Nigerian Journal of Pharmaceutical Sciences
Vol. 23, No1, 2024, ISSN: 0189-823X

SUB-CHRONIC TOXICITY OF METHANOL LEAF EXTRACT OF *CRYPTOLEPIS OBLONGIFOLIA* (MEISN) SCHLTR ON BIOCHEMICAL PARAMETERS AND MAJOR ORGANS OF WISTAR RATS

***[1]Abdussalam, U. S., [2]Maje, I. M., [2]Anafi, S. B. and [3]Jatau, I. D**

[1]Department of Pharmacology and Therapeutics, Bayero University, Kano State, Nigeria.

[2]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

[3]Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

***Author for correspondence**: usabdussalam.pha@buk.edu.ng; +2348066930736

ABSTRACT

Medicinal plants are used for primary healthcare needs of many populations across the globe. Efficacy and safety of these plants must be established in order to maximize their therapeutic benefits and minimize harmful effects from their long-term intake. *Cryptolepis oblongifolia* has been used in traditional medicine of African countries including Nigeria for the treatment of fever, malaria, diarrhoea and stomachache. There is no adequate literature to suggest previous scientific work on subchronic toxicity profile of the plant on biochemical parameters and internal organs. This study aims to investigate the toxic effect of methanol leaf extract of *Cryptolepis oblongifolia* (MLECO) on liver and kidney function parameters, haematological indices and histology of major organs of Wistar rats after administration of the extract for 28 days. Fresh leaves of *Cryptolepis oblongifolia* were collected and extracted with 70% (v/v) methanol. Acute toxicity study and preliminary phytochemical screening were carried out. Twenty-four (24) Wistar rats of either sex were used for the study. The rats were grouped into four groups of 6 rats each. Rats in group I received 1 ml/kg of distilled water (Vehicle) and rats in groups II-IV received 375, 750 and 1500 mg/kg graded doses of the extract. The rats were monitored for signs of toxicity, weight changes, food and water consumption; and on 29th day the rats in the four groups were humanly euthanized and blood samples were collected for liver and kidney function tests, heamatological parameters determination and major organs: heart, brain, liver, kidney, lung and spleen were harvested for histopathological examination. The oral median lethal dose (LD_{50}) of the extract was > 5000 mg/kg; and preliminary phytochemical screening revealed the presence of alkaloid, phenols, terpenoids, steroids, flavonoids, saponins, tannins and cardiac glycosides. There was no mortality during the 28-day repeated oral toxicity study. The extract, at the doses of 375, 750 and 1500 mg/kg, showed no significant effect on the body weight of the treated rats. The extract showed significant increase in the serum total protein at 375 mg/kg with no significant effect on other liver function parameters. The extract also showed significant reduction ($p < 0.05$) in serum chloride, total white cell counts and bicarbonate at the doses of 375 and 750 mg/kg. The MLECO showed various degrees of histopathological changes in the heart, brain, kidney, liver, spleen and lungs after 28-day oral administration of the extract. The MLECO was acutely non-toxic to the rats but has lowered the serum chloride, bicarbonate and result in leucopenia after 28 days administration. The extract also induced various degrees of histopathological changes in major organs of the treated rats.

Keywords: *Cryptolepis oblongifolia*, Haematological indices, Hepato-renal function, Sub-Chronic, Toxicity

INTRODUCTION

Medicinal plants are used by many populations across the globe for the treatment and prevention of diseases. Many orthodox medicines in clinical practice today, such as aspirin (Mamadalieva and

Mamedov, 2020), metformin, digitalis (Bailey, 2017), paclitaxel (Mahdi, 2010), among others, were obtained from medicinal plants. The perception of the general public is that medicinal plants are safe and can be taken continuously owing to their natural source (Bemidinezhad *et al*., 2023). However, many toxicological studies have shown that some medicinal plants can be harmful to the body and some may cause death from their toxicities on organ-systems. (Basaran *et al.,* 2022). The kidney is an important organ for excretion of drug metabolites and one-fourth of all medications given to the patients in the hospital were reported to have potential nephrotoxicity (Kane-Gill *et al*., 2017). The liver is a major organ that handles metabolism of drugs and other xenobiotics and drug-induced hepatic damage was reported to cause oxidative stress, mitochondrial damage and hepatocellular dysfunction (Garcia-Cortes *et al.,* 2020).

The haematological system is prone to toxicity by drugs, chemicals; and it was reported that some medicinal plants caused anaemia through alteration of red blood cell production or by primary bone marrow toxicity (Putra and Rifa'i, 2019).

By their positions and roles, both kidney and liver are at risk of drug-induced injury following ingestion of toxic substance or drug for a long period of time. This calls for the need to scientifically investigate the potential toxicity of medicinal plants used in traditional medicine. The plant, *Cryptolepis oblongifolia*, has been used in traditional medicine for the treatment of cough, fever, malaria, diarrhoea and stomachache. To the best of our search of data bases, there was no previous scientific report on its safety data. This necessitates the need for this study in order to document the sub-chronic toxicity profile of the plant in Wistar rats.

MATERIALS AND METHODS

Plant Collection

Fresh leaves of *Cryptolepis oblongifolia* were collected from Karau-Karau Village, Giwa Local Government Area, Kaduna State, Nigeria. The plant was identified and authenticated by a Botanist in the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria. A voucher specimen number was collected (ABU03359).

Extract Preparation

The leaves of *Cryptolepis oblongifolia* were washed with tap water, sorted and then shade dried for two weeks until constant weight was obtained. The leaves were ground into fine powder using pestle and mortar. One kilogram of the powdered plant was macerated with 5 L of 70% methanol (v/v) for one week with regular shaking. The extract was filtered using Whitman's filter paper and then evaporated to dryness using water bath maintained at a temperature of 45˚C.

Experimental Animals

Twenty-four Wistar rats (115-160 g) of either sex (male to female ratio: 1:1, kept in separate cages) were used for the study. The female rats were nulliparous and non-pregnant. The rats were obtained from the animal house facility of Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were kept under standard condition, fed with regular laboratory diet made of chikun animal feeds (Vita plus, Jos Nigeria) and allowed access to water *ad libitum.* The study protocol was reviewed and approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with an ethics approval number: ABUCAUC/2022/010.

