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ANALGESIC AND TOXICOLOGICAL EFFECTS OF LEAF EXTRACT OF *CLERODENDRUM VOLUBILE* LINN. IN WISTAR ALBINO RATS

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ABSTRACT

Clerodendrum volubile is commonly found in the Southern part of Nigeria and it is known for its food and medicinal purposes. This study was aimed to investigate the phytochemical constituents and toxicological effects of the leaf extract of *C. volubile* on Wistar rats. The plant was screened for qualitative and quantitative phyto-constituents using standard procedures. Toxic effect of the plant was determined after a 28-day sub-acute toxicity study in Wistar albino rats of both sexes. Treatment groups received a daily dose of the plant extract at 1, 10, 100 mg/kg, acetaminophen 100 mg/kg, aspirin 100 mg/kg and distilled water as control for 28 days. The toxicity was evaluated by using haematological, histological and biochemical parameters. Alkaloids, saponins, tannins, anthraquinones, phenols and flavonoids were appreciably present in methanol and diethyl-ether extracts when compared with aqueous extract. This study showed no lethality of animals after 28 days. A significant increase in haemoglobin concentration was observed (8.92±0.01 g/dL) when compared with acetaminophen and distilled water. The spleen and heart architectures were normal compared with the standard drugs. The study concludes that the methanol extract of *C. volubile* showed minimal morphological alterations on various organs examined. Further study is recommended to isolate the active ingredient responsible for its activities.

Keywords*:* Medicinal plant; pain relief; secondary metabolites; biochemical; haematological; toxicity
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INTRODUCTION

The usage of herbal products in traditional African medicine is spreading largely in virtually many African communities most especially in the rural areas and many medically isolated villages in Africa where there are no established and functioning medical centres (Ebomoyi, 2009). In Nigeria, herbs and traditional medicine remain a popular and sometimes the only source of remedy open to millions of people. This particularly holds true for the poor, the uneducated, semi-literate and the rural dwellers who lack access to orthodox medicine or cannot afford the prohibitive cost of Western medication (Herbert, 2012). The scientific information on effectiveness and toxicological effects of such medicinal plants is limited.

Clerodendrum volubile is commonly found in the Southern part of Nigeria. It is known for its food and medicinal purpose with insufficient scientific database. It is a climbing shrub of 3 m, glabrous except the inflorescences. It is found in the deciduous forest and secondary jungle (Burkill, 1985). This plant is a noted medicinal plant used for the treatment of several diseases such as red eye infections (Okiei *et al*., 2009). According to Iwu *et al*. (1999), it is used for venereal disease, topically on sprained joints and bruises and as a general tonic for physical and nervous debilities. It is also used for the treatment and management of fractures. Bark preparations of the plant are taken as a tonic against bodily and nervous exhaustion. The leaf sap mixed with shear butter is rubbed on the body against pain and stiffness of limbs. A wet dressing made from the leaves

is applied to furuncles and abscesses. In Nigeria, stem maceration together with stems and roots of several other plants is drunk against convulsion. In Kenya, the stem is roasted and eaten to treat convulsions in infants. In Uganda, the plant is used effectively for the treatment of dementia, snakebites and epilepsy. The plant is also used by people in the treatment of malaria, cough and bleeding (Mosango, 2008).

The present study was undertaken to investigate the secondary metabolites responsible for the acclaimed medicinal uses of *C. volubile* and toxicological effects on blood serum and organs of rats using haematological, histological and biochemical parameters.

MATERIALS AND METHODS

Plant Collection

The leaves of *Clerodendrum volubile* were collected during the rainy season (between April and August) in 2012 and authenticated at Forestry Research Institute (FRIN) Herbarium, Jericho, Ibadan, Oyo State, Nigeria, where the voucher specimen was kept and voucher Number (FHI - 108884) was allocated.

Phytochemical Screening

The leaves were cleaned, washed with clean water and dried under shade for a period of 14 days. The dried leaves were later milled into a fine powder with a blender. The fine powder was weighed and kept in a clean container for further use. The powdered plant sample was tested by standard phytochemical screening (qualitative and quantitative) procedure for the presence of alkaloids, anthraquinones, flavonoids, tannins, saponins, terpenes, steroids, cardenolides and chalcones (Harbone, 1973; Trease and Evans, 1989; Sofowora, 1993).

