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Antioxidant capacity of *Moringa oleifera* seed oil against CCl_4-induced hepatocellular lipid peroxidation in wistar albino rats

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ABSTRACT

Hepatocytes are reportedly susceptible to the injurious effects of oxidants when exposed to toxic substances such as Carbon-tetrachloride (CCl_4). The widespread claims of the medicinal efficacy of various parts of Moringa oleifera plant have been well documented in literature. So far, less attention has been focused particularly on the seed oil extract of the plant; hence this study was aimed at investigating the effects of Moringa oleifera seed oil as a possible therapy in treatment of chemical-induced hepatic disorders. Twenty-four albino rats (male, Wistar) were divided into 4 groups of six. Group1 served as Controls and group2 received CCl_4 only. Group3 animals were pre-administered daily with Moringa seed oil (2ml/Kg) for 10 days before receiving CCl_4 (1ml/Kg) once; group4 animals received Moringa seed oil only for 10 days. Thereafter, liver tissues were excised for biochemical assay of tissue homogenates. Markedly raised MDA ($p<0.01$) was observed in CCl_4-intoxicated animals (group2) compared to those pre-treated with the Moringa seed oil (group3). The group4 animals also showed a slightly but significantly ($p<0.01$) lower MDA (malondialdehyde) levels than the Controls. Furthermore, CCl_4-induced alterations in activities of antioxidant enzymes Catalase and SOD (superoxide dismutase) were significantly ($p<0.01$) prevented in the group3 animals. These results are a clear indication that Moringa oleifera seed oil possesses strong antioxidant capacity to protect against CCl_4-induced hepatic oxidative damage.

Keywords: CCl_4, Hepatic disorders, *Moringa oleifera* plant, Seed oil, Antioxidants

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INTRODUCTION

Carbon tetrachloride (CCl_4) is one of the most potent hepatotoxins, and is widely used in scientific research to evaluate hepatoprotective agents [1].

CCl_4 is metabolized by cytochrome P450 systems in hepatocyte endoplasmic reticulum and mitochondria with the formation of CCl_3^- (trichloromethyl free radical), which subsequently interacts with molecular oxygen to form CCl_3OO^- (trichloromethyl peroxy radical). Interactions of these reactive oxygen species lead to the development of pathological situations often characterized by compromised membrane integrity and thus malfunctioned cellular transport system [2]. Lipid peroxidation leads to a cascade of reactions, thereby not only destroys membrane lipids but also generates endogenous toxicants that can readily react with adjacent molecules like membrane proteins or diffuse to more distant molecules like DNA, which may lead to more hepatic complications and functional anomalies [3].

For instance, the end-product malondialdehyde (MDA) reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts to them, primarily M_1G (pyrimidol[1,2-a] purin-10(3H)-one) [4].

In an attempt to protect the cell membrane, living organisms have different mechanisms that speed up termination of lipid peroxidation by scavenging free radicals. One important such is the antioxidant defence system made up of endogenous antioxidants, e.g. superoxide dismutase (SOD), catalase (CAT) and various peroxidases which basically constitute the first line of protection against attack by free radicals; and exogenous antioxidants derived mainly from the diet. However, under conditions, which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions [5].

Recent reports suggest that cruciferous vegetables act as a good source of natural antioxidants due to the high levels of carotenoids, tocopherols and ascorbic acid and strong epidemiological evidence shows that these compounds may help to protect the human body against damage by reactive oxygen damage by reactive oxygen species [6].

Animal studies have shown that dietary phytochemical antioxidants are capable of removing free radicals [7]. There exist various sources of dietary antioxidants, one of which is *Moringa oleifera*, a plant which has most recently attracted much interest due to its vast medicinal potentials. *Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics [8]. It is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil [9].

Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine [10]. In view of this, the present study focuses mainly the potential of *Moringa oleifera* seed oil to act as an exogenous source of antioxidants to protect against CCl_4-induced hepatocellular lipid peroxidation in Wistar albino rats.

