Original Article

Effect of partially purified sub-fractions of Pterocarpus mildbraedii extract on carbon tetrachloride intoxicated rats

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ABSTRACT

Background: This study was designed to determine the hepatoprotective effect of partially purified fractions from Pterocarpus mildbraedii extract on carbon tetrachloride (CCL4) intoxicated rats.

Methods: The methanol extract of P. mildbraedii was subjected to solvent partitioning using n-hexane, chloroform, ethylacetate and water. Separation of fractions with proven antioxidant activity was achieved by chromatographic techniques. Acute toxicity and hepatoprotective studies of the methanol sub-fraction 6 (Me 6), methanol sub-fraction 7 (Me 7) and methanol sub-fraction 8 (Me 8) from P. mildbraedii extract on carbon tetrachloride (CCL4) intoxicated Wister rats.

Results: Intoxication of rats with CCl4 resulted in significant (p < 0.05) increase in the activities of aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), level of malondialdehyde (MDA) while glutathione (GSH) concentration, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were decreased. Administration of Me6, Me7 and Me8 sub-fractions of P. mildbraedii caused a significant reduction (p < 0.05) in the activities of the liver enzymes, MDA level, total and direct bilirubin in dose-dependent manner. There were significant (p < 0.05) increases in GSH concentration, SOD, CAT, and Gpx activities in the treated groups. The subfractions also restored the damaged hepatic-architecture in the treated groups.

Keywords:
Pterocarpus mildbraedii
Carbon tetrachloride
Intoxicated rats
Sub-fractions
Hepatoprotective

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1. Introduction

The liver is the central organ of metabolism involved in bile production, storage of iron, vitamins, trace elements and detoxification of drugs. These crucial roles make the liver susceptible to different injury leading to several diseases. These diseases are mainly caused by toxic chemicals like carbon tetrachloride, acetalaminophen, excess consumption of alcohol, infections and autoimmune/disorder in which excess reactive oxygen species is implicated. Liver diseases are still serious health problems in both developed and developing world.

The effectiveness of orthodox medicines is inconsistent in the treatment of liver diseases and most of these drugs are often accompanied by devastating adverse reactions such as renal dysfunction, poor tolerance and anemia. Most often, the treatment is worse than the disease. These drugs only provide symptomatic relief, without significant changes to the progression of the disease. They are laden with high relapse rate and are expensive.

Therefore, the search for natural antioxidants for the prevention and treatment of liver diseases arising from oxidative stress becomes very necessary in drug discovery and formulation of nutraceuticals. It has been established that some vegetables and fruits contain antioxidants that could be harnessed in the treatment and management of diseases arising from oxidative stress such as cancer, liver diseases, diabetes, ulcer, ageing process, arthritis, asthma, inflammation, ischemia, mongolism, neurodegeneration, Parkinson’s disease and fungal diseases.

Pterocarpus mildbraedii belong to the family Papilionaceae and is found majorly in some West African countries like Nigeria, Ghana, Liberia, Cameroun, and Cot devoire. It is commonly called paduk and with different local names (“Oha” in Igbo, “Madobiyar rafi” in Hausa, “Urube” in Edo, “Geneghar” in Ijaw and “Kakupupu” in Urhobo. The leaves of P. mildbraedii are used as cooked vegetable in southern Nigeria. In Ghana the trees have been planted in cocoa plantation to provide shade. P. mildbraedii is widely exploited for its timber in Tanzania and the wood which is whitish are widely used for carving cutlass handles, tie-rod and mortar making. Apart from their food value, traditional vegetables are useful for other purposes, such as medicine, cash income and in cultural observance In Eastern and Southern Nigeria, the leaf extracts from P. mildbraedii are used in the treatment of headaches, pains, fever, convulsions, respiratory disorders and as antimicrobial agents. Despite the extensive use of this plant as vegetable and herbal medicine there are very few validated scientific reports regarding the pharmacological activities, therefore, this study evaluates the hepatoprotective properties of P. mildbraedii.

