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# Ethanol Leaf Extract of *Pterocarpus mildbraedii* Ameliorates Hepatotoxicity, Oxidative Stress and Dyslipidemia in Cadmium Chloride Induced Male Wistar Rats

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## ABSTRACT

**Aim**: Cadmium toxicity is mediated by free radicals, thereby, necessitating studies on the antidotal benefits of medicinal plants with anti-oxidative properties. *Pterocarpus mildbraedii* leaf extract was studied to evaluate its protection against hepatotoxicity, oxidative stress and dyslipidemia in Wistar rats intoxicated with cadmium chloride.

**Methods**: Male Wistar rats (24), weighing 140 – 180 g, used for the study were randomized into groups (1 - 4) with each group consisting of 6 animals. Group 1 was the control while 10 mg/kg bw of cadmium chloride was administered to Group 2. Ethanol leaf extract of *Pterocarpus mildbraedii* (400 mg/kg bw) was administered to animals in Group 3. Group 4 was treated with cadmium chloride before the extract for a period of 21 days. Indices of hepatotoxicity and lipid profile were determined in serum. Parameters of oxidative stress were analyzed in liver homogenate. The leaves were obtained in December 2020 while experimentation and data analysis were carried out in January/February 2021.

**Results**: Hepatotoxicity induced by cadmium chloride was evidenced as significantly increased (p□0.05) activities of marker enzymes in serum and increased serum bilirubin concentration with a significant reduction in total protein. Cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol concentrations in serum increased significantly ($p<0.05$) while HDL-cholesterol concentration decreased. Antioxidant enzyme activities also decreased following cadmium chloride administration. The adverse effects of cadmium chloride were ameliorated by *Pterocarpus mildbraedii* leaf extract.

**Conclusion**: Ethanol leaf extract of *Pterocarpus mildbraedii* attenuated cadmium chloride toxicity suggesting the need for further assessment of its pharmacological benefits.

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## INTRODUCTION

Cadmium is a soft, bluish-white toxic metallic element that is widely distributed in the biosphere.[1,2] Natural sources of cadmium include rocks, fossil fuels, volcanic eruptions and forest fires.[3,4] Industrial processes that utilize cadmium include electroplating, production of alloys, pigments, batteries and production of agricultural fertilizer.[5-7] Non occupational exposure of humans to cadmium is predominantly via the consumption of contaminated foods, drinks or cigarette smoke.[8,9] In occupational population, exposure to cadmium could occur through the inhalation of workplace dust or fumes.[7,10] Tissue accumulation of cadmium is facilitated by its inefficient excretion from the body[11] in addition to having 10 to 30-years half-life.[12]

Cadmium does not play any useful function in man. It is a cumulative toxin in the liver.[13,14] Cancer, hypertension, anemia, osteomalacia, osteoporosis, atherosclerosis and nephropathy are some of the diseases attributed to cadmium toxicity.[15] Cadmium induced systemic effects have been proposed to follow the free radical reaction principles characterized by increased lipid peroxidation and dyslipidemia.[16-18] Antioxidant vitamins and minerals have been found to modulate the harmful effects of cadmium.[19] Medicinal plants and spices with antioxidant potentials have also been evaluated as antidote against cadmium toxicity.[2,20]

KEYWORDS:
Cadmium chloride, Hepatotoxicity, Lipid Profile, Oxidative Stress, *Pterocarpus mildbraedii*

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*Pterocarpus mildbraedii* Harms is a popular plant in Eastern Nigeria. It is locally known as 'oha' in Ibo language and 'mkpa' or 'mkpafere' in Efik/Ibibio languages. The young leaves of *Pterocarpus, milbreadii* are components of some indigenous soups.[21] Chemical composition of *Pterocarpus mildbraedii* leaves have been described.[22] Phytochemicals present in the leaves include alkaloids, flavonoids and tannins.[23,34] In traditional medical practice, the leaf extract of *Pterocarpus mildbraedii* has been applied in managing various diseases and microbial infections.[24] These authors[24] also reported that *Pterocarpus milbreadii* leaves exhibit free radical scavenging activity *in vitro*. Other authors[25,26] have documented the use of *Pterocarpus mildbraedii* leaf extract in ameliorating hepatotoxicity induced by carbon tetrachloride. Furthermore, Fajobi *et al*.[27] observed that the leaves of *Pterocarpus mildbraedii* exhibit antioxidative and cardioprotective activities.

