

HISTOLOGICAL AND HISTOCHEMICAL EVALUATION OF- ANTI-CADMIUM
TOXICITY EFFECTS OF *MORINGA OLEIFERA* SEED OIL AND *ANACARDIUM
OCCIDENTALE* NUT OIL IN THE HIPPOCAMPUS OF JUVENILE MALE WISTAR RATS

Abstract

The hippocampus is the part of the brain that is 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. 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 the effects of cadmium toxic effects particularly on the hippocampus. A total of 35 juvenile male Wistar rats were used for the study. They ~~were the~~ rats were randomly grouped into **seven** A, B, C, D, E, F and G; each containing 5 animals. Group A, Group B and Group C animals were given ~~3mL3ml~~ per kg body weight of 0.9% NaCl solution intraperitoneally for 3weeks; toxicity of Cadmium was induced in each rat of in-group B ~~was induced intraperitoneally~~ with a single dose of 2.5mg/kg body weight of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ intraperitoneally, and in group C, each rat was given 5mg/kg body weight vitamin C dissolved in 0.9% NaCl solution along with ~~and~~ 6mg/kg body weight vitamin E dissolved in olive oil orally for 3 weeks. Group D animals were given 5mg/kg body weight vitamin C dissolved in 0.9% NaCl solution and 6mg/kg body weight vitamin E dissolved in olive oil orally for 3 weeks, after which each rat was induced intra-peritoneally with a single dose of 2.5mg/kg body weight of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. Groups E, F and G were first given 2.5mg/kg body weight of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, and after 48 hours, treatment began. Group E rats were treated with 4mg/kg body weight *Moringa oleifera* ~~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 2mg/kg body weight *Moringa oleifera* ~~Oleifera~~ seed oil + 2mg/kg body weight *Anacardium occidentale* ~~Occidentale~~ nut oil. 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 on neuronal morphology and relative population cum distribution, *Moringa* ~~moringa~~ seed oil was the most potent in reducing the formation of senile plaques in the hippocampus. The potency combined effect of *Moringa* ~~moringa~~ *oleifera* seed oil and *Anacardium occidentale* nut oil in reducing or treating the oxidative damage caused by cadmium on the hippocampus is also observed. The natural oils were more potent than the selected regularly used antioxidants.

Key Words: Hippocampus, Cadmium toxicity, ~~Toxicity~~, Antioxidants, *Moringa oleifera*, *Anacardium occidentale*

Introduction

The hippocampus is believed to play important roles in several functions of the body. 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, 5]. The alveus is the deepest layer and contains the axons from pyramidal neurons, passing on towards the fimbria/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 to CA. 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 LD₅₀ of 5.7mg/Kg per 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 Cd-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-phosphate-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 Cd [10].

Moringa oleifera (*Moringa*) is a rich source of antioxidant [15] and has an LD₅₀ of 340 – 400mg/Kg body weight intraperitoneally on rats [15]. It has been reported that aqueous extracts of leaf, fruit and seed of *Moringa* act as an antioxidant. 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 activity with 65.1 and 66.8%, respectively [16, 17]. [16]; Siddhuraju and Becker, 2003[17]). It was also reported that the major bioactive compounds of phenolics, such as quercetin and kaempferol are responsible for antioxidant activity [17].; Siddhuraju and Becker, 2003[17]). During another

study, quercetin and kaempferol have shown good antioxidant activity on hepatocyte growth factor (HGF) induced methionine phosphorylation with IC₅₀ 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* seed are superiors for radical scavenging [18].(Ogbunugafor *et al.*, 2011 [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].(Jaiswal *et al* 2010 [19]). Cashew nut oil has an LD₅₀ of 1230mg/kg body weight on rats intraperitoneally [19].(Jaiswal *et al* 2010 [19]). Effects of immature cashew nut-shell liquid (*Anacardium occidentale*) against oxidative damage in *Saccharomyces cerevisiae* and inhibition of acetylcholinesterase activity were reported. A study showed immature cashew nut shell liquid (iCNSL), a source of unsaturated long-chain phenols, and may have a potential role in protecting DNA against oxidative damage [20].(Isao, 2005 [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 analysis. 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.