2. Methods

2.1. Collection of samples and identification of samples

P. mildbraedii vegetable was collected from a farm in Anambra State, Nigeria and identified by Dr. Ugbabe, G. of National Institute of Pharmaceutical Research, Abuja, Nigeria (NIPRD). The vegetable was assigned a Voucher/Specimen number of NIPRD/H6713 and deposited at NIPRD herbarium.

2.2. Plant preparation and extraction

The extraction was done according to the method of Ogbadoyo et al. The debris were removed from the vegetable, washed and air-dried at room temperature with adequate ventilation. The dried sample was pulverized using a Binatone blender (Binatone model BLG-555, China). Fifty gram (50 g) of the powdered sample was weighed into 400 mL of methanol in a reflux flask and refluxed for 2 hours. The extract was filtered with a muslin cloth and subsequently evaporated using a rotary evaporator (Bibby Steriling Ltd, Stone Staffordshire ST 15OSA, UK). The dried extract was weighed, placed in sterile sample bottles and stored in a refrigerator until required for use.

The crude extract of P. mildbraedii (100 g) was dissolved in 100 mL of distilled water in a beaker and was poured into a liter of separatory funnel and partitioned using n-hexane, chloroform, ethyl acetate to give four fractions (n-hexane, chloroform, ethyl acetate and aqueous fractions). The aqueous fraction of P. mildbraedii with the highest in vitro antioxidant properties was then subjected to vacuum liquid chromatography (VLC). The methanol fraction obtained from VLC that was observed to have highest in vitro-antioxidant activity were further subjected to column chromatography to give eight subfractions (Me1–Me8). The partially purified active sub-fractions (Me6–Me8) were chosen for this study.

2.3. Experimental animals

Wistar albino rats weighing between 120 and 180 g were obtained from the Animal house, Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna. They were fed on standard rat pellet diet (Ladokun Feeds, Nigeria) and water was provided ad libitum and the animals were allowed to adapt to the laboratory environment for two weeks in order to acclimatize.

2.4. Laboratory animal ethics

The principles governing the use of laboratory animals were followed as approved by the Ethical Committee on the use of laboratory animals of Federal University of Technology, Minna.
which is also in accordance to the Guidelines of National Research Council Guide for the Care and Use of Laboratory Animals\textsuperscript{18} and principles of Good Laboratory Procedure.\textsuperscript{15}

### 2.5. Acute toxicity test

The acute toxicity study of sub-fractions Me6, Me7 and Me8 of *P. mildbraedii* was performed by the method of Organization of Economic Co-operation and Development (OECD) guideline 423.\textsuperscript{16}

### 2.6. Experimental design and animal groupings

The effect of purified sub-fractions; Me6, Me7 and Me8 on carbon tetrachloride induced hepatotoxicity in rats were determined as described by Mayuren et al.\textsuperscript{17} Forty five rats weighing between 120 and 180 g were divided randomly into nine groups of five rats each. The 2 mL/kgbw of Carbon tetrachloride (CCl₄) in olive oil (1:1) was used to induce hepatotoxicity in rats at intraperitoneal region (i.p) every 72 hours followed by the treatments for 10 days daily.

Group I: received normal saline (2 mL/kgbw, i.p), Group II: CCl₄ only, Group III: 50 mg/kgbw of silymarin + CCl₄, Group IV: 50 mg/kgbw of Me6 sub-fraction of *P. mildbraedii* + CCl₄, Group V: 100 mg/kgbw of Me6 sub-fraction of *P. mildbraedii* + CCl₄, Group VI: 50 mg/kgbw of Me7 sub-fraction of *P. mildbraedii* + CCl₄, Group VII: 100 mg/kgbw of Me7 sub-fraction of *P. mildbraedii* + CCl₄, Group VIII: 50 mg/kgbw of Me8 sub-fraction of *P. mildbraedii* + CCl₄, Group IX: 100 mg/kgbw of Me8 sub-fraction of *P. mildbraedii* + CCl₄.

