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# Hepatoprotective properties of methanol leaf extract of *Pterocarpus mildbraedii* Harms on carbon tetrachloride-induced hepatotoxicity in albino rats (*Rattus norvegicus*)

Thelma Ebele Ihedioha[1], Rita Ifeoma Odo[1], Uwakwe Simon Onoja[2], Chikaodili Adaobi Nwagu[1], John Ikechukwu Ihedioha[3]

[1]Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka 410001 Nigeria

[2]Department of Nutrition and Dietetics, Faculty of Agriculture, University of Nigeria, Nsukka 410001 Nigeria

[3]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka 410001, Nigeria

## ABSTRACT

**Aim:** This study evaluated the effects of methanol leaf extract of *Pterocarpus mildbraedii* Harms on carbon tetrachloride ($CCl_4$)-induced sub-acute hepatotoxicity in albino rats.

**Methods:** Fresh leaves of *P. mildbraedii* were collected in December 2016. Thirty male albino rats were randomly assigned to six groups (A–F) of five rats each. Sub-acute hepatotoxicity was induced in groups A, B, C, D, and E by intraperitoneal injection of 1 ml/kg $CCl_4$ in equal volume of olive oil at 3-day intervals for 12 days. Group A was given 10 ml/kg distilled water placebo and served as untreated (negative) control, groups B, C, and D were treated with 100, 200, and 400 mg/kg *P. mildbraedii* methanol leaf extract (PME), respectively, group E was treated with 100 mg/kg silymarin as positive control, while group F was given 10 ml/kg distilled water placebo and served as normal control. Treatment with PME and silymarin was done orally twice daily for 15 days. Blood samples were collected on the 15th day for evaluation of liver enzyme markers [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase activities] and liver function (total serum protein, albumin, globulin, total cholesterol, and bilirubin), following standard procedures. Relative liver weight was calculated.

**Results:** Treatment with PME and silymarin significantly ($p < 0.05$) decreased the elevated ALT and AST, and thus restored hepatocellular integrity, and also ameliorated inflammatory liver enlargement in the rats given $CCl_4$.

**Conclusion:** These findings imply that treatment with PME as used in this study led to significant hepatoprotection against $CCl_4$-induced hepatotoxicity.

## ARTICLE HISTORY

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## KEYWORDS

*Pterocarpus mildbraedii* extract; hepatoprotection; carbon tetrachloride; hepatotoxicity

## Introduction

The liver is one of the most complex and most important organs in the human body. It plays a major role in the metabolism of food substances and detoxification of drugs and xenobiotics [1]. It is also concerned with the deamination of excess proteins, storage of iron, vitamins and glycogen, production of urea, bile, and vital enzymes in the body. The liver is constantly and variedly exposed to xenobiotics which may lead to liver damage or hepatotoxicity [2]. Toxic liver damage is a major health problem; the manifestations of which are highly variable ranging from an asymptomatic elevation of liver enzymes to fulminant liver failure. Despite notable developments in modern medicine, liver diseases remain a global health challenge [3].

Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for liver diseases. Traditionally, a variety of plants have been recommended for the treatment of liver diseases [4]. The efficacy of most of these medicinal plants has not yet been scientifically validated.

**Contact** Thelma Ebele Ihedioha ✉ thelma.ihedioha@unn.edu.ng Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka 410001 Nigeria.

