Original Research Article

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

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 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 [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 enzyme's activities significantly; these effects were ameliorated by the anti-oxidant contents of the oils to varying extents.

Key Word: oxidative markers, hippocampus, antioxidant and cadmium.

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 #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, 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. The cashew fruit is eaten to treat scurvy and diarrhea [11].

They are also effective in preventing cholera and can be used as remedy to treat neurological damage, pain and rheumatic fever. The seeds 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 (promotes energy release and bone growth), phosphorus (builds bones and teeth) and zinc (essential to 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 antioxidant effects of *Moringa oleifera* seed oil and Cashew nut oil on cadmium toxic effects in the hippocampus of Wistar rats using in vivo biochemical activities and parameters.

Materials and Methods

The plant materials were collected from Bingham University, Nigeria and Moringa oleifera 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 *adlibitum*. 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 has specific rationale for the treatment employed. Group A served as control and normal reference; Group B' 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.

Animas 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 $4^{o}C$ for antioxidants bioassay. During the animal sacrifice, blood samples were collected from the heart via cardiac puncture using suitable needle and syringe and placed in serum bottles; serum was separated from the blood sample and preserved at -4 ^{0}C for bioassays. Sigma-Aldrich® kits 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.

Results

Oxidative Stress Enzymes Assay

Table 1: Mean ± Standard Error of Mean (SEM) of oxidative stress enzymes activity in the brain tissue and serum; all treated groups have 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	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 $p<0.05$

Enzymes of Carbohydrate Metabolism Assay

Table 2: Mean ± Standard Error of Mean (SEM) of carbohydrate metabolism in brain tissue. LDH levels were significantly elevated in all treated groups relative to the control, except in the Group. G6PDH levels were significantly increased in Groups B, C, E and G and significantly lowered in Groups D and F.

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 $p<0.05$

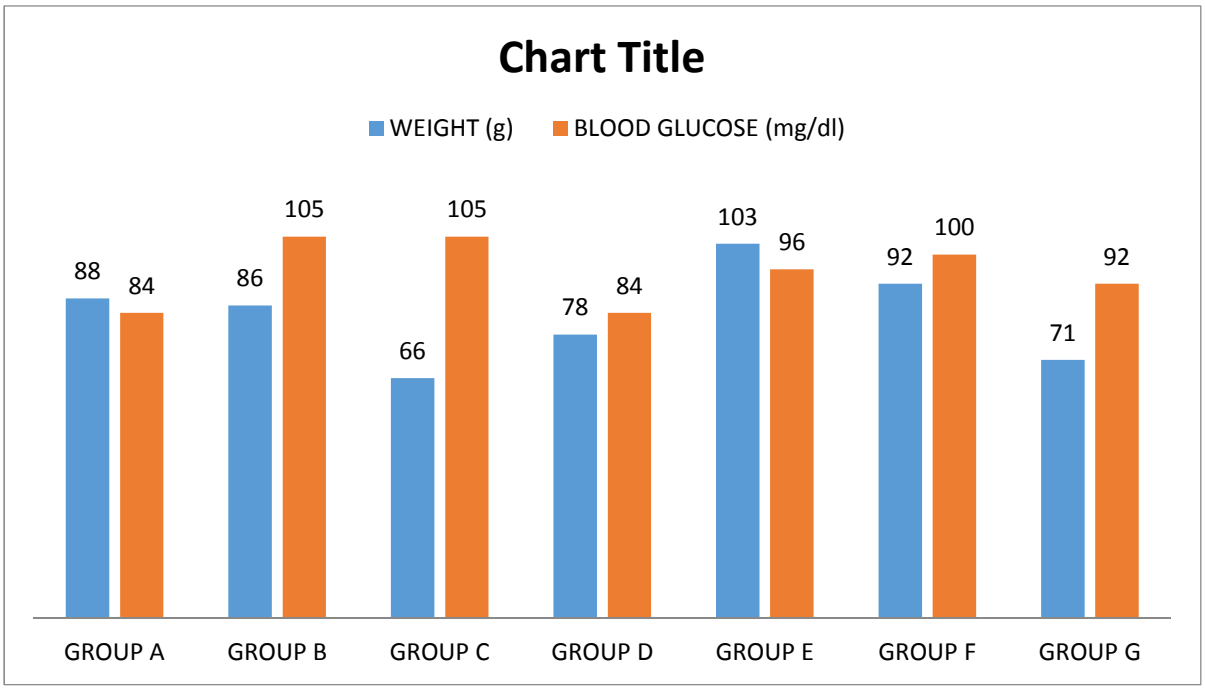

Figure 1: Bar chart showing weight and blood glucose at the third week; blood sugar levels were altered in the treated groups relative to the control

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 groups. The variations however have certain notable features and implications per group. The highest level f 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 has lower level of the enxyme 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 has a lower levels of the enxyme relative to the control in the tissue homogenate; this is also a positive expression since increase in the enzyme could be beneficial for combating increase 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 required validation. Group F has 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 also factual that the administered oils affected this oxidative stress conditions in various ways in the treated Group. While it is logical to suggest that the antioxidants in the oils have potentials of influencing 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 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 Grouops had significantly high levels of G6PD; this enzyme is strongly associated with the hexose penthose pathway- by implication, surviving cells would actively require rapid glucose metabolism to ensure surbvival and enrrgey production to meet neural functions' demands. Only Grop 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 enxymes of morphologically deformed and traumatized cells, in other 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 lelevs were altered in the treated groups relative to the control. This observation suggest 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 to 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 of Moringa seed oil and Cashew nut oil strongly suggest that bioactive constituents present in both extracts [such as flavonoid, terpenoids, saponin, tannins, steroid and glycoside] which are antioxidant agents could act positively against factors causing inflammation and toxicity [including diabetes,

cardiac failure, hypertention, bacterial infection, cancer cells, diarrhea, scurvy and membrane lipid perioxidation] as reported [19] 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 [20].

