Hepatoprotective effect of hydromethanolic leaf extract of *Musanga cecropioides* (Urticaceae) on carbon tetrachloride-induced liver injury and oxidative stress

Lucky L. Nwidu, PhD\(^a\)*, Yibala I. Oboma, MSc\(^b\), Ekramy Elmorsy, PhD\(^c\) and Wayne G. Carter, PhD\(^d\)

\(^a\) Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Nigeria

\(^b\) Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Mansoura University, Egypt

\(^c\) Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

\(^d\) School of Medicine, University of Nottingham, Royal Derby Hospital Centre, Derby, UK

Received 8 February 2018; revised 5 April 2018; accepted 12 April 2018; Available online 17 May 2018

Abstract

Objective: Natural antioxidant products are gaining popularity as treatments for various pathological liver injuries. *Musanga cecropioides* (Urticaceae) leaf extract is used in ethnomedicine for the management of jaundice and other hepatic ailments in Ibibio, Nigeria. This study evaluated the hepatoprotective and antioxidant effects of *M. cecropioides* hydromethanolic leaf (MCHL) extract against carbon tetrachloride (CCl\(_4\))-induced hepatotoxicity in rats.

Methods: Liver damage was induced by administering CCl\(_4\) dissolved in liquid paraffin (2 mL/kg bw 1:1 intraperitoneally) after pretreatment with MCHL extract for 7 days. The rats were sacrificed 24 h post-treatment for histopathological evaluation and biochemical assessment.

Results: The liver histopathology and biochemical parameters of rats treated with MCHL extract showed a significant attenuation in the liver injury and oxidative stress induced by CCl\(_4\) treatment compared to the positive control group.

Conclusion: The results of this study indicate that *M. cecropioides* extracts have potential hepatoprotective and antioxidant properties, which may make them useful in the management of liver diseases.
Musanga cecropioides (Cecropiaceae) is commonly called African cork wood tree or umbrella tree. The local names are Aga aghawo (Yoruba), Ulu (Igbo), and Odzuma (Ghana). It is an erect, rapidly growing tree of the deciduous tropical West African rainforest. It is abundant in swampy forests, riverside, or lakeside at altitudes of 700–1200 m. The tree grows up to 20 m in height with an umbrella-shaped crown, straight and cylindrical trunk (up to 2 m in girth), and stilt adventitious roots of up to 3 m above ground level. The botany of M. cecropioides has already been reported.1–4

In the traditional medicine of the Ibibio of the South–South region of Nigeria, infusion of the M. cecropioides leaf and stem bark is used for the treatment of fever, jaundice, acute gastric poisoning, and liver diseases. A M. cecropioides decoction is used in the management of menstruation pain, induction of labour, and lowering of elevated blood pressure and high blood sugar levels, and as a dehydrant, expectorant, anthelmintic, anti-dysenteric, and analgesic; it is also used in ethnomedicine to treat asthma in infants and restore appetite.5,6 The sap is drunk as a blood-purifier; for cleansing the stomach; for management of blemorrhoea, cough, and chest infections; as a galactagogue; and commonly as a wash for persons with sleeping sickness, leprosy, and fever to relieve aches and pains and rheumatism.5–7

Pharmacological studies have established the uterotonic,8 oral hypotensive,5,9 hypoglycaemic, and anti-diabetic effects9 of M. cecropioides. The compounds isolated from M. cecropioides include kaica acid, triterpenoid acids, and bioactive compounds such as alkaloids, flavonoids, tannins, free and bound anthraquinone, saponin, and cardiac glycosides; anthocyanosides and cyanogenic glycosides were reported to be absent.9 M. cecropioides leaf extract contains isovitexin, vitexin, chlorogenic acid, catechin, and procyanidins.10

Despite its enormous and profound uses, no scientific evidence is available to justify the use of M. cecropioides leaf extract in Ibibio traditional medicines. This study was therefore designed to validate the claim of hepatoprotective efficacy of M. cecropioides hydromethanolic leaf (MCHL) extract in the treatment of liver diseases and to elucidate the possible mechanism(s) of actions. In this investigation, an experimental rat model of carbon tetrachloride (CCl4)-induced acute liver injury was utilised, as it is known to generate lipid radicals that initiate the chain reactions of lipid peroxidation within and outside hepatic cells, causing liver damage.

Materials and Methods

General reagents and chemicals

CCl4, silymarin, diethyl ether, and methanol were purchased from Sigma–Aldrich, St. Louis, Missouri, USA. Diagnostic kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated bilirubin (CBIL), and total bilirubin (TBIL) were purchased from Randox Laboratories Ltd. London, UK. Other chemicals and solvents were of the highest (analytical) grade commercially available and obtained from either Sigma–Aldrich or Merck, UK.

