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Safety Assessment of Repeated Oral Administration of *Motandra paniculata* (Poir) I.M. Turner (Apocynaceae) Leaf Ethanol Extract in Sprague-Dawley Rats

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ABSTRACT

***Background*:** *Motandra paniculata* (Poir.) I.M. Turner (Apocynaceae) is used traditionally in some African communities to treat nervous disorders and malaria fever. It has also been reported in literature to have antiproliferative activity. This may increase the use of this plant in local populations and a paucity of information on its toxicity profile thus calls for its investigation. The study was undertaken to carry out a 30-day safety assessment of repeated use of the crude ethanol leaf extract (MPLE) of the plant.

***Methods*:** Three doses of MPLE were administered to 24 adult albino rats at 3 doses (50, 100, 200 mg/kg). Biochemical parameters from plasma, haematological parameters from blood, histopathological and *in vivo* antioxidant parameters were obtained from tissue samples and statistically compared using Analysis of variance (ANOVA) with Dunnett post hoc comparison.

***Results*:** Findings show that there was no significant difference ($p > 0.05$) in body weight of the animals, organ weight of animals, biochemical parameters (except non-consequent ALP fluctuations), haematological parameters (apart from red blood cell count consistent with observations from a recent sub-acute toxicity study), histological characteristics of brain, liver, kidney and heart tissues isolated from the animals after oral administration of the extract and no marked changes in the levels of antioxidant parameters in the studied organs (except in the heart).

Conclusion: These observations corroborate the relative sub-chronic safety of *Motandra paniculata* from previous studies due to the repeated safe oral administration of MPLE in rats.

***Keywords*:** Megastigmane-type terpenes, *Motandra paniculata*, rats, repeated-dose toxicity, safety assessment.

1. INTRODUCTION

The African continent offers a rich variety of plants that serve as a source of income and medicine to the native and indigenous people. These plants have been used for ages to manage ailments that plague man and animals. The knowledge of these medicinal plants has been passed down from generation to generation through oral tradition [1]. *Motandra paniculata* (Poir.) I.M. Turner also known as *Motandra guineensis* (Thonn) A.DC is a plant that is native to the wet rainforest biome of Southwest Nigeria [2]. Its leaves have been used to manage mouth abscesses and sedation [2], to manage malaria and fever [3] in some indigenous African populations. The crude extract showed metabolic effects in rabbits [4], had cytotoxic activity against brine shrimp nauplii, human ovarian cancer, skin melanoma [5-6] and displayed relative safety in normal cells [6]. Chemical compounds, mainly meantime-type terpenes were isolated from the crude extract by means of high-performance liquid chromatography (HPLC) [5]. The compounds isolated from the plant were

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shown to be effective in an *in vitro* study to arrest the growth of skin melanoma and ovarian cancer cell lines [5]. A recent study in rats showed that the plant extract was safe after a 14-day repeated oral administration [5]. These recent information on the antiproliferative activity of the plant in addition to the traditional use of the plant may prompt increased use of the plant and its products to treat various ailments including chronic disorders. Hence, this has necessitated this investigation to assess how safe this plant may be upon repeated use beyond short term. The present study aims to carry out a 30-day safety assessment of continuous oral use of the crude ethanol leaf extract of the plant.

2. MATERIALS AND METHOD

2.1 Materials

2.1.1 Biological materials: Plant Collection, Identification and Extraction

Motandra paniculata leaves were collected in May 2025 according to the World Health Organisation (WHO) Good Agricultural and Collection Practices (GACP) from Kajola Village, Osun State, Nigeria (7.55°N, 4.529°E; Altitude: 297m). The plant material was identified and authenticated by Mr. Ifeoluwa Ogunlowo at the Faculty of Pharmacy, Ile-Ife (FPI) Medicinal Plants Herbarium, Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria and assigned the voucher number, FPI 2658 and also deposited there (https://sweetgum.nybg.org/science/ih/herbarium-details/?irn=253433). Leaves of the plants were oven dried at 40°C, pulverised to obtain the dried plant material, extracted by maceration using 95% ethanol for 72 hours (7 L). Extracts were dried under reduced pressure at 30°C using a rotary evaporator (Buchi) to obtain crude extracts.

2.1.2 Chemicals and reagents

95 % ethanol, Tween 80, distilled water, formalin, haematoxylin, eosin.

2.1.3 Equipment and other materials

Rotary evaporator (Buchi), cages, beddings, rat feed,oral cannula, syringes, surgical knives, forceps, surgical blade, EDTA sample bottles, non-heparinised centrifuge sample bottles, biochemical assay kits, antioxidant assay kits, centrifuge, light microscope, analytical balance, spectrophotometer

2.2 Methods

2.2.1 Toxicity Testing

2.2.1.1 Animals

The animals were handled according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines. Twenty-four Sprague-Dawley rats (12 males, 12 females) were purchased from the Nigerian Institute of Medical research, Yaba, Lagos. The animals were restrained with 3 animals per cage for male group and 3 animals per cage for female group. The animals were kept in 12 hours light, 12 hours dark and fed at room temperature and given water *ad libitum.* Acclimatisation of the animals to their surroundings was done for 14 days as per the International Standard for Animal Handling Practice.

