Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2024 June Vol 6 Issue 2: 113-120
©2024 College of Veterinary Medicine,
Michael Okpara University of Agriculture, Umudike, Nigeria
Original Research

Acute and subacute toxicity profiling of methanol extract of *Combretum dolichopetalum* leaf in albino rats

[*1]Madubuike, K.G., [2]Daniel-Igwe, G., [1]Ukwueze1, I.J. & [1]Nganya, C.

[1]*Department of Veterinary Physiology and Pharmacology,* [2]*Department of Veterinary Pathology Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria*

**Correspondence:* madubuike.kelechi@mouau.edu.ng; + 2348036689778

ABSTRACT

The leaf of *Combretum dolichopetalum* is widely used in ethnomedicine to treat cases of diarrhea, inflammation and open wound. This study was hence, undertaken to determine the acute and subacute toxicity profile of the plant in albino rat model. A total of 426 g of dried and pulverized leaves of the plant was extracted with 2.5 L of 80% methanol by cold maceration method. A modified up-and-down method was employed for the oral acute toxicity study, with the extract administered at a maximum dose of 4000 mg/kg. For the subacute toxicity study, 18 rats were assigned into 3 groups (n = 6). Group A (control) received distilled water (5 ml/kg), while groups B and C were given 200 and 400 mg/kg of the extract, respectively. All the treatments were delivered orally for 28 days, after which blood samples were collected for hematology and serum biochemistry. Some vital organs were harvested for histological examination. The result of the acute toxicity recorded neither death nor morbidity even at the highest dose of the extract. For the subacute study, 400 mg/kg of the extract caused significant ($p < 0.05$) increase in total cholesterol and low density lipoprotein cholesterol (LDL-C); and significant ($p < 0.05$) decrease in high density lipoprotein cholesterol (HDL-C) in the treated rats when compared with the control. The histology slides showed only a mild fatty infiltration of hepatocytes in the group treated with 400 mg/kg of the extract. The results indicate a high safety index of the *Combretum dolichopetalum* leaf, however prolonged administration of high doses may cause hyperlipidemia.

Keywords: *Combretum dolichopetalum*; toxicity; extract; antioxidant; hematology.

INTRODUCTION

Dependence on medicinal plants by man for healthcare delivery has been on before civilization (Banes *et al*., 2008). The World Health Organization (WHO) estimates that about 80% of the human population particularly in developing countries rely on herbs for their primary health challenges (Shizha & Charema, 2011). Herbal remedies are generally perceived to be safer (less toxic) when compared to orthodox medication (Sumner, 2000; Cohen & Erst, 2010; Ijioma *et al*., 2014), however, there are reports of serious and sometimes life-threatening adverse effects after administration of natural health products. Toxicity studies on medicinal plants, using appropriate animal models therefore, constitute an integral and very essential component of pre-clinical studies in drug development (Arthur *et al*., 2011, Sana *et al*., 2023).

Combretum dolichopetalum (family, *Combretaceae*) is a wild tropical plant, widely distributed in Nigeria and neighboring countries. The plant is reputed in folklore medicine for treating diarrhea, gastric ulcer, wounds and inflammatory conditions. Extracts of the roots and leaves of *C. dolichopetalum* have been reported to possess various pharmacological properties, including: antidiarrheal (Asuzu *et al*., 1992; Onoja *et al*., 2015), antidiabetic (Uzor *et al*., 2014), antitrypanosomal (Nnadi *et al*., 2021), antioxidant and wound healing properties (Barku *et al*., 2016). However, there is paucity of information on effects of the plant on the various organs of the body, especially when used repeatedly. This study was therefore designed to determine the acute and subacute toxicity profile of *C. dolichopetalum* in an albino rat model.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of *Combretum dolichopetalum* were collected from its natural habitat in Amaokwe Ugwu Nkpa, in Bende

http://doi.org/10.54328/covm.josvas.2024.186

Local Government Area of Abia State, Nigeria. Identification of the plant was done by Prof. M. C. Dike of the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Nigeria. A voucher specimen with identification number: MOUAU/VPP/2016/16 was deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

PREPARATION OF PLANT EXTRACT

The leaves were dried under shade (22 - 29°C) and pulverized into coarse powder. A total of 426 g of the powdered leaves was macerated in 2.5 L of 80% methanol for 72 h at room temperature (22 - 26°C), with intermittent shaking. The residue was removed by filtration and the filtrate (extract) was concentrated in a rotary evaporator and dried in hot air oven (40 °C). The yield was determined and the extract was stored in a refrigerator (4 °C) as MECD (methanol extract of *Combretum dolichopetalum*) until time of use (Madubuike *et al*., 2014).

