

Original Research Article

ANTIOXIDANT PROPERTIES OF *MORINGA OLEIFERA* OIL AND *ANACARDIUM OCCIDENTALE* OIL ON CADMIUM INDUCED LIVER DAMAGE IN ADULT WISTAR RATS.

Abstract

Background: Cadmium is one of the most toxic heavy metals found in the environment and exposure of the liver to cadmium has been shown to cause hepatic dysfunction in addition to the oxidative damage it causes to cells. This study was aimed at investigating the combined effects of *Moringa oleifera* oil and Cashew nut oil on cadmium-induced liver damage in wistar rats.

Methods: Thirty-five (~~35~~ 35) wistar rats (80-180 ~~grams~~ g) of both sexes were used for the study. The rats were randomly selected and divided into seven (7) groups of five rats each. Group A received 5 ml of 0.9% w/v phosphate buffer; Group B received 3.5 mg/kg bw 3CdSO₄.8H₂O; Group C received 100 mg/kg Vit C and 300 mg/kg Vit E; Group D received 100mg/kg Vit C and 300 mg/kg Vit E and 3.5 mg/kg bw Cd; Group E received 3.5 mg/kg bw Cd and 40 mg/kg *Moringa oleifera* seed oil; Group F received 3.5 mg/kg bw Cd and 40 mg/kg Cashew nut oil and Group G received 3.5 mg/kg bw Cd *Moringa oleifera* seed oil and Cashew nut oil 20 mg/kg each.

Results: Histochemical studies carried out showed that Cadmium significantly increased the activities of the antioxidant defense system enzymes such as MDA, GPx and Catalase and reduced the activities of SOD when compared with the Group A control rats which thus suggests the generation of free radicals and the treated groups also– significantly

increased the activities of the antioxidant ~~defence~~defense system enzymes such as MDA, GPx and Catalase when compared with the control group. This suggests that both *Moringa oleifera* oil and Cashew nut oil extracts helped to restore the activities of the antioxidant ~~defence~~defense system.

Conclusion: These show ameliorative effect of *Moringa oleifera* seed oil and Cashew nut oil on Cadmium induced liver damage.

Key words: Histochemical, Antioxidants, *Moringa oleifera*, *Anacardium occidentale*, Oxidative stress, Liver, Cadmium.

Introduction

Cadmium is one of the most toxic metal ions of our environment bound in the air, food and water [17]. It is found in foods (such as vegetables, grains and cereals) and also produced as a byproduct of zinc and lead mining and smelting [28]. Cadmium metal is used mainly as an anticorrosive, electroplated onto steel. Cadmium sulfide and selenide are commonly used as pigments in plastics. Cadmium compounds are used in electric batteries, electronic components and nuclear reactors [8][15]. It is absorbed by most tissues of the body and becomes more concentrated in the liver and kidney. It is slowly excreted from the body and has a biological life span of 17-30 years [14]. It has been associated with cancers of the lung, prostate, pancreas, and kidney [16]. *Moringa oleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [11]. It is a small, fast-growing, evergreen, or deciduous tree that usually grows up to 10 or 12 m in height. It is distributed among Sub Himalayan Tracts, Assam, Bengal and Peninsular India [11]. It is a perennial softwood tree with timber of low quality, but which for

centuries has been advocated for traditional, medicinal and industrial uses [9]. Leaves are longitudinally cracked leaves, 30-75 cm long main axis and its branch jointed, glandular at joints, leaflets are glabrous and entire [11][19]. The leaflets are finely hairy, green and almost hairless on the upper surface, paler and hairless beneath, with red-tinged mid-veins, with entire (not toothed) margins, and are rounded or blunt-pointed at the apex and short-pointed at the base [11][19]. The twigs are finely hairy and green. Flowers are white, scented in large axillary down panicles, pods are pendulous, ribbed, seeds are 3-angled [11][19]. Moringa seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil that resists rancidity. It has been used in salads, for fine machine lubrication, and in the manufacture of perfume and hair care products [30]. *Anacardium Occidentale* is a native of Brazil and the Lower Amazons. The cashew has been introduced and is a valuable cash crop in the Americas, the West Indies, Madagascar, India and Malaysia [7]. *Anacardium occidentale* L. (Family cultivation) grows well in the tropical Anacardiaceae), is a multipurpose tree of the tropics which attains a height of about 15m. They grow on relatively dry soil in nature but in cultivation grow in tropical rain forest. Tyman and Morris [31] described the composition of Cashew Nut Shell Liquid (CNSL). The economic importance of this special tree is such that while the tree is native to Central and South America, it is now widely distributed throughout the tropics, particularly in many parts of Africa and Asia. The Cashew tree will tolerate a wide range of condition including drought and poor soil, but cannot withstand cold frost. The major producing countries of Cashew are Tanzania, India, Mozambique, Srilanka, Kenya, Madagascar, Thailand, Malaysia, Indonesia, Nigeria, Senegal, Malawi and Angola. World Bank data estimates that 97% of production is from wild trees and only 3% is from established plantations [26]. Gibbon *et al.*, [10] reported that many trees are found growing wild and that the plant germinates poorly, those that are