MATERIALS AND METHODS

2.1 Experimental Animals

Twenty four (24) male Wistar albino rats (weights ranging from 150-180g) were obtained from the Department of Animal science, University of Ibadan, Nigeria. In full compliance with the internationally recognized Directive [11] on the protection of animals used for scientific purposes, the experimental animals were acclimatized for 2 weeks at the animal house of the Department of Biochemistry, University of Benin, Benin City, Nigeria.

Thereafter, the animals were assigned into four (4) groups of six (6) animals each and maintained under standard conditions with unrestricted access to standard diet (grower's mash) and distilled water.

2.2 Moringa Seed Oil Extract

The extract was purchased from Millennium Quality Oil factory in Gombe, Gombe state, Nigeria.

2.3 Dosage Administration

	Group	Dosage
1.	Control	-
2.	CCl_4 Only	1g CCl_4/kg body weight, once (on the 10th day)
3.	CCl_4 + Oil	2ml of Moringa oil/Kg body weight for 10 days and intoxicated with CCl_4 (1ml/Kg), once (on the 10th day)
4.	Oil Only	2ml of Moringa oil/Kg body weight for 10 days

The route of administration was by oral (*per orem*). Twenty-four hours after the last day of treatments, the experimental animals were sacrificed and liver tissues removed for homogenization and centrifugation. The clear supernatant obtained was thereafter subjected to biochemical analysis.

2.4 Malondialdehyde Assay

Liver tissues were homogenized using 5mls of 0.09% normal saline in a mortar and were assayed by the method of Beuge and Aust [12].

In practice, TBARS are expressed in terms of Malondialdehyde equivalents. MDA forms a 1:2 adduct with thiobarbituric acid (TBA) and produces a complex which can be measured spectrophotometrically. This complex absorbs maximally at 532nm.

2.4.1 Assay Protocol

A stock solution of trichloroacetic acid (TCA), thiobarbituric acid (TBA) and Hydrochloric acid (HCl) containing 15g TCA, 0.375g TBA and 0.25N HCl was prepared. The solution was heated mildly to assist the dissolution of TBA. To 1ml of the tissue sample, 2ml of TCA-TBA-HCl mixture was added and heated in a boiling water bath for 15 minutes. It was cooled and then centrifuged at 3600rpm for 10 minutes. The absorbance of the supernatant was read at 532nm against a reagent blank which contained 3ml of the TCA-TBA-HCl solution.

The malondialdehyde concentration was calculated as follows:

$$\text{TBARS} = \frac{\text{Absorbance} \times V \times 100}{A \times v \times Y}$$

Where: Absorbance is read at 532nm; V= total volume of the reaction mixture
A= molar extinction coefficient which is 1.5×10^5
Y= Weight of tissue; v= volume of sample used.

2.5 CATALASE ACTIVITY ASSAY

The tissues were homogenized using 0.09% normal saline solution in a mortar. The homogenate was then centrifuged at 3600rpm for 10 minutes and the supernatant used for assay according to the method of Cohen et al. [13].

Catalase prevents the accumulation of H_2O_2 by converting it to H_2O and O_2 according to the reaction shown below:

$$H_2O_2 \longrightarrow 2H_2O + O_2$$

2.5.1 ASSAY PROTOCOL

0.5ml aliquot of extract homogenate was added to ice cold tubes. Reaction was started by adding 5ml of 30mM H_20_2. Tube contents were then mixed thoroughly by inversion. Reaction was stopped after 3 minutes with 1ml of 6M H2S04. 7ml of 0.01M potassium permanganate ($KMnO_4$) was then added and absorbance read at 480nm within 30-60 seconds.

$$\text{Catalase Enzyme activity} = \frac{\text{Absorbance/min} \times V \times 1000}{M \times v \times Y}$$

Absorbance is read at 480nm
V= total volume of the reaction mixture; A= molar extinction coefficient which is 40
Y= Weight of tissue; v= volume of sample used

2.6 Superoxide Dismutase Activity Assay

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. The assay of SOD in the liver was determined by the method of Misra and Fredovich [14].

An aliquot, 0.4mls of the diluted supernatant of the tissue homogenate was added to 5mls of 0.05M carbon buffer, pH 10.2 to equilibrate in the spectrophotometer and the reaction started by the addition of 0.6mls of freshly prepared 0.3mM epinephrine as the substrate to the buffer supernatant mixture, which was quickly mixed by inversion.

