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Histological and Histochemical Evaluation of Anticadmium Toxicity Effects of *Moringa oleifera* Seed Oil and *Anacardium occidentale* Nut Oil in the Hippocampus of Juvenile Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ODO, BJD, EAA and SAA designed the study and wrote the protocol. Authors YJS and ODO 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

The hippocampus is a part of the brain involved in memory formation, organization and storage; being a limbic system structure that is particularly important in forming new memories and connecting emotions and senses, such as smell and sound, to memories. Cadmium on the other hand is a metallic poison that affects the brain tissues as a whole, producing seriously deleterious effects. Antioxidants could ameliorate the negative effects of toxic substances on body tissues.

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Since *Moringa oleifera* seed and *Anacardium occidentale* nut oils are natural edible oils that are greatly rich in antioxidants and medicinal phytochemicals, it is important to observe their potentials in helping the body to combat the effects of cadmium toxicity particularly on the hippocampus. Thirty five juvenile male Wistar rats were grouped into seven: A, B, C, D, E, F and G. Group A served as the control and the animals were fed *ad libitum*; other groups had each animal administered 2.5 mg/kg body weight of $3CdSO_4.8H_2O$ to induce cadmium toxicity prior to treatment with the agents employed. Each Group B rat was left untreated to observe the effects and nature of cadmium toxicity; each Group C animal received 5 mg/kg body weight Vitamin C dissolved in 0.9% NaCl solution orally. Group D animals were given a daily dosage of 6 mg/kg body weight Vitamin E dissolved in olive oil each. Group E rats were treated with 4 mg/kg body weight *Moringa oleifera* seed oil each; Group F rats were treated with 4 mg/kg body weight *Anacardium occidentale* nut oil and group G rats were treated with 2 mg/kg body weight *Moringa oleifera* seed oil plus 2 mg/kg body weight *Anacardium occidentale* nut oil. Cadmium intoxication has observable deleterious effects on brain tissues. All agents used produced positive anti-cadmium effects, however to varying degrees. The natural oils were more potent than the selected regularly used antioxidants.

Keywords: Hippocampus; cadmium toxicity; antioxidants; Moringa oleifera; Anacardium occidentale.

1. INTRODUCTION

The hippocampus is believed to play important roles in several functions of the brain. The hippocampus is the part of the brain that is involved in memory formation, organization and storage [1,2]. Hippocampus is a limbic system structure that is particularly important in forming new memories and connecting emotions and senses, such as smell and sound, to memories. The hippocampus acts as a memory indexer by sending memories out to the appropriate part of the cerebral hemisphere for long-term storage and retrieving them when necessary [1,2]. Cadmium on the other hand is a metallic poison that affects the brain tissues as a whole producing seriously deleterious effects. It is believed that antioxidants could ameliorate the negative effects of toxic substances on body tissues. Since *Moringa oleifera* seed oil and *Anacardium occidentale* nut oil are natural edible oils which are greatly rich in antioxidants and medicinal phytochemicals, it is important to observe their potentials in helping the body to combat cadmium toxic effects particularly on the hippocampus.

The hippocampus is composed of multiple subfields. Though terminology varies among authors, the terms most frequently used are dentate gyrus and the cornu ammonis literally "Amun's horns", abbreviated CA. The dentate gyrus contains the fascia dentata and the hilus, while CA is differentiated into fields CA1, CA2, and CA3. The horse shoe appearance of the hippocampus is caused by cell density differentials and the existence of varying degrees of neuronal fibers. The CA regions are also structured depth-wise in clearly defined strata [3,4]. The alveus is the deepest layer and contains the axons from pyramidal neurons, passing on towards the fimbria or fornix, one of the major outputs of the hippocampus. Stratum oriens is the next layer superficial to the alveus [5]. The cell bodies of inhibitory basket cells and horizontal trilaminar cells, named for their axons innervating the three layers-the oriens, pyramidal, and radiatum are located in this stratum [5]. The basal dendrites of pyramidal neurons are also found here, where they receive input from other pyramidal cells, septal fibers and commissural fibers from the contra lateral hippocampus usually recurrent connections, especially in CA3 and CA2 regions [5].

Stratum pyramidale contains the cell bodies of the pyramidal neurons, which are the principal excitatory neurons of the hippocampus. This stratum tends to be one of the more visible strata to the naked eye [5]. In region CA3, this stratum contains synapses from the mossy fibers that course through stratum lucidum. This stratum also contains the cell bodies of many interneurons, including axo-axonic cells, bistratified cells, and radial trilaminar cells. Stratum lucidum is one of the thinnest strata in the hippocampus and only found in the CA3 region. Mossy fibers from the dentate gyrus granule cells course through this stratum in CA3, though synapses from these fibers can be found in stratum pyramidale. Stratum radiatum like stratum oriens, contains septal and commissural fibers. It also contains Schaffer collateral fibers, which are the projection forward from CA3. Some interneurons that can be found in more superficial layers can also be found here,

including basket cells, bistratified cells, and radial trilaminar cells. Stratum lacunosum is a thin stratum that too contains Schaffer collateral fibers, but it also contains perforant path fibers from the superficial layers of entorhinal cortex. Due to its small size, it is often grouped together with stratum moleculare into a single stratum called stratum lacunosum-moleculare. Stratum moleculare is the most superficial stratum in the hippocampus. Here the perforant path fibers form synapses onto the distal, apical dendrites of pyramidal cells. The hippocampal sulcus or fissure is a cell-free region that separates the CA1 field from the dentate gyrus. Because the phase of recorded theta rhythm varies systematically through the strata, the fissure is often used as a fixed reference point for recording EEG as it is easily identifiable.

The dentate gyrus is composed of a similar series of strata namely; Polymorphic layer is the deepest layer of the dentate gyrus and is often considered a separate subfield [5]. This layer contains many interneurons, and the axons of the dentate granule cells pass through this stratum on their way to CA3 [5]. Stratum granulosum contains the cell bodies of the dentate granule cells. Stratum moleculare, outer third is where both commissural fibers from the contralateral dentate gyrus run and form synapses as well as where inputs from the medial septum terminate on the proximal dendrites of the granule cells [6]. Stratum moleculare, external two thirds is the deepest of the strata, sitting just superficial to the hippocampal fissure across from stratum moleculare in the CA fields. The perforant path fibers run through this stratum, making excitatory synapses with the distal apical dendrites of granule cells [6].