Acute Toxicity Study

The oral acute toxicity study was conducted on Wistar rats, it was done to determine the

median lethal dose (LD_{50}) of the extract. The OECD 425 guidelines (2008) was adopted. In this study, the limit test was done, where one female rat was administered with 5000 mg/kg of the extract and observed for 24 hours. On survival after 24 hours, two more rats were administered with the same dose of the extract and monitored for 24 hours. Both rats survived and the three rats were monitored for 14 days for physical signs of toxicity, such as changes in skin, eyes, mucous membranes, body secretions, abnormal movement/pattern of respiration, bizarre behaviour, and/or death.

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of MLECO was done using method of Trease and Evans (2009).

28-Day oral toxicity study

The 28-day oral toxicity study of MLECO was done using OECD guidelines 407 (2008).

Animal groupings and treatment

Twenty-four (24) Wistar rats of either sex were randomly grouped into four (4) groups of six (6) rats each (male to female ratio per group: 1:1, kept in separate cages). Rats in group I were treated with 1 ml/kg of distilled water (vehicle) and served as negative control while rats in groups II-IV were treated with 375, 750 and 1500 mg/kg of the extract. The treatment was done daily at the same time of the day via oral gavage.

Monitoring for physical signs of toxicity and mortality

The rats in the four groups were monitored for clinical signs of toxicity such as restlessness, skin changes, presence of secretions and excretions, lacrimation, changes in respiration, eye changes, abnormal gait/posture, convulsion, bizarre behaviour and or death.

Monitoring of body weight changes, food and water consumption

The body weight of the rats in the four groups was measured at the beginning of the study, once weekly and on the last day of the study. A calculated amount of feed (1000 mg) and water (1L) were administered each day and the quantity consumed were determined once in 24 hours by subtracting the leftover from the amount given the previous day.

Measurement of liver and kidney function parameters

On the last day of the study, 29th day, all the rats in the four groups were humanly euthanized under inhalational chloroform anesthesia. Blood samples were collected by cardiac puncture, 7 ml from each rat, 5 ml was put into a plain sample bottle and 2 ml into an EDTA bottle. The blood samples in the plain bottles were placed in a centrifugation machine and centrifuged at 1000 RPM for 10 minutes. After centrifugation, the serum was separated and used for analysis of liver (Alanine transaminase (ALT), Aspartate Transaminase (AST), alkaline phosphatase (ALP), direct and total bilirubin (DBL & TB) and albumin (Alb) and kidney (serum sodium, potassium, chloride, bicarbonate, urea and creatinine) function parameters. The analysis was done using spectrophotometer method.

Measurement of haematological parameters

The 2 ml blood sample collected into the EDTA bottles was used for determination of haematological indices using Bk 6100 Heamatology auto analyzer (Shehani *et al.* 2018). The haematological parameters measured include total white blood cells count, total red blood cells count, haemoglobin concentration, packed cell volume, lymphocyte count, granulocyte count, platelets count, mean corpuscular

volume and mean corpuscular haemoglobin concentration.

Determination of relative organ weights

The rats in the four groups were carefully dissected via a ventral incision using surgical bleed; and the heart, lungs, kidneys, liver, spleen and brain were identified, removed and their wet weight was recorded in grams. The relative organ weight of each rat was determined using the following formula: (OECD, 2008).

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of the rat (g)}} \times 100$$

Histopathological study of the major organs

The harvested organs which include heart, brain, lungs, kidney, liver and spleen were examined for any morphological changes such as bleeding, swelling or change in texture. They were placed in specimen bottles containing 10% buffered formalin. A tissue section was taken from each organ and placed separately in ascending grades of alcohol for dehydration. Each tissue was placed in three changes of 70%, 95% and 100% methanol for 2 hours each (total of 6 hours). The tissues were cleared using xylene in two changes of xylene for 1 hour and then infiltrated with 2 changes of molten paraffin wax for 2 hours each. The tissues were then embedded in molten paraffin wax using plastic embedding cassette and cut into 5 microns size using a microtome (Leica RM 2125 RTS, Germany). The sections were dried on a slide dryer and stained with Haematoxylin and Eosin stain (Atik, *et al*., 2019). After staining, the tissue sections were examined under microscope (Leitz Wetzler 962134, Germany).

DATA ANALYSIS

Data analysis was done using statistical package for the social sciences (SPSS) version 20.0. Results were presented as Mean ± Standard Error of Mean (Mean ± SEM) and analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's *post hoc* test. Statistical significance was set at $p \leq 0.05$.

RESULTS

Median Lethal (LD_{50}) Dose of MLECO in Rats

The oral median lethal dose (LD_{50}) in rats was found to be > 5000 mg/kg.

Preliminary Phytochemical Screening of MLECO

The MLECO was found to contained alkaloid, phenols, terpenoids, steroids, flavonoids, saponins, tannins and cardiac glycosides.

Monitoring for Physical Signs of Toxicity and Mortality in Rats Treated with MLECO for 28 Days

In the acute toxicity study, the rats were found to be restless with hyperventilation in the first four hours after extract administration. There were no obvious signs of toxicity and/or mortality during the 28-day repeated oral administration of the extract.

Monitoring of Body Weight Changes in Rats Treated with MLECO for 28 Days

The MLECO, at the doses of 375, 750 and 1500 mg/kg, showed no significant change on body weight of the treated rats after 28-day repeated oral administration compared to the negative (control) group (Figure 1).

