Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat

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Abstract

**Objectives:** *Musanga cecropioides* stem-bark extract is frequently used in folkloric medicine in Nigeria for management of some chronic ailments like hypertension, diabetes mellitus, memory loss etc. The aim of this study is to evaluate long time safety profile of *Musanga cecropioides* hydromethanolic (MCH) stem-bark extract using acute and sub-chronic model in rats.

**Materials and Methods:** Acute toxicity test was instituted with single oral doses (100 - 5000 mg/kg) of MCH stem-bark extract in rats which was observed from 48 hours to 14 days for behavioral sign of toxicity and death. The sub-chronic study utilizes doses (125, 250 and 500 mg/kg) of MCH stem-bark extract for 96 days. Blood samples were collected by cardiac puncture to evaluate serum biochemistry, hematology, lipid profile, lipid peroxidation and *in vivo* antioxidant activities. Evaluation of the effects of the MCH extract on brain, kidney and liver histopathology were executed.

**Results:** No signs of behavioral toxicity and deaths were observed during the acute toxicity test. Sub-chronic study revealed a significant increase of mean weights of male (P < 0.001) and female (P < 0.05) animals but no significant effects on organ weights. There were no significant effects on hematology, lipid profile, lipid peroxidation and antioxidant activities. The MCH extract dose dependently demonstrate mild to severe degeneration of liver and kidney histopathological cytoarchitecture while no significant impingement of the brain and hematological tissues across the investigated doses.

**Conclusion:** The result of this study provided evidence that oral dosing of 125 mg/kg of MCH is relatively safe in rats and may not exert any severe toxic effects.

**Keywords:** *Musanga cecropioides; Oral Chronic Toxicity; Hematology; Histopathology; Serum Biochemistry; Antioxidant Profile*

Introduction

The worldwide patronages of plant based products are on the increase. Currently, an estimated 80% of the world’s populations depend on medicinal plants or derivatives to meet basic health care needs [1]. The World Health Organization (WHO) have posited that herbal and plant derived remedies are the most frequently utilized therapies worldwide. In 2003, WHO estimated that the global markets for plant derived medicine stood over $6 billion US dollars annually. In Africa, WHO estimated that up to 80% of the population utilizes traditional medicine including herbal preparation for their primary health care need and in China traditional herbal preparation account for 30 - 50% of all medicinal consumption [2]. As a result of preponderance of side-effects associated with orthodox pharmacotherapy, alternative and complimentary medicine has emerged to fill this gap [3]. The positive points for patronizing native medicine is the belief

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that it is safer and of less toxic insults to the organism than synthetic molecules [4]. Besides litany of reports abound in literatures incriminating long term usage which might be advantageous in terms of toxicity consideration. Likely, bioactive agents obtained from natural products have relatively low animal and human toxicity [5]. It is against this background that the World Health Organization (WHO) have recognized and advocated the patronage of traditional medicine as alternative and complimentary medicine and empowered member nations to make policies inculcating this form of medicine into their primary health care [6]. Most time patients are confused and assuming that herbs are natural alternative to synthetic drugs and devoid of toxic effects; ignorantly not appreciating the armamentarium of phytotoxins contained therein which could be of serious toxicity implication as report actually confirm toxicity of medicines derived from herbs [7,8]. Though many herbal products are innocuous, many are not devoid of toxicity implication with their usage. The heterogeneous bioactive constituents of herbal products might induce multiple toxicity effects on multiple organ systems such as the renal, hepatic, nervous, cardiovascular, gastrointestinal and hematologic system [9-11]. Report abounds attesting to the fact that many herbal medicine preparations are potentially toxic and even carcinogenic [11-18] therefore should be cautiously used [19]. For example, aristolochic acid derived from Aristolochia spp. is reported to be associated with the development of nephropathy and urothelial cancer [20,21]. This claim cannot be over-ruled since only a few plants/herbs used in herbal medicine have scientific validation for any claimed medicinal effect, thereby slowing down the pace of drug discovery from medicinal plants. Therefore, high premium is placed on herbs with a high therapeutic value and minimal side effects as prelude for scientific evaluation of plants or plant products for pharmaceuticals. This approach is both scientific and logical because over the years research outcomes have questioned the popular notion that “things of natural origin are safe”. From the above mentioned facts, it has become expedient to execute reasonable and safe toxicological investigation to provide the highly prize preclinical scientific data needed to justify safety profile of herbal preparations before obtaining regulatory approval for marketing drug products [22].

Experiments with non-human subjects such as rodents and primates were used over the years to generate these data. The findings gotten from these studies are largely reliable and usually serve as reference in clinical studies in humans since the animals and humans have similarly related biological activities [23]. The herbal medicines toxicity might in part be associated with factors such as: (i) the presence of phytotoxins in some pure herbal medicines; (ii) errors in botanical identification; (iii) inappropriate recipes of plants; and (iv) utilization of plants that antagonize orthodox pharmacotherapy etc., therefore the necessity for institution of chronic toxicity screening of medicinal plants in addition to numerous other investigations can not be over-emphasized.

The utilization of traditional medicine of herbal origin abounds among all ethnic groups from the six geopolitical regions of Nigeria. Musanga cecropioides is an indigenous plant in folkloric medicine of the Effik and Ibibio tribes of South-South, Nigeria. It belongs to the urticateae family. It is commonly called African cork wood tree or umbrella tree (English). The indigenous names are Aga agbawo (Yoruba), Ulu (Igbo), Odzuma (Ghana). The plant is an upright, swiftly developing tree of the deciduous tropical West African rainforest; ample in swampy forest, river or lake side; situated at an altitude of 700 - 1200m. It breeds up to 20 m tall with umbrella-shaped crown, erect and tubular trunk (up to 2 m in girth) and still adventitious roots of up to 3 m above ground level. Fruit is yellowish green and succulent [26]. The common ethnomedicinal uses of the plant in Nigeria is utilization of the infusion of the plant leaf and stem bark for treatment of fever, jaundice, acute gastric poisonings, liver diseases, menstrual pain, induction of labour, depressing of high blood pressure and elevated blood sugar levels. It is also used as dehydrant, expectorant, anthelminthic, anti-dysenteric and analgesic, treatment of asthenia in infants and for restoration of appetite [27-33].