EXPERIMENTAL ANIMALS

Mature albino rats, bred in the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, were used for the experiments. The animals weighed between 110 and 120 g, and were housed in steel cages made of stainless metals. They were given standard pelleted feed (Vital feed®, Nigeria Limited) and clean drinking water, *ad libitum*. The experimental protocols were approved by the institution's Ethics Committee for use of Laboratory Animals (Approval No.: MOUAU/CVM/REC/201918).

ACUTE TOXICITY STUDY

An oral acute toxicity testing of MECD was done following a modified up-and-down-procedure. Three (3) albino rats were dosed orally with 4000 mg/kg of MECD, while another 3 rats (untreated control) received equivalent volume of water orally. The rats were given feed and drinking water *ad libitum* for 14 days during which they were observed for signs of toxicity and death (OECD, 2008).

SUBACUTE TOXICITY STUDY

A total of 18 male rats (n=6), were randomly assigned to three (3) groups, (A – C). Rats in group A received distilled water (5 ml/kg), while rats in groups B and C were treated with MECD 200 and 400 mg/kg, respectively. All the treatments were given orally, once daily for 28 days. Blood was obtained from the retro-orbital plexus of each of the treated rats into EDTA and plain sample bottles for hematological and serum biochemical analysis. Afterwards, the rats were humanely sacrificed and vital organs (liver, kidney, lung and heart) were harvested for histopathological examination.

BODY WEIGHTS AND RELATIVE ORGAN WEIGHTS (ROW)

The body weights of the rats as well as the relative weights of the liver, kidney, lung and heart were determined using a sensitive weighing balance.

HEMATOLOGICAL PARAMETERS

The hemoglobin concentration was determined by the Cyanomethemoglobin method (Kachmar, 1970). The packed cell volume was estimated using the microhematocrit method as described by Coles (1986). The hemocytometer method described by Schalm *et al*. (1975) was employed for the white blood cell and red blood counts.

LIVER MARKER ENZYMES

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were evaluated following the method of Reitman & Frankel (1957); alkaline phosphatase (ALP) was measured using the method of Kind & King (1972); total bilirubin was assayed by the method of Jendrasik & Grof (1938) while the direct biuret method (Gornall *et al*., 1948) was adopted for estimation of total protein.

KIDNEY FUNCTION PARAMETERS

The method of Bauer *et al*., (1982) was followed to determine serum urea concentration. Urea was hydrolyzed to ammonia in the presence of urease, and read photometrically at 546 nm. Serum creatinine level was estimated using the method of Cockroft & Gault (1976). Creatinine reacts with picric acid in an alkaline medium to produce creatinine alkaline picrate, a colored compound, which was read photometrically at 546 nm.

LIPID PROFILE

The enzymatic colorimetric chod-pad test as described by Allain *et al*., (1974) was employed for the estimation of total cholesterol (TC), while triglyceride (TG) was evaluated by the method of Tietz (1990). High density lipoprotein (HDL) assay was done by the method of Grove (1979) as described in Randox kit. The method of Bergmenyer (1984) was employed to determine the low density lipoprotein cholesterol (LDL-C), as the difference between total cholesterol and the cholesterol contained in the supernatant, following precipitation of the LDL-C fraction of polyvinyl sulfate in the presence of polyethylene-glycol monomethyl ether. Very low density lipoprotein cholesterol (VLDL-C) was calculated as the one-fifth of TG (Wilson *et al*., 1981).

LIPID PEROXIDATION/ *IN VIVO* ANTIOXIDANT EFFECT OF MEPS

Lipid peroxidation was evaluated by determining the level of malondialdehyde (MDA), a product of lipid peroxidation in living tissues. Thiobarbituric acid reacts with MDA to form a pink or red colored complex which was read photometrically

(Draper & Hadley, 1990). Catalase was estimated based on the method of Aebi (1984), in which decomposition of hydrogen peroxide (H_2O_2) with catalase resulted in decreases in the ultraviolent absorption of H_2O_2. The method of Sun *et al*., (1988) was followed to determine superoxide dismutase (SOD) activity. Xanthine-xanthine oxidase system was used to generate a superoxide flux while nitroblue tetrazolium (NBT) was used as the indicator of superoxide production. The activity of SOD was estimated by the degree of inhibition of the reaction unit of enzyme providing 50% inhibition of NBT reduction.

HISTOPATHOLOGY

Tissue samples from the vital organs (liver, kidney, lung and heart) were fixed in 10% formalin for 24 h. They were afterwards washed in ascending grades of ethanol, cleared with xylene and embedded in paraffin wax, then sectioned with a microtome, stained with hematoxylin and eosin (H & E) and examined under a microscope (Bancroft & Stevens, 1997). Using an Olympus photomicroscope, photomicrographs of observed histopathological lesions were obtained.

