Toxicology International, *32(1) : 65-80; 2025. DOI: 10.18311/ti/2025/v32i1/44462*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Investigating the Phytochemical Profile and Safety of *Musanga cecropioides* Root Extracts: An *In Vivo* Evaluation in Rats

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Abstract

Originating in Africa, *Musanga cecropioides* (MC) possesses a rich heritage of application in indigenous medicinal systems. This study investigates the phytochemical makeup of MC root extracts and their safety profile in rats. Hydromethanolic (Me70) and aqueous (Aq) extracts of MC roots were prepared using standard techniques. The plant was screened for the presence of bioactive secondary metabolites with potential bioactivity. Acute toxicity was assessed through oral administration of varying doses (10, 1000, and 5000 mg/kg) to rats over two weeks. Sub-acute toxicity was assessed through a repeated-dose oral gavage study using escalating doses (500, 1000, and 2500 mg/kg) administered daily for 28 days. Clinical signs, biochemical markers, and histological examination were employed to assess toxicity in both experiments. Phytochemical analysis revealed the presence of promising chemical constituents within the MC root extracts. Notably, Rats exhibited no adverse effects or behavioural changes following oral administration of the test substance (0-1000 mg/kg), indicating no acute toxicity within this dose range. Sub-acute toxicity testing in rats demonstrated no adverse clinical symptoms, significant biochemical changes, or histological abnormalities at doses up to 2500 mg/kg (compared to controls, $p<0.05$). Based on the experiment, the LD_{50} of MCr Aq extract is 1000 mg/kgwhile the LD_{50} for MCr Me70 extract is 5000 mg/kg. This study demonstrates a lack of apparent toxic effects in animals treated with MC Me70 root extracts, suggesting its potential safety in animal models at doses up to 2500 mg/kg. Further research is warranted to explore the efficacy of these extracts for their intended therapeutic applications.

Major Findings: Phytochemical analysis: *Musanga cecropioides* (MC) root extracts contain promising chemical constituents with potential bioactivity. Acute and sub-acute toxicity: Oral administration of MC root extracts showed no adverse effects in rats at doses up to 2500 mg/kg, indicating a lack of apparent toxic effects. Safety profile: The LD50 of the substance exceeded 2500 mg/kg, suggesting potential safety in animal models at doses up to 2500 mg/kg.

Keywords: *Musanga cecropioides*, LD_{50}, Phytochemical, Root Extract, Sub-Acute Toxicity

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Article Received on: 12.06.2024 Revised on: 21.10.2024 Accepted on: 05.11.2024

1. Introduction

Plants hold immense potential for drug discovery due to their unique capacity for secondary metabolism[1]. This specialized metabolic pathway generates a rich tapestry of bioactive compounds, many of which possess therapeutic properties[2]. These natural products serve as valuable starting points, or "lead compounds," for novel drug discovery[2]. Scientists can then use these natural scaffolds as a foundation for further chemical modifications and optimization, ultimately leading to the creation of more potent and specific therapeutic agents[3]. Many of these compounds possess inherent pharmacological activity, offering potential for direct use in treating or preventing diseases[4]. Among these intriguing botanical resources is MC, the umbrella tree, belonging to the *Urticaceae* family[5]. Native to Africa's tropical regions, this fast-growing, large deciduous tree is easily recognized by its characteristic expansive leaves resembling an umbrella. Traditionally, MC extracts from stem bark and leaves offer a diverse range of potential benefits, including anti-inflammatory, analgesic, antimicrobial, antioxidant, antidiabetic, and antihypertensive properties[5,6].

The scientific basis for these traditional applications is gaining momentum, with research delving into the phytochemical composition of MC and its potential biological activities[7]. Several investigations have revealed a diverse array of bioactive compounds within MC, encompassing flavonoids, alkaloids, phenols, steroids, coumarins and terpenoids, present in leaves, stem bark, root bark and sap[5, 8]. The pharmacological profile of these plant-derived constituents encompasses a diverse spectrum of bioactivities, including: anticholinesterase, antimicrobial, anti-inflammatory, analgesic and antioxidant properties[7, 8]. The observed effects suggest the potential for compounds to exhibit antioxidant properties. This is evidenced by their ability to decrease malondialdehyde levels and elevate superoxide dismutase activities[9,10].

While anecdotal reports suggest potential benefits of *Musanga cecropioides* root (MCr) extracts against various dysfunctions, the lack of scientific validation raises concerns about potential toxicity. Therefore, this research explores the preliminary toxicity profile and phytochemical constituents of hydromethanolic (Me70) and aqueous (Aq) extracts derived from MC roots using a rat model.

2. Materials and Methods

2.1 Reagents

The study utilized high-purity chemicals obtained from Randox Ltd. (USA) through a reputable local vendor.

2.2 Verification and Processing of Plant Specimens

Approximately 3.0 kg of root samples from the plant species MC were procured from privately owned agricultural land situated in the village of *Ulorin*, Ovia North Local Government Area, Edo State, Nigeria. The taxonomic identity of the collected specimens was authenticated by Prof. MacDonald Idu, a renowned taxonomist affiliated with the Department of Plant Biology and Biotechnology at the University of Benin, Benin City. A voucher specimen assigned the accession number UBH-M500, was subsequently deposited within the same departmental repository for future reference. The harvested root samples underwent a meticulous post-collection processing protocol. The extraneous materials were meticulously removed, and the roots were subjected to ambient air drying for two weeks. After drying, the roots were ground into a fine powder using a Gallehamp British Milling Machine. The resulting powder was weighed and stored in airtight, light-protected, and waterproof containers until extraction. Preparation of hydromethanolic (Me70) and aqueous crude extract.

About 200 g of the powdered sample was extracted using the Soxhlet extraction procedure to obtain both a hydromethanolic (Me70) and aqueous (Aq) extract for 72 h. The Me70 extraction utilized 70% methanol, while the Aq extraction used 4.0 litres of distilled water. Following extraction, the filtrates from each process were concentrated using a rotary evaporator (IG-100 Pro, iGene Labserve, New Delhi) to yield a light brown solid residue. After the extraction process, the resulting residual materials were meticulously preserved at a temperature of 4°C, ensuring optimal conditions for further analytical investigations. The extraction procedure employing the 70% methanolic solvent system yielded a substantial quantity of 12.01 g of the concentrated extract, henceforth designated as the Me70 extract. Concurrently, the extraction protocol utilizing an aqueous solvent system yielded a relatively lower quantity of 5.8 g of the concentrated extract, denoted as the Aq extract. Both extracts were transferred to light-protected containers and placed in a Freezer (HMG-Iqf,

India) maintained at -21°C until required for subsequent pharmacological investigations[11,12].

2.3 Phytochemical Analysis

The phytochemical analysis and quantitative estimation of chemical constituents were performed employing a comprehensive array of methodologies. Qualitative analysis was performed to identify the presence of saponins, tannins, anthraquinones, alkaloids, triterpenes, flavonoids, glycosides, reducing sugars, and phlobatannins in the plant material. Established protocols from Trease and Evans[13] and Sofowora[14] were employed.

The quantitative determination of alkaloid content was accomplished through a spectrophotometric technique involving the reaction with bromocresol green, as meticulously delineated by Erdenechimeg *et al.*[15]. This approach facilitated the precise estimation of the alkaloid concentration present within the samples under investigation.

The total phenolic content was quantified through the implementation of the widely acclaimed Folin-Ciocalteu assay, a robust and reliable methodology as described by Lawage*t al.*[16]. This approach leverages the redox reactions involving the phenolic moieties to provide an accurate assessment of their cumulative abundance.