The reference cuvette contains 5mls of the buffer, 0.6mls of the adrenaline and 0.4mls of distilled water. The increase in absorbance at 480nm due to the adrenochrome formed was monitored every 30seconds for 120seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of epinephrine to adrenochrome during 60seconds.

CALCULATION: $\%\ \text{inhibition} = \frac{\text{Absorbance test} - \text{Absorbance reference}}{\text{Absorbance test}} \times 100$

$$\text{Units of activity/mg protein} = \frac{\%\ \text{inhibition}}{50 \times Y}$$

Absorbance test= Absorbance of sample

Absorbance reference= absorbance of blank; Y= weight of protein in the volume of sample used.

2.7 STATISTICAL ANALYSIS OF DATA

Using SPSS version 17 for Windows, Analysis of Variance (ANOVA) method was employed to compare the mean differences observed among the various groups. The results are therefore presented as mean ± SD (standard deviation) with the level of significance set at $p< 0.05$.

RESULTS AND DISCUSSION

The concentration of malondialdehyde in the liver was taken as an index of hepatic lipid peroxidation induced by toxicant CCl_4, while levels of activity of both superoxide dismutase and catalase were determined as indices of hepatic antioxidant status. The results are presented in Table 1.

CCl_4 is a well-known model compound for producing chemical hepatic injury [15]. This study investigated the protective role of *Moringa oleifera* seed oil against CCl_4-induced hepatocellular oxidative damage. It was found that CCl_4 intoxication caused a markedly elevated hepatic MDA level (Table 1) which is an indication of increased oxidative stress; this finding correlates with previous reports [16]. Previously, *Moringa oleifera* seed oil had been suggested to be capable of reversing or inhibiting lipid peroxidation in liver cells [17]. Increased production of intracellular ROS plays a major role in CCl_4-induced hepato-cellular damage [16]. In the present study, animals pre-administered with the oil had significantly lower MDA compared with those treated only with the toxicant CCl_4, an indication of the attenuating effect of *Moringa oleifera* seed oil on CCl_4-induced liver injury. Moringa seed oil has been reportedly shown to contain a surplus of tocopherols for quenching free radicals and performing its other antioxidant functions in the human body [18]. Tocopherols are natural lipid soluble antioxidants and potent free radical scavengers present in the oil [19].

Animals pre-treated with the oil also showed significantly higher and relatively normal SOD and Catalase activities as against those treated with only CCl_4. SOD and Catalase are antioxidant enzymes considered to play crucial roles in cellular defence against chemical- or xenobiotic-induced oxidative damage. Thus, Moringa oil acting as a dietary (exogenous) antioxidant source, appeared to complement the antioxidant enzymes in maintaining the hepatocellular integrity.

Table 1. Liver MDA Concentrations, Catalase and SOD Activities among the Different Treatment Groups

Group	MDA (mmole/g) x 10^{-2}	Catalase (unit/ml) x 10^{-2}	SOD (unit/mg) x10^{-1}
1 (Control)	4.407 ± 0.458^{a}	89.09 ± 0.09971^{a}	100.8 ± 18.08^{a}
2 (CCl4)	$5.793\pm0.182^{b,d}$	$89.40\pm0.04917^{b,c}$	$60.60\pm15.75^{a,b}$
3 (CCl4+Oil)	5.121 ± 0.337^{a}	89.38 ± 0.07940^{a}	108.9 ± 15.70^{a}
4 (Oil Only)	$3.875\pm0.430^{a,c}$	$89.05\pm0.06515^{a,d}$	$127.3\pm7.734^{a,c}$

n=6 for each group; b and c values are significantly different from 'a' (control) values at p<0.01

CONCLUSION

The findings from this study strongly suggest that *Moringa oleifera* seed oil possesses strong hepatoprotective effect through prevention of CCl_4-induced hepatocellular membrane damage. This potential of the Moringa seed oil extract is therefore believed to be largely attributable to its natural antioxidant constituents.

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