STATISTICAL ANALYSIS

Data obtained from the study were presented as mean (± S. E. M.) and analyzed using One Way Analysis of Variance (ANOVA). The Least Significance Difference (LSD) of the different groups was used to separate the variant means, and significance was accepted at the level of $P \leq 0.05$.

RESULTS

EXTRACTION

The extraction process yielded 65 g of dried crude extract of *Combretum dolichopetalum*, giving a 15.2% yield. The extract was greenish brown, pasty and has a sweet smell.

ACUTE TOXICITY STUDY

Within the 14 days observation period neither death nor any other sign of toxicity was recorded in both the control and the extract-treated groups.

SUBACUTE TOXICITY STUDY

VISUAL OBSERVATION

The rats appeared healthy and there was no observable sign of toxicity throughout the 28 days experimental period.

BODY WEIGHTS AND RELATIVE ORGAN WEIGHTS

There were continuous increases in body weight of rats across the groups, however, significant difference was not observed between the control and the MECD-treated groups ($P > 0.05$) throughout the duration of the study (Table I). Relative weights of the vital organs (liver, kidney, heart and lung) also, did not show significant difference ($P > 0.05$) between the control and the groups treated with MECD (Table II).

HEMATOLOGY

The hematological indices (hemoglobin concentration, packed cell volume, red blood cell count and white blood cell count) of rats treated with both doses of the extract (200 and 400 mg/kg) did not differ significantly ($P > 0.05$) from those of the control group (Table III).

LIVER ENZYMES MARKERS

The extract, at the doses tested did not cause any significant change in any of the concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) or total protein (TP) of the extract-treated groups when compared with the control. However, the level of total bilirubin was significantly increased in the treated groups when compared with the control (Table IV).

KIDNEY FUNCTION PARAMETERS

The result showed that the extract at the doses tested did not cause any significant difference ($P > 0.05$) in the level of urea and creatinine in the treated groups when compared to the control.

LIPID PROFILE

The result showed that treatment with 400 mg/kg of MECD caused significant ($P < 0.05$) increase in the concentrations of total cholesterol and low density lipoprotein-cholesterol in the extract-treated when compared with the control. The levels of triglycerides and very low density lipoprotein-cholesterol were increased significantly ($P < 0.05$) while high density lipoprotein was decreased in the groups treated with 200 and 400 mg/kg of MECD, when compared with the control (Table V).

***IN VIVO* ANTIOXIDANT ACTIVITY**

The result showed that treatment with MECD for 28 days caused significant ($P < 0.05$) increase in the activities of catalase and superoxide dismutase in the extract-treated rats when compared with the control. However, the level of malondialdehyde in the extract-treated groups did not differ significantly ($p > 0.05$) from that of the control group (Table IV).

HISTOPATHOLOGY

Histological examination of the vital organs showed that treatment with 200 and 400 mg/kg of MECD did not cause any lesion in the lungs, heart and kidney of the rats. However, there was fatty infiltration in the liver of rats treated with 400 mg/kg of MECD (Plates 1A-C).

Table I. Body weight of albino rats treated with MECD for 28 days

Treatment	Mean body weight ± S.E.M. (g)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Water (5 ml/kg)	111.0 ± 3.78	120.3 ± 4.62	131.5 ± 4.81	142.4 ± 6.64	153.9 ± 5.90
MECD (200 mg/kg)	107.6 ± 7.74	116.8 ± 6.71	125.0 ± 7.12	132.1 ± 9.33	140.0 ± 13.23
MECD (400 mg/kg)	114.0 ± 2.63	122.2 ± 4.50	136.7 ± 5.11	151. 5 ± 9.96.	174.9 ± 7.03

Note: Values are presented as Mean ± SEM (Standard Error of Mean) MECD = methanol extract of *Combretum dolichopetalum*

Table II. Relative organ weights of albino rats treated for 28 days with MECD

Treatment	Relative organ weight (x 10^{-3} g)			
	Heart	Liver	Kidney	Lung
Water (5 ml/kg)	4.1 ± 0.56	37.0 ± 3.13	6.3 ± 0.41	5.8 ± 0.80
MECD (200 mg/kg)	3.9 ± 0.21	38.9 ± 2.46	6.1 ± 0.33	6.1 ± 0.37
MECD (400 mg/kg)	4.0 ± 0.23	35.1 ± 1.55	6.2 ± 0.30	6.4 ± 0.63