Animals and Experimental Design

Male and female Wistar albino rats, weighing 130–150 g were used for the acute toxicity assay. The rats were obtained from Babcock University, Ilisan, Ogun State, Nigeria. They were kept in the animal house at Department of Pharmacology, Olabisi Onabanjo University, Sagamu, Ogun State. All the rats were acclimatised with a 12 h light-dark cycle, for a period of seven days prior to the commencement of the experiment. The rats were supplied with water and normal rat feed *ad libitum* during the acclimatisation and experimental periods. The chronic toxicity test was performed following the procedure described by OECD guideline (2000) for testing chemicals. Rats of both sexes were randomly assigned into six groups: three treatments and three controls. The methanol extracts of *C. volubile* were dissolved in distilled water and administered orally on daily basis for a period of 28 days at single doses of 1, 10, 100 mg/kg while the control groups received distilled water, acetaminophen (100 mg/kg) and aspirin (100 mg/kg). Visual observations for mortality, behavioural pattern and changes in physical appearance as well as signs of illness were conducted once daily during this period. At the end of this phase, the rats were anaesthetised using chloroform and animals were sacrificed. Blood samples were also collected and organs were excised and examined macroscopically. Principal organs such as liver, kidney, lung, heart and spleen were preserved in a fixation medium of 10% solution of formaldehyde, for histological study. Biochemical, haematological and histological analyses were performed at Department of Histology, College of Health Sciences, Olabisi Onabanjo University Teaching Hospital, Sagamu.

Biochemical Assay

Biochemical parameters were determined using photometrim procedure according to the methods described by Morgan and Iwana (1997) and Tietz (1990). Parameters determined include levels of the liver enzymes-Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) as well as serum determination of Total Bilirubin (T-BIL), Direct Bilirubin (D-BIL), Indirect Bilirubin (I-BIL),

Triglyceride (TGC), Albumin, High Direct Lipid (HDL), Low Direct Lipid (LDL), Blood Urea Nitrogen (BUN) and creatinine using a commercially available kit (Randox Laboratory Limited, United Kingdom).

Haematological Assay

Blood samples were collected by cardiac puncture into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles. Haematological parameters including Red Blood Cells (RBC), White Blood Cells (WBC), Packed Cell Volume (PCV) and Haemoglobin (Hb) were evaluated using the methods of Dacie and Lewis (1984). The Erythrocytes indices including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated following Stockham and Scott (2002) and Samson (2013) as follows:

MCV = (PCV÷ RBC) × 10
MCH = (Hb ÷ RBC) × 100
MCHC = (Hb ÷ PCV x 100

Keys: PCV = Packed Cell Volume, RBC = Red Blood Cell, Hb = Haemoglobin

Histological Assay

The histopathological analysis of the kidney, the liver, the spleen and the heart excised from the experimental animals was carried out using standard procedure according to OECD (2000) guidelines. The various organs were cut and placed in embedded cassettes. Thereafter, they were fixed with 10% formalin for 1 hour and then dehydrated using different concentrations of methanol (70, 90 and 100%) in ascending order at different times in order to remove water from the tissues. Thereafter, clearing with xylene was done for 2 hours to remove alcohol and to prepare the tissue for waxing. Embedding was done using paraplast wax by impregnating cassettes with molten wax at 60^{o}C for 3 hours. Slicing was done at 5 microns using a manual rotary microtome. The slide was dyed for 20 minutes on a hot plate. Afterwards, de-waxing and hydration were done using xylene and various percentages of alcohol. Thereafter, staining was done with Cole's hematoxylin for 10 minutes to stain the nucleus while eosin was used to stain the cytoplasm for 3 minutes. Re-dehydration was carried out in alcohol which was later cleared with xylene. A mounting medium, Dibutylphthalate Xylene (DPX) was dropped on the tissue sections and the slides were viewed through the microscope.

RESULTS

The preliminary qualitative phytochemical screening of the crude powder of *C. volubile* leaves was done to assess the presence of bioactive components (Table 1). The methanol extracts contained phlobatannin, terpenes, steroids, glycosides and flavonoids in lower amount (+) while alkaloids, phenols, saponins, tannins and anthraquinones were present in appreciable quantity (+++).

Majority of the bioactive components were absent in water extract except for saponins, alkaloids, phenols and glycosides. Diethyl ether extract contained all the secondary metabolites in appreciable amount (+++) except cardenolides, chalcones and flavonoids, which were present in trace amount (+).

Table 2 shows the result of the preliminary quantitative phytochemical constituents of leaf extracts of *Clerodendrum volubile*. Total alkaloids content was higher in methanol and diethyl ether extracts. Phenolic content was higher in diethyl ether extracts followed by methanol extract. Flavonoids content present in methanol extract was higher (0.0130) than that of diethyl ether extracts (0.0097).

Table 3 shows the effect of methanol leaf extract of *Clerodendrum volubile* on serum biochemical parameters. The result of the serum biochemistry revealed that urea and creatinine levels of rats administered with aspirin was significantly higher (58.00±0.29, 0.81 ±0.01 Mg/dl) when compared with the control (52.17±0.03, 0.76±0.00 Mg/dl) and 100 mg/kg methanol leaf extract of *C. volubile* (55.64±0.42, 0.78±0.01

mg/dl). Urea and creatinine levels of *C. volubile* at 1 mg/kg were significantly lower (51.72±0.21, 0.73±0.00) compared to 100 mg/kg and the control.