*Pterocarpus mildbraedii* Harms is an evergreen or semi-deciduous tree belonging to the family *Papilionaceae*. It is a medium-sized to large tree up to 35 m tall, with smooth gray or pale brown bark, and small rounded crown [5]. Two species are recognized locally, *P. mildbraedii* Harms (oha) and *Pterocarpus santalinoides* DC (nturukpa) [6]. *Pterocarpus mildbraedii* is commonly called "African rosewood." Its tender leaves are locally used as a vegetable in the preparation of soup [6,7]. The leaves are a good source of beneficial nutrients like amino acids, calcium, iron, potassium, vitamins A, B, and C, and also possess antioxidant, antibacterial, antispasmodic, and diuretic properties [8]. In South-Eastern Nigeria, leaf and stem bark extracts of *P. mildbraedii are* used for the treatment of a variety of ailments and disorders [7,9]. Studies in our laboratory and also by other researchers have shown that extracts from two species belonging to the genus *Pterocarpus* (*P. santalinoides* and *Pterocarpus erinaceus*) possess hepatoprotective activity [10,11]. The need then arose to evaluate *P. mildbraedii* for hepatoprotective activity, as it is the specie that is widely available, commonly consumed as vegetable in soups, and generally more acceptable. The present study, therefore, evaluated the effects of methanol leaf extract of *P. mildbraedii* on $CCl_4$-induced hepatotoxicity in albino rats.

## Materials and Methods

### *Assay kits, chemicals, solutions, and reagents*

The assay kits for evaluation of the serum enzyme activity concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and serum levels of total proteins, albumins, and total cholesterol (TC) are products of Quimica Clinica Aplicada (QCA), Spain. Total bilirubin was assayed using the Randox® bilirubin test kit (Randox Laboratories Ltd, County Antrim, United Kingdom). Methanol, silymarin, and carbon tetrachloride were sourced from Sigma-Aldrich (St. Louis, Missouri). Thiopentone sodium was obtained from Chandra Bhagat Pharma Pvt., Ltd., Mumbai, India. All other routine chemicals and reagents were of analytical grade.

### *Gathering of plant material, plant identification, and extract preparation*

The study was carried out in 2017. Fresh leaves of *P. mildbraedii* were collected from Orifite town in Ekwusigo Local Government Area of Anambra State, Nigeria, in December 2016. The plant was identified by Mr. A.O. Ozioko, a plant taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka [(Voucher Specimen Number—UNH (University of Nigeria Herbarium) No. 158]. The *P. mildbraedii* leaves collected were allowed to dry under the shade, and later ground into powder. Five hundred grams (500 g) of the ground leaves were extracted with 80% methanol using the cold maceration extraction method, with intermittent shaking at 2-hour intervals for 48 hours. The extract obtained was filtered with Whatman size 1 filter paper, concentrated to dryness in a rotary evaporator (Buchi, Switzerland), and referred to as *P. mildbraedii* extract (PME).

### *Experimental animals*

Forty-two (42) adult male albino rats (*Rattus norvegicus*) of 12 weeks of age and body weights between 220 g and 250 g were obtained from the Department of Veterinary Physiology and Pharmacology Laboratory Animal Unit, University of Nigeria, Nsukka, and used for the study. Twelve (12) of the albino rats were used to study the acute toxicity of the extract, while thirty (30) were used to study the hepatoprotective activity of PME. The albino rats were kept in stainless steel cages in a fly proof Animal House at room temperature (27°C–29°C) and allowed for 2 weeks to acclimatize. They were fed commercial pelletized rat chow (Grand Cereals Nig. Ltd, Jos, Nigeria), composed of 13% crude protein, 8% fat, 15% crude fiber, 0.9% calcium, 0.35% phosphorus, and 2,600 Kcal/kg metabolizable energy, and provided with clean water *ad libitum* all through the study. Guidelines for the use of animals for laboratory experiment were strictly adhered to [12]. The animal experimental protocol was approved by the Experimental Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

## Methods

### *Acute toxicity test*

Oral acute toxicity and median lethal dose ($LD_{50}$) of PME was done using the OECD Acute Toxic Class method [13]. The Annex 2b OECD test procedure with a starting dose of 50 mg/kg was followed. Twelve male albino rats were used, three rats each for testing at 50, 300, 2,000, and the limit dose of 5,000 mg/kg, respectively. The rats were fasted for 12 hours before the commencement of the test, but

drinking water was made available all through [13]. The varied doses of the extract were each dissolved in 1 ml of water and administered using an intubation cannula. The rats were observed for 14 days for any signs of toxicity.