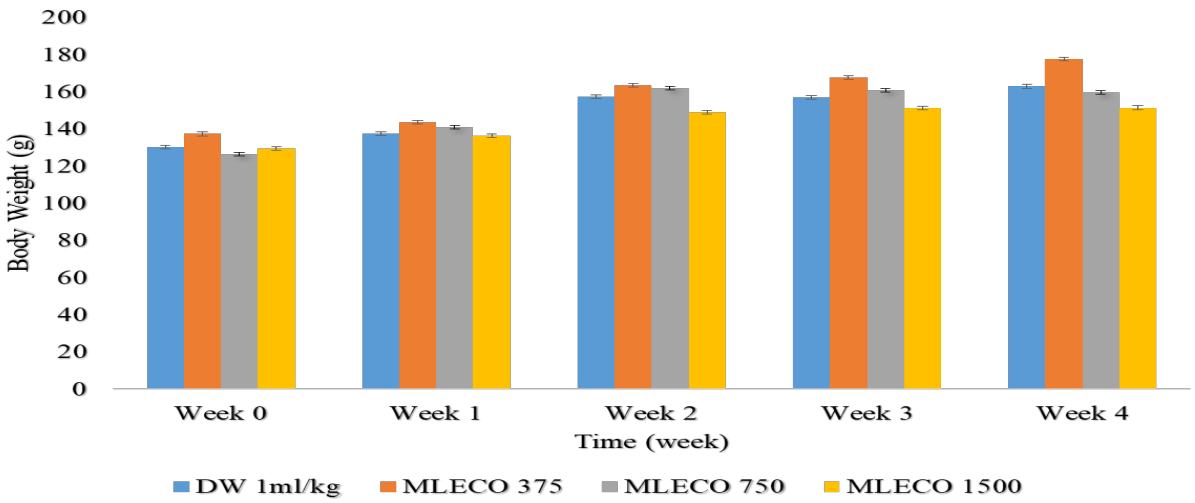

Figure 1: Effects of 28-Day Repeated Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Body Weight of Wistar Rats.
Values are presented as Mean ± SEM. n = 6, DW = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia*.

Determination of Relative Organ Weights of Rats Treated with MLECO for 28 Days

The (MLECO) showed significant ($p < 0.001$) reduction in the relative weight of the lungs and significant ($p < 0.001$) increase in the relative weight of the heart in the group that was treated with 1500 mg/kg of the extract compared to the distilled water treated group. The extract showed no significant change in the relative weight of the brain, liver, kidney and spleen (Table 1).

Daily Monitoring of Food Intake in Rats Treated with MLECO for 28 Days

It was observed that both male and female rats, treated with 375 mg of the extract, have significantly ($p < 0.01$ and 0.001) consumed more food compared to the distilled water treated group (Figure 2).

Table 1: Effect of 28-Day Repeated Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Relative Organ Weight of Wistar Rats

	Mean Relative organ weight (%)			
Organs	**D/W 1ml/kg**	**MLECO (375)**	**MLECO (750)**	**MLECO (1500)**
Brain	0.97 ± 0.07	0.97 ± 0.03	1.06 ± 0.06	1.01 ± 0.06
Liver	3.3 ± 0.29	3.2 ± 0.22	2.8 ± 0.11	2.9 ± 0.19
Kidney	0.64 ± 0.05	0.63 ± 0.02	0.63 ± 0.05	0.72 ± 0.03
Lung	0.82 ± 0.09	1.01 ± 0.07	0.93 ± 0.08	0.41 ± 0.09*
Heart	0.39 ± 0.03	0.36 ± 0.01	0.33 ± 0.02	0.68 ± 0.10*
Spleen	0.55 ± 0.06	0.48 ± 0.04	0.42 ± 0.01	0.45 ± 0.03

Values are presented as Mean ± SEM, n = 6, *significantly different from the distilled water group at $p < 0.001$, using one way ANOVA followed by Dunnett's *post hoc* test. D/W = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia.* % = Percentage

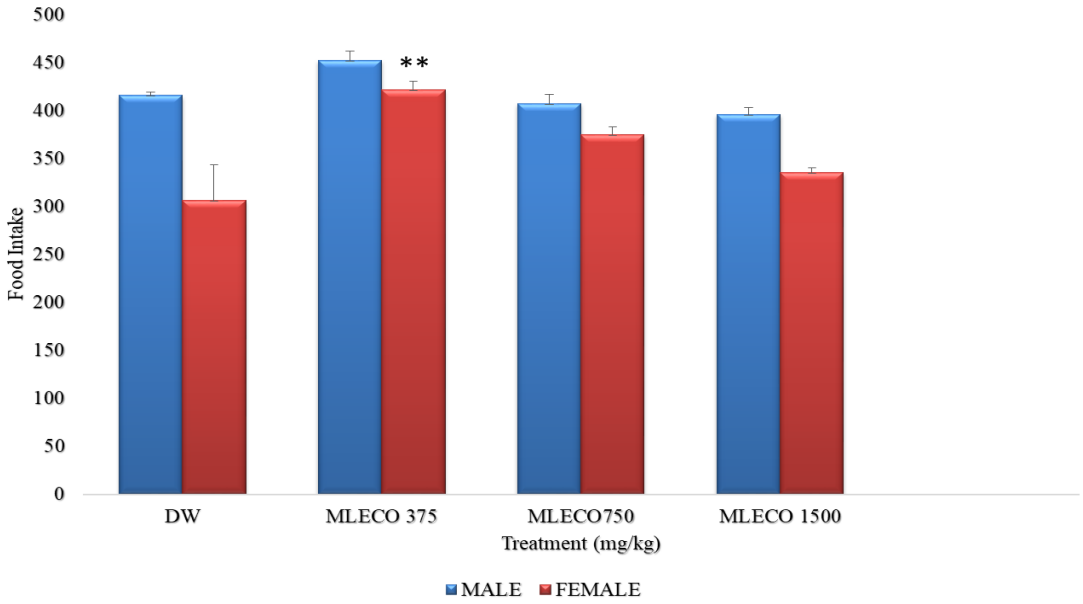

Figure 2: Effects of 28-Day Repeated Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Food Intake of Wistar Rats.
Values are presented as Mean ± SEM. n = 6, * and ** significantly different from the distilled water group at $p = < 0.01$ and 0.001 respectively, using one way analysis of variance followed by Dunnett's *post hoc* test. DW = Distilled water, MLECO = Methanol Leaf extract of *Cryptolepis oblongifolia*

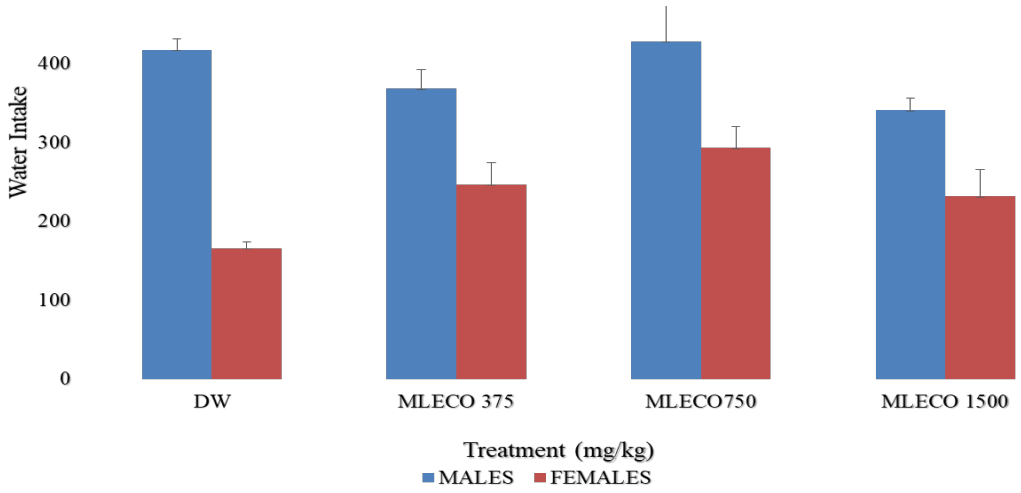

Figure 3: Effect of 2- Day Repeated Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Water Intake of Wistar Rats.
Values are presented as Mean ± SEM. n = 6, test. DW = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis*