Cadmium is a soft, malleable, ductile, bluish-white divalent metal. It is similar in many respects to zinc but forms complex compounds [7]. Unlike other metals, cadmium is resistant to corrosion and as a result it is used as a protective layer when deposited on other metals. As a bulk metal, cadmium is insoluble in water and is not flammable; however, in its powdered form, it may burn and release toxic fumes [8]. Cadmium cannot penetrate the adult blood brain barrier (BBB), although it might diffuse across the BBB with the help of a vehicle such as ethanol [9]. Cadmium can more effectively pass the BBB during the developmental stage in an organism and is more toxic in newborns [10,11]. Cadmium has an LD_{50} of 5.7 mg/kg body weight intraperitoneally on rats and once inside, it accumulates in different areas of the brain, induces lipid peroxidation and weakens the antioxidative defense [10,12]. In battery workers cadmium-induced oxidative stress was demonstrated to cause amyotrophic lateral sclerosis due to reduced brain SOD activity. Cadmium (0.4 mg CdAc2/kg body weight) injected intraperitoneally to young albino rats for 30 days generated free radicals in the brain causing region-specific membrane changes, which in turn led to significant alterations in membrane fluidity, intracellular Calcium concentrations and phospholipid composition [13]. It also resulted in a decreased GSH/GSSG ratio as well as activities of GR (glutathione reductase) and glucose-6-phospate-dehydrogenase (G6PDH) in various brain regions, although the decrease in GSH/GSSG was not seen in the hippocampus and midbrain [14]. Cadmium-induced oxidative damage also induced enhanced lipid peroxidation and protein carbonylation in male Swiss albino mice that received 4 mg CdCl2/kg body weight orally for three days [10]. The oxidative impairment was characterised by increased ROS (reactive oxygen species) production, reduction of total thiols and the GSH pool together with an increase in GSSG level. In addition, also activities of antioxidant enzymes such as SOD, CAT, GST, GR, GPx and G6PDH were diminished. The authors also showed the protective abilities of taurine (single oral dose of 100 mg/kg body weight for five days before Cd treatment) and vitamin C (single oral dose of 100 mg/kg body weight for five days before Cd treatment) against oxidative impairment in brain tissue caused by Cadmium [10].

Moringa oleifera (moringa) is a rich source of antioxidants [15] and has an LD_{50} of 340 – 400 mg/Kg body weight intraperitoneally on rats [15]. It has been reported that aqueous extracts of leaf, fruit and seed of moringa act as antioxidants. During a study reporting antioxidant property of freeze dried moringa leaves from different extraction procedures, it was found that methanol and ethanol extracts of Indian origin moringa have the highest antioxidant activities-65.1% and 66.8%, respectively [16,17]. It was also reported that the major bioactive compounds of phenolics, such as quercetin and kaempferol are responsible for the antioxidant activities [17]. In another study, quercetin and kaempferol showed good antioxidant activities on hepatocyte growth factor (HGF) induced methionine phosphorylation with IC_{50} value for 12

and ~6 µM/L, respectively. Another recent study comparing palm oil with moringa seeds for their antioxidant potential found out that moringa seeds are superior for radical scavenging [18].

The antioxidant properties and the effect on nitric oxide (NO) production of various extracts of the leaves of *Anacardium occidentale* have been reported. The result suggested that the leaves of *A. occidentale* are a potent source of natural antioxidants [19]. Cashew nut oil has an LD_{50} of 1230 mg/kg body weight on rats intraperitoneally [19]. Effects of immature cashew nut-shell liquid (*Anacardium occidentale*) against oxidative damage in Saccharomyces cerevisiae and inhibition of acetylcholinesterase activity were reported. A previous study showed that immature cashew nut shell liquid (iCNSL), a source of unsaturated long-chain phenols, may have a potential role in protecting DNA against oxidative damage [20].

The current study is an attempt to investigate the potential ameliorative effects of the two oils- *Anacardium occidentale* nut oil and *Moringa oleifera* seed oil, on cadmium toxicity in the hippocampus of the brain using histological and histochemical procedures of demonstration and analyses. Results from this investigation would help to examine further the possible use of the natural anti-oxidants-rich edible oils in combating toxic effects of substances on the brain. It will also provide information to users of the oils on its effects on the brain tissues- particularly the hippocampus. Very importantly, results would contribute greatly to knowledge in the fields of toxicology and phytomedicine, as well as several other several biomedical sciences.

2. MATERIALS AND METHODS

Moringa seed, 2000 g, was obtained from Kuchikau town, Nassarawa state, Nigeria. *Moringa oleifera* seed oil was extracted by heating the seed inside an oven at 40℃ and pounded with mortar and pestle to separate the chaff from the seeds. The seeds were grinded into powder with a grinding mill, dissolved in 400 mL of water at ambient temperature for two days and later filtered. The aqueous extract was poured into molten mesh and was placed on an oil extractor machine, seed oil was removed at high temperature and pressure and oil extract was kept at 20℃ before administration. Cashew nut, 2000 g, was produced from Kuchikau town, Nassarawa state, Nigeria. The seeds were heated inside an oven at 40℃ and grinded into powder with a grinding mill. The extract was poured into molten mesh and was placed on oil extractor machine, seed oil was removed at high temperature and pressure; the oil extract was kept at -20℃ before administration.

The cadmium sulphate solution was prepared by dissolving the cadmium sulphate salt in normal saline solution. The Ascorbic acid (Vitamin C) was dissolved in normal saline solution and the Vitamin E (tocopherol) was dissolved in olive oil. All other chemicals were of the highest purity commercially available. The Cadmium sulphate was administered at sub lethal dose according to the body weights of the rats intraperitoneally using insulin syringes. The ascorbic acid and alpha tocopherol were administered orally using an oral cannula.

