

Acute and subchronic toxicity profiles of *Melastomastrum capitatum* (Vahl) Fern. (Melastomataceae) root aqueous extract in Swiss albino mice

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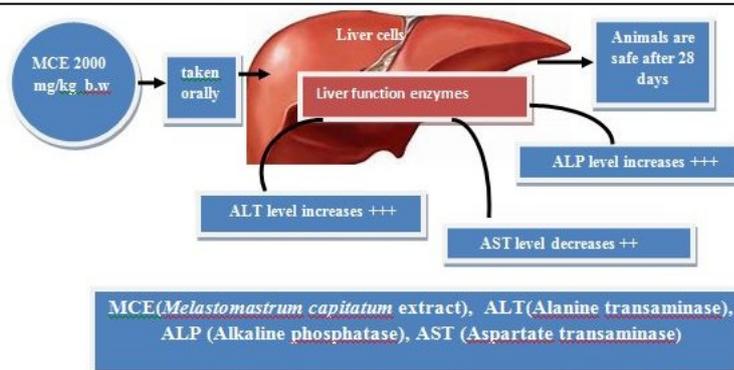
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GRAPHICAL ABSTRACT



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ABSTRACT

Melastomastrum capitatum is a plant whose leaf extract is popularly known for its ability to cure cancer of the ovary in Mambila plateau towns in Nigeria. Apart from the leaves, the root extract has been used to manage various diseases such as bacterial infections, pains, and diabetes. As a result of these health benefits, liver and vital organ damage are often associated with short (acute) or long (subchronic) intake of this plant decoction in traditional medicines. This present study was carried out to determine short and long (subchronic) terms effect of the root aqueous extract for the treatment of diseases especially diabetes by the Fulani tribe in Mambila plateau in Taraba State, Nigeria. Acute and subchronic toxicity studies were carried out following the guidelines stipulated by the Organization for Economic Cooperation and Development (OECD). In the acute toxicity study, a limit test dose of 2000 mg/kg body weight (b.w) of aqueous root extract was administered by oral route into five Swiss albino mice consisting of five groups of one mouse per group. Observations were carefully made for signs of toxicity for the first 4 hours and then once daily for 2 weeks. A lower dose of 300 mg/kg b.w administered to the mice do not show any sign of acute toxicity unlike the higher dose which produced signs such as reddish eyes, itching and restlessness which last only a few minutes of extract administration. Subchronic toxicity study revealed that root extract of the plant is slightly toxic as had shown by results of most of blood parameters investigated such as WBC, PCV, ALT, AST, ALP, serum electrolytes, etc. However, our results showed that root aqueous extract of *M. capitatum* is well tolerated at the doses investigated as there was no major damage to vital organs like the liver, kidney and heart of the animals. The study therefore showed that the root extract of the plant is safe for use as an ethnomedicinal prescription for diseases in traditional medicine.

1. Introduction

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs of peoples, and experiences indigenous to different cultures used in the maintenance of health, prevention of diseases and improvement of physical and mental illness. In practice, traditional medicine refers to the following components: acupuncture, Ayurveda, Unani, traditional birth attendant's medicine, mental healer's medicine, herbal medicine, and various forms of indigenous medicine. Complementary or alternative medicine, on the other hand, refers to a broad set of healthcare practices that are not part of a country's own tradition and are not integrated into the popular healthcare system. Traditional medicine has maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries. The number of medicinal plants has been estimated to be on the order of 40,000 to 70,000 [1], which means that almost 25% of all plant species have some sort of medicinal use somewhere in the world.

Thus, medicinal plants are used in crude or pure form in the preparation of drugs in different systems. In countries like India, China and others with well-established traditional systems of herbal medicine, plant-based formulations occupy an important place in health management [2-4]. The therapeutic use of herbs is as old as human civilization and has evolved along with it. The vast majority of people on this planet still rely on their indigenous system of medicine and use herbal drugs. The Indian and Chinese systems of medicine are well established with written records going back around 3000 years. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases, and neurological disorders. Interest in herbal drugs and natural medicine is undergoing a renaissance at the present time. The use of medicinal plants as remedies for diseases is extensive, increasing and complex. A recent study showed that 20–33% of the UK population use herbal medicines alone or in combination with conventional medicines for disease treatments. This usage is particularly frequent amongst those who are over-the-counter medicines-users. In general outlook, there is not a wide understanding of the usefulness of herbal medicines to many people in the developed countries. Despite this, healthcare professionals and students commonly use herbal products for managing and treating various ailments. In the United States, approximately 38% of adults and approximately

12% of children are using herbal medicines or herbal products [5]. For the US alone, recent study has shown that 38 million adults in the US (18.9% of the population) used herbal medicines or supplements [6]. Data for other regions are even more limited, but the usage of herbal medicines is widespread in countries like India, Indonesia, Australia, and China, to name just a few.