Twenty hours after the last administration of CCl₄ which signify the end of the experiment, the rats were euthanized by cervical dislocation and blood samples were collected by cardiac puncture from the inferior vena cava of the heart into serum bottles.\textsuperscript{19} The blood samples were allowed to clot at room temperature and sera were obtained by centrifuging at 3000 rpm for 10 minutes.\textsuperscript{19,20}

### 2.7. Assessment of liver function

Biochemical parameters such as AST, ALT,\textsuperscript{21} ALP,\textsuperscript{22} albumin,\textsuperscript{23} total bilirubin and total protein\textsuperscript{24} were analyzed using Randox diagnostics kits (United Kingdom) according to individual kits manual.

### 2.8. Preparation of liver homogenate and assessments of oxidative stress

The preparation of liver homogenate was done as reported by Erukainure et al.\textsuperscript{25} A section of the rat’s liver was rinsed in ice-cold 1.15% KCl, blotted and weighed. They were then homogenized in four volumes of ice-cold isotonic phosphate buffer (100 mM, pH 7.4) in pre-cooled laboratory mortar and pestle placed in a bowl of ice chips. The homogenates were centrifuged at 12,500 x g for 15 minutes at 4 °C in a high speed refrigerated ultra-centrifuge (Model LR10-2.4A, Shendi, and Shanghai). The supernatants were used for assay for superoxide dismutase,\textsuperscript{26} catalase\textsuperscript{27} and glutathione peroxidise\textsuperscript{28} enzyme activity. The homogenate was also used for the assay of determination of lipid peroxidation (LPO) by measuring the release of malondialdehyde (MDA) by the method of Varshney and Kale\textsuperscript{29} and the estimation of reduced glutathione enzyme.\textsuperscript{30}

### 2.9. Histopathological studies

A section of the liver tissues were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with hematoxylin–eosin. Histological observations were made under light according to the method described by Wallace.\textsuperscript{71}

### 2.10. Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis was performed by one-way Analysis of Variance (ANOVA) and individual comparisons of the group mean values were performed using post hoc Duncan multiple range test. SPSS software was used in the data analysis.

### 3. Results

#### 3.1. Acute toxicity (LD₅₀)

No lethality was observed in rats administered with single dose of subfractions Me6, Me7 and Me8 of *P. mildbraedii* extract at 2000 mg/kgbw. Animals did not show any significant changes in behavioral, physiological and physical activities. There were also no signs of late toxicity in rats when observed up to 14 days as shown in Table 1

| Samples | No of animal used | Mortality | Dose | Toxicity signs |
|---------|------------------|-----------|------|----------------|
| Me6     | 5                | 0         | 2000 mg/kgbw | No observable sign of toxicity |
| Me7     | 5                | 0         | 2000 mg/kgbw | Heavy breathing and inactive between 1 and 30 min |
| Me8     | 5                | 0         | 2000 mg/kgbw | Inactive within 1st – 30 min |

Key: Normal = control, CCl₄ = toxic and not treated, Silymarin = treated with standard drug, Me6₅₀ = treated with sub-fraction Me6 at 50 mg/kgbw, Me6₁₀₀ = treated with sub-fraction Me6 at 100 mg/kgbw, Me7₅₀ = treated with sub-fraction Me7 at 50 mg/kgbw, Me7₁₀₀ = treated with sub-fraction Me7 at 100 mg/kgbw, Me8₅₀ = treated with sub-fraction Me8 at 50 mg/kgbw, Me8₁₀₀ = treated with sub-fraction Me8 at 100 mg/kgbw. LD₅₀ > 2000 mg/kgbw if 3 animals survived. Therefore LD₅₀ of Me6, Me7 and Me8 is greater than 2000 mg/kgbw.
3.2. Marker enzymes of liver damage

The effect of Me6, Me7 and Me8 subfractions of P. mildbraedii leaf extract on the activities of marker enzymes of liver damage is presented in Table 2. Significant elevations (p < 0.05) were observed in the activities of serum ALT, AST and ALP in the group induced with CCl4 when compared with control. However, simultaneous administration of standard drug (silymarin) and different sub-fractions of leaves of P. mildbraedii with CCl4 significantly (p < 0.05) reduced the activities of these enzymes when compared with the toxic group.