A total of 35 juvenile male Wistar rats (100-200 g) were used for the study. They were maintained under standard laboratory condition in the animal house (National Academy Press, 1996). The juvenile male Wistar rats were grouped into seven: A, B, C, D, E, F and G. Group A served as the control and the animals were fed *ad libitum*; other groups had each animal administered 2.5 mg/kg body weight of $3CdSO_4.8H_2O$ to induce cadmium toxicity prior to treatment with the agents employed. Each Group B rat was given 5 mg/kg body weight Vitamin C dissolved in 0.9% NaCl solution and each Group C animal, 6 mg/kg body weight vitamin E dissolved in olive oil orally. Group D animals were given 5 mg/kg body weight vitamin C dissolved in 0.9% NaCl solution and 6 mg/kg body weight vitamin E dissolved in olive oil. Group E rats were treated with 4 mg/kg body weight *Moringa oleifera* seed oil each, Group F rats were treated with 4mg/kg body weight *Anacardium occidentale* nut oil and Group G rats were treated with 2 mg/kg body weight *Moringa oleifera* seed oil plus 2 mg/kg body weight *Anacardium occidentale* nut oil.

The animals were treated for three (3) weeks; twenty four hours after the last administration, the rats were sacrificed by cervical dislocation. The skulls of the rats were then fractured open with forceps, the brains were removed into specimen bottles containing 40% formal calcium solution for histological and immunohistochemical analyses. Tissues were demonstrated using the Haematoxyline and Eosin staining technique, Cresyl Fast Violet staining technique, Bielschowsky staining technique; as well as the immunohistochemical

demonstrations of Cathepson D and p53 expressions. Ethical approval to conduct research was obtained from the Department of Anatomy, Bingham University, Nigeria. Results were interpreted using qualitative histological and histochemical principles [21].

3. RESULTS

Group A

Photomicrographs of the hippocampus of experimental animals administered 3 ml of 0.9% NaCl solution per kg body weight as the control [N= Neurons; G = Glia; Senile plaque is being encircled].

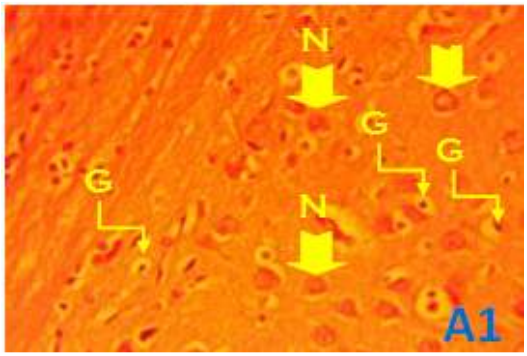

Fig. A1. Histological demonstration of the hippocampus using the H&E staining technique [x400] showing normal neurons, glia and neuropil

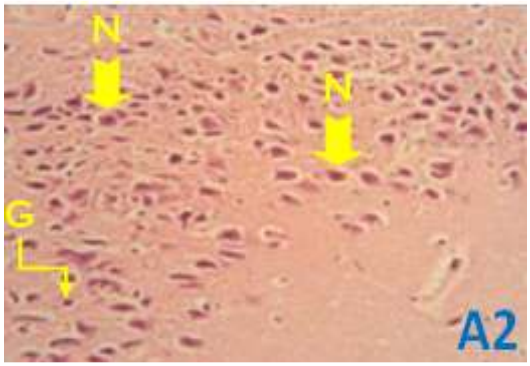

Fig. A2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; showing numerous pyramidal cells and stellate cells as well as glia with normal morphology and normal pattern of distribution. Neuropil also appears normal

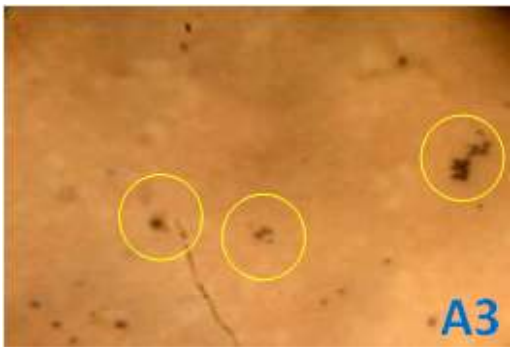

Fig. A3. Histochemical demonstration of the hippocampus, using the Bielschowsky staining technique [x400] showing quite few senile plaques typical of a normal tissue

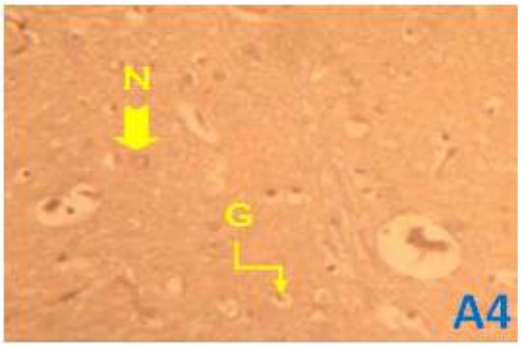

Fig. A4. Histochemical demonstration of Cathepson D expression [x400]; showing normal expression of Cathepson D in the hippocampus

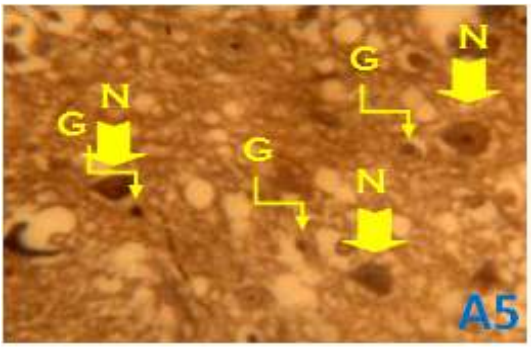

Fig. A5. Histochemical demonstration of p53 expression [x400]; showing moderate expression of p53 in the hippocampus

Group B

Photomicrographs of experimental animals administered a single dose of 2.5 mg/kg body weight of 3CdSO4.8H2O at the onset of experiment and left untreated for the rest of the treatment period [N= Neurons; G = Glia; Senile plaque is being encircled].