The use of herbal medicines in Africa especially, Nigeria is becoming rampant due to their affordability, accessibility and easy to use as well as cultural beliefs. Traditional medicine practice in Nigeria is not fully regulated because of high proliferation many herbalists that are not licensed. Because of this, the administration of herbal medicines by traditional medicine practitioners in most developing countries is without accurate or proper drug doses. This has resulted in many side effects as a result of short (acute) or long (chronic) term usage of herbal drugs [7]. In most continental European countries, such phytomedicines are licensed medicinal products and are used under medical supervision to avoid any side effect or adverse reaction of herbal drugs. However, the widespread use of herbal medicines by the general public raises several important issues bordering on the precise dosage of the drugs to be administered to patients. Some of these issues also relate to how individuals, whether consumers or healthcare professionals, perceive and use these preparations; other concerns relating to the quality, safety, and efficacy of the herbal medicines themselves [8-10].

Among medicinal plants used in traditional and complementary (folkloric) medicines in Nigeria is *Melastomastrum capitatum*. It belongs to the family *Melastomataceae* a taxon of dicotyledonous flowering plants commonly found in the tropics [11]. It is an annual or a perennial herb, shrub or small tree with simple opposite leaves having characteristic variation pattern and multi-colored when matured. It can grow up to 1.25 m high in wetlands and stream-banks especially in Mambila plateau Taraba State Nigeria [12], Guinea, Mali, Uganda, and Angola. In Nigeria, it is locally called "Belkon" by the Fulani Village tribe of Mambila plateau Nigeria where the leaf decoction is used to manage and treat ovarian cancer by traditional medicine practitioners. A large part of the leaf has sweet to sour taste. The leaf-sap diluted into a little water is used in Nguroje Village of Mambila plateau as a sedative. Its leaf extract can reduce cholesterol, pains, and as antidiabetic agent [13], and purifies blood vessels. Preliminary phytochemical screening of the leaf methanol extract revealed the

presence of various types of glycosides and alkaloids including carbohydrates [12, 14]. The short (acute) and long (subchronic) term administration of root aqueous extract in disease management without proper recourse to dose limit may present some problems if not investigated. In this present study, we determined the acute and subchronic toxic profiles of root aqueous extract of *M. capitatum* in Swiss albino mice.

2. Materials and Experimental Methods

2.1. Chemicals and Apparatus

Some of the solvents used are methanol, hematoxylin & eosin stains, 10 % formalin, dissecting kits, liver function, and lipid profiles kits, Mindray BC-2800 auto hematology analyzer (Guangzhou Medsinglong medical equipment Co., Ltd, China), Roche Reflotron plus autoanalyzer, scanning biological microscope, etc.

2.2. Experimental Animals

Thirty-five Swiss albino mice of opposite sexes weighing 15-20.5 g were obtained from the animal houses of the Departments of Pharmacology, University of Jos, Nigeria. The animals were housed in stainless steel cages, and supplied with clean drinking water, and fed with food (*ad libitum*) with standard animal feeds (Royal Feeds, Nigeria Ltd) in an environment of ambient temperature (26 °C) and lighting period of 12 h per day, with relative humidity of 60 %. The animals were allowed to acclimatize to the laboratory condition where the study took place for 1 week. The experimental procedures for this study were approved by the research ethics committee of the above-mentioned universities, and their guidelines were strictly followed.

2.3. Collection and Identification of Plant

Roots of *Melastomastrum capitatum* were collected from Mambila plateau Sarduana Local Government Area, Taraba State, Nigeria, in the evening hour corresponding with the time the traditional medicine practitioners collect the plant for medicinal purposes. It was authenticated at the herbarium of Department of Botany, Ahmadu Bello University and Zaria by a taxonomist Mr. Namadi Sunusi with a voucher specimen number ABU2761 deposited at the herbarium for future reference.

2.4. Preparation of Plant Material

Roots of *M. capitatum* were air-dried under shade for 3 weeks and were pulverized into a fine powder using an electronic blender (Model 5000 MH, Japan). The powder

was then sieved using sieve number 20 mesh to obtain the fine powder, and remove any unwanted debris, It was then weighed using an electronic scale balance to obtain the initial weight of the powdered leaves. The powdered plant material was stored in a clean and dried polythene bag protected from direct sunlight using aluminium foil sheet and kept in the refrigerator (37 °C) for further use.

