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Original Article

# Hepatoprotective and antioxidant activities of *Spondias mombin* leaf and stem extracts against carbon tetrachloride-induced hepatotoxicity

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**الملخص**

**أهداف البحث:** تستخدم ورقة نبات سبونديا‌س مومبين (بطمية) في الطب الشعبي في نيجيريا لعلاج التهاب الكبد. هذه الدراسة تقيم نسبيا تأثيرات الحماية الكبدية ومضادات الأكسدة في الجسم الحي لورقة نبات سبونديا‌س مومبين ومستخلصات الميثانول في جذع النبات في نموذج الفئران لتسمم الكبد.

**طرق البحث:** تم تقسيم اثنين وأربعين فأرا إلى سبع مجموعات. أعطيت المجموعة أ الماء، وأعطيت المجموعة ب الماء، كما أعطيت المجموعتين س و د ورقة نبات سبونديا‌س مومبين بجرعة ٥٠٠ و١٠٠٠ مجم/كجم من وزن الجسم، على التوالي، وأعطيت المجموعتين إ و ف جذع نبات سبونديا‌س مومبين ٥٠٠ و ١٠٠٠ مجم /كجم من وزن الجسم، على التوالي، و أعطيت المجموعة ج سيليمارين بجرعة ١٠٠ مجم/كجم. أعطيت جميع المستخلصات والأدوية يوميا بواسطة غشاء فموي لمدة سبعة أيام، ثم تم حث تسمم الكبد الحاد للمجموعتين ب و ج بإعطاء $CCl_4$. بعد ٤٨ ساعة تم ذبح الفئران وفحص المؤشرات النسيجية والكيميائية الحيوية لتسمم الكبد.

**الاستنتاجات:** أحدث $CCl_4$ إصابة بالكبد بزيادة كبيرة في مستويات علامات الإصابة الكبدية: ALT,AST, TBIL,CBIL, بالإضافة إلى خفض كبير في البروتين الكلي في الدم. حسنت المستخلصات النباتية لورقة نبات سبونديا‌س مومبين وجذع نبات سبونديا‌س مومبين عند ٥٠٠ و١٠٠٠ مجم/كجم قبل العلاج ب $CCl_4$ بشكل ملحوظ إصابة الكبد، وخفضت مستويات ALT,AST,TBIL, CBIL. مستخلصات ورقة نبات سبونديا‌س مومبين أو جذع نبات سبونديا‌س مومبين زاد كثيرا المستويات الخلوية للجلوتاثيون، ونشاطات الكتالاز وديسموتاز الفائق، وخفض كثيرا المواد المتفاعلة لحمض ثيوباربيتوريك.

**النتائج:** تقدم هذه الدراسة أدلة أولية تدعم الفوائد المحتملة لنبات سبونديا‌س مومبين لعلاج تسمم الكبد -الناجم عن الاكسيوبيوتيك.

**الكلمات المفتاحية:** ورقة نبات سبونديا‌س مومبين؛ تسمم الكبد؛ حماية الكبد؛ الأكسدة

**Abstract**

**Objective:** *Spondias mombin* L. is a tree used in folk medicine in Nigeria for the treatment of hepatitis. This study was carried out to comparatively evaluate the hepatoprotective and antioxidant effects of *S. mombin* leaf and stem (SML and SMS) methanolic extracts in a rat model of carbon tetrachloride ($CCl_4$)-induced hepatotoxicity.

**Methods:** Forty-two rats were distributed into seven groups. Groups A and B received water; groups C and D received 500 and 1000 mg/kg SML extract, respectively; groups E and F received 500 and 1000 mg/kg SMS extract, respectively; and group G received 100 mg/kg silymarin. Water, the two extracts, and silymarin were administered daily by oral gavage for 7 days. Hepatotoxicity was induced in groups B to G by the administration of $CCl_4$ once on the seventh day. After 48 h, rats were sacrificed, and tissues and serum samples were examined for histological and biochemical indices of hepatotoxicity.

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**Results:** Administration of $CCl_4$ resulted in liver injury with significant elevation in the hepatocellular injury markers alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL), and conjugated bilirubin (CBIL), associated with a significant reduction in total circulatory protein. Pretreatment with SML and SMS extracts at both doses significantly ameliorated liver injury; lowered ALT, AST, ALP, TBIL, and CBIL levels; elevated cellular glutathione levels as well as catalase and superoxide dismutase activities; and decreased the levels of thiobarbituric acid reactive substances.

**Conclusion:** This study provides preliminary evidence supporting the potential therapeutic benefit of *S. mombin* in xenobiotic-induced hepatotoxicity.

**Keywords:** Hepatoprotection; Hepatotoxicity; Oxidative stress; *Spondias mombin* L.



## Introduction

Liver diseases are major causes of illness and death worldwide,[1–4] and constitute a public health challenge that requires the development of new therapeutic options. Investigating the hepatoprotective effects of medicinal plants in laboratory animals is an important initial step in evaluating the safety of new biomolecules.[5–9] Natural products from ethnomedicine have provided safe and effective alternatives for the treatment of hepatotoxicity. Many previous reports have demonstrated the hepatoprotective effects of local phytoextracts rich in natural antioxidants,[5–15] and several bioactive compounds and plant extracts have been investigated for their hepatoprotective and antioxidant effects.[16,17]

Phenolic compounds found in several plants are usually associated with multiple biological activities such as free radical scavenging activities.[18–20] It has been suggested that natural antioxidants found in food, such as phenolic compounds or flavonoids, might play an important role in the prevention of oxidative stress-related disorders and in the reduction of premature mortality.[21,22] Flavonoids are certainly ubiquitous in the epidermal cells of many plant parts and exist in both glycosidic and non-glycosidic forms.[23]