The sap is drunk as a blood-purifier; for cleansing stomach, for blennorrhoea, cough and chest infections, as a galactagogue, and commonly as a wash for persons with sleeping sickness, leprosy and fevers to relieve aches and pains; it is of use in management of rheumatism, inflammatory conditions and has analgesic properties [29,33,34]. Pharmacological studies evaluated includes: uterotonic and oral hypnotensive effects [29,35], oxytocic effects; hypoglycemic and antidiabetic activities [28,36] amongst others. Isolated compounds includes kalaic acid [30], triterpenoid acids [33] and other bioactive compounds such as alkaloids, flavonoids, tannins, free and bound anthraquinone, saponin and cardiac glycosides but anthocyanosides and cyanogenic glycosides are absent [37]. The leaves extract contain isovitexin, vitexin, chlorogenic acid, catechin, and procyanidins [38].

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Currently, preliminary data posit memory enhancing effects justifying that the plant could be subjected to chronic exposure in management of neurodegenerative conditions. Though, preliminary acute and subchronic toxicity studies for 28 days on *M. cecropioides* stem aqueous bark extract have been investigated [33], there is no data on chronic exposure. The aim of the study is to provide data from *M. cecropioides* stem-bark chronic exposures in Wistar albino rats for 3 months on hematological parameters, liver function, lipid profile, antioxidant enzymes, lipid peroxidation and to examine any histopathological alteration of target organs of toxicity: brain, blood, kidney and liver.

**Materials and Methods**

**Drugs and Chemicals**

Isoniazid (Alpha LabLtd, India), Rifampicin (Ziel Medicare Ltd), silymarin (Microlab Ltd, India), analytical grade xylene (Sigma Aldrich Chemmie, Germany), absolute alcohol (BDH chemicals Ltd, Poole England), intermediate melting point paraffin (Alexandra, Egypt). The equipment used include, analytical balance CPA 3245 (Sartonus AG, Gogningen, Germany), spectrophotometer (Spectrum Labs, USA), water bath (TT-6 Techmel and Techmel USA), centrifuge 412B (Techmel and Techmel, USA).

**Plant collection, identification and preparation**

Fresh stem bark of *Musanga cecropioides* were obtained from Itak Ikpa Local Government area of Akwa Ibom State, Nigeria in January 2014 by a herbalist Mr. Etefia Okon in the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, University of Uyo. Identification and authentication was done by Mrs. Margret Bassey of Botany Department of University of Uyo, Akwa Ibom State. The Herbarium specimen with voucher number UUPH 20(a) was deposited at Department of Pharmacognosy, University of Uyo, Akwa Ibom State, Nigeria for referencing. After shade drying for one week, the stem bark was pulverized using a mechanical grinder. Then 250.0g of the powdered stem bark was extracted using 2.0L of 50% methanol. Maceration was carried out for 3 days with continuous agitation, after which filtration was carried out using a double layered gauge. The filtrate was evaporated to dryness using a water bath (TT-6 Techmel and Techmel USA) set at 55°C. The yield is 4.18%. The dried extract was then transferred into a clean container and stored at 4°C in a refrigerator until used.

**Phytochemical screening**

Phytochemical analysis was executed on the stem bark extract of *Musanga cecropioides* following the procedure of Trease and Evans [39]. The various phytochemical agents analysed includes: saponins, tannins, alkaloids, flavonoids, cardiac glycosides, reducing sugars and anthraquinones.

**Experimental animals**

A total of forty (40) Wistar albino rats were used for this study. The animals were obtained from the Animal house at the Niger Delta University (NDU), Bayelsa State, Nigeria. The animals were housed in a good laboratory practice (GLP) facility with controlled temperature (22 ± 1.5°C), humidity (50 ± 12.5), ventilation (10 - 15 times/h), light (12:12h light: dark cycle), and good illumination. The animals were fed with Vita feed (Ibadan) and clean water was provided *ad libitum*. The animals were randomly allocated to one control group and three treatment groups. All experimental protocols follow the "Guide to the care and use of animals in research and teaching (NIH, 1996) with approval of University of Port Harcourt Ethical Committee on 20/02/2017 via a circular UPH/2017/048.

**Acute Toxicity studies**

The LD$_{50}$ of the *Musanga cecropioides* stem extract was evaluated adopting the procedure described [40,41]. Twelve healthy male Wistar albino rats (175 - 200g) divided into 4 groups of 3 animals were used. The rats were deprived of food but allowed access to water *ad libitum* for 18 hours prior administration of extract on day 0. The stem extract was administered orally in doses of 500 - 5000 mg/kgbw to determine the range which toxicity lies. All the treated animals were observed every 2 hours for 12 hours for signs of toxicity such as ataxia, dullness, alertness, convulsion, and nervousness. The animals were monitored for a period of fourteen days for signs of toxicity.

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and death. The number of surviving animals after the 14 days period was documented. The animals were subjected to narcolepsy, the kidneys and liver gross anatomy were observed macroscopically. The LD₅₀ was observed on the basis of mortality [42]. The LD₅₀ was then calculated as the geometric mean of the lowest dose causing death and the highest dose causing no death.

**Treatment of Animals**

The animals were divided into 4 groups and allowed to acclimatize for a period of 14 days. All procedures were carried out in accordance with the principles of Good Laboratory Practice issued by the Organization for Economic Cooperation and Development (OECD) with approval of University of Port Harcourt Ethical Committee on 20/02/2017 via a circular UPH/2017/048. The *Musanga cecropioides* hydromethanolic stem bark extract was suspended in water for injection (WFI), usually prepared once a week in accordance with the results from stability analysis assessed previously. Three doses for the *Musanga cecropioides* hydromethanolic extract were chosen since the LD₅₀ of the previous work by Adeneye, *et al.* [33] is reported to be safe above 3000 mg/kg.