Measurement of Liver Function Parameters in Rats Treated with MLECO for 28 Days

The extract showed significant ($p < 0.05$) increase in the serum total protein of the rats treated with 375 mg/kg of the extract compared to the distilled water treated group. The extract showed no significant effect on serum liver enzymes, albumin and bilirubin (Table 2).

Measurement of Kidney Function Parameters in Rats Treated with MLECO for 28 Days

The extract showed significant ($p < 0.05$) reduction of serum chloride in the group treated with 750 mg/kg of the extract. It was also observed that the extract significantly ($p < 0.05$) lowered the serum bicarbonate of the rats treated with 375 mg/kg. The extract showed no significant change in the serum urea, creatinine, sodium and potassium of the treated rats compared to the rats treated with distilled water (negative control) (Table 3).

Measurement of Haematological Parameters in Rats Treated with MLECO for 28 Days

The MLECO showed significant ($p < 0.001$) reduction in the total white blood cells count of the rats treated with 750 mg/kg compared to the distilled water treated group. The extract showed no significant effect on the other haematological parameters of the Wistar rats after 28- day repeated oral administration (Table 4).

Table 2: Effect of 28-Day Daily Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Liver Function Parameters of Wistar Rats

Parameters	Treatment (mg/kg)			
	DW	MLECO (375)	MLECO (750)	MLECO (1500)
ALT (IU/L)	12.50 ± 1.90	17.70 ± 2.12	15.83 ± 2.00	18.5 ± 2.60
AST (IU/L)	95.00 ± 10.87	114.20 ± 16.40	92.50 ± 16.8	96.00 ± 9.80
ALP (IU/L)	33.83 ± 1.91	36.23 ± 3.30	36.5 ± 3.30	28.00 ± 4.60
TB (mmol/L)	13.85 ± 1.05	13.93 ± 0.82	15.32 ± 0.94	15.52 ± 1.24
DB (mmol/L)	6.30 ± 0.44	7.53 ± 1.17	9.33 ± 1.41	8.73 ± 1.00
TP (g/dl)	3.77 ± 0.25	4.68 ± 0.17*	3.92 ± 0.36	3.98 ± 0.19
ALB (g/dl)	2.92 ± 0.12	3.23 ± 0.14	2.97 ± 0.16	3.00 ± 0.26

Values are presented as Mean ± SEM, n = 6, * significantly different from DW at $p < 0.05$ using one way ANOVA and Dunnett's *post hoc* test, DW = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia*, ALT = Alanine Transaminase, AST = Aspartate Transaminase, ALP = Alkaline Phosphatase, TB = Total Bilirubin, DB = Direct Bilirubin, TP = Total Protein, ALB = Albumin

Table 3: Effect of 28-Day Daily Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Renal Function Parameters of Wistar Rats

Parameters	Treatment (mg/kg)			
	DW	MLECO (375)	MLECO (750)	MLECO (1500)
Urea (mg/dl)	102.76 ± 19.1	101.91 ± 26.65	64.53 ± 8.87	96.13 ± 22.38
Cr (mEq/L)	0.96 ± 0.183	1.02 ± 0.13	0.93 ± 0.15	0.97 ± 0.14
Na+ (mmol/L)	167.80 ± 52.27	90.98 ± 2.94	92.30 ± 3.43	119.23 ± 31.21
K+(mmol/L)	20.70 ± 2.74	24.43 ± 3.96	23.10 ± 3.33	21.33 ± 1.73
Cl^- (mg/dl)	36.00 ± 3.36	32.83 ± 3.26	24.26 ± 1.22*	29.76 ± 3.22
$HC0_3$ (mg/dl)	98.26 ± 7.70	76.5 ± 4.20*	86.50 ± 5.80	89.30 ± 5.52

Values are presented as Mean ± SEM, n = 6, * significantly different from DW at $p < 0.05$ using one way ANOVA and Dunnett's *post hoc* test, DW = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis oblongifolia*, Cr = Creatinine, Na^+ = sodium, K^+ = Potassium, Cl^- = Chloride, HCO_3 = Bicarbonate

Table 4: Effect of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Haematological Parameters of Wistar Rats after 28-Day Oral Administration

Parameters	Treatment (mg/kg)			
	DW	MLECO (375)	MLECO (750)	MLECO (1500)
WBC (10^3/u L)	5.12 ± 0.19	4.60 ± 0.34	3.90 ± 0.47*	4.37 ± 0.28
LMY (10^3/u L)	6.33 ± 0.15	5.87 ± 0.14	5.70 ± 0.44	6.13 ± 0.39
GRA (10^3/u L)	2.70 ± 0.15	2.47 ± 0.32	2.75 ± 0.20	2.62 ± 0.20
LMY %	64.40 ± 1.83	57.96 ± 1.72	59.40 ± 2.03	62.02 ± 3.33
GRA %	30.73 ± 1.74	37.23 ± 1.31	35.43 ± 2.22	33.83 ± 3.15
RBC (10^6/u L)	5.72 ± 0.18	5.22 ± 0.51	5.05 ± 0.56	5.53 ± 0.48
Hb (g/dl)	13.83 ± 0.47	12.9 ± 1.07	12.3 ± 1.04	11.9 ± 0.17
PCV (%)	42.67 ± 1.65	40.00 ± 3.84	36.33 ± 2.40	34.00 ± 1.24
MCV (f L)	85.80 ± 1.54	88.8 ± 2.66	90.33 ± 2.99	92.80 ± 1.62
MCH (*p*g)	31.13 ± 1.54	30.50 ± 1.85	30.13 ± 0.33	30.20 ± 0.11
MCHC (g/dl)	33.50 ± 0.25	34.63 ± 2.22	35.23 ± 1.03	34.20 ± 0.43
PLT (10^3/u L)	190.33 ± 14.95	224.00 ± 21.52	206.26 ± 34.82	193.33 ± 20.46