*Spondias mombin* L. (Anacardiaceae) is commonly known as hog plum (English), akika (Yoruba), ijikara (Igbo), tsadarmaser (Hausa), chabbuh (Fulani), nsukakara (Efik), and atoa (Ashanti).[24] It is a deciduous erect tree, which grows up to 15–20 m high, with a trunk that is 60–75 cm wide.[25,26] *S. mombin* is commonly found in the tropical Americas, including the West Indies, and has also been naturalized in parts of Africa, including Ghana, and some parts of Asia.[26] In ethnomedicine, *S. mombin* parts, including the stem bark, leaves, and roots, have been used for the treatment of various conditions. *S. mombin* possesses antimicrobial[27,28] and antiviral activities.[29] Its leaves show anti-inflammatory,[30] anthelmintic,[31] hematinic,[32] and sedative[33] activities, while its stem bark possesses anti-mycobacterial activity.[34] In a previous study, phytochemical screening indicated that *S. mombin* leaf (SML) contains tannins, saponins, alkaloids, flavonoids, and phenols.[35] It is also rich in ascorbic acid and niacin, and contains riboflavin and thiamine.[35]

The hepatoprotective effects of *Ocimum gratissimum* and SML have been previously evaluated in rats after intoxication with dimethylnitrosamine.[36] However, the effects of SML and *S. mombin* stem (SMS) on carbon tetrachloride ($CCl_4$)-induced hepatotoxicity have not yet been assessed. Thus, the aim of this study was to establish whether SML and SMS methanolic extracts show hepatoprotective effects against $CCl_4$-induced hepatotoxicity in rats.

## Materials and Methods

### *Chemicals and reagents*

$CCl_4$, 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. All other chemicals and solvents were of the highest commercially available grade (analytical grade) and were obtained either from Sigma–Aldrich or Merck, UK.

### *Plant collection and validation*

Fresh leaves and stems of *S. mombin* L. were collected from Obafemi Awolowo University campus in January 2015. The plant was identified and authenticated by Dr. Oladele Adekunle, a Taxonomist at the Forestry Department, University of Port Harcourt, Nigeria, where specimens of SML (20015) and SMS (20016) were deposited.

### *Preparation of S. mombin leaf and stem methanolic extracts*

Three hundred grams of *S. mombin* L. fresh leaves and stem barks were weighed, air dried, and powdered. Then, powdered leaves and stems were extracted by the cold extraction method (maceration) using methanol as a solvent; SML and SMS powders were soaked in one liter of 50% methanol for 3 days, during which the mixture was shaken twice daily to promote extraction. The solvent was filtered over a layer of gauge and the filtrate was evaporated to dryness *in vacuo* at 55 °C. The weights of the dried extracts were 21.3 g and 9.4 g, and the obtained yields were 7.1% and 3.1% for SML and SMS extracts, respectively. The extracts were stored in a refrigerator for up to 4 weeks for subsequent use in assays.

### *Phytochemical screening*

The methanolic extracts of SML and SMS were quantitatively assayed for the presence of phytochemicals such as saponins, tannins, alkaloids, terpenoids, cardiac glycosides, and flavonoids using standard procedures.[36]

*Experimental animals*

Forty two healthy Wistar rats of both sexes (21 male rats and 21 female rats) weighing 320–355 g were purchased from the animal house of the Pharmacology Department, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria. Animals were acclimatized for one week prior to experimentation. All animals were fed a standard chow diet and were given access to water *ad libitum*. Experimental techniques and protocols used in this study follow the "Guide to the Care and Use of Animals in Research and Teaching"[37] as adopted and approved by Niger Delta University Institutional Animal Care and Use Committee on 20/02/2015 with an approval number NDU/2014/007.

*Acute toxicity study*

An acute toxicity study was carried out to determine the approximate median lethal doses of SML and SMS extracts in Albino mice (25–30 g) of both sexes. Mice were distributed into eight groups, three mice per group, and were administered single doses of SML and SMS extracts (100, 500, 1000, 2000, 3000, 4000, and 5000 mg/kg) intraperitoneally. Treated animals were monitored for 24 h for mortality and behavioral changes consistent with toxicity.[38,39]

*Experimental design*

A total of 42 rats were weighed and distributed into seven groups, six rats per group (three males and three females). In groups A (negative control) and B (positive control), rats received 0.2 mL/kg distilled water. In groups C and D, rats received 500 and 1000 mg/kg SML extract, respectively, dissolved in distilled water. In groups E and F, rats received 500 and 1000 mg/kg SMS extract, respectively, dissolved in distilled water. In group G, rats received 100 mg/kg silymarin suspended in distilled water. Distilled water, SML and SMS extracts, and silymarin were administered daily by oral gavage for 7 days. On the seventh day, 1 h after administration of the last dose, all groups except group A received a 1:1-mixture of freshly prepared $CCl_4$ in liquid paraffin (2 mL/kg) intraperitoneally. Body weights of all rats were measured and recorded daily throughout the 7 days of the experiment.