2.5. Preparation of Aqueous Root Extract

Exactly 300 g of the powdered roots were weighed and defatted with 800 mL petroleum ether by cold maceration for 24 h to remove fat, latex and non-polar compounds of high molecular weights. The defatted plant residues were then macerated in 1000 mL water distilled for 92 h to obtain aqueous root extract (MCRE). The collected extract was filtered through Whatman № 1 filter paper. Finally, the filtrate was concentrated *in vacuo* using rotary evaporator to obtain the gel-like dark extract. The extract was weighed and the final weight was noted. The extract was then stored in a clean neatly labelled sample bottle protected with aluminium foil and kept in a refrigerator for further use.

2.6. Acute Toxicity Study of *Melastomastrum capitatum* Leaf Methanol Extract

Acute toxicity study was carried out in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines paragraph 453. Five Wistar albino rats of opposite sexes were weighed and fasted overnight. A test dose of *M. capitatum* leaf methanol extract was calculated in terms of the animal's body weights. A limit test dose of 2000 mg/kg body weight was administered to all the rats via oral gavages. After this, the animals were regularly observed for behavioral changes and general toxicity signs such as restless, hypoactive, itching and reddish eyes, as well as death especially within the first 4 h of administration and for 24 h. After these, observations were continued daily for a total of 14 days with free access to water and food [15, 16].

2.7. Determination of Body Weights of Mice After 28 Days of Extract Administration

The initial body weights of the mice were measured on an electronic scale balance. Subsequently, the body weight of each mouse was measured three days in a week for changes in body weights for 28 days.

2.8. Subchronic Toxicity Study of *Melastomastrum capitatum* Aqueous Root Extract

Thirty Swiss albino mice of opposite sexes were weighed and divided into five groups of six each. The animals were kept in separate cages and labelled accordingly. Animals in Group I served as the normal control group and were administered distilled water throughout the study period while mice in groups II, III, IV, and V were orally administered doses of 250, 500, 1000 and 2000 mg/kg body weight *M. capitatum* root extract (MCRE) respectively, once daily for 28 days. The body weights of all the mice were recorded weekly throughout the experimental period. After 28 days of treatment, the mice were fasted for 8 hours and gently anesthetized using chloroform. Thereafter, the blood samples were immediately collected by cardiac puncturing for hematological and biochemical parameters [17].

2.9. Determination of Organ Weights of Mice After 28 Days

After 28 days, the mice were sacrificed under chloroform anaesthesia, and the weights of some vital organs such as the heart, lungs, liver, kidneys, testes, and ovaries were determined by dissecting carefully each organ from the sacrificed rats into 10 % (v/v) formalin in a clean petri dish. Water was drained from the organs using cotton wool and thereafter weighed on a micro scale balance. Three replicate measurements were taken and the average was taken as the weight of the organ.

2.10. Determination of Hematological Parameters

Hematological parameters were determined using Mindray BC-2800 auto hematology analyzer (Guangzhou medsinglong medical equipment Co., Ltd, China) at a temperature of 37 °C and normal atmospheric pressure [18].

2.11. Determination of Serum Biochemical Parameters and Electrolytes of Mice

Biochemical parameters such as liver function and kidney function as well as serum electrolytes were determined using Reflotron® Plus (Roche, Germany) auto-analyzer with various kits [19].

2.12. Histopathological Examination of Vital Organs of Mice after Oral Administration

Histopathological examination of these organs liver, kidney, heart, lung, pancreas, testes, and ovary of the rat from each group was carried out by fixing the tissues in 10

% formalin solution. The organs were then partially dehydrated, and a thin section of the organs was on clean slides. The tissue of the organs then stained with hematoxylin and eosin stain and viewed with an Olympus microscope under 40x magnification [20, 21].

2.13. Statistical analysis

Data obtained were subjected to one-way analysis of variance (one-way ANOVA). Data were shown as the means of three individual experiments represented as the mean \pm SD. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Acute Toxicity Study

In acute toxicity study, there was no death; however, slight signs of toxicity were observed in the mice administered with a limit dose of 2000 mg/kg of MRCE after 14 days. Clinically, the mice do not show any sign of toxicity at 300 mg/kg b.w within the observed period. They were active, and their eyes were reddish with slight body itching in the first 4 h after the high dose was given.