The determination of total flavonoid content was accomplished through the aluminium trichloride ($AlCl_3$) colourimetric assay, a well-established technique that employs catechin as a reference compound, as elucidated by Fattahi *et al.*[17]. This method exploits the complexation of flavonoids with aluminium ions, resulting in a distinctive colour change that can be quantified spectrophotometrically.

The total saponin content was evaluated through the implementation of the vanillin-sulfuric acid assay, a time-honoured protocol outlined by Trease and Evans[13]. This method capitalizes on the specific reaction between vanillin and saponins, yielding a coloured product that can be quantified through spectrophotometric measurements.

2.4 Animal and Management

Wistar rats (100-200 g, both sexes) were used for *in vivo* experiments. These animals were procured from Ambrose Alli University, Ekpoma, and subsequently allowed a two-week acclimatization period within the animal housing facilities of the Department of Animal and Environmental Biology at the University of Benin, Benin City. The rats were housed in standard plastic cages and provided *ad libitum* access to commercially available animal pellets (Vital Feeds, Nigeria) and potable water. The environmental conditions within the animal housing facilities were meticulously controlled, with the animals exposed to a 12-h light/dark cycle and an ambient temperature of 28.0 ± 1.0°C. All animal handling procedures adhered to the stringent protocols outlined by the National Institute of Health (2002) for the ethical use of laboratory animals. Furthermore, the research protocols involving animal handling and experimentation were reviewed and endorsed by the animal use and care committee at the University of Benin, Benin City, with the assigned approval number UBAUCC H0842/66. The administration of the plant extracts to the experimental animals was facilitated through the oro-gastric gavage technique, a widely accepted method that ensures precise dosing and minimizes the potential for exposure-related variability.

2.5 Toxicity Assessment

Toxicity assessments were carried out as described below:

The acute oral toxicity assessment of the hydromethanolic (Me70) and aqueous (Aq) root extracts derived from the plant species MC was conducted in Wistar rats, following the methodological framework delineated by Lorke[18] with minor modifications as described by Usman *et al.*[19] Twenty-four Wistar rats (equal numbers of male and female) were randomly divided into four groups of six animals each (n=6) for the experiment. The control group received tap water orally at 10 ml/kg body weight to serve as a baseline for comparison. In contrast, the remaining three experimental groups were subjected to single oral doses of 10, 1000, and 5000 mg/kg body weight of the Me70 and aqueous extracts of MC roots, respectively. After the extract administration, the animals were meticulously observed at hourly intervals for the initial 12 h, and thereafter, once daily for the ensuing two-week period. Animals were observed for signs of intoxication such as salivation, abnormal posture, laboured breathing, lethargy, and mortality.

2.6 Sub-Acute Toxicity Assessment

The subacute toxicity assessment of the MC Me70 and aqueous root extracts was performed according to the methodological frameworks outlined by Oshomoh *et al.*[11] and Olaniyan *et al.*[20]. The experimental design

encompassed the utilization of Wistar rats of mixed gender, segregated into four distinct cohorts, each comprising six individuals.

The experimental groups were subjected to oral administrations of graded doses of the MC root extracts, specifically 500, 1000, and 2500 mg/kg body weight, respectively. Concomitantly, a control group was established, wherein the animals received a standardized dietary regimen devoid of the investigational extracts. This controlled experimental paradigm was maintained for a duration of 28 days, adhering to the rigorous guidelines prescribed in the OECD 407 protocol[21].

At the end of the 28 days of exposure, the animals underwent an overnight fasting regimen. Subsequently, they were anaesthetized utilizing isoflurane administered in a controlled chamber environment. Following anesthesia, the animals were humanely sacrificed, and approximately 5.0 ml of blood was meticulously collected via cardiac puncture for comprehensive hematological and biochemical analyses.

Furthermore, the organ weights were precisely documented, and vital organs were aseptically harvested for subsequent histological examinations. These analytical procedures were conducted with utmost precision and adherence to established protocols, ensuring the acquisition of accurate and reliable data for the comprehensive evaluation of the subacute toxicity profile of the investigated plant extracts.

2.7 Relative Organ and Body Weights Study

To monitor physiological changes, the animals' body weights were meticulously recorded weekly. Following the experiment, vital organs (liver, kidneys, brain, and heart) were aseptically excised and weighed precisely on calibrated balances, and their relative weights were calculated using established protocols by Wachtel-Galor and Benzie[22].

Relative organ weight (%) = (Organ weight (g) / Body weight (g)) × 100

2.8 Haematological Evaluation

Blood tests assessed immune function and blood cell health in animals. 2 mL of blood was collected aseptically and analyzed for white blood cell types (lymphocytes, monocytes, granulocytes) and red blood cell counts, haemoglobin, hematocrit, and platelets. An automated hematology analyzer (BC-2800) was used.

2.8.1 Biochemical Analysis

Five millilitres (mL) aliquots of whole blood were collected in 5.0 mL lithium heparinized tubes. Following centrifugation at 2500 rpm for 10 min, the plasma supernatant was carefully transferred to sterile plain tubes for subsequent biochemical analysis.

Liver function was evaluated through enzyme activity assays (ALT, AST, ALP, GGT) and measurements of bilirubin (total and conjugated), and total protein using established protocols[23]using a commercially available test kit from BioVision Inc. (USA).

Creatinine and urea were assayed using commercial kits and by principles previously reported by Taylor and Vadgama[24]. Sodium, chloride bicarbonate and potassium were measured using the standard Flame Atomic Absorption Photometry procedure as described by Howe *et al.*[25].

Total cholesterol was determined enzymatically by hydrolysis and oxidation as described by Trinder[26]. Triacylglycerol was estimated after enzymatic hydrolysis by lipases following the Tietz[27] methods. High-density lipoprotein was estimated using a Randox kit. Levels of Low-Density Lipoprotein (LDL) and Very Low-Density Lipoprotein (VLDL) cholesterol were determined indirectly using the Friedewald formula[28].

Lipid peroxidation, a marker of oxidative stress, was evaluated by measuring the serum concentration of Malondialdehyde (MDA), a well-established byproduct of this process. The method employed involved a chromogenic reaction between MDA and Thiobarbituric Acid (TBA), yielding a red-coloured complex with a characteristic absorbance maximum of 535 nm. The intensity of this absorbance is proportional to the MDA concentration, allowing for indirect quantification of lipid peroxidation. Furthermore, the activities of key antioxidant enzymes were assessed to evaluate the cellular defense system against oxidative stress. Superoxide Dismutase (SOD) activity, responsible for dismutating superoxide radicals, was measured using the method described by Misra and Fridovich[29]. Catalase activity, which decomposes hydrogen peroxide, was determined following the protocol outlined by Cohen *et al*[30]. Additionally, the activity of Glutathione Peroxidase (GPx), an enzyme that detoxifies hydrogen peroxide using glutathione, was assessed using the method established by Flohe and Gunzler[31]. Finally, the activity of Glutathione Reductase (GR), responsible for regenerating reduced glutathione, a vital cofactor for GPx, was determined following the method described by Teitze[32].

2.9 Histology of Vital Organs

Following sacrifice, vital organs were carefully removed from the rats and their weights were recorded using a digital balance. To preserve the tissue structure for microscopic examination, the organs were then immersed in a 10% formalin solution for 24 h (fixation). Next, the fixated tissues underwent a dehydration process using graded alcohol solutions followed by xylene, to remove water and prepare them for embedding. The dehydrated tissues were then embedded in paraffin wax to provide support for sectioning. Thin slices (sections) were cut from the paraffin-embedded organs and stained with hematoxylin and eosin dyes. Finally, microscopic photographs were captured from these stained sections for further analysis and record-keeping. The organs considered include the heart, liver, lungs, and kidney.

