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ACUTE AND SUBCHRONIC TOXICITY STUDIES OF THE CARBONATED DRINK EXTRACT OF *ENANTIA CHLORANTHA* STEM BARK

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ABSTRACT

Enantia chlorantha is a plant known for its medicinal properties, particularly in traditional medicine across West Africa. The acute and sub chronic toxicity studies of the extract were evaluated. Group 1 animals were given distilled water while Groups 2 – 4 were administered 50, 150 and 250 mg/kg body weight of the extract respectively. The LD50 values from the acute toxicity study of the extracts were greater than 5000 mg/kg body weight. The RBC, Hb, HCT, MCV, MCH, MCHC, and platelets levels were higher ($p > 0.05$) in all the extract treated groups. There was no significant difference ($p > 0.05$) in the serum TC, TG, HDL, LDL, VLDL - cholesterol levels in all the treated groups when compared to the control group. This showed that the extracts did not affect the lipid profile of the mice. There were no significant ($p > 0.05$) difference in ALP, AST, ALT and GGT activities. The Potassium levels were non-significantly ($p > 0.05$) higher in the extracts treated groups when compared to the control group. Significant ($p < 0.05$) increased in GSH concentration was observed in the mice administered 250 mg/kg body weight of the extract when compared with the control group. No prominent hepatocellular or glomerular damages were observed in the vital organs. The findings suggest that the carbonated drink extract of *E. chlorantha* stem bark is safe for use and therefore supports the traditional use of the plant in treating various disease condition.

Keywords: Toxicity, Carbonated drink, *Enantia chlorantha*, Liver, Kidney

INTRODUCTION

Herbal remedies have historically been esteemed for their natural sources and believed minimal adverse effects, constituting a vital component of local and regional healing practices across the globe (WHO, 2019; Rizvi et al., 2022). *Enantia chlorantha* (EC) is widely used in herbal medicine for the treatment of several ailments of the human body. Olasehinde & Ojeaburu in 2024 reported the traditional use of *E. chlorantha* in managing diabetes and its complications. However, its toxicity profiles are not well documented (Olamide and Agu, 2013). Like other therapeutic agents, *E. chlorantha* may not be devoid of side effects or toxicity in both human and animal studies (Ikpeme et al., 2011). Several in vivo studies were conducted where both aqueous and ethanolic extracts of EC were tested using oral and sub-cutaneous routes of administration. The effect of the leaf and bark extracts on reticulocyte and haematocrit values as well as nucleated cell numbers in the spleen, bone marrow, peritoneum, liver and peripheral blood, are indications of possible acute and chronic toxicity of these extracts (Agomo et al., 1992). The mean LD_{50} values were reported as 0.7g/kg and 43.7g/kg for the ethanolic and aqueous extracts respectively, but no fatalities were recorded in the sub-acute toxicity test (3–5 mg/kg aqueous extract for five weeks) and no significant damage to the body organs was observed. Tan et al. (2007) showed that the aqueous extracts of *E. chlorantha* were not acutely toxic at doses of up to 5000mg/kg body weight, but could cause lung, hepatic and kidney disorders following medium-to-long term use at doses greater than 500mg/kg. Adebiyi and Abatan (2013b) studied the effects of high doses of the ethanolic extract (500 – 3000 mg/kg body weight) and reported that high doses (e.g. > 1000 mg/kg body weight) caused congestion in the heart and kidneys of the experimental animals, as well as significant decreases in haemoglobin concentration and red blood cell counts. Moody et al. (2007) conducted acute and sub-chronic toxicity studies using alkaloidal fractions and suggested the relative safety of short-term use of preparations containing *E. chlorantha*.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study were of analytical grade.

Collection of Plant Materials

The plant samples used in this study was sourced locally from herb seller at Lagos Street, Benin City, Edo State, Nigeria and it was identified by the Botanist Dr. H. A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City for authentication and the voucher specimen of the plant was deposited at the herbarium of the University of Benin, Benin City.

Preparation and Extraction of Plant Materials

The fresh stem bark of *Enantia chlorantha* were washed under clean tap water to remove contaminants, debris and dust particles and were cut into pieces after which they were air-dried under shade at room temperature. Thereafter, the dried plant materials were pulverized using an electric blender. One thousand grams (1000g) of the pulverized stem bark were macerated with 5L of the carbonated drink (soft drink). The resulting extracts were filtered using Whatman No.42 filter paper (125mm) into clean containers and the filtrates were concentrated using a freeze dryer at the National Centre for Energy and Environment, University of Benin, Benin City and the extract was thereafter stored in sterile containers and kept at 2 - 8^0C till when needed (Sotade et al., 2024).