3.2. Body Weights of Mice After 28 Days of Root Extract Administration

The body weights of the mice slightly increased in Groups I-V at doses 250, 500, 1000 and 2000 mg/kg of MCRE orally from day one to day 28 of the study. However, these increases were not significantly different ($p < 0.05$) from the normal control group that received distilled water within the same period (Table 1).

3.3. Organ Weights of Mice after 28 Days

There was an increase in relative weights of the vital organs after 28 days of MCRE administration in the treatment groups orally except the liver and the kidneys. Despite these increase at doses of 250, 500 and 1000 mg/kg of MCRE. These increases were not significantly different ($p < 0.05$) from the negative control group (Table 2).

3.4. Effects on Hematological Parameters

The results of hematological parameters obtained showed that most of the parameters increase in a dose-dependent fashion in all the groups (Table 3; Figure 1). White blood cell differentials decrease in doses dependent fashion, but the WBC differentials, do not show a significant increase at doses 250 to 2000 mg/kg.

Table 1: Effect of *M. capitatum* root aqueous extract on body weight of mice after 28 days of oral administration

Animal (g)	Week of administration (days)			
	1	2	3	4
GP I (control)	15.00±0.01	16.40±0.01	16.50±1.01	16.98±0.10
GP II 250 mg/kg	15.00±0.01	14.81±0.02	14.61±0.02	14.11±0.01
GP III 500mg/kg	18.00±0.02	18.16±0.02	18.21±0.01	16.02±0.01
GP IV 1000mg/kg	18.50±0.02	18.26±0.01	18.20±0.01	14.55±0.02
GP V 2000mg/kg	20.52±0.02	20.33±0.02	17.22±0.01	12.30±0.01

Values are mean±SD, n= 6 mice per group, GP (group) .

Significant level was determined at p< 0.05 (one-way ANOVA).

Table 2: Effect of *M. capitatum* root aqueous extract on organ weights of mice after 28 days of oral administration

Animal (g)	Week of administration (days)				
	control	250	500	1000	2000
Heart	0.24±0.01	0.26±0.01	0.24±0.02	0.28±0.01	0.22±0.01
Liver	2.40±0.01	2.43±0.10	2.38±0.10	2.33±0.01	2.31±0.01
Kidney	1.37±0.01	1.37±0.01	1.36±0.01	1.32±0.01	1.31±0.01
Lung	1.92±0.01	1.90±0.01	1.91±0.01	1.93±0.01	1.90±0.01
Testis	1.48±0.01	1.47±0.01	1.49±0.01	1.53±0.01	1.55±0.01
Ovary	1.53±0.01	1.53±0.01	1.54±0.01	1.50±0.01	1.48±0.01

Values are mean±SD, n= 6 mice per group.

Significant level was determined at p< 0.05 (one-way ANOVA).

Table 3: Effect of *M. capitatum* root extract on hematological parameters of albino mice after 28 days oral administration

Parameter	<i>M. capitatum</i> root extract (mg/kg)				
	Control	250	500	1000	2000
PCV(%)	37.60±0.20	37.51±0.04	35.25±0.04	35.50±0.02	35.81±0.20
MCV(fL)	8.31±0.02	8.30±0.01	8.26±0.01	8.28±0.01	8.32±0.01
MCH(pg)	5.22±0.01	5.21±0.01	5.18±0.01	5.16±0.01	5.10±0.01
MCHC(g/L)	10.44±0.02	10.42±0.02	10.38±0.02	10.34±0.01	10.30±0.02
RBC(x 10 ¹² /L)	4.81±0.01	4.84±0.01	4.88±0.01	4.92±0.01	4.96±0.01
RDW(%)	16.02±0.02	16.11±0.02	16.15±0.02	16.18±0.02	16.24±0.02
WBC(x10 ⁹ /L)	6.63±0.01	6.60±0.01	6.58±0.01	6.54±0.01	6.48±0.01
Hb (g/dL)	12.48±0.02	12.49±0.02	12.52±0.02	12.58±0.02	14.12±0.02
PLT (x 10 ⁹ /L)	133.12±0.12	135.10±0.04	135.12±0.02	138.10±0.02	140.50±0.08

Values are mean ± SD of six mice per group, p< 0.05 (one-way ANOVA). PLT(platelets), WBC(white blood cells), PCV(packed cell volume),RDW(red blood cell distribution width)

