



## Preliminary Phytochemical Screening and Acute Toxicity Study of Methanol Leaf Extracts of *Musanga cercropioides*

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### Abstract

*Musanga cercropioides* is a plant that is used to treat a variety of ailments in many African countries. However, just a few studies have been conducted to assess the toxicity of the various sections of this plant. The goal of this study was to determine the toxicity profile of *Musanga cercropioides* methanol leaf extract in albino rats. Lorke's approach was used to conduct an acute toxicity study. In the first phase, nine (9) albino rats were randomly divided into three (3) groups of three (3) animals each. Each of the animals were orally administered 10, 100 and 1000 mg/kg body weight of the extract. In the second phase, twelve (12) animals were used, which were divided into four groups of three (3) animals each. The first three groups received single doses of 1500, 3000, and 5000 mg/kg, respectively, while the fourth group which served as a control was given distilled water orally. After observation for 14 days, no signs of mortality were identified. The maximum dose supplied (5000 mg/kg body weight) had no effect on the test animals' mortality or general behavior. When the treated groups were compared to the control groups, there was no significant difference ( $p > 0.05$ ) in biochemical markers, body weight, or relative organ weights of liver, kidney, heart, and spleen. These findings show that oral administration of *Musanga cercropioides* methanol leaf extract is safe at a dose of up to 5000 mg/kg body weight in Wistar albino rats.

**Keywords:** *Musanga cercropioides*, methanol extracts, phytochemicals, toxicity, LD<sub>50</sub>

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### Introduction

The use of plants to sustain human health is as old as mankind and this, as reported by Akindele *et al.* [1], is underscored by the words of the Swiss German philosopher and physician, Philippus Aureolus Theophrastus Bombastus von Hohenheim (Paracelsus) that "All that man needs for health and healing has been provided by God in nature, the challenge of science is to find it." Because of their intricate secondary metabolism, plants have produced a vast array of therapeutic chemicals and lead candidates for new drug discoveries.

Over the past three decades, the use of herbal medicinal products and supplements has increased tremendously with not less than 80% of people worldwide relying on them for some part of primary healthcare [2]. African traditional healers are well known for employing a wide range of plants to treat parasite illnesses like malaria. A significant percentage of the herbal remedies that traditional healers provide are also widely believed to be successful by their patients. This corroborated the findings of scholars as reported by Adeniyi [3] that medicinal plants have helped in the management of

mild, chronic or seemingly incurable ailments/diseases, and several ethnobotanical studies have been conducted to document traditional medical practices, plants used, method of preparation as well as the mode of administration. Compounds of plant origin have been and still are an important source of compounds for drugs and their discovery [4].

Though many medicinal plants have great therapeutic benefits, there has been an increasing worry recently regarding their safety. Some of these plants are intrinsically toxic by virtue of their constituents and can cause adverse reactions if inappropriately used [5]. Furthermore, it is well recognized that consuming medicinal herbs without first determining their efficiency and safety might have unintended or harmful consequences that impact the physiology of several organs in the human body. Because they are involved in the metabolism and excretion of chemical substances, the liver and kidney are the primary targets in toxicological examination. The usage of medicinal herbs in the treatment of various disorders has also been linked to renal damage [6].



Cough, constipation, schizophrenia, chest infection, rheumatism, leprosy, *trypanosomiasis*, high blood pressure, toothache, malaria, wounds, and jaundice are among the diseases that *Musanga cercropioides* (Cecropiaceae) is commonly used to treat (mediated through boosting of immune system) in Cameroon, Gabon, Nigeria, and also, the Democratic Republic of Congo [7]. Its extracts have been proven to have anti-hypertension, anti-hyperglycemia, anti-diabetes, anti-diarrhea, and anti-bacterial properties in several scientific studies [8-11]. Shemishere *et al.* [12] and Sowemimo *et al.* [13] reported that the leaves of *M. cercropioides* have been shown to have oxytocic, antihypertensive, antiglycaemic, antioxidant and anti-inflammatory properties. Aqueous extract of the stem bark and the adventitious root sap have shown to have hypotensive and antimicrobial activity as well [14].