Therefore, the influence of leaf extract of *Pterocarpus mildbraedii* Harms on some serum biomarkers of oxidative stress, liver function and lipid profile in cadmium chloride induced male Wistar rats was investigated in the present study. It was expected that the results would provide additional information on the medicinal value of this plant.

## MATERIALS AND METHODS

### Assay kits

Reagent kits manufactured by Randox Laboratories Ltd, County Antrim, United Kingdom and Fortress Diagnostics Ltd, Antrim, United Kingdom were used for the assays. Sigma-Aldrich (USA) produced the cadmium chloride used in the study. Analytical grade chemicals were also utilized in the course of the study.

### Preparation of ethanol extract of *Pterocarpus Mildbraedii* leaves

Fresh *Pterocarpus mildbraedii* leaves were acquired in December, 2020 from a vegetable market at Okom, Onna, Akwa Ibom State, Nigeria. The leaves were authenticated by a plant taxonomist, Professor Margaret Bassey of University of Uyo, Uyo and a voucher specimen (UUPH 34) was registered at the institution's herbarium. The leaves were cleaned, air-dried and pulverized prior to extraction. The powdered sample (500 g) was macerated in ethanol (80%) for 72 hours, with intermittent agitation. Whatman No. 1 filter paper was used to filter the mixture to obtain a filtrate which was concentrated using a rotary evaporator. The crude extract obtained was preserved at 4°C.

### Experimental animals used in the study

Faculty of Basic Medical Sciences Animal House facility in University of Uyo, Uyo, was used to house the twenty-four(24) Wistar rats used for the study. The animals were kept in standard polypropylene cages at room temperature of 23-28°C, 50-55% humidity and 12-hour cycle of light and darkness. A 10-day period of acclimatization was allowed before commencement of studies. Rat chow manufactured by Guinea Feeds Nigeria Ltd. Nigeria, was fed to the animals and they were allowed access to clean drinking water throughout the study. International guidelines[28] as well as recommended procedures of Animal Ethics Committee, University of Uyo, Uyo, Nigeria were applied in the course of studies.

### Experimental design

The experimental animals were randomized into 4 treatment groups (1–4) containing six rats each and treated by a modification of the protocol reported in.[29] Rats in Group 1 (control) received rat chow and normal drinking water. Cadmium chloride (10 mg/kg bw) was administered to animals in Group 2. *Pterocarpus mildbraedii* leaf extract (400 mg/kg bw) was given to rats in Group 3. The rats in Group 4 received rat chow and were administered 10 mg/kg cadmium chloride before 400 mg/kg bw of *Pterocarpus mildbreadii* leaf extract, three hours later. *Pterocarpus mildbreadii* and cadmium chloride were orally administered for 21 consecutive days. The rats were then fasted overnight and anaesthetized by intra-peritoneal injection of ketamine hydrochloride (10 mg/kg) on the 22nd day.

### Methods

Blood samples (3 mL each) were obtained from the rats by cardiac puncture, dispensed into plain sample containers then kept 30 minutes for coagulation to take place. Serum was obtained after centrifuging at 3000 revolutions per minute for 10 minutes and dispensed into fresh sample containers for the determination of the indices of liver function as well as lipid profile. Liver homogenate was prepared as described by Abdel-Wahab[30] and used for assay of some indices of oxidative stress.