Aspertate Transaminase (ASL) was significantly higher at 100 mg/kg (23.00±0.00 U/l), 10 mg/kg (26.00±0.58 U/l) and 1 mg/kg (13.00±1.73 U/l) at $p<0.005$ compared with the control (10.00±0.58 U/l). Alanine phosphate (ALT) at a dose of 1 mg/kg (22.00 ±0.00 U/l) was similar to the control (23.00 ±0.58 U/l) when compared with 10 mg/kg (24.67 ±0.33 U/l) and 100 mg/kg (26.00 ±0.58 U/l), respectively. Triglyceride at 10 mg/kg and 100 mg/kg were high but similar to the control (193.00 ±0.58 Mg/dl).

Table 4 shows the effect of *Clerodendrum volubile* on the haematological indices of Wistar albino rat treated for 4 weeks. There was a significant decrease in the red blood cell counts of animals treated with 100 mg/kg of *C. volubile* (1.95±0.15 µL) compared with the control (rats treated with distilled water) (3.03±0.03 µL). There was a significant increase in haemoglobin concentration of the latter (8.92±0.01 g/dL) when compared with acetaminophen and control.

Plates 1 and 2 show the photomicrographs (x 400) of methanol leaf extracts of *C. volubile* on histopathological slides of liver and kidney of tested animals, respectively. Animals administered with 10 mg/kg extract showed congested veins and loss of cellular outlay; however, there was no observable necrosis found in the structure of the liver and kidney of animals administered with 1 mg/kg and 100 mg/kg of *C. volubile* compared with acetaminophen.

Plates 3 and 4 show the photomicrographs (x 400) of methanol leaf extracts of *C. volubile* on histopathological slides of heart and spleen of tested animals. The histopathology of the heart of animals administered with methanol leaf extract of *C. volubile* showed normal spleen and heart architectures even at the highest concentration used.

Table 1: Preliminary qualitative phytochemical constituents of leaf extract of *Clerodendrum volubile*

Constituents	Methanol Extract	Aqueous Extract	Diethyl ether
Alkaloids	+++	++	+++
Saponins	++	+++	+++
Tannins	++	+	+++
Phlobatannins	+	+	++
Phenols	+++	++	+++
Anthraquinones	++	-	+++
Terpenes	+	-	++
Cardenolides	-	-	+
Steroids	+	-	++
Glycosides	+	++	+++
Chalcones	-	-	+
Flavonoids	+	-	+

Key:
+++ = Present in appreciable amount
++ = Present in moderate amount
+ = Present in minute or trace amount
- = Completely absent

Table 2: Preliminary quantitative phytochemical constituents of leaf extracts of *C. volubile*

Constituents	Methanol Extract (g/ml)	Aqueous Extract (g/ml)	Diethyl ether g/ml
Alkaloids	1.1037	0.2823	1.1037
Saponins	0.3853	0.4687	0.4237
Tannins	0.0673	0.0062	0.0880
Phenols	0.0337	0.0230	1.0950
Anthraquinones	0.9797	-	0.0357
Terpenes	0.0270	-	0.0037
Phylobatanninns	0.0110	0.0023	0.0167
Steroids	0.0160	-	0.0130
Glycosides	0.0470	0.0270	0.0403
Chalcones	0.0090	-	0.0000
Flavonoids	0.0130	-	0.0097
Cardenolides	0.0053	-	-

Table 3: Effect of methanol leaf extract of *Clerodendrum volubile* on serum biochemical parameters

Parameters	CONRTOL	AMP	ASP	CVL1	CVL2	CVL3
T-BIL (Mg/dl)	10.67±0.09^{a}	4.90±0.26^{c}	5.20±0.40^{c}	4.80±0.06^{c}	6.30±0.21^{b}	6.43±0.03^{b}
D-BIL (Mg/dl)	14.47±0.03^{a}	0.47±0.09^{c}	3.33±0.30^{b}	3.37±0.07^{b}	0.23±0.09^{c}	0.04±0.06^{c}
I-BIL (Mg/dl)	3.80±0.12^{c}	4.27±0.37^{b}	2.07±0.69^{c}	1.40±0.12^{c}	4.93±0.47^{ab}	6.00±0.06^{a}
TGC (Mg/dl)	193.00±0.58^{a}	121.67±0.67^{a}	109.33±13.91^{a}	24.33±0.67^{b}	159.67±48.93^{a}	177.33±43.06^{a}
T-CHOL (Mg/dl)	207.00±0.58^{d}	154.67±1.20^{e}	211.00±1.00^{d}	275.33±1.76^{b}	263.67±0.33^{c}	397.33±3.71^{a}
CREATININE (Mg/dl)	0.76±0.00^{bc}	0.76±0.01^{bc}	0.81±0.01^{a}	0.73±0.00^{c}	0.75±0.01^{cd}	0.78±0.01^{b}
UREA (Mg/dl)	52.17±0.03^{d}	54.39±0.31^{bc}	58.00±0.29^{a}	51.72±0.21^{d}	53.14±1.03^{cd}	55.64±0.42^{b}
ALBUMIN(g/dl)	4.53±0.03^{a}	2.00±0.00^{c}	1.73±0.03^{d}	2.67±0.20^{b}	2.20±0.00^{b}	2.03±0.03^{b}
HDL (Mg/dl)	207.00±0.58^{a}	80.67±0.33^{c}	93.67±0.33^{b}	80.67±0.33^{c}	78.00±0.58^{d}	73.33±1.20^{e}
LDL (Mg/dl)	152.67±1.45^{c}	50.00±1.16^{e}	93.33±2.40^{d}	191.67±0.88^{b}	252.00±11.02^{a}	252.00±1.16^{a}
ALT (U/l)	23.00±0.58^{de}	26.67±0.33^{a}	24.00±0.58^{cd}	22.00±0.00^{e}	24.67±0.33^{bc}	26.00±0.58^{ab}
AST (U/l)	10.00±0.58^{d}	10.00±0.00^{d}	7.67±0.33^{d}	13.00±1.73^{c}	26.00±0.58^{a}	23.00±0.00^{b}

