

TOXICOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF ETHANOL EXTRACT OF CLERODENDRUM VOLUBILE IN RODENTS

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Abstract

Clerodendrum volubile is commonly known and consumed among other leafy vegetables in south-west and south-south Nigeria. This study was designed to evaluate the toxicological, biochemical and histopathological effects of Clerodendrum volubile ethanol extract in rodents. The toxicity profile of the ethanol extract of Clerodendrum volubile by determining its effects after acute and sub-chronic oral administration in mice and rats, respectively using standard methods. No death and signs of toxicity and no negative behavioral changes were observed in the extract at 5000 mg/kg in mice during the acute toxicity test. In rats, single oral alternate daily doses of Clerodendrum volubile extract (250, 500 and 1000 mg/kg), given for 30 days, resulted in increased body weights in treated rats compared to controls. No significant changes in organ weights were observed. There was no significant difference in the plasma activity of ALT and ALP in the treated rats compared with the control group. However, AST levels were significantly lower in the higher dose levels

(500 and 1000 mg/kg) compared with the control group. The administration of the extract did not alter significantly AST and ALT activities in the liver. Kidney uric acid and creatinine concentrations revealed the extract had no deleterious effect on the kidney. No treatment-related histoarchitectural changes were observed in the liver and kidney. *Clerodendrum volubile* is safe for human consumption. However, it is advisable to apply some caution with its chronic use.

Keywords: *Clerodendrum volubile*, liver enzymes, kidney parameters, sub-chronic toxicity

Introduction

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo et al., 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Medicinal plants are still an indispensable source of medicaments in the contemporary health care delivery system because they are still being used for both conventional and traditional medicine. Examples of conventional medicines developed or derived from plants include aspirin (first obtained from willow), taxol® (extracted from yew) and digitoxin obtained from foxglove (Newman and Cragg, 2007). Some outstanding medicinal drugs which have been developed from the ethnomedicinal uses of plants include vinblastine and vincristine from *Catharanthus roseus* (the periwinkle). Vinblastine is used for the treatment of Hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer while vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of hodgkins, lymphosarcoma, cervical and breast cancer (Barrales-Cureño, 2015). Additionally, reserpine from *Rauwolfia serpentina* (Indian snake root) is used for treating hypertension, aspirin from *Salix purpurea* (willow) is used for treating inflammation, pain and thrombosis and quinine from *Cinchona pubescens* (cinchona) used for treating malaria (Williams, 2006). Currently used modern drugs such as quinine, artemisinin derivatives, digoxin glycosides, tubocurarine, aspirin etc. are good examples of drugs from plant medicine. Medicinal plants in Africa are

used to cure common ailments like malaria, fever, headache, burns, ulcer, cough, snake and scorpion poison, rheumatism, infertility etc. Scientific evaluation and validation of traditional medicinal plants is however, essential for their possible utilization or in elimination of harmful practices (Makonnen et al., 2003).

Clerodendrum volubile is commonly known among other leafy vegetables in south-south Nigeria, with dearth of nutritional data base. It is commonly known among the Urhobo and Itsekiri tribes of the Niger-Delta as Obenetete, the south- west Yoruba tribe as Dagba. It is a climbing shrub to 3 m high, glabrous except the inflorescences, flowers numerous, 1½ cm long, greenish-white, yellow in throat, in subcorymbose terminal inflorescences; of deciduous forest and secondary jungle, across the region, Senegal to Fernando Po (Erukainure et al., 2011). *Clerodendrum volubile* has been used in the treatment of different forms of ailments such as arthritis, rheumatism, dropsy, swellings, oedema and gout. It is also used as analgesic, pregnancy tonic, anti-abortionifacient, sedative and to aid labour in pregnant women (Burkill, 1985; Erukainure et al., 2011a, b; Olanrewaju et al., 2018).