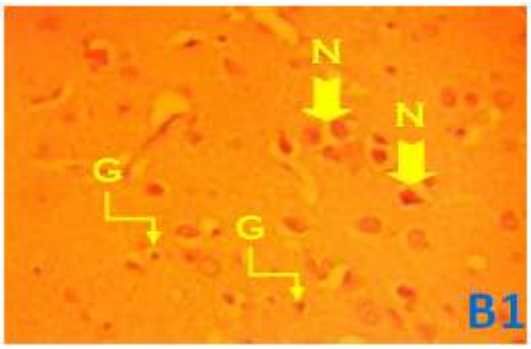

Fig. B1. Histological demonstration of the hippocampus of Group B animals using the H&E staining technique [x400] showing areas of clustered neurons with heterogeneous appearance

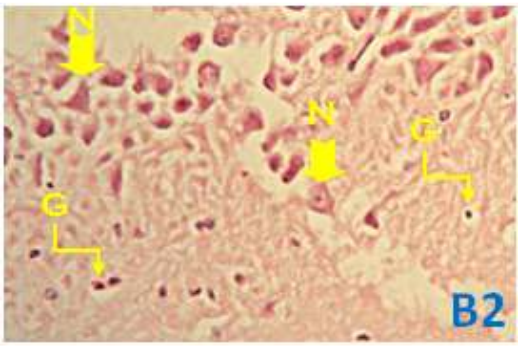

Fig. B2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; showing differential pattern of distribution of neurons and glia; an area of densely packed predominantly pyramidal neurons and another area of prominent glia- predominantly astrocytes

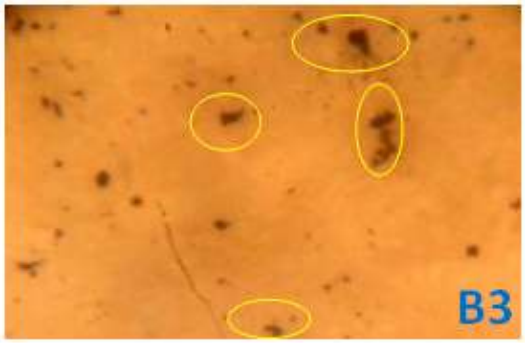

Fig. B3. Histochemical demonstration of the hippocampus using the Bielschowsky staining technique [x400] showing quite numerous senile plaques of various and randomly distributed

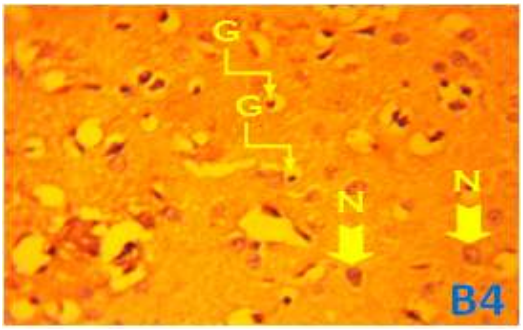

Fig. B4. Histochemical demonstration of Cathepson D expression [x400] in the hippocampus; showing marked expression of Cathepson D in glia and relatively low expression in neurons

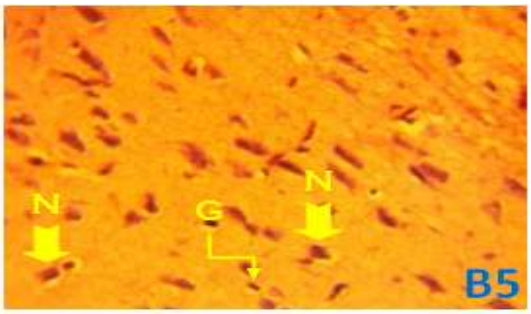

Fig. B5. Histochemical demonstration of p53 expression [x400]; neurons take on unusual morphology and pattern of p53 expression in the hippocampus

Group C

Photomicrographs of the hippocampus of experimental animals administered 5 mg/kg body weight vitamin C orally, daily, throughout the duration of experiment [after cadmium intoxication] [N= Neurons; G = Glia; Senile plaque is being encircled].

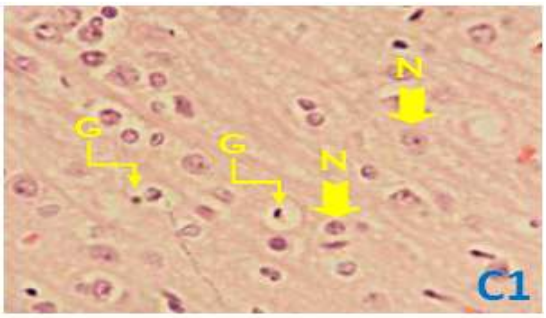

Fig. C1. Histological demonstration of the hippocampus using the H&E staining technique [x400] showing normal neuron morphology and pattern of distribution; glia and neuropil

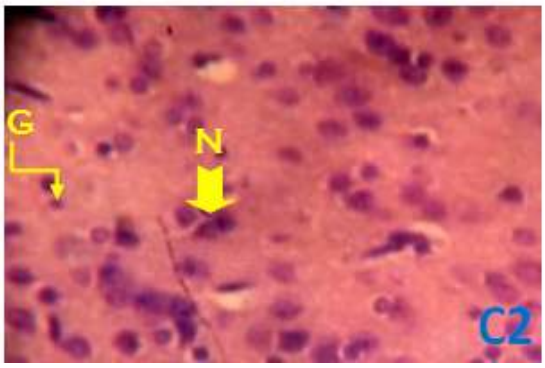

Fig. C2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; showing numerous neurons as well as glia with normal morphology and normal pattern of distribution. Neuropil also appears normal

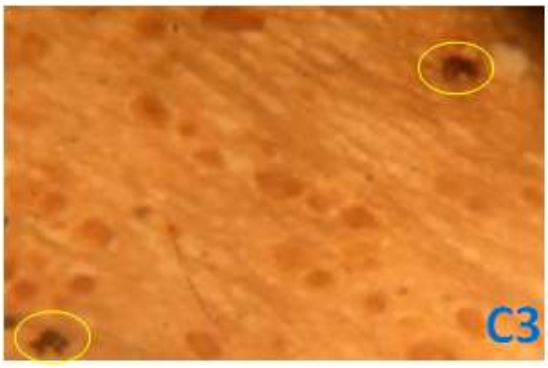

Fig. C3. Histochemical demonstration of the hippocampus, using the Bielschowsky staining technique [x400] showing relatively few senile plaques