Plant collection and extraction

Fresh leaves of M. cecropioides were collected from Itak Ikpa Local Government area of Akwa Ibom State, Nigeria. The plants were collected by Mr. Etefia Okon and identified by Mrs. Margaret Bassey of the Botany Department of the University of Uyo, Uyo, Nigeria. The leaf was collected in January 2015 from the wild and the extract was stored for two months before use. A voucher specimen (UUH 2001) of the leaf was deposited at the herbarium of the Department of Pharmacognosy, University of Uyo, Uyo, Nigeria.

Preparation of M. cecropioides leaf extract

Fresh leaves of M. cecropioides were air-dried and powdered. The dry powder (300 g) was subjected to cold extraction via maceration in 50% methanol (solvent). The dry powder was soaked for a period of 72 h. The resultant mixture was shaken twice daily for proper extraction. Methanol was separated from the marc by filtration through a double-layer gauge. The filtrate was evaporated to dryness in vacuo at 55 °C. The yield obtained was 4.5%. The extract was stored in a refrigerator for up to four weeks for use in assays.

Introduction

Musanga cecropioides (Cecropiaceae) is commonly called African cork wood tree or umbrella tree. The local names are Aga aghawo (Yoruba), Ulu (Igbo), and Odzuma (Ghana). It is an erect, rapidly growing tree of the deciduous tropical West African rainforest. It is abundant in swampy forests, riverside, or lakeside at altitudes of 700–1200 m. The tree grows up to 20 m in height with an umbrella-shaped crown, straight and cylindrical trunk (up to 2 m in girth), and stilt adventitious roots of up to 3 m above ground level. The botany of M. cecropioides has already been reported.1–4

In the traditional medicine of the Ibibio of the South–South region of Nigeria, infusion of the M. cecropioides leaf and stem bark is used for the treatment of fever, jaundice, acute gastric poisoning, and liver diseases. A M. cecropioides decoction is used in the management of menstruation pain, induction of labour, and lowering of elevated blood pressure and high blood sugar levels, and as a dehydrant, expectorant, anthelmintic, anti-dysenteric, and analgesic; it is also commonly used in ethnomedicine to treat asthma in infants and restore appetite.5,6 The sap is drunk as a blood-purifier; for cleansing the stomach; for management of blemorrhoea, cough, and chest infections; as a galactagogue; and commonly as a wash for persons with sleeping sickness, leprosy, and fever to relieve aches and pains and rheumatism.5–7

Pharmacological studies have established the uterotonic,8 oral hypotensive,5,9 hypoglycaemic, and anti-diabetic effects9 of M. cecropioides. The compounds isolated from M. cecropioides include kaica acid, triterpenoid acids, and bioactive compounds such as alkaloids, flavonoids, tannins, free and bound anthraquinone, saponin, and cardiac glycosides; anthocyanosides and cyanogenic glycosides were reported to be absent.9 M. cecropioides leaf extract contains isovitexin, vitexin, chlorogenic acid, catechin, and procyanidins.10

Despite its enormous and profound uses, no scientific evidence is available to justify the use of M. cecropioides leaf extract in Ibibio traditional medicines. This study was therefore designed to validate the claim of hepatoprotective efficacy of M. cecropioides hydromethanolic leaf (MCHL) extract in the treatment of liver diseases and to elucidate the possible mechanism(s) of actions. In this investigation, an experimental rat model of carbon tetrachloride (CCl4)-induced acute liver injury was utilised, as it is known to generate lipid radicals that initiate the chain reactions of lipid peroxidation within and outside hepatic cells, causing liver damage.

Materials and Methods

General reagents and chemicals

CCl4, silymarin, diethyl ether, and methanol were purchased from Sigma–Aldrich, St. Louis, Missouri, USA. Diagnostic kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated bilirubin (CBIL), and total bilirubin (TBIL) were purchased from Randox Laboratories Ltd. London, UK. Other chemicals and solvents were of the highest (analytical) grade commercially available and obtained from either Sigma–Aldrich or Merck, UK.

Plant collection and extraction

Fresh leaves of M. cecropioides were collected from Itak Ikpa Local Government area of Akwa Ibom State, Nigeria. The plants were collected by Mr. Etefia Okon and identified by Mrs. Margaret Bassey of the Botany Department of the University of Uyo, Uyo, Nigeria. The leaf was collected in January 2015 from the wild and the extract was stored for two months before use. A voucher specimen (UUH 2001) of the leaf was deposited at the herbarium of the Department of Pharmacognosy, University of Uyo, Uyo, Nigeria.