Values are the means ± S.E.M of measurements (n=5). Means with different superscripts within a row are significantly different at $p< 0.05$. Control represents untreated animals while ASP, AMP, CVL1, CVL2, CVL3 represent animals with aspirin, Acetaminophen, *Clerodendrum volubile* at 1 mg/kg, 10 mg/kg and 100 mg/kg, respectively. T-BIL=Total Bilirubin, D-BIL Direct Bilirubin, I-BIL= Indirect Bilirubin, TCG=Triglyceride, T-CHOL = Total Cholestorol, LDL= Low-Density Lipoprotein, ALT= Alanine Transferase, AST= Apartate Transferase

Table 4: Effects of *Clerodendrum volubile* on the haematological indices of wistar albino rat treated for 4 weeks

Treatment	WBC $10^3/\mu L$	RBC $10^6/\mu L$	HGB (g/dL)	PCV (%)	MCV fL (μm^3)	MCH (pg)	MCHC (g/dL)
Distilled water	4.10±0.05[a]	3.03±0.03[a]	7.63±0.15[e]	31.43±2.09a	81.94±4.60 [c]	21.56±0.33[d]	27.05±1.94[c]
AMP	2.91±0..04[c]	3.371±1.8[a]	7.89±0.02[b]	27.02±1.31[b]	74.27±6.69 [c]	21.57±1.14[d]	29.55±1.44[c]
ASP	3.38±0.23bc	3.91±0.04[a]	7.50±0.02[d]	30.75±0.82[a]	78.66±2.04 [c]	19.19±0.22[d]	23.34±0.95[d]
CVL_1	4.21±0.07 [a]	2.39±0.05[b]	7.78±0.0[c]	25.08±1.40[bc]	115.97±3.94[b]	32.73±0.73[c]	31.55±1.89[bc]
CVL_2	3.83±0.13[ab]	2.17±0.11[bc]	8.88±0.02[a]	21.67±0.88[c]	101.21±8.24[b]	41.55±2.22[b]	41.60±1.60[a]
CVL_3	3.33±0.29[bc]	1.95±0.15 [c]	8.92±0.01[a]	26.17±1.45[b]	133.53±6.96[a]	45.55±0.34[a]	34.62±1.98[b]

Values are means ± S.E.M. of six measurements (n = 6). Means with different superscripts within a row are significantly different at $p< 0.05$. Control represents untreated animals while CVL1, CVL2, CVL3 represent animals treated with *Clerodendrum volubile* at1mg/kg, 10 mg/kg and 100 mg/kg, respectively. WBC, RBC, HGB, PCV, MCV, MCH and MCHC represent white blood cell counts, red blood cell counts, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration, respectively.