The root sap is used in the treatment of rheumatism, blood problems, and sexual illnesses, as well as a topical lotion for lung congestion, lactation stimulants, and pain relievers [11]. From all parts of this plant, many chemical substances have already been separated. Many investigations have identified triterpenoid acids such as kalaic, musangic, and cecropioidic acid, as well as a variety of other alternative organic chemistry substances [11, 15]. However, there paucity of information in the acute toxicity status hence the lethal dose 50 (LD<sub>50</sub>) of the methanol leaf extracts of the plant. Thus, the aim of this study was to investigate the acute toxicity study of the methanol leaf extract of the plant.

## Materials and Methods

### Collection and identification of plant materials

In order to identify the plant samples, fresh leaves of *Musanga cercropioides* were collected from the rain forest in Aladja, Delta State, Nigeria. Herbarium specimens bearing voucher numbers MC/PBB/209/15 were identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Nigeria. Prior to use, both plant samples were air-dried at room temperature, ground into a powder, and stored in airtight containers.

### Preparation of methanol extract

A measured quantity of 500 g of the crushed sample was weighed in a specific volume (2.5 litres) of methanol for 72 h and then filtered using Muslin cloth. The extracts were then concentrated by using a rotary evaporator at 40°C and further freeze-dried.

### Animals

In this investigation, male Wistar albino rats weighing 120-250 g were employed. The animals were kept in a well-ventilated cage with unlimited access to food and water. The animals were given 14 days to acclimate to laboratory conditions before the test began. All animal experimental techniques were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, which outlines human animal care requirements.

### Experimental design

The modified Lorke's approach was used to conduct an acute toxicity study [16]. A total of sixteen animals

were used in the investigation, which was conducted in two parts. Prior to receiving the extract, the albino rats were fasted overnight. Twelve animals were separated into four groups of three albino rats in the first phase. To determine the possible range of doses eliciting hazardous effects, Group 1, 2, and 3 animals were given a single dose of 10, 100, and 1000 mg/kg of the extract orally. Group 4, the control group, was given water ad libitum instead of the extract.

Three animals were employed in the second phase, which were divided into three groups of three animal each to ascertain the exact LD<sub>50</sub>. Single doses of 1500, 3000, and 5000 mg/kg were given to Groups 1, 2, and 3. After that, the animals were kept under surveillance for 24 h for observance of behavioral changes. For two weeks, the surviving animals were evaluated daily for signs of acute poisoning. Recovery and weight gain were regarded as signs of surviving acute poisoning. The formula was used to calculate the geometric mean of the highest dose at which the rats survived and the lowest dose at which the albino rat died in order to determine the LD<sub>50</sub>:  $LD_{50} = \sqrt{D_0 \times D_{100}}$

D<sub>0</sub> = Maximum dosage without causing death

D<sub>100</sub> = Lowest dosage causing death.

### Evaluations of weight

The Wistar albino rats in all of the experimental groups were weighed before and after medication delivery, as well as after 7 and 14 days.

### Blood collection and Tissue homogenate preparation for bioassay

The animals were fasted overnight prior to anaesthesia, although they were given access to water ad libitum. In a desiccator, the animals were anesthetized with mild doses of chloroform, blood was collected immediately and labeled appropriately. The rats' liver were excised, weighed, and refrigerated in ice cold 0.9% NaCl after 14 days. The liver homogenized in 10-part homogenizing buffer (0.1 M phosphate buffer) and then centrifuged at 10,000 g for 10 min to obtain a supernatant. The supernatant was kept in the refrigerator until further use for biochemical assay.

### Biochemical assays

#### Estimation of malondialdehyde (MDA)

MDA concentration was evaluated using Janero's TBARS test [17].

#### Estimation of liver enzymes

The serum enzymes of the samples, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were tested using describe by Reitmann [18].

### Qualitative phytochemical screening

The extracts were subjected to chemical analyses for the screening of bioactive chemical contents in the plants that were investigated, as reported by Sofowora [19], Treason and Evans [20] and Harborn [21].

### Results analysis

Mean ± standard error of the mean (SEM) is how the findings are displayed. Tukey's multiple comparison test was performed after a one-way analysis of variance (ANOVA) to identify significant differences between groups. The results were deemed significant at  $p < 0.05$ .