Preparation of M. cecropioides leaf extract

Fresh leaves of M. cecropioides were air-dried and powdered. The dry powder (300 g) was subjected to cold extraction via maceration in 50% methanol (solvent). The dry powder was soaked for a period of 72 h. The resultant mixture was shaken twice daily for proper extraction. Methanol was separated from the marc by filtration through a double-layer gauge. The filtrate was evaporated to dryness in vacuo at 55 °C. The yield obtained was 4.5%. The extract was stored in a refrigerator for up to four weeks for use in assays.
**Phytochemical screening**

The extract was qualitatively screened for the presence of alkaloids, saponins, anthraquinones, tannins, flavonoids, and cardiac glycosides by using standard methods described in the literature.11

**Experimental animals**

Thirty-six healthy adult Wistar rats of either sex and with an average weight of 230–250 g were purchased from the animal house of the Department of Pharmacology, Niger Delta University, Bayelsa State. The animals were acclimatised for one week prior to the experiment. They were maintained under standard laboratory conditions (a 12:12-h dark/light period, 23 ± 2 °C temperature, and 55 ± 5% humidity). The animals were fed with a standard diet (normal commercial pellet diet) and with water ad libitum. All animal experiments conformed with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985) and were approved by the Niger Delta University Ethical Committee on the use of laboratory animals on February 20, 2015, via a circular (NDU/2014/007).

**In vivo pharmacological study**

**Acute toxicity studies**

The median lethal dose (LD₅₀) was determined by the method described by Lörke12 with modification from the report of Pereek et al.13 The Wistar albino rats used in this study were starved for 24 h with free access to water except for 2 h prior to experimentation. Varying doses (1000, 2000, 3000, and 5000 mg/kg i.p.) of the MCHL extract were administered to four groups of rats (n = 3) to establish the range of doses of the extract that would elicit toxic effects. The mice were observed for 24 h after treatment for signs of excitement, sluggishness, nervousness, alertness, convulsions, ataxia, or even death. The LD₅₀ was estimated by determining geometric means of the dose that caused 100% mortality and the dose that did not cause lethality.

**Experimental design**

Wistar albino rats of both sexes were randomised into six groups, each comprising six rats (three males and three females). The protocol used for the study was as follows: Group A was termed the negative control and received distilled water (0.2 mL/kg, p.o.) once daily for six days. Group B received distilled water (0.2 mL/kg bw by oral dosing). Groups C–E received 70.7, 141.4, and 282.8 mg/kg p.o. MCHL extract, respectively, once daily for seven days. Group F was treated with silymarin 100 mg/kg once daily by gavage for seven days. On the seventh day, groups B to F were treated with a mixture of freshly prepared CCl₄ in liquid paraffin (1 mL/kg bw, 1:1 intraperitoneally), the dose effective in a pilot study, 1 h after administration of the last treatment dose was administered. The body weights of all rats were recorded daily throughout the seven days of treatment. Blood was obtained by cardiac puncture and collected into an EDTA vacutainer for determination of haematological parameters by using an automated SYSMEX – KX21 Hematological Analyzer (SYSMEX Corporation, Japan). The haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, and platelet count (PLC) were thus determined. For a biochemical assessment, blood was spun at 3000 rpm for 10 min at 4 °C to separate serum; serum was stored in vacutainer vials at 4 °C until analysis. Livers were immediately extracted and perfused with ice-cooled normal saline (0.9% sodium chloride) before utilisation in further analyses.

**Estimation of biochemical parameters**

The serum collected was used to determine ALT, AST, ALP, albumin (ALB), CBIL, TBIL, and total protein (TP) by using Randox diagnostic kits. These analyses were performed at the Department of Chemical Pathology, Niger Delta Teaching Hospital (NDUTH), Okolobiri, Bayelsa State, Nigeria.

The hepatoprotective activity (%) was determined using the following formula:

\[
\text{Hepatoprotective activity (\%)} = \frac{1 - ((\text{HALA} - \text{W})/(\text{RN} - \text{W}))) \times 100
\]

where MC, C, and W are the measured variables in rats treated with MCHL extract plus CCl₄, CCl₄, and distilled water, respectively.

**Measurement of hepatic antioxidants/markers of lipid peroxidation**

Liver tissues from the experimental animals were perfused with ice-cold saline and transported from the laboratory of Faculty of Pharmacy Pharmacology, Niger Delta University, on dry ice to the School of Medicine, University of Nottingham, Royal Derby Hospital Centre, Derby, UK, and stored at −80 °C until used for the assay of hepatic antioxidant enzymes. Liver pieces (100 mg) were diced and homogenised in 100 mL of 5 mM Tris/HCl buffer (pH 7.4), 1 mM EDTA and complete mini protease inhibitor cocktail (Roche). The homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C and the clear supernatant used for the estimation of antioxidant parameters (glutathione [GSH], superoxide dismutase [SOD], catalase [CAT], and thiobarbituric reacting substances [TBARS]).