Values are presented as Mean ± SEM, n = 6, *significantly different from DW group at p <0.001 using one Way ANOVA and Dunnett's post hoc test, DW = Distilled water, MLECO = Methanol Leaf Extract of *Cryptolepis Oblongifolia,* WBC = White blood cells, LMY = Lymphocytes, GRA = Granulocytes, LMY % = Lymphocytes percentage, GRA % = Granulocytes percentage, RBC = Red blood cells, Hb = Hemoglobin, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, PLT = Platelets

Histopathology of Major Organs of Rats Treated with MLECO for 28 Days

The MLECO showed various degree of histopathological changes in the heart, brain, kidney, liver, spleen and lungs of Wistar rats after 28-day repeated oral administration compared to the distilled water group. There was slight cellular congestion and

myocardial inflammation on the heart of rats treated with 375 and 1500 mg/kg, respectively (Plate I). There was slight neural inflammation on the brain of rats treated with 1500 mg/kg of the extract (Plate II). On kidney, the extract produced slight glomerular and tubular necrosis at tested doses of 375 and 750 mg/kg respectively; and lymphocytic hyperplasia at dose of 1500 mg/kg (Plate III). The liver showed lymphocytic hyperplasia and sinusoidal congestion in the group treated with 375 mg/kg and sinusoidal and vascular congestion in the group treated with 750 mg/kg of the extract. Kupper cells hyperplasia was observed on the liver of the rat treated with 1500 mg/kg (Plate IV). Mild and moderate lymphocytic hyperplasia was observed on the spleen of the rats treated with 750 and 1500 mg/kg respectively (Plate V). Moderate alveolar congestion and lymphocytic hyperplasia were seen on the lungs of the rats treated with 375 and 750 mg/kg of the extract respectively (Plate VI).

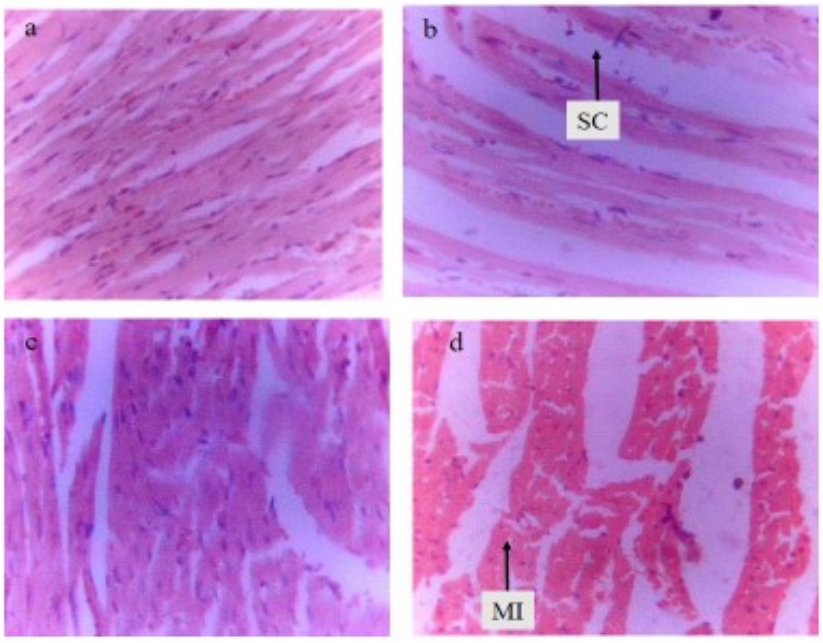

Plate I: **Photomicrograph Showing Histology of the Heart After 28-Day Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia***

a, b, c and d represent groups treated with Distilled water and plant extract at 375, 750 and 1500 mg/kg respectively. Heart histology of group b and d showed slight congestion and myocardial inflammation (figure b and d, arrow heads: SC & MI). H and E stain, Magnification: X 400

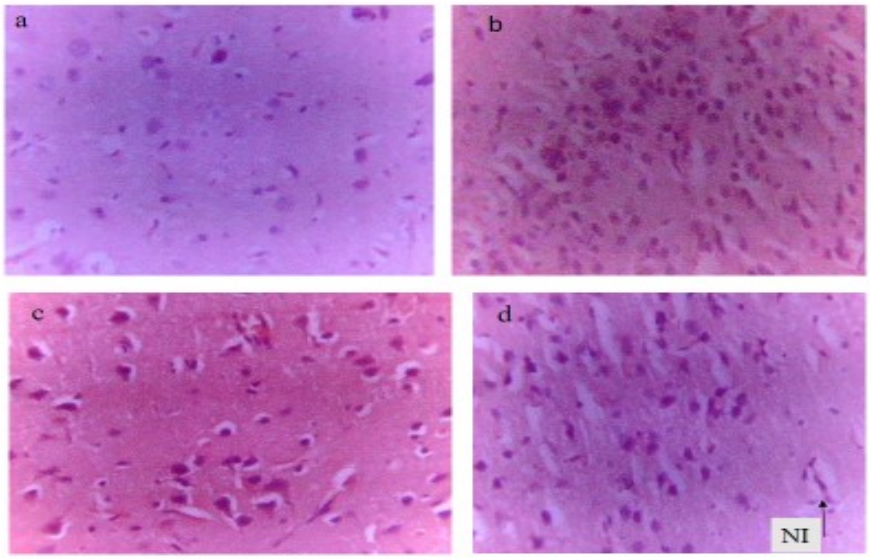

Plate II: **Photomicrograph Showing Histology of the Brain after 28-Day Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia***,

a, b, c and d represent groups treated with Distilled water and plant extract at 375, 750 and 1500 mg/kg respectively. Brain histology of group d showed slight neuronal inflammation (figure d, arrow head: NI). H and E stain, Magnification: X 400

