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Positive Anti-Cadmium Poisoning Anti-Oxidant Effects of Moringa Seed Oil and Cashew Nut Oil in Juvenile Wistar Rats Hippocampus

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ODO, JOO, HBA, EAA and SAA designed the study and wrote the protocol. Author BJD managed the animals, collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Author JOO did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Moringa oleifera seed oil and *Anacardium occidentale* oil contain certain antioxidants including ascorbic acid, phenolics, flavonoids and carotenoids; these antioxidants have potentials to act against oxidative stress generated by cadmium accumulation and activities in tissues. This study was aimed at investigating some antioxidant effects of moringa seed oil and cashew nut oil on cadmium toxic effects in the hippocampus of Wistar rats. Thirty five juvenile male Wistar rats were divided into seven groups (A, B, C, D, E, F and G) of five (5) animals. Group A served as control

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[fed *ad libitum*]; Groups B, C, D, E, F and G were injected intraperitoneally with 2.5 mg/kg of cadmium sulphate ($3CdSO4.8H_2O$) to induce cadmium poisoning. After cadmium administration: Group B animals were left untreated; Group C and D animals received daily oral administration of 100 mg/kg vitamin C and 30 mg/kg vitamin E respectively; Groups E and F were administered 4 mg/kg of Moringa seed oil and 4 mg/kg of Cashew nut oil respectively- orally and daily, while Group G received daily oral administration of 2 mg/kg of *Moringa oleifera* oil and 2 mg/kg of Cashew nut oil [combined]. The experiment lasted three weeks [21 days]; animals were sacrificed and the hippocampus and blood were assayed for enzymes LDH, G6PDH, SOD and GPx. Average weights and blood sugar were also measured. Results indicate that cadmium caused oxidative stress in the hippocampus by upsetting the tested enzymes' activities significantly; these effects were ameliorated by the anti-oxidant contents of the oils to varying extents.

Keywords: Oxidative markers; hippocampus; antioxidant and cadmium.

1. INTRODUCTION

Cadmium, a heavy metal is a highly deleterious environmental pollutant that can lead to many health problems that affect people across the world [1]. It has been classified as a Group 1 category human carcinogen by the International Agency for Research on Cancer of USA because of its carcinogenic properties [2,3]. Cadmium is a potent human carcinogen and has been associated with cancers of the lung, prostate, pancreas and kidney. Cadmium-induced nephrotoxicity is clearly a frequently occurring ailment in humans as a result of chronic exposure to the metal [4]. The most sensitive cellular targets of cadmium seem to be ion transport and signal transduction [5]. These include intracellular mobilization of second messengers such as inositol triphosphate and calcium [5], inhibition of plasma membrane calcium channels [6], and inhibition of Ca^{2+} ATPases of the sarcoplasmic reticulum [7].

Although orthodox medicine is the mainstream medicine in Western countries, application of herbal medicines or phytomedicine is growing worldwide for many reasons, in particular, the side effects or inefficacy of modern drugs [8]. For centuries, people in many countries have used *Moringa oleifera* [moringa] seed oil and *Anacardiun occidentale* [cashew] nut oil as traditional medicine for common ailments [9]. Various parts of these plants such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants and they possess antitumor, antipyretic, antiepileptic, anti-inflammatory, anti-ulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial and antifungal activities; and are being employed for the treatment of different ailments in the indigenous system of medicine [10].

The phytochemicals are the prime and bioactive compounds of plants that are responsible for the extended biological properties. Cashew fruit is eaten to treat scurvy and diarrhea [11]. It is also effective in preventing cholera and can be used as remedy to treat neurological damage, pain and rheumatic fever. The nuts are consumed orally to cure impotency and as aphrodisiac [12]. Cashew nuts have various health advantages as they are significant sources of iron (essential for red blood cell function and enzyme activity), magnesium (which promotes energy release and bone growth), phosphorus (which builds bones and teeth) and zinc (essential for digestion and metabolism). In addition, the nuts contain significant amounts of phytochemicals with antioxidant properties that protect the human body from cancer and heart diseases [13]. In tropical medicine, cashew nut shell liquid (CNSL) is used to treat leprosy, elephantiasis, psoriasis, ringworm, diabetes, warts and corns [14].