**GSH levels**

GSH levels were determined based on the method described by Ellman,14 with slight modifications. A homogenate (0.2 mL) was mixed with 25% TCA and centrifuged at 3000 rpm for 10 min. The supernatant (0.2 mL) was mixed with 10 mM 5,5′-dithiobis(2-nitrobenzoic acid (DTNB) in the presence of phosphate buffer (0.1 M, pH 7.4) and absorbance was read at 420 nm.
**CAT activity measurements**

A CAT assay was performed according to the method described by Aebi\textsuperscript{15} with slight modifications. The assay relies upon the ultraviolet absorption of hydrogen peroxide that can be measured at 240 nm. The decomposition of hydrogen peroxide in the presence of CAT on a quartz plate allows quantitation of catalase activity. Assays were performed in the presence of 50 mM phosphate buffer. Hydrogen peroxide decomposition was monitored in a 96-well quartz plate using a Spectramax (Thermo Fisher) microplate reader. CAT activity was expressed as units/mg protein.

**SOD activity measurements**

Liver cytosolic SOD activity was measured according to the method described by Kakkar et al.\textsuperscript{16} Cytosol (0.05 mL) was mixed with sodium pyrophosphate buffer (0.052 M, pH 8.3, 1.2 mL), phenazine methosulphate (0.186 mM, 0.1 mL), nitroblue tetrazolium chloride (0.3 mM, 0.3 mL), and NADH (0.78 mM, 0.2 mL). The reaction was stopped after 90 s by the addition of glacial acetic acid. The colour intensity of the chromogen was extracted in butanol solution (2.0 mL) with vigorous shaking. The mixture was then centrifuged at 3000 rpm for 10 min, the supernatant was extracted, and the absorbance at 560 nm was determined using the Spectramax microplate reader.

**Determination of TBARS**

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA), as described by Draper and Hadley.\textsuperscript{17} MDA reacts with thiobarbituric acid (TBA) to form a red/pink-coloured complex, which absorbs maximally in an acid solution at 532 nm. Spectrophotometric measurements were recorded using the Spectramax microplate reader.

**Histopathological studies**

A portion of the liver was cut into pieces of approximately 6 mm\(^3\) in size and fixed in 10% phosphate-buffered formaldehyde solution. These liver pieces were embedded in paraffin wax before thin sections of 5 \(\mu\)m in size were made into permanent slides and examined under a high-resolution microscope (Olympus BX60MF, Japan), after which photomicrographs were taken at a magnification of 400×.

**Statistical analysis**

All statistical measures were performed using PRISM 5 (GraphPad Software Inc., San Diego, California USA). Unless specified otherwise, results are expressed as the mean ± standard error of the mean (SEM) values. One-way analysis of variance (ANOVA) was used to compare group data, followed by Tukey’s multiple comparisons test. A \(p\) value of <0.05 was considered significant.

**Results**

**In vivo pharmacological assay**

**Haematological analysis**

The effects of administration of MCHL extracts on liver biomarker enzymes are shown in Table 2. ALT, AST, and ALP levels significantly (\(p < 0.001\)) increased by 49%, 64%, and 38% respectively. TP and ALB levels significantly (\(p < 0.001\)) decreased by 30% and 132% respectively in the 70.7 mg/kg MCHL extract-treated group. CBIL and TBIL significantly increased by 187% and 163%, respectively, following intoxication with CCl\(_4\) compared to the values in the negative control group (\(p < 0.05\) and \(p < 0.01\), respectively). However, following pretreatment with MCHL extracts at doses of 70.7, 141.4, and 282.8 mg/kg and silymarin at 100 mg/kg, TP levels significantly (\(p < 0.01-0.001\)) increased compared to those in the CCl\(_4\) group; the percentage hepatoprotection observed

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test</th>
<th>Observations</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>2 mL of MCHL extract + 2 mL of Dragendorff reagent</td>
<td>Dark orange precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>2 mL of extract shaken vigorously to observe the reaction</td>
<td>Persistent froth unbroken upon standing</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>2 mL of extract + 10 mL of benzene, followed by the addition of 5 mL of 10% ammonia solution to the filtrate</td>
<td>Presence of reddish colour</td>
<td>–</td>
</tr>
<tr>
<td>Tannins</td>
<td>2 mL of MCHL extract + FeCl(_3)</td>
<td>Resultant solution turns woolly brown</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>2 mL of MCHL extract + 2 mL of 10% lead acetate</td>
<td>Yellowish green precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>2 mL of MCHL extract mixed with 2 mL of chloroform + CH(_2)SO(_4) carefully added</td>
<td>Deep reddish-brown colour at the interface and a steroid ring</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) to (+++) = detected in scant to moderate quantities.
MCHL: *Musanga cecropioides* hydromethanolic leaf.
was 102%, 100%, 137%, and 137%, respectively. Similarly, ALT, AST, ALP, CBIL, and TBIL levels decreased significantly ($p < 0.05$–$0.001$) following pretreatment with the MCHL extract. In the group treated with the 70.7 mg/kg MCHL extract, the percentage hepatoprotection for ALT, AST, ALP, CBIL, and TBIL was 130%, 128%, 127%, 130%, and 124%, respectively. The corresponding values for the group treated with the 141.4 mg/kg MCHL extract were 182%, 140%, 104%, 127%, and 116%. The group treated with the highest dose of the MCHL extract (282.8 mg/kg) showed no significant hepatoprotection for these enzyme biomarkers. The percentage hepatoprotection afforded by the pure drug silymarin at 100 mg/kg was 168%, 102%, 116%, 105%, 127%, 103%, and 100% for TP, ALT, AST, ALP, ALB, CBIL, and TBIL, respectively.