Assay kits supplied by Randox Laboratories Ltd, United Kingdom, were used to assay some indices of liver function such as bilirubin, protein, ALP, AST and ALT in serum. Lipid profile was also estimated using reagents from Randox laboratories Ltd. VLDL-cholesterol was calculated from the concentration of triglyceride while the concentration of LDL-cholesterol was calculated from other components of lipid profile according to Friedewald *et al*.[31] The methods described by Ohkawa *et al*. [32] and Marklund[33] were employed in assay of MDA concentration and SOD activity respectively. Catalase activity was measured as described by Aebi.[34] For histological studies, sections of the liver tissue fixed in 10% formalin were processed, stained and observed under light microscope set at 100x magnification.[35]

### Analysis of data

One-way analysis of variance (ANOVA) as well as Post hoc multiple comparison test (Least Significance Difference) were the statistical tools used in the analysis of data with the aid of SPSS statistical software version 20.0. Group means were considered statistically significant at $p < 0.05$.

## RESULTS

The parameters of liver function in the experimental animals are presented in Table 1. The activities of liver enzymes (alanine aminotransferases, aspartate aminotransferase, and alkaline phosphatase) were significantly increased ($p < 0.05$) following cadmium chloride administration.

Cadmium chloride also induced a significant ($p<0.05$) increase in serum concentration of bilirubin with decreased concentration of total protein. This table also shows that ethanol leaf extract

**Table 1:** Liver function parameters in Wistar rats treated with cadmium chloride and *Pterocarpus mildbreadii* leaf extract

| Groups* | *ALT (U/L)* | *AST (U/L)* | *ALP (U/L)* | *Total Protein (mg/dL)* | *Tota Bilirubin (mg/dL)* |
|---|---|---|---|---|---|
| 1 | 20.50 ± 1.32 | 77.83 ± 3.44 | 120.53 ± 0.86 | 5.06 ± 0.46 | 0.45 ± 0.02 |
| 2 | 40.63 ± 1.53a | 105.15 ± 3.01a | 146.63 ± 0.56a | 2.35 ± 0.62a | 0.72 ± 0.02a |
| 3 | 19.58 ± 6.59b | 62.65 ± 5.06ab | 77.90 ± 1.04ab | 4.40 ± 0.23ab | 0.41 ± 0.33ab |
| 4 | 17.57 ± 1.23b | 98.56 ± 2.71ac | 95.09 ±1.63abc | 4.23 ± 0,22ab | 0.42 ± 0.24ab |

Values are presented as Mean ± SEM. Groups*: 1 – control; 2 – 10 mg/kg bw of cadmium chloride; 3 – 400 mg/kg bw of *Pterocarpus milbraedii* leaf extract; 4 – 400 mg/kg bw of extract and 10 mg/kg bw of cadmium chloride.
Significant difference between means of groups at $p < 0.05$ are indicated with alphabetical superscripts; a = comparison with Group 1; b = comparison with Group 2; c = comparison with Group 3.

**Table 2:** Lipid profile of Wistar rats administered cadmium chloride and *Pterocarpus mildbraedii* leaf extract

| *Groups** | *Total Cholesterol (mg/dL)* | *Triglyeride (mg/dL)* | *HDL-Chol (mg/dL)* | *LDL-Chol (mg/dL)* | *VLDL-Chol (mg/dL)* |
|---|---|---|---|---|---|
| 1 | 129.50 ± 5.47 | 39.63 ± 1.68 | 17.30 ± 0.80 | 104.27 ± 5.91 | 7.93 ± 0.34 |
| 2 | 154.63 ± 7.53 | 50.68 ± 3.70 | 11.99 ± 0.56a | 132.50 ± 7.29 | 10.14 ± 0.74 |
| 3 | 122.75 ± 6.59ab | 34.85 ± 1.66ab | 14.87 ± 1.37b | 100.91 ± 6.38ab | 6.97 ± 0.33b |
| 4 | 127.88 ± 6.14c | 42.51 ± 3.81 | 18.30 ± 0.59bc | 101.07 ± 5.24 | 8.50 ± 0.76b |

Values are presented as Mean ± SEM. Groups*: 1 – control; 2 – 10 mg/kg bw of cadmium chloride; 3 – 400 mg/kg bw of *Pterocarpus milbraedii* leaf extract; 4 – 400 mg/kg bw of extract and 10 mg/kg bw of cadmium chloride.
Significant difference between means of groups at $p < 0.05$ are indicated with alphabetical superscripts; a = comparison with Group 1; b = comparison with Group 2; c = comparison with Group 3.

