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Antioxidant Properties of *Moringa oleifera* Oil and *Anacardium occidentale* Oil on Cadmium Induced Liver Damage in Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ODO, SAA, NOA and UPI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SAA, ODO, NOA and UPI managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors SAA and ODO managed the experimental process and authors SAA and ODO identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Cadmium (Cd) is one of the most toxic heavy metals found in the environment. This study aimed at combined effects of *Moringa oleifera* (Mo) oil and Cashew nut oil on cadmium-induced liver damage in wistar rats.
Thirty-five wistar rats of both sexes were randomly divided into seven groups of five rats each. Group A received 5ml of phosphate buffer single dose intraperitoneally (ITP); group B received 3.5 mg/kg $3CdSO_4.8H_2O$ ITP; group C received 100 mg/kg Vit C and 300mg/kg Vit E orally; group D

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received 100 mg/kg Vit C and 300 mg/kg Vit E orally and 3.5 mg/kg Cd ITP; group E received 3.5 mg/kg Cd and 40 mg/kg *Mo* seed oil orally; group F received 3.5 mg/kg Cd ITP and 40 mg/kg Cashew nut oil orally and group G received 3.5 mg/kg Cd ITP, *Mo* seed oil and Cashew nut oil 20 mg/kg each. At the end of the experiment liver was harvest, immersed in sucrose and homogenized for biochemical analysis. The supernatants obtained were used with appropriate salts for enzyme activities such as superoxide dismutase (SOD), Glutathione Peroxidate (GPx), Malondialdehyde (MDA) and Catalase spectrophotomerically with the use of spectrophotometer.

This studies showed that Cadmium significantly increased the activities of the antioxidant defense system enzymesat p≤ 0.05 such as MDA, GPx and Catalase and reduced the activities of SOD at p≤ 0.05 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 defense system enzymes such as MDA, GPx and Catalaseat p≤ 0.05 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 defense system.

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

Keywords: Histochemical; antioxidants; Moringa oleifera; Anacardium occidentale; oxidative stress; liver; cadmium.

1. INTRODUCTION

Cadmium is one of the most toxic metal ions of our environment bound in the air, food and water [1,2,3]. It is found in foods (such as vegetables, grains and cereals) and also produced as a byproduct of zinc and lead mining and smelting [4,5]. 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 [6,7]. 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 [8]. It has been associated with cancers of the lung, prostate, pancreas, and kidney [9,10]. *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 perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional, medicinal and industrial uses [12]. Leaves are longitudinally cracked, 30-75 cm long main axis and its branch jointed, glandular at joints, leaflets are glabrous and entire [11,13]. 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 [11,13]. The twigs are finely hairy and green. Flowers are white, scented in large axillaries down panicles, pods are pendulous, ribbed, seeds are 3-angled [11,13]. 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 [14]. *Anarcardium 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 alaysia [15]. *Anacardium occidentale*. Grows well in the tropical, is a multipurpose tree of the tropics which attains a height of about 15 m. They grow on relatively dry soil in nature but in cultivation grow in tropical rain forest. Tyman and Morris [16] 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 [17]. Gibbon et al. [18] reported that many trees are found growing wild and that the plant germinates poorly, those that are cultivated are propagated by seed which are planted at a rate of 2-3 per hole due to poor germination rates.

The commercial that phytochemicals activity importance of cashew is due to its richness in nutrient of CNSL. that constitutes of 47% fat, 21% protein and 22% carbohydrate, vitamins and all essential amino acids especially thiamine [19]. It is not a triglyceride and contains a high proportion of phenolic compound. It is used in industry as a raw material for brake lining compounds, as a water proofing agent, a preservative and in the manufacturing of paints and plastics. It is toxic and corrosive to the skin. Edible oil can be extracted from cashew nuts but no evidence of it being carried out commercially has been found. The ability of cashew apples to supply and fortify the nutritional requirement for vitamin C, particularly in Africa was reported by Akinwale [20]. Cashew apple juice has been found to contain the highest amount of vitamin C (203.5 mg/100 ml) of edible portion and when the cashew apple was blended with other tropical fruits it boosted their nutritional quality. The importance of the Cashew Nut Kernel Oil and Cashew Nut Shell Liquid (CNSL**)** cannot be overemphasized. The fat of nut is completely natural and unprocessed which is best for the body. It is especially rich in Linoleic acid (Omega-3) and is least damaging to heart and arteries. It constitutes about 47% of the total weight of the nut. Nuts often produce oil half their weight. Cashew has what is called the 'good fat. Cashew has the right combination of fat and the ratio of saturated to monounsaturated and 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 [21].

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 [22]. 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.

2. MATERIALS AND METHODS

2.1 Plants Extract Preparation

2330 g 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 heated inside an oven at a temperature of 40ºC and were pounded using a mortar and pestle in order to separate the chaff from the seeds. The seed extract was then grinded 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 [23]. The *Anacardium occidentale*nut 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 40ºC and were grinded 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 [23].