Group A: control (WFI, 5ml/kg/day)
Group B: 125 mg/kg of *Musanga cecropioides* stem bark extract
Group C: 250 mg/kg of *Musanga cecropioides* stem bark extract
Group D: 500 mg/kg of *Musanga cecropioides* stem bark extract

All doses were orally administered by gavage 5 days per week for 14 weeks. The experimental design followed the method of Pongri and Igbe [41].

**Weight evaluations**

The weights of the Wistar albino rats in all the experimental groups were weighed initially before drug administration and subsequently on a daily basis throughout the duration of experiment and on the 96th day. The weight of the liver was recorded after sacrificing the animal on the 96th day.

**Blood sampling /organ collection**

Feed was withdrawn from the animals cages 24 hours after the last treatment and the animals were anaesthetized with light doses of diethyl ether in a desiccator. The thoracic region was opened to expose the heart, and blood was quickly collected via cardiac puncture with a 10 ml syringe and needle into appropriately labelled containers. Organs such as the brain, the liver and kidney were excised for histopathological studies.

**Blood sampling**

The rats were deprived of food but allowed acess to water *ad libitum* for 18 hours prior to anaesthetization using diethyl ether. Blood was obtained by cardiac puncture, centrifuge at 3000 rpm for 5 minutes. The sera was collected into vacutainers using micropipette in aliquots, stored at -20°C until used for the various liver function analyses within 12 hours of collection. Blood samples for hematological analysis were transferred to EDTA bottles.

**Hematological analysis**

Hematological analysis was evaluated using an automated hematology analyzer (Sysmex kx-21N, Sysmex corporation, Kube, Japan). The various hematological parameters analyzed include: packed cell volume (PCV), hemoglobin (HB), mean corpuscular volume (MCV), Red Blood Cell (RBC), Mean Corpuscular Hemoglobin Concentration (MCHbC), Mean Corpuscular Hemoglobin (MCH), Platelet (PLT), White Blood Cell (WBC), Neutrophils (NEU), Lymphocytes (LYM), Monocytes, Eosinophil and Basophils (MEBs). The analysis was executed adopting standard protocol of the Hematology Unit of Braithwaite Memorial Specialist Hospital (BMSH) Port Harcourt.

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Biochemical measurements

Serum enzymes and other biomarkers were determined using standard protocol. The activity of serum aminotransferase (alanine aspartate transaminase (AST) and alanine transaminase (ALT) were determined following kinetic protocol described by Reitman and Frankel [43]. Serum alkaline alanine phosphatase (ALP) was assayed using the procedure of Roy [44]. Serum bilirubin was determined calorimetrically using the method of Young [45]. Plasma albumin (ALB) concentration was determined by photometric method of Doumas, et al [46]. Total protein (TP) assay was achieved by Biuret method [47,48]; high density lipoprotein-cholesterol (HDL-C) by the method of Lopes-Virella, et al [49]; total cholesterol (TC) by the procedure of Roeschlau, et al [50]; triglycerides (TG) by the method of Tietz [51]; electrolytes like Chloride (Cl-), potassium (K+), sodium (Na+) and bicarbonate (HCO₃⁻) were estimated spectrophotometrically.

Determination of markers oxidative stress

**Estimation of reduced Glutathione (GSH):** Glutathione level was determined using spectrophotometric method [52]. Briefly, two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 35 ml of 50 % trichloroacetic acid (TCA) and 0.5 ml distilled water; while T2 contained 35 ml of 50% trichloroacetic acid (TCA) and 0.5 ml of test sample. The solution was mixed and centrifuged at 2000 xg for 5 minutes, and then supernatant (1 ml) was transferred into another test tube containing 2 ml of 0.01 M DTNB reagent (Elliott's reagent). The solution was kept away from direct light for 15 - 20 minutes and absorbance was read at 412 nm. For the standard: The test tubes labeled T3 contained 0.5 ml of standard glutathione, 1.5 ml phosphate buffer and 2 ml of 0.01M DTNB reagent. The absorbance was read at 412 nm after 15 minutes. The concentration of glutathione (µg/ml) was traced from the standard curve for glutathione.

**Glutathione peroxidase (GSH-Px) level:** The level of GSH-Px was assessed following the procedure of Rotruck, et al [53]. The assay mixture containing 0.5 ml of sodium phosphate buffer; 0.1 ml sodium azide, 0.2 ml of reduced glutathione, 0.1 ml of H₂O₂ and 0.5 ml 1:10 cell extract was taken and the total volume was made up to 2.0 ml with distilled water. The tubes were incubated at 37°C for 3 minutes and the reaction was terminated by the addition of 0.5 ml 10% TCA. To determine the residual glutathione content, the phosphate (0.3M) solution and 1 ml of the DTNB reagent were added. The colour developed was read at 412 nm against a reagent blank containing only phosphate solution and DTNB reagent on a spectrophotometer. Suitable aliquots of the standard were also treated similarly.

**Estimation of superoxide Dismutase (SOD) levels:** Superoxide dismutase activity was determined using auto-oxidation method [54]. Tissues homogenates was centrifuge at 12 000g and an aliquot of supernatant was diluted with water to make a 1:10 dilution. The diluted sample (200 µl) was added to 2.5 ml of 0.05 ml carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by addition of 0.3 ml of freshly prepared 0.3 mM epinephrine to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer; 0.3 ml of substrate (epinephrine) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored every 30s for 2.5 minutes.

**Estimation of catalase levels:** Catalase (CAT) levels were determined using hydrogen peroxide as a substrate according to the method of Clairborne [55] with slight modification. For determination of catalase activity, 0.2 ml of sample (equivalent to 50-100 µg protein) was added to 50 mM of phosphate buffer (pH 7.4) containing 100 mM (v/v) of H₂O₂ (sigma, St Louis, USA) in a total of 1 ml. The reaction mixture was incubated for 2 minutes at 37°C and the rate of absorbance change (Δ A/min) at 240 nm was recorded, which indicate the decomposition of H₂O₂. Activities were calculated using the molar extinction coefficient of H₂O₂ at 240 nm, 43.59 L/mol cm. One unit of catalase activity equals the amount of protein that converts 1 µmol H₂O₂ /min. All samples were measured in quadruplicates.

