ACUTE AND SUB-ACUTE TOXICITY EVALUATION OF METHANOLIC LEAF EXTRACT OF *CORCHORUS OLITORIUS* IN EXPERIMENTAL ANIMAL MODELS

ABSTRACT

Aim: In this study, the oral toxicity potential of *Corchorus olitorius* leaf extract was evaluated in rats.

Methods: Acute and sub-acute toxicity studies were carried out on different sets of rats treated with graded doses of the extract. In the acute toxicity study, forty five albino mice assigned to nine groups of five mice each were administered different doses of the extract ranging from 500 mg/kg body weight to 8000 mg/kg body weight and were observed for toxicity signs and mortalities. For the sub-acute toxicity study, four groups of rats were assigned treatments. While group 1 rats served as the control, groups 2, 3 and 4 were the test groups and were administered 250, 500 and 1000 mg/kg body weight of the extract respectively for 28 days.

Results: The results of acute toxicity evaluation indicated an LD_{50} value of 7100 mg/kg. In the sub-acute toxicity study, no significant difference was observed between relative organ weights for test groups at lower dose treatments ($P>0.05$). Basal Metabolic Index values were significantly lower in all test groups ($P<0.05$). RBC count, PCV values and haemoglobin concentrations were significantly increased following treatment except in the 1000 mg/kg treated group where a decline was observed with a concurrent increase in white blood cell count. Platelet count was also significantly lowered in the 1000 mg/kg body weight treatment group ($P<0.05$). Serum urea, creatinine, AST, ALT, ALP, total protein and serum electrolyte concentrations were all significantly elevated in the 1000 mg/kg extract treated group ($P<0.05$). Liver sections showed some degree of liver damage when compared with control, while kidney sections were intact in all test groups.

Conclusion: Hence, low to moderate consumption of *Corchorus olitorius* leaves extract may cause no toxic effects to the blood, liver or kidney. However, high dose administrations over a long period of time may pose threats of toxicity to the blood and liver.

KEYWORDS: *Corchorus olitorius*, toxicity, haematology, biochemical, histopathology.

1. Introduction

The use of plants and plant based materials as medicine has of late become an area of global interest, such that over 80% of the world's population are said to be relying on herbal medicine [1]. Plants indeed are enriched with numerous phytochemical substances which in most cases are responsible for their healing properties [2]. Although reports of efficacy are, by far, more numerous than those on toxicity, toxicity evaluation of plants has become a vital aspect of modern researches since findings from such researches may help to keep dosage formulations and illicit use under check and also ensure effectual open communication of such toxicity findings for the purpose of enhancing knowledge [3,4].

Toxicity effects due to consumption of plants and plant based materials may range from mild changes in body weights to severe damages of vital tissues and organs such as blood, liver, kidney, heart and brain. Deaths have been reported in most of such cases. Therefore, necessitates a need to further the investigation of herbal remedies and phytochemicals to incorporate the observations of short and long-term toxic manifestations [5].

Corchorus olitorius is only one of the numerous plants that are currently being studied. *C. olitorius* is called jute mallow or bush okra. It is a green leafy vegetable popularly consumed among the Yorubas of southwestern Nigeria where it is commonly called '*Ewedu*'. While the Igbos of Southeast, Nigeria call it '*Ahihara*' [6]. Among the medicinal uses of *C. olitorius* are; demulcent, diuretic, purgative, bitter tonic, laxative, refrigerant, carminative and lactagogue. The leaf extract has given positive results in the management of chronic cystitis, dysuria, hyperglycaemia, dysentery, fever and gonorrhea [7,8].

Corchorus olitorius leaves are well known as an emolient and for purifying human body and are very rich in proteins, β-carotene, iron, calcium, vitamins (A, B, C, E), folic acid, amino acid and essential minerals. The leaves are used as an herbal pharmacopoeia against malaria or typhoid fever. The leaves of *C. olitorius* were reported to have hypoglycemic effect and high antibacterial activity. Consumption of jute mallow provides indispensable antioxidants needed for good health [9].

In a previous work, we reported that preliminary phytochemical screening of *Corchorus olitorius* leaves extract showed the presence of flavonoids (4.00 ± 0.035 mg/100 g), steroids (0.89± 0.031 mg/100 g), terpenes (1.27 ± 0.016 mg/100 g), phenolic compounds (2.05 ± 0.514 mg/100 g), alkaloids (3.10 ± 0.026 mg/100 g), saponins (4.00 ± 0.054 mg/100 g), tannins (0.32 ± 0.044 mg/100 g) and cardiac glycoside (1.61 ± 0.068 mg/100g). In that study, the result of GCMS analysis of the extract showed the presence of 46 different bioactive compounds with 2-Dodecenal, 3-Methyl-1-penten-4-yn-3-ol, 2,4-Decadienal, and Ethanone being in high amounts. No mortality was observed during the acute toxicity study period carried out up to a dose of 5000 mg/kg body [6].