Note: Values are presented as Mean ± SEM (Standard Error of Mean), MECD = methanol extract of *Combretum dolichopetalum*

Table III. Hematological parameters of albino rats treated for 28 days with MECD

Treatment	HB (g/dl)	PCV (%)	RBC (x 10^6)	TWBC (x 10^3)
Water (5 ml/kg)	12.6 ± 0.33	32.4 ± 0.77	74.1 ± 1.41	7.1 ± 1.51
MECD (200 mg/kg)	11.9 ± 0.69	30.2 ± 2.18	71.3 ± 3.19	6.3 ± 0.56
MECD (400 mg/kg)	13.6 ± 0.68	34.7 ± 1.77	79.5 ± 3.99	7.8 ± 0.75

Note: Values are presented as Mean ± SEM (Standard Error of Mean). HB = Hemoglobin, PCV = Packed cell volume, RBC = Red blood cell, TWBC = Total white blood cell, MECD = methanol extract of Combretum dolichopetalum

Table IV. Liver function parameters of albino rats treated for 28 days with MECD

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	TB (mg/dl)
Water (5 ml/kg)	27.7 ± 4.04	75.1 ± 14.39	46.0 ± 8.15	4.8 ± 0.66	0.02 ± 0.01
MECD (200 mg/kg)	27.3 ± 6.65	69.2 ± 13.49	52.4 ± 5.96	4.4 ± 0.39	0.10 ± 0.01*
MECD (400 mg/kg)	24.4 ± 3.84	79.8 ± 8.08	60.2 ± 12.70	5.4 ± 0.09	0.10 ± 0.03*

Note: Values are presented as Mean ± SEM (Standard Error of Mean). Superscript (*) shows significant ($P > 0.05$) difference, when compared with control. ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline Phosphatase, TP = Total protein, TB = Total bilirubin, MECD = methanol extract of Combretum dolichopetalum

Table V. Lipid profile of albino rats treated with MECD for 28 days

Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Water (5 ml/kg)	90.6 ± 2.3	98.2 ± 21.3	29.5 ± 2.5	41.5 ± 4.4	19.6 ± 4.3
MECD (200 mg/kg)	85.2 ± 4.8	119.1 ± 20.6	20.0 ± 3.0*	41.4 ± 8.7	23.8 ± 4.1
MECD (400 mg/kg)	123.5 ± 7.9*	117.6 ± 13.3	25.9 ± 1.4*	74.1 ± 8.9*	23.5 ± 2.7

Note: Values are presented as Mean ± SEM (Standard Error of Mean). Superscript (*) shows significant ($P < 0.05$) difference, when compared with control. TC = Total cholesterol, TG = Triglyceride, HDL = High-density lipoprotein; VLDL: Very-low-density lipoprotein; LDL: Low-density lipoprotein, MECD = methanol extract of Combretum dolichopetalum.

Table VI. *In vivo* antioxidant profile of albino rats treated for 28 days with MECD

Treatment	Creatinine (mg/dl)	Urea (mg/dl)
Water (5 ml/kg)	0.87 ± 0.06	44.28 ± 2.62
MECD (200 mg/kg)	0.90 ± 0.07	38.97 ± 5.66
MECD (400 mg/kg)	0.83 ± 0.09	47.02 ± 3.45

Note: Values are presented as Mean ± SEM (Standard Error of Mean). MDA = Malondialdehyde, SOD = Superoxide dismutase, CAT = Catalase, MECD = methanol extract of Combretum dolichopetalum

