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Short term Safety Profile of Methanol Stem bark Extract of *Ficus vallis-choudae* (Moraceae) in Mice and Rats

[1]Abdullahi Ibrahim Doma *, [1]Adamu Yusuf Maitama, [1]Abdullahi Rahana Amira, [1]Abdullahi Rabiu Abubakar, [1]Abdullahi Hamza Yaro

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

Email: ibdomaa@yahoo.com

Abstract

The study evaluated the toxicological profile of methanol stem bark extract of Ficus vallis-choudae in animal models. The oral acute and sub-chronic toxicity of the 70 % methanolic extract was investigated through the OECD guidelines by examination of mortality rate, body and organ weight changes, and biomarkers of hepatic and renal functions. The LD_{50} was higher than 5000 mg/kg (p.o) and the result of haematological parameters determination after 28days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FvMSE did not show significant difference from that of the distilled water group. No significant effect ($p > 0.05$) on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities. There was no significant effect ($p > 0.05$) on serum total protein (TP), blood urea nitrogen (BUN), albumin (ALB) and creatinine (CREA) levels for all treatment groups. There was a statistically significant ($p<0.05$) difference in respect of sodium electrolytes at 500 and 1000 mg/kg doses for FvMSE administered groups compared to the distilled water group. The result revealed that FvMSE doses did not produced significant change in relative organs weight compared to distilled water treated groups. Histopathological examinations revealed slight hepatic necrosis, slight myocardial necrosis and slight hyperplasia of inflammatory cells in the kidney of the FvMLE treated rats after 28 days of daily oral administration. The slight changes observed in the organs though not accompanied by any significant change in the biochemical markers of measured liver and kidney injury may still constitute a source of concern as to the safety profile of the extracts especially in long term use.

Keywords: *Ficus vallis-choudae*, toxicity, safety, sub chronic, methanol

INTRODUCTION

Medicinal plants possess bioactive compounds which are responsible for their pharmacologic impacts that is usually known to enhance health exclusively, additively or synergistically (Azaizeh, 2005). The identification and isolation of bioactive compounds from plants as a bedrock of important drugs in contemporary medicine instigated the enthusiasm of researchers in the assessment of these compounds for chemotherapy (Wintola *et al*. 2010). The World Health Organization (WHO) in recognition of the enormous importance of herbal medicine to primary health care delivery has recommended for actual identification, empirical utilization and development of medicinal herbs for their safety use and efficacy in medical care (Newman and Cragg, 2007). Several incidences of organs toxicity resulting from prolong ingestion of herbal medicine have already been reported (Ahmad, 2006, Bandaranayake,

*Author for Correspondence

2006). However, the demand and use of herbal medicine is rising possibly owing to its availability, affordability and the acclaimed safety (Hosseinzadeh *et al*. 2015; Mahomoodally, 2013). The rising patronage is not without its attendant consequences as some people tend to abuse it (Olayode *et al* 2020). Evidences of toxicity resulting from ingestion of drugs of plant origin have been documented (Elufioye and Agbedahunsi, 2004; Salawu *et al*. 2008; Salawu *et al*. 2009).This therefore justify the need to establish and document the safety profiles. Moreover, the continuous call for translating herbal medicine into conventional medicinal (Agostinho, 2018, Krah, 2018) make it necessary to determine and document the safety profiles of every plant used for medication.

Ficus vallis-choudae is a deciduous plant found mainly in the savanna region of West Africa. It belongs to the family Moraceae, locally called Dulu/Gimi in Hausa (Lawan *et al*. 2008), Oguro in Yoruba (Gbile, 1984). It ripen fruits are called "figs". The figs are edible and are really appreciated by children (Vivien and Faure, 1996). The stem bark is chewed with kola nut either to relieve thirst or as remedy for sore throat (Dalziel, 1995). The various parts of the plant have been reported to be used in the treatment of stomach pain, paralysis, convulsion, epilepsy, jaundice, nausea, bronchial, gastro intestinal troubles and malaria (Burkill, 1985; Adekunle *et al*. 2005; Olowokudejo *et al*. 2008; Bello *et al*. 2017). Previous studies reported that the stem bark extract possess antifungal and anticonvulsant activities (Adekunle *et al*. 2005; Malami *et al*. 2010) as well as anti-inflammatory and anti-nociceptive effects (Lawan *et al*. 2008). It is also reported that the ripe figs of the plant possess *in vitro* antiplasmodial activity (Chouna *et al*. 2022). It is generally known that every drug is bound to be toxic at certain dose. Therefore, knowing the toxic status of any therapeutic agent is as important as its pharmacological effect. For the plant use in the local treatment of various ailments, it was essential to examine, confirm and make available its toxicity profiles.

MATERIALS AND METHODS

Experimental Animals

Swiss albino mice (16-24 g) and Wistar strain rats (170-200 g) of either sex were obtained from the animal house facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. They were housed in standard propylene cages and kept under natural day and light cycle at the Department of Pharmacology and Therapeutics, Bayero University, Kano. They were fed on standard mouse feed (Vital Feeds, Jos, Nigeria) and were allowed access to water *ad libitum*. The animals were allowed to acclimatize to the laboratory conditions for at least 3 days before being subjected to the experiments.

Drugs and Chemicals

The drugs and chemicals used for the studies include: Ethyl acetate (MERCK Eurolab); N-Butanol (KESHI, USA); Chloroform (Sigma Aldrich, St. Louis Mo, USA); Hydrochloric acid, Sulphuric acid (May and Baker, UK), Ferric chloride anhydrous (Avishkar, India), ammonia (Loba chemie, India). Agappe diagnostic kit (Switzerland), Randox diagnostic kit (UK), Distilled Water, Giemsa solution, Immersion Oil, Methanol (JHD Sci-Tech. Co. Ltd, China).etc.

Equipment

Thermostat Oven (DHG-9101, USA), Water Bath (HH-4 ENGLAND Lab science), Electric Weighing Balance (FA2104A, Gulfed Medical and Scientific England), Digital (DB-1A, PEC MEDICAL USA), Animals weighing balance (SF-400), Animal cages, Pestle and mortar, Syringes (1 ml, 2 ml, 5 ml and 10 ml), What man's Filter Paper No. 1, Crucibles, Separating funnel, Conical flask, Beakers and Retort stand. Electronic balance, Microscope, Microscope slides, syringes, Mortar and Pestle, Animal cages, Spatula, EDTA bottles, Whatman No. 1 filter

paper (1mm mesh size), Vacutainer syringe, Heparanised capillary tubes, Plasticine, , Eppendorf micro pipettes, Desiccator, Centrifuge (England), Thermostat oven (DHG-9101-ISA), Micro-hematocrit reader (Hawksley-15006, England), Biobase auto hematological analyser (BK 6300). etc.