The most extended study on *C. volubile* is its action on diabetes (Molehin, et al; 2018, Molehin and Oloyede 2018 and Erukainure, et al; 2018). This plant has a reputable usage in traditional folkloric treatment of ailments especially in Southern Nigeria. It also elicited wide range of biological activities such as antidiabetic, antihyperlipidemic, anti-hypertension, antioxidant, anticancer, anti-inflammatory, hepatoprotective, antimicrobial, and analgesic. (Ajao, et al., 2018). It is also used as spices or cooked with other vegetables such as *Telfaria occidentalis* and *Amaranthus* (Adefegha and Oboh, 2011; Happy, 2016). The management of some neurodegenerative diseases with *C.volubile* has been reported in folklore medicine (Oboh et al., 2017). Furthermore, this plant is regarded as an underutilized indigenous vegetable of utmost nutritive and pharmacological importance (Ajao et al., 2018). Although the acute toxicity of and sub-acute toxicity of aqueous extract of *C. volubile* leaves have been reported (Akinpelu et al., 2016; Molehin and Oloyede, 2018), the scientific information on its toxicity profile is not adequate considering its extent of use and relevance in folkloric medicine. Thus, the evaluation of the safety of short and long term consumption of *C. volubile* is

necessary. This study was therefore designed to investigate the toxicological and biochemical indices of *C. volubile* ethanol extract after sub-chronic administration in albino rats to predict the safety of its consumption in inflammatory and chronic conditions.

Methods

Plant material and authentication

Fresh *Clerodendrum volubile* leaves were collected from Ogudu in Ife East Local Government Area of Osun State, Nigeria. The plant was identified and authenticated at Department of Botany Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. A voucher specimen (Ife-7673) was deposited in the herbarium. The leaves were air-dried for twenty one (21) days at room temperature.

Preparation of extract for administration

Five hundred gram (500 g) of *Clerodendrum volubile* leaves was extracted by maceration with 70 % ethanol in ratio of 1:5. The mixture was filtered using muslin cloth followed by Whatman filter paper. The resultant filtrate was evaporated to dryness under reduced pressure using a rotary evaporator at 400 C to give a dark brown extract. The resulting extract was put in desiccators containing a self-indicating silica gel. The drug was prepared by constituting the plants extracts in distilled water.

Experimental animals

Albino Wistar rats (20) of both sexes weighing between 150-200 g and fifteen albino mice weighing between 18- 22 g were used for toxicology studies. They were obtained from Animal House Department of Pharmacology, Obafemi Awolowo University, Ile-Ife, Osun State and they were given standard feed (Bendel flour mills, broilers mash) and water. They were allowed to acclimatize with the environment for a period of two weeks. All animal experiments complied with the Guide for the Care and Use of Laboratory Animals and the 'Principles of Laboratory Animal Care' (NIH publication No. 82-83, revised in 2010).

Acute toxicity (LD50 determination)

Acute toxicity study of the leaves of *Clerodendrum volubile* was determined using the method of Lorke, (1983). The study was carried out in two phases, in the first phase, nine mice according to their body weight were divided into three groups of three mice per group, each group was given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The mice were observed for signs of toxicity and mortality for 24 h and then for 14 days. From the results of the phase one study, the procedure was repeated using another set of six mice divided into three groups of two mice each, given 1600, 2900 and 5000 mg extract/kg body weight respectively. These mice were also observed for signs of toxicity which include but not limited to paw-licking, salivation, stretching, rubbing of nose on the floor and on wall of cage and death for the first critical four hours and there after daily for fourteen days. The oral median lethal dose was calculated as the geometric mean of doses that caused 0 and 100% mortality respectively.

Evaluation of Sub-chronic toxicity

Sub-chronic toxicity study was evaluated in wistar rats using the method of Salawu et al., 2010. Twenty rats of either sex were divided into four groups of five rats each, based on their body weight. The extract was orally administered every other day for 30 days. Group one which served as the control received distilled water, while Groups two, three and four were given 250, 500 and 1000 mg/kg of the extract respectively. The body weights were recorded at the beginning, then once every three days during the study. Water and food consumption were noted daily and recorded throughout the experimental period. At the end of the treatment period, the animals were sacrificed by cervical dislocation 24 hrs after the last dose. Blood samples were collected by ocular puncture for biochemical analyses. Also liver and kidney were harvested for biochemical and histopathological analyses.

