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Original Research Article

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

Abstract

It has been shown that Moringa oleifera oil and Anacardium occidentale contain some antioxidant properties such as ascorbic acid, phenolics, flavonoids and carotenoids, against oxidative stress generated as a result of excess accumulation of cadmium in the tissue. This study was aimed at investigating the combined anti-oxidant effects of Moringa oleifera oil and Cashew nut oil on cadmium toxic effect in the hippocampus of wistar rats. Thirty five developing male wistar rats (80g - 120g) were used in this study. The rats were divided into seven groups (A, B, C, D, E, F and G) of five (5) animals each. Normal control (group A), induced control (group B) and positive control (group C). Each rat in group A received 3mg/kg body weight of 0.9% NaCl solution intraperitoneally a single dose and were maintained at normal laboratory condition for three weeks; each rat in group B, E, F and G were induced intraperitoneally with a single dose of 2.5mg/kg body weight of $3CdSO_4.8H_2O$, group B animals were left untreated but were maintained at normal laboratory condition for three weeks and the other groups E, F and G rats were treated with different doses of moringa and cashew oil extract for three weeks while group C rats were treated with standard drugs (100mg/kg vitamin C and 30mg/kg vitamin E) simultaneously. The results revealed that in the hippocampus of rats, enzyme activities of LDH and G6PD were significantly high at ($P< 0.05$) in group B animals while the enzyme activity of group G was found to be the lower relatively compared to group A rats at ($P< 0.05$) while enzyme activities of oxidative stress markers, SOD and GPx show significant difference in group C, D, E, F and G as compared to group A animals. These findings indicate that cadmium has

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ability to cause oxidative stress on the hippocampus and these effects can be curbed or reduced with the combined anti-oxidant activities of Moringa oleifera and Anacardium occidental oil extracts which are dose dependent.

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 in the world [22]. 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 [10, 11]. Cadmium is a potent human carcinogen and has been associated with cancers of the lung, prostate, pancreas, and kidney. Cd-induced nephrotoxicity is clearly the most important and the most frequently occurring ailment in humans as a result of chronic exposure to the metal [7]. The most sensitive cellular targets of cadmium seem to be ion transport and signal transduction [18]. These include intracellular mobilization of second messengers such as inositol triphosphate and calcium [18], inhibition of plasma membrane calcium channels [9], and inhibition of Ca2+ ATPases of the sarcoplasmic reticulum [4].

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 [23]. 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 [14]. 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, antiulcer, 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 [3].

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 [16]. They are also effective in preventing cholera and can be used as remedy to treat neurological

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damaged, pain and rheumatic fever. The seeds are consumed orally to cure impotency and as aphrodisiac [5]. 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 disease [2]. In tropical medicine, cashew nut shell liquid (CNSL) is used to treat leprosy, elephantiasis, psoriasis, ringworm, diabetes, warts and corns [15].

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 [17]. 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 [8]. Damage to the hippocampus can also result from oxygen starvation (hypoxia), oxidative stress, encephalitis, or medial temporal lobe epilepsy [8].

Materials and Methods

The plant material was collected from Bingham University, Nigeria and Moringa oleifera seeds and Cashew nuts were identified by botanist in the department of Biological Science of Bingham University. The plant material was air dried at room temperature for three weeks and grounded into fine powder and the extract was obtained following standard procedure [21].

Thirty- five (35) wistar rats were used for the study. The animals were made to acclimatize for two weeks and were maintained under standard condition 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 A, B, C, D, E, F and G each containing five animals. Group A served as control, Groups B, E, F and G were injected intra-peritoneally

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with 2.5mg/kg of cadmium sulphate ($3CdSO4.8H_2O$) [19]. Groups E and F were administered orally with 4mg/kg of Moringa oleifera oil, 4mg/kg of Cashew nut oil respectively. Group G received oral administration of 2mg/kg of Moringa oleifera oil and 2mg/kg of Cashew nut oil simultaneously while group C and D animals received 100mg/kg vitamin C and 30mg/kg vitamin E simultaneously for the period of three weeks. 72 hrs before the last administration group D rats were injected intraperitoneally with 2.5mg/kg of $3CdSO4.8H_2O$. An equivalent volume of phosphate buffer was administered to groups A which served as normal control for the period of two weeks.

Animas were sacrificed after three weeks of last administration of the Moringa oleifera oil and Cashew nut oil extract by cervical dislocation. The brain tissues were carefully excised and brain tissues were homogenized in 5% sucrose solution at 4^oC for histochemistry and antioxidant bioassay. During the animal sacrifice, blood were collected from the heart via cardiac puncture using needle and syringe and placed in plain serum bottles, serum were separated from the blood sample and preserved at -4^0C for antioxidant activities. 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].