Fourty eight hours after CCl4 administration, rats were anesthetized using diethyl ether and then sacrificed. Blood was collected by cardiac puncture into an EDTA vacutainer for determination of hematological parameters using the Automated Hematology Analyzer KX-21 (SYSMEX Corporation, Japan). The hemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, white blood cell count, and platelet count were determined. For biochemical assessment, blood samples were centrifuged at 3000 rpm for 10 min at 4 °C, and serum was separated into vacutainer vials and stored at 4 °C for subsequent analysis. Livers were immediately collected, perfused with ice cold normal saline (0.9% sodium chloride), and transported on dry ice from the Pharmacology laboratory, Faculty of Pharmacy, Niger Delta University, Nigeria, to the School of Medicine, University of Nottingham, Royal Derby Hospital Centre, Derby, UK, where they were stored at −80 °C for subsequent use in further analyses.

*Assessment of biochemical parameters*

Serum levels of ALT, AST, ALP, CBIL, TBIL, and total protein (TP) were assessed using Randox diagnostic kits. These analyses were performed at the Department of Chemical Pathology, Niger Delta University Teaching Hospital, Okolobiri, Bayelsa state, Nigeria.

*Measurement of oxidative stress markers*

Liver sections (100 mg) were diced and homogenized in 100 mL of 5 mM Tris/HCl buffer (pH 7.4), 1 mM EDTA, and complete, Mini, EDTA-free Protease Inhibitor Cocktail tablet (Roche). Homogenates were then centrifuged at 10,000 rpm for 10 min at 4 °C and the clear supernatant was collected for the estimation of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid reactive substances (TBARS). Assessment of GSH was carried out based on the method published by Ellman et al.[40] with slight modifications as follows: the homogenate (0.2 mL) was mixed with 25% trichloroacetic acid and centrifuged at 3000 rpm for 10 min, then the supernatant (~0.2 mL) was mixed with 10 mM DTNB in the presence of phosphate buffer (0.1 M, pH 7.4), and the absorbance was read at 420 nm. Determination of CAT was performed based on the method described by Aebi,[41] which relies upon the ultraviolet absorption of hydrogen peroxide that can be measured at 240 nm in the presence of 50 mM phosphate buffer. Hydrogen peroxide decomposition was monitored in a 96 well quartz plate using a Spectramax microplate reader (ThermoFisher, Stafford, UK). Catalase activity was expressed as units/mg protein. Liver cytosolic SOD activity was measured according to the method described by Kakkar et al.[42] Finally, extent of lipid peroxidation was determined spectrophotometrically by measuring malondialdehyde levels, as described by Draper and Hadley.[43]

*Histopathological investigation*

Liver specimens from each rat were cut into pieces (approximately 6 $mm^3$ in size), fixed in phosphate buffered 10% formaldehyde, and embedded in paraffin wax. Then, 5-μm-thick sections were cut, fixed onto glass slides, and stained with hematoxylin and eosin (H&E). Slides were examined under a high-resolution microscope (Olympus BX60MF, Japan), and photomicrographs were taken at a magnification of ×400.

*Statistical analysis*

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

## Results

### *Phytochemical constituents in SML and SMS*

Preliminary phytochemical screening of SML and SMS extracts revealed the presence of alkaloids, reducing sugars, saponins, and tannins [Table 1]. The SMS extract was found to contain more saponins and tannins than the SML extract. However, regarding other qualitative assays, both extracts were similar.

### *Acute toxicity study of SML and SMS in mice*

Treated mice were monitored for mortality and no signs of toxicity were observed up to 24 h after extract admnistration of single dose (intraperitneally) of either SML or SMS extracts (100−5000 mg/kg). Consequently, we chose two doses, 1/10 and 1/5 of the maximal dose examined (5000 mg/kg), i.e., 500 and 1000 mg/kg, for both extracts as the experimental doses.

### *Body weight changes throughout the study period*

The effect of $CCl_4$ administration as well as pretreatment with SML and SMS extracts on the body weights of rats throughout the course of the experimental study is presented in Figure 1.

Significant changes in the body weights of the rats were observed in each group throughout the time course of the experiment (two-way ANOVA, $p < 0.0001$). However, when investigating the effects of the treatments, no significant differences were found between body weight changes in the treated groups and the $CCl_4$-induced group ($p = 0.506$). Pretreatments with 500 and 1000 mg/kg SMS extract were associated with 3.5% and 4.3% increase in body weights, respectively.

### *Effect of SML and SMS on biochemical and histological markers of hepatotoxicity*

To assess the hepatoprotective effects of SML (500, 1000 mg/kg) and SMS (500, 1000 mg/kg) extracts in $CCl_4$-induced hepatotoxicity, serum ALT, AST, ALP, TP, CBIL, and TBIL levels were assessed [Table 3]. Administration of $CCl_4$ resulted in hepatocellular toxicity demonstrated by a significant ($p < 0.001$−$0.05$) elevation in serum ALT (102%), AST (58%), ALP (27%), and TBIL (62%) levels, and a significant ($p < 0.001$) decrease in TP (54%), compared to the negative control group.

Pretreatment with 500 and 1000 mg/kg SML extract decreased serum levels of ALT by 7% and 46%; AST by 54% and 91%; ALP by 33% and 31%; TP by 97% and 116%; CBIL by 75% and 88%; and TBIL by 62% and 41%, respectively, compared to the positive control ($CCl_4$-induced) group. Regarding SMS extract, pretreatment with 500 and 1000 mg/kg lowered serum levels of ALT by 4% and 37%; AST by 47% and 83%; ALP by 27% and 23%; TP by 111% and 119%; CBIL by 8% and 31%; and TBIL by 59% and 55%, respectively, compared to the positive control group. Moreover, pretreatment with SML and SMS extracts counteracted the $CCl_4$-induced decrease in TP; pretreatment with 500 and 1000 mg/kg SML extract significantly ($p < 0.001$) increased serum TP levels by 97% and 116%, respectively, while 500 and 1000 mg/kg SMS extract sigificantly ($p < 0.001$) increased serum TP levels by 111% and 119%, respectively, compared to the positive control group. The standard antioxidant silymarin (100 mg/kg) significantly ($p < 0.001$) increased the serum level of TP by 116% and significantly ($p < 0.05$−$0.001$) decreased serum levels of ALT, AST, ALP, CBIL, and TBIL ($p < 0.05$) by 22%, 90%, 22%, 31%, and 36%, respectively, compared to the $CCl_4$-induced group.