Collection and verification of plant

The fresh stem barks of *Ficus vallis-choudae* were collected from Toro district, Toro Local Government Area of Bauchi State, Nigeria. The plants were identified and authenticated by Baha'uddeen Said Adam of the herbarium unit of the department of Biological Sciences, Bayero University, Kano, Nigeria. A voucher specimen number BUKHAN 0447 was collected.

Preparation of plant extract

Fresh stem bark of *Ficus vallis – choudae* were collected from the plant, rinsed with clean water and shade-dried. The plant materials were then pulverized into fine powder using porcelain mortar and pestle and sieved. Powdered plant material weighing 2kg was macerated with 7L of 70%v/v methanol at room temperature for 7 days with occasional agitation of the mixture. At the end of the extraction, the crude methanol extract was filtered using Whatman's filter paper (1mm mesh size) and then concentrated on water bath maintained at 45^{o}C until brownish residues were obtained and stored in a desiccator.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the crude methanol stem bark extract of *Ficus vallis-choudae* (FvMSE) as described by Trease and Evans (2009).They were screened for the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins, anthraquinones, steroids and triterpenes.

Quantitative Analysis

Total Alkaloids

The sample (5g) of *Ficus vallis-choudae* crude extract was taken into a 250ml beaker. Then 200 ml of 10% acetic acid in ethanol was transferred, covered and allowed to stand for 4 hrs. The mixture was filtered and then concentrated over water bath to ¼ of the initial volume. Later, few drops of concentrated HN_4OH were added to the extract until complete precipitate was formed. The whole mixture was allowed to settle and the precipitate was collected and washed with dilute HN4OH and then filtered. The residue produced was dried and weighed as alkaloid (Harbone, 1973).

Total Flavonoids

In this test, powdered sample (2.5g) of *Ficus vallis-choudae* crude extract was mixed with 50ml of 80% aqueous methanol in 250ml beaker, and allowed to stand for 24 hours at room temperature. The supernatant layer was discarded, and the residue was re-extracted three times with 50ml of ethanol. The solution produced were filtered using Whatman filter paper number 42 (125mm). The filtrates were later evaporated to dryness over a water bath. The content was cooled in a desiccator and weighed until constant weight was obtained (Boham and Koupai-Abyazan, 1974). The percentage yield for flavonoid was calculated as follows: % Yield = Weight of flavonoid / Weight of sample ×100.

Total Saponins

Ficus vallis-choudae crude extract, 20 g was placed in conical flask and 100ml of 20% aqueous ethanol was added. The mixture was heated on water bath at 55o C for 4 hours with continuous stirring. It was filtered and the residue re extracted with another 200 ml of 20%

ethanol. The combined extract was reduced to 40 ml over water bath at about 90o C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether added and vigorously shaken. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was purified and 60 ml of n-butanol was added. The n-butanol extract washed twice with 10 ml of 5 % aqueous NaCl. The remaining solution was evaporated and dried in the oven to a constant weight (Obadoni and Ochuko, 2002). The saponin content was calculated using the formula below: % Yield = Weight of saponin / Weight of sample ×100.

Total Cardiac Glycosides

In this test, 10% of *Ficus vallis-choudae* extract was mixed with 10 mL freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). The mixture was allowed to stand for 1 hour. This is followed by dilution with 20 mL distilled water and the absorbance was measured at 495 nm using UV spectrophotometer (Solich *et al*., 1992). The percentage yield of cardiac glycosides was calculated as follows: Weight of sample (g): Absorbance of sample/Absorbance of standard x Concentration of standard. % Yield = Weight of saponin / Weight of sample ×100.

Acute toxicity study in mice (LD_{50})

Fixed dose method of acute toxicity study in rats and mice

LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD, 2001) guidelines in rats and mice. In this method, two groups each of three animals were fasted prior to dosing (food but not water was withheld overnight for the rats and for 3 hours for mice). The fasted body weight was determined for each animal and the dose was then calculated according to the body weight. Food was then further withheld for 3-4 hours in rats and 1-2 hours in mice after the extract had been administered. The test substance was administered in a single oral dose using an oral cannula. A start dose of 2000 mg/kg was used for each animal in the first phase. Animals dosed in the first phase were observed for 48 hours after which there was no death and the test proceeded to the second phase. The same procedure was used but at a dose of 5000 mg/kg. Animals were observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours with careful observation during first 4hours and then daily for 14 days. Observations included changes in skin and fur, eyes and mucous membranes, somatic activity and behaviour pattern, autonomic and central nervous systems etc. Animals were also observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted.

Individual weights of animals were determined at least weekly. At the end of the test, surviving animals were weighed and then sacrificed using chloroform. Organs of the animals such as liver, kidney, lungs, heart and gastrointestinal system were freshly harvested and fixed in 10% v/v formalin for Histological studies.

Sub chronic (28 days daily administration)

This test was conducted according to the method described by OECD, guidelines 407 (OECD, 2008) with slight modification. Twenty Wistar rats (10 males and 10 females) weighing (170-200g) were selected and divided into four groups of 5 rats each. The doses of FvMSE were selected based on the result of acute toxicity testing. The highest dose (1000 mg/kg) which was 20% of LD_{50} was administered to group I, then 500 mg/kg to group II and 250 mg/kg to group III while the last group received distilled water 1 ml/kg. The administration was done every 24 h for 28 days (OECD, 2008). The rats were observed for sign of toxicity and behavioural changes on daily basis. At the 29th day, the rats were anaesthetized and sacrificed.

Blood samples were collected for haematological analysis using EDTA bottles (1 mg/ml of blood). The biochemical analysis was done after the blood sample was centrifuged at 1500 rpm for 15 minutes. The analysis was carried out using auto analyser (Biobase auto hematological analyser (BK 6300). Subsequently, the rats were dissected and internal organs such as liver, heart and kidney were removed, weighed and kept for histological studies (OECD, 2008).