Biochemical Analyses

Blood samples from each animal, collected into lithium heparin bottles, were centrifuged at 3000 rpm for 15 min in Denley BS-400

centrifuge at room temperature. The plasma samples were collected into sterile sample bottles and kept in the freezer at -4 °C for further analyses.

One gram (1 g) of liver was homogenized in 10 ml of 0.01M phosphate buffer with pKa of 6.86 and pH 7.2 and centrifuged at 4000 rpm for 15 min at room temperature. The supernatant was collected into clean sterile bottles, labeled and kept in the freezer at -4 °C for further analysis.

Enzyme assays

Determination of Alanine aminotransferase activity (ALT) and Aspartate transaminase activity (AST)

The plasma and liver homogenate alanine aminotransferase activity and aspartate aminotransferase activity were estimated as described by Reitman and Frankel (1957) using the Randox kit. The assay mixture consisted of plasma each (0.1 ml) and 0.5 ml R1 buffer (phosphate buffer, L-aspartate and α -oxoglutarate). The mixture was incubated at 37 °C in a water bath for exactly 30 min. The reaction was terminated by adding 0.5 ml of solution R2 (2, 4-dinitrophenylhydrazine) and allowed to stand for 20 min at room temperature. The colour was developed by adding 0.4 mol/l NaOH (5.0 ml) and allowed to stand for 5 min; absorbance was read at 546 nm. The reagent blank was prepared similarly but contained 0.1 ml distilled water in place of plasma or liver homogenate in the assay mixture.

Determination of Alkaline Phosphatase Assay (ALP)

The quantitative and equalization in vitro determination of alkaline phosphatase (ALP) in plasma and liver homogenate using Randox commercial kit.

Estimation of plasma total bilirubin

The plasma and liver total bilirubin was estimated as described by Jendrassik and Grof (1938). The assay mixture contain R1 (Sulphanilic acid and Hydrochloric acid) 200 μ l, R2 (Sodium nitrite) 50 μ l, R3 (Caffeine and Sodium benzoate) 1000 μ l, plasma each 200 μ l. The mixture was incubated for 10 min at room temperature. The colour was develop by the addition of reagent R4 (Tartrate and Sodium hydroxide) 1000 μ l. The

mixture was further incubated for 5-30 min. The absorbance was read at 578 nm. The blank was prepared similarly but did not contain R2 (sodium nitrite) as contain in the assay mixture.

Estimation of total protein concentration in the plasma and liver homogenate.

The total protein concentration in plasma and liver was estimated using the Randox kits.

Estimation of kidney function indices

Plasma and kidney creatinine

The plasma and kidney creatinine activities were estimated as described by Bartels and Bohmer, (1972) using the Randox kit.

Plasma uric acid

The plasma uric acid activities was estimated as described by Fossati et al., (1980) using the Randox kit.

Histopathological analysis

After rats were sacrificed, their liver and kidney were carefully excised and weighed. Sections were immediately taken from the organs and fixed in 10% neutral buttered formalin. Tissue sections were stained with hematoxylin and eosin (H & E) for light microscopic examination.

Statistical analysis

Data were expressed as mean $\pm$ SEM. Statistical analysis was performed by one way ANOVA followed by Turkey-Kramer multiple comparison test to ascertain differences between treatment groups. All analysis was performed using Graph Pad Instat 3 (version 1.1, 2007). Differences were considered to be significant if $p \leq 0.05$.

Results

Acute toxicity study

Table 1: Acute toxicity study of *C. volubile* ethanol extract in mice

Groups	Dose(mg/kg)	No. of Dead rats	% Mortality
Stage1			
1	10	0/3	0.00
2	100	0/3	0.00
3	1000	0/3	0.00
Stage 2			
1	1600	0/2	0.00
2	2900	0/2	0.00
3	5000	0/2	0.00

No death and signs of toxicity were observed in the ethanol extract at 5000 mg/kg (Table 1). Acute toxicity testing at 5000 mg/kg body weight of ethanol extract of *C. volubile* did not produced any gross negative behavioral changes such as excitement, restlessness, convulsions and coma in the treated mice.