**Estimation of Malondialdehyde (MDA):** Malondialdehyde is the most copious aldehyde from breakdown products of lipid peroxidation and its measurements is an indirect guide of lipid peroxidation. Malondialdehyde (MDA) was estimated according to the method of Ohkawa and Ohishi [56]. An aliquot of 0.4 ml of supernatant was added to 1.6 ml of Tris-KCL buffer to which 0.5 ml of 30% TCA was added and vortexed. After which 0.5 ml of 0.75% thiobarbituric acid (TBA) was introduced and placed in boiling water for one hour. This was then cooled on ice and centrifuged at 3000g. The clear supernatant was collected and the absorbance measured against a reference blank

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of distilled water at 532 nm. The level of MDA was estimated following the procedure of Todorova, et al. [57] and expressed as µmol MDA/mg protein.

Histopathology studies

Brain, liver and kidney tissues harvested were fixed in 10% formal saline and were processed into paraffin embedded diagnostic tissue blocks using analytical grade xylene purchased from Sigma Aldrich chemie (GmbH, Germany), Absolute alcohol (BDH chemicals Ltd, Poole England) and Intermediate melting point paraffin wax was supplied by Alexandra (Egypt). The tissue sections harvested from control and test experimental Wistar albino rats were fixed for 48 hours. Tissue specimens were then dissected and representative tissue blocks were taken for standard processing into paraffin embedded tissue blocks. Tissue sections were cut at 4µm using a Leica RM 125RT rotary microtome. Optimised haematoxylin and eosin staining protocol was carried out on all the slides. Well stained slides were examined under the light microscope for morphological changes after mounting in DPX mountant obtained from Atom Scientific (Manchester, UK).

Statistical analysis

The data are expressed as Mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Turkey multiple comparison test. All statistical analysis and graphical representation of the data were performed by using Graphpad Prism, version 5 software. The level of significance were mentioned as *P < 0.05; **P < 0.01, ***P < 0.001.

Results

Phytochemical screening

The screening of the hydromethanolic extract revealed the presence of saponins, flavonoids, alkaloids, tannins, phlobatannins, cardiac glycosides, reducing sugars and anthraquinones (Table 1).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td></td>
</tr>
<tr>
<td>(i) Benedict’s test</td>
<td>+</td>
</tr>
<tr>
<td>(ii) Emulsion test</td>
<td>+</td>
</tr>
<tr>
<td>(iii) Frothing test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td></td>
</tr>
<tr>
<td>(i) Bromine water test</td>
<td>++</td>
</tr>
<tr>
<td>(ii) Ferric chloride test</td>
<td>++</td>
</tr>
<tr>
<td>(iii) Phlobatannins</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
</tr>
<tr>
<td>(i) Draggendoff’s test</td>
<td>++</td>
</tr>
<tr>
<td>(ii) Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td>(iii) Wagner’s test</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
</tr>
<tr>
<td>(i) Lead acetate test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Ferric chloride test</td>
<td>+++</td>
</tr>
<tr>
<td>(iii) Sodium chloride test</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td></td>
</tr>
<tr>
<td>(i) Keller–Kelliani test</td>
<td>+++</td>
</tr>
<tr>
<td>(ii) Salkowski’s test</td>
<td>++</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td></td>
</tr>
<tr>
<td>(i) Hexose sugar</td>
<td>+</td>
</tr>
<tr>
<td>(ii) Ketosugar</td>
<td>+</td>
</tr>
<tr>
<td>(iii) Pentosugar</td>
<td>+</td>
</tr>
<tr>
<td>(iv) Monosaccharide</td>
<td></td>
</tr>
<tr>
<td>Anthraquinones</td>
<td></td>
</tr>
<tr>
<td>(i) Free anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>(ii) Bound anthraquinones</td>
<td>++</td>
</tr>
<tr>
<td>(iii) Anthocyanides</td>
<td>-</td>
</tr>
</tbody>
</table>

| Table 1: Chemical constituents of Musanga cecropioides stem bark methanolic extract. |
| -: not detected; +: present in low concentration; ++: present in moderate concentration |
Acute oral Toxicity

The results of acute toxicity (LD₅₀) showed that the extract at 5 g/kg did not produce any oral toxicity in rats after fourteen days treatment. No behavioral sign of toxicity. Narcolepsy did not reveal any visible signs of toxicity. Therefore we utilized doses of 1/50, 1/10 and 1/5 of 5g/kg as the experimental doses.

Body and organ weights analysis

The initial mean body weight and mean weight changes of Wistar albino rats are shown in figure 1. The Musanga cecropioides hydromethanolic extract administered to rats for 14 weeks of this study caused no significant (P > 0.05) weight gain in female animals at the first month of treatment but a significant (P < 0.05) gain in weight in 2nd and 3rd months of treatment; but in the male group there was a significant (P < 0.001) gain in weight at all months of treatment when compared to control. The effects of the hydromethanolic extract on the weights of some vital body organs in rats is shown in table 2. Similarly, the extract administration did not cause any significant difference in the organ weights of the test animals when compared to the control.

Figure 1: Effects of M. cecropioides stem bark extract on weights of female rats during chronic toxicity test for 3 months.
Data is presented as mean ± SD with significance changes in body weight at Mo denoted with aP < 0.05; cP < 0.001.
Abbreviations: Mo: Initial Month of Acclimatization; M1: First Month of Treatment; M2: Second Month of Treatment; M3: Third Month of Treatment; Grp A: Control; Grp B: 125 mg/kg; Grp C: 250 mg/kg; Grp D: 500 mg/kg.
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Table 2: Effects of MCH stem-bark extract on organ weights following 3 months continuous dosing.