Biochemical studies

After the animals were anaesthetized in a chloroform chamber, they were opened up surgically, blood samples were collected by cardiac puncturing into plain samples bottles. The blood was then allowed to clot and centrifuged at 3500 rpm for 10 minutes. The serum was separated and stored at -4°C until used. The serum was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), serum bilirubin, serum urea, creatinine, chloride, sodium, potassium, bicarbonate using standard kits (Agappe Diagnostic kit, Switzerland and Randox Diagnostic kit, UK).

Hematological analysis

The blood sample was collected in EDTA bottles to prevent clotting. The parameters were white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT). The hematological analysis was performed using Biobase automatic hematological analyzer (BK6300).

Histopathological studies

Tissues slice of 3 cm thickness was cut and fixed in 10% buffered formalin saline and put in automatic tissue processor and then fixed in 10% buffered formalin-saline solution for 6 hours. They were then dehydrated for 2 hours in each of the ascending grades of alcohol- 70%, 90% and 100% v/v. The dehydrated tissues were cleaned with toluene for 2 hours after which the tissues were embedded in paraffin wax and left to cool. The blocks were trimmed and sectioned on the microtone at 5 microns. The ribbons of sections were floated in a warm water bath. Suitable sections were selected, attached to slide and dried on a hot plate and stained with Haematoxylin and Eosin stain. Sections were dewaxed in xylene, rehydrated in descending grades of alcohol 100%, 90% and 70% v/v, then stained in haematoxylin for 5 minutes, differentiated in 1% acid alcohol, glued in Scott's tap water and stained with eosin for 3 minutes. Sections were rinsed and dehydrated in ascending grades of alcohol 70%, 90% and 50 100%, then finally dewaxed in xylene and mounted in a box. The slides were then examined microscopically for pathological lesions (Arthur and John, 1978).

Statistical Analysis

Results were expressed as mean ± standard error of mean and presented as graphs and tables. Data were analysed using one way analysis of variance (ANOVA) followed by Dunnett t-test and repeated measures ANOVA followed by Bonferroni test for multiple comparison. Results were considered significant at $p< 0.05$.

RESULTS AND DISCUSSION

Analysis of the chemical components found in the stem bark extract of *Ficus vallis-choudae*.

The results of the preliminary phytochemical screening of *Ficus vallis-choudae* methanol stem bark extract (FvMSE) revealed the presence of various phytochemicals such as cardiac glycosides, tannins, flavonoids, alkaloids, saponins, steroids and terpenoids (Table 1).

Table1: Phytochemical Constituents of Methanol Stem bark Extract of *Ficus vallis-choudae*

Chemical constituents	FvMSE
Alkaloids	+
Anthraquinone	-
Steroids	+
Terpenoids	+
Cardiac glycosides	+
Saponins	+
Tannins	+
Flavonoids	+

+ =present, **- =** absent, FvMSE = *Ficus vallis* methanol stem bark extract.

Quantity of Preliminary Phytochemical Constituents

The quantitative analysis revealed that Crude extract contains 10 mg/g alkaloids, 10 mg/g flavonoids, 24.5 mg/g saponins, and 0.87 mg/g cardiac glycosides. The result of quantitative analysis is shown in Table 2:

Table 2: Quantity of Phytochemicals in Crude Extract and Fractions of *Ficus asperifolia*

	QUANTITY (mg/g)
CONSTITUENT	FvMSE Crude
Alkaloids	1.00
Flavonoids	22.00
Saponins	35.50
Cardiac Glycoside	2.28

FvMSE= Methanol stem bark extract of *Ficus vallis-choudae*

Median Lethal Dose (LD_{50}) Values

The oral median lethal dose (LD_{50}) of FvMSE in mice and rats was above 5000 mg/kg (Table 3).

Table 3: Median Lethal Dose (LD_{50}) of FvMSE

Extract	Animal Specie	Route	Value (mg/kg)
FVMSE	Mice	Oral	>5000
FvMSE	Rats	Oral	>5000

FvMSE = *Ficus vallis-choudae* Methanol stem bark extract.

Sub chronic Toxicity

Haematological Parameters

Results of haematological parameters determination after 28days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FvMSE did not show any statistically significant difference from that of the distilled water group (Figure 1).

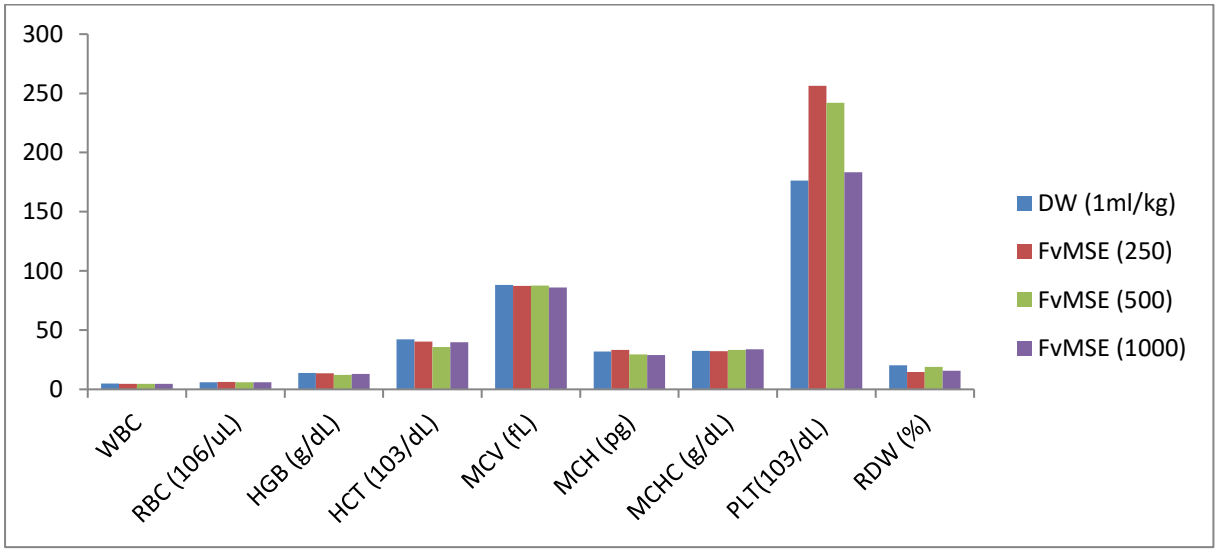

Figure 1: Effect of 28 days Oral Administration of FvMSE Extract on Hematological indices in Rats Key: White blood cell (WBC), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red distribution width (RDW), Platelet (PLT), Distilled Water (DW), Methanol Stem bark Extract of *Ficus vallis-choudae* (FvMSE), n= 5.