Sub-chronic toxicity study of *C. volubile* ethanol extract in rats

Table 2: Effect of sub-chronic administration of *Clerodendrum volubile* ethanol extract on body weight of rats

Groups	Doses(mg/kg)	Body weight(g) before treatment	Body weight (g) after treatment
Control	-	125.30±5.94	152.96±3.35
<i>C. volubile</i>	250	132.02±2.28	155.80±5.48
	500	141.62±2.46	169.44±4.02
	1000	130.35±4.54	156.18±4.15

Data are expressed as mean ± SEM. Not significant when compared to control $p > 0.05$ $n = 5$.

The rats exposed to ethanol leaf extract of *C. volubile* for thirty (30) days experienced increase in their growth and relative body weights comparable to their corresponding control groups (Table 2). The relative organ weight of rats is presented in Table 3. There is no significant ($p > 0.05$) change in the relative weight of the liver and kidney when compared with control

Table 3: Effect of *C. volubile* ethanol extract on relative organ weight in rats.

Organ	Control	250mg/kg	500mg/kg	1000mg/kg
Liver	2.82±0.02	2.90±0.04	3.00±0.10	2.92±0.06
Kidney	0.59±0.01	0.58±0.02	0.59±0.02	0.58±0.01

Data are expressed as mean ± SEM.

Table 4: Effect of Clerodendrum volubile ethanol extract on Biochemical Parameters in Plasma of Rats

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Total Protein (g/dl)	8.26±0.12	8.14±0.22	7.95 ± 1.10	7.52 ± 0.83
AST (U/I)	69.9±7.2	48.7±9.7	38.8 ± 1.9*	38.8 ± 4.2*
ALT (U/I)	26.52±2.21	21.28±2.68	24.78 ± 2.49	27.55 ± 4.17
ALP (U/I)	3.37±0.00	3.66±0.00	3.71 ± 0.00	3.01 ± 0.00
Total Bilirubin (µmol/l)	3.37±0.86	2.96±0.32	5.99 ± 3.29	3.42 ± 0.92
Creatinine (µmol/l)	20.22±1.74	19.94±2.143	19.28 ± 0.60	23.66 ± 2.8

Values are expressed as mean ±SEM (standard error of mean) for five animals in each group. Values with * are statistically significant at $p < 0.05$ from the normal control group as compared.

AST - Aspartate aminotransferase

ALT - Alanine aminotransferase

ALP - Alkaline phosphatase

The effect of the extract on biochemical parameters are shown in Table 4. There was no significant ($P > 0.05$) difference in the plasma total protein and total bilirubin of treated animals when compared with the control group. There was no significant ($p > 0.05$) difference in the plasma activity of liver function marker enzymes ALT and ALP in the treated rats when compared with the control group. However, AST levels were significantly ($p < 0.05$) lower in the higher dose levels (500 and 1000 mg/kg) compared with the control group. Plasma creatinine levels were higher in 1000 mg/kg group but the difference was not significant ($p > 0.05$).

Table 5: Effect of Clerodendrum volubile ethanol extract on Biochemical Parameters in Liver of Rats.

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Total Protein(g/dl)	4.49±0.40	4.75±0.75	1.48±0.78*	0.94±0.16**
AST (U/I)	234.15±15.44	226.20±24.67	203.30±4.31	194.44±3.56
ALT (U/I)	122.92±5.57	123.94±6.75	126.74±2.51	134.08±5.41
ALP (U/I)	0.83±0.00	3.82±0.00	1.36±0.00	0.60±0.00

Values are expressed as mean ±SEM (standard error of mean) for five animals in each group.

* $p < 0.05$, ** $p < 0.01$ compared with control..