2.2.1.2 Toxicity Testing Protocol

The dosages selected were 50 mg/kg, 100 mg/kg and 200 mg/kg derived from the sub-acute toxicity evaluation of this plant [5]. Experimental animals were divided into 4 groups [1 control group and 3 dosage groups - 50, 100, 200 mg/kg as earlier stated [7] administered per group. Body weights were taken on designated days (1st, 8th, 15th, 22nd, 29th and 31st days). The doses were administered orally once daily at a 24-hour interval for 30 days and the vehicle (2% Tween 80 in distilled water) was administered to the control group in the experiment. Upon completion of the study, the animals were fasted for 24 hours with access to water only. Blood samples were thereafter collected via ocular puncture using capillary tubes into EDTA bottles and non-heparinised centrifuge tubes for haematological and biochemical analyses, respectively. Body weights of the animals were adequate before sacrifice because there was no extreme weight loss compared to the control group as the animals experienced a steady weight gain (40 - 60 %) during the study. Animals were humanely euthanised in accordance with the approved institutional protocol prior to tissue collection. Animals were subsequently sacrificed by cervical dislocation and target organs — including the brain, kidneys, liver, and heart — were excised, weighed, and processed for histological examination [8]. Tissue homogenates prepared from the isolated organs were analysed for *in vivo* antioxidant activity [9]. Tissue sections were stained with haematoxylin and eosin and examined under ×100 magnification [5].

2.3 Statistical Analysis

All data were processed and analysed using Microsoft Excel and presented as as mean ± standard error of meanwhile statistical comparisons were performed via one-way Analysis of Variance (ANOVA) for organ weight, haematology, biochemistry and *in vivo* antioxidant analysis while two-way repeated-measures ANOVA analysis was done for body

weight at a 95% significance level ($p < 0.05$) indicating statistical significance respectively. Post hoc analysis was done using Dunnett's test for multiple comparisons (GraphPad Prism 9.5, San Diego, CA) [5].

3. RESULTS

3.1 Extract yield

The yield of crude extract was 8.47 % (150.84 g) from was 1.78 kg of dried plant material.

3.2 Sub-chronic Toxicity Testing

The animals were grouped according to Table 1. At the end of the 30 days study duration, there was no death across all groups.

3.2.1 Body Weight of Rats

No statistically significant difference was observed in animal body weights throughout the study period on comparing the treatment groups to the control ($p > 0.05$). The obtained weight values plotted against time (in days) is illustrated in Figure 1.

Table 1: Grouping of rats for animal study

Group	Dose	Number of males	Number of females
I	Control (2 % Tween 80	3	3
II	50 mg/kg	3	3
III	100 mg/kg	3	3
IV	200 mg/kg	3	3
Total		12	12

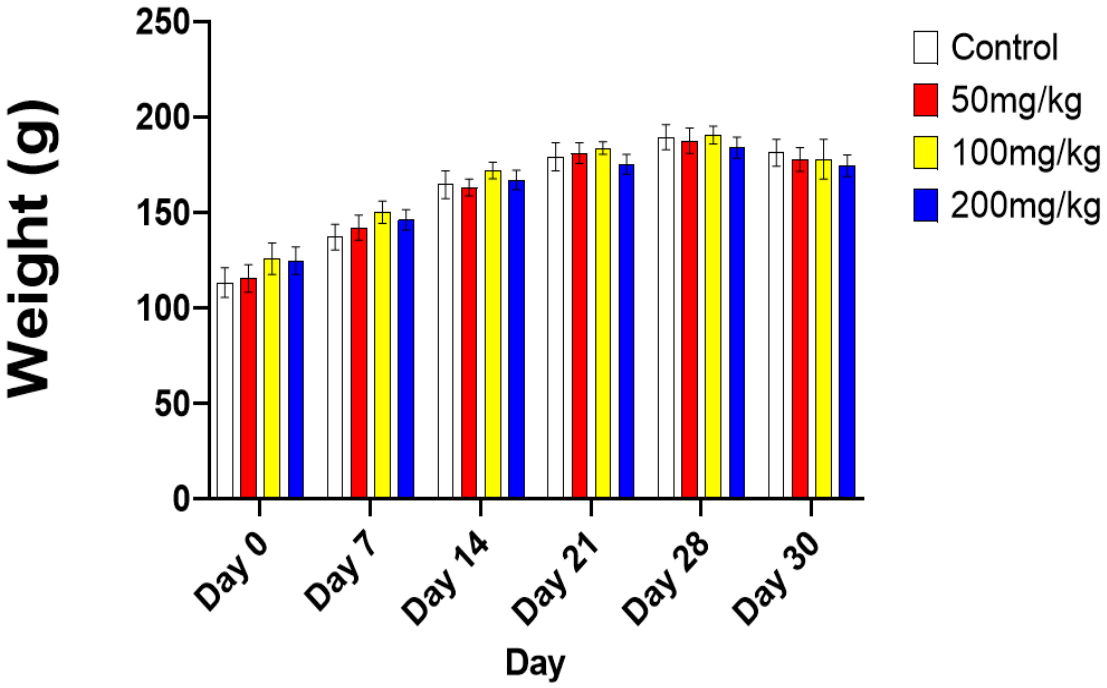

Figure 1: Body weight of rats after 30-day treatment with *M. paniculata* leaf crude extract Organ Weight of Rats

Organ weights showed no statistically significant differences across the various treatment groups compared to the control as observed in Figure 2 and table 2.