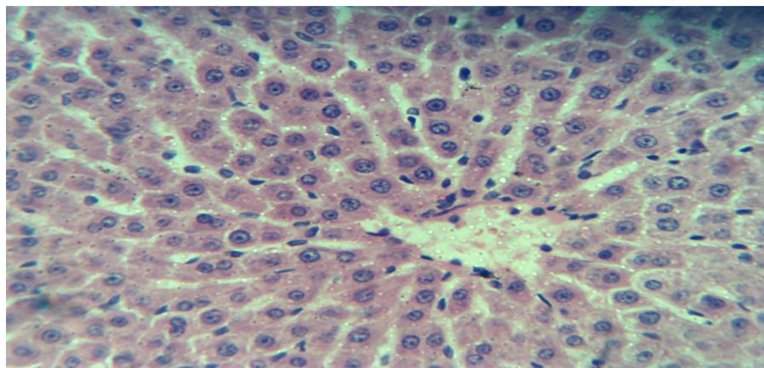

Plate 1A: Histologic section of rat's liver

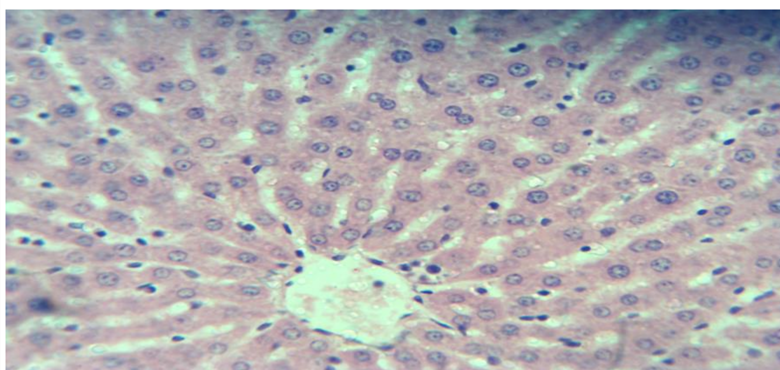

Plate 1B: Photomicrograph of rats' liver section *after 28 days treatment with 200 mg/kg of methanol extract of Combretum dolichopetalum* (H&E ×400). The section shows normal liver

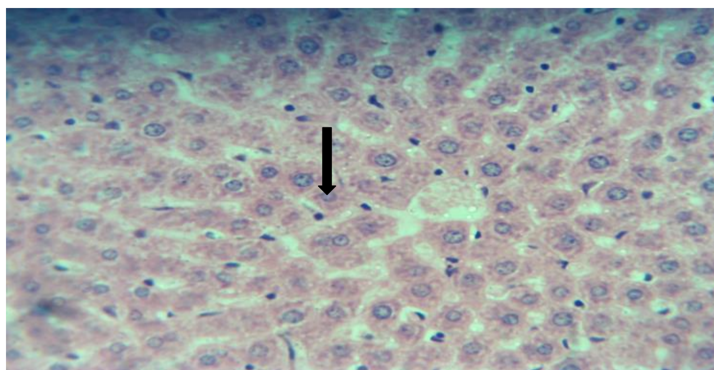

Plate 1C: Photomicrograph of rats' liver section after 28 days treatment with 400 mg/kg of methanol extract of Combretum dolichopetalum (H&E ×400). The arrow shows fatty infiltration of hepatocytes

DISCUSSION

Evaluation of the toxicity profile of *Combretum dolichopetalum* involved both acute and subacute toxicity studies because treatment with herbal medications and chemotherapeutic agents may involve single or repeated administration (Madubuike *et al*., 2020). The use of hydromethanolic (80% methanol) extract for the study was to mimic the traditional way of using the plant for healing purposes.

Behavioral changes (such as abdominal contortions, piloerection, respiratory distress, convulsion etc.) and death are vital indices for toxicological evaluations (Costa *et al*., 2011). There was neither death nor any observable sign of toxicity during both the acute and chronic toxicity studies suggesting that the extract was tolerated by the rats. Nisha *et al.* (2009) identified changes in body and organ weights as useful indicators for predicting the toxicity of a compound or plant extract. In the subacute toxicity study, body weight-gain was recorded in all the extract-treated groups as well as the control group. This is attributed to normal growth of the rats with age. The hematopoietic system is a very sensitive target for poisonous compounds; hence hematological indices provide important information on the response of the body to toxic substances (Mukinda & Eagles, 2010). The administration of MECD at 200 and 400 mg/kg for 28 days did not cause any significant ($P > 0.05$) variation in hematological parameters of treated rats when compared to the control. This may indicate that the extract, at the doses tested had no toxic effect on the haematopoietic system of the rats.

The liver is the principal organ responsible for detoxification of drugs and xenobiotics (Bagban *et al*., 2012), hence the levels of serum liver biomarker enzymes (ALT, AST, ALP etc.) are vital indicators of hepatotoxicity. Although AST is less specific than ALT as a marker of liver damage, elevation in the serum levels of these two enzymes indicate hepatic tissue damage and altered membrane permeability (Satpal & Punia, 2010; Kolawole *et al*., 2013). Alkaline phosphatase and bilirubin are markers of biliary function and cholestasis, while total protein reflects the liver's synthetic function (Davern & Scharschmidt, 2002; Harris, 2005). In this study, the extract did not cause any significant ($P > 0.05$) difference in the levels of ALT, AST, ALT and total protein in the treated rats, when compared to the control, indicating that subacute administration of MECD at the doses used did not cause hepatocellular damage.