Materials and Methods

Moringa seed, 2000g, was produced from kuchikau town, Nassarawa state, Nigeria. 2000g of the powdered *Moringa oleifera* seed-oil was extracted by the following procedure. The seeds were heated inside an oven at temperature of 40°C-40°C and were later pounded with mortar and pestle to separate the chaff from the seeds. Finally the seed extract was grinded into powder with a grinding mill. The powdered extract of *Moringa oleifera* seed was dissolved in 400mL-400ml of water at ambient temperature for two days and was 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°C-20°C before administration. 2000g of Cashew nut was produced from kuchikau town, Nassarawa state, Nigeria. 2000g of the powdered Cashew nut-oil was extracted by the following procedure. The seeds were heated inside an oven at temperature of 40°C-40°C and the seed extract was 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 and oil extract was kept at -20°C-20°C before administration.

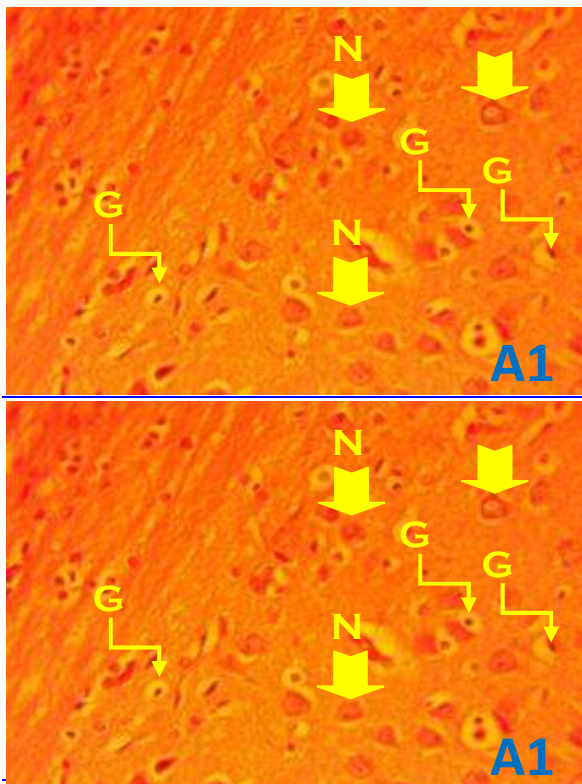
The Cadmium Sulphate solution was prepared by dissolving the Cadmium Sulphate salt in normal saline solution. The Ascorbic acid (Vitamin C) was dissolved in normal saline solution and the Vitamin E (tocopherol) was dissolved in olive oil. All other chemicals were of the highest purity commercially available. 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 with a ball point at the tip.

A total of 35 juvenile male Wistar rats (100-200g) were used for the study. They were maintained under standard laboratory condition in the animal house (National Academy Press, 1996) the rats were randomly grouped into **seven** A, B, C, D, E, F and G, each containing 5 animals. Group A, Group B ~~and Group C~~ animals were given ~~3mL3ml~~ per kg body weight of 0.9% NaCl solution intraperitoneally for 3weeks; toxicity of Cadmium was induced in each rat ~~of in~~ group B ~~was induced intraperitoneally~~ with a single dose of 2.5mg/kg body weight of 3CdSO₄.8H₂O intraperitoneally, and in group C, each rat was given 5mg/kg body weight vitamin C dissolved in 0.9% NaCl solution and 6mg/kg body weight vitamin E dissolved in olive oil orally for 3 weeks. Group D animals were given 5mg/kg body weight vitamin C dissolved in 0.9% NaCl solution and 6mg/kg body weight vitamin E dissolved in olive oil orally for 3 weeks, after which each rat was induced intraperitoneally with a single dose of 2.5mg/kg body weight of 3CdSO₄.8H₂O, and after 48 hours, the rats were sacrificed. Groups E, F and G ~~were~~ were first induced intraperitoneally with 2.5mg/kg body weight of 3CdSO₄.8H₂O, and after 48 hours, treatment began. Group E rats were treated with 4mg/kg body weight *Moringa oleifera* ~~Oleifera~~ seed oil each, group F rats were treated with 4mg/kg body weight *Anacardium occidentale* ~~Occidentale~~ nut oil and group G rats were treated with 2mg/kg body weight *Moringa oleifera* ~~Oleifera~~ seed oil + 2mg/kg body weight *Anacardium occidentale* ~~Occidentale~~ nut oil.

