up to 30cm long (Iwu, 1993). The milky latex of the plant is applied to wounds in Nigeria and Senegal. Tea made from the leaves serves as a strengthen portion that relieves fatigue and shortness of breath. It is also used to prevent premature childbirth and to treat painful menstruation (Sofowora, 1993). In Senegal, a leaf decoction is drunk as a tonic and against fatigue. In Cote d'ivoire a decoction of the leaves is applied as a wash against oedema and is used as a friction and is drunk in the treatment of leprosy. The pulp from the leaves or stem bark is applied to soothe convulsions in children and the juice is put in the nostrils as a tranquillizer (Burkill et al, 1985). The dried and powdered roots without the outer bark are mixed with porridge and taken against kidney troubles and menstruations problems in women. The wood is used to make musical instruments and also used for firewood. Good fiber can be obtained from the bark and is made into rope. (Tona et al, 1998). In the present study, the subchronic toxicity of the aqueous extract of the leaves was evaluated to assess its safety, since the findings are important

Corresponding author Email: igbe.ighodaro@uniben.edu

considering the usage of the plants by human beings.

MATERIALS AND METHODS

*Plant Materials and Extraction***:** The leaves of *Voacanga africana* were collected from Okhoro village in Benin City, Nigeria in the month of March, 2015. The plant was identified and authenticated by Prof. Macdonald Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City were a voucher specimen was deposited. The leaves were sun dried to a constant weight over a 14 day period. The dried leaves were then powdered using a mechanical grinder. The powdered material (500g) of *Voacanga africana* was boiled in 2.5L of distilled water for 30 minutes, and allowed to cool and then filtered. The filtrate was then concentrated in a rotar vapor to give a total yield of 13.3% (w/w). The dried extract was preserved in an air tight clean glass container and kept in a refrigerator maintained at -4°C until use.

*Experimental Animals***:** Experiments were performed using albino wistar rats of either sex (180-220g). The animals were obtained from Animal House, School of Basic medical Science, Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were acclimatized for two weeks and fed with standard feed (from Ewu feed and flours mills Ltd, Ewu, Edo state, Nigeria) and tap water ad libitum. Animals were exposed to natural lighting conditions and were handled in accordance with international principles guiding the use and handling of experimental animals (National Institutes for Health, USA, 2002) approved by the Faculty of Pharmacy Ethics Committee on Animals.

*Toxicity Study***:** Animals were randomly allotted to 4 groups of 10 rats per group containing five males, five females. Animals received distilled water in group I while groups II to IV received 100mg/kg, 400mg/kg and 800mg/kg of the extract respectively, The extract was orally administered daily at single doses for 28 days and the rats were closely observed for the general and behavioral signs of toxicity, body, weight changes and mortality during the entire period of the experiment. At the end of the 28-day treatment period, animals were sacrificed under chloroform anaesthesia. Blood samples were withdrawn by cardiac puncture into plain or lithium heparinized sample bottles.
Selected internal organs such as liver, heart, spleen, kidney and lungs were collected, weighed and stored in 20ml sample bottles (Axiom, Zhanjiang Gong Jong medical technology Co. Ltd, China) containing 10% formyl saline. Thereafter, they were processed for histopathological studies.

Effect of Voacanga africana on body weight.**:** In the course of the 28-day oral treatment, body weights of rats were regularly taken at a 7day interval. Weight changes were calculated in respect of the initial body weights on day zero (0).

*Effect of Voacanga africana on organ weight index***:** The organs (heart, lungs, liver, spleen and kidney) were carefully dissected out and freed from adjoining supporting connective tissues. The organs were gently rinsed in normal saline, blotted with filter paper and weighed. The organ index was then calculated using the formula:

$$\text{Organ weight index} = \frac{\text{Weight of organ}}{\text{Weight of the animal}}$$

*Haematological assay***:** The blood samples collected into the lithium heparinized samples bottles were for determination of the red cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), and platelet count (PLT), total white blood cell counts and its differentials using Automated Haematology System. (Diatron Abacus junior hematology analyzer).

*Biochemical assay***:** Biochemical analysis was performed on serum obtained after centrifugation of white blood (without Anticoagulant) at 2500rpm for 5 minutes. Standardized diagnostic kits (Randox by Randox laboratories LTD., United Kingdom). Determination of the following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, alkaline phosphatase, urea, bicarbonate, total proteins, albumin, total and conjugated bilirubin.