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 group rats [21]. The results of antioxidant enzymes activities showed an increase in SOD and GPx activities in cadmium treated group of rats, as compared with animals in normal control and other treatment animal groups. Results also suggest the protective activities of antioxidant (vitamin C and Vitamin E) against cadmium toxicity in tissue homogenate of hippocampus [22]. The treatment group animal's shows ameliorative evidences as a result of antioxidant agents found in Moringa oleifera oil extract and Cashew nut oil extracts [23].

Conclusion

This study has provided additional crucial information on the ameliorative or rejuvenative properties of Moringa oleifera oil and Anacardium occidentale oil extracts- especially their antioxidant activities- against cadmium poisoning which can disrupt cells and tissues of the hippocampus. This also generally points to the potentials of these oils in managing cases of cadmium poisoning. It also suggest 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.

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Reference

[1] W.H.O. . Inorganic mercury Geneva, Switzerland: World Health Organization, International Programme on Chemical Safety. 1999; Vol. 118. 168

[2] IARC. . Cadmium and cadmium compounds. In Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 58. Lyon, France: International Agency for Research on Cancer. 1993; pp. 119-239.

[3] IARC. *Cadmium and cadmium compounds (Group1).* IARC monographs Vol. 58:1993; International Agency for Research on Cancer, Lyon. IPCS (1992). Cadmium. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria. 1993; 134; http://www.inchem.org/documents/ehc/ehc/ehc134.htm).

[4] Goyer RA and Cherian MG . Renal effects of metals In: Goyer, R.A., Klaassen, C.D., Waalkes, M.P. (Eds.), Metal Toxicology. Academic Press San Diego. 1995; pp:389–412.

[5] Rayment GE. Sources of Cadmium in Agricultural Products. *National Cadmium Workshop*, DPIE/NFA, Canberra. Brisbane, Queensland: Queensland Department of Natural Resources. 1995.

[6] Huang Y, Quayle JM, Worley J.F, Standen N.B and Nelson MT . External cadmium and internal calcium block of single calcium channels in smooth muscle cells from rabbit mesenteric artery. *Biophys. J.* 1989; 56:1023–1028.

[7] Carol M, Herak K, Ivan S, Maja B, Peter JS, Dennis B and Sylvie B. Cadmium inhibits vacuolar H+ATPase-mediated acidification in the rat epididymis *Biol. Reprod*. 2000; 63:599-606.

[8] Willow JH L . Traditional Herbal Medicine Research Methods. John Wiley & Sons, Inc. 2011; 101: 1140-1144.

[9] Lee C, Hsi CS . Recycling of Scrap Cathode Ray Tubes. Environmental Science and Technology. 2002; 36 (1): 69 doi:10.1021/es010517qPMID 11811492.

[10] Anwar F, Latif S, Ashraf M, Gilani AH. . Moringa oleifera: a food plant with multiple medicinal uses. Phytother Res. 2007; (1):17-25. Source Department of

[11] Onwuka FC, Erhabor O, Eteng MU and Umoh IB . "Ameliorative effect of cabbage extract on cadmium induced changes on hematology and biochemical parameters of albino rats". J. Toxicol. Environ. Health Sci.. 2010: 2.

[12] Evans J and Hastings L. . Accumulation of Cd(II) in the CNS depending on the route of administration: intraperitoneal, intratracheal, or intranasal. Fundam Appl Toxicol. 1992; 19: 275-278.

[13] Algarwal, R. and Abdelhaq, R. . Indian Journal of Clinical Biochem. 2003; 18(2): 64-70.

[14] Meydani M. . *Vitamin E*. Lancet. 1995; 345: 170–5.

[15] Pearce J . *The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish*. J Neurol Neurosurg Psychiatry. 2001; 71 (3): 351.

[16] Hampel H, Bürger K, Teipel SJ, Bokde AL, Zetterberg H, Blennow K . Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. Alzheimers Dement. 2008; 4 (1): 38–48.

[17] Ugwu-Okechukwu PC, Nwodo O FC, Joshua PE, Bawa A, Ossai EC and Odo CE . Phytochemical and Acute Toxicity Studies of Moringa oleifera Ethanol Leaf Extract. Int. J. Life Sc. Bt & Pharm. Res. 2013;(2): 2.

[18] Salawu EO, Adeeyo OA., Ige, SD(. The effect of cadmium on renal system of wistar rats *J. Med. Sci.* 2009.

[19] Isao K , Noriyoshi M, Tae J H, Kazuo T . Antioxidant activity of anacardic acids. Department of Environmental Science, Policy and Management, University Of California, Berkeley, Ca 94720-3112, USA; 2005.

[20] Fuglie LJ . Moringa stem planting. 1999, 2000; Church World Service, Dakar Senegal

[21] Toxicol let. 1990. Dec; 54(92-3): 199-205.

[22] Akerele O. . *Nature Medical Bounty: don't throwit away.* World health forum. 2007; 14: 390-395.

[23] Kasparova S, Brezova V, Valko M, Horecky J and Mlynarik V . Study of theoxidative stress in a rat model of chronic brain hypoperfusion. *Neurochem Int*. 2005; 46:601- 611