AST - Aspartate aminotransferase

ALT - Alanine aminotransferase

ALP - Alkaline phosphatase

In the liver, administration of the extract caused a dose-dependent decrease and a dose-dependent increase in the activities of AST and ALT respectively but these differences were not statistically significant ($p > 0.05$) (Table 5). A significant ($p < 0.05$) decrease in total protein concentration occurred at the higher doses.

Table 6: Effect of ethanol extract of Clerodendrum volubile on Biochemical Parameters in Kidney of Rats

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Uric Acid (mg/dl)	8.53±2.24	6.71±0.98	6.46±1.29	5.97±1.13
Creatinine (μmol/l)	9.87±2.52	3.08±0.22**	2.46±0.27**	1.52±0.22**

Values are expressed as mean ±SEM (standard error of mean) for five animals in each group. Values with ** are statistically significant at $p < 0.01$ from the normal control group as compared.

A significant ($p < 0.05$) decrease in creatinine levels was seen in the extract treated groups compared with the control group (Table 6). A decrease in uric acid concentration was observed in the treated groups although this difference was not significant ($p > 0.05$).

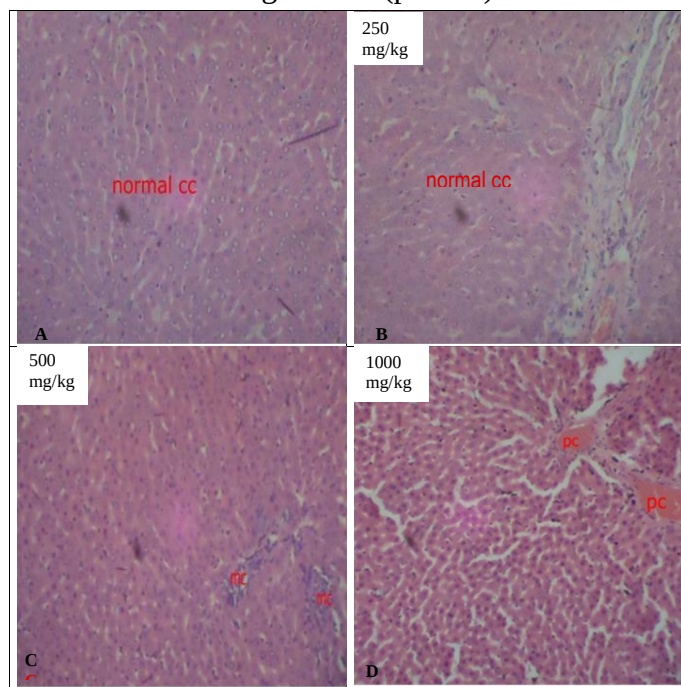


Figure 1: Effect of ethanol extract of *Clerodendrum volubile* ethanol extract on liver of rats.

A. Control showing well-outlined normal cytoarchitecture of hepatocytes
B. normal cytoarchitecture of hepatocytes
C. showing normal hepatocytes with areas of mild to moderate cellular infiltration (mc) by mononuclear cells.
D. Liver section showing otherwise normal hepatocytes with areas of portal congestion (pc) of red cells.