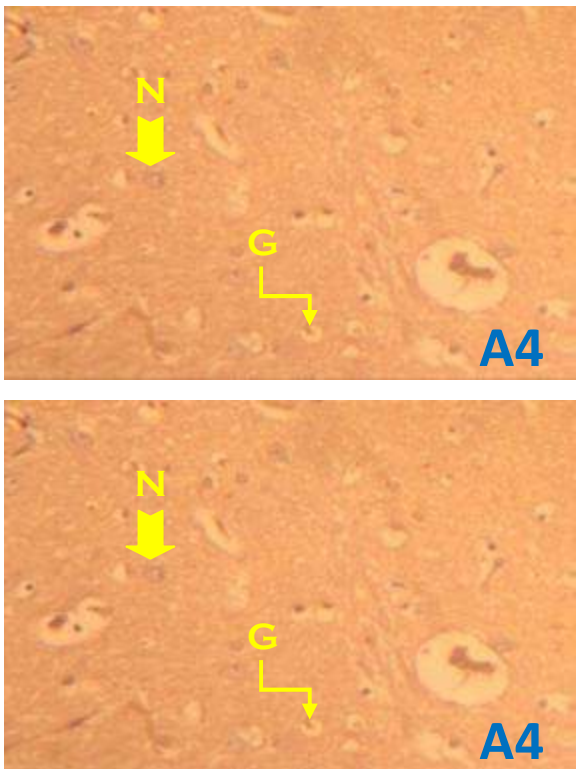
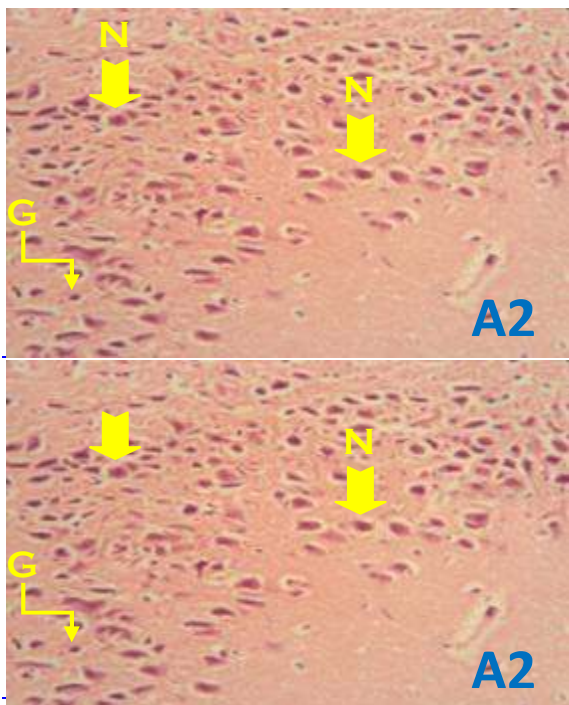
The animals were treated as for three (3) weeks; twenty four hours after the last administration, the rats were sacrificed by cervical dislocation. The skulls of the rats were then fractured open with forceps, the brains were removed into specimen bottles containing 40% formal calcium solution for histological and immunohistochemical analyses. Ethical approval to conduct research was obtained from the Department of Anatomy, Bingham University, Nigeria.