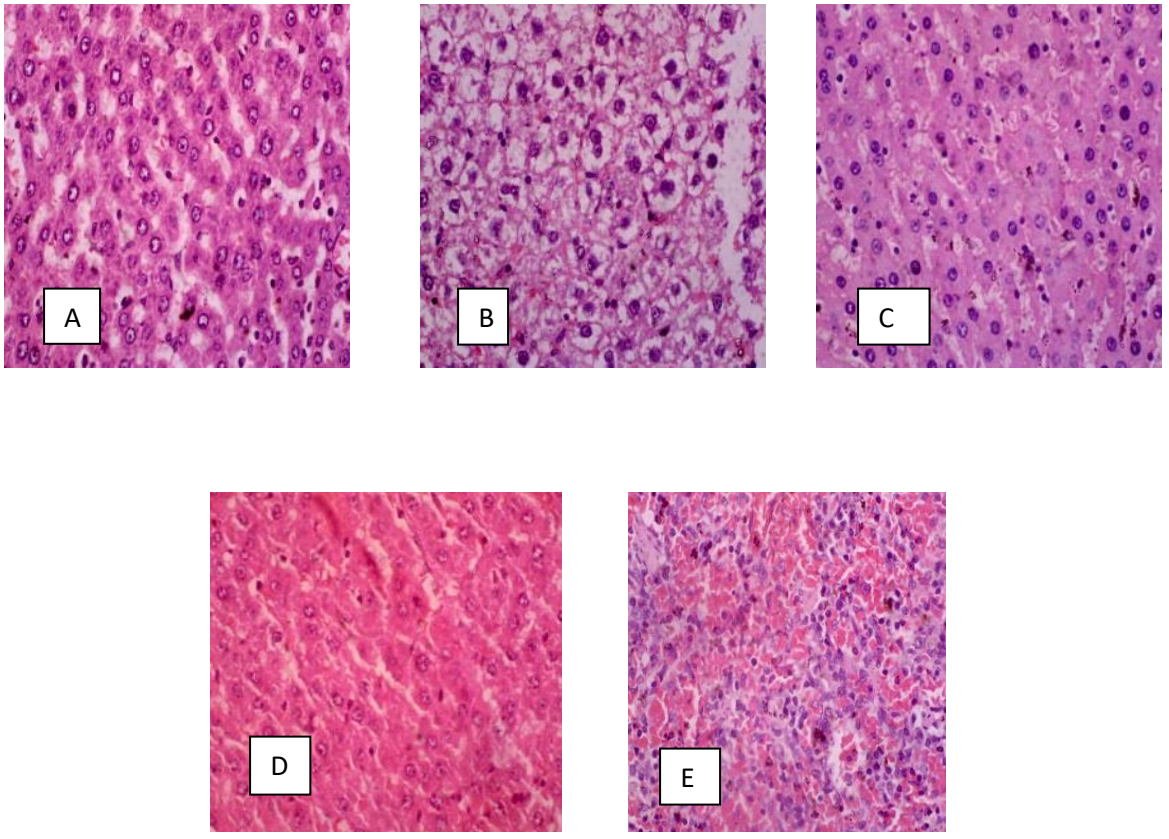

Plate 1: Photomicrograph of the sections of the liver (x 400) of Wister rat treated orally with 1 mg/kg (A), 10 mg/kg (B), 100 mg/kg (C) of methanol leaf extracts of *Clerodendrum volubile* (D), aspirin (E) acetaminophen