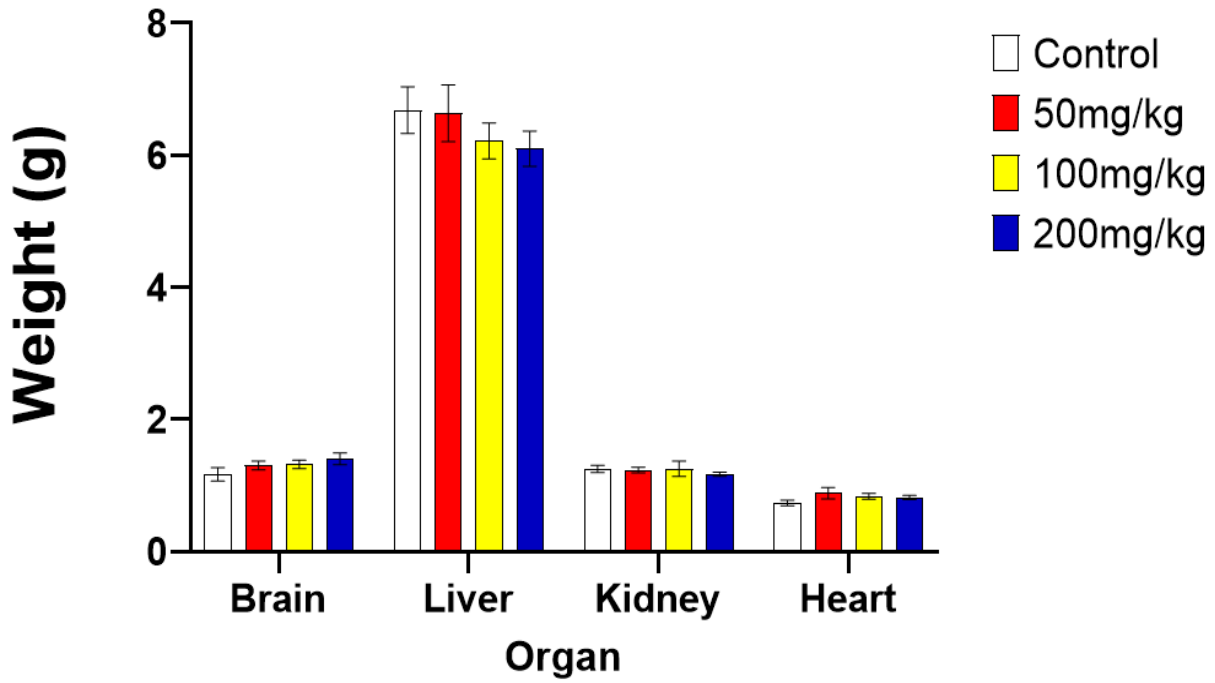

Figure 2: Organ weight of rats after 30-day treatment with *M. paniculata* leaf crude extract

Table 2: Organ weight of rats after 30-day treatment with *M. paniculata* leaf crude extract

Organ weight	Brain	Liver	Kidney	Heart
Control	1.17±0.14	6.68±0.50	1.25±0.14	0.73±0.10
50 mg/kg	1.30±0.10	6.63±0.61	1.23±0.10	0.88±0.12
100 mg/kg	1.32±0.09	6.22±0.67	1.25±0.29	0.83±0.10
200 mg/kg	1.40±0.13	6.10±0.38	1.17±0.08	0.82±0.04

3.2.2 Biochemical Parameters Following Oral Administration

A majority of biochemical parameters showed no statistically significant difference following treatment with *M. paniculata* leaf crude extract except for alkaline phosphatase whose standard deviation at 100 mg/kg is very large, which may be due to an outlier [Table 3].

Table 3: Effect of 30-day treatment of *M. paniculata* leaf crude extract on biochemical parameters in rats

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg
AST (U/L)	166.53±2.63	174.70±19.23	170.93±21.11	161.82±13.40
ALT(U/L)	37.33±3.06	40.80±3.48	37.78±5.16	36.12±4.38
ALP (U/L)	223.12±25.06	199.69±23.36	307.68±102.23***	223.14±31.66
PROT (g/L)	72.45±1.83	75.07±2.75	73.64±1.88	70.71±6.05
ALB (g/L)	43.71±1.41	43.20±0.87	43.03±2.29	43.79±2.28
T BIL (umol/L)	7.43±0.37	6.75±0.17	7.01±0.07	7.58±0.20
DBIL (umol/L)	1.86±0.06	1.86±0.01	1.80±0.04	1.83±0.03
UREA (mmol/L)	5.24±0.21	6.41±0.64	5.71±0.43	4.88±0.66
CREAT (umol/L)	75.17±9.55	89.94±8.23	73.49±8.47	78.32±12.56
Na (mmol/L)	148.05±0.69	152.43±0.82	147.68±0.69	147.58±0.54
K (mmol/L)	5.67±0.12	5.91±0.53	5.19±0.22	5.00±0.32
Cl (mmol/L)	103.05±0.75	104.40±0.67	103.38±0.63	103.93±0.44
HCO_3 (mmol/L)	18.68±1.46	11.88±1.77	14.38±2.12	15.78±0.34
TCHOL(mmol/L)	2.25±0.13	1.92±0.17	1.85±0.10	2.24±0.24
TRIG (mmol/L)	0.80±0.18	1.17±0.29	0.85±0.04	0.54±0.20
HDL (mmol/L)	1.85±0.10	1.31±0.28	0.77±0.04	0.96±0.11
LDL (mmol/L)	0.88±0.19	0.54±0.20	0.50±0.12	0.60±0.16