2.9.1 Data Analysis

Statistical tests were performed to assess differences between groups. One-way Analysis of Variance (ANOVA) was used to determine if there were overall significant differences ($p<0.05$) between the treatment groups and the control group. If the ANOVA indicated a significant difference, Dunnett's multiple comparison test was employed for further analysis. This post-hoc test allowed for pinpointing which specific treatment groups differed from the control group. All data are presented as mean values accompanied by Standard Error of the Mean (SEM) to show variability within each group. A p-value less than 0.05 ($p<0.05$) was considered statistically significant. GraphPad Prism software (version 6.01) was used to perform all statistical analyses.

3. Results

3.1 Screening of Phytoconstituents

The qualitative phytochemical analysis of MCr aqueous (Aq) and hydromethanolic (Me70) extracts revealed the presence of terpenoids, alkaloids, saponins, tannins, phenols, and flavonoids in both extracts (Table 1). However, cyanogenic glycosides were not detected in either extract. Anthraquinones and oxalates were identified only in the MCr Me70 extract, while sterols were found exclusively in the aqueous extract.

3.1.1 Quantitative Phytochemical Screening

The quantitative phytochemical analysis (Table 2) revealed that alkaloids, phenols, flavonoids, and phenols were abundant in both MCr aqueous (Aq) and hydromethanolic (Me70) extracts. Notably, the total phenolic content was significantly higher ($p<0.05$) in the

Table 1. Qualitative phytochemical screening of MCrAq and Me70 extracts

Phytochemicals	MCrAq	MCr Me70
Alkaloid	+	+
Tannin	+	+
Saponin	+	++
Flavonoid	+	+
Phenol	++	++
Terpenoid	++	++
Cyanogenic Glycoside	-	-
Anthraquinone	-	+
Sterol/Steroid	+	-
Oxalate	-	+

-negative; + positive; ++ strongly positive
MCr: *Musanga cecropioides* root; Aq: Aqueous extract; Me70: Hydromethanolic extract

Table 2. Quantification of phytochemical constituents in MCr methanolic (Me70) and aqueous extracts

Phytochemicals	Me70 extract	Aq extract
Flavonoids (mg RE/ 100g)	41.55 ± 0.04^{a}	39.02 ± 0.02^{ab}
Phenols (Mg GAE / 100g)	16.78 ± 0.02^{a}	8.00 ± 0.11^{b}
Saponins (mg DE / 100 g extract)	20.02 ± 0.01^{b}	18.98 ± 0.31^{b}
Alkaloids (%)	11.44 ± 0.41^{b}	10.54 ± 0.46^{b}

Data were analyzed following triplicate measurements and presented as mean ± Standard Deviation (SD). Statistical comparisons between groups within each column were performed using a one-way ANOVA test. Values shown as superscript letters are considered statistically significant at $p<0.05$.

Table 3. Result of acute toxicity assessment on MCrextracts in rats

STAGE 1				
Extract	**Dose (mg/kg)**	**Number of death**	**Mortality (%)**	**Symptoms**
Aqueous extract	10	0/6	0	None
	1000	0/6	0	None
	5000	6/6	100	Death within 1 hour
Me70 extract	10	0/6	0	None
	1000	0/6	0	None
	5000	0/6	0	None

Table 4. Changes in whole body weight of rats treated daily with aqueous extracts of MCroots for 28 days

Treatment groups/ Dose	The mean weight of animals (g) during the experiment				
	Day 1	Day 7	Day 14	Day 21	Day 28
Control 1 ml/kg DW	133.2 ± 33.67	141.8 ± 28.88	163.2 ± 23.52	167.3 ± 26.40	182.9 ± 29.69
MCRAq 500 mg/kg	180.4 ± 11.05	182.9 ± 11.49	174.8 ± 13.04	179.4 ± 11.22	191.8 ± 9.03
MCRAq 1000 mg/kg	173.9 ± 17.83	181.6 ± 19.87	179.6 ± 19.77	169.3 ± 17.17	175.5 ± 22.27
MCRAq 2500 mg/kg	170.0 ± 20.36	178.1 ± 21.10	159.2 ± 7.49	168.6 ± 13.66	172.3 ± 13.86

Data are presented as mean ± Standard Error of the Mean (SEM). Statistical significance was set at $p<0.05$ (n = 6). MCR refers to *Musanga cecropioides*root, Aq denotes the aqueous extract, and DW indicates distilled water. No statistically significant differences ($p\geq0.05$) were observed compared to the control group.

Table 5. Effect of daily oral Me70 extract of MC root on body weight in rats over 28 days

Treatment groups/Dose	The mean weight of animals (g) during the experiment				
	Day 1	One week	Two weeks	Three weeks	One month
Control 1 ml/kg DW	133.2 ± 33.67	141.8 ± 28.88	163.2 ± 23.52	167.3 ± 26.40	182.9 ± 29.69
MCR Me70 500 mg/kg	170.7 ± 21.03	157.2 ± 16.73	159.1 ± 17.86	162.7 ± 19.75	166.2 ± 21.07
MCR Me70 1000 mg/kg	158.2 ± 5.19	150.8 ± 2.25	153.9 ± 2.32	156.3 ± 2.19	160.4 ± 2.70
MCR Me70 2500 mg/kg	141.3 ± 7.68	139.6 ± 7.62	139.9 ± 8.37	140.7 ± 9.10	141.0 ± 11.27

Data are presented as mean ± Standard Error of the Mean (SEM), with statistical significance set at $p<0.05$ (n = 6). MCr refers to MC root, Me70 denotes the hydromethanolic extract, and DW indicates distilled water. No statistically significant differences ($p\geq0.05$) were observed compared to the control group.

MCr Me70 extract compared to the aqueous extract. The concentrations of other identified phytochemicals were comparable between the two extracts.

3.2 Acute Toxicity Assessment

The acute toxicity assessment for MCr extracts (Table 3) showed no mortality or behavioural changes in rats administered either 10 mg/kg or 1000 mg/kg body weight of either the aqueous (MCrAq) or methanolic (MCr Me70) extracts. However, all animals (6/6) in the group receiving 5000 mg/kg MCrAq extracts died within 1 h. Conversely, no mortality was recorded in the group given 5000 mg/kg of the MCr Me70 extract. Based on these observations, the estimated median lethal dose (LD50) for MCrAq extract is considered to be above 1000 mg/kg body weight, while the LD50 for MCr Me70 extract is likely higher than 5000 mg/kg body weight.

3.3 Sub-Acute Toxicity Assessment

3.3.1 Changes in Whole Body Weight

The changes in whole body weight of animals treated with MCrAq and Me70 extracts (Tables 4 and 5) in the 28-day sub-acute toxicity assessment respectively are shown above. No significant difference in weight change was observed at $p<0.05$ throughout the 28 days of the study in groups treated with both MCr extracts at 500, 1000 and 2500 mg/kg body weight, respectively.

Table 6. Organ-to-body weight ratios of rats treated daily with MCrAq extract for 28 days

Treatment groups/ Dose	Ratios ($\times10^{-3}$)			
	LV:BW	H:BW	K:BW	L:BW
Control 1 ml/kg DW	35.88 ± 2.30	3.45 ± 0.13	6.12 ± 0.44	8.03 ± 0.97
MCRAq 500 mg/kg	34.08 ± 1.33	3.00 ± 0.43	6.37 ± 0.25	7.45 ± 0.99
MCRAq 1000 mg/kg	33.83 ± 3.01	3.98 ± 0.35	6.11 ± 0.36	6.02 ± 0.32
MCRAq 2500 mg/kg	32.83 ± 1.66	4.05 ± 0.53	6.01 ± 0.26	6.75 ± 0.81

Values are expressed as Mean ± SEM, $p<0.05$; n=6, MCR: *Musanga cecropioides* root; Aq: Aqueous extract; DW: distilled water; LV: Liver; H: Heart; K: kidney; L: Lungs; Treatment with MCr did not result in significant changes compared to the control group ($p\geq0.05$).