Kidney Function Test.

Results of kidney function test after 28days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FvMSE showed a statistically significant ($p<0.05$) difference in respect of sodium electrolytes at 500 and 1000 mg/kg doses for FvMSE administered groups while the remaining parameters did not show any significant difference (Table 4).

Table 4: Effect of Methanol Stem bark Extract of *Ficus vallis-choudae* on Serum Urea, Creatinine and Electrolytes in Rats after Twenty Eight Days Daily Oral Administration

Parameters	Treatments (mg/kg)			
	DW 1ml/kg	FvMSE (250)	FvMSE (500)	FvMSE (1000)
Urea (mg/dL)	79.00±4.19	67.50±6.41	72.25±4.10	72.00±2.17
Sodium (mmol/L)	116.18±8.44	95.85±9.73	250.28±7.91*	423.23±10.83*
Potassium (mmol/L)	14.11±3.02	13.39±2.52	13.58±2.76	13.57±2.16
Creatinine (meq/L)	0.88±0.07	1.08±0.09	0.85±0.07	1.00±0.04
Chloride (mg/dL)	26.75±3.37	30.50±1.55	24.75±3.84	30.25±3.75
Bicarbonate (mg/dL)	92.75±5.23	100.50±3.50	87.75±5.63	100.25±7.27

Data presented as Mean ± SEM, * significantly different from negative analysed using one-way ANOVA at $p<0.05$ followed by Dunnettes post hoc test: DW = distilled water (1 ml/kg), n = 5, FvMSE = *Ficus vallis-choudae* methanol leaf extract.

Liver Function Test

Results of liver function test after 28days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FvMSE did not show any statistically significant difference from that of the distilled water group in all the liver function indices (Figure 2).

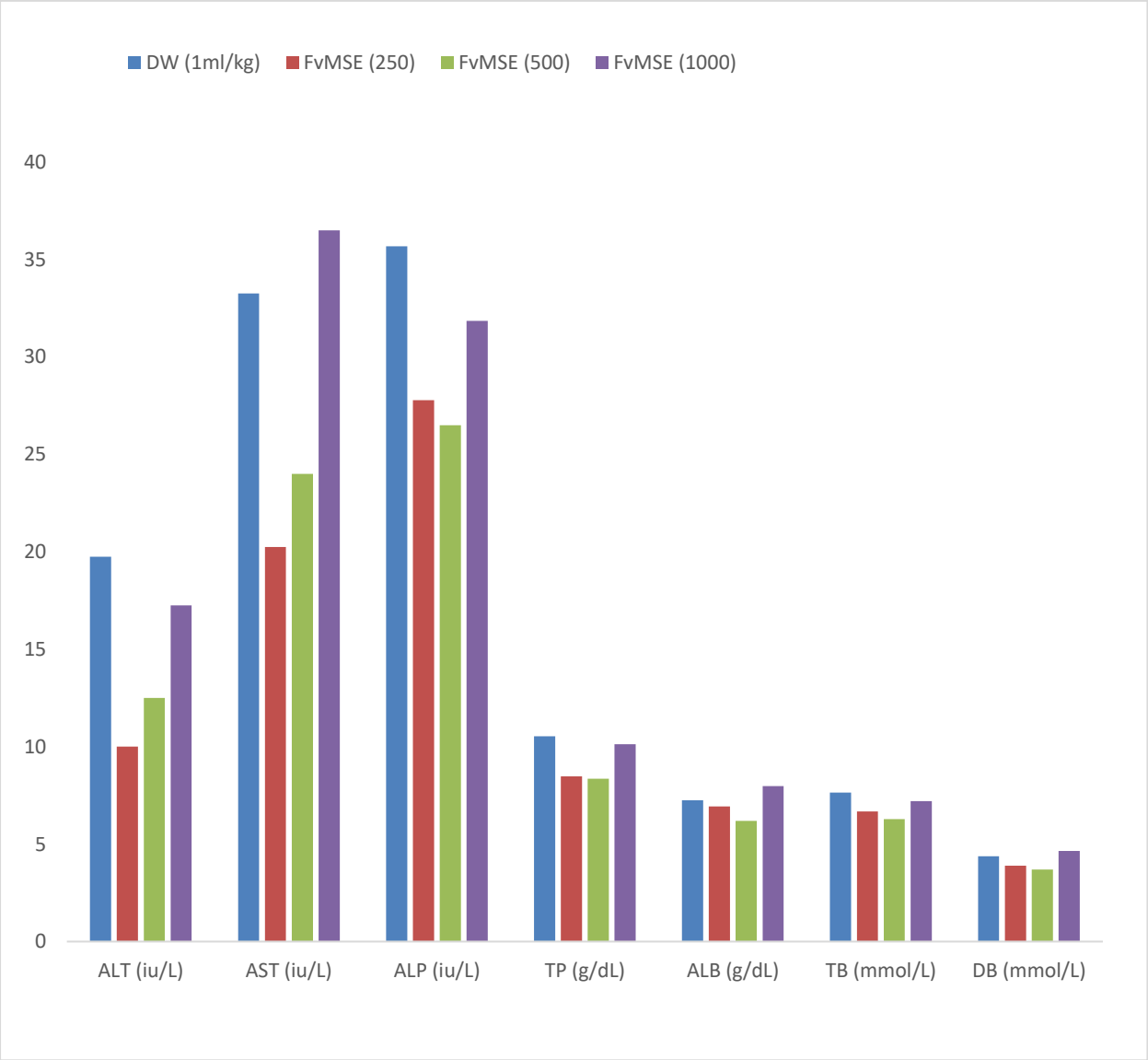

Figure 2: Effect of 28 days Oral Administration of FvMSE Extract on Liver function Test in Rats. Key: Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Stem bark Extract of *Ficus vallis-choudae* (FvMSE), Distilled Water (DW), No significant different from control at $p<0.05$ analysed using one-way ANOVA followed by Dunnettes post hoc test. n=5.