*** = significantly different from control ($p < 0.001$)

3.2.3 Haematological Parameters Following Oral Administration

Most haematological parameters showed no statistically significant difference between the treatment and control groups following oral administration of *M. paniculata* leaf crude extract. However, red blood cell count was significantly different across all treatment groups when compared to the control ($p < 0.0001$) [Table 4].

Table 4: Effect of 30-day treatment of *M. paniculata* leaf crude extract on haematological parameters in rats

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg
WBC x 10^3/µL	7.35±3.02	7.27±1.86	8.11±2.65	11.18±2.59
L %	75.78±2.47	73.15±1.62	73.10±4.95	76.73±2.41
M %	8.08±0.61	9.70±0.75	9.55±1.70	8.20±0.76
N %	16.15±1.94	17.15±1.32	17.35±4.47	15.08±1.67
RBC x 10^6/µL	6.56±1.37	8.10±0.56****	8.17±0.25****	7.92±0.18***
Hb (g/dL)	12.73±2.78	16.40±0.51	16.33±0.56	16.20±0.64
HCT %	43.15±9.44	55.78±1.94	51.73±1.84	49.70±1.70
MCV (fL)	64.83±1.54	69.45±3.14	63.30±0.39	62.70±0.91
MCH pg	19.15±0.60	20.45±0.98	19.95±0.18	20.48±0.56
MCHC (g/dL)	29.53±0.65	29.48±1.01	31.55±0.16	32.60±0.55
PLT x 10^3/µL	636.50±148.01	760.75±44.77	777.50±81.84	863.75±31.82

*** = significantly different from control ($p < 0.001$), **** = significantly different from control ($p < 0.0001$)

3.2.4 In Vivo Antioxidant Parameters of Isolated Organs

Analysis of *in vivo* antioxidant parameters revealed no statistically significant differences in the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), and catalase (CAT) across all doses following treatment with *M. paniculata* leaf crude extract relative to the control groups. However, statistically significant differences were recorded in heart tissues across doses compared to the control ($p < 0.05$) with *in vivo* antioxidant parameter levels generally higher than those of the control group (Table 5).

Table 5: Effect of 30-day treatment of *M. paniculata* leaf crude extract on *in vivo* antioxidant parameters

		In vivo antioxidant parameters			
Organ	Treatment (mg/kg)	GSH	SOD	CAT	MDA
Kidney	Control	0.62±0.03	0.69±0.06	1.80±0.16	4.30±0.12
	50	0.91±0.01	0.66±0.03	2.22±0.01*	6.85±0.29****
	100	0.88±0.01	0.78±0.02	2.13±0.05	6.44±0.07****
	200	0.84±0.01	0.72±0.02	2.08±0.05	6.28±0.18****
Liver	Control	0.67±0.11	0.53±0.03	1.53±0.33	4.81±0.85
	50	0.78±0.04	0.63±0.08	1.81±0.09	5.90±0.07
	100	0.73±0.16	0.56±0.10	1.78±0.47	5.71±1.52
	200	0.56±0.01	0.64±0.01	1.94±0.05	4.82±0.09
Brain	Control	0.66±0.03	0.79±0.05	1.77±0.21	4.95±0.65
	50	0.77±0.09	0.83±0.02	1.59±0.13	4.48±0.35
	100	0.73±0.02	0.73±0.03	2.07±0.10	4.30±0.29
	200	0.85±0.10	0.80±0.15	2.27±0.15	4.72±0.48
Heart	Control	0.67±0.02	0.37±0.02	0.66±0.01	1.92±0.17
	50	0.86±0.02*	0.51±0.01	1.00±0.02***	1.32±0.02****
	100	1.07±0.01***	0.67±0.01**	1.23±0.02****	1.33±0.03****
	200	1.40±0.00****	0.10±0.01**	1.59±0.03****	1.94±0.09

* = significantly different from control ($p < 0.05$) ** = significantly different from control ($p < 0.01$), ***= significantly different from control ($p < 0.001$), ****= significantly different from control ($p < 0.0001$)

3.2.5 Histological examination of isolated organs

Microscopical examination of cut and stained sections of kidney, liver, brain and heart tissues show no structural damage or pathological changes in the tissues as seen in plates 1 - 4.