### *Phytochemical analysis*

Semi-quantitative phytochemical analysis was conducted to determine the phytochemicals present in PME, following the procedures described by Trease and Evans [14], and Harborne [15]. Tests for the presence of tannins, flavonoids, alkaloids, saponins, carbohydrates, glycosides, starch, sterols, and terpenes were conducted on PME. One gram (1 g) of PME was dissolved in 100 ml of distilled water in a beaker. The solution was filtered with Whatman no 1 filter paper to obtain a clear filtrate which was used to test for the presence of the phytochemicals. High levels of specific phytochemicals were scored +++, moderate levels were scored ++, low level was scored +, while phytochemicals that were absent were not scored [14,15].

### *Evaluation of the hepatoprotective activity of PME in albino rats*

The thirty (30) male albino rats used for the hepatoprotection study were randomly assigned to six groups (A–F) of five rats each. Sub-acute hepatotoxicity was induced in groups A, B, C, D, and E by intraperitoneal injection of 1 ml/kg $CCl_4$ in equal volume of olive oil (50% volume/volume) at the beginning of the experiment (day 0), and at every 3 days for the next 12 days (3, 6, 9, and 12). Group A was given 10 ml/kg distilled water as placebo and served as negative control (untreated), groups B, C, and D were treated with 100, 200, and 400 mg/kg PME, respectively, group E was treated with silymarin (a standard hepatoprotective drug) at the dose of 100 mg/kg as positive control, while group F was given 10 ml/kg distilled water as placebo and served as normal control. Treatment started from day 1 of the experiment and was done orally twice daily for 15 days. Blood samples were collected from the albino rats using the orbital technique [16], on day 15 (at the end of the experiment) for serum biochemistry assay. The blood samples were allowed 30 minutes to clot. The serum biochemistry assay was done immediately upon separation of the serum from blood clot following standard procedures using Quimica Clinica Applicada® (QCA) test kits (QCA, Spain), and Randox® bilirubin test kit (Randox Laboratories Ltd, United Kingdom).

Liver damage marker enzymes, such as serum ALT and serum AST activity levels, were evaluated following the Reitman–Frankel method [17]. The ALT in the serum sample and ALT standard catalyzed the reaction of L-alanine and alpha-ketoglutaric acid to form pyruvic acid and L-glutamic acid, while the AST catalyzed the reaction of L-aspartic acid with alpha-ketoglutaric acid to form oxaloacetic acid and L-glutamic acid. These ketonic acids produced were reacted with 2-4, dinitrophenyl hydrazine to form corresponding colored hydrazone, the optical density of which was then measured and ALT/AST quantified at 505 nm wavelength using the semi-automated analyzer (Daitek Instruments Co. Ltd., Wuxi, China).

The serum ALP activity was quantified using the QCA alkaline phosphatase test kit, which is based on the phenolphthalein monophosphate method [17]. In this method, alkaline phosphatase in the serum and a standard (containing 30 IU/l alkaline phosphatase) hydrolyzed a colorless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH turned to pink color, the optical density of which was measured and alkaline phosphatase activity quantified at 546 nm wavelength, using the semi-automated analyzer.

Assay of serum total protein was by the direct Biuret method [18]. This procedure involved a reaction of the proteins in the serum samples and a standard (containing 5g/dl of proteins) with copper ions in the Biuret reagent in an alkaline medium, which resulted in the formation of a stable colored complex, the optical density of which was measured and the serum total protein quantified at 546 nm wavelength using the semi-automated analyzer. The serum albumin was quantified using the QCA albumin test kit, which is based on the bromocresol green method [18]. This procedure involved the reaction of the albumin in the serum samples and standard (containing 5 g/dl of albumin) with bromocresol reagent at acid pH to form a colored complex, the optical density of which was measured and albumin quantified at 630 nm wavelength using the semi-automated analyzer. The globulin levels were calculated by subtracting the serum albumin levels from the serum total protein levels [18].