Experimental Animals

Healthy female albino mice of Swiss strain weighing (25.00 ± 2.00 g) were purchased from the Animal House of the Department of Pharmacology, University of Benin, Benin City, Nigeria. The animals were housed under standard laboratory conditions at a temperature of 27 ± 2°C and were allowed to acclimatize for seven days under standard environmental conditions with a 12-hour light/dark cycle; maintained on standard feed and water ad libitum. The laboratory animals were treated according to the Canadian Council on Animal Care Guidelines and Protocol Review (Olfert et al., 1993).

Acute toxicity study

Acute toxicity of the extracts were performed in two phases as described by Lorke (1983). Eighteen (18) Swiss albino mice (25.00 ± 2.00 g) were used for the first and second phases of nine (9) mice per phase.

Phase 1

Animals were divided into 3 groups of 3 animals each and the extracts were administered orally at doses of 10, 100 and 1000mg/kg body weight respectively. The animals were observed for 24 hours for signs of toxicity due to the treatment. These include: behavioural changes (dizziness, reaction to contact and noise, movement, aggressiveness and reaction to food supply), feeding, water intake, death etc.

Phase 2

In the second phase, the mice were divided into three (3) groups of three (3) mice each and they were administered with more specific doses of 1600, 2900 and 5000 mg/kg body weight of the extracts respectively and observed for signs of toxicity and death in 24 hours. The final LD_{50} which is calculated as the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived was calculated.

Sub-Chronic Toxicity Study

From the results of the acute toxicity study, graded doses were selected for sub chronic toxicity test. Twenty (20) Swiss albino mice were divided into four (4) groups of five (5) animals each and the carbonated drink extract (CDE) was given at 8 am on alternate days for 28 days.

Group 1 (n = 5): Animals were given distilled water (10ml/kg) and feed ad libitum (Control).

Group 2 (n = 5): Animals were administered 50mg/kg body weight of CDE.

Group 3 (n = 5): Animals were administered 150mg/kg body weight of CDE.

Group 4 (n = 5): Animals were administered 250mg/kg body weight of CDE.

On the last day of the administration, the animals were fasted (feeding was stopped by 7pm on the 28th day) overnight after which they were anaesthetized using chloroform and blood samples were collected using needles and syringes via cardiac puncture, into already labelled plain and K_2 EDTA bottles and the samples were mixed by gentle inversion. The samples in the K_2 EDTA anticoagulant bottles were immediately sent for haematological analysis using automated haematology analyzer for full/complete blood counts. The samples in the plain bottles were centrifuged at 3000 rpm for 15 minutes to obtain serum which was used for biochemical assays in this study.

Biochemical Assays

All the assay kits used in these assays were from RANDOX® Laboratories Limited, Ardmore, United Kingdom.

The total cholesterol (TC), triacylglycerol (TAG), and high-density lipoprotein cholesterol (HDL-c) were estimated using Randox kits, LDL - cholesterol = Total cholesterol – HDL-cholesterol – Triglycerides/5 (Friedewald et al., 1972), VLDL-cholesterol = TRIGS/5 (Satheesh and Pari, 2008), Castelli's risk index 1: (TC/HDL-C), Castelli's risk index 11: (LDL-C/HDL-C), Castelli's risk index 111: (TG/HDL-C) (Ikewuchi and Ikewuchi, 2009), Atherogenic coefficient (AC) **:** (TC) - (HDL-C)/HDL-C (Bherdwaj et al., 2013), Atherogenic index of serum (AIS): Log (TG/HDL-C) (Dobiasova and Frohlich, 2001).

The ALP, AST, ALT, GGT, T.BIL, D.BIL, TP, ALB, GLO tests were respectively conducted according to the methods previously reported by (Englehardt, 1970; Reitman and Frankel, 1957; Szasz, 1976; Jendrassik and Grof, 1938; Tietz, 1995; Doumas et al., 1971; Busher, 1990).