<table>
<thead>
<tr>
<th>Organs Weight (g)</th>
<th>Control (WFI 5 ml/kg)</th>
<th>MCH 125 mg/kg</th>
<th>MCH 250 mg/kg</th>
<th>MCH 500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>6.32 ± 0.4</td>
<td>6.21 ± 0.37</td>
<td>6.08 ± 0.31</td>
<td>5.67 ± 0.28</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.27 ± 0.07</td>
<td>1.18 ± 0.08</td>
<td>1.25 ± 0.25</td>
<td>1.11 ± 0.09</td>
</tr>
<tr>
<td>Brain</td>
<td>1.19 ± 0.04</td>
<td>1.31 ± 0.06</td>
<td>1.35 ± 0.08</td>
<td>1.23 ± 0.09</td>
</tr>
<tr>
<td>Heart</td>
<td>0.77 ± 0.06</td>
<td>0.67 ± 0.03</td>
<td>0.64 ± 0.04</td>
<td>0.69 ± 0.10</td>
</tr>
<tr>
<td>Ovary</td>
<td>0.42 ± 0.13</td>
<td>0.18 ± 0.03</td>
<td>0.37 ± 0.18</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Testes</td>
<td>3.75 ± 0.86</td>
<td>2.82 ± 0.12</td>
<td>2.68 ± 0.22</td>
<td>2.03 ± 0.26</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.72 ± 0.07</td>
<td>0.62 ± 0.04</td>
<td>0.79 ± 0.08</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>Stomach</td>
<td>2.26 ± 0.12</td>
<td>2.04 ± 0.12</td>
<td>2.60 ± 0.30</td>
<td>2.28 ± 0.19</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.67 ± 0.08</td>
<td>1.74 ± 0.14</td>
<td>2.05 ± 0.20</td>
<td>1.82 ± 0.23</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; (n = 6). Values were not statistically significant (P > 0.05) when compared to the control. Abbreviation: MCH - *Musanga cecropioides* stem-bark extract; WFI - Water for injection. The body weights were measured right before necropsy after overnight fasting.

Hematological responses of rats on chronic dosing of *Musanga cecropioides*

The effect on hematological indices following chronic exposure of stem bark hydromethanolic extract of *Musanga cecropioides* is shown in table 3. The extract caused no statistically significant (P > 0.05) difference on the hematological parameters being investigated at the tested doses in the treated group when compared to control.

Table 3: Effects of MCH stem-bark extract on hematology following 3 months continuous dosing.

<table>
<thead>
<tr>
<th>Organs Weight (g)</th>
<th>Control (WFI 5 ml/kg)</th>
<th>MCH 125 mg/kg</th>
<th>MCH 250 mg/kg</th>
<th>MCH 500 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>48.11 ± 2.6</td>
<td>48.22 ± 1.3</td>
<td>46.83 ± 2.0</td>
<td>50.50 ± 2.7</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>13.26 ± 0.6</td>
<td>13.81 ± 0.3</td>
<td>13.87 ± 0.5</td>
<td>12.95 ± 2.1</td>
</tr>
<tr>
<td>WBC (×10^3 cells/µL)</td>
<td>4.78 ± 0.9</td>
<td>6.14 ± 0.8</td>
<td>9.74 ± 1.8</td>
<td>9.78 ± 1.6</td>
</tr>
<tr>
<td>PLT (×10^3 cells/µL)</td>
<td>652.9 ± 87.3</td>
<td>735.3 ± 60.0</td>
<td>767.0 ± 81.7</td>
<td>927.3 ± 10.1</td>
</tr>
<tr>
<td>RBC (×10^6 cells/µL)</td>
<td>7.6 ± 0.5</td>
<td>7.5 ± 0.4</td>
<td>7.5 ± 0.4</td>
<td>8.0 ± 2.4</td>
</tr>
<tr>
<td>MCV(%)</td>
<td>64.3 ± 1.5</td>
<td>65.1 ± 1.7</td>
<td>62.1 ± 1.3</td>
<td>63.3 ± 0.3</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>17.7 ± 0.3</td>
<td>18.7 ± 0.4</td>
<td>18.7 ± 0.3</td>
<td>16.3 ± 2.5</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>27.8 ± 0.3</td>
<td>28.6 ± 0.9</td>
<td>29.8 ± 1.1</td>
<td>25.8 ± 3.9</td>
</tr>
<tr>
<td>NEU (%)</td>
<td>21.9 ± 2.8</td>
<td>14.9 ± 2.3</td>
<td>23.7 ± 3.6</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>LYM (%)</td>
<td>68.7 ± 3.4</td>
<td>74.8 ± 2.5</td>
<td>63.8 ± 3.9</td>
<td>81.8 ± 1.5</td>
</tr>
<tr>
<td>MEB (%)</td>
<td>11.1 ± 1.9</td>
<td>10.3 ± 1.6</td>
<td>12.5 ± 1.8</td>
<td>8.03 ± 1.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD; (n = 6). Values were not statistically significant (P > 0.05) when compared to the control. PCV: Packed Cell Volume; HB: Hemoglobin; WBC: White Blood Cell; PLT: Platelet; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; NEUT: Neutrophils; Lymph: Lymphocytes; MEB: Monocytes; Eosinophils and Basophils; MCH-Musanga cecropioides Hydromethanolic Extract; WFI: Water for Injection.

**Citation:** Nwidu Lucky Legbosi and Teme Raphael Ellis. “Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat”. *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat

Liver enzymes and proteins assays

The result (Table 4) shows that the hydromethanolic extract of *Musanga cecropioides* did not provoke any significant changes in the levels of liver proteins and enzymes biomarkers, total proteins (TP), albumin (ALB), AST, ALT and ALP. There was no significant (P > 0.05) alteration of the level of all the parameters investigated when compared to the control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TP (g/dl)</th>
<th>ALB (mg/dl)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.17 ± 1.6</td>
<td>27.0 ± 3.0</td>
<td>28.67 ± 6.4</td>
<td>66.66 ± 16.3</td>
<td>102.16 ± 33.4</td>
</tr>
<tr>
<td>MCH (125 mg/kg)</td>
<td>57.67 ± 3.6</td>
<td>37.6 ± 2.6</td>
<td>29.83 ± 5.7</td>
<td>53.33 ± 16.01</td>
<td>119.67 ± 25.1</td>
</tr>
<tr>
<td>MCH (250 mg/kg)</td>
<td>66.86 ± 2.7</td>
<td>39.43 ± 2.1</td>
<td>27.71 ± 6.1</td>
<td>56.85 ± 20.2</td>
<td>122.85 ± 47.2</td>
</tr>
<tr>
<td>MCH (500 mg/kg)</td>
<td>65.50 ± 6.3</td>
<td>38.0 ± 2.53</td>
<td>28.16 ± 6.68</td>
<td>67.33 ± 28.5</td>
<td>144.83 ± 87.1</td>
</tr>
</tbody>
</table>

*Table 4: Effect of *M. cecropioides* stem-bark extract on chronic dosing for 3 months on liver proteins and enzymes.*

Values are expressed as Mean ± SD; n = 6; P > 0 when compared to control.