**Table 3:** Some oxidative stress indices in Wistar rats treated with cadmium chloride and *Pterocarpus mildbreadii* leaf extract

| *Groups** | *MDA Level (nmol/ml)* | *SOD Activity (units/mg protein)* | *Catalase Activity (units/mg protein)* | *GPx Activity (units/mg protein)* |
|---|---|---|---|---|
| 1 | 5.92 ± 0.21 | 9.85 ± 0.07 | 24.93 ± 0.89 | 72.23 ± 1.62 |
| 2 | 12.68 ± 0.29acd | 4.49 ± 0.12acd | 12.01 ± 1.25acd | 58.96 ± 6.14acd |
| 3 | 5.98 ± 0.13b | 7.12 ± 0.13b | 23.34 ± 0.95b | 70.62 ± 8.41b |
| 4 | 5.33 ± 0.33b | 9.70 ± 0.08b | 22.70 ± 1.21b | 71.77 ± 1.12b |

Values are presented as Mean ± SEM. Groups*: 1 – control; 2 – 10 mg/kg bw of cadmium chloride; 3 – 400 mg/kg bw of Pterocarpus milbraedii leaf extract; 4 – 400 mg/kg bw of extract and 10 mg/kg bw of cadmium chloride.
Significant difference between means of groups at $p < 0.05$ are indicated with alphabetical superscripts; a = comparison with Group 1; b = comparison with Group 2; c = comparison with Group 3.

of *Pterocarpus mildbraedii* reversed the changes in the indices of liver function produced by cadmium chloride.

Total cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol were observed to increase while HDL-cholesterol reduced significantly when compared to the control following administration of cadmium chloride as shown in Table 2. Ethanol leaf extract of *Pterocarpus mildbraedii* modulated the cadmium chloride induced dyslipidemia.

In Table 3, cadmium chloride instigated an increase in MDA concentration while activities of SOD, catalase and GPx decreased significantly ($p < 0.05$). *Pterocarpus mildbraedii* leaf extract was observed to ameliorate the changes induced by cadmium chloride on the biomarkers of oxidative stress.

Histological examination of sections of the liver tissue (Figure 1) revealed normal hepatic architecture in the control animals (L1), extract treated animals (L3) and animals that received a combination of the extract and cadmium chloride (L4). Hepatic necrosis was observed in animals treated with cadmium chloride alone (L2).

## DISCUSSION

Cadmium is an established hepatotoxic agent [16,36]. The hepatotoxic effect of cadmium chloride is demonstrable by the observed deleterious changes in biomarker enzymes, total proteins, bilirubin as well as histopathology.[37]

The aminotransferase enzymes (ALT and AST) occur abundantly in hepatic tissues. ALT and AST are present in cytosolic compartment but mitochondria of the hepatocytes also contain AST. These enzymes usually leak into circulation when the cells are damaged. Hence, increased activities of these enzymes beyond the normal range in serum, serve as a marker of hepatic injury.[38] Thus, the higher activities of ALT and AST seen in the cadmium chloride intoxicated animals could be attributed to cadmium induced membrane damage resulting in the leakage of these enzymes into extra hepatic circulation. [39]. Serum alkaline phosphatase activity is also associated with the status and function of the hepatocytes.[40] Increased serum activity of ALP is usually associated with cholestasis.[41] The obstruction to bile flow (cholestasis) has been observed to enhance the synthesis and release of ALP.[42]. Cadmium induced hepatotoxicity resulting in increased activities of AST, ALT and