### Results and Discussion

The conventional wisdom that medicinal plants that are found in or close to nature are always harmless is no longer accepted. The advent of technological advancements has enabled scientists to detect minute amounts of potential carcinogens and toxic chemicals in these herbs and as well as recognize or evaluate potentially hazardous effects of some of the herbs used in traditional medicines since centuries [22].

The biological/pharmacological activity and possible toxicity of a plant are closely linked to the kind, amount, and kind of secondary metabolites that the plant contains. As a result, it is critical to test for the existence of potential phytochemicals in plants [23]. Table 1 shows the results of the preliminary phytochemical screening. Flavonoids, tannins, cardiac glycosides, steroids, phenols, alkaloids, carbohydrates, reducing sugars, coumarins, and Phlobatannins were found in the *Musanga cercropioides* methanol leaf extract, but Anthroquinone was not detected.

**Table 1: Phytochemical constituent of methanol leaf extract of *M. cercropioides***

Phytochemicals	<i>Musanga cercropioides</i>
Flavonoids	+
Tannins	+
Cardiac glycosides	+++
Saponins	+++
Steroids	++
Alkaloids	++
Reducing sugars	+
Carbohydrates	+
Anthroquinone	-
Coumarin	+
Phlobatannins	-
Phenols	++
Terpenoids	+++

+ = moderately detected, ++ = Detected, +++ = highly detected, - = not detected

**Table 2: Effect of oral administration of methanol leaf extract of *Musanga cercropioides* on body weight of rats**

Exper.	Doses (mg/kg)	Mean weight (g) at Day 0	Mean weight (g) at Day 7	Mean weight (g) at Day 14	Weight Diff. (%)
control	0	180.73±4.46	186.71±5.73	206.95±20.7	+14.51%
Phase 1	10	158.89±1.21	165.4±1.12	176.9±1.48	+11.33%
	100	184.04±8.05	186.88±9.05	191.22±10.24	+3.90%
	1000	159.137±36.24	192.86±19.52	258.92±17.14	+62.71%
Phase 2	1500	189.01±0.81	192.94±0.224	201.04±14.4	+6.36%
	3000	216.82±24.9	240.59±1.31	250.25±0.26	+15.41%
	5000	131.25±1.02	204.84±1.02	238±0.02	+81.42%

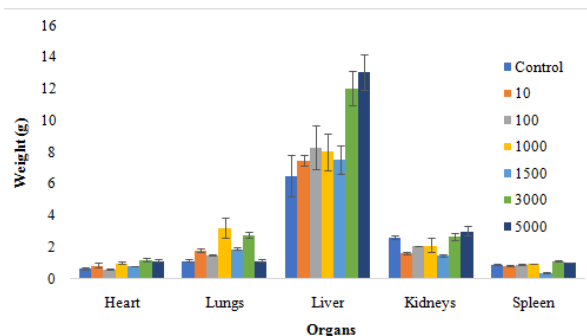
The results of this study showed that an acute dose of 5000 mg/kg of *M. cercropioides* given orally did not cause any toxicity in terms of appearance, behavior, or death. All of the animals given the extract lived to see

the end of the 14-day observation period. This indicates that the extract's median acute toxicity value (LD<sub>50</sub>) is greater than 5000 mg/kg body weight. Substances with an LD<sub>50</sub> of more than 5000 mg/kg by oral route are basically non-toxic [22]. As a result, the acute toxicity of *M. cercropioides* methanol leaf extract appears to be devoid of acute oral toxicity.

Also, in this investigation, all of the experimental animal groups gained weight, indicating that the administration of the crude extract has a minor effect on the animals' growth (Table 2). Furthermore, determining food and water consumption is critical in the investigation of a product's safety for therapeutic purposes, as correct nutrient intake is critical to the animal's physiological status and the achievement of a proper reaction to the medications examined [24, 25]. Food intake and water consumption were not affected by the administration of methanol leaf extract of *M. cercropioides* in this study, and it did not cause appetite suppression or have any negative consequences.