**Phytochemical studies**

A preliminary phytochemical screening of the MCHL extract revealed the presence of alkaloids, saponins, anthraquinone, tannins, flavonoids, and cardiac glycosides. The extract contained these bioactive agents in moderate amounts as shown in Table 3.

**Acute toxicity**

The MCHL extract was found to be toxic at doses of $>2000$ mg/kg. At 24 h, the numbers of dead/live animals after treatment with 100, 200, 300, and 500 mg/kg were 0/3, 3/3, 3/3, and 3/3, respectively. The LD$_{50}$ was found to be 1414.2 mg/kg. This justifies the use of MCHL extract doses of 70.7, 141.8, and 282.8 mg/kg as the 1/20th, 1/10th, and 1/5th LD$_{50}$ dose used in this study.

**Histopathological examination**

The effects of 70.7, 141.8, and 282.8 mg/kg MCHL extracts and 100 mg/kg silymarin on the liver histology of CCl$_4$-intoxicated rats are shown in Figure 1. The section of the control rat liver shows normal sinusoidal spaces with a

### Table 3: Effect of MCHL extract on biochemical parameters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Body weight (g/dl)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
<th>ALB (g/dl)</th>
<th>CBIL (mg/dL)</th>
<th>TBIL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>17.7 ± 0.7</td>
<td>52.3 ± 2.4</td>
<td>41.7 ± 3.8</td>
<td>56.7 ± 1.2</td>
<td>8.6 ± 0.3</td>
<td>10.9 ± 0.2</td>
<td>16.7 ± 0.9</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 mL/kg CCl$_4$</td>
<td>13.6 ± 0.3***</td>
<td>102.9 ± 1.1***</td>
<td>116.7 ± 0.9***</td>
<td>91.0 ± 3.2***</td>
<td>3.7 ± 0.3***</td>
<td>31.3 ± 0.2***</td>
<td>44.0 ± 0.6***</td>
</tr>
<tr>
<td>MCHL extract</td>
<td>CCl$_4$ + 70.7</td>
<td>17.8 ± 0.7***</td>
<td>67.3 ± 0.3***</td>
<td>62.6 ± 0.8***</td>
<td>66.0 ± 1.2***</td>
<td>8.3 ± 0.7***</td>
<td>17.0 ± 1.1***</td>
<td>22.0 ± 0.9***</td>
</tr>
<tr>
<td></td>
<td>CCl$_4$ + 141.4</td>
<td>18.2 ± 0.3***</td>
<td>43.1 ± 1.7***</td>
<td>30.2 ± 1.8***</td>
<td>55.3 ± 3.0***</td>
<td>8.2 ± 0.2***</td>
<td>16.5 ± 0.2***</td>
<td>20.2 ± 0.6***</td>
</tr>
<tr>
<td></td>
<td>CCl$_4$ + 282.8</td>
<td>16.2 ± 0.2*</td>
<td>93.1 ± 1.7</td>
<td>100.2 ± 1.6</td>
<td>82.2 ± 3.9</td>
<td>5.6 ± 0.3</td>
<td>28.8 ± 2.0</td>
<td>42.9 ± 3.1</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CCl$_4$ + 100</td>
<td>16.4 ± 0.3*</td>
<td>53.3 ± 0.1***</td>
<td>53.7 ± 0.3***</td>
<td>55.0 ± 2.6***</td>
<td>7.3 ± 0.6***</td>
<td>10.2 ± 0.1***</td>
<td>17.2 ± 0.6***</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM, n = 6. Significant results of extracts and pure drug are displayed relative to positive control values; and positive control displayed relative to healthy (negative) control; results with significant changes from controls are marked with asterisks. For significance: $p < 0.05$; $**p < 0.01$ and ***$p < 0.001$. Statistical analysis was performed using one-way ANOVA.

**Abbreviations**: MCHL extract, *Musanga cecropioides* hydromethanolic extract; TP: total protein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; ALB: albumin; CBIL: conjugated bilirubin; TBIL: total bilirubin.
non-congested portal tract, differentiated blood vessels, and viable hepatocytes (Figure 1A). Liver sections of rats intoxicated with CCl$_4$ indicate marked extensive necrosis, steatosis, ballooning degeneration, gross mononuclear infiltration, and loss of cellular boundaries of the hepatocytes (Figure 1B). Pretreatment with the 70.7 mg/kg MCHL extract showed minor amelioration of the histopathological architecture truncated by CCl$_4$ intoxication. Hepatocytes with an area of localized and complete necrosis amidst viable hepatocytes were seen, indicating that the hepatocyte insult (induced by CCl$_4$) was not yet fully resolved at this dose of MCHL extract (Figure 1C). Following pretreatment with the 141.4 mg/kg MCHL extract, areas of fibrin deposition and numerous mitotic bodies were observed, indicating a healing process and signs of hepatoprotection at this dose (Figure 1D). Hepatocytes of the rats treated with 282.8 mg/kg MCHL extract showed multiple microvesicles, few lipid cells, and numerous inflammatory cells within the portal tract, indicating injury and that this dose is not fully hepatoprotective (Figure 1E). Following treatment with the pure drug, silymarin, at 100 mg/kg, liver tissue with numerous mitotic bodies was observed, demonstrating hepatocyte regeneration and that silymarin is hepatoprotective.