The kidney function indices which included urea, creatinine, sodium, potassium, chloride and bicarbonate were respectively conducted according to the methods previously reported by (Weatherburn, 1967; Bartels and Bohmer, 1972; Tietz et al., 1996; Tiezt, 1976; Davidson and Henry, 1979).

The MDA concentration, GPx, SOD and CAT activities were conducted according to the methods reported by (Buege and Aust, 1979; Nyman, 1959; Misra and Fridovich, 1972; Cohen et al., 1970) respectively while the GSH, Vit C and E concentrations were by (Ellman, 1959; Omaye et al., 1979; Desai, 1984).

Histopathology

After sacrificing the mice, vital organs (liver and kidneys) were harvested and preserved using 10% formal saline, processed through a graded ethanol and finally with xylene, and embedded in paraffin wax. Paraffin blocks were then prepared and sectioned into 6 µm thick slices using a rotary microtome. The paraffin-embedded tissue sections were mounted on glass slides and deparaffinized with xylene. The sections were rehydrated through a descending series of ethanol concentrations and finally rinsed in distilled water. For general histological evaluation, the sections were stained with haematoxylin and eosin (H & E). Slides were immersed in haematoxylin solution for 5-10 minutes to stain cell nuclei, followed by brief differentiation in 1% acid alcohol. The sections were then placed in running tap water for 10 minutes to enhance nuclear staining. Subsequently, the slides were stained with eosin solution for 1-2 minutes to provide a counterstain, highlighting the cytoplasmic and extracellular matrix components. After staining, the slides were dehydrated through ascending grades of ethanol, cleared in xylene, and mounted with a coverslip using a synthetic resin mounting medium. The stained sections were then examined under a light microscope at various magnifications to evaluate

histopathological changes. Photomicrographs were captured to document representative histopathological findings (Dahiru and Obidoa, 2008).

Statistical Analysis

Data are presented as mean ± SEM. Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to compare differences between means. Both ANOVA and Tukey's post-hoc tests were performed using GraphPad Prism, version 7. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity Study of the Carbonated Drink Extract of *E. Chlorantha* Stem Bark

The median lethal dose (LD_{50}) value of the carbonated drink extracts of *E. chlorantha* stem bark was greater than 5000 mg/kg body weight. Table 1 shows the results of the acute toxicity studies of CDE of *E. chlorantha* stem bark extracts administered orally to the mice within 24 hours. There were no visible signs of toxicity such as muscle weakness, drowsiness, tremor, hair erection, loss of hair, shivering, lacrimation, heartbeat, reduced feeding and water intake, breathing, movement or death observed in all the animals when the extracts were administered at different doses.

Table 1: Acute Toxicity Evaluation of the CDE of *E. Chlorantha* Stem Bark

Phase	Extracts	Dose (mg/kg)/body weight	No. of animals	Mortality ratio
I	CDE	10	3	0/3
		100	3	0/3
		1000	3	0/3
II	CDE	1600	3	0/3
		2900	3	0/3
		5000	3	0/3

Key: (0/3) 0 = number of deaths, 3 = number of mice used; CDE = carbonated drink extract.

There was a slight increase ($p > 0.05$) in red blood cell counts, haemoglobin concentration, haematocrit, mean corpuscle volume, mean corpuscle haemoglobin, mean corpuscle haemoglobin concentration and platelets in all the extract treated groups when compared to the untreated group (control group). However, no significant difference ($p > 0.05$) was observed in white blood counts in all the groups when compared to the control group (Table 2).

There was no significant difference ($p > 0.05$) in serum total cholesterol, triglyceride, high density lipoprotein, low density and very low-density lipoprotein cholesterol levels in all the treated groups when compared to the control group. This showed that the extracts did not affect the lipid profile of the mice (Table 3).