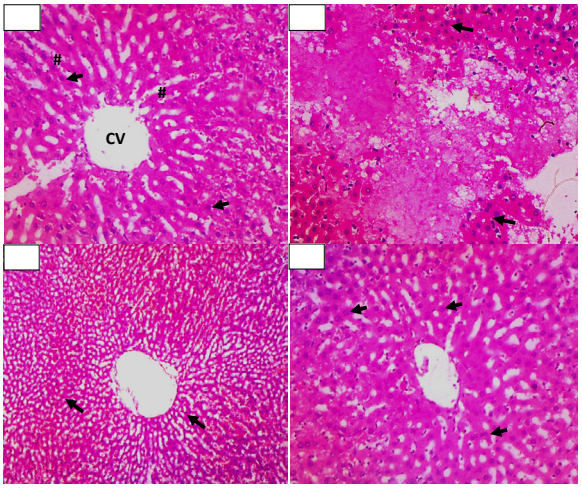


**Fig. 1:** Photomicrograph of liver sections (x100). L1: control rat with normal array of hepatocytes (arrow), sinusoid (#) and average sized central vein (CV). L2: cadmium chloride exposed rat with haemorrhagic necrosis (N). L3: extract treated animal with normal array of hepatocytes (arrow) and average sized central vein (CV). L4: animal exposed to the extract and cadmium chloride with no pathological lesion.

ALP has been reported by other authors.[43,44] The reversal of the activities of these enzymes by ethanol leaf extract of *Pterocarpus mildbraedii* would imply that this extract has protective activity against cadmium induced hepatotoxicity by conferring stability on the membranes of cells thereby preventing passage of the marker enzymes into circulation from the liver.[45]. The ability of the extract of this vegetable to protect against liver injury has been reported by other authors.[25,26]

Serum protein concentration is an important marker of liver's anabolic capacity.[42] The decreased concentration in serum protein observed, is therefore, an indication of compromised synthetic capacity of the hepatocytes which is in tandem with earlier reports.[1,46] The restoration of serum protein concentration to normal levels by the ethanol leaf extract of *Pterocarpus mildbraedii* could be attributed to the regeneration of the hepatocytes and stimulation of protein synthesis as postulated.[47]

Bilirubin is a breakdown product of heme in the reticuloendothelial system.[48] Conjugation and excretion of bilirubin through bile was reported as one of the major functions performed by the liver.[49] Consequently, the determination of serum concentration of bilirubin is a vital test of the excretory function of the liver.[50,51] Under normal conditions, little quantity of bilirubin is present in blood. However, when the liver is damaged, the serum concentration of bilirubin increases remarkably.[52] The ability of *Pterocarpus mildbraedii* leaf extract to mitigate the cadmium chloride induced derangement in the excretion of bilirubin by the liver is a demonstration of its hepatoprotective potential.

There are indications that increased production of lipid peroxides and elevated tissue concentrations of MDA are characteristics of cadmium induced liver damage.[53,54] Disruption of fluidity, permeability and electrical potential of biological membranes may be instigated by lipid peroxides resulting in the movement of specific enzymes from the hepatocytes to general circulation.[55] In this study, animals exposed to cadmium chloride exhibited higher concentrations of MDA in liver tissues, which could be due to increased peroxidation of lipids. This finding corroborates with previous reports on cadmium induced oxidative stress. [29,53,54,56] The reported antioxidant activity of leaf extract of *Pterocarpus mildbraedii*[24] may be responsible for the decreased malondialdehyde concentration following treatment with the extract.