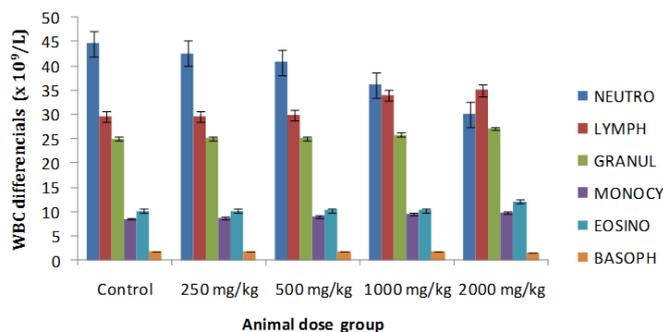


Fig. 1: Effect of *M. capitatum* root extract on WBC differentials of mice

3.5. Effects on Liver Function Enzymes

The effects of subchronic administration of *M. capitatum* crude leaf extract on Swiss albino mice showed that alkaline phosphatase (ALP) had the highest dose-dependent manner. After 28 days administration of MCE orally to the mice, aspartate transaminase (AST), and bilirubin (BIL) showed a progressive decrease in values from dose 250 mg/kg to 2000 mg/kg body weights. These declines were not statistically different from those of the control group at $p < 0.05$ (Table 4).

3.6. Effects of *M. capitatum* Aqueous Root Extract on Serum Electrolytes Level

After 28 days administration, MCRE to the mice at different doses, serum electrolytes such as Na^+ , Cl^- , Ca^{2+} , K^+ , urea, and creatinine showed a significant increase in a dose-dependent manner, except few that decreased at

2000 mg/kg body of MCRE (Figure 2). Uric acid also showed a slight increase at doses of 500 and 1000 mg/kg body weights but reduced significantly further at 2000 mg/kg b.w.

3.7. Effects of *M. capitatum* Aqueous Root Extract on Lipid Profiles

The results of subchronic administration of the root extract of *M. capitatum* are shown in figure 3. The extract caused elevated values of high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), total cholesterol (TCH), triglycerides (TRG), and plasma glucose (GLU) in a dose-dependent fashion, while the value of low-density lipoproteins (LDL) decreased as the doses increase. There were no significant differences at $p < 0.05$ between the treated and the control groups.

3.8. Histopathological Examination of Vital Organs

There was slight organ congestion as well as necrosis of the liver, kidney, lung, and testis as a result of subchronic administration of *M. capitatum* aqueous root extract orally. Photomicrograph sections of the organs revealed cell lumen as compared with the normal control group (Figure 4; a-d).

Table 4: Effect of *M. capitatum* root aqueous extract on serum biochemical parameters of Swiss albino mice after 28 days oral administration

Parameter	M. capitatum extract dose (mg/kg)				
	Control	250	500	1000	2000
ALP(U/L)	40.51±0.04	41.52±0.12	42.33±0.10	45.12±0.01	49.23±0.10
AST(U/L)	25.13±0.02	25.14±0.02	25.28±0.20	25.86±0.20	36.11±0.02
ALT(U/L)	20.42±0.01	21.42±0.10	21.44±0.00	21.48±0.10	22.66±0.01
ALB(g/L)	3.76±0.01	3.88±0.01	3.89±0.01	3.92±0.01	4.12±0.01
BIL(mg/dL)	0.21±0.01	0.44±0.01	0.48±0.01	0.52±0.01	0.89±0.01
GLO(g/dL)	1.49±0.01	1.52±0.00	1.64±0.01	1.88±0.01	2.32±0.01
TPRN(g/dL)	5.55±0.01	5.56±0.01	5.58±0.01	5.64±0.01	6.14±0.01

Values are mean ± SD, n= 6 mice per group, Significant level was determined at $p < 0.05$ (one-way ANOVA), ALP (alkaline phosphatase), AST(aspartate transaminase), ALT(alanine transaminase), ALB(albumin), BIL(bilirubin), GLO(globulin), TPRN(total protein).

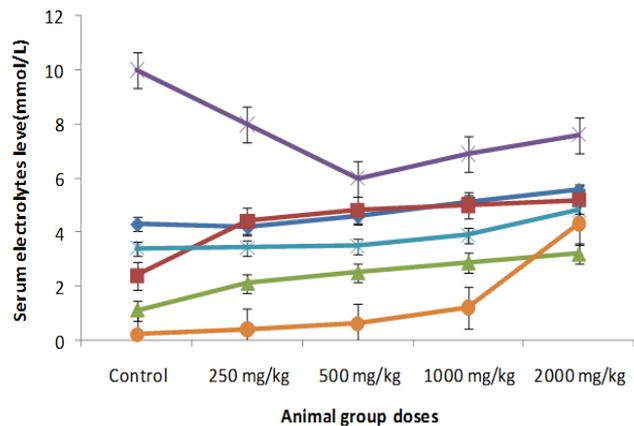


Fig. 2: Effect of *M. capitatum* root extract on serum electrolyte levels of mice, n= six mice per group, CRE(Creatinine)