3.3. Biochemical parameters in normal and CCl4 induced hepatotoxic rat

Treatment of rats with CCl4 alone significantly (p < 0.05) reduced serum total protein when compared with control (Table 3). However, group treated with CCl4 with a simultaneous administration of 50 mg/kg bw and 100 mg/kg bw of sub-fractions significantly (p < 0.05) increased the protein concentration. A significant decrease (p < 0.05) in albumin concentration was also observed in rats treated with CCl4 when compared with control. The change effected on albumin by the treatment with CCl4 was significantly (p < 0.05) reversed by the administration of 50 mg/kg and 100 mg/kg bw of sub-fractions of leaf of P. mildbraedii. The dose of 100 mg/kg bw however caused a more significant increase in the concentration of serum total protein and albumin than the 50 mg/kg bw for all the treated groups.

A significant (p < 0.05) increase in the levels of total bilirubin and direct bilirubin in the hepatotoxic group was observed when compared with control. However, all the groups treated with subfractions Me6, Me7 and Me8 significantly (p < 0.05) reduced the total bilirubin and direct bilirubin concentration when compared with group treated with CCl4. The groups treated with subfractions Me6 (1.60 ± 0.27 μmol/L) and Me8 (1.70 ± 0.32 μmol/L) at 100 mg/kg showed a better decrease in direct bilirubin concentration than the standard drug silymarin (2.10 ± 0.10 μmol/L).

3.4. Superoxide dismutase (SOD) activity

A significant reduction by 71% in SOD activity was observed in hepatotoxic group compared to control normal (Fig. 1A). An increase in SOD activity was observed in rats that received either silymarin or Me6, Me7 and Me8 subfractions of P. mildbraedii at 50 and 100 mg/kg bw compared with the hepatotoxic group. The subfractions Me6 (24.00%) and Me8 (24.00%) at 50 mg/kg bw showed the same effect as the standard drug (24.05%) while the groups treated with the subfractions at 100 mg/kg bw showed a significant (p < 0.05) increase in SOD activity of 34.67–38.02% compared to the standard drug (24.00%).

3.5. Catalase activity

All the treatment groups showed an increase in catalase activity significantly (p < 0.05) compared with the hepatotoxic group.
Fig. 1 – Effects of Me6, Me7 and Me8 subfrations of *P. milbraedii* on (A) superoxide dismutase activity, (B) catalase activity, (C) glutathione concentration, (D) glutathione peroxidase activity, (E) MDA level in normal and CCl₄ induced rats. Results are mean of five determinations ± SEM. Bar with different superscripts are significantly different (*p* < 0.05).
Subfractions Me7 and Me8 of P. mildbraedii at a dose of 100 mg/kg bw were more effective in enhancing the activity of this enzyme by 60 and 62.37% respectively in comparison with the CCl4 hepatotoxic group. The standard drug, silymarin showed only 58.82% increments in enzyme activity compared to the toxic group.

3.6. Glutathione activities

Fig. 1C showed that Me6, Me7 and Me8 subfractions of P. mildbraedii at a dose of 50 and 100 mg/kg bw were able to increase GSH concentration significantly (p < 0.05) when compared with the hepatotoxic group. The sub-fraction Me8 at both doses of 50 and 100 mg/kg body weight showed a more significant increase by 97 and 98% respectively when compared to hepatotoxic control group. This significant increase in GSH concentration by Me8 at 50 and 100 mg/kg body weight was observed to be better than that of Silymarin by 12 and 13%.

3.7. Glutathione peroxidase activity

The effect of sub-fractions Me6, Me7 and Me8 of P. mildbraedii on glutathione peroxidase activity is presented in (Fig. 1D). A significant (p < 0.05) decrease in the enzymatic activity of glutathione peroxidase in the hepatotoxic group (1.80 μmoles NADPH oxidized/mg protein) was observed when compared to the normal group (5.68 μmoles NADPH oxidized/mg protein). All the treatment groups offered some protection at 50 and 100 mg/kg bw against CCl4-induced but not treated group. However, the sub-fraction Me7 at 100 mg/kg body weight showed the highest increment in glutathione peroxidase activity compared to other treatment groups.