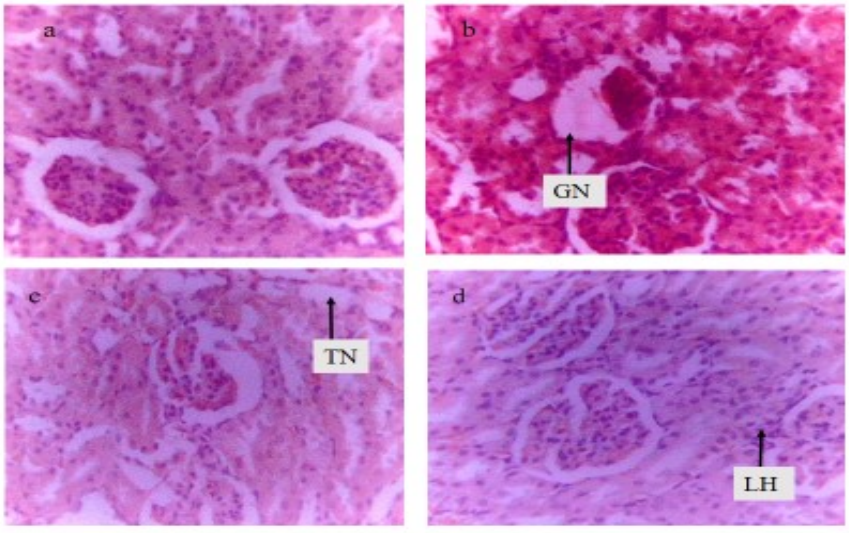

Plate III: **Photomicrograph Showing Histology of the Kidney after 28-day Oral administration of Methanol Leaf Extract of *Cryptolepis oblongifolia***,

a, b, c and d represent groups treated with Distilled water and plant extract at 375, 750 and 1500 mg/kg respectively. Kidney histology of group b showed slight glomerular necrosis (figure b, arrow head: GN), group c showed slight tubular necrosis (figure c, arrow head: TN), and group d shows lymphocytic hyperplasia (figure d, arrow head: LH). H and E stain. Magnification: X 400.

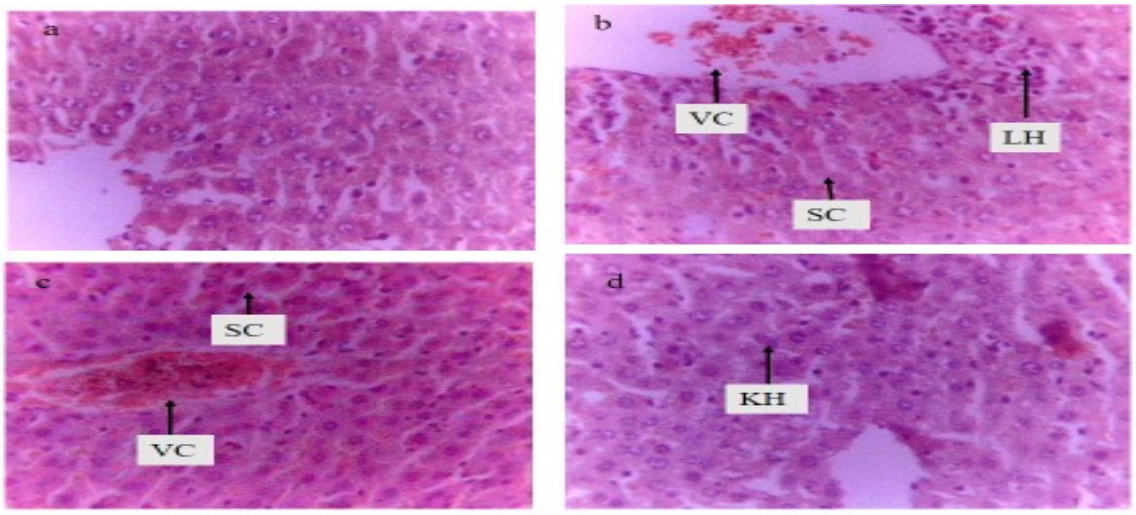

Plate IV: **Photomicrograph Showing Histology of the Liver after 28-Day Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia***

a, b, c and d represent groups treated with Distilled water and plant extract at 375, 750 and 1500 mg/kg respectively. Liver histology of group b shows lymphocytic hyperplasia and sinusoidal congestion (figure b: LH and SC, arrow heads), that of group c showed sinusoidal and vascular congestions (figure c: arrow heads: SC & VC,), that of group d showed Kupper cells hyperplasia (figure d: arrow head: KH). H and E stain. Magnification: X 400

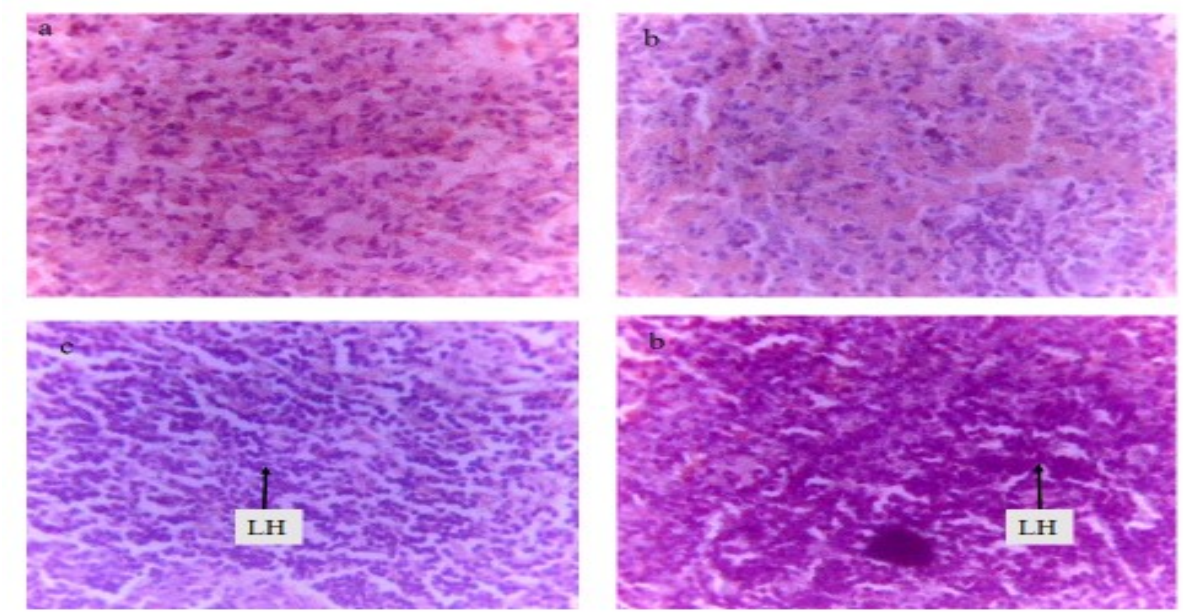

Plate V: **Photomicrograph Showing Histology of the Spleen after 28-Day Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia***,

a, b, c and d represent groups treated with Distilled water and plant extract at 375, 750 and 1500 mg/kg respectively. Spleen histology of groups c and d showed mild and moderate lymphocytic hyperplasia, (figure c and d, arrow heads: LH), respectively. H and E stain. Magnification: X 400