3.3.2 Organ-to-Body Weight Ratio

The organ-to-body weight ratios of rats administered MCrAq and Me70 extracts (Tables 6 and 7), respectively, following the 28-day sub-acute toxicity assessmentare shown above. On the 28th day of extract administration

Table 7. Organ-to-body weight ratios of rats treated daily with MCr Me70 extract for 28 days

Treatment groups/Dose	Ratios (×10-3)			
	LV:BW	H:BW	K:BW	L:BW
Control 1 ml/kg DW	35.88 ± 2.30	3.48 ± 0.13	6.12 ± 0.44	8.03 ± 0.97
MCR Me70 500 mg/kg	32.19 ± 0.80	4.04 ± 0.32	6.38 ± 0.24	7.53 ± 0.47
MCR Me70 1000 mg/kg	34.13 ± 1.12	3.46 ± 0.10	6.02 ± 0.40	6.77 ± 0.32
MCR Me70 2500 mg/kg	32.58 ± 1.31	4.00 ± 0.39	6.00 ± 0.34	7.49 ± 0.45

Values are expressed as Mean ± SEM, $p<0.05$; n=6, MCR: *Musanga cecropioides* Root; Me70: Hydro-methanolic extract; DW: distilled water; LV: Liver; H: Heart; K: kidney; L: Lungs; No Significant difference observed at $p<0.05$, when compared with the control group.

at 500, 1000 and 2500 mg/kg, no significant difference ($p<0.05$) was observed in the liver, heart, kidney and lungs-to-body weight ratio when compared to the control group.

3.4 Haematology

The haematology analysis of animals treated with MCr aq and Me70 extract (Tables 8 and 9) in the 28-day sub-acute toxicity assessment, respectively is shown above. Administration of MCr aq and Me70 extract (at 500, 1000 and 2500 mg/kg) did not significantly alter the white blood cell count, lymphocytes, monocytes, granulocytes, red blood cell count, haemoglobin concentration, haematocrit and platelet count when compared to the control group.

Table 8. Effect of aqueous extract of MC root on the haematological parameters of rats in 28 days sub-acute toxicity study

Parameters	WBC 10^{-3}/uL	LYM 10^{-3}/uL	MON 10^{-3}/uL	GRAN 10^{-3}/uL	RBC 10^{-6}UL	HGB g/dL	HCT %	PLT 10^{-3}/uL
Control 1ml/kg DW	10.83 ± 3.02	9.00 ± 2.65	0.80 ± 0.30	1.03 ± 0.60	5.55 ± 0.44	12.50 ± 0.87	48.55 ± 0.59	693.8 ± 95.39
MCRAq 500 mg/kg	6.60 ± 0.30	6.18 ± 0.29	0.15 ± 0.03	0.28 ± 0.03	5.54 ± 0.25	13.33 ± 0.57	48.25 ± 2.00	653.0 ± 65.94
MCRAq 1000 mg/kg	10.05 ± 4.17	8.78 ± 4.10	0.48 ± 0.18	0.80 ± 0.37	5.21 ± 0.19	12.48 ± 0.57	44.13 ± 1.21	676.3 ± 130.9
MCRAq 2500 mg/kg	7.45 ± 1.19	6.10 ± 1.34	0.48 ± 0.09	0.88 ± 0.23	6.09 ± 0.41	12.43 ± 1.35	50.35 ± 2.30	863.3 ± 235.5

Table 9. Effect of Me70 extract of MCroot on the haematological parameters of rats in 28 days sub-acute toxicity study

Parameters	WBC 10^{-3}/uL	LYM 10^{-3}/uL	MON 10^{-3}/uL	GRAN 10^{-3}/uL	RBC 10^{-6}UL	HGB g/dL	HCT %	PLT 10^{-3}/uL
Control1 ml/kg DW	10.83 ± 3.02	9.00 ± 2.65	0.80 ± 0.30	1.03 ± 0.60	5.55 ± 0.44	12.50 ± 0.87	48.55 ± 0.59	693.8 ± 95.39
MCR Me70 500 mg/kg	6.93 ± 0.44	6.08 ± 0.40	0.35 ± 0.13	0.50 ± 0.18	6.24 ± 0.17	14.65 ± 0.42	50.05 ± 0.94	809.3 ± 116.9
MCR Me70 1000 mg/kg	6.73 ± 1.20	5.43 ± 0.76	0.43 ± 0.11	0.88 ± 0.36	6.09 ± 0.12	14.40 ± 0.21	49.58 ± 0.38	667.8 ± 104.0
MCR Me70 2500 mg/kg	7.20 ± 1.94	4.55 ± 1.30	0.70 ± 0.27	1.95 ± 0.41	4.09 ± 0.65	11.38 ± 1.35	33.23 ± 5.93	534.5 ± 157.1

Data are presented as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Me70 denotes the hydromethanolic extract, and DW indicates distilled water. Haematological parameters assessed include White Blood Cell count (WBC), with differential counts for lymphocytes (LYM), Monocytes (MON), and Granulocytes (GRAN), as well as Red Blood Cell count (RBC), haemoglobin concentration (HGB), Hematocrit(HCT), and Platelet Count (PLT). Importantly, no statistically significant differences were observed in any of these haematological parameters compared to the control group ($p\geq0.05$).

Data are presented as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Aq denotes the aqueous extract, and DW indicates distilled water. Haematological parameters assessed include White Blood Cell count (WBC), with differential counts for Lymphocytes (LYM), Monocytes (MON), and Granulocytes (GRAN), as well as Red Blood Cell Count (RBC), Haemoglobin Concentration (HGB), Hematocrit (HCT), and Platelet Count (PLT). Importantly, no statistically significant differences were observed in any of these parameters compared to the control group ($p \geq 0.05$).

3.5 Liver Function

The liver function tests of animals treated with MCrAq and Me70 extracts (Tables 10 and 11), in the 28-day sub-acute toxicity assessment, respectivelyare shown above. Table 10 shows a significant increase ($p<0.01$) in alkaline phosphatase activity in the group administered 2500 mg/kg body weight (21.12 ± 2.34 U/L) compared to the control group (15.41 ± 0.43 U/L).Table 11 reveals several effects on liver enzymes: Alanine aminotransferase (ALT) activity reduced ($p<0.05$) when given 1000 mg/kg body weight (25.87 ± 1.27 U/L) compared to control (29.59 ± 0.75 U/L). Aspartate aminotransferase (AST) activity declined ($p<0.05$) at 500 mg/kg body weight (43.52 ± 0.21 U/L) compared to control (48.21 ± 0.48 U/L). Total protein levels were significantly decreased ($p<0.05$) at 1000 mg/kg body weight (11.90 ± 0.68 g/dL) compared to control (15.28 ± 0.78 g/dL).