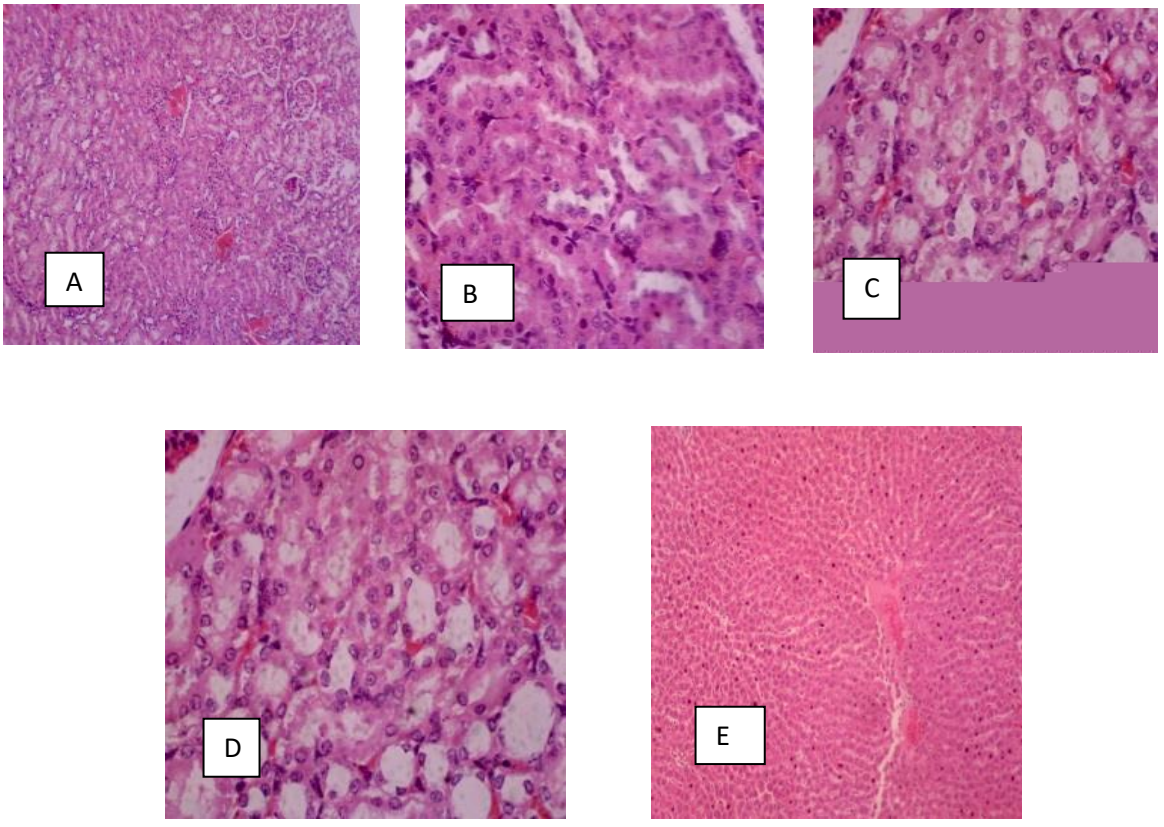

Plate 2: Photomicrograph of the sections of the kidney (x 400) of Wister rat treated orally with 1 mg/kg (A), 10 mg/kg (B), 100 mg/kg (C) of methanol leaf extracts of *Clerodendrum volubile* (D), aspirin (E) acetaminophen

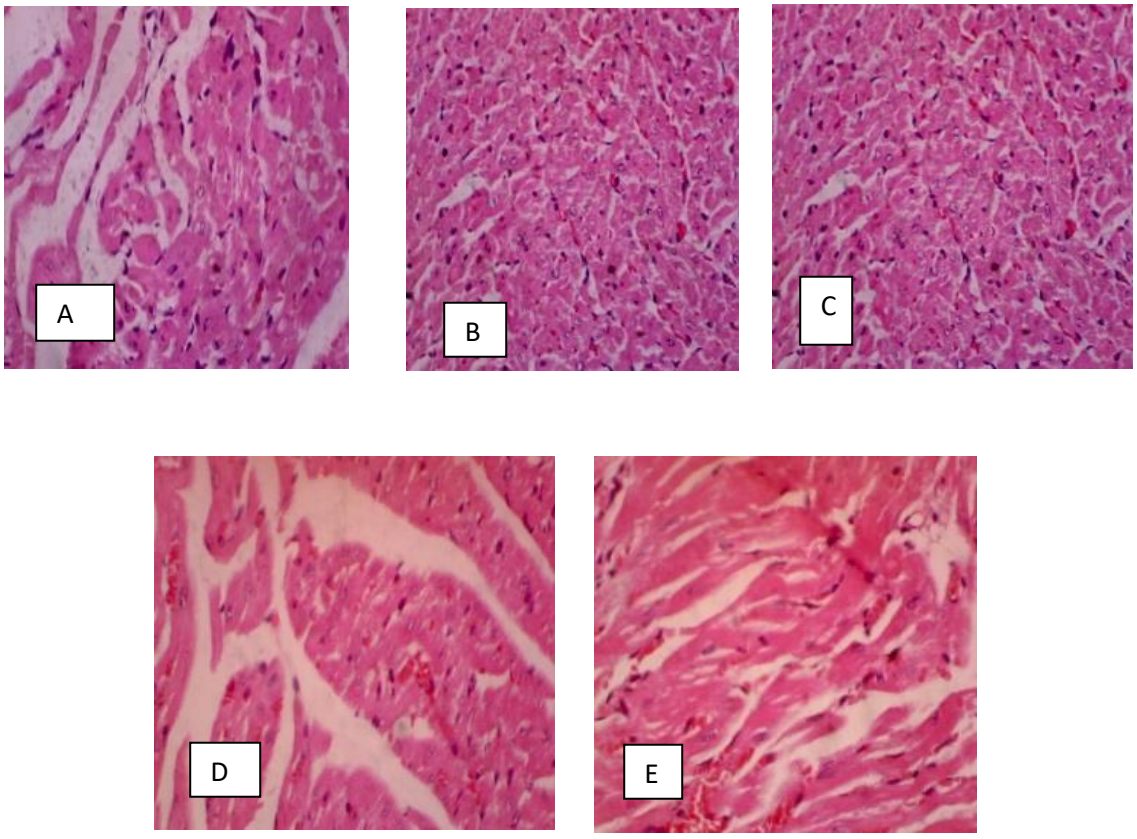

Plate 3: Photomicrograph (x 400) showing heart of Wister rat treated orally with 1 mg/kg (A), 10 mg/kg (B), 100 mg/kg (C) of methanol leaf extracts of *Clerodendrum volubile* (D), aspirin (E) acetaminophen