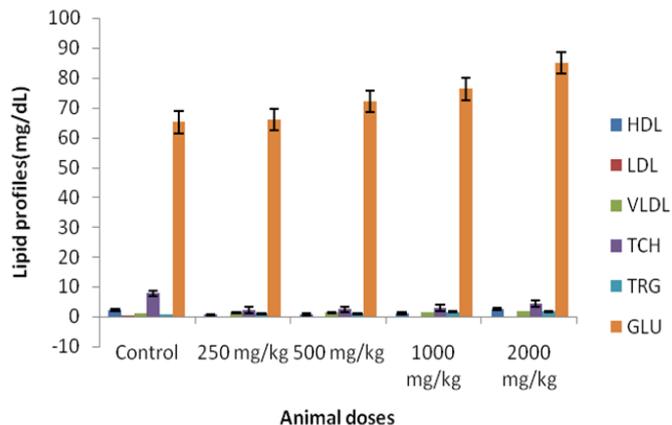
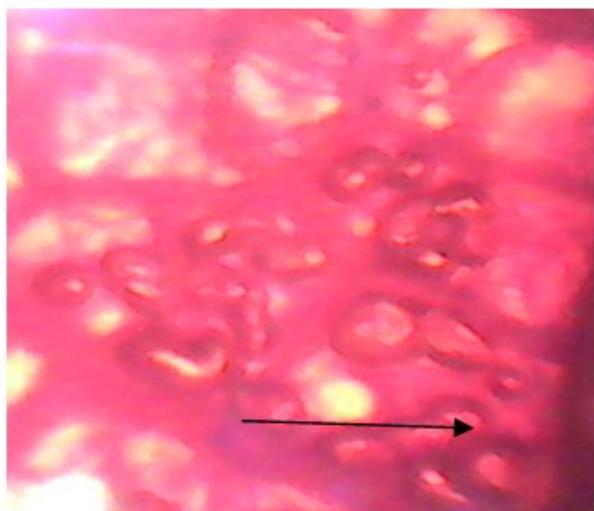
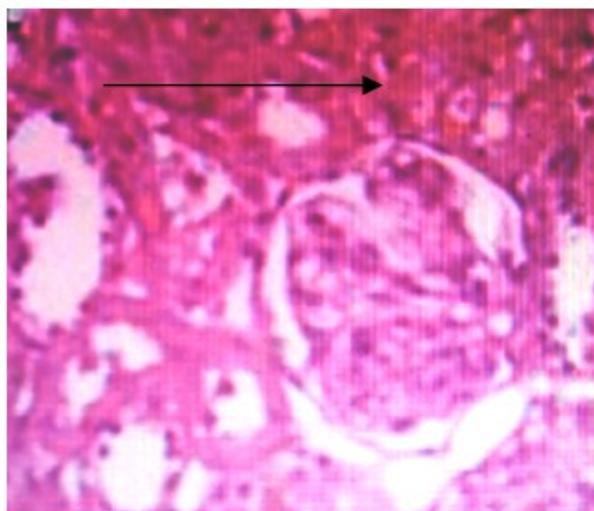


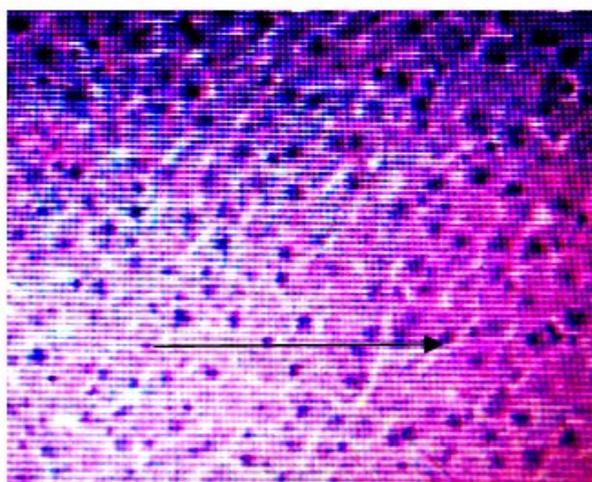
Fig. 3: Effect of *M. capitatum* root extract on lipid profiles of mice. HDL (high density lipoproteins), LDL (low density lipoproteins), VLDL (very low density lipoproteins), TCH(total cholesterol), TRG(triglycerides), GLU(plasma glucose), n= 6 mice per group.