Lipid profile assay

The result (Table 5) shows that the hydromethanolic extract of *Musanga cecropioides* has no profound significant effect on TTG, TCHO, HDL and LDL levels. Increased serum levels of TTG (500 mg/kg), TCHO and LDL were obtained for the test animals groups while decreased serum level was obtained for HDL when compared to the control group. The values of TCHO, HDL and LDL were not statistically significant (P > 0.05) but that of TTG (500 mg/kg) was statistically significant (P < 0.05) when compared to the control animals group. A dose-effect relationship was observed in the values obtained for the lipid profile parameters in the test animals groups with exceptions for the values obtained for HDL and LDL.

<table>
<thead>
<tr>
<th>Group</th>
<th>TTG (mmol/L)</th>
<th>TCHO (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69 ± 0.3</td>
<td>1.47 ± 0.2</td>
<td>0.57 ± 0.1</td>
<td>0.58 ± 0.4</td>
</tr>
<tr>
<td>MCH 125 mg/kg</td>
<td>0.62 ± 0.1</td>
<td>1.48 ± 0.2</td>
<td>0.53 ± 0.1</td>
<td>0.65 ± 0.2</td>
</tr>
<tr>
<td>MCH 250 mg/kg</td>
<td>0.63 ± 0.4</td>
<td>1.61 ± 0.4</td>
<td>0.56 ± 0.1</td>
<td>0.76 ± 0.4</td>
</tr>
<tr>
<td>MCH 500 mg/kg</td>
<td>1.05 ± 0.2*</td>
<td>1.69 ± 0.6</td>
<td>0.53 ± 0.1</td>
<td>0.68 ± 0.6</td>
</tr>
</tbody>
</table>

*Table 5: Effect of the stem bark extract of *M. cecropioides* on oral chronic dosing for 3 months on lipid profile in Wistar rats.*

All values are expressed as Mean ± SD; n=6; *P < 0.05 when groups are compared to control.

Abbreviations: TTG: Total Triglycerides; TCHO: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

In-vivo antioxidant enzymes and lipid peroxidation

The result of the effect of hydromethanolic extract of *Musanga cecropioides* on antioxidant and lipid peroxidation biomarkers did not reveal any significant alterations at 125 and 250 mg/kg of antioxidant enzymes, CAT (Figure 2), SOD (Figure 3), GSH (Figure 4) and GPx (Figure 5) when compared to the control group. The lipid peroxidation product, MDA (Figure 6) was also not significantly (P < 0.05) elevated in all extract treated group compared to the control group. The increased in level of GPx was not statistically significant (P > 0.05) when compared to the control group.

Citation: Nwidu Lucky Legbosi and Teme Raphael Ellis. “Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat”. *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat

**Figure 2:** Effect of chronic administration of MCH stem bark extract on CAT in wistar rats for 90 days.

Data presented as mean ± SD. No significance changes compared to control (P > 0.05).

**Figure 3:** Effect of chronic administration of MCH stem bark extract on SOD in wistar rats for 90 days.

Data presented as mean ± SD. No significance changes compared to control (P > 0.05).
Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat

**Figure 4:** Effect of chronic administration of MCH stem bark extract on GSPx in wistar rats for 90 days.

Data presented as mean ± SD. No significance changes compared to control (P > 0.05).

**Figure 5:** Effect of chronic administration of MCH stem bark extract on GSH in wistar rats for 90 days.

Data presented as mean ± SD. No significance changes compared to control (P > 0.05).

*Citation:* Nwidu Lucky Legbosi and Teme Raphael Ellis. “Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat”. *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
Histopathological studies of brain, kidney and liver

In the vehicle treated group there was impingement in cytoarchitecture of the brain (Figure 7a), kidney (Figure 7b) and liver (Figure 7c). However, a dose dependent impingement was observed for all the organs. The low dose appears to present no adverse distortion of the cytoarchitecture of the organs investigated. In other words, the low dose group of the liver, kidney and brain are normal. The medium and highest doses however poses moderate to severe distortion of the histological features. At the middle and high doses there are moderate expansion of the interstitium and moderate infiltration with lymphocytes with focal inflammation.

Citation: Nwidi Lucky Legbosi and Teme Raphael Ellis. "Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of Musanga cecropioides (Urticaceae) in Rat". EC Pharmacology and Toxicology 6.3 (2018): 76-95.
Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat

Figure 7b: Photomicrograph of rat kidney tissues (x400).

Tissue sections of rat kidney show similar but varying degree of morphological changes characterized by focal inflammation, necrosis and renal tubular fibrosis.

A: Photomicrograph of control rat treated with WFI, 5ml/kg/day, shows essentially normal renal tubules. The renal glomeruli and papilla are also normal.

B: Photomicrograph of test rats treated with 125mg/kg of MCH stem bark extract shows normal features with focal inflammation of renal tubules.

C: Photomicrograph of test rats treated with 250mg/kg of MCH stem bark extract shows inflammation mild tubular necrosis and fibrosis.

D: Photomicrograph of test rats treated with 500mg/kg of MCH stem bark extract shows moderate renal tubular fibrosis.

Figure 7c: Photomicrograph of rat liver tissues (x400).