In animals, organ weight is also a useful indicator of physiological and pathological condition. The relative organ weight is critical in determining whether or not the organ was injured. The principal organs impacted by toxicant-induced metabolic reactions are the heart, liver, kidney, spleen, and lungs. Organ weight can be the most sensitive indicator of an effect of drug toxicity and its changes is often in combination with pathological background [26].

The organ weight of the extract-treated group was not significantly different from that of the control group in this investigation, indicating that the extract had no negative impact on organ weight (Fig. 1).



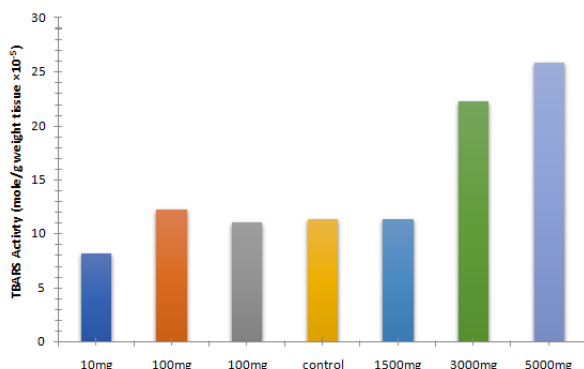
**Fig. 1: Effect of oral administration of methanol leaf extract of *Musanga cercropioides* on absolute organ weight**

**Table 3: Effect of methanol leaf extract on liver function indices in rats**

Experiment	Doses (mg/kg)	ALT (U/L)	AST (U/L)
Control	0	5.60±0.80	7.93±0.53
Phase 1	10	6.48±0.19 <sup>a</sup>	7.12±1.1 <sup>a</sup>
	100	7.04±1.65 <sup>a</sup>	7.35±0.19 <sup>a</sup>
	1000	7.15±1.42 <sup>a</sup>	13.18±2.43 <sup>b</sup>
	Phase 2	1500	14.8±0.25 <sup>b</sup>
3000		14.4±2.02 <sup>b</sup>	29.10±1.10 <sup>b</sup>
5000		15.28±1.14 <sup>b</sup>	29.55±1.04 <sup>b</sup>



Results are reported as Mean  $\pm$  SEM. Results with superscript “a” shows no significant difference ( $p > 0.05$ ) when compared to the control group while results with superscript “b” shows significant difference ( $p < 0.05$ ) when compared to the control group.



**Fig. 2: A chart showing the various concentration of MDA in the both control and treated group**

The liver is a critical organ in the human body that is responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, vitamin storage among other functions [27]. The level of biochemical, liver function markers such as aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase can be used to assess the degree of liver damage caused by a chemical compound or plant extract (ALP). After cellular liver injury, ALP is released into circulation from the cytoplasm. ALT and AST are enzymes that are generated when the liver is injured, particularly when the mitochondria of the liver cells are damaged. Elevated levels of these enzymes may indicate cellular injury, leakage, and loss of the hepatic cell membrane's functional integrity. When the extract-treated groups are compared to the control group, the results show no significant variations in the levels of liver enzymes (AST and ALT) (Table 3).

Following chronic dosing, a considerable increase in MDA level at lethal dose of the extract indicates extensive lipid peroxidation of polyunsaturated fatty acids and the amount of MDA is proportional to the degree of lipid peroxidation (Figure 2) [28]. The oxidative breakdown of lipids is known as lipid peroxidation. Alkaloids, saponins, tannins, triterpenes, flavonoids, and other phytochemical elements are found in *Musanga cercropioides*. It's likely that high doses of the leaf extract led to higher concentrations of phytochemical elements with pro-oxidant potential on lipid membranes. According to the findings, subacute and chronic toxicity studies should be conducted in order to establish the potential harmful effects of long-term leaf extract con-

### Conclusion

In conclusion, the acute toxicological study of the methanol leaf extract of *Musanga cercropioides*, when given orally, may be deemed largely free of toxicity

because it caused no mortality/lethality or created any notable biochemical adverse effects in all of the albino rats treated. Furthermore, oral administration of the extract did not create any significant changes in body weights or organ weights, nor did it cause any clinical toxicity signs such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, or coma.

**Conflict of interest:** The authors declare that no competing interest to disclose.

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