In this study, we evaluated further the acute effect of the extract and also studied the sub-acute toxicity effects of the extract with a view to assessing possible systemic toxicity effects and other mortalities as expected in the acute study phase.

2. Materials and methods

2.1. Collection and identification of plant material

Fresh leaves of *C. olitorius* were collected from a bush fallow in Umudike, Ikwuano Local Government Area of Abia State, Nigeria and identified in the Department of Forestry, College of

Natural Resources and Environmental Management, Michael Okpara University of Agriculture. Umudike. A voucher number MOUAU/VPP/17/009 was assigned and a sample was deposited in the herbarium of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

2.2. Preparation of plant extract

The collected leaves of *C. olitorius* were air dried at room temperature for 14 days before being ground into powder in a locally fabricated milling machine. One hundred (100) grams of the powdered material was introduced into the extraction chamber of the soxhlet extractor for extraction using methanol as solvent and maintained at 60^0C. At the end of 48 hrs, the extract in solution was dried at low temperature in a hot air oven to obtain a crude extract which weighed 8.18 g and represented a percentage yield of 8.18%. The extract so prepared was preserved in the refrigerator until needed. The extract is hereafter referred to as *C. olitorius* leaf extract (COLE).

2.3. Preparation of Experimental Animals

Apparently healthy Albino mice (25-30 g) and Wistar Albino rats of about 10 weeks old and weighing 150- 200g were procured from the laboratory animal unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike were used for the study. Animals were handled in accordance with the guidelines for the care and use of laboratory animals as approved by the animal ethical committee of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike. The animals were housed in metal cages and were allowed access to feed and drinking water *ad libitum*. The acclimatization period allowed was 14 days within which the animals were exposed to 12 hrs light/dark cycle.

2.4. Acute toxicity evaluation of COLE

Acute toxicity evaluation of the COLE was carried out in accordance with methods used by Akomas et al,[10]. Forty-five albino mice were assigned to 9 groups of 5 mice each and each group was assigned a particular dose level of the extract. Groups 1, 2, 3, 4 and 5 received 500, 1000, 2000, 3000 and 4000 mg/kg body weight of the extract, respectively, while groups 6, 7, 8 and 9 were administered 5000, 6000, 7000 and 8000 mg/kg body weight of the extract respectively. After treatment, animals in all groups were placed under watch for the manifestations of toxicity signs and deaths within a 24-hr period and a further 7 days. The number of deaths recorded was used to evaluate the LD_{50} value of the extract. Karber's formula written below was applied:

$$LD_{50} = LD_{100} - \frac{\sum DDxMD}{5}$$

Where LD_{50} = The dose that produced mortality of 50% of animals in a given population
LD_{100} = The dose that produced mortality of 100% of animals in a given population
$\sum DDxMD$ = Sum of the products of dose difference and mean death

2.5. Oral Sub-acute toxicity study

Forty albino rats of both sexes were divided into four groups of 10 rats each in separate cages. While group 1 rats served as the control, groups 2, 3 and 4 were administered 250, 500 and 1000 mg/kg body weight of COLE via the oral route for 28 days. Changes in body weights were noted by recording body weights at the beginning and also at the end of administration before all rats were sacrificed on the 29^{th} day for blood collection by cardiac puncture for haematological and biochemical analysis. Vital body organs (liver, kidney, heart, spleen, lung) were harvested from each animal and were weighed to determine their relative organ weight (ROW). The liver and kidney were immediately fixed in 10% formalin for the histological study. The ROW was determined using the expression:

$$ROW = \frac{\text{Organ weight}}{\text{Body weight}} \times \frac{100}{1}$$

2.6. Basal Metabolic Index (BMI)

Basal Metabolic Index (BMI) for each rat was estimated after measuring their respective body weights and heights.

The expression: $BMI = \frac{\text{Body weight in kilograms}}{\text{Square of height in meters}}$

was used to calculate the BMI for each rat and expressed in kg/m^2.