Serum ALT, AST and ALP were measured using the enzyme kinetic method of Reitan and Franklin (1957). Urea was measured using urease-Berthelot method (Fawcett and Scott,1960). Estimation of creatinine was done by the Jaffe's reaction method (Biod and Sirota, 1948). The total protein was estimated by Biuret method (Tietz, 1970) while that of albumin was determined by Bromocresol green (Lowry et al, 1957). The total bilirubin and the conjugated bilirubin were determined by Jendrassik-Grof method (Spencer and Price, 1977).

*Histopathology***:** Spleen, liver, lungs, heart and kidney were fixed immediately in 10% formalsaline for routine histopathological examination. The

tissues were embedded in molten paraffin wax and then sectioned, stained with haematoxylin and eosin and were examined under light microscope. Photomicrographs of the microscopic sections were taken with the help of a photomicroscope (Motic, Canada) provided with motic images plus 2.0 software.

Statistical analysis Data were expressed as mean±SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's post-hoc test using the Graph prism 6, Software, inc., USA). Statistical significance were considered at $p<0.05$.

Table 1: Effect of administration of *Voacanga Africana* on some haematological parameters in Wistar rats

	Treatment groups			
	Control	100 mg/kg	400 mg/kg	800 mg/kg
FEMALE				
RBC ($x10^6$/ul)	6.74±0.84	6.58±0.18	6.75±0.73	6.99±0.49
Hb (g/dl)	13.4±0.63	14.0±0.63	14.2±1.00	13.95±1.06
HCT %	40.2±1.82	46.13±2.80	42.03±2.97	41.3±2.08
PLT (x 10^3/ul)	539.7±44.5	550.3±25.31	553.7±112.0	584.5±58.61
MPV (fl)	7.57±0.34	8.50±0.40	7.93±0.696	7.30±0.20
LY %	75.0±3.97	66.7±13.2	70.1±20.7	68.6±10.70
MI %	5.13±0.18	6.0±1.92	6.20±2.48	8.78±3.08
GR %	19.8±3.70	27.23±11.3	23.7±18.3	22.6±7.75
MCV (fl)	59.7±1.96	70.2±4.60	62.8±2.67	59.3±1.97
MCH (pg)	19.9±0.49	21.23±-0.9	21.13±0.90	19.9±0.62
MCHC (g/dl)	34.4±0.24	30.4±0.85	33.63±0.53	33.63±0.99
RDWC %	15.8±0.68	19.73±1.97	15.7±0.30	17.85±0.96
MALE				
RBC ($x10^6$/ul)	7.19±0.04	6.48±0.59	6.08±0.95	6.04±0.44
Hb (g/dl)	14.9±0.35	14.4±0.87	13.8±1.00	12.45±0.25
HCT %	44.7±0.80	41.6±2.70	38.8±5.21	36.9±0.10
PLT (x 10^3/ul)	521±21.0	443.3±70.22	475.0±32.04	404.5±32.5
MPV (fl)	7.7±0.006	8.0±0.41	8.23±0.47	7.55±0.45
LY %	55.7±25.7	62.7±12.90	46.4±10.2	67.2±11.1
MI %	11.75±3.85	8.30±3.27	11.03±1.79	9.90±5.90
GR %	32.5±21.85	29.08±10.9	37.4±8.38	22.9±5.20
MCV (fl)	62.2±1.45	65.3±4.88	64.5±3.46	61.4±4.65
MCH (pg)	20.8±0.35	22.5±1.92	23.4±2.31	20.60±1.10
MCHC (g/dl)	33.4±1.40	34.6±0.736	36.3±3.01	33.7±0.80
RDWC %	16.15±0.35	17.7±1.62	18.1±0.65	15.9±1.05

Values are expressed as mean ± SEM (n=10). Hb, hemoglobin; hematocrit; RBC, red blood cells; WBC, white blood cells; PLT, platelets; MPV, mean platelet volume; GR, granulocytes count; IM, monocytes/eosinophil; LY, lymphocytes; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; Control group received 4ml/kg distilled

Table 2: Effect of administration of *Voacanga africana* on some biochemical parameters on Wistar rats