Table 2: Effect of *E. Chlorantha* Stem Bark Extracts on the Haematological Indices of the Mice

Treatment groups	WBC ($x10^3/\mu L$)	RBC ($x10^3/\mu L$)	Hb(g/dL) (g/dL)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dL)	PLT ($x10^3/\mu L$)
CONTROL	6.12 ± 0.34^a	5.81 ± 0.21^a	13.17 ± 0.64^a	39.65 ± 1.23^a	52.65 ± 1.23^{ab}	17.52 ± 0.96^a	32.21 ± 0.01^a	502 ± 3.78^a
CDE 50	6.23 ± 0.31^a	5.91 ± 0.26^a	13.82 ± 0.83^a	41.32 ± 1.89^a	51.12 ± 1.11^a	17.02 ± 1.32^a	32.42 ± 0.04^a	550 ± 4.78^a
CDE 150	6.45 ± 0.40^a	5.93 ± 0.27^a	13.87 ± 0.92^a	40.21 ± 1.40^a	51.72 ± 1.21^a	17.22 ± 1.08^a	33.23 ± 0.09^a	560 ± 5.34^a
CDE 250	7.02 ± 0.69^a	6.01 ± 0.29^a	13.80 ± 0.85^a	42.12 ± 1.92^b	53.65 ± 1.30^b	17.87 ± 1.22^a	33.08 ± 0.06^a	600 ± 6.72^b

Key: CDE = carbonated drink extract; WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; PLT = platelet; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

Table 3: Effect of *E. Chlorantha* Stem Bark Extracts on Serum Lipid Profile of the Mice

Treatment groups	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Control	60.05 ± 7.22^a	45.63 ± 0.56^a	35.47 ± 0.64^b	15.46 ± 1.02^a	9.12 ± 0.21^a
Cde 50	58.60 ± 6.00^a	47.60 ± 0.70^a	35.00 ± 0.12^b	14.08 ± 0.54^a	9.52 ± 0.04^a
Cde 150	54.14 ± 4.00^a	41.26 ± 0.90^a	35.10 ± 0.25^b	10.79 ± 0.00^a	8.25 ± 0.70^a
Cde 250	60.18 ± 5.44^a	50.17 ± 1.73^a	38.36 ± 0.92^a	11.79 ± 0.47^b	10.03 ± 1.32^b

Key: CDE = carbonated drink extract; TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; TG = triglyceride; VLDL-C = very low-density lipoprotein cholesterol; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

There was no significant difference ($p > 0.05$) in total CRI - 1, CRI - 11 and CRI - 111 in the extract treated groups when compared to the control group (Table 4). This showed that the extracts did not affect the lipid profile in the mice. There was no significant change ($p > 0.05$) in the atherogenic coefficient and atherogenic index in the serum of the treated groups when compared to the control group. The cardiovascular risk ratios of the control group and extracts treated groups were moderate.

Table 4: Effect of *E. Chlorantha* Stem Bark Extracts on the Cardiac Risk Ratios of the Mice

Treatment groups	Cardiac Risk Ratios				
	CRI - I	CRI – II	CRI - III	AC	AIS
CONTROL	1.69 ± 0.02^{b}	0.43 ± 0.07^{b}	1.28 ± 0.03^{b}	0.69 ± 0.02^{b}	0.17 ± 0.08^{b}
CDE 50	1.67 ± 0.03^{b}	0.25 ± 0.07^{a}	1.11 ± 0.05^{a}	0.48 ± 0.08^{a}	0.04 ± 0.03^{a}
CDE 150	1.54 ± 0.08^{a}	0.40 ± 0.03^{b}	1.36 ± 0.03^{b}	0.44 ± 0.09^{a}	0.13 ± 0.05^{b}
CDE 250	1.57 ± 0.09^{a}	0.30 ± 0.02^{a}	1.18 ± 0.06^{a}	0.54 ± 0.01^{a}	0.07 ± 0.08^{a}

Key: CDE = carbonated drink extract; CRI = castelli's risk index; AC= atherogenic coefficient; AIS = atherogenic index of serum; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

There was no significant difference ($p > 0.05$) in the activities of ALP, AST, ALT, GGT and the levels of total and direct bilirubin in all the treated groups when compared to the control group (Table 5).

There was no significant difference ($p > 0.05$) in the levels of serum total protein, albumin and globulin concentrations in all the treated groups when compared to the control group (Table 6). The albumin globulin ratio of the treated groups are comparable to that of the standard.