2.7. Haematological, Biochemical and histological Examination

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China). The parameters which were evaluated included: red blood cells (RBC) count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); leukocytes (WBC) count and differential WBC counts were obtained at once for each blood sample. Biochemical parameters were also estimated for each sample using the respective commercial test kit for each test (Randox, UK). Biochemical parameters assayed include total protein (TP), bilirubin (BL), albumin (ALB), creatinine, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Total cholesterol, high density lipoprotein-cholesterol (HDL), triglycerides (TAG), Electrolytes(K^+, Na^+, Cl^-, HCO_3^-) and calcium ion (Ca^{2+}). Histological examination was also carried out on the liver and kidney samples harvested from the test groups and control and were examined by a moticam microscope attached to a computer.

2.8. Statistical Analysis

Results were expressed as means ± standard error of mean (SEM). Statistical analysis was done using one-way analysis of variance (ANOVA). Students' t-test at 95%, the level of significance was used to access significant difference between control and treated groups. P values less than 0.05 were considered as significant. Computer software package, SPSS version 21 was employed.

3. Results

3.1. Acute toxicity evaluation of COLE

No toxicity behaviors and mortality were observed during the acute toxicity study period of 24 hrs and a further 7 days in groups treated with 500-6000 mg/kg of the extract. However, two mortalities were observed in group 8 treated with 7000 mg/kg, while all animals in group 9 to which 8000 mg/kg were administered died. The number of deaths in each group was used to calculate the LD_{50} value in the application of Karber's formula and was found to be 7100 mg/kg

Table 1.Result of acute toxicity evaluation of COLE

Group	Dose (mg/kg)	Number of deaths	Percentage mortality	Dose Difference(DD)	Mean Death (MD)	DD x MD
1	500	0	0	500	0	0
2	1000	0	0	1000	0	0
3	2000	0	0	1000	0	0
4	3000	0	0	1000	0	0
5	4000	0	0	1000	0	0
6	5000	0	0	1000	0	0
7	6000	0	0	1000	1	1000
8	7000	2	40	1000	3.5	3500
9	8000	5	100	……	…….	…….

$$LD_{50} = LD_{100} - \frac{\sum DDxMD}{5}$$

$$= 8000 - \frac{4500}{5}$$

$= 7100mg/kg$

3.2. Effect of COLE on relative organ weight (ROW) in rats

No significant difference was observed between relative organ weights for all organs under study in all test groups at 250 and 500 mg/kg body weight (P>0.05) except for the lungs in the 500 mg/kg treatment group which was lower. However, for test groups treated with 1000 mg/kg body weight of the extract, ROW for the heart was significantly increased when compared with control (P<0.05) while that of the lungs was significantly lower than that of control group (Table 2).

Table 2. Effect of COLE on relative organ weights

Organs	Relative Organ Weight			
	Normal	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Liver	0.0338 ± 0.0009	0.0342 ± 0.0007	0.0351 ± 0.0012	0.0356 ± 0.0006
Heart	0.0030 ± 0.0002	0.0033 ± 0.0001	0.0033 ± 0.0001	0.0034 ± 0.0002*
Kidney	0.0075 ± 0.0002	0.0080 ± 0.0002	0.0076 ± 0.0001	0.0077 ± 0.0002
Spleen	0.0047 ± 0.0003	0.0049 ± 0.0002	0.0045 ± 0.0004	0.0044 ± 0.0001
Lung	0.0073 ± 0.0002	0.0068 ± 0.0001	0.0064 ± 0.0004*	0.0062 ± 0.0002*

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at P<0.05

3.3. Effect of COLE on the Basal Metabolic Index (BMI) of treated rats

Treatment with COLE significantly lowered BMI values in all test groups when compared with control (P<0.05). The effect of COLE on BMI was dose dependent in nature. i.e increasing with increasing doses (Table 3).

Table 3. Effect of COLE on the Basal Metabolic Index (BMI) of treated rats

	COLE doses			
Parameters	**Normal**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Initial BMI (g/cm^3)	0.39 ± 0.01	0.38 ± 0.01	0.46 ± 0.01*	0.43 ± 0.01*
Final BMI (g/cm^3)	0.42 ± 0.01	0.41 ± 0.01	0.46 ± 0.01*	0.42 ± 0.01
Change in BMI	0.03 ± 0.01	0.03 ± 0.00	0.00 ± 0.01*	-0.01 ± 0.01*