Results

Oxidative Stress Enzyme Assay

Table 1: Mean±Standard Error of Mean (SEM) of oxidative stress enzymes activity in the brain tissue and serum

GROUP	SOD 1 (U/ml) Mean±SEM	SOD 2 (U/ml) Mean±SEM	GPx 1 (U/L) Mean±SEM	GPx 2 (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*

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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 1- superoxide dismutase activity in brain tissue
SOD 2- superoxide dismutase activity in serum
GPx 1- glutathione activity in brain tissue
GPx 2- glutathione activity in serum
* indicates a statistically significant mean difference when the value is compared to the control at $p<0.05$

Histochemical Assay

Table 2: Mean±Standard Error of Mean (SEM) 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 $p<0.05$

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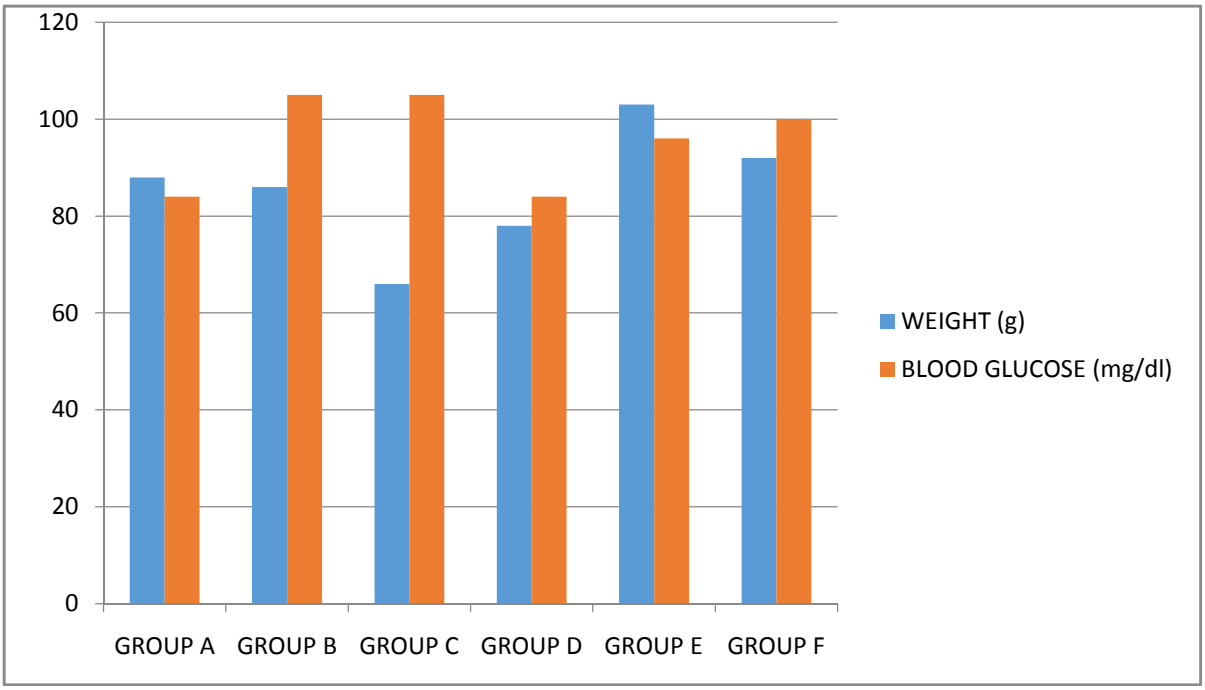