3.8. Lipid peroxidation

The extent of lipid peroxidation was measured by assaying thiobarbituric acid reactive substances (MDA) for all groups (Fig. 1E). A significant (p < 0.05) increase in lipid peroxidation was observed in the CCl4 hepatotoxic rats group. Treatment with sub-fractions of P. mildbraedii (50 and 100 mg/kgbw) significantly (p < 0.05) decreases the MDA level to almost normal. Specifically, the effect of sub-fraction Me6 at both doses were comparable with that of standard.

3.9. Histopathology

Histopathology profile of Control showed normal hepatic architecture. However, CCl4 induced nontreated animals exhibited severe hepatotoxicity indicated by massive infiltration (fatty liver) caused by marked hepatocellular necrosis and vascular degeneration. All sub-fractions and silymarin treated groups showed reduction in hepatic damage in line with biochemical analysis results. Treatment with the subfractions of P. mildbraedii reduced the severity of hepatic damage in a dose dependent manner (Fig. 2).

4. Discussions

Toxicity studies are useful parameters in the investigation of therapeutic index of drugs and xenobiotics. The acute toxicity test showed that oral administration of Me6, Me7 and Me8 caused no death in the test animals. Therefore, it is inferred that oral LD50 of the sub-fractions was greater than 2000 mg/kg bw. The tested sub-fractions can be categorized as having high safety margins since substances possessing LD50 higher than a minimum allowable dose of 2000 mg/kg bw are considered acutely nontoxic. According to guidelines of the Organization for Economic Cooperation and Development, substances possessing LD50 dose of 2000 mg/kg bw or higher are categorized as unclassified nontoxic.

The significant increase (p < 0.05) in the activities of liver marker such as serum ALT, AST and ALP showed hepatotoxicity in the experimental animals. Increased levels of serum ALT, AST, ALP have been reported to be sensitive indicators of liver injury. The leakage of these enzymes from the cells through peroxidative damage of the membrane into the bloodstream usually caused increase in their activities. ALT is specifically produced in the hepatocytes to catalyze the transfer of amino group from l-alanine to α-ketoglutarate to form l-glutamate and oxaloacetate thus making it relatively a better determinant of hepatic injury than AST, which is observed in other tissues. AST elevation indicate cellular leakage as well as loss of functional ability of cell membrane in liver. Alkaline phosphatase increase gives an assessment of the integrity of the plasma membrane and diagnosis of hepatobiliary effects for instance high serum ALP is associated with biliary disorder such jaundice and cirrhosis.

Sub-fractions Me6, Me7 and Me8 of the P. mildbraedii leaf extract significantly decreased the level of ALT, AST and ALP in a dose dependent manner. Sub-fraction Me6 at 100 mg/kg bw body weight reduced these enzyme activities better than all others including the standard drug, silymarin. The lowering of enzyme levels or activities is in agreement with results of a previous study in which n butanol fraction of Gongronema latifolium showed a similar effect. The present finding may represent a pioneering effect of sub-fractions of P. mildbraedii leaf extract to offer protection against the toxicity of CCl4. These protective effects may be due to the healing of hepatic parenchyma and regeneration of hepatocyte by the presence of phytochemicals such as flavonoids and phenols present in P. mildbraedii these secondary metabolites may be acting alone or synergistically in quenching free radicals generated in the toxic groups.

The low serum levels of total proteins and albumin due to CCl4 intoxification may be attributed to the initial damage of the endoplasmic reticulum resulting in the loss of Cyt Normal = control, CCl4 = toxic and not treated, Silymarin = treated with standard drug, Me6 = treated with subfraction Me6 at 50 mg/kg bw, Me6100 = treated with subfraction Me6 at 100 mg/kg bw, Me7 = treated with subfraction Me7 at 50 mg/kg bw, Me7100 = treated with subfraction Me7 at 100 mg/kg bw, Me8 = treated with subfraction Me8 at 50 mg/kg bw, Me8100 = treated with subfraction Me8 at 100 mg/kg bw.
**Fig. 2** – Liver sections of (a) control group (normal), (b) untreated group, (c) standard drug (Silymarin) group, (d) subfracton Me6 (50 mg/kg), (e) subfraction Me6 (100 mg/kg), (f) subfraction Me7 (50 mg/kg), (g) subfraction Me7 (100 mg/kg), (h) subfraction Me8 (50 mg/kg), (i) subfraction Me8 (100 mg/kg).