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at P<0.05

3.4. Effect of COLE on haematological values of treated rats

Red blood cells count (RBC), packed cell volume (PVC) and haemoglobin (Hb) concentration were significantly increased in test groups treated with 250 and 500 mg/kg body wt of the extract when compared with control (P<0.05). However, at 1000 mg/kg body wt, the values of these parameters experienced severe decline being significantly lower than control (P<0.05). White blood cells count significantly increased in test group treated with 500 and 1000 mg/kg body weight of the extract, and it was not significantly altered in the group administered 250 mg/kg of the extract (Table 4). Platelet count did not significantly differs in the test groups when compared with the control except in the 1000 mg/kg body weight treated group where the value was lower (P<0.05). MCH values in all test groups remained unaltered following treatment, but MCV and MCHC experienced a decline in the extract treated groups when compared with the control (Table 4). Neutrophils and monocytes counts were significantly reduced with increasing dose of the extract while lymphocytes count increased along the test groups (Table 5)

Table 4. Effect of COLE on haematological values of treated rats

		COLE doses		
Parameters	**Normal**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
RBC ($x10^{12}$/L)	8.40 ± 0.17	8.44 ± 0.11*	8.95 ± 0.13*	6.45 ± 0.06*
PCV(%)	49.13 ± 0.55	48.98 ± 0.88	57.83 ± 1.42*	39.92 ± 0.17*
Hb (g/dl)	13.09 ± 0.18	12.48 ± 0.18*	13.96 ± 0.20*	10.30 ± 0.14*
WBC ($x10^{9}$/L)	6.34 ± 0.36	6.84 ± 0.30	8.34 ± 0.41*	7.46 ± 0.17*
Platelets ($x10^{9}$/L)	758.10 ±20.96	746.80 ± 19.11	7491.90 ± 26.91	241.40 ± 9.82*
MCV (*fl*)	58.59 ± 0.58	62.33 ± 0.39*	64.51 ± 0.80*	61.90 ± 0.60*
MCH (*pg*)	15.56 ± 0.21	15.92 ± 0.11	15.60 ± 0.11	15.86 ± 0.20

MCHC (g/L)	26.64 ± 0.18	25.56 ± 0.23*	24.20 ± 0.33*	25.80 ± 0.30*

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at P<0.05

Table 5. Effect of COLE on differential white blood cells count in treated rats

		COLE doses		
Parameters	**Normal**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Neutrophils (%)	63.20 ± 0.73	59.00 ± 0.39*	58.10 ± 0.53*	41.40 ± 0.86*
Lymphocytes (%)	28.30 ± 0.70	32.30 ± 0.42*	34.40 ± 0.48*	51.60 ± 0.82*
Monocytes (%)	5.00 ± 0.26	4.40 ± 0.16	3.20 ± 0.25*	3.30 ± 0.15*
Eosinophil (%)	2.70 ± 0.26	3.70 ± 0.30*	2.90 ± 0.35	2.80 ± 0.36
Basophil (%)	0.80 ± 0.13	0.70 ± 0.15	0.90 ± 0.10	0.80 ± 0.13

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at P<0.05

3.6. Effects of COLE on biochemical parameters in rats

Most biochemical parameters were not significantly altered following treatment with COLE at lower doses (250 and 500 mg/kg body weight) when compared with control (P<0.05), except for the bilirubin concentration in the 500 mg/kg body weight treatment group which was significantly elevated. However, the group treated with 1000 mg/kg body weight of the extract had significantly elevated urea, creatinine, AST, ALT and ALP concentrations (Table 5). Total protein concentration also in all test groups treated with the extract was significantly raised when compared with the control (P<0.05).

Table 7. Effects of COLE on biochemical parameters in rats

		COLE doses		
Parameters	**Normal**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Total protein (g/dl)	5.90 ± 0.13	6.65 ± 0.27*	7.14 ± 0.10*	6.81 ± 0.35*
Albumin (g/dl)	3.44 ± 0.15	3.65 ± 0.18	3.87 ± 0.11	3.64 ± 0.20
Urea (mg/dl)	15.13 ± 0.31	14.49 ± 0.37	14.87 ± 0.37	17.43 ± 0.32*
Bilirubin (mg/dl)	0.76 ± 0.04	0.76 ± 0.03	0.89 ± 0.01*	1.10 ± 0.06*
Creatinine (mg/dl)	0.59 ± 0.01	0.58 ± 0.01	0.62 ± 0.01	0.68 ± 0.02*
AST (U/L)	22.10 ± 0.78	22.30 ± 0.58	23.90 ± 0.57	30.10 ± 0.69*
ALT (U/L)	13.20 ± 0.51	12.70 ± 0.42	14.20 ± 0.53	17.70 ± 0.94*
ALP (U/L)	69.64 ± 0.66	71.05 ± 0.61	70.81 ± 0.35	74.42 ± 0.93*

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at $P<0.05$

3.7. Effects of COLE on Lipid profile of treated rats

Total cholesterol and very low density lipoprotein cholesterol were significantly lower in all test groups treated with the extract at all doses while low density lipoprotein cholesterol concentration was significantly lower in groups treated with 500 and 1000 mg/kg body weight of the extract, though triglycerides concentration increased significantly in these groups too. High density lipoprotein cholesterol concentration was not significantly altered in the 250 and 500 mg/kg body weight treated groups but increased significantly in the group treated with 500 mg/kg body weight of the extract (Table 8).