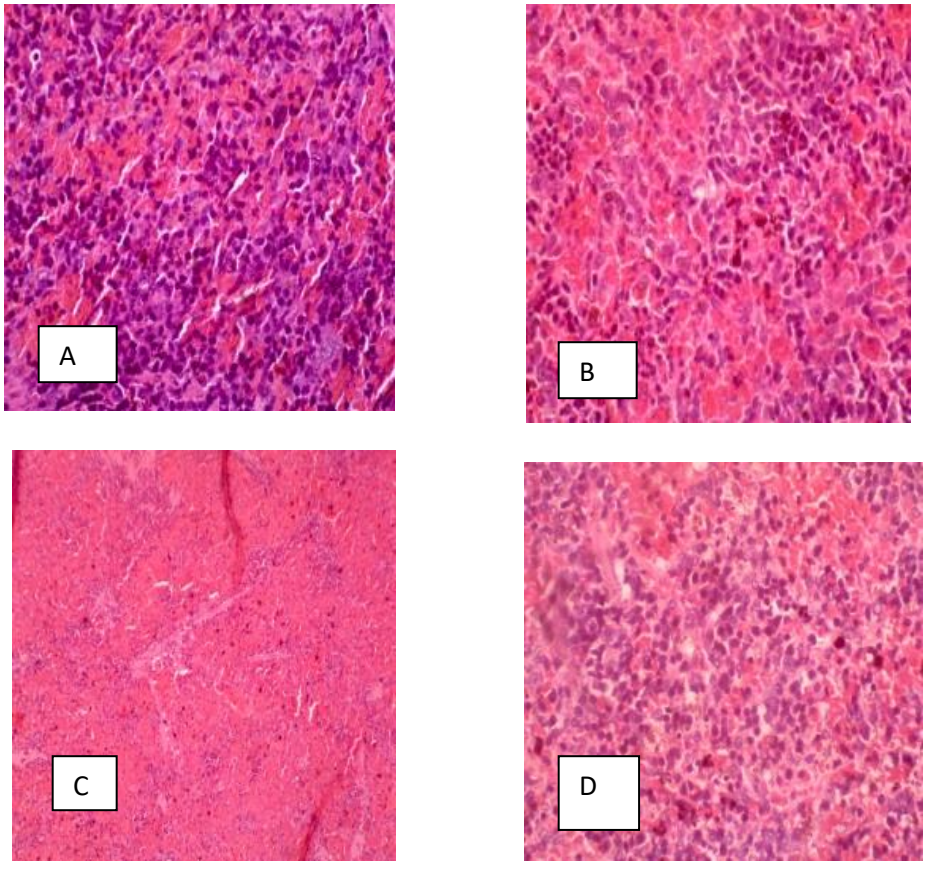

Plate 4: Photomicrograph (x 400) showing the spleen of rat treated orally with 1 mg/kg (A), 10 mg/kg (B), 100 mg/kg (C) of methanol leaf extracts of *Clerodendrum volubile*, (D) acetaminophen.

DISCUSSION

Clerodendrum volubile diethyl ether extract contained alkaloids, saponins, tannins, phenols, anthraquinones, terpenes, phlobatannin, steroids, glycosides and flavonoids. The methanol phyto-constituent was similar. Its water extract contained alkaloids, saponins, tannins, phlobatannin, phenols and glycosides and this is almost similar to the report of Erukainure *et al.* (2011). The alkaloids content was higher than that reported by Erukainure *et al.* (2011). Alkaloids are used in the pharmaceutical industries in the production of analgesics due to their analgesic properties (Okwu and Ndu, 2006; Erukainure *et al.*, 2011).

The 28-day toxicological assessment of methanol leaf extracts of the studied plant revealed that plasma enzymes (AST and ALT) and other biochemical parameters such as albumin, creatinine and Direct Bilirubin (D-BIL) were not significantly increased in *C. volubile*. Elevated values of AST and ALT may be attributable to pathological changes such as necrosis of hepatocytes, which caused an increase in the permeability of the cell membrane. This resulted in the release of aminotransferases into the blood stream, and is suggestive of liver or bile duct disease such as myocardial infarctions (Dufour, 2001; Giboney, 2005; Singh, 2011). Increase in the activity of AST is usually directly proportional to the infarction size (Robert *et al*., 1975; Hawcroft, 1987; Nwindu *et al*., 2012). A typical myocardial infarction indicates an AST/ALT ratio greater than 1 in most cases. As the results showed, AST/ALT ratio was less than 1 in all the tested plant extracts, indicating that *C. volubile* extract was not hepatotoxic even at the highest dose used; the results of standard drugs: acetaminophen and aspirin with AST/ALT showed a ratio greater than 2:1.

Assessment of pathological imbalances generated in laboratory animals by novel drugs represents the basis of their safety before they could be used in the clinical set-up. This assessment is based chiefly on histopathological techniques (Donatus, 2015). Consequently, histopathological assay was employed to further examine the pathological effect of the extracts on the various organs of treated animals. The spleens for all the treated animals were normal since there were no observable lesions. *Clerodendrum volubile* extracts showed no observable necrosis of the liver and the heart at 1 mg/kg and 100 mg/kg.

Based on the results derived from this study, it is suggested that further chronic toxicity studies should be carried out on fractions of *C. volubile* extract using rats and other animal species. This is to ascertain the safety of the plant since this is the first documented report on its toxicity.