Hepatic tissue of test rats show varying degree of inflammation and hepatocellular necrosis in some areas.

A: Photomicrograph of control rat treated with WFI, 5ml/kg/day, shows essentially normal hepatocytes, intact sinusoids and portal tracts.

B: Photomicrograph of test rats treated with 125mg/kg of MCH stem bark extract shows normal hepatocytes with mild hemorrhagic necrosis and focal portal and periportal inflammations.

C: Photomicrograph of test rats treated with 250mg/kg of MCH stem bark extract shows focal areas of mild hepatocellular necrosis.

D: Photomicrograph of test rats treated with 500mg/kg of MCH stem bark extract shows moderate hepatocellular necrosis in areas.

Citation: Nwidi Lucky Legbosi and Teme Raphael Ellis. “Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat.” *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
Discussion

There is however, dearth of reports of subchronic toxicity effects of oral administration of MCH stem-bark extract in experimental animals in scientific and academics literatures. This is significant as this herbal medicine is pharmacological proven to be effective in management of chronic diseases like diabetes mellitus and hypertension [27,29]. Its usage in chronic diseases might necessitate consumption for prolong period of time, therefore there is need to examine untoward effects that might develop with longtime treatment. This study was design for 90 days to assess the safety profile of the hydromethanolic (1:1) extract of stem-bark extract of *M. cecropioides* in rats.

Impingements following chronic oral administration were appraised on hematology, serum biochemistry, lipid profile, lipid peroxidation and histology of liver, kidney and brain. The effect of extract on general body weights, and organs weights, general behavioural signs of toxicity and effects on pulps and litters were monitored for five months postnatally.

Medicinal plants safety profile has recently been questioned due to reports of illnesses and fatalities [58]. "Drug Induced Liver Injury (DILI)" presents a major challenge in the pharmaceutical industry and public health since DILI is the commonest grounds of drug development cessation, drug restrictions and post-marketing drug withdrawal [59]. Hence, toxicity studies are conducted on herbal products to determine and mitigate potential hazards associated with the prolonged consumption.

The investigation revealed significant increase on body weights of male than female rats indicating valuable effect on appetite, secondary effects on feeling of emptiness or fullness; indicating potential extract effect on the body fat and protein metabolism. Similarly no significant differences were observed in the organ weights of treated rats compared to the control group confirming the safety of the extract. Gross internal organ weight changes serve as indicator of adverse drug effects [60] as well as an important index of the physiological and pathological status of animals. The relative organ weights help to indicate if the organs were exposed to injury or not [61].

Hematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems [62]. The normal range of these parameters can be altered by the consumption of some toxic plants or plant products contamination [63]. They can also be used to establish hematotoxicity effects of phytochemical compound or extract. It is a highly sensitive, accurate, and reliable bedrock of ethical and rational research, disease diagnosis, prevention and treatment [64,65].

*Musanga cecropioides* extract exhibited statistically significant (P > 0.05) elevation or depression in hematological indices in treated rats (125 - 500 mg/kg) compared to the control group. The result revealed that the extract possess no significant insults to PCV, RBC and related indices, Hb, MCV, MCH and MCHC. The extract lacks the potential to arouse erythropoietin system of the kidneys and bone marrow [66,67]. Values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia; thus, the extract lack potential to induce anemia or polycythemia. Likewise the extract is devoid of adverse effect on the bone marrow, kidney and hemoglobin metabolism [68]. The extract caused no significant change in total white blood cells count, lymphocyte, neutrophil, monocyte and eosinophil probably indicating lack of immunosuppression [69,70] and no effect on the anti-allergic and anti-parasitic infectious responses of the body respectively.

The liver exerts a fundamental role on drug or xenobiotic biotransformation, protein synthesis and in homeostasis of organisms. Hence, liver enzymes are used as biomarkers in assessment of drug or plant extract safety or toxicity [71,72]. Hepatotoxins elevate AST, ALT and ALP levels in the serum and are commonly associated with hepatotoxicity [73]. Nonetheless, ALT is more confine to the liver and thus a well favoured parameter for identifying liver injury; AST is also associated with diseases of other organs such as heart and muscle [74]. ALP is present mostly in cells lining the biliary duct of the liver and is used to diagnose obstruction to the biliary system. Thus, its upsurge in the blood is a confirmation of cholestatic ailments such as gallstone or tumor obstructing the bile duct [75]. Oral dosing of the *M. cecropioides* extract (125 - 500 mg/kg) for 14 weeks normalizes the level of ALT with the untreated control at all doses, while the AST level deceases dose dependently (125 - 250 mg/kg) and normalize slightly above the untreated normal control. ALP revealed a dose dependent insignificant increase when compared with the untreated control. The effect on total proteins was not significantly affected by the doses of extract used on sub-chronic dosing. However, the TP increases slightly as well as ALB dose dependently but insignificantly. This might in part be due to increased production of globulin in the lymphoid organ and albumin with possibility of liver involvement [76].

Hyperlipidemia is widely reported as the chief risk factor underpinning atherosclerosis and is a precursor of coronary artery disease (CAD) [77]. The exposure of the rats to MCH stem-bark extract for 14 weeks did not provoke any significant alteration of TCHO, HDL and LDL except T TG which was significantly (P < 0.05) elevated at 500 mg/kg when compared to the untreated control. Elevated levels of LDL-cholesterol are associated with an increased risk of atherosclerosis and coronary heart disease [78]. In contrast, higher levels of HDL cholesterol are protective [79].