Histopathological Examination after the Sub chronic Toxicity Studies

Relative body organ weight

Body organs harvested include Brain, liver, heart, lungs, kidney and spleen. The result revealed that FvMSE at 250, 500 and 1000 mg/kg doses did not produced statistically significant change in relative organs weight compared to distilled water treated groups (Table 5).

Table 5: Effect of Methanol Stem bark Extract of *Ficus vallis-choudae* on Body and Organ Weight of Rats Following Twenty Eight Days Daily Oral Administration

Parameters	Treatments (mg/kg)			
	Control	**FvMSE (250)**	**FvMSE (500)**	**FvMSE (1000)**
Initial Weight (g)	119.00±1.13	116.33±2.20	114.15±2.22	118.00±1.43
Final Weight (g)	149.50±3.21	147.00±2.68	142.17±3.11	143.83±1.30
Weight Change (g)	30.50±1.34	30.67±2.25	28.02±4.31	25.83±2.79
Brain (g)	1.50±0.01	1.45±0.06	1.52±0.08	1.48±0.04
Heart (g)	0.62±0.01	0.59±0.06	0.61±0.15	0.64±0.09
Kidneys (g)	0.80±0.11	0.76±0.04	0.77±0.09	0.79±0.06
Liver (g)	4.35±0.22	4.23±0.14	4.01`±0.18	4.41±0.31
Lungs (g)	1.32±0.13	1.24±0.08	1.09±0.14	1.21±0.09
Spleen (g)	0.74±0.10	0.81±0.06	0.72±0.09	0.73±0.05

Data presented as Mean ± SEM, analysed using one-way ANOVA followed by Dunnettes post hoc test: Control = distilled water (10 mL/kg), n = 5, FvMSE = *Ficus vallis-choudae* methanol stem bark extract.

Liver

The group of rats that received distilled water showed normal hepatocytes (Plate I). It was also observed that the FvMSE treated rats showed slight hepatic necrosis in the liver compared to the distilled water treated rats after 28 days of daily oral administration (Plate II).

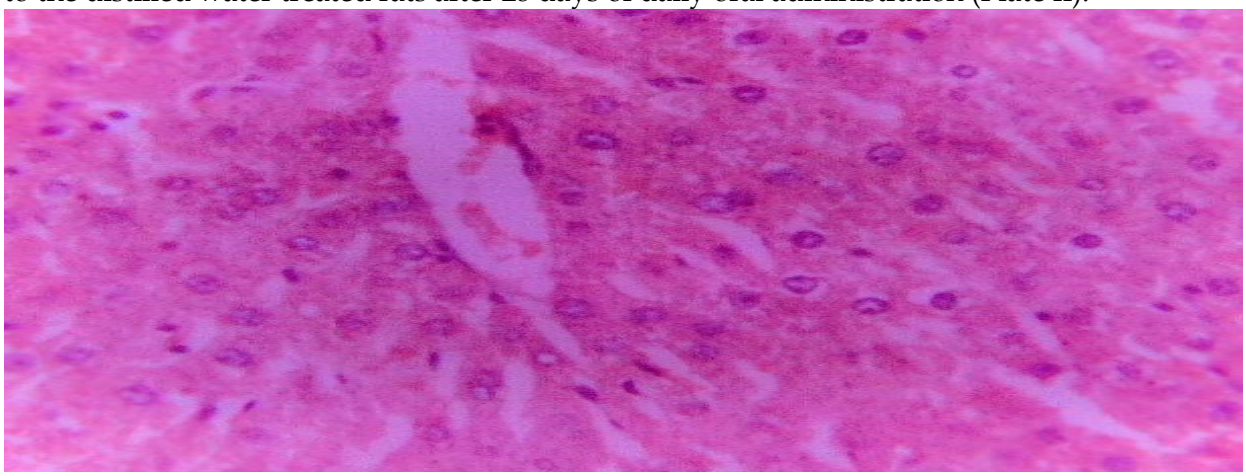

Plate I: Photomicrograph of liver section of distilled water 28 days oral daily treated rats showing normal hepatocytes. Hematoxylin & Eosin (H&E) stain (× 400).

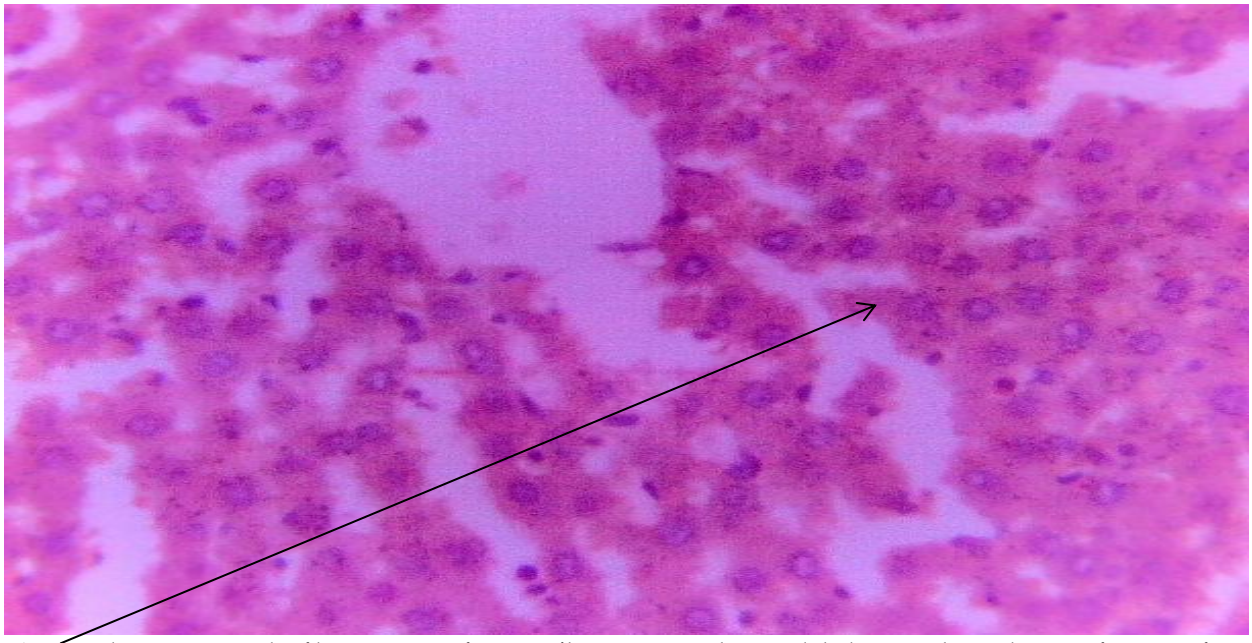

Plate II: Photomicrograph of liver section of 250 mg/kg FvMSE 28 days oral daily treated rats showing features of slight hepatic necrosis. H&E stain (× 400).