The serum TC was determined using the QCA TC test kit, which is based on the enzymatic colorimetric method [19]. In this procedure, TC in the serum samples and a standard (containing 200 mg/dl of cholesterol) was enzymatically hydrolyzed by

cholesterol esterase and further oxidized by cholesterol oxidase contained in the QCA TC working reagent. The reactions led to the formation of a colored quinonic derivative, the optical density of which was measured and TC quantified at 505 nm wavelength using the semi-automated analyzer.

The total bilirubin levels in the serum samples were assayed using the Randox® bilirubin test kit (Randox Laboratories Ltd, County Antrim, United Kingdom), which is based on the Jendrassik and Grof method [20]. In this determination, the serum samples were reacted with diazotized sulfanilic acid in the presence of caffeine to produce an azo pigment, the optical density of which was measured and total bilirubin quantified at 578 nm using the semi-automated analyzer.

After the collection of blood samples, the rats were euthanized by intra-peritoneal injection of 250 mg/kg thiopentone sodium [21]. The livers were eviscerated and weighed, and the relative liver weight of individual rats was calculated.

### *Statistical analysis*

Data obtained from the study were subjected to one-way analysis of variance, and variant means were separated *post hoc* using the least significant difference method. Statistical Package of the Social Sciences software, version 16.0, was used for the analysis. Significance was accepted at $p < 0.05$.

## Results

### *Acute toxicity test*

The varied doses of PME used for the acute toxicity test had no adverse clinical effect on the behavioral responses of the tested rats during the 14 day monitoring period. Physical observation also indicated that all the rats behaved essentially normal, with no signs of changes in the skin, fur, eyes, mucous membrane, and behavioral patterns. There was no mortality in all the treated rats. The rats tolerated the extract up to 5,000 mg/kg; therefore, the $LD_{50}$ of the extracts is above 5,000 mg/kg ("Category 5—Unclassified" of the Global Harmonized Classification System [13].

### *Phytochemical analysis*

Results of the phytochemical analysis showed varied degrees of bioactive phytochemicals in the PME. It showed high levels (+++) of carbohydrates, saponins, glycosides, fats and oil; and moderate levels (++) of flavonoids and tannins.

### *Hepatoprotective activity of PME in albino rats*

The serum ALT activity levels of rat groups B, C, D, E, and F were significantly lower ($p < 0.05$) than that of group A, and there were no significant differences ($p > 0.05$) between groups C, D, and E in their serum ALT activity levels (Table 1). The serum AST activity levels of groups C, D, E, and F were significantly lower ($p < 0.05$) than that of groups A and B, and there were no significant differences ($p > 0.05$) in serum AST activity of groups C, D, and E (Table 1). The serum ALP activity levels of group F was significantly lower ($p < 0.05$) than that of groups A, B, C, D, and E, and there were no significant differences ($p > 0.05$) in serum ALP activity between groups A, B, C, D, and E (Table 1).

There were no significant variations in the serum total protein levels among the groups, but the serum albumin levels of groups E and F were significantly higher ($p < 0.05$) than that of groups

**Table 1.** Effects of PME on serum enzymes of rats given sub-acute toxic doses of $CCl_4$.

| Groups* | Mean ± standard error | | |
|---|---|---|---|
| | ALT (U/l) | AST (U/l) | ALP (U/l) |
| Group A | 102.24 ± 2.82[a] | 139.73 ± 4.74[a] | 242.00 ± 1.19[a] |
| Group B | 92.25 ± 7.34[b] | 138.34 ± 9.17[a] | 240.68 ± 0.50[a] |
| Group C | 65.79 ± 3.48[c] | 113.01 ± 2.69[b] | 239.48 ± 1.74[a] |
| Group D | 67.82 ± 5.11[c] | 109.49 ± 4.72[b] | 237.90 ± 1.79[a] |
| Group E | 64.31 ± 3.05[c] | 105.49 ± 2.98[b] | 233.65 ± 3.34[a] |
| Group F | 28.07 ± 1.73[d] | 61.56 ± 2.94[c] | 172.02 ± 12.43[b] |