Bilirubin is mainly derived from the breakdown of haem in the red blood cells. This unconjugated bilirubin then binds to albumin and is taken up by the liver where it is conjugated, making it water soluble, which allows for its excretion

(Giannini *et al*., 2005). In this study, both doses of MECD significantly ($P < 0.05$) increased total bilirubin in the treated groups when compared to the control group. It may have been that MECD decreased hepatic function with respect to conjugation and excretion of bilirubin. It may also be that the extract caused increase in the rate of haem degradation, hence elevating the concentration of unconjugated (indirect) bilirubin in the rats.

Proteins are synthesized in the liver and form the major portion of dissolved substances in the plasma. Liver disorders are sometimes associated with decreased levels of serum protein concentrations (Kiple, 2003). In this study, the concentration of total protein in the extract-treated rats did not vary significantly ($P > 0.05$) from that of the control, suggesting that the liver's synthetic function was not affected by MECD.

Creatinine and urea are vital indicators of kidney function (Mukinda & Eagles, 2010). Treatment with MECD in this study did not cause significant ($P > 0.05$) difference in the levels of urea and creatinine suggesting that subacute administration of MECD did not interfere with renal function.

The administration of MECD to the experimental rats for 28 days resulted in significant ($p < 0.05$) increase in total cholesterol, triglycerides, LDL-C and VLDL-C, and significant ($p < 0.05$) decrease in HDL in treated rats when compared to control rats. This result showed that the extract may enhance the pathway of endogenous cholesterol synthesis and inhibit the synthesis of hepatic LDL-C receptors which are responsible for taking up LDL-C into the liver, resulting in increased LDL-C/HDL-C ratio in the MECD-treated rats (Rang *et al*, 2007). This hyperlipidemic effect of MECD suggest that it may have the potential to induce or worsen already existing health conditions associated with hyperlipidemia such as diabetes mellitus and cardiovascular diseases associated with atherosclerosis (Fuster *et al*., 2005).

The antioxidant activity of MECD following subacute (28 days) administration was evaluated by measuring the concentration of MDA, SOD and catalase in serum. Malondialdehyde (MDA) is an endogenous genotoxic product of enzymatic and reactive oxygen species (ROS)-induced lipid peroxidation. Its level is widely used as a marker of lipid peroxidation in conditions of elevated oxidative stress (Niedernhofer, 2003). On the other hand, catalase, a hemoprotein, which catalyzes the reduction of H_2O_2, is known to be involved in detoxification of H_2O_2 concentrations (Bakirel *et al*., 2008). Superoxide dismutase is considered an important defense enzyme, being involved in the direct elimination of ROS. It also catalyzes the dismutation of superoxide radicals and hence diminishes the toxic effect of these radicals (Arulsevan & Subramaniam, 2007). The results showed that the MECD did not cause significant ($P > 0.05$) change in the level of any of the antioxidant parameters assayed, in the treated groups, when compared with the control. This finding suggests that MECD, at the doses used had no effect on the antioxidant defensive mechanism of the body (Miao *et al*., 2003).

Histopathological examination of some vital organs revealed fatty infiltration of hepatocytes in the group treated with 400 mg/kg of MECD. This could be as a result of fat deposits in the liver caused by the high cholesterol level. This is a mild degenerative change and so, is easily reversible (Sleisenger, 2009).

In conclusion, the acute toxicity testing of methanol extract of *Combretum dolichopetalum* leaf showed neither morbidity nor mortality in the rats. The subacute toxicity study did not record any deleterious effect in any of the vital organs examined, except a mild degenerative change (fatty infiltration) of hepatocytes observed in the liver of rats treated with the high dose of the extract.

ACKNOWLEDGEMENT:

We wish to acknowledge Mr. Egeonu of the Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike for his assistance in preparing the histology slides.

CONFLICT OF INTEREST:

We wish to declare that there is no known conflict of interest associated with this publication.

REFERENCES

Aebi, H.E. (1984). Catalase: In Bergmeyer (Ed). Methods of Enzymatic Analysis (3rd ed., pp. 273-285). Weinheim, Germany: Verlag Chemie.

Allain, C.C., Poon, L.S., Chan, L.S., Richmond, C.S.G. & Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clinical. Chemistry, 20*, 470-475.

Arthur, F.K.N., Woode, E., Terlabi, E.O. & Larbie, C. (2001). Evaluation of acute and subchronic toxicity of *Annona muricata* (Linn) aqueous extract in animals. *European Journal of Experimental Biology, 1*, 115-124.

Aruselvan, P. & Subramanian, S.P. (2007). Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra-structural changes of pancreatic β-cells in experimental diabetes in rats. *Chem. Biol. Interact., 165*, 155-164.

Asuzu, I.U. & Njoku, J.C. (1992). The pharmacological properties of the methanolic root extract of *Combretum dolichopetalum*. *Phytotherapy Research*, *6*(3), 125-128.