**Citation:** Nwidi Lucky Legbosi and Teme Raphael Ellis. “Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat”. *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
An insignificant elevation of the level of CAT, SOD and GSH at the highest dose of the stem bark extract (500 mg/kg) was noted. This implies that the elevated level of CAT will rapidly catalyze the decomposition of hydrogen peroxide ($H_2O_2$) into less-reactive gaseous oxygen ($O_2$) and water ($H_2O$) molecules, as the peroxide is a harmful byproduct of many normal metabolic processes and needs to be converted into innocuous substances, to prevent damage to cells and tissues. Similarly the rise in SOD level will favor significant dismutation of the superoxide ($O_2^-$) radical (produced as a byproduct of oxygen metabolism) into either ordinary molecular oxygen ($O_2$) or $H_2O_2$ with aim of preventing damage to cells and tissues from the superoxide radical ($O_2^-$). Moreover, the significant elevation of GSH level implies that there is significant action of the molecule in preventing damage to important cellular components caused by reactive oxygen species (ROS). Reduction of disulfide bonds by GSH formed within cytoplasmic proteins to cysteines implies its role as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG), also called L-(−)-glutathione, which can be reduced back by glutathione reductase, using NADPH as an electron donor. The elevated Gpx level though not significant infers reduction of lipid hydroperoxides to their corresponding alcohols and reduction of free hydrogen peroxide.

The significant increase in MDA level at middle dose of the extract (250 mg/kg), denotes significant lipid peroxidation of polyunsaturated fatty acids [80] could occur following chronic dosing, and the amount of MDA is related to the degree of lipid peroxidation [80]. Lipid peroxidation is the oxidative degradation of lipids. *Musanga cecropioides* possess many phytochemical constituents such as alkaloids, saponins, tannins, triterpenes, flavonoids, etc. (detected by simple qualitative and quantitative methods of Trease and Evans [57] and Sofowora [81]), it is possible that high doses of the stem bark extract resulted in increased concentration of the phytochemical constituents with capacity to be pro-oxidant on lipid membrane.

The biochemical evaluation of lipid profile, liver enzymes, liver proteins and antioxidant enzymes protection were corroborated with the histopathology of the rat liver, kidney and brain tissues revealing intact normal morphology. On the brain slices, undamaged usual morphology of the cerebellum, hippocampus and frontal cortex in the extract controlled groups matched the untreated control group. The astrocytes and the nuclei/cytoplasm of the neurons are also normal. The state of the blood, kidney and liver are very essential indicators of the health status of any organism since they serve as pathological mirrors of the entire body. The kidney is an excretory organ thus removes metabolize and non-metabolized toxic materials from the body; it would thus be exposed to high concentrations of noxious materials which could cause lesions. It should however be noted that lesions (area of abnormal tissue change) vary in severity from harmless to serious lesions. Histologic examination of the kidney showed no visible histopathological lesion in the control group. The sections of the low dose extract (125 mg/kg) treated group revealed essentially normal cortex, medulla and papilla with very mild inflammatory cells. These inflammatory cells occurred possibly due to some underlying factors such as infection. A dose dependent increase in tubular inflammation, necrosis, vascular congestion and cytoplasmic clearance were observed. The observed necrosis and vascular congestion though very mild, was more pronounced at the dose of 500 mg/kg. Nephritis is commonly associated with infection or auto-immune processes. Nephritis has the effect of damaging and closing up the microscopic filters in the kidney. This implies that in addition to various toxic waste products, the inflamed kidney filters out important proteins (large molecules) from the blood. Acute tubular necrosis is kidney injury associated with severe damage to the tubular epithelial cells caused by ischemia or less often, by nephrotoxic substances such as drugs [82]. Vascular congestion occurs due to overfilling and distention of the vascular tissues with blood as a result of mechanical obstruction.

The liver plays a major role in the detoxification of various metabolites; hence, histopathological and hematological parameters are essential in establishing the functional status of the body subsequent to toxicant exposure [83,84]. The liver is the largest organ in the human body and is mainly involved in metabolism and detoxification of drugs and environmental chemicals [85]. It is reported that many toxic plants compound transverse the liver where they are detoxified by biotransformation [86]. A histopathological study of the liver is useful in assessing toxic effects of medicinal plants on the liver cytoarchitecture. Histopathologic examination of the liver are focused on identifying tissues with marked necrosis or significant lesion and possible elevation of liver enzymes (hepatic biomarkers) in the blood serum. The absence of these effects on hepatic tissues and enzymes demonstrate safety and non-toxic nature of the evaluated plant extract on the hepatocytes. A cross-section of the control group histopathological analysis revealed normal portal triad, central vein and hepatocytes. The histologic presentation of the test groups (125, 250 and 500 mg/kg) respectively were similar. Sections showed normal hepatic lobes and architecture with focal hepatic and portal inflammation with chronic inflammatory infiltrates and areas of sinusoidal dilatation. The degree of alteration of hepatic cytoarchitecture was mild, moderate and severe dose dependently. The lesions observed in these studies could have resulted from impingement of various bioactive constituents like saponins, tannins, alkaloids, flavonoids and glycosides.

Citation: Nwidi Lucky Legbosi and Teme Raphael Ellis. “Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat”. *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat

However, no effect was noticed on littermate obtained during the study as they were observed to be very active even after about 5 months postnatal observation. Many phytochemicals have been elucidated from bioactive extract and fractions of *M. cecropioides* [87-91]. Phytochemical analysis carried out on the MCH stem-bark extract revealed saponins, flavonoids, alkaloids, tannins, phlobatannins, cardiac glycosides, reducing sugars and anthraquinones [27].

**Conclusion**

The study demonstrated that MCH stem-bark extract might be well tolerated as it possesses no toxicity on acute dosing but with long time use higher doses of the extract are likely to constitute toxicity implication to the kidney and liver but not the brain and hematological functions. The study has also shown that the uterotonic properties of the extract on the female animals were not affected since the conception potentials of the animals remained intact over time. The pulps or littermates observed for five months postnatal did not reveal any birth defects. Therefore to ensure maximal benefits from long term therapy in chronic diseases utilization of lower dose below 125 mg/kg should be considered to avoid toxicological impingement of the liver and kidney.

**Disclosure**

The authors report no conflict of interest in this work.

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**Bibliography**


_Citation_: Nwidu Lucky Legbosi and Teme Raphael Ellis. "Sub-Chronic Toxicity of Hydromethanolic Stem Bark Extract of *Musanga cecropioides* (Urticaceae) in Rat". *EC Pharmacology and Toxicology* 6.3 (2018): 76-95.
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