*Groups. Group A—$CCl_4$ alone, no treatment; Group B—$CCl_4$ +100 mg/kg PME; Group C—$CCl_4$ + 200 mg/kg PME; Group D—$CCl_4$ + 400 mg/kg PME; Group E—$CCl_4$ + 100 mg/kg silymarin; Group F—No $CCl_4$, no treatment.

[a,b,c]Alphabetical superscripts in a column indicate significant differences between the groups, $p < 0.05$.

A, B, C, and D (Table 2). The serum globulin level of group F was significantly lower ($p < 0.05$) than that of groups A, B, C, D, and E, while the serum globulin level of group E was significantly lower ($p < 0.05$) than only that of groups A and B (Table 2).

The serum TC of group F was significantly lower ($p < 0.05$) than only that of groups A and B, and the serum TC of groups C, D, and E was lower, but not statistically different ($p > 0.05$) from that of groups A and B. There were no significant differences ($p > 0.05$) between groups C, D, and E in their serum TC (Table 3).

The serum total bilirubin of group F was significantly lower ($p < 0.05$) than only that of groups A and D, while the serum total bilirubin of groups B, C, and E was lower than that of groups A and D but not found to be statistically significant ($p > 0.05$). There were no significant differences ($p > 0.05$) between groups B, C, and E in their serum total bilirubin (Table 3).

The relative liver weight of group F was significantly lower ($p < 0.05$) than that of groups A, B, C, D, and E, while the relative liver weight of groups B and E was significantly lower ($p < 0.05$) than only that of group A. The relative liver weight of groups C and D was lower, but not statistically different ($p > 0.05$) from that of group A (Table 3).

## Discussion

The result of the acute toxicity suggests that PME is not acutely toxic, and is thus considered safe for the treatment of ailments and disorders for which it is effective. This result is in agreement with the report of Ihedioha et al. [11], that methanol leaf extract of *Pterocarpus santalinoides*, a specie in the same genus *Pterocarpus*, is acutely non-toxic.

**Table 2.** Effects of PME on serum proteins of rats given sub-acute toxic doses of $CCl_4$.

| Groups* | Mean ± standard error | | |
|---|---|---|---|
| | Total proteins (g/dl) | Albumins (g/dl) | Globulins (g/dl) |
| Group A | 5.64 ± 0.03 | 3.15 ± 0.08 [a] | 2.48 ± 0.04 [a] |
| Group B | 5.64 ± 0.05 | 3.23 ± 0.06 [a] | 2.42 ± 0.05 [a] |
| Group C | 5.68 ± 0.04 | 3.31 ± 0.04 [a] | 2.37 ± 0.03 [ab] |
| Group D | 5.57 ± 0.04 | 3.32 ± 0.11 [a] | 2.26 ± 0.06 [ab] |
| Group E | 5.69 ± 0.02 | 3.59 ± 0.02 [b] | 2.11 ± 0.03 [b] |
| Group F | 5.69 ± 0.05 | 3.89 ± 0.05 [c] | 1.86 ± 0.04 [c] |

*Groups. Group A—$CCl_4$ alone, no treatment; Group B—$CCl_4$ +100 mg/kg PME; Group C—$CCl_4$ + 200 mg/kg PME; Group D—$CCl_4$ + 400 mg/kg PME; Group E—$CCl_4$ + 100 mg/kg silymarin; Group F—No $CCl_4$, no treatment.

[a,b,c]Alphabetical superscripts in a column indicate significant differences between the groups, $p < 0.05$.