The hippocampus is a major component of the brains of humans and other vertebrates. It belongs to the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation [15]. In Alzheimer's disease, the hippocampus is one of the first regions of the brain to suffer damage; memory loss and disorientation are included among the early symptoms [16]. Damage to the hippocampus can also result from oxygen starvation (hypoxia), oxidative stress, encephalitis, or medial temporal lobe epilepsy [16]. It is therefore very important to observe possible means of combating cadmium poisoning effects on this vital part of the brain using natural antioxidants rich products such as the oils employed in this investigation. More specifically, the primary aim of this investigation is to observe some antioxidants effects of moringa seed oil and Cashew nut oil on cadmium toxic effects in the hippocampus of

Wistar rats using *in vivo* biochemical activities and parameters.

2. MATERIALS AND METHODS

The plant materials were collected from Bingham University, Nigeria and moringa seeds and Cashew nuts were identified by a botanist in the department of Biological Science of Bingham University. The plant materials were air dried at room temperature for three weeks and grounded into fine powder and the extracts were obtained following standard procedures [17].

Thirty- five (35) Wistar rats were used for the study. The animals were made to acclimatize for two weeks and were maintained under standard animal holding facility conditions in Bingham University animal house holding, they were housed in well ventilated cages and kept under controlled light schedule (12 hour light and 12 hour dark) cycle and were fed with standard laboratory feed and water *ad libitum*. The rats were randomly grouped into seven groups labeled A, B, C, D, E, F and G; each group consisting of five animals. Group A served as control [animals were simply fed *ad libitum*]; Groups B, C, D, E, F and G were injected intra-peritoneally with 2.5 mg/kg of cadmium sulphate ($3CdSO4.8H_2O$) to induce cadmium poisoning; with rationale based on existing literatures [18]. After cadmium administration: Group B animals were left untreated; Group C and D animals received daily oral administration of 100 mg/kg vitamin C and 30 mg/kg vitamin E respectively; Groups E and F were administered 4 mg/kg of Moringa seed oil and 4 mg/kg of Cashew nut oil respectively- orally and daily while Group G received daily oral administration of 2 mg/kg of *Moringa oleifera* oil and 2 mg/kg of Cashew nut oil [combined]. The experiment lasted three weeks [21 days].

Each group had specific rationale for the treatment employed. Group A served as control and normal reference; Group B's rationale was to observe the sole effects of cadmium poisoning on the hippocampus; Group C was to observe the possible specific effects of Vitamin C only on cadmium poisoning in the hippocampus [being a conventional antioxidant]; Group D was to observe the possible sole effects of Vitamin E on cadmium poisoning in the hippocampus [another conventional antioxidant]; Group E was treated with moringa seed oil to observe its effects after cadmium intoxication; Group F was treated with cashew nut oil to observe its effects after cadmium intoxication while Group G was treated with both oils combined to observe their combined effects against cadmium poisoning in the hippocampus.

Animals were sacrificed six hours after the last day or time of treatment by cervical dislocation. The animals' skulls were dissected and the brain tissues of interest were carefully excised and homogenized in 5% sucrose solution at 4^oC for antioxidants bioassay. The homogenate was centrifuged at 26,000 × g for 15 min at 4℃. The supernatant was taken through another round of centrifugation to ensure purity. The resulting supernatant was assayed for the selected enzymes as this would supposedly provide information on the tissue-in-situ enzymes; the results of which could be compared with the serum-carrying quantities of the same enzymes [19,20]. During the animal sacrifice, blood samples were collected from the heart via cardiac puncture using suitable needle and syringe and placed in serum bottles. The collected blood samples were left to clot, undisturbed at room temperature; this lasted approximately30 minutes. Centrifuging at 1,000-2,000 x g for about10 minutes in a refrigerated centrifuge helped to obtain the serum from the blood as the supernatant. After centrifugation the serum from each sample was transferred into a clean polypropylene tube using a Pasteur pipette. The samples were maintained at 2-8℃ while handling [21,22]. Sigma-Aldrich® Bioassay kits [23] were suitably used for the biochemical assays. Data from results were collated, represented using suitable descriptive statistics and the SPSS 22.0 Version was used to carry out the Analysis of Variance tests- as inferential statistics for the results [P values being $P \leq 0.05$; results that showed levels of statistical significance were asterisked [*] as presented on the table. Ethical approval for the research was obtained from the Department of Anatomy, Bingham University, Nigeria; being a standard institutional requirement for biomedical investigations.

3. RESULTS

3.1 Antioxidants Enzymes Assay

Table 1 Mean±Standard Error of Mean (SEM) of antioxidant enzymes activity in the brain tissue and serum; all treated groups had their SOD levels upset relative to the control to varying extents. All treated group had their GPx levels significantly altered relative to the control.