	Treatment groups			
	Control	100mg/kg	400mg/kg	800mg/kg
FEMALE				
TP (mg/dl)	6.66±0.58	7.50±0.36	7.40±0.38	5.52±0.37
ALB (mg/dl)	3.72±0.24	3.62±0.33	3.80±0.21	2.78±0.08
TB (mg/dl)	0.50±0.77	0.58±0.16	0.64±0.07	0.38±0.07
CB (mg/dl)	0.24±0.02	0.30±0.08	0.22±0.02	0.14±0.02
ALP (IU/L)	48.0±14.2	18.2±1.16	36.8±2.13	22.0±2.69
ASP (IU/L)	70.2±16.74	50.2±5.36	60.2±20.1	45.6±6.92
ALT (IU/L)	29.0±5.13	17.4±2.36	20.2±4.36	21.4±2.49
Glucose (mg/dl	92.94±0.45	83.88±0.25	72.24±0.27	82.74±0.30

MALE				
TP (mg/dl)	8.20±0.33	8.26±0.14	7.72±0.27	7.22±0.35
ALB (mg/dl)	3.66±0.23	4.26±0.14	4.22±0.15	3.68±0.16
TB (mg/dl)	0.52±0.06	0.04±0.06	0.64±0.05	0.57±0.07
CB (mg/dl)	0.24±0.02	0.24±0.02	0.22±0.02	0.24±0.02
ALP (IU/L)	40.2±13.51	16.2±1.53*	27.2±3.48*	22.0±3.21*
ASP (IU/L)	56.0±17.51	46.8±5.41	76.8±16.31	69.8±8.74
ALT (IU/L)	17.6±3.71	16.4±3.14	18.20±5.04	18.4±1.66
Glucose (mg/dl)	74.54±0.56	85.00±0.05	83.50±0.23	73.46±0.19

Values are expressed as mean ± SEM (n=5); Control group received 4ml/kg distilled water. ALP, Alkaline Phosphatase; AST, Aspartate transaminase; ALT, Alanine transaminase; TB, Total Bilirubin; CB, conjugated Bilirubin.

Table 3: Effect of administration of *Voacanga africana* on organ weight index on Wistar rats.

	Treatment groups			
	Control	100 mg/kg	400 mg/kg	800 mg/kg
FEMALE				
Liver	0.0309±0.0025	0.0322±0.0013	0.0288±0.0010	0.0295±0.0010
Kidney	0.0028±0.0023	0.0026±0.0001	0.0044±0.0002	0.0026±0.0001
Lungs	0.0092±0.0015	0.0055±0.0001	0.0093±0.0019	0.0055±0.0003
Spleen	0.0044±0.0033	0.0055±0.0004	0.0034±0.0003	0.0045±0.0004
Heart	0.0029±0.0024	0.0033±0.0001	0.0034±0.0002	0.0032±0.0002
MALE				
Liver	0.0317±0.0021	0.0311±0.0008	0.0319±0.0016	0.0320±0.0016
Kidney	0.0027±0.0001	0.0024±0.0001	0.0028±0.0002	0.0313±0.0003
Lungs	0.0066±0.0011	0.0059±0.0002	0.0075±0.0005	0.0059±0.0002
Spleen	0.0037±0.0029	0.0039±0.0002	0.0044±0.0004	0.0053±0.0009
Heart	0.0028±0.0001	0.0033±0.0002	0.0035±0.0001	0.0036±0.0002

Values are expressed as mean ± SEM (n=5), Control group received 4ml/kg distilled water

Table 4: Effect of administration of *Voacanga africana* on body weights on Wistar rats

	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
Female						
Control	-	237±18.54	21.00±23.37	20.00±15.13	29.00±9.02	29.00±4.89
V. africana	100	178±6.04	61.00±6.20	9.00±5.09*	7.00±4.84*	5.00±4.98*
	400	194±22.93	95.00±19.45	2.00±19.78*	14.00±12.0*	8.00±18.54*
	800	190±12.74	70.00±26.12	8.00±17.00*	11.00±16.91*	12.00±14.88*
Male						
Control	-	216±20.57	70.00±20.70	9.00±21.85	2.00±23.80	8.00±24.82
V. africana	100	148±10.19	99.00±22.89	21.00±8.57	25.00±7.00	37.00±8.37
	400	237±39.35	66.00±30.72	4.00±28.75	14.00±26.53	5.00±25.48
	800	184±19.46	81.00±12.24	14.75±11.16	17.25±8.44	41.00±10.80

*[1]*IGHODARO, IGBE; SILVANUS, INNIH[2]; STEPHEN, VINCENT OGEDENGBE[1]; LUCY, AMAMINA [2]*

Values are expressed as mean ± SEM (n=5), Control group received 4ml/kg of water.*P < 0.05 Compared to Control.