Interestingly, alkaline phosphatase activity was also significantly higher ($p<0.001$, $p<0.01$, and $p<0.05$) at

Table 10. Sub-acute toxicity investigation on the impact of MCaqueousroot extracts on liver function of rats

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	TB × 10^{-2} (mg/dL)	CB × 10^{-2} (mg/dL)	UB × 10^{-2} (mg/dL)	TP (g/dl)
Control 1 ml/kg DW	29.59 ± 0.75	48.21 ± 0.48	15.41 ± 0.43	0.61 ± 0.01	5.50 ± 0.65	3.25 ± 0.48	2.25 ± 0.25	15.28 ± 0.78
MCRAq 500 mg/kg	29.56 ± 0.90	46.57 ± 0.89	17.81 ± 1.29	0.56 ± 0.04	7.25 ± 0.48	3.75 ± 0.25	3.50 ± 0.50	13.92 ± 0.23
MCRAq 1000 mg/kg	29.01 ± 1.54	46.31 ± 1.36	18.78 ± 1.81	0.59 ± 0.05	6.50 ± 1.04	3.50 ± 0.65	3.00 ± 0.41	12.69 ± 0.75
MCRAq 2500 mg/kg	27.41 ± 1.22	44.95 ± 1.24	21.12 ± 2.34**	0.54 ± 0.05	6.25 ± 0.63	3.25 ± 0.25	3.00 ± 0.41	12.07 ± 0.60

Values are expressed as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Aq denotes the aqueous extract, and DW indicates distilled water. Liver function parameters assessed include Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Total Bilirubin (TB), Conjugated bilirubin (CB), Unconjugated Bilirubin (UB), and Total Protein (TP). ** Significant values at $p<0.01$, when compared to the control group

Table 11. Sub-acute toxicity investigation on the impact of MC Me70root extracts on liver function of rats

Parameters	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	TB × 10^{-2} (mg/dL)	CB × 10^{-2} (mg/dL)	UB × 10^{-2} (mg/dL)	TP (g/dl)
Control 1 ml/kg DW	29.59 ± 0.75	48.21 ± 0.48	15.41 ± 0.43	0.61 ± 0.01	5.50 ± 0.65	3.25 ± 0.48	2.25 ± 0.25	15.28 ± 0.78
MCR Me70 500 mg/kg	27.07 ± 0.64	43.52 ± 0.21*	25.23 ± 0.55***	0.59 ± 0.13	4.50 ± 0.50	2.00 ± 0.00	2.50 ± 0.50	13.17 ± 0.75
MCR Me70 1000 mg/kg	25.87 ± 1.27*	45.84 ± 1.17	21.04 ± 0.99**	0.52 ± 0.03	4.25 ± 0.63	2.00 ± 0.41	2.25 ± 0.25	11.90 ± 0.68*
MCR Me70 2500 mg/kg	27.45 ± 0.36	45.74 ± 1.22	20.40 ± 0.74*	0.52 ± 0.03	4.25 ± 0.48	2.00 ± 0.00	2.25 ± 0.48	12.59 ± 0.92

Data are presented as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Me70 denotes the hydromethanolic extract, and DW indicates distilled water. Liver function parameters assessed include Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), TotalBilirubin (TB), Conjugated bilirubin (CB), Unconjugated Bilirubin (UB), and Total Protein (TP). Statistically significant differences compared to the control group are denoted by superscript numbers: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

doses of 500 mg/kg (25.23 ± 0.55 U/L), 1000 mg/kg (21.04 ± 0.99 U/L), and 2500 mg/kg (20.40 ± 0.74 U/L) body weight, respectively.

3.6 Renal Function

The renal function tests of animals treated with MCr Aq extract and Me70 extracts (Tables 12 and 13) in the 28-day sub-acute toxicity assessment, respectivelyare shown above.

In Table 12, Urea concentration was higher ($p<0.05$) at all doses (500 mg/kg: 31.95 ± 1.85 mg/dL, 1000 mg/kg: 31.39 ± 2.89 mg/dL, 2500 mg/kg: 32.30 ± 2.25 mg/dL) compared to control (25.24 ± 0.75 mg/dL).

Creatinine and sodium levels were elevated ($p<0.01$) only at the 500 mg/kg dose (creatinine: 0.46 ± 0.03 mg/dL, sodium: 108.5 ± 3.21 mmol/L) compared to control (creatinine: 0.36 ± 0.01 mg/dL, sodium: 99.49 ± 0.48 mmol/L).

Interestingly, potassium concentration declined ($p<0.05$) at the highest dose (2500 mg/kg: 21.60 ± 1.77 mmol/L) compared to control (25.80 ± 0.24 mmol/L).

In Table 13, Potassium and bicarbonate levels decreased ($p<0.001$ and $p<0.05$, respectively) at all doses (500, 1000, and 2500 mg/kg body weight) compared to control. Chloride levels were also significantly decreased.

3.7 Lipid Profile

The lipid profile of animals treated with MCr Aq extract and Me70 extracts (Tables 14 and 15) in the 28-day sub-acute toxicity assessment respectively is shown above.

In Table 14, MCr Aq extracts significantly reduced low-density lipoprotein (LDL) levels compared to control: 500 mg/kg: 15.85 ± 0.40 mg/dL ($p<0.001$), 1000 mg/kg: 23.80 ± 6.29 mg/dL ($p<0.01$), and 2500 mg/kg: 17.89 ± 5.34 mg/dL ($p<0.001$).

Table 12. Effect of aqueous extract of MCr on the renal function parameters of rats in 28 days sub-acute toxicity study

Parameters	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control 1 ml/kg DW	25.24 ± 0.75	0.36 ± 0.01	99.49 ± 0.48	25.80 ± 0.24	65.95 ± 0.47	13.05 ± 0.67
MCRAq 500 mg/kg	31.95 ± 1.85*	0.46 ± 0.03**	108.5 ± 3.21**	22.73 ± 1.24	66.85 ± 2.13	10.58 ± 0.12
MCRAq 1000 mg/kg	31.39 ± 2.89*	0.41 ± 0.03	102.1 ± 2.08	23.64 ± 1.67	67.71 ± 3.69	10.60 ± 0.09
MCRAq 2500 mg/kg	32.30 ± 2.25*	0.42 ± 0.02	102.5 ± 1.44	21.60 ± 1.77*	62.54 ± 3.80	10.55 ± 0.12

Data are presented as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Aq denotes the hydromethanolic extract, and DW indicates distilled water. Statistically significant differences compared to the control group are denoted by superscript numbers: *p<0.05, **p<0.01.

Table 13. Effect of Me70 extract of MCroot on the renal function parameters of rats in 28 days sub-acute toxicity study

Parameters	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control 1 ml/kg DW	25.24 ± 0.75	0.36 ± 0.01	99.49 ± 0.48	25.80 ± 0.24	65.95 ± 0.47	13.05 ± 0.67
MCR Me70 500 mg/kg	26.87 ± 3.21	0.35 ± 0.02	97.70 ± 2.13	16.84 ± 0.90***	41.35 ± 5.59***	10.20 ± 0.08*
MCR Me70 1000 mg/kg	26.01 ± 0.10	0.36 ± 0.01	98.31 ± 1.33	18.81 ± 0.59***	46.85 ± 6.37***	10.31 ± 0.07*
MCR Me70 2500 mg/kg	26.48 ± 0.48	0.36 ± 0.02	99.28 ± 1.56	18.86 ± 0.95***	48.37 ± 5.85**	10.26 ± 0.07*

Data are presented as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Me70 denotes the hydromethanolic extract, and DW indicates distilled water. Statistically significant differences compared to the control group are denoted by superscript numbers: *p<0.05, **p<0.01, ***p<0.001.

In Table 15, MCr Me70 extracts significantly reduced LDL levels compared to control ($p<0.001$) at all doses (500, 1000, and 2500 mg/kg). For both extracts (Tables 14 and 15): HDL (high-density lipoprotein) and total cholesterol levels were similar to the control group.

3.8 Plasma Antioxidant

The plasma antioxidant concentration of animals treated with MCrAq extract and Me70 extracts (Tables 16 and 17) in the 28-day sub-acute toxicity assessment, respectively is shown above.