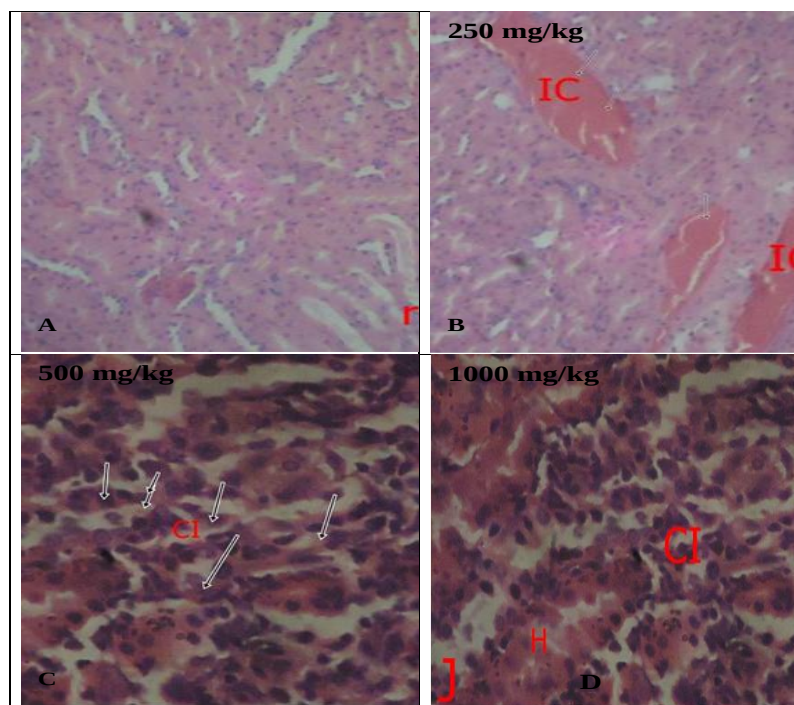


Figure 2: Effect of *Clerodendrum volubile* ethanol extract on kidney of rats.

A- Control shows normal cellular architecture of the kidney with the renal corpuscles (rc). B - moderate interstitial congestion is seen within the cortex (IC). C-shows areas of mononuclear cellular infiltration (CI) accompanied with mild hemorrhage at a higher concentration. D-areas of mononuclear cellular infiltration (CI), accompanied with mild hemorrhage (H) at a higher concentration, are evident.

Histological analysis of the liver revealed moderate cellular infiltration of mononuclear cells at 500 mg/kg and a congestion of red blood cells at 1000 mg/kg (Figure 1). Cellular infiltration and interstitial congestion was observed at higher doses in the histological examination of the kidney (Figure 2).

Discussion

Diabetes is an inflammatory disease. The antidiabetic and other pharmacological effects of this plant have been reported (Molehin, et al; 2018, Molehin and Oloyede 2018 and Erukainure, et al; 2018). The ethanol extract of *Clerodendrum volubile* exhibited anti-inflammatory effect (Olarenwaju et al 2018). The toxicological evaluation of *C. volubile* therefore is very useful and necessary for its safe use for medicinal purposes and herbal medicine production. This is expected to reduce the high cost of obtaining health care since the plant is readily available and the cost of preparation is relatively cheaper. This study was carried out to investigate the possible toxic effects of *C. volubile* leaf ethanol extract in mice and adult albino rats. Overexposure to toxic substances and an inability to properly metabolize them can impact negatively on one's health, producing a wide variety of symptoms such as headaches, muscle and joint pain, fatigue, and allergy or flu-like symptoms.

Extraction with alcohol in this research is in consonance with folkloric practices (Ubolom et al., 2011). In addition, a polar organic solvent (e.g. ethanol, methanol, or an aqueous alcoholic mixture) is employed in an attempt to extract as many compounds as possible. This is based on the ability of alcoholic solvents to increase cell wall permeability, facilitating the efficient extraction of large amounts of polar and medium- to low-polarity constituents (Lavie et al., 1987; Seidel, 2005). Thus, the extraction with aqueous ethanol would have captured more constituents than the aqueous extraction.

Many health problems are solved using medicinal plants, however, a number of these plants may be toxic if not properly prepared or dispensed (Nasri and Shirzad, 1997). A scientific approach was taken to evaluate the safety of *C. volubile* plant based on the fact that it is used in managing ailments especially in developing countries where the level of literacy is low, the status of health management is poor and about 80% of the population patronize herbal drugs (Ekor, 2014). Furthermore, there is a general misconception that natural products are not toxic and are devoid of adverse effects. This leads to improper use and unrestrained intake and this has also resulted in severe poisoning and acute health problems (UNESCO, 2013).