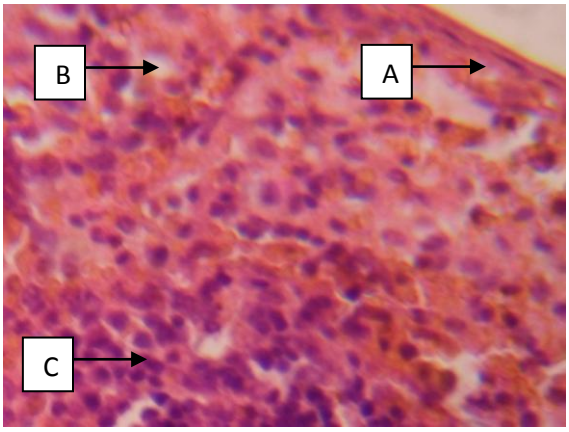

Fig 1: Control: Rat Spleen showing thin capsule A red pulp B and white pulp C (H&E x 40)

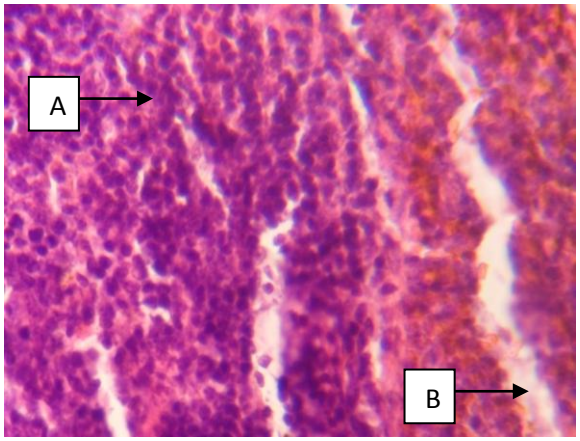

Fig 2: : Rat Spleen treated with 800mg/kg *V. africana* showing mild activation of white pulp A and mild tissue separation B (H&E x 40)

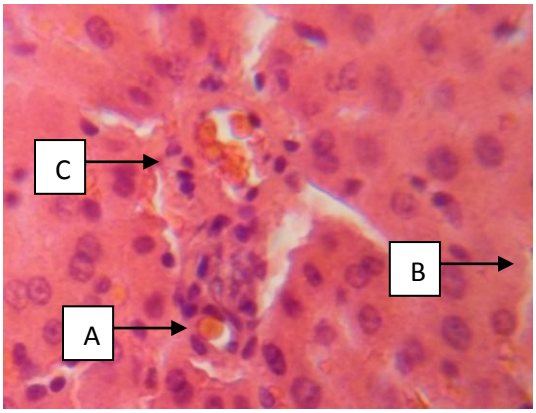

Fig 3: Control: Rat liver showing portal triad A, hepatocytes B and sinusoids C (H&E x 40)

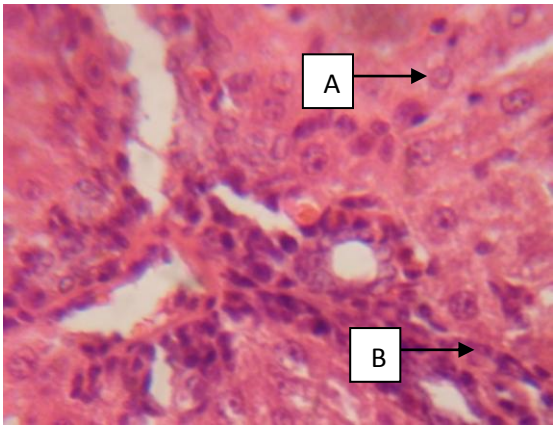

Fig 4: Rat Liver treated with 800mg/kg *V.africana* showing mild tissue separation A and kupffer cell activation B (H&E x 40)

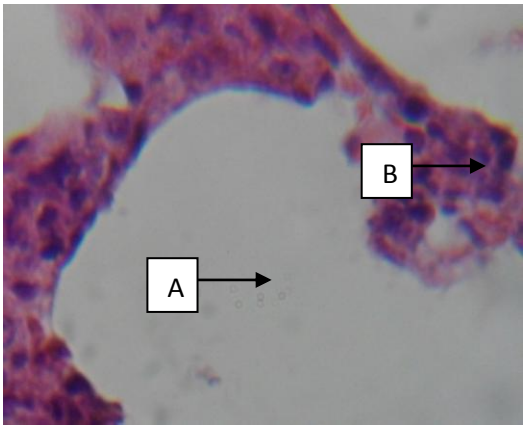

Fig 5: Control: Rat Lungs composed of alveoli A surrounded by interstitial space B (H&E x 40)