Table 5: Effect of *E. Chlorantha* Stem Bark Extracts on Liver Function Indices of the Mice

Treatment groups	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	T.BIL(mg/dl)	D.BIL(mg/dl)
CONTROL	26.50 ± 1.38^{a}	12.42 ± 1.94^{a}	1.32 ± 0.31^{a}	21.02 ± 0.23^{b}	0.74 ± 0.06^{b}	0.26 ± 0.02^{b}
CDE 50	28.64 ± 1.28^{a}	20.27 ± 1.32^{b}	1.42 ± 0.29^{a}	26.12 ± 0.33^{c}	0.53 ± 0.02^{a}	0.19 ± 0.01^{a}
CDE 150	29.67 ± 1.38^{a}	27.90 ± 1.69^{cd}	1.36 ± 0.26^{a}	15.12 ± 0.11^{a}	0.25 ± 0.02^{a}	0.12 ± 0.02^{a}
CDE 250	28.83 ± 1.03^{a}	21.05 ± 1.80^{bc}	1.40 ± 0.81^{a}	14.27 ± 0.12^{a}	0.64 ± 0.04^{b}	0.22 ± 0.07^{a}

Key: CDE = carbonated drink extract; ALP = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyltransferase; T.BIL = total bilirubin and D.BIL = direct bilirubin; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

Table 6: Effect of *E. Chlorantha* Stem Bark Extracts on Serum Total Protein, Albumin and Globulin Concentrations of the Mice

Treatment groups	TP (g/dl)	ALB (g/dl)	GLO (g/dl)	ALB/GLO
CONTROL	3.97 ± 0.72^{ab}	2.31 ± 0.50^{a}	1.66 ± 0.12^{a}	1.39 ± 0.03^{a}
CDE 50	3.36 ± 0.07^{a}	2.02 ± 0.32^{a}	1.34 ± 0.09^{a}	1.51 ± 0.02^{a}
CDE 150	2.41 ± 0.06^{a}	1.40 ± 0.54^{a}	1.01 ± 0.08^{a}	1.39 ± 0.08^{a}
CDE 250	3.72 ± 0.37^{ab}	2.20 ± 0.30^{a}	1.52 ± 0.36^{a}	1.44 ± 0.02^{a}

Key: CDE = carbonated drink extract; TP = total protein; ALB = albumin; GLO = globulin; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

The effect of sub-chronic administration of the extract at doses of 50 mg/kg, 150 mg/kg and 250 mg/kg body weight did not produce any significant difference ($p > 0.05$) in the serum urea and creatinine levels in the treated groups relative to the control group (Table 7).

There was no significant difference ($p > 0.05$) on the electrolytes of the mice when compared to the control group, However, the potassium levels were higher when compared to the control group but was not statistically significant ($p > 0.05$) while the decrease in sodium levels when compared to the control group were also not statistically significant ($p > 0.05$) (Table 8).

There was no significant difference ($p > 0.05$) in SOD activity and MDA concentration in all the treated groups when compared to the control group (Table 9).

There was no significant difference ($p > 0.05$) in vitamin C and E concentrations in all the treated groups when compared to the control group (Table 10). GSH concentration was however increased significantly ($p < 0.05$) in the group treated with CDE (250 mg/kg body weight) when compared to the control group.

Table 7: Effect of *E. Chlorantha* Stem Bark Extracts on Serum Urea and Creatinine Levels of the Mice

Treatment groups	UREA (mg/dl)	CREAT (mg/dl)
CONTROL	30.23 ± 0.92^{a}	0.37 ± 0.40^{a}
CDE 50	32.10 ± 0.60^{ab}	0.41 ± 0.05^{a}
CDE 150	33.64 ± 0.13^{b}	0.27 ± 0.37^{a}
CDE 250	28.64 ± 0.17^{a}	0.35 ± 0.02^{a}

Key: CDE = carbonated drink extract; CREAT = creatinine; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

Table 8: Effect of *E. Chlorantha* Stem Bark Extracts on Serum Electrolytes of the Mice

Treatment groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
CONTROL	1.40 ± 0.05[a]	5.13 ± 0.10[a]	46.28 ± 1.24[ab]	30.23 ± 0.92[a]
CDE 50	1.25 ± 0.01[a]	5.82 ± 0.18[a]	44.23 ± 1.15[a]	30.12 ± 0.87[a]
CDE 150	1.15 ± 0.01[a]	5.64 ± 0.17[a]	45.45 ± 1.14[a]	29.97 ± 0.81[a]
CDE 250	1.20 ± 0.01[a]	5.32 ± 0.15[a]	47.32 ± 1.03[b]	29.98 ± 0.82[a]

Key: CDE = carbonated drink extract; Na^+ = sodium; K^+ = potassium; Cl^- = chloride; HCO_3^- = bicarbonate; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$)