**Effect of MCHL extracts on hepatic antioxidants and markers of lipid peroxidation**

To assess the effects of pretreatment with the MCHL extract (70.7, 141.4, and 282.8 mg/kg) on oxidative stress markers following acute CCl$_4$ intoxication in rats, we assayed liver homogenates for antioxidant capacity by measuring CAT, GSH, SOD, and TBARS.
Liver GSH, CAT, and SOD levels were significantly ($p < 0.001$) reduced by 48%, 41%, and 31%, respectively, after CCl4 intoxication compared to the corresponding levels in the positive controls. However, only pretreatment with MCHL (141.4 mg/kg) significantly ($p < 0.01$) increased GSH, CAT, and SOD levels to 53%, 41%, and 21% while silymarin (100 mg/kg) treatment significantly ($p < 0.01$) increased GSH, CAT, and SOD levels by 75%, 50%, and 22%, respectively, compared to the levels in the CCl4-intoxicated group. The effects of other doses of the extract were not significant (Figure 2).

Lipid peroxidation activity in the liver significantly ($p < 0.001$) increased to 70% after CCl4 intoxication compared to that in the negative control; however, pretreatment with the 141.1 mg/kg MCHL extract significantly ($p < 0.05$) decreased the lipid peroxidation level by 18%. On the other hand, pretreatment with silymarin significantly ($p < 0.001$) reduced lipid peroxidation by 26% of that in the positive controls. The lowest and highest doses of the extract had no significant effect on lipid peroxidation levels.

**Body weight evaluation**

The effects of MCHL extracts on body weight gain in the rats are shown in Figure 3. The MCHL extracts led to a dose-dependent and non-significant ($p > 0.05$) increase in body weight gain compared to the initial depression in weight following the CCl4 insult.

**Discussion**

Hepatotoxic insults commonly occur due to environmental factors, industrial chemicals, drugs, and diseases such as viral hepatitis, which account for the profound morbidity and mortality from liver diseases. The participation of hepatic cells in a variety of metabolic activities including biotransformation and excretion of chemical agents makes the liver more vulnerable and susceptible to toxicity from these agents. Liver damage is predicated on lipid peroxidation and cell necrosis. This may be an emerging threat in the Niger Delta environment, where there is a

![Figure 2: Effects of MCHL extract on markers of oxidative stress.](image)

**Figure 2:** Effects of MCHL extract on markers of oxidative stress. Levels of oxidative stress markers (GSH, CAT, SOD, and TBARS) were measured in homogenised liver samples. Histograms represent mean ± SEM values, with significant changes marked with asterisks. For significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. GST: glutathione; CAT: catalase; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; MCHL: *Musanga cecropioides* hydromethanolic leaf; CCl4: carbon tetrachloride.

![Figure 3: The effect of CCl4, MCHL extract, and silymarin on weight gain in rats through the course of the experiment.](image)

**Figure 3:** The effect of CCl4, MCHL extract, and silymarin on weight gain in rats through the course of the experiment. Data presented above demonstrates that there were no significant effects of the MCHL extract on the weights of the animals. MCHL: *Musanga cecropioides* hydromethanolic leaf; CCl4: carbon tetrachloride.
massive release of hydrocarbon toxicants from petroleum exploration and exploitation and natural gas-flaring activities are ubiquitous. Some of the hydrocarbons released are likely to be hepatotoxins, as anicteric hepatotoxicity has been reported as a potential health effect of long-term occupational exposure in the petroleum refining and distribution industry in Nigeria.23 The paucity of prognosis of hepatotoxicity is aggravated by poor treatment or untreated infective hepatitis, late presentation at hospitals for treatment, alcoholism, and drug abuse and misuse, which account for the condition becoming a medical emergency.24

There is an acute lack of availability of effective drugs that can stimulate liver function and protect the liver from damage or help regenerate hepatic cells.25 Herbal drugs have received considerable attention recently because of their safety, efficacy, and cost-effectiveness.26 One of the important and well-documented uses of plant products is their use as hepatoprotective agents.27–29 There is a growing need for safe hepatoprotective agents; hence, several medicinal plants have been extensively studied for the management of liver disorders.26,29,30 The literature indicates that hepatoprotection is possible with plant extracts rich in antioxidants.31,32 Some plant extracts have already been reported to possess strong antioxidant activities,33 and their protective effects are considered, in part, to be related to the various constituent antioxidants.34