Figure 1: Bar chart showing weight and blood glucose at the third week

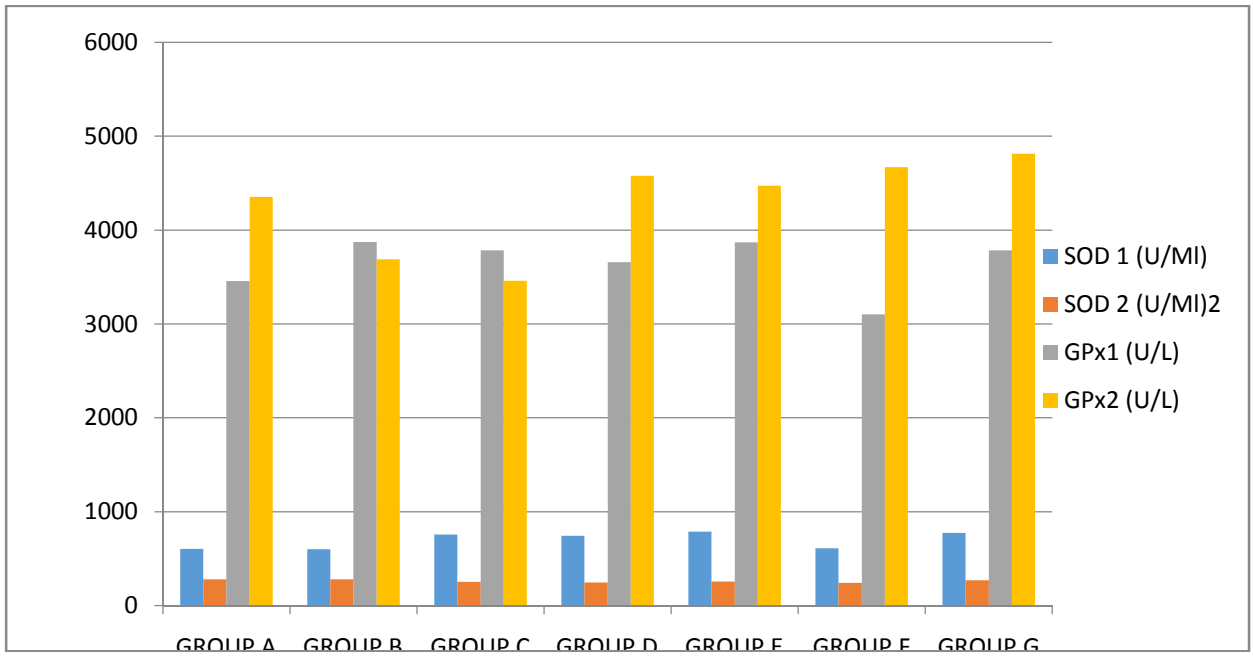

Figure 2: bar chart showing SOD1, SOD2, GPx1 AND GPx2 activities for each group

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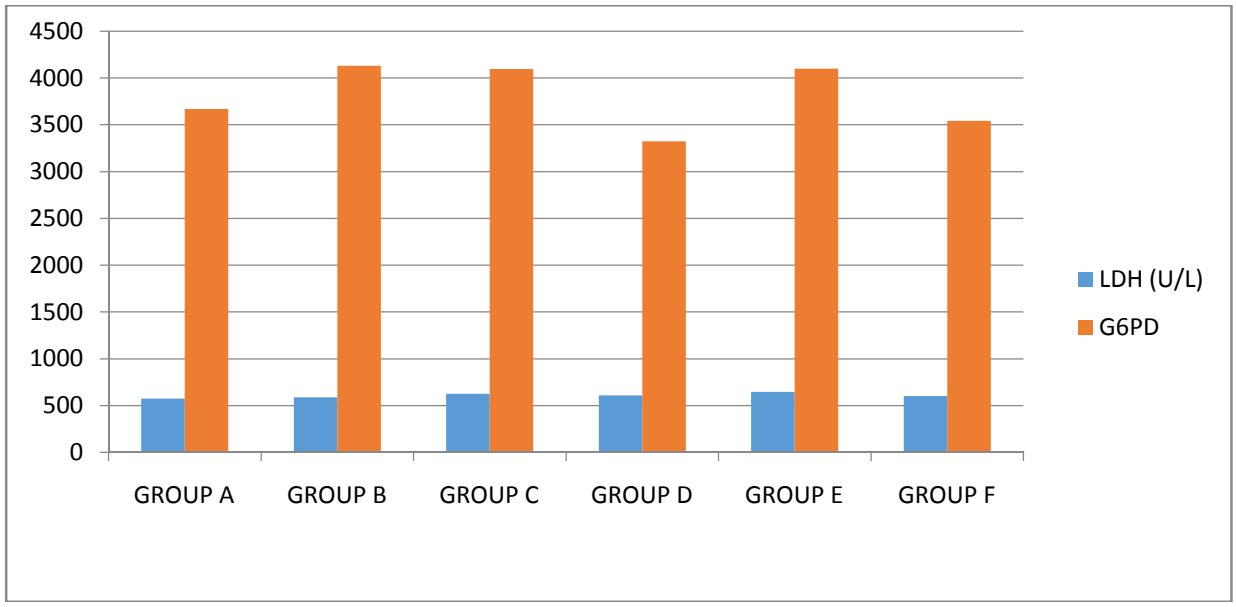

Figure 43: bar chart showing LDH and G6PD activities for each group

Discussion

The Current investigation on phytochemical properties- and potential protective effects on hippocampus- of Moringa oleifera seed and Cashew nut extracts strongly suggest that bioactive constituents present in both extracts such as flavonoid, terpenoids, saponin, tannins, steroid and glycoside which are antioxidant agent against factors causing inflammation and toxicity [including diabetes, cardiac failure, hypertention, bacterial infection, cancer cells, diarrhea, scurvy and membrane lipid perioxidation as reported [12] were present in the administered substances. The above phytochemical findings provide information on the protective ability of the extracts against factors that might have affected the hippocampus to cause cell damage [6].

Observation on the enzymes of carbohydrate metabolism such as LDH and G6PDH shows a significant difference 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 [20] and antioxidant enzyme (SOD and GPx) also so similar characteristic, increase in superoxide dismutase and gluthathione peroxidase were evident in cadmium treated group rats as compared with animals in normal control and other treatment animal groups which is significant, hence, there was a decrease in the SOD, GPx, G6PDH and LDH in group D animals which is relatively similar to activities seen in group A

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rats, which suggest the protective activities of antioxidant (vitamin C and Vitamin E) against cadmium toxicity in tissue homoginate of hippocampus [1]. The treatment group animal's shows restoring evident as a result of antioxidant agents found in Moringa oleifera oil extract and Cahew nut oil extracts [13]..

Conclusion

This study has provide crucial information on the curative and preventive properties of Moringa oleifera oil and Anacardium occidentale oil extracts and their antioxidant activities against factors that can implicate cells and tissues of hippocampus.

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