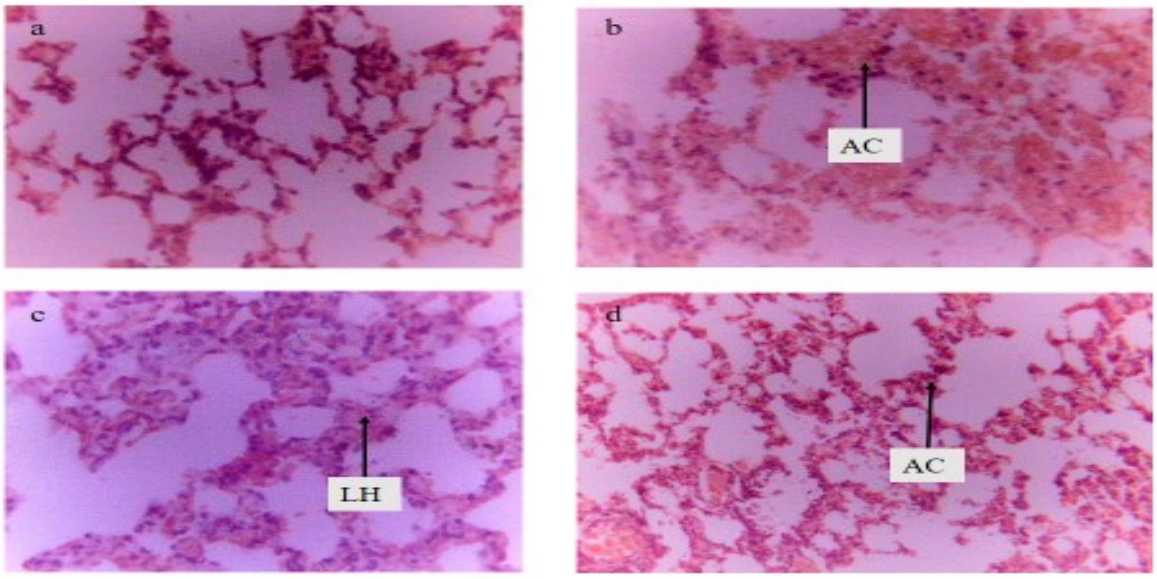

Plate VI: **Photomicrograph Showing Histology of the Lungs after 28-Day Oral Administration of Methanol Leaf Extract of *Cryptolepis oblongifolia***,

a, b, c and d represent groups treated with Distilled water and plant extract at 375, 750 and 1500 mg/kg respectively. Lung histology of group b showed moderate alveolar congestion (figure b: arrow head: AC), that of group c showed lymphocytic hyperplasia and group d showed slight alveolar congestion (figure d: arrow head: AC). H and E stain. Magnification: X 100

DISCUSSION

Detail evaluation of toxicity profile of plants used in traditional medicine is an essential requirement in drug development. This will establish the possible range of toxicities of the plants on organ-systems of the body. Toxicity data are used to either recommend or withdraw a particular medicinal plant for potential usage in humans.

The preliminary phytochemical screening of MLECO revealed the presence of alkaloid, phenols, terpenoids, steroids, flavonoids, saponins, tannins and cardiac glycosides. These are secondary metabolites with numerous pharmacological activities as reported in our previous work (Abdussalam *et al.*, 2022).

Acute toxicity study is used to evaluate the adverse effects of a test compound/extract after a single administration within short period of time. The median lethal dose (LD_{50}) estimation provides toxicological information on the toxicity profile of an extract/compound prior to its administration to a larger group of animals or humans (Rispin, *et al.*, 2002). The result of LD_{50} serves as the basis for determining the dosage levels for the 28-day repeated oral toxicity study of the extract.

In acute toxicity evaluation of MLECO, the treated rats showed restlessness and hyperventilation in the first four hours after extract administration. This could be due to acute toxicity of the plant on the nervous and respiratory systems. The treated rats showed no signs of toxicity and/or death throughout the observation period. This showed that the initial signs of toxicity observed were transient and completely reversible. The LD_{50} value (> 5000 mg/kg) of MLECO indicates that the extract is non-

toxic to the rats after single exposure (OECD, 2008).

Repeated daily treatment of rats with MLECO for 28 days did not result in noticeable signs of clinical toxicity such as skin changes, watery secretions and excretions, lacrimation, abnormal respiration, eye changes, abnormal gait or posture, convulsion or bizarre behaviour. There was no mortality during the 28-day repeated oral administration of the extract. These indicate that repeated administration of the extract was not associated with change in the physical wellbeing of the treated rats.

Body weight development, appetite and food intake are regulated in a very complex manner. Body weight of experimental animal is a simple parameter which can provide information for severity assessment and decision about toxicity of a new drug or chemical (Heisler and Lam, 2017). Changes in body weight of experimental animals, after repeated administration of drug, is the first sign of drug-related toxicity (Jatsa, *et al*., 2018). Loss of body weight can be a symptom of different diseases associated with reduced appetite, metabolic alterations, increased energy expenditure or malabsorption (Andermann and Lowell, 2017). The MLECO at the tested doses did not result in weight loss during the 28-day study period. The rats treated with the extract were found to have normal body weight gain compared to those in the negative control group. This indicates that the extract has no suppressive effect on appetite, food absorption and energy metabolism of the treated rats.