68 cultivated are propagated by seed which are planted at a rate of 2-3 per hole due to poor
69 germination rates. The commercial that phytochemicals entails in the antifungal activity
70 importance of cashew is due to its richness in nutrient of CNSL; that constitutes of 47% fat, 21%
71 protein and 22% carbohydrate, vitamins and all essential amino_acids especially thiamine
72 (Ohler,1979) [24]. Cashew nut is a high value edible nut. It yields two “Oils” one of these found,
73 between the seed coat (or pericarp) and the nuts, is called the Cashew Nut Shell Liquid (CNSL).
74 It is not a triglyceride and contains a high proportion of phenolic compound. It is used in industry
75 as a raw material for brake lining compounds, as a water proofing agent, a preservative and in
76 the manufacturing of paints and plastics. It is toxic and corrosive to the skin. Cashew apples are
77 sometimes made locally into fruit drinks, wines and pickles. In some countries they are also
78 Osmo-Sol dried to produce a date- like caramel. An edible oil can be extracted from cashew nuts
79 but no evidence of it being carried out commercially has been found. The ability of cashew
80 apples to supply and fortify the nutritional requirement for vitamin C, particularly in Africa was
81 reported by Akinwale [2]. Cashew apple juice has been found to contain the highest amount of
82 vitamin C (203.5mg/100ml) of edible portion and when the cashew apple was blended with other
83 tropical fruits it boosted their nutritional quality. The importance of the Cashew Nut Kernel Oil
84 and Cashew Nut Shell Liquid (CNSL) cannot be overemphasized. The fat of nut is completely
85 natural and unprocessed which is best for the body. It is especially rich in Linoleic acid (Omega-
86 3) and is least damaging to heart and arteries. It constitutes about 47% of the total weight of the
87 nut. Nuts often produce oil half their weight. Cashew has what is called the ‘good fat’. Cashew
88 has the right combination of fat and the ratio of saturated to monounsaturated and
89 polyunsaturated is 1:2:1 which is ideal for human consumption. The relative abundance of

monounsaturated fatty acids in cashew nut is conducive to the promotion of good health and that the relative abundance of fat in cashew nut in no way poses a nutritional risk [1].

It has been reported that continuous exposure and intoxication of liver to different types of exogenous compounds on a daily basis may lead to hepatic dysfunction [21]

Materials and Method

Extract preparation;

2330g of Moringa seed was purchased from Mararaba market in Nasarawa State. The oil was extracted using the following procedures. The husks were removed from the seeds and they were ~~were~~ heated inside an oven at a temperature of 40 °C and were pounded using a mortar and pestle in order to separate the chaff from the seeds. The seed extract was then ~~grinded-ground~~ into powder using a grinding mill. The powdered extract of *Moringa oleifera* was dissolved in 466 ml of water at ambient temperature for two days and was later filtered. The aqueous extract was then poured into molten mesh and was placed on an oil extractor machine. The seed oil was removed at high temperature and pressure.

2000_g of Cashew nut was purchased from Kuchikau in Nasarawa state. The oil was extracted using the following procedure: The nuts were heated inside an oven at a temperature of 40 °C and were ~~grinded-ground~~ into powder using a grinding mill. The extract was poured into a molten mesh and placed in an oil extractor machine. The oil was removed at high temperature and pressure and both oil extract were kept at room temperature.

Drug Preparation

~~The~~ Cadmium ~~s~~Sulphate Solution ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) was prepared by dissolving 9.9198_mg of Cadmium ~~s~~Sulphate salt (CdS) in 5_ml of 0.9% w/v Phosphate buffer. The Ascorbic acid

(Vitamin C) was prepared by dissolving 5_{mg} of Vitamin C in 10_{ml} of 0.9% w/v Phosphate buffer. The Vitamin E (Alpha Tocopherol) was prepared by dissolving 6_{mg} of Vitamin E in 20 ml of olive oil.

Experimental animals:

Thirty five (35) Wistar rats weighting between 80g-180g were used for this research work. The rats were randomly selected into seven (7) groups as follow A, B, C, and D, E, F and G each group containing five rats. They were kept in the animal house of Bingham University, Nigeria and given feed and water *ad libitum*—*libitum*. The treatment for the various groups was administered accordingly.