2.2 Drug Preparation

The Cadmium Sulphate Solution ($3CdSO_4.8H_2O$) was prepared by dissolving 9.9198 mg of Cadmium Sulphate salt (CdS) in 5ml of 0.9% w/v (PH 7.4) 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 6mg of Vitamin E in 20 ml of olive oil.

2.3 Experimental Animals

Thirty five (35) wistar rats weighing between 80 g-180 g 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*. All experimental investigations were done in compliance with humane animal as stated in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub. No NIH 86 – 23 [24] and in accordance with ethical approval of the Anatomy Department, Bingham University, Karu, Nigeria.

2.4 Chemical and Extract Administration

Moringa oleifera of 0.32 ml (40 mg/kg) and Cashew nut seed oil, 0.16 ml (20 mg/kg) each of *Moringa oleifera* seed oil and Cashew nut oil, 0.8 ml (100 mg/kg) of Vitamin C, O.3 ml of Vitamin E (300 mg/kg) and 5 ml of 0.9% w/v of (PH 7.4) 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.

2.5 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
B	5	3.5 mg/kg 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 Cd
E	5	3.5 mg/kg Cadmium+ 40 mg/kg *Moringa oleifera* seed oil (0.32 ml)
F	5	3.5 mg/kg Cadmium+ 40 mg/kg Cashew nut oil (0.32 ml)
G	5	3.5 mg/kg Cadmium+ *Moringa oleifera* seed oil + Cashew nut oil 20 mg/kg each (0.16 ml each).

2.5.1 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 3.5mg/kg $3CdSO_4.8H_2O$. The Positive Control Group (C) received 100 mg/kg (0.8 ml) Vitamin C and 300 mg/kg (0.3ml) Vitamin E orally for a period of four (4) weeks.

2.5.2 Prophylactic treatment group

The animals in Group D were the prophylactic group that received 100 mg/kg (0.8 ml) Vitamin C and 300 mg/kg (0.3 ml) Vitamin E for a period of four (4) weeks followed by intraperitoneal injection of 3.5mg/kg body weight $3CdSO_4.8H_2O$. The rats were then sacrificed after 24 hours.

2.5.3 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 $3CdSO_4.8H_2O$. 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.

2.6 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 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 symphisis. 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. The supernatants obtained were used to carry out by first principle methods with the use of appropriate salts for enzyme activities such as superoxide dismutase (SOD) [25], Glutathione Peroxidate (GPx), Malondialdehyde (MDA) [26] and Catalase [27] spectrophotomerically with the use of spectrophotometer.

2.7 Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean (S.E.M), One- Way ANOVA using Medical software packages to determine the level of significance $P < 0.05$ (95% CI) was taken as the significant level.

3. RESULTS AND DISCUSSION

Lipid peroxidation is one of the main manifestations of oxidative damage, which plays

an important role in the toxicity of many xenobiotics [28,29]. The prevention of lipid peroxidation is essential for all aerobic organisms and the organism is well equipped with antioxidants that directly or indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals [30,31]. The Groups C, D, E, F and G showed an increased in the level of MDA activity in the liver tissue supernatant, which was significant at $p < 0.05$ when compared with Group A rats. This means that the combined antioxidant properties of the drugs and both plants oil extract 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 [32] that antioxidants have shown protective functions against cadmium induced toxicity in different animal models where their supplementation reduced Reactive Oxygen Species (ROS) levels and lipid peroxidation.

Group B showed a significant increase in the level of MDA activity in the liver supernatant when compared with Group A at $p \leq 0.05$. This means that free oxygen radicals were generated by cadmium in the liver. This thus confirmed as suggested by [33] that increased accumulation of Cd in the liver induces lipid peroxidation and increases the production of malondialdehyde (MDA).

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 [34,35]. The Groups C, D and E showed statistical significant increased in MDA activity in 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 is in accordance with [36] 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.

The superoxide dismutase is a primary antioxidant enzyme and is essential to the organism to fight against oxidative effects of free radicals [34,35]. 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 [37] that supplementation of antioxidants reduced ROS levels, enzymatic and non-enzymatic components of antioxidant defense system.

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 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_2water and oxygen per second [6,38]. The Groups B, C, D and E showed an increased level of activity in the liver

Table 2. Histochemical analysis for enzyme assay results (antioxidant defense system enzymes)

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

*The Mean± the 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. * indicates statistical significance when compared with the normal control values*

homogenate, which was significant at $p \leq 0.05$ when compared with group A and there was an increase in CAT activities in group F which was not significant at $p \leq 0.05$. This implies that the activity of Catalase was increased in the liver by the combined action of the antioxidants present in the drugs and in both oil extracts of *Moringa Oleifera* seed oil and Cashew nut oil in order to efficiently scavenge the free radicals which were generated [36].

4. CONCLUSION

This study therefore revealed ameliorative effect of *Moringa oleifera* seed oil and Cashew nut oil on Cadmium induced liver damage.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the authors hereby declare that all the experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in line with the ethical procedure laid down in 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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