Table 8. Effects of COLE on Lipid profile of treated rats

		COLE doses		
Parameters	**Normal**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
HDL (mg/dl)	56.50 ± 1.15	57.38 ± 0.49	59.51 ± 0.72*	55.83 ±1.14
TAG (mg/dl)	89.87 ± 0.56	91.63 ± 0.32	95.92 ± 0.43*	119.70 ± 1.03*
VLDL (mg/dl)	17.97 ± 0.11	18.33 ± 0.06	19.18 ± 0.09*	23.95 ± 0.21*
LDL (mg/dl)	25.47 ± 1.84	12.61 ± 0.76*	7.95 ± 0.73*	6.93 ± 1.17*
Total Cholesterol (mg/dl)	99.95 ± 1.37	88.32 ± 1.21*	86.64 ± 0.92*	86.74 ± 1.74*

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at P<0.05

3.8. Effects of COLE on serum electrolytes in rats

Serum calcium, sodium, chloride and bicarbonate were all significantly increased following treatment with the extract when compared with control (P<0.05), while potassium concentration was not significantly altered (P>0.05). The data for serum electrolytes are presented in table 9.

Table 9. Effects of COLE on serum electrolytes in rats

		COLE doses		
Parameters	**Normal**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Ca^{2+} (mEq/L)	8.27 ± 0.09	9.24 ±0.13*	9.04 ± 0.11*	9.20 ± 0.14*
Na^{+} (mEq/L)	120.52 ± 0.63	125.07 ±0.16*	126.35 ± 0.41*	128.48 ± 0.25*
Cl^{-} (mEq/L)	91.27 ± 0.35	93.28 ± 0.23	93.81 ± 0.96*	107.34 ± 1.30*
K^{+} (mEq/L)	4.00 ± 0.05	4.20 ± 0.02	4.25 ± 0.05	3.87 ± 0.31
HCO_3^{-} (mEq/L)	22.51 ± 0.47	23.60 ± 0.20*	24.22 ± 0.29*	25.21 ± 0.15*

Values are expressed as mean±SEM, n=10, Means marked * are significantly different from control at P<0.05.

3.9. Effect of COLE on liver histology in rats

Photomicrographs of the cross section of control liver showed well preserved liver architecture with evenly spaced portal triads around a central vein and no portal inflammation (Plates 1a&b). The group 2 liver when compared with control had mild portal inflammation, interface hepatitis, mild lobular hepatitis with focal confluent necrosis. No steatosis was observed (Plates 2a&b). For the group 3, there was severe portal inflammation, interface hepatitis, moderate lobular hepatitis with confluent necrosis without any steatosis (Plates 3a&b). Liver histology in group 4 was similar to that of group 3 showing severe portal inflammation, interface hepatitis, moderate lobular hepatitis with confluent necrosis and no steatosis (Plates 4a&b).

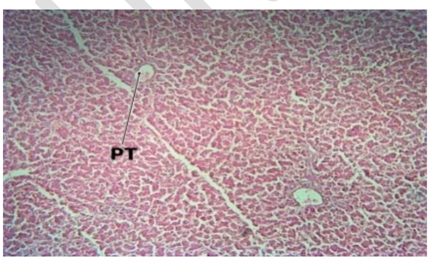

Plate 1a (control group)

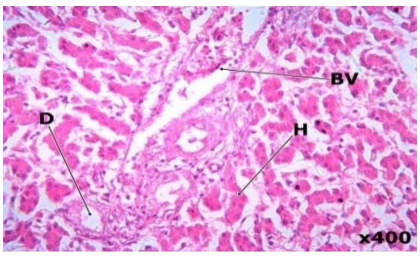

Plate 1b (control group)