Heart

The group of rats that received distilled water showed normal features of heart section (Plate III). However slight myocardial necrosis and hyperplasia of inflammatory cells was observed in the heart features of the FvMSE treated rats after 28 days of daily oral administration (Plate IV).

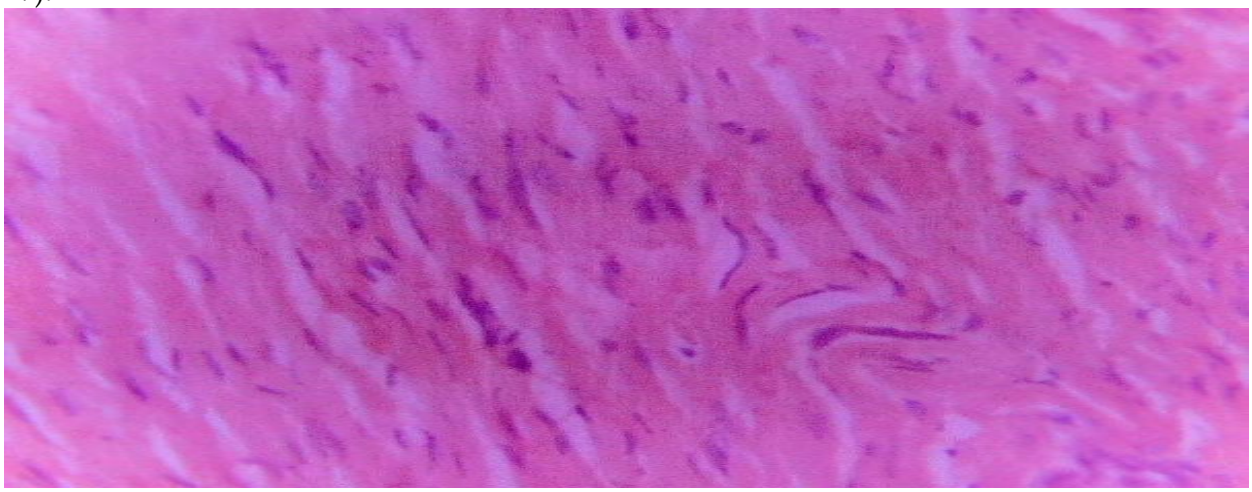

Plate III: Photomicrograph of heart section of 28 days distilled water oral daily treated rats showing normal myocardium. H&E stain (× 400).

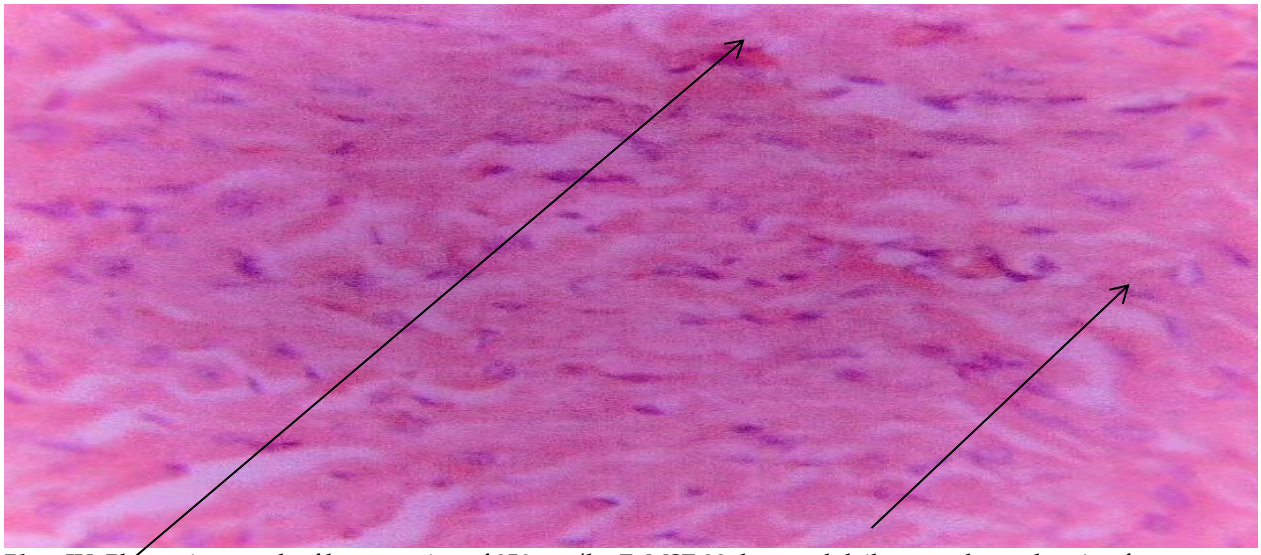

Plate IV: Photomicrograph of heart section of 250 mg/kg FvMSE 28 days oral daily treated rats showing features of slight myocardium necrosis and hyperplasia of inflammatory cells. H&E stain (× 400).

Kidney

The group of rats that received distilled water showed normal features of kidney section (Plate V) while the FvMSE treated rats after 28 days daily oral administration showed features of moderate hyperplasia of inflammatory cells and slight tubular necrosis (Plate VI).