Liver sections of (a) control group (normal; group I) showing normal liver histoarchitecture, (b) CCl4 induced untreated group (group II) showing distorted liver histoarchitecture and severe inflammation; fatty change, congested central vein; infiltration of inflammatory cells, necrosis, vascular degeneration, (c) silymarin treated (group III) showing mild distortion of the liver histoarchitecture; moderate fatty change, macro and micro vesicles, hepatocytes, central vein, and sinusoids with few necrotic cells, (d) subfraction Me6 (50 mg/kg) treated group showing mild distortion of the liver histoarchitecture; micro vascular fatty change, infiltration of inflammatory cells, (e) subfracton Me6 (100 mg/kg) treated group showing mild distortion of the liver histoarchitecture, mild to moderate bridged inflammation, (f) subfraction Me7 (50 mg/kg) treated group showing mild distortion of the liver histoarchitecture, moderate inflammation and bridging with bile ductular reaction, infiltration of inflammatory cells, (g) subfraction Me7 (100 mg/kg) treated group showing mild distortion of the liver histoarchitecture, subtle fatty change, and mild bridging inflammation with ductular reaction, (h) subfraction Me8 (50 mg/kg) treated group showing very mild distortion of the liver histoarchitecture almost normal; subtle bile ductular reaction, (i) subfraction Me8 (100 mg/kg) treated group showing moderate fatty change, few macro vesicles, numerous micro vesicles and no inflammation.

P-450 leading to fatty livers. This decrease in serum protein levels observed in CCl4 intoxication correspond to extensive liver damage as a result of impaired protein turnover. Albumin is a useful indicator of hepatic synthetic function. Liver injury is associated with a decrease in the concentration of serum albumin, and hypoalbuminemia has also been considered to be significant in the decreased rate of hepatic synthesis of essential protein in both humans and animals. Concomitant treatment with sub-fractions of *P. mildbraedii* leaf extract remarkably increased the concentration of total protein and albumin in the serum. Values obtained in this study are higher than those obtained from a previous study. The higher dose was generally more effective and similar to the standard drug, silymarin. Me7 and Me8 sub-fractions were much better than the standard drug in terms of total protein and albumin levels respectively. Therefore, sub-fractions from *P. mildbraedii* extract possess the ability to promote protein synthesis and this plays significant role in the overall hepatoprotective effect.

The significant rise in the levels of total and direct bilirubin observed in CCl4-intoxication may be attributed to hepatic dysfunction induced. Bilirubin is the major bile pigment that is formed from the breakdown of hem in red blood cells. It is transported to the liver and secreted into the bile. Elevated bilirubin level may also be associated with reduced hepatocyte uptake of bilirubin, impaired conjugation of bilirubin and impaired hepatocyte secretion of bilirubin. The significant reduction (p < 0.05) in the level of serum total and direct bilirubin following administration of sub-fractions of leaves of *P. mildbraedii* is indicative of the hepatoprotective potential of
this vegetable. The reduction in bilirubin concentration, liver marker enzymes, elevation of protein and albumin levels in serum is in agreement with views that their levels revert to normal with the healing of hepatic parenchyma cells as well as the regeneration of hepatocytes.47,48

Oxidative stress resulting from enhanced free radical formation and/or defects in antioxidant defense cause severe tissue damage which have been implicated in a number of diseases, like liver diseases, coronary artery diseases, atherosclerosis, cancer and diabetes.49 Antioxidant enzymes SOD, CAT and GPx are the three major scavenging enzymes that represent protection against oxidative tissue-injury.50

SOD catalyzes the dismutation of superoxide anion to a hydrogen peroxide. CAT and GPx converts H2O2 to nontoxic products H2O and O2. The CCl4 induction of rats lead to reduced antioxidant capacity as evident in the decreased activity of the antioxidant enzymes. These results are consistent with earlier reports.59,61 Treatment with sub-fractions of P. mildbraedii leaf restored the antioxidant enzyme profiles and reversed the oxidative hepatic damage. The higher dose was generally more effective than the standard drug. These positive effects could be associated with the phytoconstituents in these fractions as it has earlier been reported that some phytochemicals like tannins, terpenoids, phenols steroids and flavonoids enhance antioxidant enzyme activities in vivo.51 Enhanced antioxidant activity as enumerated by these enzymes in the sub-fractions of P. mildbraedii may be attributed to their phytochemical constituents.