**Table 3.** Effects of PME on serum total cholesterol, bilirubin and relative liver weight of rats given sub-acute toxic doses of $CCl_4$.

| Groups* | Mean ± standard error | | |
|---|---|---|---|
| | Total cholesterol (mg/dl) | Total bilirubin (mg/dl) | Relative liver weight (%) |
| Group A | 35.52 ± 3.34 [a] | 0.63 ± 0.04 [a] | 4.64 ± 0.16 [a] |
| Group B | 35.23 ± 3.86 [a] | 0.58 ± 0.03 [ab] | 4.16 ± 0.14 [b] |
| Group C | 28.29 ± 2.10 [ab] | 0.58 ± 0.09 [ab] | 4.26 ± 0.13 [ab] |
| Group D | 33.07 ± 4.01 [ab] | 0.64 ± 0.08 [a] | 4.46 ± 0.16 [ab] |
| Group E | 32.21 ± 2.32 [ab] | 0.60 ± 0.10 [ab] | 4.15 ± 0.11 [b] |
| Group F | 24.10 ± 2.34 [b] | 0.41 ± 0.28 [b] | 3.56 ± 0.10 [c] |

*Groups: Group A—$CCl_4$ alone, no treatment; Group B—$CCl_4$ +100 mg/kg PME; Group C—$CCl_4$ + 200 mg/kg PME; Group D—$CCl_4$ + 400 mg/kg PME; Group E—$CCl_4$ + 100 mg/kg Silymarin; Group F—No $CCl_4$, no treatment.

[a,b,c]Alphabetical superscripts in a column indicate significant differences between the groups, $p < 0.05$.

The findings in this study of the presence of varied levels of flavonoids, glycosides, saponins, tannins, carbohydrates, fats, and oil in PME, is in agreement with the reports of Akindahunsi and Salawu [22], and Usunobon and Igwe [23], who had earlier reported that the leaves of *P. mildbraedii* are good sources of flavonoids, tannins, saponins, and glycosides. Onyeka and Nwambakwe [24] also reported that *P. mildbraedii* leaves are rich in these phytochemicals. According to Okwu [25], these phytochemicals are commonly found in various medicinal herbs and they exhibit a wide range of biological activities.

The ability of $CCl_4$ to induce liver damage was evident by the significantly higher serum enzyme activity levels of ALT, AST, and ALP in all the groups that were given $CCl_4$ [26]. The damage to the liver hepatocytes caused by $CCl_4$ was evident by the alterations in the activity levels of these liver damage marker enzymes. When cell membranes of hepatocytes are damaged, enzymes, such as ALT, AST, and ALP, are released into the blood from the cytosol of hepatocytes [27]. A rise in serum transaminases is a sensitive indicator of cell membrane damage of hepatocytes. Serum activity concentrations of AST and ALT are the most commonly used biochemical markers of hepatocellular necrosis [28,29]. The finding in this study that rat groups treated with PME at all doses (B, C, and D), and those treated with 200 mg/kg and 400 mg/kg (groups C and D), respectively, had serum ALT and AST activities significantly lower than that of group A and comparable to that recorded for group E, showed that administration of PME at these doses protected the integrity of the hepatocytes, and compared favorably with silymarin which is a standard hepatoprotective drug. These findings are in agreement with the reports of Offor *et al.* [30] who recorded decreases in ALT and AST activity levels in albino rats treated with ethanol leaf extract of *P. santalinoides.* Aja et al. [31] and Ihedioha et al. [11] also recorded significant decreases in ALT and AST activity levels in albino rats in which hepatotoxicity was induced with $CCl_4$, and acetaminophen, respectively, and treated with leaf extract of *P. santalinoides*, a related specie in the genus *Pterocarpus*. The ability of PME to enhance hepatocellular integrity as seen in this study may be due to the presence in this extract of phytochemicals like flavonoids, glycosides, and tannins, which are known natural antioxidants [32]. Reports have shown that these phytochemicals possess the ability to reduce free radical formation and also to scavenge free radicals [32]. Carbon tetrachloride is known to induce hepatotoxicity by the generation of free radicals which induce oxidative stress [33]. Since free radicals play such an important role in $CCl_4$-induced hepatotoxicity, it seems logical that compounds that neutralize such free radicals may have hepatoprotective activity as a consequence of their antioxidant properties, and as such may be necessary for protection against free radical damage by $CCl_4$ in the liver. Flavonoids, tannins, and water-soluble glycosides also function in protection against allergies, inflammation, platelet aggregation, tumors, and hepatotoxins [25]. Other natural products that possess antioxidant properties have also been reported to protect against $CCl_4$-induced hepatotoxicity [34].