Chemical and Extract Administration;

0.32_{ml} (40_{mg}/kg body weight) of *Moringa Oleifera* and Cashew nut seed oil, 0.1_{6ml} (20 mg/kg body weight) each of *Moringa oleifera* seed oil and Cashew nut oil, 0.8ml (100_{mg}/kg body weight) of Vitamin C, 0.3_{ml} of Vitamin E (300_{mg}/kg body weight) and 5_{ml} of 0.9% w/v of Phosphate buffer were administered orally to the experimental rats according to their individual groups for a period of four (4) weeks using a 2_{ml} syringe with an oral cannular at the tip. The administration was done by holding the rats with a glove using the left hand in such a way that the neck region was being held so that the rats would be stable while the extracts were being administered orally. The treatment was done every morning after which the animals were fed.

Animal Treatment

The animals were treated as shown below in the table

Table 1: Number of Animals in each group and Dosage of treatment given:

Groups	Number of Animals	Dosage
A	5	5 ml of 0.9 % w/v Phosphate buffer per kg bw
B	5	3.5 mg/kg bw Cadmium only
C	5	100 mg/kg Vitamin C (0.8 ml) and 300 mg/kg Vitamin E (0.3 ml)
D	5	100 mg/kg Vitamin C (0.8 ml) and 300 mg/kg (0.3 ml) Vitamin E + 3.5 mg/kg bw Cd
E	5	3.5 mg/kg bw Cadmium+ 40 mg/kg <i>Moringa oleifera</i> seed oil (0.32 ml)
F	5	3.5 mg/kg bw Cadmium+ 40 mg/kg Cashew nut oil (0.32ml)
G	5	3.5 mg/kg bw Cadmium+ <i>Moringa oleifera</i> seed oil + Cashew nut oil 20 mg/kg each (0.16 ml each).

Control Groups

The animals were grouped into three control groups which are; The normal control (A), The negative control (B) and the positive control (C). The Normal Control Group (A) received 5 ml of 0.9 % w/v Phosphate buffer orally for a period of four (4) weeks. The Negative Control Group (B) was induced intraperitoneally with 3.5 mg/kg $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. The Positive Control Group (C) received 100 mg/kg (0.8ml) Vitamin C and 300 mg/kg (0.3ml) Vitamin E orally for a period of four (4) weeks.

Prophylactic Treatment Group

The animals in Group D were the prophylactic group that received 100mg/kg (0.8 ml) Vitamin C and 300 mg/kg (0.3ml) Vitamin E for a period of four (4) weeks followed by intraperitoneal injection of 3.5 mg/kg body weight $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. The rats were then sacrificed after 24 hours.

Therapeutic Treatment Groups

The animals in Groups E, F and G were the therapeutic control group. The animals in each group were injected intraperitoneally with 3.5 mg/kg $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. After 48 hours of the injection, the animals in Group E and F received 40 mg/kg body weight of *Moringa Oleifera* Seed Oil and Cashew Nut Oil for a period of four (4) weeks. The animals in Group G received 20 mg/kg each (0.16 ml each) of both *Moringa Oleifera* Seed oil and Cashew nut oil.

Animal Sacrifice

The animals were sacrificed twenty four (24) hours through cervical dislocation after which food and water had already being withdrawn from them. The animals were laid in a supine position on the dissecting board, the two hind limbs were held and the tail was pulled until a sound was held heard which indicated that the animal was dead. The thoracic and abdominal cavities were exposed adequately by using a surgical blade to make a midline incision through the skin of the abdominal wall from the xiphisternum to the pubic symphysis. After the abdominal cavity had been adequately exposed, the liver was removed and immersed in sucrose for biochemical analysis and homogenized thereafter. They were then processed for histochemical analysis.

RESULT

HISTOCHEMICAL ANALYSIS FOR ENZYME ASSAY RESULTS (ANTIOXIDANT DEFENSE SYSTEM ENZYMES)

Table 2: The Mean $\pm$ Standard Error of Mean (SEM) of antioxidant enzymes for Oxidative Stress Markers in the Liver at P<0.05. Significant Level at 95% Confidence Interval.

GROUP	MDA(10^{-8} units/mg) Mean $\pm$ SEM	GPx (μ moles/mg) Mean $\pm$ SEM	SOD (units/mg) Mean $\pm$ SEM	CAT (μ moles/mg) Mean $\pm$ SEM
A	1.8 $\pm$ 0.22	0.41 $\pm$ 0.01	2.8 $\pm$ 0.20	1.36 $\pm$ 0.03
B	4.13 $\pm$ 0.37*	0.74 $\pm$ 0.07*	2.4 $\pm$ 0.40	2.25 $\pm$ 0.20*
C	4.16 $\pm$ 0.49*	0.69 $\pm$ 0.03*	1.2 $\pm$ 0.20*	1.89 $\pm$ 0.11*
D	3.63 $\pm$ 0.13*	0.69 $\pm$ 0.03*	1.2 $\pm$ 0.49*	2.16 $\pm$ 0.09*
E	3.25 $\pm$ 0.06*	0.47 $\pm$ 0.01*	0.6 $\pm$ 0.24*	1.30 $\pm$ 0.02*
F	4.32 $\pm$ 0.56*	0.57 $\pm$ 0.07	1.2 $\pm$ 0.49*	1.65 $\pm$ 0.22
G	8.05 $\pm$ 1.75*	0.34 $\pm$ 0.04	1.6 $\pm$ 0.24*	1.01 $\pm$ 0.11*