In Table 16, MCrAq Extract increased malondialdehyde (MDA) concentration ($p<0.05$) at 500 mg/kg (46.43 ± 1.87 mmole/ml) compared to control (36.12 ± 0.83 mmole/ml). Superoxide dismutase (SOD) at all doses ($p<0.01$ for 500 mg/kg, $p<0.05$ for 1000 mg/kg, $p<0.01$ for 2500 mg/kg). Catalase ($p<0.001$) at all doses. Glutathione peroxidase (GPx) at 500 mg/kg ($p<0.001$), 1000 mg/kg ($p<0.001$), and 2500 mg/kg ($p<0.01$).

In Table 17, MCr Me70 Extract reduced antioxidant enzyme activity at 500 mg/kg:

MDA ($p<0.05$), SOD ($p<0.05$), Catalase ($p<0.001$) and GPx ($p<0.05$). Higher doses (1000 and 2500 mg/kg)

Table 14. Effect of aqueous extract of MCroot on the lipid profile parameters of rats in 28 days sub-acute toxicity study

Parameters	Cholesterol (mg/dL)	TAG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control 1 ml/kg DW	59.15 ± 0.60	217.6 ± 0.47	23.25 ± 0.48	38.88 ± 0.99	43.52 ± 0.09
MCRAq 500 mg/kg	67.92 ± 0.67	204.1 ± 4.90	21.25 ± 0.18	15.85 ± 0.40***	40.82 ± 0.98
MCRAq 1000 mg/kg	69.94 ± 7.62	224.2 ± 9.15	21.30 ± 0.45	23.80 ± 6.29**	44.85 ± 1.83
MCRAq 2500 mg/kg	70.93 ± 7.45	211.8 ± 13.29	21.67 ± 0.58	17.89 ± 5.34***	42.36 ± 2.66

Data are presented as mean ± standard error of the mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Aq denotes the aqueous extract, and DW indicates distilled water. Statistically significant differences compared to the control group are denoted by superscript numbers: *p<0.05,**p<0.01, ***p<0.001. Lipid parameters assessed include Triacylglycerol (TAG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL).

Table 15. Effect of Me70 extract of MCroot on the lipid profile parameters of rats in 28 days sub-acute toxicity study

Parameters	Cholesterol (mg/dL)	TAG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control 1 ml/kg DW	59.15 ± 0.60	217.6 ± 0.47	23.25 ± 0.48	38.88 ± 0.99	43.52 ± 0.09
MCR Me70 500 mg/kg	65.11 ± 1.94	205.1 ± 7.23	25.56 ± 0.83	13.54 ± 1.04***	41.01 ± 1.45
MCR Me70 1000 mg/kg	68.14 ± 2.70	217.4 ± 4.39	24.93 ± 1.05	19.74 ± 3.16***	43.48 ± 0.88
MCR Me70 2500 mg/kg	71.85 ± 3.08	208.9 ± 2.01	24.50 ± 0.62	20.59 ± 3.74***	41.76 ± 0.41

Data are presented as mean ± Standard Error of the Mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Me70 denotes the hydromethanolic extract, and DW indicates distilled water. Lipid parameters assessed include Triacylglycerol (TAG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL). Statistically significant differences compared to the control group are denoted by superscript numbers: **p<0.01, ***p<0.001.

Table 16. Effect of aqueous extract of MCroot on the plasma antioxidant parameters of rats in 28 days sub-acute toxicity study

Parameters	MDA (mmole/ml)	SOD (U/ml)	CAT (U/ml)	GPX (U/ml)	GRX (U/ml)
Control 1 ml/kg DW	36.12 ± 0.83	7.66 ± 0.21	255.3 ± 1.04	150.3 ± 0.86	2.92 ± 0.04
MCRAq 500 mg/kg	46.43 ± 1.87*	4.87 ± 0.86**	202.4 ± 14.56***	113.3 ± 5.75***	2.63 ± 0.19
MCRAq 1000 mg/kg	39.58 ± 6.52	5.43 ± 1.27*	202.4 ± 14.23***	115.1 ± 5.74***	2.77 ± 0.15
MCRAq 2500 mg/kg	37.72 ± 6.22	4.96 ± 0.35**	186.1 ± 16.61***	128.1 ± 2.66**	2.47 ± 0.18

Data are presented as mean ± standard error of the mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Aq denotes the aqueous extract, and DW indicates distilled water. Oxidative stress parameters assessed include Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), and Glutathione Reductase (GRX). Statistically significant differences compared to the control group are denoted by superscript numbers: *p<0.05, **p<0.01, ***p<0.001.

Table 17. Effect of Me70 extract of MCroot on the plasma antioxidant parameters of rats in 28 days sub-acute toxicity study

Parameters	MDA (mmole/ml)	SOD (U/ml)	CAT (U/ml)	GPX (U/ml)	GRX (U/ml)
Control 1 ml/kg DW	36.12 ± 0.83	7.66 ± 0.21	255.3 ± 1.04	150.3 ± 0.86	2.92 ± 0.04
MCR Me70 500 mg/kg	24.32 ± 0.38*	5.45 ± 0.08*	167.8 ± 4.21***	130.1 ± 0.88*	3.18 ± 0.33
MCR Me70 1000 mg/kg	29.22 ± 1.83	6.87 ± 0.28	156.8 ± 8.15***	118.3 ± 2.91***	2.96 ± 0.08
MCR Me70 2500 mg/kg	27.29 ± 1.35	6.36 ± 0.35	172.6 ± 10.07***	122.3 ± 0.84***	2.56 ± 0.32

Data are presented as mean ± standard error of the mean (SEM) with n=6 animals per group. *Musanga cecropioides* Root (MCr) refers to the plant material, Me70 denotes the hydromethanolic extract, and DW indicates distilled water. The following oxidative stress parameters were assessed: Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), and Glutathione Reductase (GRX). Statistically significant differences compared to the control group are denoted by superscript numbers: *p<0.05, ***p<0.001.

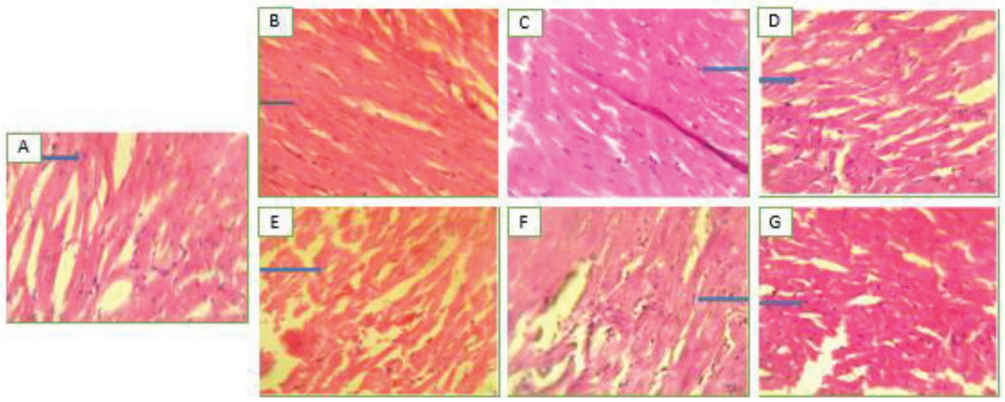


Figure 1. Histological determination of MCroot extract exposure on the heart in a sub-acute (28-day) rat toxicity study, H and E×400. **(A)** Normal control shows normal myocyte; **(B)**MCrAq (500 mg/kg) shows normal myocyte; **(C)**MCrAq (1000 mg/kg) arrow shows normal myocyte; **(D)**MCrAq (2500 mg/kg) arrow shows normal myocyte; **(E)**MCr Me70 (500 mg/kg) arrow shows normal myocyte; **(F)**MCr Me70 (1000 mg/kg) arrow shows normal myocyte; **(G)**MCr Me70 (2500 mg/kg) arrow shows normal myocyte.