The lack of significant variation between the groups in their serum levels of total proteins indicates that sub-acute administration of $CCl_4$ as used in the study did not significantly affect total protein synthesis in the liver. This may be because the duration of the study (sub-acute) was not long enough for the total protein to be affected, as hypoproteinemia has been reported to occur mainly in chronic liver diseases [35]. The significantly lower serum albumin in the groups that were given $CCl_4$ is a further indication of liver dysfunction caused by $CCl_4$ administration [36]. Serum albumin is the major plasma protein synthesized in the liver. It is a clinically useful marker of hepatic synthetic function [29]. The liver is an important site for the synthesis of serum albumins and the administration of $CCl_4$ in this study adversely affected hepatic synthesis of albumins. Treatment with silymarin led to significant elevation in serum albumin level and thus enhanced hepatic synthetic activity. The significant elevation in serum globulin levels in groups A and B when compared to groups E and F is an indication of a high priority for globulin in the damaged liver [37].

Administration of $CCl_4$ altered the serum lipid profile by increasing the serum levels of TC of group A and others that were given $CCl_4$. The liver is the principal site for the formation and clearance of lipoproteins. Hence, it is not surprising that toxic liver damage can affect plasma lipid levels in a variety of ways. Cholestasis is associated with hypercholesterolemia as the major excretory pathway of cholesterol is blocked in liver disorder [38]. Treatment with PME at 200 and 400 mg/kg, and silymarin at 100 mg/kg (groups C, D, and E), slightly ameliorated this cholestasis and dyslipidemia induced by $CCl_4$ administration by a slight reduction of serum TC level at these doses. Adegbite and Ezekwesili

[39] recorded a reduction in serum TC level in $CCl_4$-induced rats treated with ethanol and aqueous leaf extracts of *Pterocarpus milbraedii*.

Treatment with PME at 100 mg/kg and 200 mg/kg (groups B and C), and silymarin at 100 mg/kg slightly enhanced hepatic clearance of bilirubin. Otuechere and Farombi [40] reported that *P. mildbraedii* methanol extract significantly reduced serum total bilirubin level in propanil-induced hepatotoxicity in albino rats. Hamza et al. [41] also recorded a significant reduction in serum bilirubin level in albino rats whose livers were damaged with $CCl_4$ and treated with sub-fractions of *P. mildbraedii* extracts.

The higher relative liver weight seen in group A is an indication of inflammation/degeneration which is a consequence of $CCl_4$-induced hepatotoxicity [42,43]. The reduction in relative liver weight after treatment with PME at all the doses used in the study (groups B, C, and D), and silymarin at 100 mg/kg (group E), suggests their amelioration of this inflammatory enlargement of the liver caused by $CCl_4$ toxicity.

## Conclusion

Based on the results obtained from this study, it was concluded that the administration of *P. mildbraedii* methanol leaf extract to rats at the doses used in the study was protective of hepatocellular integrity, ameliorated inflammatory enlargement of the liver, and compared positively with silymarin which is a standard hepatoprotective drug. These findings imply that *P. milbraedii* methanol leaf extract possesses hepatoprotective activity against carbon tetrachloride-induced liver damage.

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## Conflict of interest

The authors declared that they have no conflict of interest.

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