The effects of pretreatment with SML and SMS extracts (500 and 1000 mg/kg) and silymarin (100 mg/kg) on liver histology of $CCl_4$-induced rats are presented in Figure 2.

### *Effect of SML and SMS on Haematological indices*

Extracts at either 500 or 1000 mg/kg did not have any significant effect on the haematological indices evaluated, except SMS at 500 mg/kg which induced a significant ($p < 0.05$) change in PCV when compared to the $CCl_4$ intoxicated group (Table 2).

### *Effect of SML and SMS on oxidative stress markers*

Glutathione levels decreased by 48%, CAT and SOD activities decreased by 59% and 30%, respectively, and TBARS levels increased by 67% following intoxication with $CCl_4$ (Figure 3). In contrast, pretreatment with SML and SMS extracts (1000 mg/kg) significantly increased GSH levels by 42% ($p < 0.05$) and 50% ($p < 0.01$), respectively, while pretreatment with silymarin (100 mg/kg) significantly ($p < 0.001$) increased GSH levels by 74%. Similarly, both CAT and SOD enzyme activities were significantly

**Table 1: Phytochemical constituents of Spondias mombin leaf and stem Extracts.**

| Phytochemicals | Observations | Extract | |
|---|---|---|---|
| | | SML Extract | SMS Extract |
| Reducing sugars | Reddish brown precipitate upon heating | + | + |
| Cardiac glycosides | Brick red precipitate | + | + |
| Saponins | Persistent froth unbroke upon standing | ++ | +++ |
| Tannins | Blue black precipitate | ++ | +++ |
| Flavonoid | Resultant solution turns yellow | +++ | +++ |

(+) to (+++) = detected in moderate to abundant quantities.

**Table 2: Effect of *Spondias mombin leaf* and *stem extracts* on hematological parameters.**

| Parameters | Group A | Group B | Group C | Group D | Group E | Group F | Group G |
|---|---|---|---|---|---|---|---|
| PCV(%) | 42 ± 3.2 | 49 ± 7.9 | 44 ± 3.5 | 42 ± 3.9 | 40 ± 6.2* | 44 ± 6.5 | 49 ± 3.1 |
| Hb(g/dl) | 12 ± 0.6 | 14 ± 2.1 | 13 ± 1.8 | 11 ± 0.7 | 12 ± 0.8 | 13 ± 2.3 | 13 ± 0.6 |
| WBC ($\times 10^3$ cells/μL) | 13 ± 4.9 | 7 ± 4.2 | 9 ± 0.4 | 11 ± 7.7 | 11 ± 4.2 | 8 ± 2.3 | 13 ± 4.5 |
| PLT ($\times 10^3$ cells/μL) | 468 ± 280 | 459 ± 366 | 761 ± 1.4 | 284 ± 316 | 500 ± 288 | 446 ± 306 | 642 ± 322 |
| RBC ($x10^6$ cells/μL) | 7 ± 1.3 | 7 ± 1.2 | 6 ± 0.0 | 6 ± 1.1 | 7 ± 0.8 | 7 ± 0.9 | 8 ± 0.3 |
| MCV (%) | 63 ± 2.5 | 65 ± 2.0 | 65 ± 7.1 | 66 ± 4.4 | 62 ± 2.6 | 63 ± 0.8 | 65 ± 4.2 |
| MCH (pg) | 17 ± 2.0 | 18 ± 0.3 | 18 ± 1.7 | 18 ± 0.5 | 17 ± 0.7 | 19 ± 1.4 | 18 ± 1.2 |
| MCHC (g/dl) | 27 ± 2.7 | 28 ± 0.5 | 28 ± 0.4 | 27 ± 2.1 | 26 ± 2.1 | 30 ± 2.5 | 27 ± 2.1 |
| NEU (%) | 32 ± 8.2 | 44 ± 13 | 37 ± 7.1 | 40 ± 14 | 52 ± 13 | 39 ± 7.2 | 38 ± 5.4 |
| LYM (%) | 63 ± 8.0 | 44 ± 13 | 57 ± 9.9 | 51 ± 15 | 42 ± 14 | 54 ± 9.3 | 54 ± 7.3 |
| MEB (%) | 6 ± 2.4 | 10 ± 4.1 | 6 ± 2.1 | 9 ± 2.1 | 6 ± 3.5 | 8 ± 3.1 | 7 ± 4.3 |

Data are presented as mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA. Group A received 0.2 mL/kg distilled water; group B was administered 1 mL/kg CCl4; groups C and D were pretreated with 500 and 1000 mg/kg SML extract, respectively, and were administered 1 mL/kg CCl4; groups E and F were pretreated with 500 and 1000 mg/kg SMS extract, respectively, and were administered 1 mL/kg CCl4; group G was pretreated with 100 mg/kg silymarin and was administered 1 mL/kg $CCl_4$. The asterisk (*), $p < 0.05$ is significantly different from Group B (positive control). Statistical analysis was performed using one way ANOVA.
**Abbreviations:** PCV: packed cell volume; Hb: hemoglobin concentration; WBC: white blood cell count; PLT: platelet count; RBC: red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; NEU: neutrophil count; LYM: lymphocyte count; MXD: mixture (monocytes, basophils, and eosinophils) count.