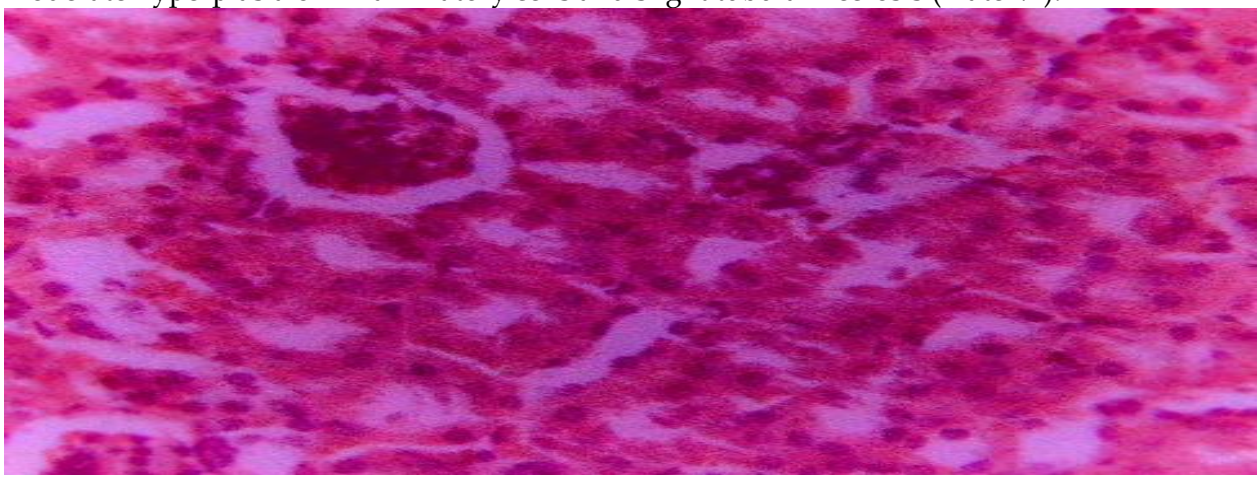

Plate V: Photomicrograph of kidney section of distilled water 28 days oral daily treated rats showing normal features. H&E stain (× 400).

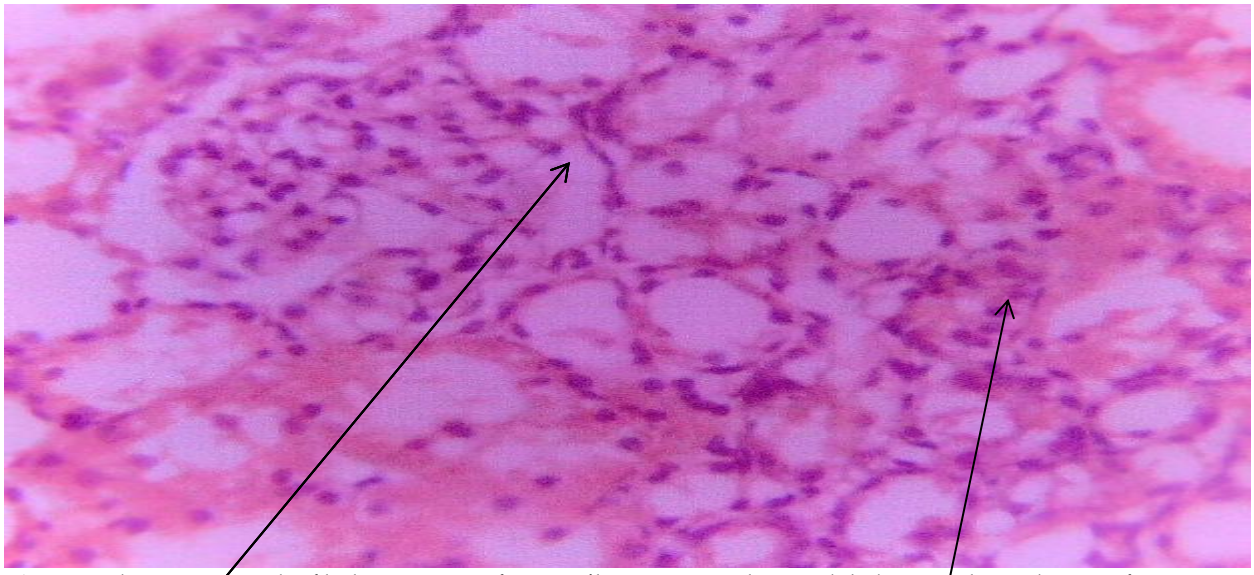

Plate VI: Photomicrograph of kidney section of 250mg/kg FvMSE 28 days oral daily treated rats showing features of moderate hyperplasia of inflammatory cells and slight tubular necrosis. H&E stain (× 400).

DISCUSSION

The preliminary phytochemical screening of the stem bark extracts *Ficus vallis-choudae* revealed the presence of saponins, flavonoids tannins, alkaloids, terpenoids, steroids and cardiac glycosides which might be responsible singly or in complement for the observed pharmacological activity of the extract. This finding is in tandem with the reported work of Lawan *et al*. (2008). Secondary metabolites such alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides and steroids are responsible for plants' biological activities (Edewor-Kuponiyi, 2013; Rungsung *et al*. 2015).

The determination of median lethal dose (LD_{50}) is a crucial aspect in acute toxicity testing, serving as a key entry point for pharmacological research (Abdullahi *et al*. 2020). This method allows for the rapid evaluation of potential hazards associated with a test substance after a single dose. LD_{50} testing is commonly employed in risk assessments for both synthetic and naturally occurring chemicals, providing valuable guidelines for subsequent clinical studies involving humans and non-target environmental organisms (Agrawal and Paridhavi, 2007). Acute toxicity studies are typically conducted to ascertain the dosage range that could be harmful to animals. Additionally, these studies enable the estimation of the therapeutic index (LD_{50}/ED_{50}) for drugs and xenobiotics (Maikai *et al*. 2008; Abdullahi *et al*. 2020). The oral LD_{50} values of the methanol stem bark extract of *Ficus vallis-choudae* (FvMSE was found to be above 5000 mg/kg, indicating relative safety (which agrees with the reported work of Lawan *et al.* 2008 and Malami *et al.* 2010). It is important to note that while LD_{50} serves as a useful measure for assessing the safety margin of a substance, it should not be regarded as an absolute value or a comprehensive evaluation of its properties. LD_{50} may not fully capture the entire range of toxicity or hazards associated with a drug or chemical (Cassarette *et al*. 1996) hence the extension of the experiment in this study to include histophatological studies. The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labeling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained for both rats and mice found to be above 5000 mg/kg, is relatively safe orally. However, a

comprehensive investigation of the substance's toxicity and hazards requires additional considerations beyond the sole reliance on LD_{50} measurements.