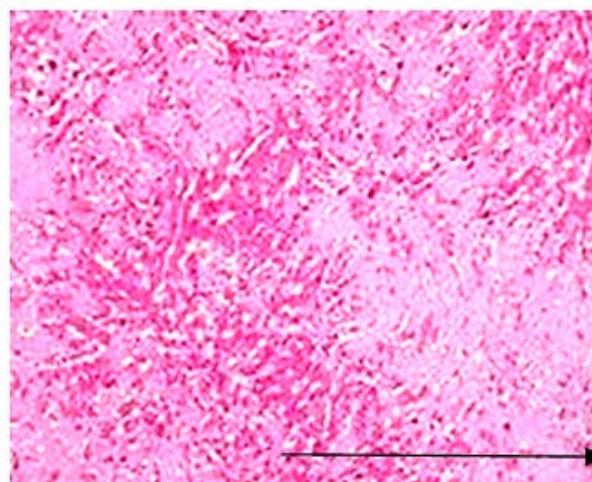
(a) Kidney



(b) Liver



(c) Testis



(d) Lung

Fig. 4: Photomicrograph of some vital organs stained with hematoxylin and eosin (H & E) stain of mice treated with highest 2000 mg/kg b.w of *M. capitatum* root extract for 28 days; the arrows indicate partial necropsies in the kidney and liver. 400x.

4. Discussion

Recently, it has been estimated that about 80 % of the population of low-income countries are fully dependent upon herbal medicines for their primary healthcare. Higher medicinal plants are the major source of drug in traditional medicine [22].

The World Health Organization (WHO) also estimated that 80 to 90 % of the world's population relies mainly on local herbal practitioners [23]. Medicinal plants are used as tinctures, poultices, powders and teas followed by formulations, and lastly as pure compounds. The extracts from these plants have been used by humans since time immemorial for treating many ailments. These plants have also provided very important drugs such as analgesics (like morphine), antitussives, antihypertensives, cardiogenic, antineoplastics, and antimalarials. Even though African traditional medicine is the oldest and probably the most diverse of all healthcare systems yet, detailed documentation on the use of medicinal plants in Africa is lacking till date. Aside, with rapid urbanization in most places, traditional oral knowledge is dwindling fast. The side effects resulting from the use of herbal drugs could be very fatal if not checked early, and this aspect of the toxicological study has not been given the needed attention.

Animals have been used as models for centuries to predict what chemicals and environmental factors would do to humans. The earliest uses of experimental animals are lost in prehistory, and much of what is recorded in early history about toxicology testing indicates that humans were the models of choice. This work consisted of dosing test animals with known quantities of agents (poisons or drugs) and included the careful recording of the resulting clinical signs and gross necropsy observations. The use of animals as predictors of potential ill effects has grown since that time. Animal experiments also have served rather successfully as identifiers of potential hazards and toxicity in humans for conventional drugs with many intended uses [24]. This is the main reason why mice were used in this study. Behavioural changes such as body itching, reddish eyes, and weight loss are salient indices for determining the effects of short-term use of herbal drugs on animals [25]. In this present study, in the acute study, there were no observable signs of deaths or mortality at 300 mg/kg b.w of *M. capitatum* aqueous root extract. But at a higher dose of 2000 mg/kg b.w MCRE, there was reddish eyes, itching and restless in the mice which only lasted for a few minutes, as seen in Table 1.

Subchronic toxicity study revealed significant weight increase from week one to week four among the treatment groups ($p < 0.05$; one-way ANOVA). These increases were significantly different from the control group, which suggest that the extract must have supplied the animals with other nutrients for their metabolism. The animals did not show much increase in the relative weights of organs in the doses administered, but there was no loss of weights from the lowest dose of 250 mg/kg b.w to the highest dose of 2000 mg/kg b.w. (Table 2) The result further suggests that the extract did not induce any form of infection in the rats at these doses. It has been reported that many herbal drugs or medicinal plants when used in the treatment of diseases usually induced infections in the animals due to unhygienic ways of preparation and storage [26].

In hematology and clinical pathology, damage to the cardiovascular systems is usually indicated by the values of the hematological parameters. From our study, all the hematological parameters increased in a dose-dependent fashion (Table 3). The monocytes and basophils do not show further increase at 1000 and 2000 mg/kg b.w. doses in the rats which suggest that high doses of the extract do not have a significant effect on these WBC differentials (Figure 1). However, these values were within the acceptable range, and any significant decrease in them could suggest a sign of anomalies in the body, hence the 28 days subchronic administration of this extract further suggests that *M. capitatum* methanol leaf extract does not possess any deleterious toxicological effects on the rats at the doses investigated.