REFERENCES

Burkill, H.M. (1985). *The useful plants of West Tropical Africa*. Families J-L. Royal Botanical Garden Kew. 3:522p.

Dacie, J.V. and Lewis, S.M. (1984). *Practical haematology*, 6th edn. ELBS and Churchill, Livingston. 445p.

Donatus, W.A. (2015). Anticonvulsant and Antidepressant effects of an Ethanolic Extract of the Leaves of *Pseudospondias microcarpa* (Engl.) A. Rich. (Anacardiaceae) in Animal Models. PhD thesis of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science & Technology, Kumasi: 168-174.

Dufour, D.R., Lott, J.A., Nolte, F.S., Gretch, D.R. and Koff, R.S. (2001). Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis and monitoring. *Clinical Chemotherapy*, 47:1133-1135.

Ebomoyi, E.W. (2009). Genomics in traditional African healing and strategies to integrate Traditional Healers into Western-Type Health Care Services. *A Retrospective Study Researcher*, **1**(6): 69-79.

Erukainure, O. L., Osaretin, A. T., Ebuehi, I. I. M., Choudhary, A. A., Rahman, M. H., Shahida, P., Aliyu, M. and Gloria, N. E. (2014). Iridoid Glycoside from the Leaves of *Clerodendrum volubile* Beauv. shows Potent Antioxidant Activity against Oxidative Stress in Rat's Brain and Hepatic Tissues. *Journal of Dietary Supplements*, Early Online: 1–11.

Giboney, P.T. (2005). Mildly elevated liver transaminase levels in the asymptomatic patient. *American Family Physician*, **71**(6): 1105–1110.

Harbone, J.B. (1973). *Textbook of Phytochemical methods*, 1st Edition. Champman and Hall Ltd., London, 279p.

Hawcroft, D. M. (1987). *Diagnostic Enzymology & Analytical Chemistry* by Open Learning. Published by permission of the controller of Her Majesty's stationary office: 186–221.

Herbert, A. O., Josephat, M. C., Agozie, C. U., Christopher, B. E. and Ikenna, K. N. (2012). Acetaminophen use (and/or misuse) in children in Enugu, South-East Nigeria. *BioMed Central Pediatrics*, 12: 103.

Iwu, M.M., Duncan, A.R. and Okunji, C.O. (1999). New antimicrobials of plant origin. In: Janich, J. (Ed.), *Perspectives on new crops and uses*. ASHS Press, Alexandria VA, 457-462.

Morgan, J. D. and Iwana, G. K. (1997). Measurement of stressed states in the field. In: Iwana, G.K., Pickering, A. D., Sumpter, J. P. and Schreck, C. B. (Editors), *Fish stress and health in aquaculture*. *Society of Exploratory Biology Seminar Series* 62:247-268.

Mosango, D. M. (2008). *Chasmanthera dependens* Hochst. In: Schmelzer, G.H. and Gurib-Fakin, A. (Editors). Prota II (1): *Medical Plants/Plantes Medicinal* I. (CD-Rom). PROTa, Wageningen, Netherlands.

Nwidu, L.L., Nnoli, M. and Nwafor, P. A. (2012). Sub-Chronic Oral Toxicity Profile of *Carpolobia lutea* Leaf Fractions in Rat. *International Journal of Bioassays*, 1(9): 42–51.

OECD (2000). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24.

Okiei, W. M., Ogunlesi, M. and Ademoye, M. A. (2009). An assessment of the antimicrobial properties of extracts of various polarities from *Chasmanthera dependens*, *Emilia coccainea* and *Cuscuta australia*, Herbal Medications for Eye Diseases. *Journal of Applied Sciences*, 4076-4080.

Okwu, D. E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, 5(4): 357-361.

Roberts, R., Ahaymada, G. and Sobel, B. (1975). Infarct Size: Its Estimation and Modification. *Atherosclerosis*, 383-387.

Samson, E. A. (2013). Evaluation of the haematology and biochemistry of *Clarias gariepinus* as biomarkers of environmental pollution in Tiga dam, Nigeria. *Brazilian Archives of Biology and Technology*, 56(3): 371-376.

Singh, A., Bhat, T. K. and Sharma, O. P. (2011). Clinical Biochemistry of Hepatotoxicity. *Journal of Clinical Toxicology*, S4:001. doi:10.4172/2161-0495.S4-001

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*, 2nd Edition. University Spectrum Books Ltd., Ibadan, Nigeria. 289p.

Stockham, S. L. and Scott. M. A. (2002). *Fundamentals of veterinary clinical pathology*. Iowa State Press, A Blackwell Publishing Company. 610p.

Tietz, N. W. (1990). *Clinical guide to laboratory tests*. Ed. Philadelphia, USA: W. B. Saunders Company: 554-556.

Trease, G. E. and Evans, W.C. (1989). *A Textbook of Pharmacognosy* (14th ed.). Bailliere Tinall Ltd., London, 579p.