Group A is the Control [fed *ad libitum]*; Groups B, C, D, E, F and G were exposed to cadmium sulphate toxicity [2.5 mg/kg]. Group B animals were untreated; Group C were treated with daily oral administration of 100 mg/kg vitamin C and Group D, 30 mg/kg vitamin E; Groups E were treated with 4 mg/kg of Moringa seed oil and Group F, 4 mg/kg of Cashew nut oil - orally and daily while Group G received daily oral administration of 2 mg/kg of *Moringa oleifera* oil and 2 mg/kg of Cashew nut oil [combined]. There are evidences of cadmium toxicity and antioxidant enzymes activities disruptions in all other Groups relative to the Control Group. Analysis of Variance Tests- as inferential statistics for the results [using the SPSS 22.0 Version; P values being P≤ 0.05] show that the asterisked [*] values are statistically significantly different relative to the Control Group values.

3.2 Enzymes of Carbohydrate Metabolism Assay

Table 2 Mean ± Standard Error of Mean (SEM) of enzymes of carbohydrate metabolism in brain tissue. LDH levels were significantly elevated in all treated groups relative to the control, except in the Group B. Group A is the Control [fed *ad libitum]*; Groups B, C, D, E, F and G were exposed to cadmium sulphate poisoning [2.5 mg/kg]. Group B animals were untreated; Group C were treated with daily oral administration of 100 mg/kg vitamin C and Group D, 30 mg/kg vitamin E; Groups E were treated with 4 mg/kg of Moringa seed oil and Group F, 4 mg/kg of Cashew nut oil - orally and daily while Group G received daily oral administration of 2 mg/kg of *Moringa oleifera* oil and 2 mg/kg of Cashew nut oil [combined]. Cadmium poisoning disrupted enzymes of carbohydrate metabolism activities in all other Groups relative to the Control Group. And this is significant for all groups except the LDH in Group B. Analysis of Variance Tests- as inferential statistics for the results was done using the SPSS 22.0 Version; P values being P≤ 0.05. G6PDH levels were significantly increased in Groups B, C, E and G and significantly lowered in Groups D and F.

Fig. 1: Bar chart showing weight and blood glucose at the third week; blood sugar levels were altered in the treated groups relative to the control. Animal Groups include A- G: Group A is the Control [fed ad libitum]; Groups B, C, D, E, F and G were exposed to cadmium sulphate toxicity [2.5 mg/kg]. Group B animals were untreated; Group C were treated with daily oral administration of 100 mg/kg vitamin C and Group D, 30 mg/kg vitamin E; Groups E were treated with 4 mg/kg of Moringa seed oil and Group F, 4 mg/kg of Cashew nut oil - orally and daily while Group G received daily oral administration of 2 mg/kg of *Moringa oleifera* oil and 2 mg/kg of Cashew nut oil [combined]. The blood sugar levels were altered in the treated groups relative to the control; it was highest in the untreated Group B and vitamin C-treated Group C; it was lowered in every other group relative to the control. Weights were taken for reference.

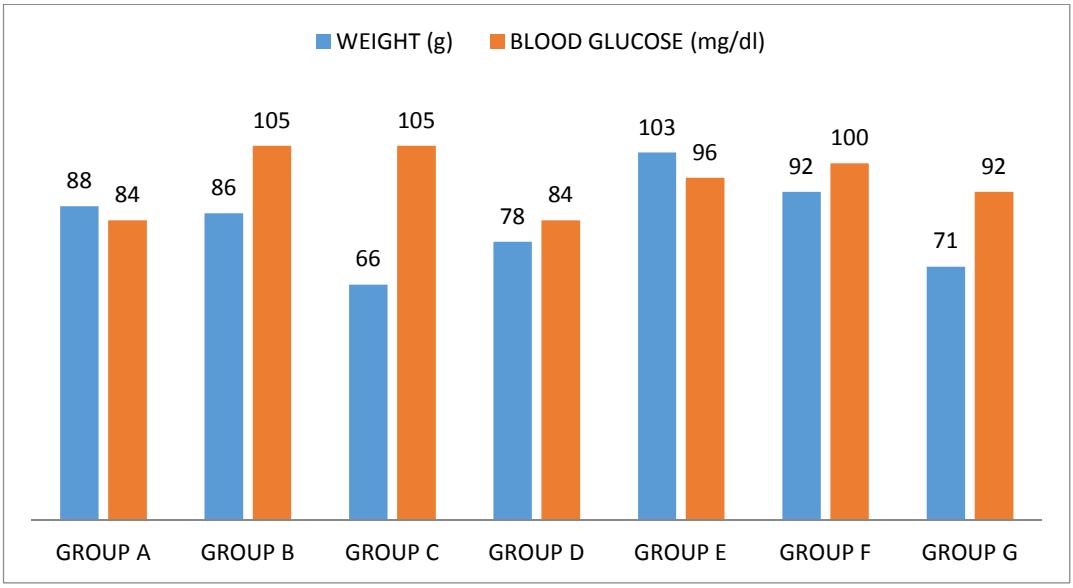

Fig. 1. Bar chart showing weight and blood glucose at the third week

Table 1. Mean±Standard error of mean (SEM) of antioxidant enzymes activity in the brain tissue and serum