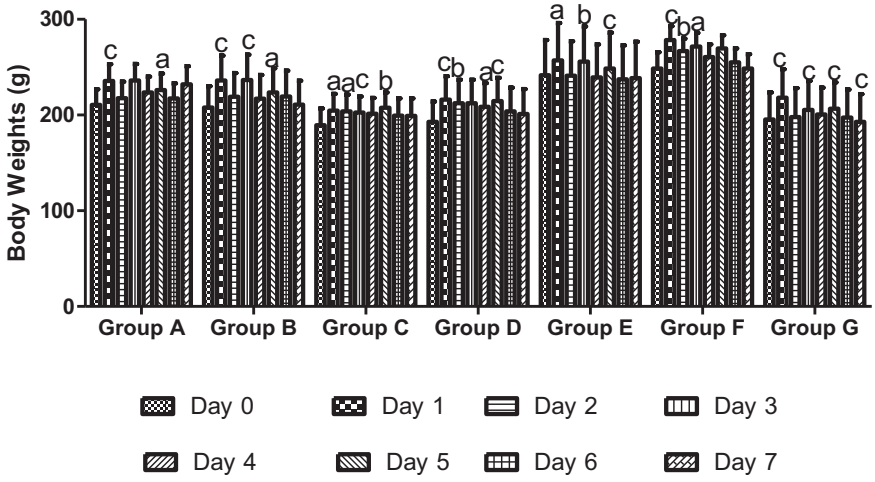


**Figure 1:** The effect of $CCl_4$ intoxication and pretreatment with *Spondias mombin* leaf and stem extracts on body weights of the experimental rats throughout the course of the experiment. Data are presented as mean ± SD. a, b, c; significant difference from body weight on day 0, $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

**Table 3: Effect of the different treatments on biochemical markers of hepatotoxicity.**

| Treatment | Dosing Regimen | ALT (U/L) | AST (U/L) | ALP (U/L) | TP (g/dl) | CBIL (μmol/L) | TBIL (μmol/L) |
|---|---|---|---|---|---|---|---|
| Control (water) | 0.0 | 115.8 ± 1.2 | 212.6 ± 3.1 | 62.0 ± 2.1 | 85.2 ± 1.9 | 1.1 ± 0.04 | 3.4 ± 0.2 |
| $CCl_4$ | 1 ml/kg $CCl_4$ | 233.5 ± $2.3^c$ | 336 ± $25.8^c$ | 79.0 ± $4.0^a$ | 38.8 ± $1.1^c$ | 1.6 ± $0.1^b$ | 8.5 ± $0.3^c$ |
| SML | 500 mg/kg | 216 ± 2.6 | 154.7 ± $2.7^c$ | 52.8 ± $1.1^c$ | 76.3 ± $3.7^c$ | 0.4 ± $0.17^c$ | 3.2 ± $0.9^c$ |
| | 1000 mg/kg | 126.7 ± $16.9^c$ | 31.3 ± $1.5^c$ | 54.3 ± $1.8^c$ | 84.0 ± $1.7^c$ | 0.2 ± $0.03^c$ | 5.0 ± $0.6^a$ |
| SMS | 500 mg/kg | 220.3 ± 5.2 | 177.3 ± $4.7^c$ | 57.3 ± $1.2^c$ | 82.0 ± $0.6^c$ | 0.2 ± $0.1^c$ | 3.5 ± $0.9^c$ |
| | 1000 mg/kg | 147.3 ± $9.4^b$ | 55.8 ± $3.3^c$ | 60.8 ± $0.9^b$ | 84.8 ± $0.9^c$ | 1.1 ± $0.1^b$ | 3.8 ± $0.3^b$ |
| Silymarin | 100 mg/kg | 182.4 ± 21.9 | 34.4 ± $1.4^c$ | 61.6 ± $4.7^b$ | 84.0 ± $1.1^c$ | 1.1 ± $0.1^b$ | 5.4 ± $0.9^a$ |

Values are represented as mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA. Results of the CCl4-induced group are compared to those of the negative control group (receiving water only), and results of the SML, SMS, and silymarin-treated groups are comparedto those of the positive control (CCl4-induced) group. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkalinephosphatase; TP: total protein; CBIL: conjugated bilirubin; TBIL: total bilirubin.