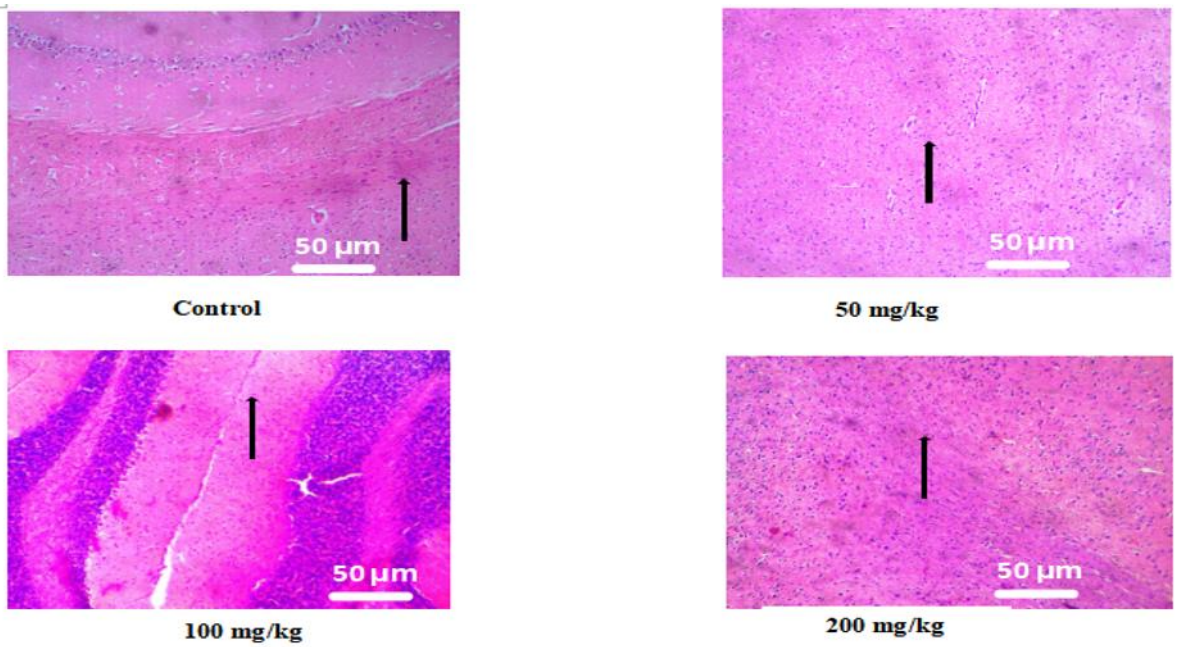

Plate 1: Histopathological sections of rat cerebral (brain) tissues following 30-day oral administration of *M. paniculata* leaf crude extract. Neuronal cell bodies, indicated by black arrows, are visible against a neuropil background, with no histopathological abnormalities detected in any of the treatment groups relative to the control. Magnification: × 100 (Haematoxylin & Eosin)

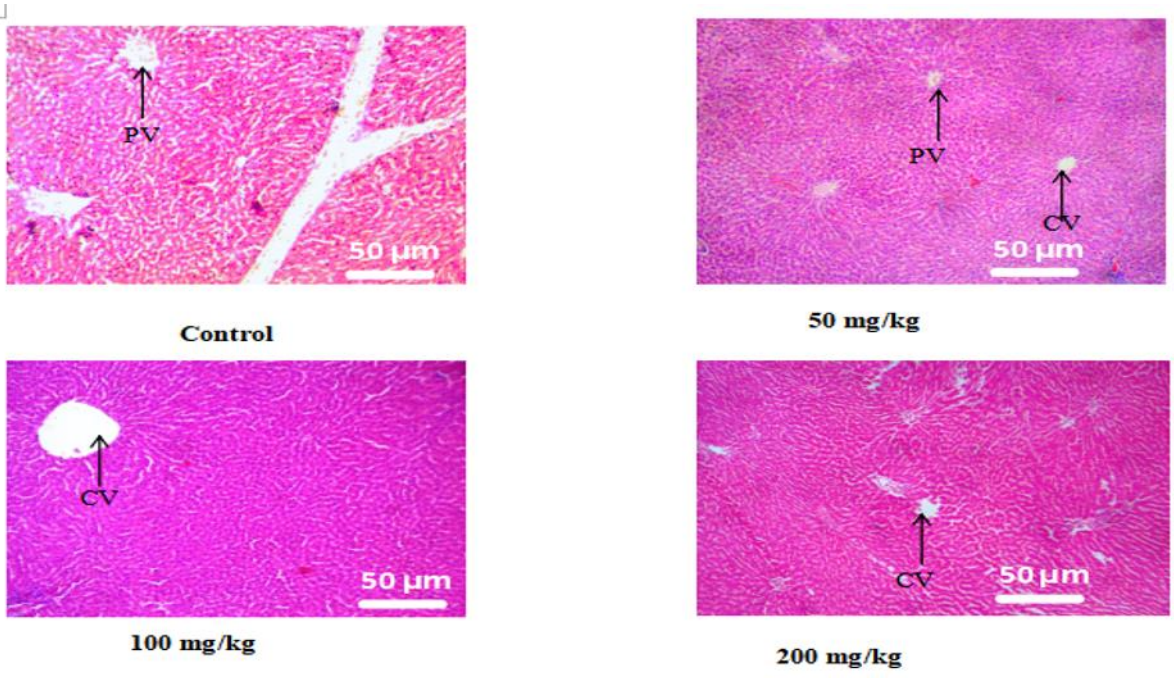

Plate 2: Histopathological sections of rat liver tissues following oral administration of *M. paniculata* leaf crude extract. Hepatocytes (indicated by black arrows) appear histologically normal with no observable abnormalities relative to the control. Key structural landmarks including the central vein (CV) and branch of the portal vein (PV) are clearly identifiable. Magnification: ×100 (Haematoxylin & Eosin stain).