Table 9: Effect of *E. Chlorantha* Stem Bark Extracts on Lipid Peroxidation and Antioxidant Enzyme Activities of the Mice

Treatment groups	MDA (nmol/mg)	GPx (U/mg)	SOD (U/mg)	CAT (U/mg)
CONTROL	3.53 ± 0.52[a]	3.15 ± 0.14[a]	2.56 ± 0.09[a]	0.019 ± 0.01[a]
CDE 50	3.67 ± 0.53[a]	3.13 ± 0.12[a]	2.54 ± 0.08[a]	0.016 ± 0.05[a]
CDE 150	3.54 ± 0.54[a]	3.30 ± 0.18[a]	2.80 ± 0.15[a]	0.018 ± 0.01[a]
CDE 250	3.49 ± 0.42[a]	4.22 ± 0.21[a]	3.23 ± 0.14[b]	0.017 ± 0.04[a]

Key: CDE = carbonated drink extract; MDA = malondialdehyde; GPx = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

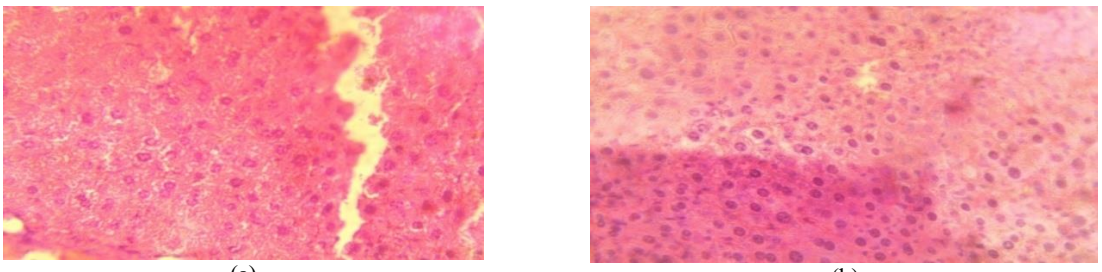
Table 10: Effect of *E. Chlorantha* Stem Bark Extracts on Non-Enzymic Antioxidant Molecules of the Mice

Treatment groups	GSH (U/mg)	VIT C (mg/ml)	VIT E (mg/ml)
CONTROL	0.030 ± 0.01[a]	0.90 ± 0.10[a]	1.20 ± 0.22[a]
CDE 50	0.035 ± 0.02[a]	1.22 ± 0.15[a]	1.80 ± 0.33[a]
CDE 150	0.037 ± 0.02[a]	1.32 ± 0.16[a]	1.82 ± 0.33[a]
CDE 250	0.054 ± 0.04[b]	1.76 ± 0.18[a]	1.97 ± 0.39[a]

Key: CDE = carbonated drink extract; GSH = reduced glutathione; VIT C = vitamin C; VIT E = vitamin E; 50, 150 and 250 represent mg/kg body weight of the extract administered. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$).

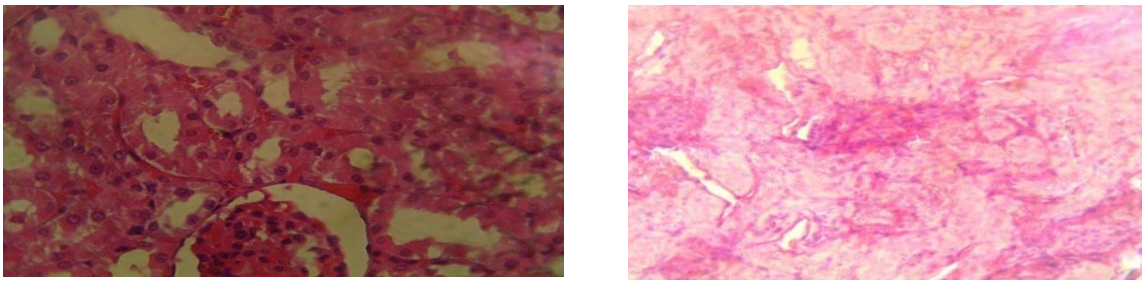
Histopathological Studies

From the liver histopathological studies, normal cellular architecture was observed after oral treatment with the extracts for 28 days (Plate 1). There were no morphological alterations seen in the livers while the histopathological studies of the kidney showed normal cellular architecture, normal glomerulus and normal tubules (Plate 2).