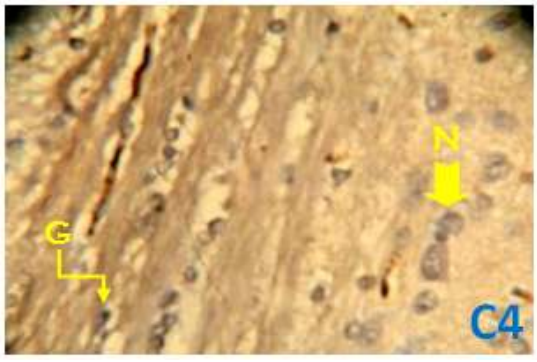

Fig. C4. Histochemical demonstration of Cathepson D expression [x400]; showing relatively normal expression of Cathepson D in the hippocampus

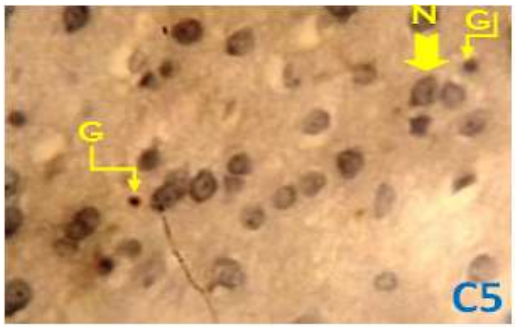

Fig. C5. Histochemical demonstration of p53 expression [x400] in the hippocampus; showing relatively moderate expression of p53 in the frontal cortex tissue

GROUP D

Photomicrographs of the hippocampus of experimental animals administered 6 mg/kg bw Vitamin E orally and daily throughout experimental period [after cadmium intoxication] [N= Neurons; G = Glia; Senile plaque is being encircled].

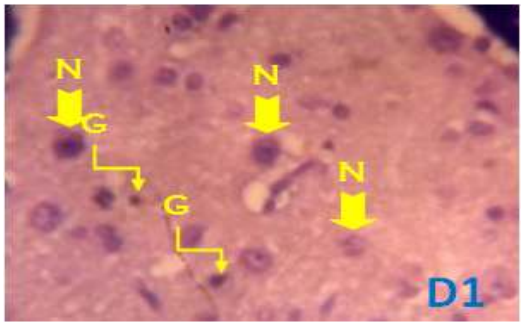

Fig. D1. Histological demonstration of the hippocampus using the H&E staining technique [x400] showing heterogeneous neuron morphology

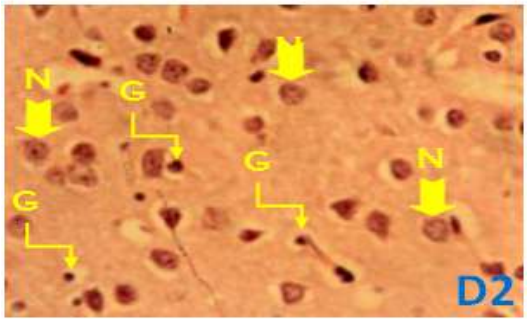

Fig. D2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; showing heterogeneous neuron morphology and prominent astrocytes

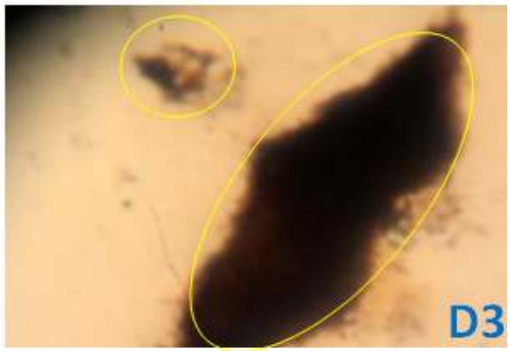

Fig. D3. Histochemical demonstration of the hippocampus, using the Bielschowsky staining technique [x400] showing quite large senile plaques

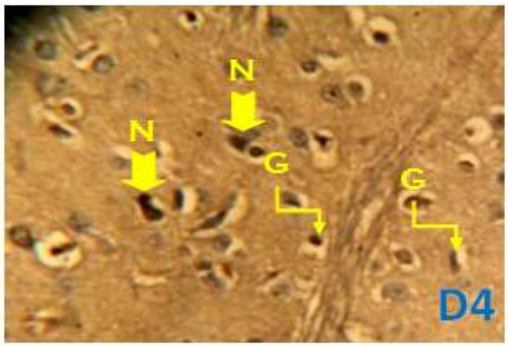

Fig. D4. Histochemical demonstration of Cathepson D expression [x400] in the hippocampus; showing heterogeneous neuron morphology and expression of Cathepson D

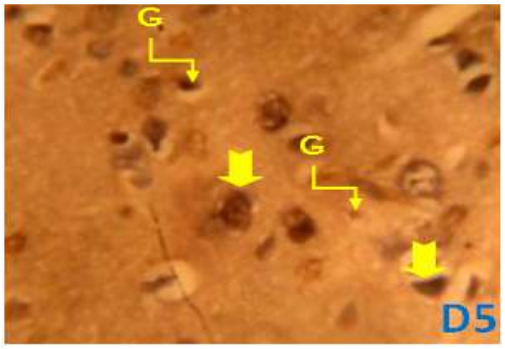

Fig. D5. Histochemical demonstration of p53 expression [x400] in the hippocampus; neurons take on heterogeneous morphology and relatively higher p53 expression in the neurons

Group E

Photomicrographs of the hippocampus of experimental animals administered 4 mg/kg body weight of *Moringa oleifera* seed oil orally and daily [after cadmium intoxication] throughout the duration of experiment.