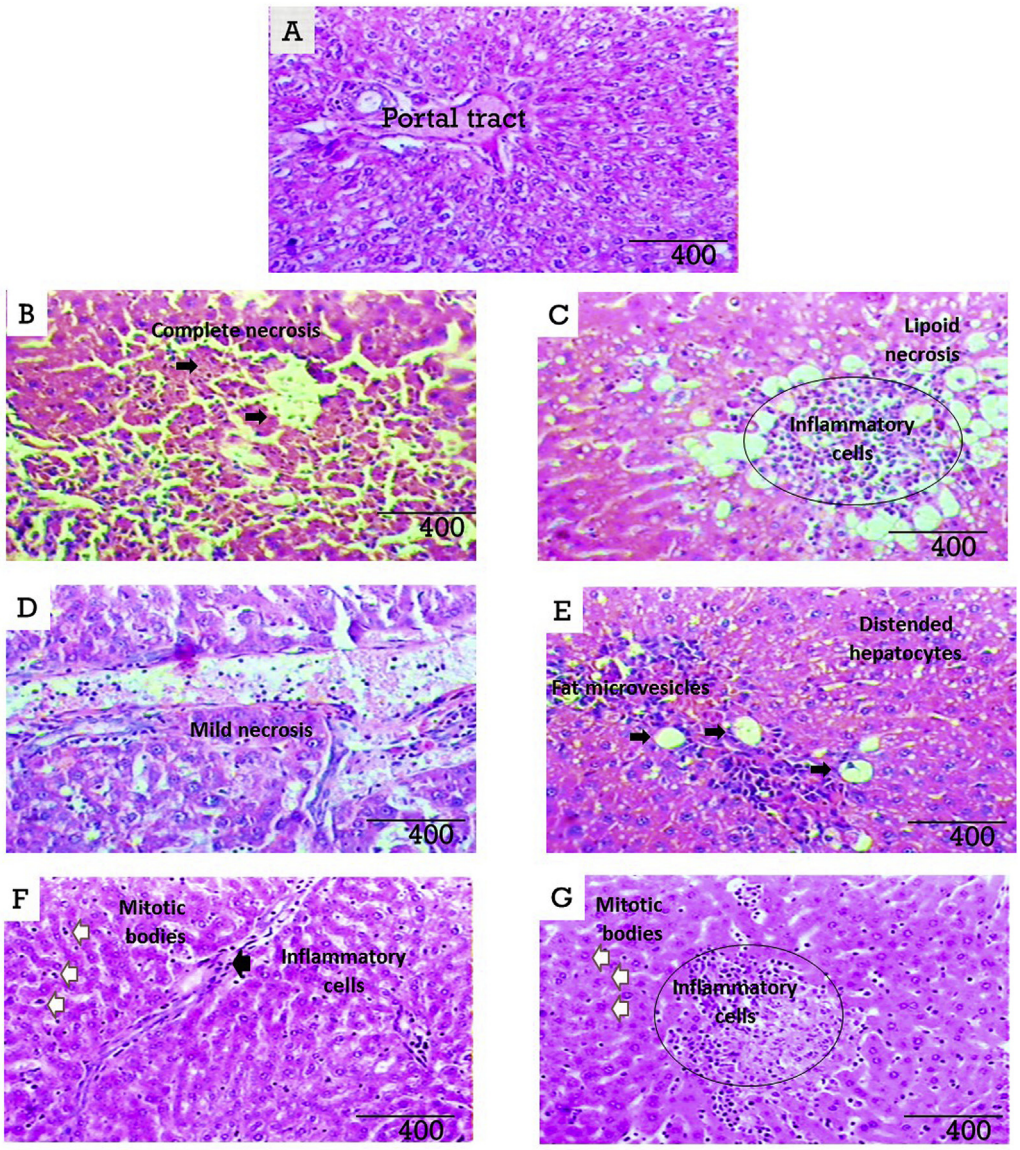


**Figure 2:** Photomicrographs of H&E-stained liver sections, magnification ×400. **A**: micrograph of liver tissue specimen from rats administered 0.2 mL/kg distilled water showing normal liver histology with prominent hepatocytes, normal hepatic artery, portal tract, and blood vessels. **B**: micrograph of liver tissue specimen from rats intoxicated with $CCl_4$ showing marked distortion of hepatocytes morphology with areas of complete necrosis, which demonstrates the hepatotoxic effect of $CCl_4$ at the concentration and route of administration used. **C**: micrograph of liver tissue specimen from rats pretreated with 500 mg/kg SML extract prior to $CCl_4$ intoxication showing prominent microvesicles with degenerating lipid cells (lipoid necrosis), which reveals incomplete resolution of $CCl_4$-induced hepatic injury by this dose of SML. **D**: micrograph of liver tissue specimen from rats pretreated with 1000 mg/kg SML extract prior to $CCl_4$ intoxication showing areas of fibrosis and localized mild necrosis, which indicates the hepatoprotective effect of SML extract at this dose. **E**: micrograph of liver tissue specimen from rats pretreated with 500 mg/kg SMS extract prior to $CCl_4$ intoxication showing microvesicles and hepatocytes with hyperchromatic nuclei, which indicates inadequate hepatoprotection provided by this dose of SMS. **F**: micrograph of liver tissue specimen from rats pretreated with 1000 mg/kg SMS extract prior to $CCl_4$ intoxication showing infiltration of inflammatory cells, mostly neutrophils, along the portal tract and abundant mitotic bodies, which indicates cellular regeneration and hepatoprotection provided by this dose of SMS. **G**: micrograph of liver tissue specimen from rats pretreated with the standard drug silymarin (100 mg/kg) prior to $CCl_4$ intoxication showing localized inflammatory reaction and areas of fibrosis with admixed mitotic bodies, which indicates healing by fibrosis and hepatoprotective effect of silymarin.

($p < 0.05$) increased by 28% and 18%, respectively, by pretreatment with SML extract (1000 mg/kg), and were significantly ($p < 0.05$) increased by 41% and 20%, respectively, by pretreatment with SMS extract (1000 mg/kg).