Sub-chronic administration of the extract result in significant reduction in relative weight of the lungs of the rats treated with the highest dose of the extract. However, the histology of the lungs showed some alveolar congestion with no evidence of necrosis or fibrosis. This reduction in the weight of the lungs was not associated with adverse respiratory event, as there was no mortality recorded in this group. There was also significant increase in the relative weight of the heart in the same group. This was also evident by the myocardial inflammation on the histology. This may indicate that the extract, which contains cardiac glycosides, might have accumulated in the heart and induced some inflammation and cardiomegaly. This effect of the extract on the lungs and heart was not dose-dependent as there was no significant difference in the relative weight of the heart and lungs in the groups treated with the lowest and middle doses compared to the negative control. The MLECO produced non-dose dependent increase in food consumption in both males and females rats treated with the middle dose of the extract compared to the negative group. This indicates that the extract has no negative effect on food intake, digestion and absorption in the treated rats compared to the negative group. The extract showed no effect on water consumption of the treated rats compared to the negative control.

At the end of the 28-day sub-chronic toxicity study, the effect of repeated administration of MLECO on liver function parameters was evaluated and it was found that the serum levels of ALT, AST, ALP bilirubin and globulin were not significantly different from those of the negative control group. Significant elevation of liver transaminases (ALT and AST) indicates liver parenchymal injury while that of ALP and conjugated bilirubin indicates cholestatic injury to the liver (Ozer *et al.,* 2008).

However, there was significant increase in the total serum albumin of the rats treated with the lowest dose of the extract. This is the same group that consumed more food

compared to the other two treated groups and the negative control group. The total protein, albumin and globulin concentration may decrease due to synthetic liver disease, malabsorption or malnutrition (Ekam, *et al.*, 2012). This effect of the extract on the serum albumin, without affecting other parameters of liver function test, may be due to increased food intake by the rats in that group. The extract showed no effect on the liver function parameters of the rats treated with the middle and highest doses.

Kidneys are essential for survival of an animal and play a central role in the excretion of drugs and other xenobiotics and it is a target of toxicity by many metabolites including repeated administration of plant extracts (Olorunnisola *et al.*, 2012). The renal function parameters which include sodium, potassium, chloride, bicarbonate, urea and creatinine of the treated rats were measured and compared with those of the negative control group. Drugs and chemicals may induce renal injury through glomerular and tubular dysfunctions and result in elevated serum levels of urea and creatinine as well as other markers of acute kidney injury (Al-Naimi *et al*., 2019).

After 28-day administration, the extract showed reduction in the serum level of chloride (Cl^-) in the group that was treated with the middle dose of the extract. The extract might have an inhibitory effect on the renal tubular reabsorption of chloride and other ions in the rat kidneys and result in increased urinary excretion of Cl- ions and corresponding low level of the ions in blood (Raghavan and Weisz, 2021). The extract also lowered serum bicarbonate of the rats treated with middle dose of the extract without effecting other parameters of renal function test. This non-dose dependent effect of MLECO on serum chloride and bicarbonate may be due to functional alteration of tubular reabsorption process in the kidney. Haematological systems are commonly affected by drugs and chemicals and alteration in the haematological indices such as packed cell volume, haemoglobin, red blood cell, total white blood cell, neutrophil, lymphocyte and platelet count may give a clue on the health status of an individual (Jorum *et al.*, 2016). The MLECO lowered the total white blood cells count, without affecting other haematological parameters of the rats treated with the middle dose of the extract. This may be due to depression of white cell production by the extract or changes in the immunological status of the treated rats (Choudhury and Sinha, 2015). The extract has not produced any change on the haematological parameters of the rats treated with the lowest and highest doses. MLECO showed no effect on red blood cell count or packed cell volume of the treated rats. Some medicinal plants were reported to have depressive effect on packed cell volume and result in anemia in the treated rats (Choudhury and Sinha, 2015; Putra and Rifa'i, 2019).

After the 28-day of MLECO administration, the heart, brain, lungs, kidney, liver and spleen were subjected to histopathological examination for the detection of any toxicological changes at tissue level. The histological examination revealed non-dose dependent myocardial congestion and inflammation in the groups treated with lowest and highest doses of the extract. The extract also produced slight inflammation of the brain neurons at the highest dose with no histological changes in the brains of rats treated with the lowest and middle doses. The kidney histology showed mild glomerular necrosis at lowest dose, mild tubular necrosis at the middle dose and lymphocytic hyperplasia at the highest dose. The extract also produced various histological changes in the liver: at lowest dose, it caused sinusoidal congestion and

lymphocytic hyperplasia, at the middle dose, the extract produced vascular and sinusoidal congestion, while the liver of the group treated with the highest dose showed Kupper cell hyperplasia. There was also mild and moderate lymphocytic hyperplasia in the spleen histology of the rats treated with middle and highest doses of the extract. The lung histology of the groups treated with the lowest and highest doses of the extract showed moderate and mild alveolar congestion, respectively. Lymphocytic hyperplasia was noted on the lung histology of the group treated with the middle dose of the extract. These findings were similar to those of a number of medicinal plants such as *Achyranthes aspera* L. (Diallo *et al.,* 2010), *Anagallis arvensis* L. (Henrich, 2009), *Calotropis procera aiton* (Henrich, 2009) and *Euphorbia helioscopa* L. (Welch *et al.*, 2020) which were reported to caused various pathological changes in the internal organs of Wistar rats. These changes include myocardial inflammation, alveolar congestion, liver necrosis and fibrosis, cellular congestions, lymphocytic infiltrations in the liver, spleen, lungs, intestine and kidneys as well as renal tubular necrosis.

These findings suggest that intake of MLECO for four weeks may result in various degrees of histopathological changes in the major organs of rats. The plant contained alkaloid, saponins, cardiac glycosides and steroids which were reported to caused various tissue injuries in the heart, brain, lungs, liver, kidney and spleen (Hueza *et al.,* 2003; Welch *et al.*, 2020).

Therefore, long term consumption of the plant extract for the treatment of fever, malaria, diarrhoea and stomachache should be done with caution.

CONCLUSION

The MLECO is relatively safe on the Hepato-renal and hematopoietic systems of Wistar rats. The extract showed various histopathological changes on the major organs of Wistar rats without causing mortality.

CONFLICT OF INTEREST

The authors have declared no conflict of interest

ACKNWOLEDGMENT

The corresponding author is grateful to Tertiary Education Trust Fund (TETFund) for the local Ph.D. scholarship grant. The authors are grateful to Mal. Abubakar Balarabe of Pharmacology laboratory and Mr. Bamidele Adetiba of Human Anatomy Department, Ahmadu Bello University, Zaria, for their kind support during the course of the work.

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