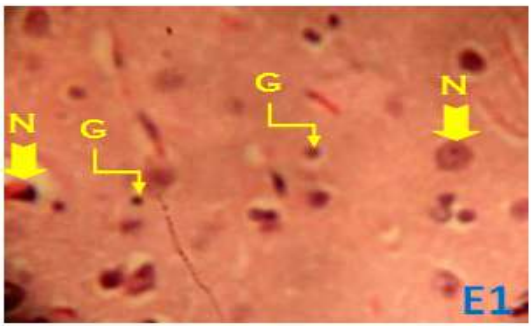

Fig. E1. Histological demonstration of the hippocampus using the H&E staining technique [x400] showing neurons and glia

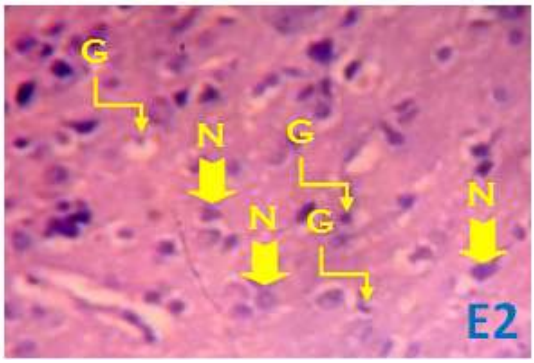

Fig. E2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; showing numerous neurons as well as glia with normal morphology and normal pattern of distribution with normal neuropil

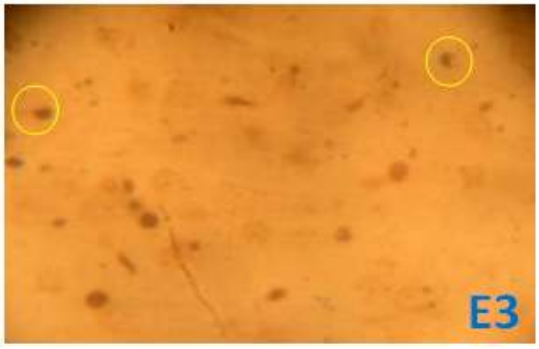

Fig. E3. Histochemical demonstration of the hippocampus tissue, using the Bielschowsky staining technique [x400] showing quite few and small senile plaques

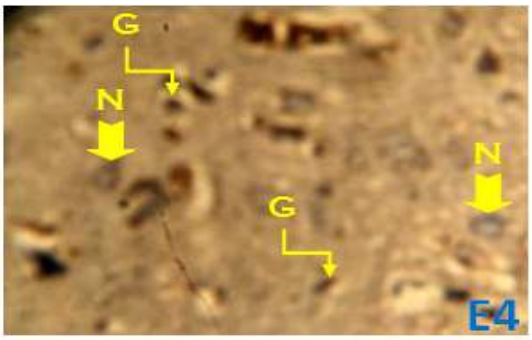

Fig. E4. Histochemical demonstration of Cathepson D expression [x400] in the hippocampus; showing areas of enhanced expression of Cathepson D in frontal cortex tissue

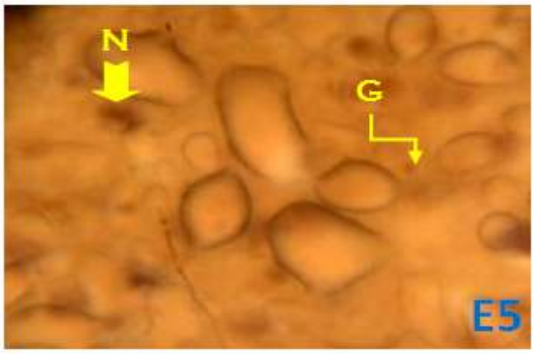

Fig. E5. Histochemical demonstration of p53 expression [x400] in the hippocampus; neurons express p53 relatively moderately

GROUP F

Photomicrographs of the hippocampus of experimental animals administered 4 mg/kgbw of *Anacardium occidentale* nut oil orally and daily [after cadmium toxicity] throughout the duration of experiment.

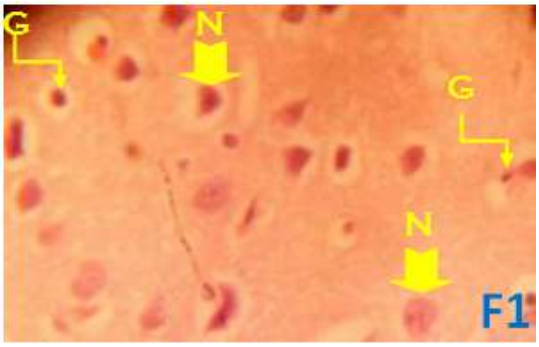

Fig. F1. Histological demonstration of the hippocampus using the H&E staining technique [x400]; neurons and glia are observable

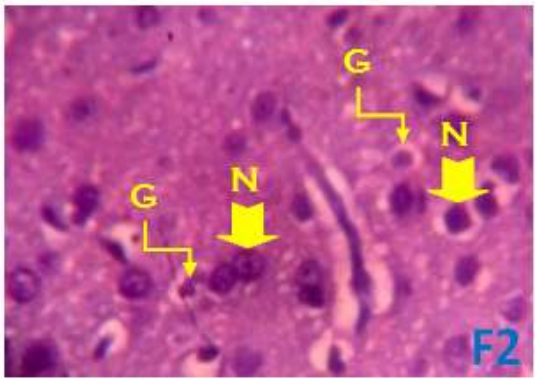

Fig. F2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; neurons and glia are observable and prominent

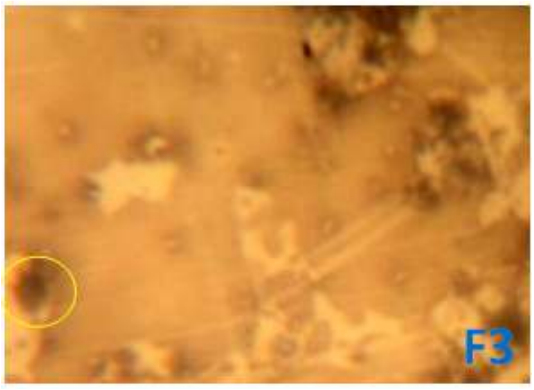

Fig. F3. Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing relatively abundant senile plaques

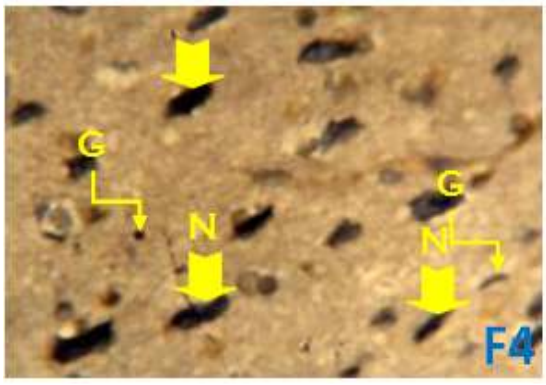

Fig. F4. Histochemical demonstration of Cathepson D expression [x400] in the hippocampus; showing highly enhanced expression of Cathepson D in the neurons