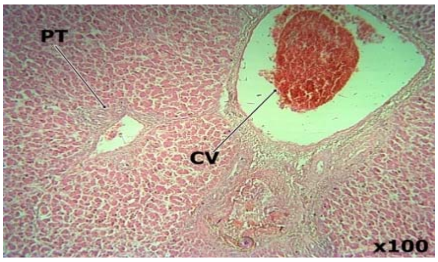

Plate 2a (250 mg/kg group)

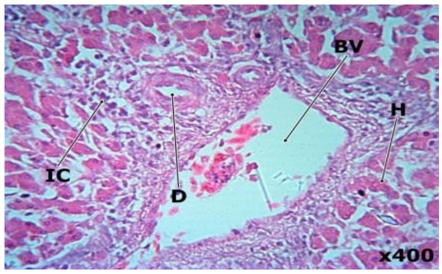

Plate 2b (250 mg/kg group)

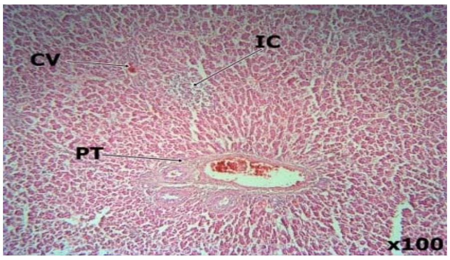

Plate 3a (500 mg/kg group)

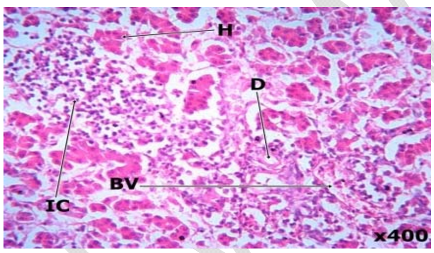

Plate 3b(500 mg/kg group)

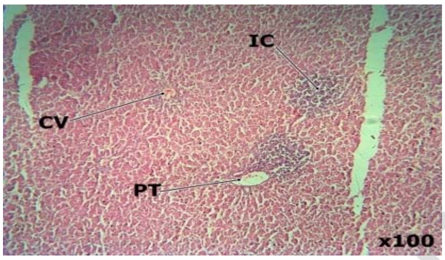

Plate 4a (1000 mg/kg group)

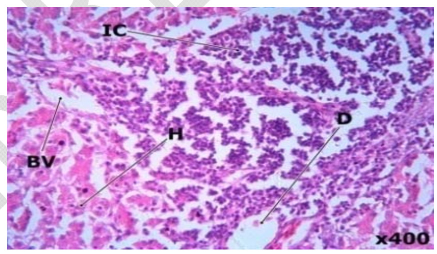

Plate 4b (1000 mg/kg group)

PT – Portal triad, CV- Central vein, BV-blood vessel, D-ductule, IC- Inflammatory cells, H-hepatocyte

3.10. Effect of oral administration of COLE on kidney histology in rats

Photomicrographs of kidney in all groups showed evenly distributed glomeruli of similar size, with increased mesangial cellularity. There were numerous open glomerular capillaries and normal endothelium. The tubules were of normal density and tubular epithelia were viable with mild haemorrhage into the interstitial (Plates 5-8).

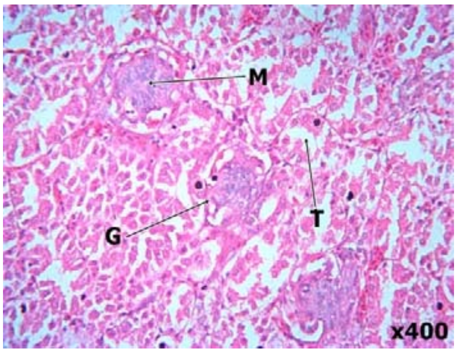

Plate 5 (control group)

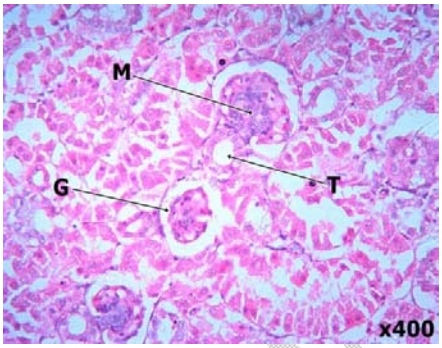

Plate 6 (250 mg/kg group)