Group	SOD I (U/ml) Mean±SEM	SOD II (U/ml) Mean±SEM	GPx I (U/L) Mean±SEM	GPx II (U/L) Mean±SEM
A	603±7	281±6	3458±35	4355±30
B	601±9	279±9	3872±40*	3689±47*
C	758±7*	252±6*	3783±47*	3460±65*
D	742±6*	244±5*	3659±11*	4578±12*
E	788±8*	254±7*	3869±66*	4473±59
F	609±4	243±5*	3101±36*	4669±66*
G	775±9*	271±6*	3784±39*	4814±52*

*SOD I- superoxide dismutase activity in brain tissue, SOD II- superoxide dismutase activity in serum, GPx I- glutathione activity in brain tissue, GPx II- glutathione activity in serum * indicates a statistically significant mean difference when the value is compared to the control at p<0.05*

Table 2. Mean±Standard error of mean (SEM) of enzymes of carbohydrate metabolism in brain tissue

Group	LDH (U/L) Mean±SEM	G6PD (U/L) Mean±SEM
A	573±6	3668±33
B	587±11	4129±34*
C	627±7*	4096±39*
D	609±4*	3322±28*
E	645±7*	4100±40*
F	602±6*	3544±48*
G	627±7*	4336±12*

LDH - Lactate dehydrogenase G6PD - Glucose-6-phosphate; indicates a statistically significant mean difference when the value is compared to the control at p<0.05*

4. DISCUSSION

The superoxide dismutase [SOD] enzyme in the brain tissue is supposedly a collection of the possible types in humans- cytoplasmic, mitochondrial and extracellular; as such, the values given should have taken into consideration the total quantities of the enzymes possibly present in the brain tissue homogenates that were assayed. Relative to the control group, Group B had lower levels of SOD in the brain and serum. This obviously would not have implied that cadmium had not produce significant oxidative stress in this group; on the other hand, the lowering of these enzymes levels could have been due to increased activities against cadmium-induced oxidative stress in the tissues, thus excessive utilisation of the enzymes and consequent depletion. The danger associated with this includes the risk of increased reactive oxygen species in the tissue and simultaneously deficiency of SOD to combat damaging activities of the species leading to increased cellular damage cum nervous tissue damage, despite the statistical insignificance, the efficiency of SOD makes slight changes in its level come with significant biological dysfunctions.

Every other group had increase in SOD in the hippocampus whereas there were reductions in the serum relative to the control. Basically, elevations in the hippocampus might be required to counter increased oxidative stress as indicated by cadmium toxicity; consequently reduced quantities would be circulated unbound in the serum [extracellular] since dismutation must have consumed larger quantities in the concerned tissues.

Glutathione peroxidase levels of activities in the hippocampus homogenate [GPx I] in all treated groups were all significantly different relative to the untreated control group. The variations however have certain notable features and implications per group. The highest level of this enzyme was recorded in the untreated Group B; noting that this enzyme would help the brain combat oxidative stress, it is expected that significant surge in the level in this group would be a response to cadmium-induced oxidative stress. Also, this Group B had lower level of the enzyme in the serum [GPx II] understandably in unbound extracellular form- indicating that such a plasma-proportion deficiency of the enzyme would be due to its increased usage in the tissues affected by oxidative stress of cadmium toxicity. All other Groups, except F have lower levels of the enzyme relative to the control in the tissue homogenate; this is also a positive expression since increase in the enzyme could be beneficial for combating increased tissue oxidative stress. This same Group F has a higher level of the serum-content of this enzyme in all groups except G- whether less of this enzyme was being used in the brain [and other tissue] and leaving relatively more to be in the

serum would require validation. Group F had an unexceptionally high level of serum GPx level; its level in the hippocampus is however not quite high relatively.