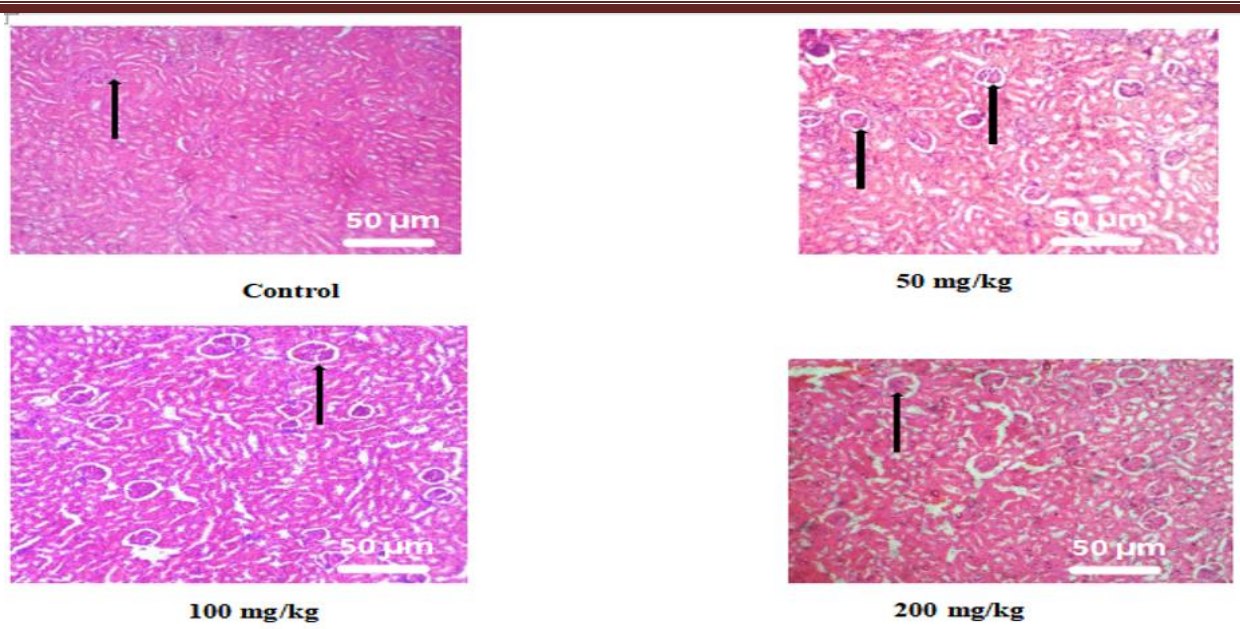

Plate 3: Histopathological sections of rat kidney tissues following oral administration of *M. paniculata* leaf crude extract. Normocellular glomerular tufts are observed against a background of intact, viable glomeruli (indicated by black arrows), with no histopathological abnormalities detected in any of the treatment groups relative to the control. Magnification: ×100 (Haematoxylin & Eosin stain).