Anecdotal information from a personal communication to Nwidu LL indicated that an infusion of MCHL extract is taken by the Effik people of Akwa Ibom state in Nigeria for liver regeneration. The effects on haematological indices revealed that the MCHL extract does not exhibit any profound effects on haematological parameters. Intoxication with CCl₄ significantly (p < 0.05) reduced the WBC count and the MCHL extract-treated groups exhibited a dose-dependent increase in leucocytes compared to that in the positive controls. This finding suggests that the extract probably contains agent(s) that upregulate the proliferation of leucocytes. The presence of such agents has been reported in Viscum album (mistletoe).35,36 This suggests that the extract may have immune-boosting effects on the animals, possibly via an increase in vascular permeability. The MCHL extract produced no significant dose-dependent increase in the PLC. However, decreased platelet survival has been reported in liver cirrhosis,37 and an increase in PLC has been reported to stimulate hepatic regeneration.38 Although increases in some haematological indices such as RBC count, PCV, Hb, MCV, MCH, and MCHC were observed, these were not statistically significant (p > 0.05). Since plants contain a combination of biomolecules, it can, therefore, be inferred that some of the active components in this plant might, in part, act on the bone marrow to stimulate the production and differentiation of haematopoietic stem cells.39,40

The liver plays a significant role in detoxification and metabolic biosynthetic processes such as synthesis of plasma proteins and gluconeogenesis.41,42 The liver hepatocyte-regenerative capacity if overwhelmed might result in a gradual loss of tissue function, leading to liver disorders.43 Acute hepatocellular injury can be induced by various hepatotoxins, including CCl₄ and high-dose acetaminophen.31,44–46 CCl₄ is biologically inactive and a stable compound.47 However, it becomes a highly reactive halogenated aromatic hydrocarbon when it undergoes biotransformation by cytochrome P₄₅₀ in the hepatic endoplasmic reticulum to form a highly reactive and unstable trichloromethyl radical.48,49 The latter in the presence of oxygen is metabolised to peroxides and chloroform, which overwhelm the antioxidant capacity of the liver, leading to oxidative denaturation of unsaturated fatty acids of lipid membranes and thereby causing severe liver damage and membrane leakage.50 This is the fundamental mechanism of lipid peroxidative degradation of membranes by CCl₄.51 Damaged liver cells release liverspecific enzymes into the bloodstream, such as AST, ALT, and ALP, which are used as biomarkers of liver damage and indicators for evaluating the effectiveness of therapeutic agents.52,53

To validate the protective effects of MCHL extract on the liver enzymes, CCl₄-induced hepatotoxicity was investigated. Significant elevations in the levels of ALT, AST, and ALP, and serum CBIL and TBIL were observed, reflecting cellular damage and hepatotoxicity.46,54 Pretreatment with the MCHL extract before application of CCl₄ led to a significant decrease in the levels of liver biomarker enzymes AST, ALT, and ALP and serum TBIL and CBIL (p < 0.001). This finding is in agreement with reports that serum levels of biomarker enzymes return to near-normal levels with the healing of the hepatic parenchyma and the regeneration of hepatocytes.55 This suggests that the MCHL extract accelerates the healing mechanism of the liver.

Adeneye et al. (2009) evaluated the hepatoprotective effect of the stem bark of M. ceropoides and observed that graded doses (125–500 mg/kg) attenuated CCl₄-induced or acetaminophen-induced hepato cellular injury in a dose-dependent fashion.44 This finding supports our findings, in that it suggests that the bioactive agents affording hepatoprotection are similar and present within both the stem bark and the leaves; however, this will need to be evaluated in future studies.

Reports indicate that TP and albumin are downregulated following CCl₄ intoxication, suggesting liver damage.46 Pretreatment with MCHL extracts increased the levels of serum TP (p < 0.001), and albumin (p < 0.01–0.001). Stimulation of protein synthesis has been postulated as a contributing hepatoprotective mechanism, which accelerates tissue production and regeneration.57

Damage to the liver might impair its ability to excrete normal amounts of bilirubin or cause obstruction of excretory ducts of the liver.58 Pretreatment with either MCHL extract or silymarin effectively limited the elevation of the biliary markers, ALP, and bilirubin levels (CBIL and TBIL). This suggests that the MCHL extract enhances the hepatic cell-secretory mechanism and has hepatoprotective potential.