Assessment of haematological parameters can be used to determine the extent of harmful effect of foreign compound on the blood. It can also be used to explain blood related functions of biochemical compounds (Yakubu *et al*. 2007). Anaemia is a common feature in the tropical regions where diseases such as malaria infection is prevalent. Some parameters used to assess anaemia include haemoglobin, red blood cell and packed cell volume (PCV) (Akanbi *et al*. 2018). In this study, the mean haemoglobin and red blood cell in FvMSE treated groups was not significantly different from that of the distilled water group. Although saponins are present in the extracts, the report that saponin has haemolytic properties and may be capable to lyse haemoglobin (Abbas *et al*. 2015) which could lead to appreciable decline in PCV, did not show significant effect. Because some conventional drugs such as primaquine presents hemolysis as side effect or as a delayed challenge in the case of artesunate, reduction of platelets leading to thrombocytopenia and decrease in WBC which may compromise immunity (Kurth *et al*. 2016), it therefore becomes imperative to determine the effect of any potential drugs on haematological indices. However, in this study the 28 day daily administration of FvMSE did not show any statistically significant difference with that of the distilled water group.

Kidney and liver play important roles in drug metabolism within the body and are necessary organs for the survival of animals which therefore necessitates the assessment of their function in toxicity studies (Olorunnisola *et al*. 2012). Damage of the kidney tubules may cause non reabsorption of electrolytes and retention of urea and creatinine in the blood. Therefore, measurement of these biochemical parameters and some electrolytes can be used to assess kidney damage (Afolabi *et al*. 2014). However, in this study the 28 day daily administration of FvMSE did not show any statistically significant difference with that of the distilled water group with the exception of sodium electrolytes which was found to have significantly increase dose dependently in the FvMSE treated groups. The observed rise in sodium electrolyte may have been as a result of intrinsic saltiness and sodium content of the extract (Zhang *et al*. 2014) or as a result of dehydration which might be due to under feeding of the animals or as sign of toxicity of the extract. Sodium chloride has many physiological functions, such as maintaining water and acid–base balance in the body. However, the excessive intake of sodium may cause fluid and electrolyte retention giving rise to increase blood pressure (He and Macgregor, 2009), thereby increasing the risk of cardiovascular (Cook *et al*. 2007) and renal disease (Cianciaruso, 1998).

In liver function test, increase in AST, ALT, ALP, bilirubin and total protein imply liver damage, but in clinical practice the most important parameter is ALP which is found in both liver and bones and is important for breakdown of proteins (Kachmar and Moss, 1976; First, 1996; Dhariyal *et al*. 2016). Increased activities of serum AST, ALT and ALT are indicative of cellular leakage and loss of functional integrity of liver cell (Sabiu *et al*. 2015). ALT is more elevated than AST in various necro-inflammatory conditions of the liver, reflecting its relative efficiency as a liver disease marker (Willianson *et al*. 1996). The pathogenesis of diseases such as malaria parasite infection involves the liver, a rich source of AST and ALT. The activities of the invading parasites in the liver in its self may lead to damage of the hepatocytes membrane and consequently release of AST and ALT into circulation resulting in elevated serum activities of the enzymes. Therefore any potential agent for such ailments must be screened for liver toxicity. However, in this study the 28 day daily administration of FvMSE did not show any statistically significant difference with that of the distilled water control group. In this study, the oral LD_{50} of the extract, which was found to be greater than 5000mg/kg, in rats,

provided an initial evidence of the potential safety of the extracts (Hodge and Sterner, 1949). Also, lack of mortality and observable adverse effects from FvMSE in the treated rats throughout the observational period of 14 days (single dose) and 28 days (repeated daily doses), coupled with general lack of significant changes in organ-body and organ-brain weights ratio, further supports the idea of potential safety of the extract. However, it should be noted that a change in body weight is an uncomplicated and sensitive index to study the detrimental effects of drugs and chemicals (Bailey *et al*. 2004). In general, toxic nature of the drug could lead to abnormalities in body weight (Nirogi *et al*. 2014; Olayode et al. 2019) and a decrease in body weight could indicate a substantial degree of toxicity, while a reduced body weight gain represents only a mild form of intoxication (Michael *et al*. 2007; Piao *et al*. 2013). Furthermore, organ weight, organ/body weight and organ/brain weight ratios are more sensitive indicators of drug toxicity, making any subtle alteration of significant importance for further investigation (Bailey *et al*. 2004; Piao *et al*. 2013). The lack of significant differences among the tested doses in organ-brain weight ratio suggests that the observed changes may not be of toxicological significance. As recommended by the OECD guidelines, comprehensive investigation of a given substance's toxicity and hazards requires additional considerations beyond the sole reliance on LD_{50} measurements (OECD, 2001). This necessitated sub chronic toxicity studies (OECD, 2008) and histological analysis where tissues of various organs mostly associated with drug handling and disposition were examined. It is reported that histological findings following single and repeated doses of extract provides some evidence of organ toxicity, similar to those associated with known hepatotoxins and nephrotoxins (Iweala *et al*. 2011; Hussain *et al*. 2018; Mukherjee and Ahmad, 2018). It also provides the much needed correlation with observed changes in biochemical indices. However, differences in liver and kidney histology do not seem to follow a specific pattern for both single and 28 days of repeated doses. The slight histophatological changes observed in rats such as slight hyperplasia of inflammatory cells (in liver, kidney and spleen) and some slight necrosis in the heart) were not accompanied by any significant change in the biochemical markers of measured liver injury although may still constitute a source of concern as to the safety profile of the extract. Furthermore, fatty changes, hydropic degeneration, fibrosis, and vascular abnormalities such as congestion of the central veins and liver sinusoids usually seen in hepatotoxicity (Khleifat *et al*. 2002; Nigatu *et al*. 2017) were not seen in any of the treated groups. However, the FvMSE, did not compare favourably with completely safe extracts because its effect on the liver, heart and kidney (sub chronic studies) revealed that it has slightly to moderately affected the histopathological features of these organs thereby suggesting that it might be potentially toxic especially in long term use.

Conclusion

The median lethal dose of methanol stem bark extract of *Ficus vallis-choudae* has demonstrated a safe acute toxicity index. Repeated administration of graded doses on daily basis for a period of four weeks also did not produced significant changes in biochemical parameters of liver and kidney function except increase in sodium ion levels which may compromise kidney function. The observed hispathological changes in the liver, kidney and heart sections of rats administered with this extract also raises concern on the safety of the extract especially in long termed administration.

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Conflict of Interest

The authors affirmed that they have no conflicts of interest related to this study or its publication.

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