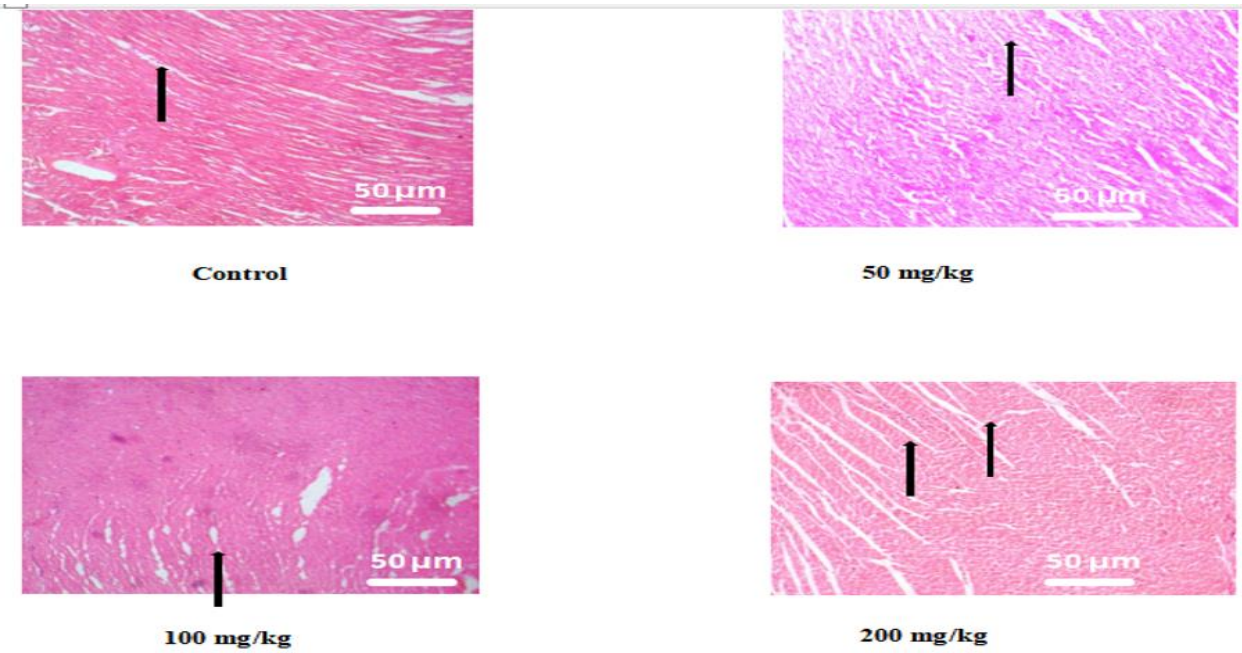

Plate 4: Histopathological sections of rat heart tissues following oral administration of *M. paniculata* leaf crude extract. Interlacing bundles of myocytes (indicated by black arrows) appear histologically normal, with no observable abnormalities detected across all treatment groups relative to the control. Magnification: ×100 (Haematoxylin & Eosin stain).

4. DISCUSSION

Plants are well documented valuable sources of therapeutic agents. However, it is widely established that their bioactive metabolites may pose toxic risks to both humans and animals [10]. This study was therefore undertaken to investigate the toxicity profile of *Motandra paniculata* leaves following 30 days of continuous oral administration. The yield of *M. paniculata* leaf crude extract in this study was low but higher than that obtained from other studies, 6.4 % [11] and 6.7 % [6] for same plant. This suggests that a large quantity of plant material will be required for research on this plant. The ability of the plant to cause toxicity *in vivo* was tested using a 30-day toxicity test on a variety of parameters in laboratory animals. When there are significant alterations in body and organ weights, these are signs of pathological changes or the presence of toxic substances [12]. In this study, animals were weighed at predetermined time points throughout the study period to monitor the effect of the extract on body weight. Administration of varying doses of the plant extract did not adversely affect body weight. Conversely, the rats recorded higher body weights at the end of the study compared to baseline suggesting that the plant exerted no adverse influence on animal growth and did not introduce any toxic agent into the animals. Similarly, no marked changes in organ weights were observed following treatment with different doses of the extract suggesting that the extract had no significant effect on vital organ mass. Biochemical parameters measured from plasma samples showed no significant alterations in many of the indices assessed, indicating that administration of the plant extract did not result in toxicity sufficient to adversely affect these parameters. Changes in electrolyte levels particularly sodium and potassium indicate a case of renal impairment [13]. Because there were no significant changes in electrolyte levels in this study, it may be suggested that the plant extract did not exert any harmful effects on kidney function. Liver function is usually assessed through the measurement of albumin, aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). A diagnosis of liver damage typically requires elevated ALP levels in conjunction with raised bilirubin concentrations [14]. In this study, ALP levels were significantly higher at the 100 mg/kg dose but there was no corresponding elevation in bilirubin indicating no suspicion of hepatotoxicity. This finding agrees with the histological results, which revealed no structural changes in liver tissue. Renal function is usually assessed using urea, creatinine, and protein levels, with the urea-to-creatinine ratio playing an important role in deciding kidney health [15]. The extract showed no adverse effects on these parameters, suggesting intact renal function further corroborated by the healthy histology of kidney tissues as well as absence of electrolyte imbalances. Lipid profile parameters such as total cholesterol (CHOL), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were normal suggesting that there was no evidence that the extract provoked dyslipidaemia or adversely affect metabolic homeostasis [16]. Haematological evaluation revealed no significant changes in most blood parameters following treatment, suggesting that the extract did not induce abnormal haematopoiesis. Continuous administration over 30 days had no consequential impact on white blood cell (WBC) but had a meaningful effect on red blood cell (RBC) production compared to the control group. Previously, a significant rise in RBC count was recently reported in a sub-acute toxicity study [5], an effect which was also observed in this present study, possibly proposing that this effect is a property of the plant. This observation requires further investigation through chronic toxicity studies. There was no notable increase in platelet count observed ruling out haemostatic dysregulation or liver-related dysfunction [17]. Haematocrit levels indicated no untoward impact on erythrocyte morphology or formation. Neutrophil counts were within normal limits suggesting an absence of infection or inflammatory responses attributable to the extract. Lymphocyte percentages were similarly within normal levels as seen in the normal L % levels indicating no disruption to normal immune cell production. Haemoglobin levels were maintained across all treatment groups showing that the extract did not compromise oxygen-carrying capacity [17]. There were no significant changes in corpuscular indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) suggesting that the extract is unlikely to cause anaemia or impair haemoglobin synthesis [17]. Superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) are powerful endogenous antioxidants. SOD is one of the most potent antioxidants in the cell which catalyses the conversion of superoxide to hydrogen peroxide. CAT converts hydrogen peroxide to water and molecular oxygen completing the work initiated by SOD [18]. GSH is responsible for detoxifying hydroperoxides, peroxynitrites, lipid peroxides and heavy metals as well as scavenging hydroxyl radical, carbon radicals, superoxide anion and nitric oxide [19]. Malondialdehyde (MDA) is a product of polyunsaturated fatty acids peroxidation and is used as a biomarker of oxidative stress [20]. The influence of the plant extract on the *in vivo* antioxidant parameters from isolated organs was also studied. This study revealed that there were no noticeable changes in the levels of these parameters in all studied organs apart from the heart. It may be suggested that the extract though showing high levels of these parameters in the heart increased protection against oxidative damage and this may be a good property. MDA, which is a sign of oxidative stress, was statistically different at low doses in the heart but showed no significant difference at the highest dose. This suggests that the initial effect on oxidative stress reduces or disappears at the highest dose. The kidney also shows significant increases of MDA (about 20 - 50 %) which indicates mild oxidative stress. However, these values are insufficient to establish nephrotoxicity. A statistically significant increase in renal MDA indicates oxidative stress in kidney tissue. However, a diagnosis of renal nephrotoxicity and tissue damage cannot be confirmed except increase in MDA levels present concurrently with other parameters such as increased creatinine, increased blood-urea nitrogen (BUN), renal tissue damage as well as reduced *in vivo* antioxidant enzyme levels [21, 22]. These are not present in this study