This study also showed an evidence of oxidative stress by the reduced level of glutathione in the CCl4 induced rats. This is in agreement with reports from hepatotoxicity studies which show that decreased levels of GSH, SOD and CAT in the liver suggests hepatic damage.52 Glutathione is a major non-enzymatic antioxidant capable of regulating intracellular redox homeostasis in all cell types.53 It scavenges free radical directly or acts as a substrate for GPx, glutathione-S-transferase (GST) during detoxification of H2O2, lipid hydroperoxides and maintains membrane protein thiol. The present results show that all sub-fractions at 100 mg/kgbw treatment restored the GSH level better than the standard drug, silymarin. Therefore, the ability of this extract to restore the GSH level will help in maintaining the body’s antioxidant defense mechanism thus protecting cell membrane integrity. The exact mechanism by which the sub-fractions enhanced GSH level is not clear but could be attributed to the presence of phytochemicals. Marked increase in MDA level, an index of lipid peroxidation observed in the liver of CCl4 induced rats is indicative of membrane damage. Treatment with subfractions Me6, Me7 and Me8 of P. mildbraedii significantly reversed these changes. Stigmasterol present in bark of Butea monosperma exhibited decrease in hepatic lipid peroxidation and increase in the activities of catalase, superoxide dismutase and glutathione thereby suggesting its antioxidant property.54 Therefore, inhibition of lipid peroxidation by P. mildbraedii sub-fractions may be attributed to their various phytochemicals.

Histopathological examination of the liver sections of control animals showed normal hepatic cells with well-preserved lobular pattern and visible central vein. Liver sections of CCl4-treated rats showed severe distortion in liver histoarchitecture with massive fatty change, necrosis, congested central vein, severe infiltration of inflammatory cells and vascular degeneration. These are signs of toxicity of CCl4 reported by other researchers.52,53 Necrosis is pathological death that occurs after abnormal stress by chemical injury or toxins. Necrotic cells are unable to maintain membrane integrity thus leak out their contents and thus eliciting inflammation in surrounding tissues.56 Inflammation is fundamentally a protective response, the ultimate goal of which to rid of the organism of both the initial cause of the cell injury (e.g., toxins) and the consequences of such injury.56

Histological architecture of liver sections of the rats treated sub-fractions of P. mildbraedii leaf extract as well as silymarin showed mild hepatotoxic change with a mild degree of fatty material, necrosis and lymphocyte infiltration comparable to the control and silymarin treated groups. Histoarchitectural preservation or protection of the hepatocytes was more effective with the 100 mg/kgbw doses. This result further validates the result of the biochemical studies showing the hepatoprotective effect of the P. mildbraedii vegetable.

The clinical impact of this study is the possession of hepatoprotective effect by P. mildbraedii leaf extract which is attributed to its ability to: reduce the rate of lipid peroxidation, enhance the antioxidant defense status and guard against the pathological changes of the liver induced by CCl4 intoxication. The strong in vivo antioxidant activity also suggests that a dietary supplement of P. mildbraedii may confer a beneficial effect against oxidative stress.

As far as we observed, the hepatoprotective of the partially purified P. mildbraedii is being reported in this research for the first time. As such, the most promising sub-fraction (Me8) as well as Me6 and Me7 that also active at 100 mg/kgbw can be purified further to obtain non-toxic, readily available and less expensive hepatoprotective compound(s) that can be used for drugs production in future. Conclusively, P. mildbraedii vegetable can be exploited as a naturally available alternative for drug supplement development in the prevention and treatment of liver damage.

Conflict of interest

The authors declare no conflict of interest.

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