Bagban, I.M., Roy, S.P., Chaudhary, A., Das, S.K., Gohil, K.J. & Bhaudhary, K.K. (2012). Hepatoprotective activity of the methanol extract of *Fagonia indica* (Burn) in carbonterachloride-induced hepatoprotectivity in albino rats. *Asian Pacific Journal of Tropical Biomedicine, S*1457-14560.

Bakirel,T., Bakirel, U., Keles, O.U., Ulgen, S.G. & Yardibi, H. (2008). *In vivo* assessment of antidiabetic and antioxidant activities of rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. *Journal of Ethnopharmacology, 116*, 64-73.

Bancroft, J.D. & Stevens, A. (1977). Theory and practice of histological techniques (1st edition). New York, U.S.A: W. B. Saunders.

Banes, P.M., Bloom, B. & Nahin, R. (2008). Complementary and alternative medicine used among adults and children. United States 2007 CDC National Health Statistics Report, No. 12.

Barku, V.Y., Yao, A., Boye, A & Erzah, F. (2016). In-vitro antioxidant and wound healing properties of *Combretum dolichopetalum. Journal of Applied Pharmaceutical Science, 6*(5), 185-192.

Bauer, J.H., Brooks, C.S. & Burch, R.N. (1982). Renal function studies in man with advanced renal insufficiency. *American Journal of Kidney Diseases, 2*, 30-35.

Cockroft, D. W & Gault, M.H. (1976). Predictions of creatinine clearance from serum creatinine. *Nephron, 16*, 31-41.

Cohen, P.A. & Ernst, E. (2010). Safety of herbal supplements: A guide for cardiologist. *Cardiovasc. Ther. 28*, 246-253.

Coles, E. H. (1986). Determination of packed cell volume. In: Coles E. H. W. Veterinary Clinical Pathology (pp. 17-19). Philadelphia: W. B. Saunders Co.

Costa, C.A., Bidinotto, L.T., Bidinotto, L.T., Takahira, R K., Salori, D.M., Barbisan, L.F. & Costa, M. (2011). Cholesterol reduction and lack of genotoxic or toxic effects in mice after 21-day oral intake of lemongrass (*Cymbopogon citratus*) essential oil. *Food Chem. Toxicol. 49*, 2268-2272.

Davern, T.J. & Scharschmidt, B.F. (2002). Biochemical liver test: In Feldman, M., Friedman, L. S. & Sliesenger, M.H. (Eds.), Sleisenger and Fordtran's Gastrointestinal and liver disease: pathophysiology, diagnosis, management (7th ed., pp. 1227-1238). Philadelphia: W. B. Saunders.

Draper, H.H.& Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol. 186*, 421-431.

Fuster, V., Eric, J. & Nabel, E.G. (2005). Patho-biology of asymptomatic atherosclerosis leading to symptomatic atherothrombosis. *J. Am. Cardiol., 46*, 937-941.

Giannini, E.G., Testa, R., Savario, V. (2005). Liver enzyme alteration: a guide for clinicians. *CMAJ, 172*, 367-379.

Gornall, A.G., Bardawill, C.S., David, M.M. (1948). Determination of serum proteins by means of the biuret reactions. *J. Biol. Chem., 177*, 551.

Grove, T.H. (1979). Effect of reagent pH on determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clin. Chem., 25*, 260-264.

Harris, E.A. (2005). Elevated liver function tests in type 2 diabetes. *Clin. Diab., 23*, 115-119.

Ijioma, S.N., Okafor, A.I., Ndukuba, P.I. & Akonas, S.C. (2014). Hypoglycaemic, haematologic and hypolipidemic activity of *Jatropha tanjorensis* ethanol leaf extract in alloxan-induced diabetic rats. *Annals Biol. Res., 5*, 15-19.

Jendrassik, L. & Grof, P. (1938). Vereinfactite photometrische methoden zur bestmmung des blutbiluribins. *Biochem. Z., 297*, 82-89.

Kachmar, S. F. (1970). Determination of blood haemoglobin by the cyanomethaemoglobin procedure. In: Tiez, N. W. (Ed.). Fundamentals of Clinical Chemistry (pp 268-269). Philadelphia: W. B. Saunders Company.

Kind, P.R.N. & King, F.J. (1954). Alkaline phosphatase determination. *Clin. Pathol., 7*, 322.

Kiple, K.F. (2003). The Cambridge Historial Dictionary of Disease. (1st Ed.). New York: Cambridge University Press.