## Discussion

Hepatotoxicity of different types and origins constitutes a major public health concern. Oxidative stress is implicated in

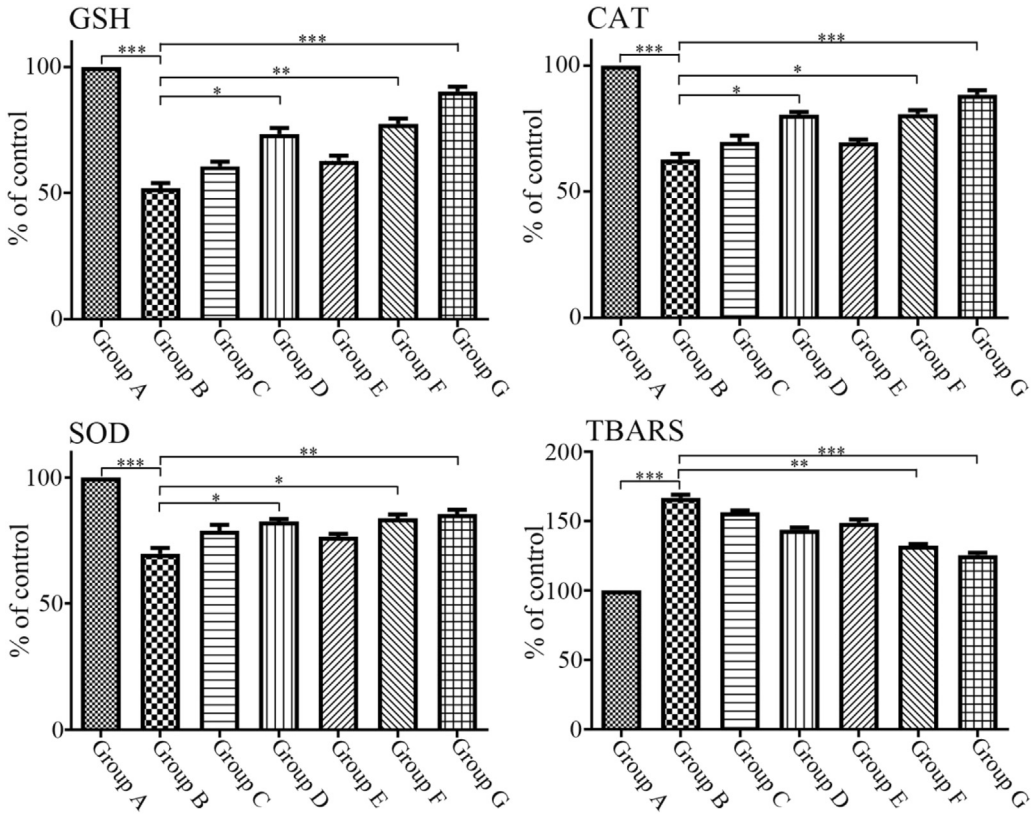


**Figure 3:** The effect of $CCl_4$ intoxication and pretreatment with *Spondias mombin* leaf and stem extracts on oxidative stress markers. Levels of GSH, CAT, SOD, and TBARS were measured in homogenized liver samples. Results of the positive control ($CCl_4$-intoxicated) group are compared to those of the negative control group (receiving water only), and results of the SML, SMS, and silymarin-treated groups are compared to those of the positive control group. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. GSH: reduced glutathione; CAT: catalase; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances.

the pathogenesis of liver diseases. Accordingly, since natural antioxidants in plants and plant extracts could ameliorate free radical-induced oxidative stress, they could be beneficial in the treatment of liver diseases. In this study, the effects of SML and SMS extracts on the body weights of rats, their hematological indices, hepatic enzymes, and the hepatic antioxidant system were examined to reveal whether biomolecules present in these plants could offer hepatoprotection against $CCl_4$-induced cellular insult.

It is important to investigate drug-induced body weight changes as they may provide an important indicator of drug effects. Previously, an increase in the body weights of laboratory animals was demonstrated following a sub-acute toxicity study of *Enantia chlorantha* aqueous extract.[44] In contrast, a decrease in the body weights of experimental rats after the administration of the ethanolic extract of *E. chlorantha* stem bark has also been reported.[45] Previous studies have used body weight changes for assessment of responses to *S. mombin* drug therapy.[33] Our results showed a dose-dependent but non-significant increase in the body weights of rats treated with SMS extract, and this is in agreement with previous reports demonstrating *S. mombin*-induced weight loss and appetite suppression.[33]

Neither the toxicant ($CCl_4$) nor the assessed hepatoprotective agents (SML and SMS extracts and silymarin) induced significant changes in hematological parameters. This indicates the absence of acute adverse effects on hematopoiesis. Low platelet counts have been associated with severe cases of liver cirrhosis.[46] However, platelet upregulation was observed only with the lower dose of SML, and was non-significant.

The effects of SML and SMS methanolic extracts on hepatic lipid accumulation in fatty liver diseases and on resolution of acute intoxication were examined by histopathological investigation of liver tissue specimens. $CCl_4$ administration resulted in damage to the normal histological architecture of hepatocytes as demonstrated by necrosis and membrane lipid peroxidation, which are common histopathological findings with $CCl_4$-induced liver damage.[47,48] Rats pretreated with SML and SMS for 7 days before challenging with $CCl_4$ demonstrated partial resolution of $CCl_4$-induced alterations in the molecular architecture of hepatocytes.

Moreover, $CCl_4$ administration resulted in hepatotoxicity with significantly elevated serum levels of AST, ALT, ALP, CBIL, and TBIL. Elevation in serum aminotransferases is a well-known indicator of liver injury. Previous studies have also shown that hepatic damage caused by $CCl_4$ administration is associated with an increase in the activities of both serum aminotransferases and ALP.[49] Both plant extracts at both doses were effective at significantly lowering CBIL, TBIL, and ALP levels compared to the positive control. However, the effect of the SML extract was stronger than that of the

SMS extract. The higher dose (1000 mg/kg) of both extracts was more effective at lowering serum ALT and AST levels than the lower dose (500 mg/kg), with SMS extract being more effective than SML extract. In addition, $CCl_4$ administration resulted in significant reduction in TP levels. However, pretreatment with SML and SMS extracts significantly normalized TP levels compared to $CCl_4$-induced rats.