therefore renal toxicity cannot be concluded to have occurred. Microscopical examination after staining with haematoxylin and eosin and viewing at × 100 magnification showed that the isolated tissues were devoid of any form of toxicity or irregularities. While this study shows relative safety of the extract, further studies need to be carried out to investigate the effect of the plant extract on the red blood cell count, fluctuations observed in ALP levels as well as fluctuations in heart MDA levels.

CONCLUSION

Motandra paniculata showed no toxicity on weight, end organs, biochemical parameters, haematological parameters, *in vivo* antioxidant parameters or abnormalities in brain, kidney, liver and heart tissues. The findings indicate that repeated oral administration of *M. paniculata* leaf ethanol extract for 30 days was not associated with overt systemic toxicity in Sprague-Dawley rats, supporting its relative sub-chronic safety. Further chronic toxicity and mechanistic studies are warranted. The results corroborate with the relative safety of the short term use of the plant as established in previous studies.

List of Abbreviations

ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
ARRIVE	Animal Research: Reporting of *In Vivo* Experiments
AST	Aspartate transaminase
CAT	Catalase
CHOL	Cholesterol
Cl	Chloride ion
CV	Central vein
D BIL	Direct Bilirubin
EDTA	Ethylene diamine tetra acetate
FPI	Medicinal Plants Herbarium Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria
GACP	Good Agricultural and Collection Practices
GSH	Reduced glutathione
Hb	Haemoglobin
HCO_3	Bicarbonate ion
HCT (PCV)	Haematocrit (Packed Cell volume)
HDL	High density lipoprotein - Cholesterol
K	Potassium ion
LDL	Low density lipoprotein - Cholesterol
L%	Lymphocytes percentage concentration
M%	Monocytes percentage concentration
N%	Neutrophils percentage concentration
paniculata	*Motandra paniculata*
MCH	Mean corpuscular Haemoglobin
MCHC	Mean corpuscular Haemoglobin concentration
MCV	Mean corpuscular Volume
MDA	Malondialdehyde
MPLE	Crude ethanol leaf extract of *M. paniculata*
Na	Sodium ion
PLT	Platelets
PROT	Protein
PV	Portal vein
RBC	Red blood cells
SOD	Superoxide dismutase
T BIL	Total Bilirubin
TCHOL	Total cholesterol
TRIG	Triglycerides
WBC	White blood cells
WHO	World Health Organisation

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Conflict of Interest

The authors declare no competing interests.

Contributions of the Authors

JOO: Conceptualisation, Methodology, Investigation, Formal analysis, Writing—original draft, Writing—review and editing, final approval of manuscript; AAO: Writing—original draft, Writing—review and editing, critical revision of manuscript; BOO: Writing—original draft, Writing—review and editing, critical revision of manuscript, AOA: Resources, Writing—original draft; VOA: Resources, Writing—original draft.

Ethics approval and consent to participate

The animal studies were done in compliance with the guidelines of the Animal Care and Use Research Ethics Committee of the College of Medicine of the University of Lagos, Nigeria (CMUL/ACUREC/06/25/2033). All experimental procedures were approved and consented to by the Animal Care and Use Research Ethics Committee of the College of Medicine of the University of Lagos, Nigeria in a letter dated 18th August 2025 as well as consent obtained from the Animal House laboratory where the animals were purchased from. A total of 24 healthy Sprague-Dawley rats of both sexes were used, with six animals per group (n = 6). All procedures were conducted in accordance to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.

AI Disclosure statement

No generative AI or large language model was used in the preparation of this manuscript.

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