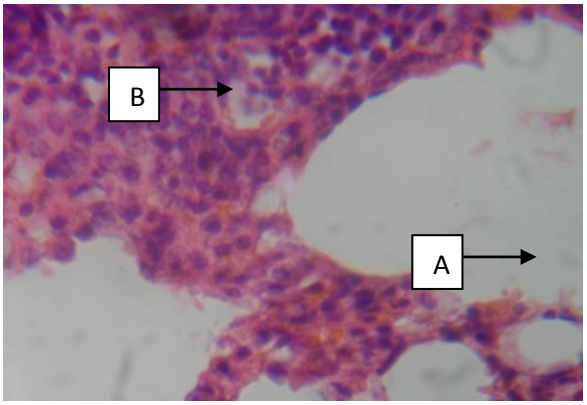

Fig 6: Rat Lungs treated with 800mg/kg *V. africana* showing moderate interstitial vascular congestion A and mild lymphoid activation B (H&E x 40)

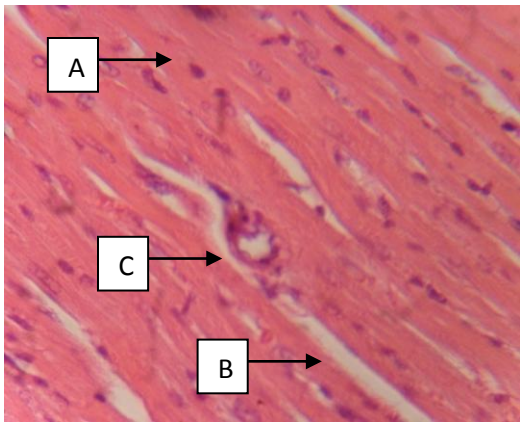

Fig 7: Control: Rat Heart showing myocardium A , interstitial space B and coronary vessel C (H&E x40)

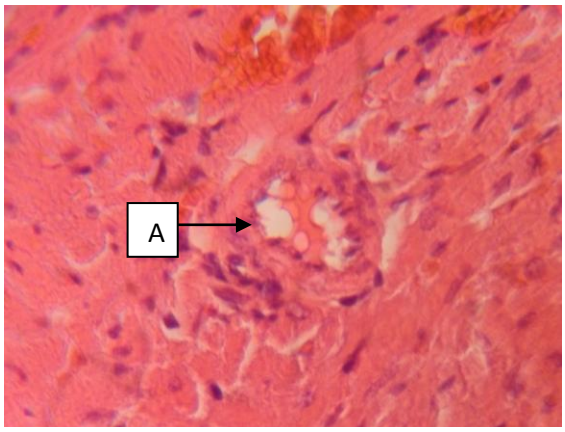

Fig 8: Rat Heart treated with 800mg/kg *V. africana* s howing mild vascular hypertrophy and congestion A (H&E x 40)

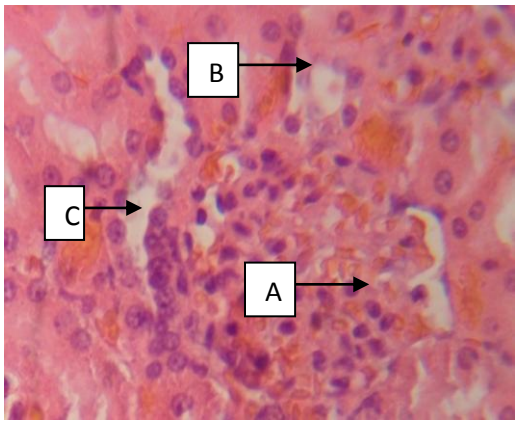

Fig 9: Control: Rat Kidney showing glomerulus A, tubules B and interstitial space C (H&E x 40)