* indicates statistical significance when compared with the normal control values

Discussion

Malondialdehyde (MDA) Activity in the liver tissue

Lipid peroxidation is one of the main manifestations of oxidative damage, which plays an important role in the toxicity of many xenobiotics (Stohs and Bagchi, 1995, Anane and Creppy, 2001)[3][27]. The prevention of lipid peroxidation is essential for all aerobic organisms and so the organism is well equipped with antioxidants that directly or indirectly protect cells against

the adverse effects of xenobiotics, carcinogens and toxic radicals (Halliwell and Gutteridge, 1999)[13].

The Groups C, D, E, F and G showed a statistically significant increased activity in the liver tissue when compared with Group A at $P \leq 0.05$ Significant levels. This means that the combined antioxidant properties of the drugs and both oil extracts of *Moringa oleifera* seed oil and Cashew nut oil were able to mop up the free oxygen radicals which were generated by Cadmium. This thus confirms the reports made by Ognjanovic *et. al.*, [23] that antioxidants have shown protection against cadmium induced toxicity in different animal models where their supplementation reduced Reactive Oxygen Species (ROS) levels and lipid peroxidation.

Group B showed a statistically significant increased activity in the liver tissue when compared with Group A at $P \leq 0.05$. This means that free oxygen radicals were generated by Cadmium in the liver tissue. This thus confirms the suggestion made by Tandon *et. al.*, [29] that increased accumulation of Cd in the liver induces lipid peroxidation and increases the production of malondialdehyde (MDA).

Glutathione Peroxidase (GPx) Activity in the liver tissue

-Glutathione peroxidase converts H_2O_2 and/or other lipid peroxides to H_2O and hydroxyl lipids and in the process glutathione is converted to oxidized glutathione (GSSG).- It has been well established that glutathione peroxidase also plays a vital role in reducing the risk of oxidative stress [18][20].

—The Groups C, D and E showed ~~showed~~ a statistically significant increased activity in the liver tissue when compared with Group A at $P \leq 0.05$ Significant Level and there was also an increase in Group F even though it was not statistically significant. This shows that the Reactive Oxygen

Species generated by Cadmium were mopped up by the combined activities of the antioxidants present in the drugs and *Moringa oleifera* Seed Oil. This agrees with the suggestion made by Flora *et al.*, [6] that antioxidant molecules are mainly free radical scavengers are thought to play a crucial role in counteracting free radical induced damage to macromolecules -by reducing the peroxide concentrations and repairing oxide membranes.

Superoxide Dismutase Activity in the liver tissue (SOD)

—The superoxide dismutase is a primary antioxidant enzyme and is essential to the organism to fight against oxidative effects of free radicals [18][20].

—The Groups C, D, E, F and G showed a statistically significant decreased activity in the liver tissue when compared with Group A at $P \leq 0.05$ Significant levels. This means that combined action of the antioxidants present in the drugs and both oil extracts of *Moringa oleifera* seed oil and Cashew nut oil were able to efficiently combat the free Oxygen radicals which were generated by Cadmium. This therefore confirms the suggestion made by Beytut *et al.*, [5] that supplementation of antioxidants reduced ROS levels, enzymatic and non-enzymatic components of antioxidant ~~defence~~defense system.

Catalase Activity in the liver tissue (CAT)

Catalase is a scavenger enzyme which is present in the peroxisomes of nearly all aerobic cells and ~~preserves to~~ protect the cell from the toxic effects of by H_2O_2 catalyzing its decomposition into molecular oxygen and water without the production of free radicals. Catalase-It is one of the most important antioxidant enzymes which cleave the toxic H_2O_2 substrate into water. One molecule of catalase can convert millions molecules of into H_2O_2 water and oxygen per second [12].

The Groups B, C, D and E showed a statistically significant increased activity in the liver tissue when compared with Group A (Normal Control) at $P \leq 0.05$ Significant level and Group F was also increased although it was not statistically significant. This means that the activity of Catalase was increased in the liver by the combined action of the antioxidants present in the drugs and both oil extracts of *Moringa oleifera* seed oil and Cashew nut oil in order to efficiently scavenge and mop up the free radicals which were generated. This therefore confirms the suggestion by Flora *et al.*, [6] that antioxidant molecules are thought to play a crucial role in counteracting free radical induced damage to macromolecules and has been found to heal the free radical mediated cell damage by directly neutralizing free radicals.

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