The lack of mortality with oral treatment at 5000 mg/kg body weight suggests that the LD50 value is more than 5000 mg/kg and the ethanol leaf extract of *C. volubile* is practically non-toxic with acute administration (Abid and Mahmood, 2019). This corroborates the reports of Akinpelu et al (2016) and Molehin and Oloyede (2018) that the ethanol and aqueous leaf extracts of *C. volubile* are safe. It could be due to wide margin of safety that *C. volubile* is consumed as a non-conventional food in various parts of South-south of Nigeria. It is therefore safe for acute ethno-therapeutic management of acute conditions ailments (Burkill, 1985). The considerable weight gain observed in animals treated with the extracts indicates that the extract does not interfere with growth processes and may have promoted growth by stimulating the synthesis of body proteins. Kluwe et al (1981) documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxics and thereafter defined nephrotoxicity as increased kidney weight either absolute or relative. The ethanol leaf extract of *C. volubile* did not induce any toxic effect on the organs tested going by this indicator since the relative weight of the organs is not significantly different from control values.

The plasma biochemical parameters were studied to evaluate the effects of *C. volubile* ethanol extract on hepatic and renal functions. Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) are well known biomarkers predicting possible toxicity thus they are good indicators of liver function (El-Hilaly and Lyoussi 2002, Ramaiah 2007). The higher the activities of these enzymes the greater the degree of liver damage (Cheesbrough, 1991; Gatising et al., 2005; Garba et al., 2006). Elevation in the levels of serum transaminase enzymes is highly indicative of hepatic impairment in animals (Alvarez and Mukherjee 2011) and there is a good correlation between its level and severity of hepatic necrosis (Lu, 1996). These enzymes are only released in significant amounts into the bloodstream from the cytosol and subcellular organelles when hepatic injuries occur (Lu, 1996). Increase in ALP level is observed in bone disorders involving osteoblastic activity. However, an elevation in ALP activity between 2 and 8 fold the normal value usually is the first clue to intra hepatic and extra hepatic cholestasis (biliary obstruction) (Moss, 1987, Young and Holland,

1995). High levels of AST and ALT indicate liver damage, cardiac infraction and muscle injury. Thus, the significant decrease in the activities of plasma AST levels at 500 and 1000 mg/kg compared with that of control suggests the extract does not have toxic effects on the liver and other organs having significant quantities of this enzyme. ALT is more hepato-specific than AST because it is more sensitive to hepatic damage (Herfindal and Gourley, 2000). This extract did not alter the ALT levels in the treated group even at higher doses, which further confirms it is not harmful to the liver. This supports the claim that methanol leaf extract of *C. volubile* is non-toxic (Molehin 2020).

A significant increase in serum or plasma total protein is an indication of tissue injury (Ekam and Udosen, 2012). In this study, *C. volubile* ethanol extract did not alter the total protein level, this further confirms its safety. Total bilirubin, exhibited no significant alterations as compared with the normal control. It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence the effects of ethanol extract of *C. volubile* on kidney function were assessed by evaluating plasma biochemical parameters such as uric acid and creatinine. There was no significant difference between uric acid levels in treated and control animals. The measurement of serum creatinine concentration was a useful index for the diagnosis of chronic kidney disease and when serum creatinine level was higher than the normal value, renal failure was most likely a possible outcome. (Edmund and David, 2006). Elevated serum creatinine level has been reported as an indicator of possible kidney dysfunction (Gross et al., 2005, Atangwho et al., 2013). Thus, the highly significant reduction in creatinine concentration in all the extract-treated groups, in this study, is a likely indication that the extract does not exert deleterious effect on renal function. *C. volubile* extract may be a useful remedy for lowering creatinine concentrations in medical conditions associated with raised serum creatinine levels or in drug-induced high serum creatinine levels. In the light of the foregoing it appears that *C. volubile* ethanol leaf extract at the doses used may have beneficial effects on the liver and kidney. These observations were further confirmed by the histological assessment of the organs.

Conclusion

This study provided valuable data on acute and sub-chronic oral toxicity profiles of *C. volubile* that should be useful for future in vivo and clinical evaluation of this plant. The sub-chronic oral administration of *C. volubile* ethanol extract was non-toxic. However, chronic administration and consumption of this extract at high doses should be used with caution.

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