Having established that cadmium induced oxidative stress in the hippocampus tissue; altering vital oxidative stress-combating enzymes considerably; it is also factual that the administered oils affected these oxidative stress conditions in various ways in the treated Groups. While it is logical to suggest that the antioxidants in the oils had potentials to influence tissues' responses to oxidative-stress induced SOD and GPx alterations; the nature of alterations and consequently, the mechanism of action would vary- most likely due to the variations in the proportions of the oils' antioxidants as well as other active phytochemicals that would be present- even nutrients. It is also true that the individual exogenous vitamins used – Vitamins C and E were helpful; it is also important to state that the nature and extents of their effects vary; suggesting that a multiple-vitamin approach might be more helpful. From the results however, moringa seed oil is favoured to be more potent than cashew nut oil especially by observing hippocampus homogenate levels of SOD and GPx first; and then relative to their levels in the serum- extracellular and inactive relative to the tissue of interest. There is not enough evidence to suggest that the 50-50 proportional combination of the two oils increased potency; yet there would have been no serious negative consequence relative to the single-oil treated groups. More investigations into the precise mechanism of these oil-contained antioxidants in terms of their amelioration of oxidative stress would be quite helpful- this is however, out of the scope of the current investigation. It would also be helpful to investigate the roles of dosage variations for each oil type.

All treated Groups had significantly high levels of G6PD; this enzyme is strongly associated with the hexose pentose pathway- by implication, recuperating cells would actively require rapid glucose metabolism to ensure survival and energy production to meet neural functions' demands. Only Group F had a higher level than the untreated Group B; expectedly, the treated tissues are largely less affected by cadmium toxicity, hence the level of glucose metabolism would have been less severely raised. LDH should provide information on tissue damage; interestingly, it is lowest in the untreated, hence unaffected Group A, but next to it, being slightly and not significantly raised is the level in Group B. First, all treated tissues appear to have suffered cellular trauma; on brain tissue damage, active tissue 'repair' done by astrocytes could lead to release of more enzymes of the destroyed, morphologically deformed and traumatized cells, in order to restore tissues' histomorphological integrity; ultimately, this would result in improved tissue integrity. But in the interim, the process might give rise to increased LDH level, thus giving a pseudo-impression of relatively higher degree of tissue damage.

Obviously, the blood sugar levels were altered in the treated groups relative to the control. This observation suggests that cadmium toxicity would also affect cellular nutrition and metabolism of glucose which is the source of cellular energy for the neural tissues; obviously, it is not only the oxidative stress managing enzymes that are altered, cellular nutrition is also affected. Weights were taken simply for reference, noting that all groups did not necessarily have the same Average Group Weight right from the start of treatment. However, it is important to still mention the fact that blood sugar was highest in the untreated Group B and vitamin-treated Group C; it was lowered in every other group relative to the control.

The current investigation on phytochemical properties and potential protective effects of moringa seed oil and cashew nut oil antioxidants on hippocampus strongly suggest that bioactive constituents present in both oils [such as flavonoid, terpenoids, saponin, tannins, steroid and glycoside] which are antioxidant agents that could act positively against factors causing inflammation and toxicity [including diabetes, cardiac failure, hypertension, bacterial infection, cancer cells, diarrhea, scurvy and membrane lipid peroxidation] as reported [24] were present in the administered substances. The current findings provide information on the potential protective abilities of the oils against factors that might have affected the hippocampus, causing cell and tissue damage [25].

Observations of the activities of the studied enzymes of carbohydrate metabolism - LDH and G6PDH, show significant differences in cadmium treated group as compared with normal control group which suggest a disruption in carbohydrate metabolism pathway and depletion

of the antioxidant defense mechanism as shown in cadmium intoxicated group of rats [26]. The results of antioxidant enzymes activities showed an increase in SOD and GPx activities in cadmium intoxicated group of rats, as compared with animals in the control and other treated animal groups. Results also showed the protective activities of antioxidant (Vitamin C and Vitamin E) against cadmium toxicity in tissue homogenate of hippocampus [27]. The treated animals showed ameliorative evidences as a result of the antioxidants found in *Moringa oleifera* oil and Cashew nut oil [28].

5. CONCLUSION

This study has provided additional crucial information on the ameliorative or rejuvenative properties of *Moringa oleifera* seed oil and *Anacardium occidentale* nut oil - especially their antioxidant activities- against cadmium poisoning which can damage cells and disrupt the hippocampus tissue functions. This also generally points to the potentials of these oils in managing cases of cadmium poisoning. It also suggests that efforts should be invested in further research that can specifically establish the possible uses of these oils against heavy metal poisoning as well as the specific benefits to general mental health. These oils could provide natural solutions to metallic poisoning especially in the developing countries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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