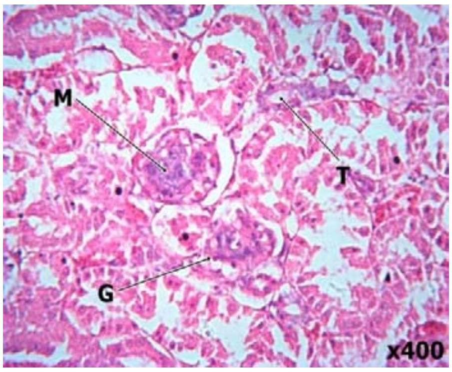

Plate 7 (500 mg/kg group)

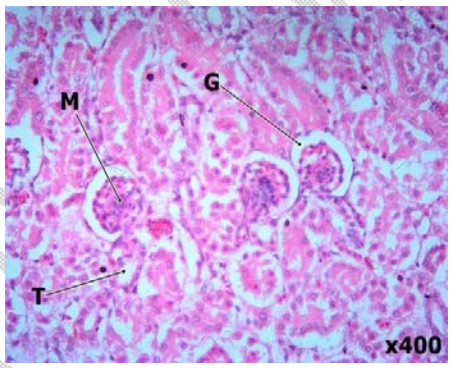

Plate 8 (1000 mg/kg group)

M= mesangium, G= glomerulus, T= tubule

4. Discussion

In this study, the acute and sub-acute toxicity effects of COLE were evaluated with results showing no toxicity at lower doses, but, with some degree of toxicity symptoms in the groups administered higher doses of the extract. The results obtained therefore suggest the COLE may be safe for consumption at low to moderate doses but toxic at higher doses. The fact that no mortality was observed following the administration of up to 6000 mg/kg body weight of the extract during the acute toxicity phase of the study suggest that the extract may be safe up to this dose limit for a single oral administration. The rats that died in groups administered 7000 and 8000 mg/kg body weight of the extract may have ingested intolerable amounts of plant phytochemicals, leading to the observed toxicity symptoms and death. Varying degrees of toxicity, including deaths due to consumption of plant materials have been reported [6,11]. OECD guidelines for acute toxicity evaluation stipulates that mortality is the most important marker in an acute toxicity study and that non observation of mortality in such study may be evidence of safety and non-existence of acute toxicity [12]. Similar conclusions were made in other acute toxicity studies involving the administration of graded doses of plant extracts given to rats [11].

Relative organ weights (ROW) when compared between test groups and control is conventionally used to evaluate the toxic effect of a test substance like plant extract and is an important marker in toxicity evaluation [13,14]. The fact that ROW was not so significantly changed in all test groups following COLE administration suggests that the extracts at low to moderate doses may be completely safe for use. The slight changes in ROW (heart and lung) observed in the 1000 mg/kg body weight treated group may be attributed to the cumulative effects of repeated administration of the extract at high dose over a 28-day period and have been associated with mild enzyme induction in these organs [14].

The fact that the blood transports materials to all parts of the body makes its evaluation a useful tool for assessing systemic toxicities arising from the consumption of medicinal plants and other substances with toxicity potential. In this study, treatment with the extract improved these RBC count, haemoglobin concentration and PCV values in the 250 and 500 mg/kg body weight COLE treated groups when compared with control, suggesting that the extract may contain substances with haematinic effects. Iron in most green plants is reportedly responsible for their usefulness as blood builders [15,16]. These plants when consumed increases the iron available for the production of more red blood cells and results in increased in RBCs count, PCV values and haemoglobin concentration following tests. The extract at these lower doses also may have improved bone marrow functions in the test rats and improvement of bone marrow function also improves directly improves erythropoietic process leading to more red cell formation. However, the fall in these parameters observed in the 1000 mg/kg body weight treated group suggests some degree of toxicity. Rat in this group may have been administered the extract beyond tolerable limits. The result of a similar study carried out on rats had shown that plant extracts at certain doses may become toxic to blood cells, which may manifest in the form of decline in the values of RBC parameters [2]. The elevations in white blood cells and lymphocytes counts observed following treatment with COLE at high doses may be due to the inflammations observed in the liver. Inflammation of body organs, especially liver is reportedly a major cause of elevated white blood cells (particularly lymphocytes) count [17]. The histological examination of the cross section of the liver tissues of rats treated with COLE had shown varying degrees of inflammations (Plates 2 to 4). Disturbances in the architecture of both the hepatocytes and portal triad with elevated enzyme levels are established indicators of liver toxicity [18]. Severe liver injuries, including acute and chronic abnormalities and even cirrhotic transformation and liver failure have indeed been reported after the ingestion of some herbal formulations [19]. The mild anti-platelet effects observed in the 250 and 500 mg/kg body weight treated groups due to decrease in platelet counts in these groups suggest that the extract may be of value in the prevention coronary thrombosis, a common cardiovascular problem amongst diabetics today. Diabetes mellitus is characterized by enhanced platelets activation and coagulation proteins with reduced fibrinolytic activity, which usually precede the development of cardiovascular complications, including thrombosis [20,21], hence the use of low doses of COLE may help prevent this disease due to its blood thinning effect. The highly significant fall in platelet count in the group administered 1000 mg/kg body weight of the extract may be considered deleterious since platelet value falls far below the normal values and may facilitate the development of bleeding problem. The serious fall in platelet count has been associated with high values of bleeding and clotting times with bleeding problems as consequences [11, 22].