The percentage hepatoprotection afforded by 70.7 and 141.4 mg/kg MCHL extract/silymarin 100 mg/kg for each biomarker is as follows: ALT, 130% and 182%/102%; AST, 128% and 140%/116%; ALP, 127% and 104%/105%; CBIL, 130% and 127%/103%; and TBIL, 124% and 116%/100%, respectively. This result indicates that the MCHL extract (70.7 and 141.4 mg/kg) has a more potent hepatoprotective effect than that of silymarin (100 mg/kg). However, the highest dose of the MCHL extract (282.8 mg/kg) extract did
not show significant hepatoprotection for these enzyme biomarkers. Insults to the liver cytoarchitecture and histomorphological alterations following acute exposure of the hepatocytes to CCl₄ are expected. The commonly reported structural changes in the hepatocytes are sinusoidal dilation, congestion, inflammation, intense degeneration, vacuolisation, pyknotic nuclei of necrotic cells with eosinophilic cytoplasm, nodular cellular damage, and hypertrophic cell structures. In this study, gross alteration of the liver cytoarchitecture was noted in the CCl₄-treated group. Cytoarchitectural irregularities were observed in the parenchymal structure, with an indistinguishable classical lobular structure. Treatment with the 141.4 mg/kg MCHL extract ameliorated the CCl₄-induced damage and induced the generation of mitotic bodies, which is indicative of a regenerative process and suggestive of hepatoprotective potential. However, the highest dose of the extract was not effective, and perhaps the extract has pro-oxidant properties at high doses.

The level of TBARS, the end product of lipid peroxidation, was upregulated in the CCl₄-intoxicated group but pretreatment with the MCHL extract (141.4 mg/kg) significantly (p < 0.05) decreased TBARS levels, which was presumably a reflection of cell membrane repair observed in the hepatocytes of the extract-treated group as evidenced in the cytoarchitecture of the liver. In response to oxidative stress, the cells affected are protected against oxidative insults via upregulation of the endogenous antioxidant system including GSH, CAT, and SOD. SOD catalyses the oxidative dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide, and CAT converts hydrogen peroxide to molecular oxygen and water. The antioxidant system can easily be overwhelmed by an excess of lipid peroxides and reactive oxygen species and a resultant decline in the antioxidant system may render hepatocytes vulnerable to toxic insults. A decrease in the levels of the antioxidant-system enzymes, CAT, SOD, and GSH in CCl₄ intoxication studies has been reported. GSH is an endogenous scavenger of free radicals and its level is a sensitive biomarker of antioxidant status. The pathophysiological consequences of a decline in GSH level have been widely investigated; a decline in the liver GSH level hinges frequently on hepatic GSH level have been widely investigated; a decline in GSH levels in CCl₄ intoxicated rats was significantly countered in a dose-dependent manner by pretreatment with the MCHL extract, as indicated by the histomorphological data and suggests tissue injury repair. The MCHL extract stimulated the recovery of antioxidant potential in the circulation, as indicated by the marked elevation of GSH level and a decrease in oxidative stress-induced damage in the histopathology data. It has been established that the liver maintains GSH even under the condition of elevated lipid peroxidation due to a supportive and compensatory mechanism.

No significant effect was observed on the body weights of the treated rats throughout the duration of the study. The effectiveness of the MCHL extract against hepatitis may be attributable to the presence of phytochemical constituents such as phenolic acids and flavonoids. Phenolics are widely reported to present excellent antioxidant effects against peroxyl and hydroxyl radicals while flavonoids act as hydrogen donors and metal chelators. Currently, further investigation is ongoing in our laboratories to elucidate the bioactive agents and the biochemical mechanism responsible for antioxidant and hepatoprotection by the MCHL extract.

Conclusion

Our study further supports the ethnomedicinal use of the components of M. cecropioides in treating jaundice and hepatitis. The stem bark, or leaf extract reported herein, may provide suitable hepatoprotection to liver toxins if utilised at an appropriate dose. The MCHL extract may be hepatotoxic at high doses; hence, caution should be exercised when choosing a dose for the management of liver injury.

Conflict of interest

The authors disclose receipt of the following financial assistance for the research, authorship, and or/publication of this article: Niger Delta University 3-month sabbatical fellowship and International Visiting Fellowship of the University of Nottingham, United Kingdom to WGC to host LLN and EE.

Authors’ contributions

Conception and design, collection and assembly of data, drafting of the article and final approval of the article, administrative, technical and logistic support: LLN. Analysis and interpretation of data, statistical expertise: EE. Histopathological analysis and interpretation of results: YIO. Critical revision of the article for the important intellectual content, provision of study materials and obtaining of funding: WGC. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Acknowledgements

We would like to acknowledge the technical support of Mr. Obi Cosmas, the technologist attached to the Pharmacology Department, and Mr. Obi Sompto for sourcing experimental animals for this project.

References

1. Adeneye AA, Ajagbonna OP, Mojiminiyi FB, Odigie IP, Ojobor PD, Etarrh RR, Adeneye AK. The hypotensive mechanisms for the aqueous stem bark extract of Musanga


41. Colark E, Ustner MC, Tekin N, Colark E, Burukoglu D, Degirmenci I, Gunes HV. The hepatoprotective effects of Cynara