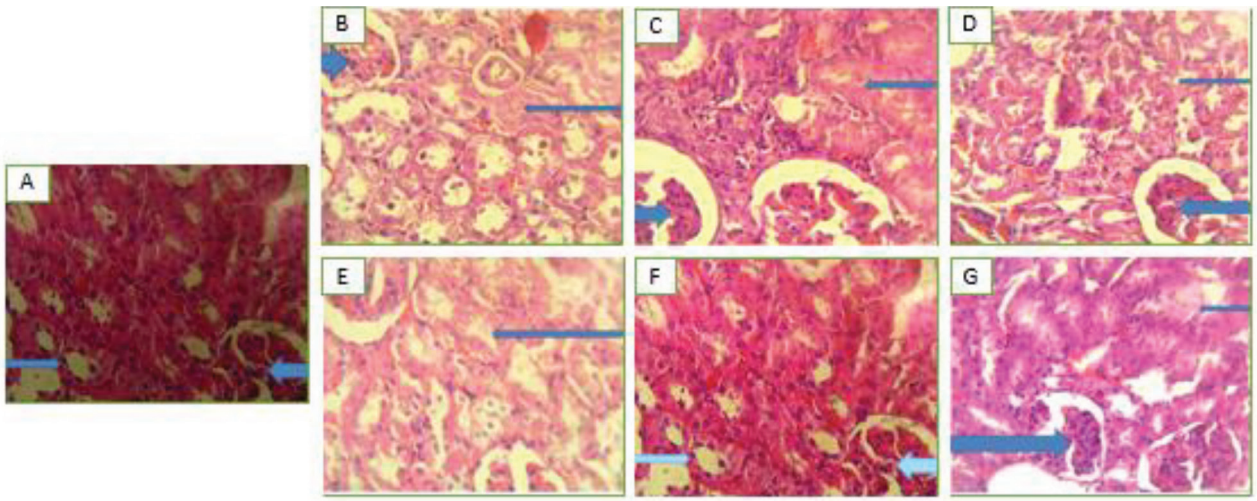


Figure 2. Renal histopathological evaluation of MCroot extract exposure in a sub-acute (28-day) rat toxicity study H and E ×400. **(A)** Normal control, thick arrow shows normal glomerulus and thin arrow shows normal tubules; **(B)**MCrAq (500 mg/kg) normal glomerulus and normal tubules seen; **(C)**MCrAq (1000 mg/kg) normal glomerulus and normal tubules seen; **(D)** MCrAq (2500 mg/kg) normal glomerulus and normal tubules seen; **(E)**MCr Me70 (500 mg/kg) normal glomerulus and normal tubules seen; **(F)**MCr Me70 (1000 mg/kg) normal glomerulus and normal tubules seen; **(G)**MCr Me70 (2500 mg/kg) normal glomerulus and normal tubules seen.

only affected catalase and GPx activity ($p<0.001$ for both enzymes at both doses).

3.9 Histology of Vital Organs

3.9.1 Histology of the Heart

The photomicrograph of sections of the heart of rats treated with MCr Aq extract and Me70 extracts (Figure 1) after the 28-day assessment respectively are shown above. Alterations observed in all treatment groups were not significant, all myocytes appeared normal after the sub-acute toxicity study.

3.9.2 Histology of the Kidney

Kidney sections from rats administered MCrAq and Me70 extracts (Figure 2) following the 28-day sub-acute toxicity study, respectively are shown. There were no visible indications of pathological alterations observed, all photomicrographs of the various treatments showed normal glomerulus and normal tubules.

3.9.3 Histology of the Liver

Liver sections from rats administered MCrAq and Me70 extracts (Figure 3) following the 28-day sub-acute toxicity

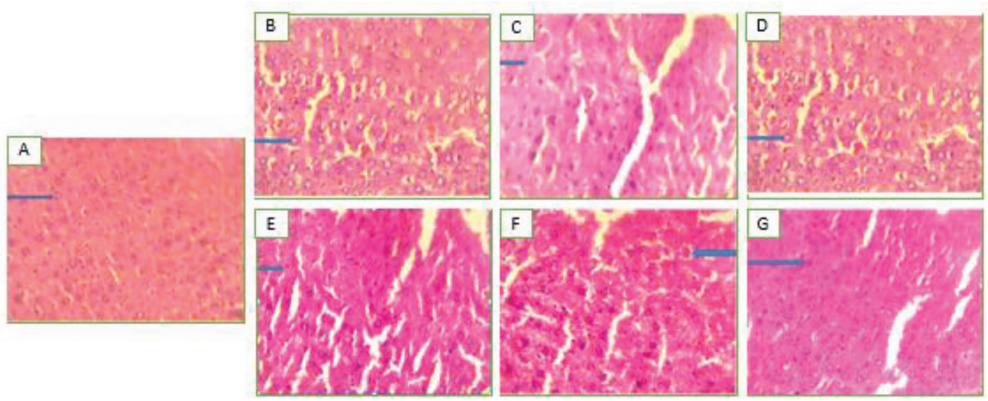


Figure 3. Hepatic histopathological evaluation of MCroot extract exposure in a sub-acute (28-day) rat toxicity study. H and E×400. **(A)** Normal control shows normal hepatocyte; **(B)**MCRAq (500 mg/kg) shows normal hepatocyte; **(C)**MCRAq (1000 mg/kg) arrow shows karyolysis of hepatocytes (a sign of necrosis); **(D)**MCRAq (2500 mg/kg) shows normal hepatocyte; **(E)** MCR Me70 (500 mg/kg) arrow shows pyknosis of hepatocyte nuclei (a sign of necrosis); **(F)**MCR Me70 (1000 mg/kg) arrow shows ballooning degeneration of hepatocytes; **(G)**MCR Me70 (2500 mg/kg) arrow shows karyolysis of hepatocyte nuclei with hypereosinophilia of cytoplasm (signs of necrosis).

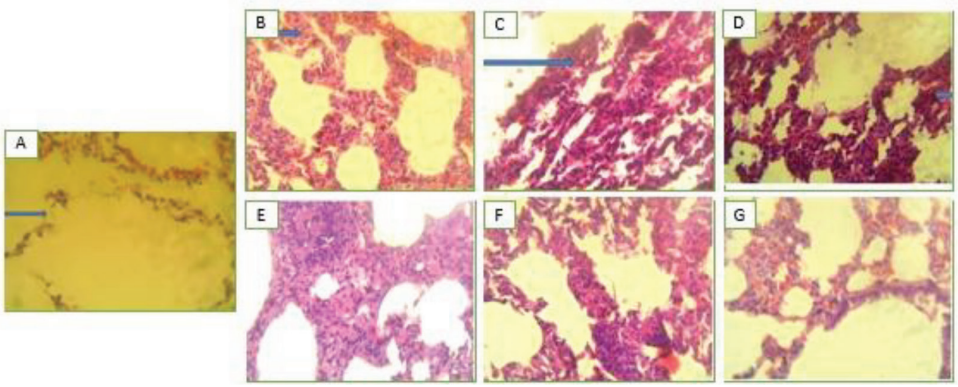


Figure 4. Pulmonary histopathological evaluation of MCroot extract exposure in a sub-acute (28-day) rat toxicity study:H and E×400. **(A)** Normal control shows normal alveoli space; **(B)**MCRAq (500 mg/kg) shows thickening of the alveoli septae; **(C)**MCRAq (1000 mg/kg) shows thickening of the alveoli septae and destruction of normal architecture; **(D)**MCRAq (2500 mg/kg) shows thickening of the alveoli septae and destruction of normal architecture; **(E)**MCR Me70 (500 mg/kg) shows thickening of the alveoli septae; **(F)**MCR Me70 (1000 mg/kg) thickening of the alveoli septae and desquamation of alveoli lining (features suggestive of diffuse alveolar damage); **(G)**MCR Me70 (2500 mg/kg) shows thickening of the alveoli septae: H and E ×400.

study respectively are shown above. All treatment groups showed signs of pathological alterations with a few exceptions.