Corchorus olitorius leaves may be rich in plant proteins which may the reason for the elevated total protein levels in all rats to which the extract was administered. A number of plants used both as food and medicines are known to be rich in protein and have been reported to increase serum protein concentrations when consumed [9,23]. The extract may also have increased the removal of water from the system via the kidney leading to dehydration and its accompanying rise in total protein values [24,25]. Although serum concentrations of ALT, AST and bilirubin in the rats administered COLE lie within a safe range, the significant elevations observed in the test rats treated with high dose of COLE when compared with the control, suggest that the liver cells may have been threatened probably due to the cumulative effect of daily administration of the extract. It is established that the threat to the liver, particularly such that destroys the hepatocytes usually results into elevated serum AST and ALT concentrations [26, 27]. Several green leafy plants have been reported to exhibit significant liver function modulatory effects. Examples of which include, *Telfairia occidentalis* [11], *Ocimum gratissimum* [10], *Venonia amygdalina* [29], *Pterocarpus soyanxii* and a host of others. Liver function modulatory effects of these plants may be linked to the antioxidant effects of flavonoids, tannins, and phenolic compounds found in the plants. While the reason for slight elevations in liver enzymes in this study is not known, there is the possibility that some alkaloids present in *Corchorus olitorius* as reported in our previous report [6] may be toxic. Alkaloids such as indole alkaloids, pyrrolizidine alkaloids, tropane alkaloids, opium alkaloids, vicine and covicine alkaloids have all been reported to show varying degrees of toxicity effects following ingestion [30]. Further work is however, required to adequately understand the nature and effects of alkaloids present in *Corchorus olitorius*.

The fall observed in serum cholesterol concentration in all rats treated with the extract suggests that COLE may have bioactive components with hypolipidaemic effects and as such may be of value in the management of hyperlipidaemia and its associated complications. Reduction in total cholesterol concentration with an increase in HDL concentration is reported to play active role in the prevention of cardiovascular diseases [10]. The hypolipidaemic effect of the extract also corroborates with the results on the effect of the extract on LDL concentration. LDL is also a bad cholesterol and mobilizes lipids to peripheral blood vessels thereby predisposing one to cardiovascular diseases [29]. The hypolipidaemic effect of the extract may be due to its saponin contents as reported in our previous work [6]. Saponin alleviate cardiac problems associated with hypertension due to its ability to bind to cholesterol in the body to inhibit the reabsorption of the later thereby facilitating its excretion from the body [6]. By increasing HDL-C concentration in the treated animals, the extract may have indirectly increased the demobilization of free fatty acids from the walls of blood vessels, transporting same back to the liver for excretion or re-utilization. This suggests that the extract may be of value in the prevention and management of cardiovascular diseases associated with decreased levels of high density lipoprotein cholesterol.

The diagnosis of renal failures is usually suspected when serum creatinine is greater than the upper limit of normal values as usually seen in muscular dystrophy and paralysis, anaemia, leukemia and hyperthyroidism while decreased values are seen in conditions such as glomerulo nephritis, congestive heart failure, acute tubular necrosis, shock, polycystic kidney disease and dehydration [31]. In this study, however, creatinine concentrations in all test groups did not fall outside the safety zone and suggest that COLE may not have induced any form of renal toxicity. The fact that serum urea concentrations in the test groups also did not fall outside the safe range further confirms the results for COLE effect on the kidneys. The increased urea level is often associated with kidney toxicity [26]. Results of histological examinations of kidney sections prepared for kidneys harvested from rats in all test groups further buttress our findings on the non-toxic effect of COLE on the kidneys.

Conclusion

If the results obtained in this study can be extrapolated to man, we then conclude that low to moderate consumption of *Corchorus olitorius* leaves extract may cause no toxic effects to either blood, liver or kidney. However, high dose administrations over a long period of time may be deleterious due to its possible toxicity effects on the blood and liver.

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