The mechanism of $CCl_4$-induced hepatotoxicity involves the generation of reactive oxygen species and depletion of antioxidant defenses like GSH, which results in a state of oxidative stress.[50] In the body, $CCl_4$ generates the free trichloromethyl radical ($CCl_3$•) which causes hepatic damage through the activation of the NADPH-Cyt $P_{450}$ system of the liver endoplasmic reticulum[51] leading to the generation of the more reactive radical, trichloromethyl peroxy radical ($CCl_3O_2$•), which provokes lipid peroxidation, disruption of calcium homeostasis, and apoptosis.[52] These functional and morphological changes in the cellular membrane and death of hepatocytes all result in leakage of hepatic enzymes. The oxidation of fatty acids by $CCl_3$• liberates lipid peroxides,[51] which are free radicals that further exacerbate the state of oxidative stress within a *milieu* deficient in antioxidants.

The mechanisms of defense against free radicals include mobilization of radical scavengers and chain terminators such as vitamins C and E, antioxidants such as GSH, and redox regulatory enzymes such as CAT, SOD, and glutathione peroxidase. Tannins, saponins, alkaloids, flavonoids, phenols, and ascorbic acid, which were reported to be abundant in SML extracts,[34] might play an important role in its antioxidant effect. Hence, we evaluated the effects of SML and SMS extracts on GSH levels, CAT and SOD activities, and TBARS levels. Our results demonstrated that SML and SMS extracts significantly reversed the $CCl_4$-induced marked elevation in TBARS levels.

Different experimental models inducing hepatic fatty infiltration have reported depletion in liver GSH stores, and investigated the pathophysiological consequences of GSH depletion in relation to free radical generation.[16,17,35] Indeed, serum GSH is a sensitive biomarker of the antioxidant status,[53] playing a pivotal defensive role against oxidative insults as an endogenous scavenger of free radicals.[54,55] Administration of $CCl_4$ resulted in a five-fold decrease in GSH levels, compared to negative control rats. However, this effect was significantly counteracted in a dose-dependent manner by pretreatment with SML and SMS extracts. This implies that SML and SMS extracts could enhance the antioxidant capacity by elevating GSH concentrations, thereby ameliorating oxidative stress-induced damage, and this reflects the presence of free radical scavengers in *S. mombin*. Certainly, the liver is reported to maintain GSH even when experiencing elevated lipid peroxidation through supportive and compensatory mechanisms.[55,56]

The role of SOD as an antioxidant is to convert superoxide to hydrogen peroxide, thereby protecting against the pervasive harmful effects of superoxide. The ability of SML and SMS extracts to elevate SOD activity, which had been decreased by $CCl_4$ intoxication, might be partly responsible for their hepatoprotective effects. Similarly, pretreatment with SML and SMS extracts induced an elevation in CAT activity, which is a hydrogen peroxide scavenger. Collectively, we hypothesize that the elevation of SOD and CAT activities, as well as GSH levels in *S. mombin*-treated groups will augment the endogenous antioxidant system.

The restorative effects of silymarin on liver cytoarchitecture after $CCl_4$ treatment may leave the liver with scar tissue due to extensive fibrosis. However, this was not observed in rats treated with SMS extract (1000 mg/kg). Hence, cellular regeneration associated with *S. mombin* treatment might have been mediated by activation of liver stem cells. Moreover, the amelioration of $CCl_4$-induced damage by SML and SMS pretreatment might be attributed to membrane stabilization, which prevents the leakage of cellular contents, as suggested by previous studies investigating the hepatoprotective properties of *Vernonia amygdalina*,[57] *Rumex crispus*,[58] *Chrysophyllum albidum*,[53] *Ocimum gratiissimum*, and *S. mombin*.[35]

The protective effects of plant extracts against $CCl_4$-induced hepatotoxicity has been attributed to the presence of endogenous phytochemicals such as flavonoids, tannins, triterpenoids, and alkaloids.[59,60] Flavonoids represent the most common and extensively distributed group of plant polyphenols, and serve as free radical scavengers and strong antioxidants that could protect against oxidative stress-induced cellular damage.[61] Flavonoids and saponins were reported to be present in *S. mombin* leaves.[62] Antioxidant chemicals in *S. mombin*, particularly polyphenols, could contribute to its antioxidant and hepatoprotective activities.[63] However, further research is required to isolate the bioactive compounds found in SML and SMS extracts and characterize the biochemical mechanisms responsible for their antioxidant and hepatoprotective activities. This work is being implemented in our laboratory and may well lead to the identification of one or more substances of potential clinical benefit in treatment of liver diseases.

## Conclusion

In this study, both SML and SMS extracts were found to exhibit hepatoprotective effects by stabilizing hepatocyte cell membranes, promoting repair of injured hepatic tissues, enhancing free radical scavenging effects, and augmenting endogenous antioxidant systems, thereby limiting oxidative insults. These results provide the premise that requires further investigation of the promising therapeutic potential of *S. mombin* in liver damage and oxidative stress-induced diseases.

## 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. 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.

## Conflict of interest

The authors have no conflict of interest to declare.

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