3.9.4 Histology of the Lungs

Lung sections from rats administered MCrAq and Me70 extracts (Figure 4) following the 28-day sub-acute toxicity study are shown above. Thickening of the alveoli septae was observed in most treatment groups with a few exceptions.

4. Discussion

The present study identified a diverse array of bioactive compounds within MC root extracts, corroborating existing literature documenting the presence of various phytochemicals across different plant parts of *M. cecropioides*[33,34]. These reported compounds included alkaloids, catechic tannins, phlobatannins, flavonoids, triterpenes, sterols, coumarins, gallic tannins, free and bound anthraquinones, anthocyanosides, oxalates, phenols, cyanogenic glycosides, saponins, and cardiac glycosides.

Furthermore, the current study contributes to existing knowledge by isolating specific phenolic compounds, saponins, and triterpenes within the MC root extracts. Previous studies have documented the isolation of these compounds from the stem bark and root bark of MC[35,36]. Acute toxicity assessment revealed an estimated Lethal Dose (LD50) for all tested extracts to be greater than 1000 mg/kg body weight. This value falls within the "practically non-toxic" classification established by Lorke[18], which categorizes substances with LD_{50} values between 500 and 5000 mg/kg body weight as having low toxicity. These findings suggest a favourable safety profile for the *M. cecropioides* root extracts.

Chronic exposure to toxic substances can often manifest as reductions in body and organ weights[37,38]. Conversely, an increase in the relative weight of vital organs might indicate hypertrophy[39]. In the present sub-acute toxicity study, there were no significant changes observed in organ or body weight in the treated animals. This lack of significant weight gain aligns with the work of Shemishere *et al.*[40], who proposed the potential application of *M. cecropioides* in weight management strategies.

The haematopoietic system, responsible for blood cell production, is highly sensitive to toxic insults and serves as a valuable indicator of overall health[33]. Analysis of various haematological parameters in all treatment groups revealed no significant deviations in comparison to the control group. The observed haematological parameters suggest that *M. cecropioides* does not directly influence the hematopoietic system of the experimental animals. This aligns with previous reports by Adeneye *et al.*[33]who observed similar findings. Liver Function Tests (LFTs) are crucial for measuring the potential toxicity of medications, providing valuable insights into hepatocellular health[41]. Among these markers, Alanine Aminotransferase (ALT) is used as an indicator of hepatotoxicity[42]. Aspartate Aminotransferase (AST), while also found in the liver is present in other tissues such as skeletal muscle and cardiac tissue. Therefore, elevated AST levels can reflect damage beyond the liver[43]. In contrast, ALT elevation is more strongly associated with liver cell injury. Furthermore, serum Alkaline Phosphatase (ALP) activity is linked to hepatic cell function, with increased levels potentially reflecting enhanced enzyme production[42].

A notable observation in this study was the rise in ALP concentration within groups receiving either the MCr aqueous extract at 2500 mg/kg or the MCr Me70 extract at doses of 500, 1000, and 2500 mg/kg. However, this finding was not accompanied by corresponding elevations in ALT or AST levels. This isolated increase in ALP may not necessarily represent significant liver damage, particularly considering the unchanged or even reduced levels of the other LFT markers[44]. However, it gives a hint of the possible toxicological properties of MCr extracts at higher doses > 2500 mg/kg.

The sub-acute toxicity study showed changes in some electrolyte and metabolite levels in animals administered *M. cecropioides* root extracts. The Me70 extract caused reductions in potassium, chloride, and bicarbonate concentrations. Conversely, the aqueous extract treatment resulted in slightly elevated levels of urea, creatinine, and sodium. These findings warrant further investigation, particularly regarding the potential renal effects of the aqueous extract. Adeneye *et al.*,[33] previously reported increased creatinine concentrations following the administration of *M. cecropioides* bark extracts, suggesting a possible trend.

Low-density lipoprotein is a form of cholesterol that has been linked to pathological conditions like atherosclerosis and heart disease when in excess; hence the interest in toxicity studies[45]. High-densityLipoprotein (HDL) acts like a scavenger, collecting extra cholesterol from tissues and bringing it back to the liver for processing

into bile salts for disposal[45]. Changes in concentrations of cholesterol, particularly HDL and triglycerides, are established indicators of an individual's susceptibility to developing cardiovascular diseases[46]. In this present study, reduced high-density lipoprotein concentrations were observed in all treatment groups, this was also reported by Tom *et al.*[47]while the groups treated with MCaqueous and methanol root extracts also showed significantly lower low-density lipoprotein relative to the control group. This shows that MCr may be incapable of interfering with mechanisms associated with the development of cardiovascular diseases or they may not in any way promote the development of related diseases[47].

Levels of Malondialdehyde (MDA), a marker of cell damage, did not differ significantly between the treatment groups and the control group. The exception was the group administered 500 mg/kg MCaqueous root extract. This finding suggests that MC Me70 root extract, even at high doses, did not initiate lipid peroxidation. Furthermore, these results support the acute toxicity findings, where the aqueous root extract caused 100% mortality at 5000 mg/kg.

Notably, the enzymatic antioxidant activities (SOD, catalase, GR, and GPx) remained unchanged across the treatment groups. Conversely, some treatment groups displayed statistically significant decreases ($p<0.05$) in these enzyme activities compared to the control group. While it may be agreed that MCdoes not initiate lipid peroxidation, the extracts may not also have a direct effect on enzymatic antioxidant activities. This may also be due to the fact that exogenous toxicants or radicals were not introduced to the experimental animals[48]. Histological examination of vital organs revealed no treatment-associated alterations in the heart or kidney. However, photomicrographs of the liver displayed signs of necrosis, while the lungs exhibited thickening of the alveolar septae. The pathological alterations observed in the liver at the various administered treatments were not significantly supported by the results obtained in the liver function tests, this may be a marker that these changes are not pathologically significant or they may be reversible[48].

Histological examination of kidney tissues would provide valuable insights into potential structural changes associated with the observed electrolyte and metabolite fluctuations. The absence of such histological evaluation makes it difficult to definitively conclude renal impairment. Furthermore, the lack of significant changes in liver function markers across all treatment groups suggests a potentially lower risk of hepatotoxicity.

Preliminary phytochemical screening of the root extracts from *Musanga cecropioides* (MCr) revealed that the hydromethanolic (Me70) extract exhibited higher concentrations of various bioactive phytochemical constituents compared to the aqueous extract. Notably, the Me70 extract was found to be enriched with total flavonoids, tannins, alkaloids, and phenolic compounds. The high content of these bioactive phytochemicals in MCr extracts is hypothesized to confer potential health-promoting effects by bolstering the body's innate defense mechanisms. The synergistic interplay of these phytochemicals may enhance the organism's ability to counteract oxidative stress, modulate inflammatory responses, and potentially exhibit antimicrobial, anticancer, or other therapeutic activities, thereby contributing to the overall well-being and maintenance of physiological homeostasis.

5. Conclusions

The oral administration of *Musanga cecropioides* (MCr) extracts can be considered relatively safe and non-toxic, particularly at doses under 1000 mg/kg body weight, as evidenced by the absence of lethal effects in both toxicological studies conducted in rats. These investigations lend support to the traditional use of MCr in folk medicine practices. However, it is crucial to conduct further preclinical evaluations to validate the therapeutic efficacy and long-term toxicological safety profile of these extracts before their potential integration into clinical applications.

6. Acknowledgements

The Authors thank the Department of Biochemistry and Biological Sciences for their support during this study.

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