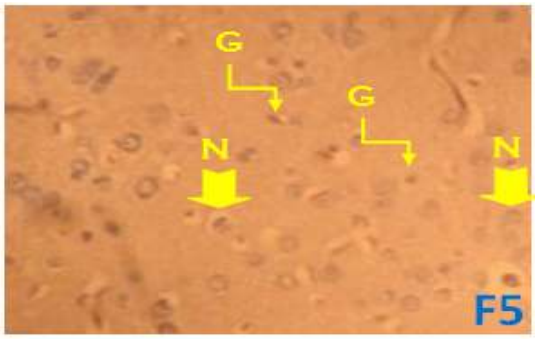

Fig. F5. Histochemical demonstration of p53 expression [x400] in the hippocampus; neurons p53 expression in the tissue is barely observable

GROUP G

Photomicrographs of the hippocampus of experimental animals administered 2 mg/kg body weight of *Moringa oleifera* seed oil plus 2 mg/kg body weight of *Anacardium occidentale* nut oil daily and orally [after cadmium intoxication] throughout the duration of experiment.

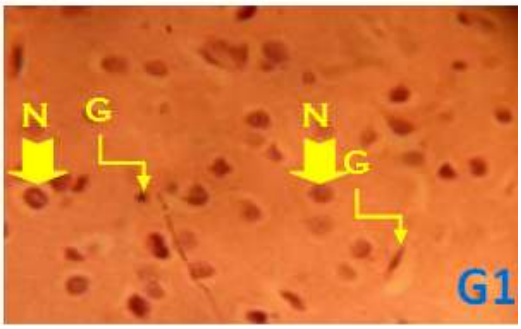

Fig. G1. Histological demonstration of the hippocampus tissue using the H&E staining technique [x400]; neurons and glia are observable and prominent

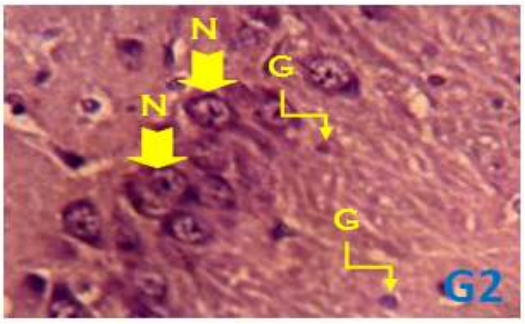

Fig. G2. Histological demonstration of the hippocampus using the Cresyl Fast Violet staining technique [x400]; showing closely packed relatively large neurons

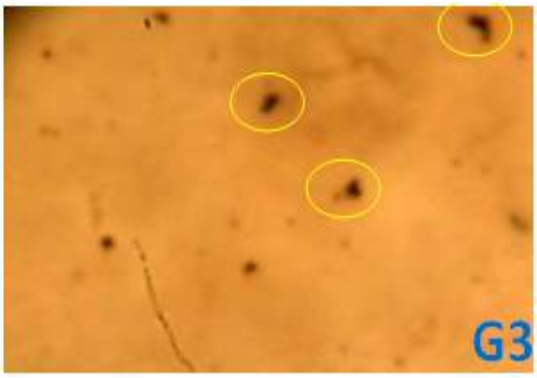

Fig. G3. Histochemical demonstration of the hippocampus, using the Bielschowsky staining technique [x400] showing relatively few small-sized senile plaques

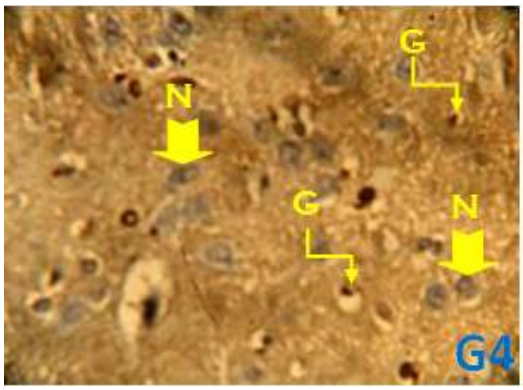

Fig. G4. Histochemical demonstration of Cathepson D expression [x400] in the hippocampus; showing relatively enhanced expression of Cathepson D especially in the glia

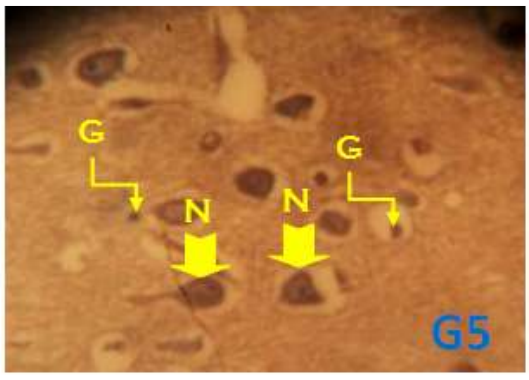

Fig. G5. Histochemical demonstration of p53 expression [x400] in the hippocampus; p53 expression in neurons and glia is barely observable

3. DISCUSSION

The present study evaluated the nature of a one-time single dose cadmium poisoning effects on the hippocampus of experimental animals; it also examined the individual effects of each of the oils- *Moringa oleifera* seed oil and *Anacardium occidentale* oil in ameliorating or neutralising such effects. The possible combined ameliorative effects of *Moringa oleifera* seed oil and *Anacardium occidentale* nut oil extracts on the destructive effects of cadmium on the hippocampus was also examined. Each of two conventional antioxidants- Vitamins C and E were administered to different animal groups to observe effects relative to the antioxidant-rich oils. The histological results showed that the control Group A animals had normal features of the hippocampal tissue. Neurons and glia have normal morphology, characteristic of normal tissues. The expressions of Cathepsin D and p53 were also quite moderate and normal. These features could serve as adequate references with respect to other animal groups.