In order to restrain the deleterious effects of free radicals, living organisms had evolved the antioxidant defense mechanism which is composed of enzymatic and none enzymatic molecules.[57] The enzymatic antioxidant pathway consists majorly of GPx, SOD and CAT enzymes.[58-60] This study demonstrated a significant decrease in the activities of these enzymes upon treatment with cadmium chloride. It has been reported that decreased antioxidant enzyme activities is a characteristic feature of oxidative stress.[60] Interestingly, reversal towards normal activity of these enzymes was observed following administration of the extract of *Pterocarpus mildbraedii* leaves. This outcome may derive from the phytochemical constituents of the *Pterocarpus mildbraedii* leaf extract, some of which have been observed to act as inducers of antioxidant enzyme activity.[23,24,61]

Serum lipid profile is used in the assessment of metabolic defects such as dyslipidemia and cardiovascular disease.[62] Cholesterol, triglyceride, LDL and HDL cholesterol concentrations are the frequently used indices of serum lipid profile.[63] In this study, total cholesterol, triglycerides, LDL and VLDL-cholesterol concentrations were increased while HDL-cholesterol concentration decreased significantly when cadmium chloride was administered. This is in agreement with earlier reports.[64-66]

Cholesterol is present in all mammalian cells especially the neurons.[67] It is an important constituent of the lipid bilayer of cellular membranes and is also used in synthesizing steroid hormones, bile salts and vitamin D.[68] High serum concentration of cholesterol is associated with cardiovascular diseases.[69] Increased expression of 3-hydroxy-3-methylglutaryl-CoA reductase was reported as a possible mechanism of cadmium induced hypercholesterolemia.[70] Hypercholesterolemia occasioned by hepatic dysfunction (as observed in this study), has also been documented[71] and is in line with the central role of the liver in cholesterol metabolism.[72]

Triglycerides are esters of glycerol and three fatty acids.[73] When calories are ingested above body requirements, the liver synthesizes triglycerides from excess acetyl-CoA and preserves them as fats.[74] Elevated serum concentration of triglycerides predisposes to cardiovascular disease.[66] Cadmium induced triglyceridemia was earlier attributed to a repression of the activity of lipoprotein lipase which is present in the blood vessels and degrades circulating triglycerides to fatty acids and glycerol before the uptake of the fatty acid component by adipocytes and skeletal muscle.[75]

LDL-cholesterol is a major transporter of cholesterol from the liver to extrahepatic tissues.[76] When the serum concentration of LDL-cholesterol is high, there is deposition of cholesterol and cholesteryl esters in the walls of blood vessels.[67] Hence, elevated serum concentration of LDL cholesterol, as observed

in the present study, would predispose to atherosclerosis and cardiovascular disease.[68]

HDL-cholesterol is often classified as 'good cholesterol' because of its involvement in reverse cholesterol transfer, a process that extracts excess cholesterol deposited in the vascular endothelium and delivers back to the hepatocytes for catabolism and excretion.[77] It has been observed that cadmium induced hepatic dysfunction could reduce the serum HDL-cholesterol content and adversely affect biological activity.[78]

Hypolipidemic properties of plant extracts are ascribed to their phytochemical constituents.[79] The bioactive compounds identified in the leaves of *Pterocarpus mildbraedii* include alkaloids, saponins, flavonoids and tannins.[23,24] Hypolipidemia triggered by alkaloids is facilitated by up regulation of the activities of lipolytic enzymes as well as the stimulation of fecal bile excretion.[79] Flavonoids are a major component of dietary polyphenols [80]. They decrease plasma lipid concentrations by up regulating LDL receptor expression and inhibiting hepatic lipid synthesis/lipoprotein secretion.[81]

Histological observation on sections of the liver confirmed the deleterious effects of cadmium and the attenuating potential of ethanol leaf extract of *Pterocarpus mildbraedii*. Oyinloye *et al*. [82] had also reported on the amelioration of cadmium induced hepatic necrosis by *Monodora myristica* leaf extract.

This study has presented additional evidence on cadmium toxicity in albino rats. Beneficial pharmacological properties of the leaf extract of *Pterocarpus mildbraedii* have also been highlighted. Hence, there is need for further pharmacological assessment of these leaves.

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