Kolawole, O. T., Akanji, M. A. & Akiibinu, M. O. (2013). Toxicological assessment of ethanolic extract of the leaves of Newbouldia laevis (P. Beauv). *American Journal of Medicine and Medical Sciences*, *3*(4), 74-80.

Madubuike, K.G., Ezeja, M.I. & Onoja, S.O. (2014). Studies on the analgesic property of the methanolic leaf extract of *Cymbopogon citratus* Stapf. *Cont. J. Pharmacol. Toxicol. Res., 7*, 1-7.

Madubuike, K.G., Anaga, A.O. & Asuzu, I.U. (2020). Chronic toxicity study of *Pterocarpus santalinoides* extract in albino rats. *Tropical Journal of Pharmaceutical Research, 19* (11), 2407-2413.

Miao, H., Jia-Wei, M., Wei-Lin, He, M., Min, J., Kong, W., He, X. Li, J. & Peng, B. (2016). A review on the pharmacological effect of vitexin and isovitexin. *Fitoterapia, 115*, 74-85.

Mukinda, J.T & Eagles, F.K. (2010). Acute and sub-chronic oral toxicity profile of the aqueous extract of *Pohygala fruticosa* in female mice and rats. *J. Ethnopharmacol., 128*, 236–240.

Niedernhofer, L.J., Daniels, J.S., Rouzer, C.A., Greene, R.E. & Marnett, L.J. (2003). Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J. Biol. Chem., 278*, 31426-31433.

Nnadi, C.O., Onuku R., Ayoka, T.O., Okorie, N. H. & Nwodo, N.J. (2022). Chemical constituents of *Combretum dolichopetalum*: characterization, antitrypanosomal activities and molecular docking studies. *Tropical Journal of Pharmaceutical Research, 21*(4), 801-808.

Nisha, A., Muthukumar, S.P. & Venkateswaran, G. (2009). Safety evaluation of arachidonic acid rich Mortierella alpine biomass in alnino rats – a subchronic study. *Reg. Toxicol. Pharmacol. 53*, 186-194.

OECD (2008). Guidelines for the testing of chemicals: Acute oral toxicity- up-and-down-procedure (UDP). https://www.oecd.org/env/test-no-425-acute-oral-toxicity-up-and-down-procedure-978926407149-en.htm. Accessed 06/06/21.

Onoja, S.O & Udeh, N.E. (2015). Antidiarrheal effects of hydromethanolic extract of *Combretum*

dolichopetalum. *Journal of Coastal Life Medicine, 3*(11), 910-913

Rang, H.P., Dale, M.M., Ritter, J.M. & Flower, R.J. (2007). Rang and Dale's Pharmacology (6th ed.). Philadelphia: Churchill Livingstone/ Elsevier.

Reitman, S. & Frankel, S. (1957). Methods of alanine and aspartate amino transferase determination. *Am. J. Clin. Pathol., 28*, 56-58.

Sana S, Fareeha A, Aslam K, Uzma T.I. (2023). *Journal of Ethnopharmacology, 311*, https://doi.org/10.1016/j.jep.2023.116447

Satpal, S.K. & Punia, J.S. (2010). Studies on biochemical changes in subacute thiodicarb toxicity in rats. *Toxicol. Int., 17*, 30-32.

Schalm, O.W., Jain, N.C. & Carrol, E.J. (1975). Veterinary haematology (3rd ed.). Philadelphia: Lea and Febieyer.

Shizha, E. & Charema, J. (2011). Health and wellness in South Africa: Incorporating indigenous and western healing practices. *Int. J. Psychol. Counsel., 3*, 167-175.

Sleisenger, P.F. (2009). Diagnosis of liver diseases. In: Sleisenger and Fordtran's gastrointestinal and liver disease pathophysiology, diagnosis, and management (9th ed., p. 212). Philadelphia: Saunders Publishing.

Sumner, J. (2000). The Natural History of Medicinal Plants. Portland, Oregon: Timber Press.

Sun, Y., Oberley, L.W. & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clin. Chem., 3*, 497-500.

Tietz, N.W. (1990). Clinical guide to laboratory test (2nd ed.). Philadelphia, U.S.A.: W. B. Saunders Company.

Uzor, P.F., Osadebe, P.O., Emeje, E.O. & Agbo, M.O. (2014). Bioassay guided isolation and isolation and evaluation of the antidiabetic principles of *Combretum dolichopetalum* root. *British Journal of Pharmacology, 4*(18), 215-71.

Wilson, P.W., Abbott, R.D., Garrison, R.J & William, P.C. (1981). Estimation of very low- density lipoprotein cholesterol from data on triglyceride concentration in plasma. *Clin. Chem., 27*, 2008–2010.