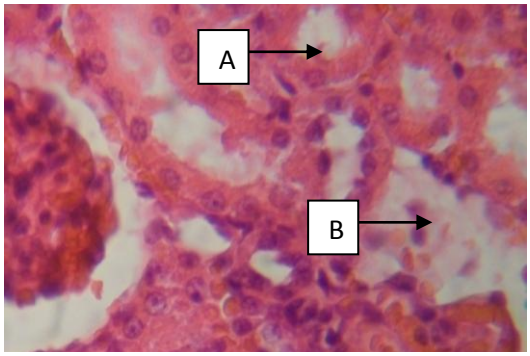

Fig 10: Rat kidney treated with 800mg/kg *V.africana* showing mild interstitial congestion A and tissue separation B (H&E x 40)

RESULTS AND DISCUSSION

In recent times, there is an increasing awareness and interest in medicinal plants and their preparations commonly known as herbal medicines in most African societies including Nigeria as well as worldwide. Traditional medicine still remains the main resources for majority (80%) of the people in developing countries (Teklehaymannt and Giday, 2007). Experimental screening method is therefore important in order to ascertain the safety and efficiency of herbal products.

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood. It can also be used to explain blood relating functions of chemical compound/plant extract. Such laboratory investigations have been reported to be highly sensitive, accurate, and reliable and it remains the bedrock of ethical and rational research, disease diagnosis, prevention and treatment (Okonkwo et al, 2004; Yakubu et al., 2007). The normal range of these parameters can be altered by the ingestion of some toxic plants (Adedapo et al., 2004). The extract at all doses did not significantly alter the red blood cells, white blood cells and platelets among the treatment groups after 28 days of oral administration. Although there was a slight decrease in the number of platelets in the male Wistar rats, this was not significant as shown in table 1. These results are in contrast to previous study (Omodamiro and Nwankwo, 2013) which reported significant elevations in MCH, MCV, PCV, red blood cells and total haemoglobin.

Liver function tests conducted through blood essays provide in depth information about the state of the liver describing its functionality (e.g. albumin, total protein), cellular integrity (e.g. amino transaminases) and its link with the biliary tract (e.g. gamma-glutamyl transferase and alkaline phosphatase) (Adeoye and Oyedepo, 2004). Liver contains a host of enzymes such as ALT, AST and ALP. The activities of these enzymes are used to assess the functional

status of the liver and as the biochemical markers of liver damage (Moss and Ralph Handerson, 1999). Hepatotoxic drugs cause damage to the liver cell membrane and these enzymes are leaked out into serum and shows increased activities (Kumar et al, 2004). In table 2, the present study showed that although there was no significant increase in AST and ALT enzymes, ALP was decreased significantly suggesting inhibition or inactivation of the enzyme by the extract (Akanji et al, 2008). However, the ALP values were still within the normal range (Wolford et al, 1986; Igbe et al, 2013)

Serum protein and albumin assay are also used as reliable and sensitive indicators of liver function status since they are synthesized and metabolized in the liver (Ganong, 2001). Estimation of the total protein is one of the most widely used means of measuring hepatocellular injury. Total protein measurement can reflect nutritional status and may be used to screen for and help diagnose kidney disease, liver disease and many other conditions. Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. High total protein level may be seen with chronic inflammation or liver infections.

Voacanga africana has no deleterious effect on the liver functions as it can be seen that no significant alterations occurred on the liver enzymes and proteins (table 2). This is a strong indication of the oral safety of *Voacanga africana* on liver function.

In the female rats, *Voacanga Africana* at oral doses of 100, 400 and 800 mg/kg showed significant difference ($p<0.05$) in the pattern of change in body weight over the 28 day period when compared to the control in table 3, which suggests adverse effects on metabolic activities of the rats treated with the leaf extract. Reduction in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to a toxic substance (Witthawaskul et al., 2003). There was reduction in the pattern of the weight gain in the female albino rats and this effect may possibly due to the effect of the extract on the female sex hormones and as such, the effect may possibly be sex dependent.

The organs such as heart, liver, spleen, lungs and liver isolated in various group did not reveal any abnormalities in their gross examinations and difference in their mean weights both in treated and control groups (table 3). In figure 4, the histological studies with heart, liver, spleen, lungs and liver did not reveal any pathological changes after treatment even with the highest dose of 800 mg dose of V. Africana extract when administered for 28 days as seen in figures 2, 4, 6, 8 and 10. Although there was mild chronic inflammation has demonstrated by mild edema, vasodilatation and congestion, thickening of the arterial walls and mild infiltration of chronic inflammatory cells in the organs of the treated group suggesting a protective response by the tissues.

In conclusion, the study suggests that subchronic administration of the aqueous leaf extract of *Voacanga africana* is relatively safe but may have the tendency to cause weight reduction in rats.

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