Our study also revealed that at the doses of 250, 500, 1000, and 2000 mg/kg b.w. serum phosphate ions and uric acid decreased progressively. Serum sodium ion (Na^+) had the highest value compared to other serum electrolytes, followed by serum chloride ion (Cl^-) (Figure 2). It is, therefore, justified on why the sweet-salty taste of the plant root is chewed by the Fulani herder's communities in Nigeria as a remedy for stomach ache.

The liver is the largest organ in the body of an animal, which is involved in the metabolism of toxic substances and drugs. To know the condition of the liver, liver enzyme biomarkers are important parameters to use in the medical diagnosis of liver related ailments. It has been reported that elevated levels of alanine transaminase (ALT), alanine phosphatase (ALP) and aspartate transaminase (AST) biomarker enzymes in the liver cells is an indication of a diseased liver condition [27]. On the other hand, ALT is a better biomarker enzyme that is specific to liver injuries, because AST is indicative of

disease condition of other body organs such as the muscles and heart. In addition, an elevated ALP value is an indication that the bile duct is not normal or there is blockage of the bile duct due to the tumor. From the results in Table 4, ALT, AST, and ALP values do not increase beyond their normal recommended range for mice. Bilirubin is a product obtained from the breakdown of hemoglobin, and it is often linked to liver diseases such as jaundice and nonmetabolic red blood cells. From our study, the progressive decrease in the value of bilirubin at doses of 250, 500, 1000, and 2000 mg/kg b.w. showed that MCRE does not present any injuries to the liver or the heart (Figure; 4a-d).

High levels of lipid a condition known as hyperlipidemia is the major cause of atherosclerosis in humans. In cardiovascular pharmacology, less attention has been paid to hyperlipidemia, unlike hypolipidemia. Our study had revealed that oral administration of *M. capitatum* root extract (MCRE) on mice for 28 days reduces low density lipoproteins (LDL) at increased doses while other lipid profiles namely: high-density lipoproteins(HDL), very low-density lipoproteins (VLDL), total cholesterol(TCH), triglycerides(TRG), and plasma glucose (GLU) showed slight dose-dependent values (Figure 3). These values were not different when compared to the untreated normal control group at $p < 0.05$ (one-way ANOVA). The low value of TCH obtained from this study further supported the previous findings by Ukwubile *et al.* (2016) when they showed that MCRE can reduce total cholesterol level in albino mice [28, 29]. The presence of cells within the lumen of vital organs has been reported to cause blockage of blood vessels thereby, obstructing the normal flow of blood [30]. In this present study, histopathological examination of some of the organs namely: kidney, liver, heart, and testis (Fig.4a-d) do not show the presence of cells in the lumen, necrosis, liver congestion and thickening of cells, which further affirmed safety of the plant extract in the treatment of certain diseases in traditional medicine in Nigeria.

Finally, we conclude that *Melastomastrum capitatum* aqueous root extract is considered safe at the doses investigated in the animals. Nevertheless, it is safer to use the extract at moderate doses because, high dose (2000 mg/kg b.w) of the root extract is slightly toxic to the mice, and this effect can be seen in humans when used in traditional medicine for the treatment of diseases.

5. Conclusion

In these present findings from our study, it is concluded that aqueous root extract of *Melastomastrum capitatum* is not toxic at lower doses and well tolerated at higher doses. This is because there were no serious clinical symptoms in the animals after oral subchronic study. There were no mortalities in all the groups, and organ necropsies as well as dense liver congestion. The initial results showed the potentials of exploring therapeutic and pharmaceutical products of interest from the root extract which has reduced or no adverse effects. It is, therefore, recommended that further study be to carry out in to determine the toxicity effect of *M. capitatum* root extract animal embryos, as well as gestation cycles of animals for comparing results. Finally, the use of *M. capitatum* root extract as an oral medication in traditional medicines for disease treatment should be done at doses which do not elicit any abnormality in its host.

6. Declaration

6.1 Conflict of Interest

We have no conflict of interest.

6.2 Funding

No available source of funding from external bodies except by the authors.

6.3 Author's Contribution

This work was designed by Cletus Anes Ukwubile, Emmanuel Oise Ikpefan, and Mathias Simon Bingari. Proper laboratory research and the first manuscript draft was done by Cletus Anes Ukwubile while Livinus Tam, Emmanuel Oise Ikpefan and Mathias Simon Bingari are collaborators.

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