In Group B which consists of animals administered cadmium to induce intoxication; hippocampus showed features of deleterious effects of cadmium on the tissues. Heterogeneous morphology of neurons as well and unusually prominent glia- especially astrocytes are indications of toxic effects of cadmium on the tissue. More so, there could have been localized rather than randomly distributed damage of tissue as observed in Fig. B2. Numerous senile plaques, quite more than usual for a normal tissue also support evidences of tissue damage. Glia expressed Cathepsin D quite prominently and at a quite high level; this also suggests the possibility of astrocytes reaction to the toxic effects of the substance on the tissue. The pattern of p53 expression was quite unusual and unconventional in Group B. All evidences- histological and histochemical, point to the toxic effects of the administered $3CdSO_4.8H_2O$ on the hippocampus. This supports existing evidences of cellular destructive or intoxicating effects of cadmium; it has already been documented that cadmium causes cellular destruction, even cellular death at high concentrations [22].

Animals in Group C which were treated with 5 mg/kg body weight Vitamin C showed normal features of a hippocampal tissue. The hippocampus is well populated by neurons and glia which are also normal in morphology. Relative to the control, the expressions of p53 and Cathepsin D are adequate and normal. Senile plaques are also few and do not show any characteristic of anomaly relative to the control.

Animals in Group D which were treated with 6 mg/kg body weight Vitamin E [after a single dose of 2.5 mg/kg body weight $3CdSO_4.8H_2O$], showed signs of altered hippocampal tissues due to toxic effects of cadmium. Neurons and glia are observable; neurons are however unusually heterogeneous and this suggests uncorrected effects of cadmium assault. The abnormally large senile plaques also point to tissue damage with serious molecular consequence. While the expression of Cathepson D might be low, that of p53 is pronounced. Neuron morphology show little improvement over the untreated case in Group D; it is however obvious that it is not a case of complete correction or healing of the effects of cadmium intoxication. Vitamin E has potential to protect critical cellular structures against damage caused by oxygen free radicals and reactive products of lipid peroxidation; lipid peroxidation is prevented by vitamin E [23].

Animals in Group E which were administered cadmium but treated with 4 mg/kg body weight of *Moringa oleifera* seed oil showed improvement in cytoarchitecture of the hippocampus. Neurons and glia as well as the neuropil are preserved and observable. Cathepson D is however prominently expressed relative to the Group D especially that were treated with selected anti-oxidants, as well as the control Group. It is important to note that there are quite few and less prominent senile plaques. It is clear that there is an improvement in this tissue over the untreated Group B. It also appears that the extent of damages that led to the formation of plaques is being greatly limited in this particular group. The heterogeneity of neuronal morphology is reduced (Fig. E) relative to Group B (Fig. B).

There are evidences that *Moringa oleifera* seed oil effects against cadmium toxicity could have limited the extent of damage; also, the consequence is that excessive development of plaques as a result of such damage is prevented [especially when compared with the untreated Group B]. In terms of potency against the formation of plaques, Moringa seed oil appears better than the selected antioxidants in terms of prevention of excessive plaque formation. These features might be due to vitamin A, vitamin C, vitamin E and oxalic acid [24]. These

constituents are said to have antioxidant effects, thus ability to remove superoxide and oxygen free radicals that are usually implicated in tissue destruction. Ascorbic acid acts as a powerful hydrosoluble antioxidant in biological fluids; and *Moringa oleifera* contains it.

The Group F animals which that were administered cadmium but treated with 4 mg/kg body weight of *Anacardium occidentale* nut extract, showed cytoarchitecture of the hippocampal tissue almost similar to those seen in group E animals which were administered cadmium and treated with *Moringa oleifera* seed oil. Neurons and glia were preserved. Cathepson D is highly expressed; senile plaques are relatively smaller than in the untreated Group B, but more prominent than that of the Group E treated with *Moringa oleifera* seed oil. This is also expectedly due to its natural antioxidant property [19]. A previous study showed that immature cashew nut-shell liquid (iCNSL) is a source of unsaturated long-chain phenols and may have a potential role in protecting against oxidative damage [20].

The Group G animals, which were administered cadmium to induce intoxication and treated with both 2 mg/kg body weight of *Moringa oleifera* seed oil and 2 mg/kg body weight of *Anacardium occidentale* oil, showed cytoarchitecture of the hippocampal tissue almost similar to those seen in Groups E and F animals due to their antioxidant properties but with more complete tissue repairs or regeneration. Neurons and glia are well preserved and they are prominent and well distributed. Senle plaques are very few and less prominent in a manner similar to the Group D treated with Moringa seed oil only. This suggests that the Moringa seed oil might be more potent in preventing the excessive formation of these plaques than other agents used. The combined effects of both oils in preserving neurons morpholgy however show possiblity of greater potency

Generally, cells in the hippocampus manifested some cadmium-induced changes ranging from altered morphology and population densities as well as the cell's relative distribution. This implies that the activity of the hippocampus in memory formation and learning will be impaired and the role of the hippocampus that involved storage and retrieval of information will also be compromised. Antioxidants exhibit positive effects that could ameliorate the damaging effects of cadmium on the hippocampus as seen in this investigation. The natural oils show improvement over the selected regularly used anti-oxidants, especially in limiting the extents of damage and the consequences in the hippocampus. Moringa seed oil produced better prevention of plaques especially, relative to *Anacardium occidentale* oil. It will be important to examine the roles of other active phytochemicals in these oils in the differential potencies of the oils, as well as the mechanism of the effects.

4. CONCLUSION

It can be concluded from the present study that cadmium intoxication has observable deleterious effects on brain tissues as a result of intra-peritoneal administration of cadmium sulphate. All agents used produced positive anti-cadmium effects, however to varying degrees. While all agents ameliorated the effects of cadmium toxicity on neuronal morphology and relative population cum distribution, moringa seed oil was the most potent in reducing the formation of senile plaques in the hippocampus. The potency combined effect of *Moringa oleifera* seed oil and *Anacardium occidentale* nut oil in reducing or treating the damage caused by cadmium on the hippocampus is also observed. The natural oils were more potent than the selected regularly used antioxidants.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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