(a) (b)

Plate 1: Photomicrograph of sections of the liver. **A** (Control), **B** (Extract). The sections showed normal cellular architecture after oral treatment for 28 days (H & E x400). There were no morphological alterations seen in the livers (normal hepatocytes).



(c) (d)

Plate 2: Photomicrograph of sections of the kidney. **C** (Control), **D** (Extract).The sections showed normal cellular architecture after oral treatment for 28 days (H & E x400). There were no morphological alterations seen in the kidneys (normal glomerulus and normal tubules).

This study provides key insights into the safety and toxicity of the carbonated drink extract of *Enantia chlorantha* stem bark. We observed that the plant extract caused an increase in the body weight of the test animals. This increase may be due to the nutritive components of the plant extract. It may also be due to the effect of the plant on the body fat metabolism. Similar results were reported by Eteng et al., 2003 and Bassey et al., 2017 who reported that secondary metabolites such as alkaloids and saponins stimulate protein synthesis and their presence in the extract among others may in part have caused the increase in the weight observed in the animals.

Acute toxicity is defined as unwanted effect (s) that occurs either immediately or at a short time interval after a single or multiple administration of such substance within 24 hours. From the results obtained in Table 1, the acute toxicity studies showed no toxic effect on the mice to which the various doses were administered, indicating the LD_{50} of E chlorantha stem bark is approximately higher than 5000 mg/kg body weight, and thus it is relatively safe and non-toxic to the mice in acute usage (Lorke, 1983). Since mortality was not observed, the extracts given to the mice showed no toxicity at the administered doses. However, mobility and aggressiveness were reduced in all the extract-treated groups when compared to the control group within 24 hours of observation.

The administration of the extract demonstrated a notable impact on various haematological parameters. Non-significant increase observed particularly at the 250mg/kg body weight in the level of RBC, Hb concentration, HCT, MCV, MCH, MCHC and platelets counts suggests an enhancement in the blood's oxygen-carrying capacity and overall haematological health. The slight increase in RBC levels observed in this study indicated that the extract may stimulate erythropoiesis. These findings aligned with the work reported by Bassey et al. (2017) who worked on the ethanolic stem bark extract of *E. chlorantha*.

The determined concentration of the total cholesterol, triglycerides, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol in both control and the treated groups suggests that the extracts did not affect the lipid profile of the mice.

The liver is the main site of metabolism in the body. Enzymes are necessary for normal cellular functions (Ramaiah, 2007). ALT, AST, and ALP are considered indicators of liver functional ability (Rezg et al., 2008). The transaminases (AST and ALT) are often used as specific markers of active hepatic injury (they both rise in the same proportion) and represent markers of hepatocellular necrosis, these liver enzymes catalyse the transfer of α-amino groups of aspartate and alanine to the α-keto group of α- ketoglutaric acid (Davern and Scharschmidt, 2002).

The electrolytes, urea and creatinine are markers of kidney function (Vasudevan and Sreekumari, 2007). From our study, we observed that the urea and creatinine levels as well as the electrolytes concentrations were normal in the groups administered with the extracts while significant ($p < 0.05$) increased was observed in GSH concentration in the group administered 250 mg/kg body weight of the extract when compared to the control group.

The histopathological analysis of the liver and kidney was considered in this study. From the results obtained, the control groups have normal liver and kidney morphology and architecture. The photomicrograph of the sections of the liver and kidney of the control and extracts treated groups showed normal cellular architecture after oral treatment for 28 days.

CONCLUSION

The acute toxicity studies of all the extract have LD_{50} greater than 5000 mg/kg body weight. The extract was able to ameliorate changes in some haematological indices, liver function enzymes, oxidative stress indices and histopathological changes in the liver and kidney. Conducting both acute and sub chronic evaluations of the carbonated drink extract of *Enantia chlorantha* stem bark is critical for ensuring consumer safety, but careful considerations must be given to dosage selection and monitoring health parameters to mitigate any potential risks associated with its consumption. From this, we can conclude that the plant *Enantia chlorantha* can safely be refined and prepared for further experiments to eventually produce different forms of it for human use. The relative stability of the biochemical and haematological parameters are indicative of its relative safety margin. This work corroborates the use of the plant by the natives of the Western and Southern regions of Nigeria.

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