Table 1: Grouping of Animals

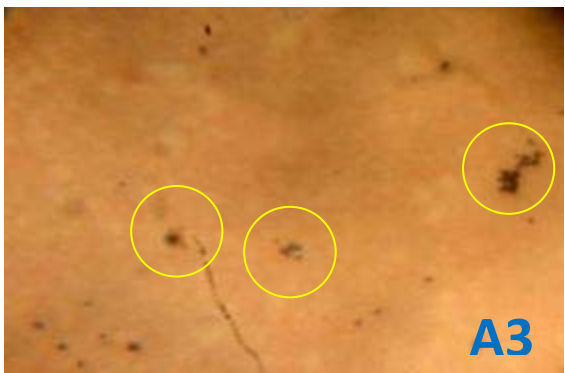
Grou	Substance Administration
p	
A	~~3mL3ml~~ of 0.9% NaCl solution per kg <u>b.w.bw</u>
B	Single dose of 2.5mg/kg bw 3CdSO ₄ .8H ₂ O
C	5mg/kg <u>b.w.bw</u> Vit. C + 6mg/kg <u>b.w.bw</u> Vit. E
D	5mg/kg <u>b.w.bw</u> Vit. C + 6mg/kg <u>b.w.bw</u> Vit. E + single dose 2.5mg/kg <u>b.w.bw</u> 3CdSO ₄ .8H ₂ O
E	4mg/kg <u>b.w.bw</u> of <i>Moringa oleifera</i> ~~Oleifera~~ seed oil
F	4mg/kg <u>b.w.W</u> of <i>Anacardium occidentale</i> ~~Occidentale~~ nut oil
G	2mg/kg <u>b.w.bw</u> of <i>Moringa oleifera</i> ~~Oleifera~~ seed oil + 2mg/kg <u>b.w.bw</u> of <i>Anacardium occidentale</i> ~~Occidentale~~ nut oil



209 **Figure A1:** Histological demonstration of the frontal
210 cortex tissue using the H&E staining technique
211 [x400] showing normal neurons, glia and neuropil.
212
213



216 **Figure A2:** Histological demonstration using the
217 Cresyl Fast Violet staining technique [x400];
218 showing numerous pyramidal cells and stellate cells
219 as well as glia with normal morphology and normal
220 pattern of distribution. Neuropil also appears normal
221



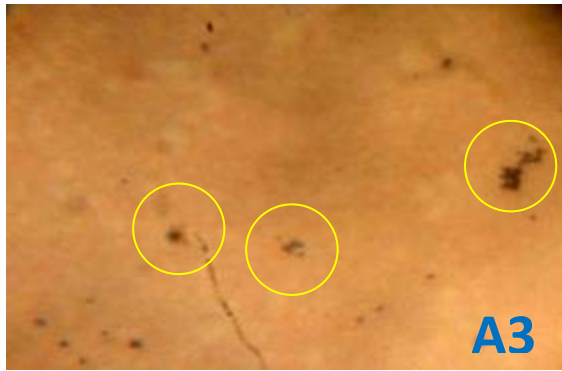


Figure A3: Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing quite few senile plaques typical of a normal tissue.

Figure A4: Histochemical demonstration of Cathepsin D expression [x400]; showing normal expression of Cathepsin D in frontal cortex tissue

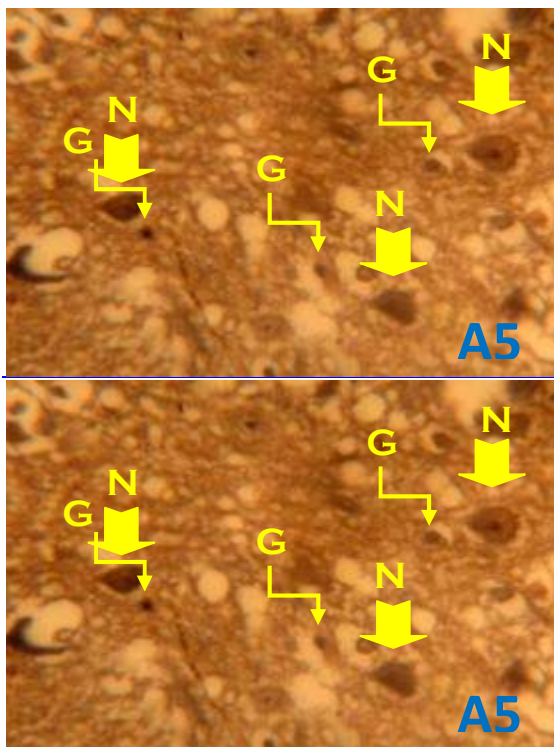


Figure A5: Histochemical demonstration of p53 expression [x400]; showing moderate expression of p53 in frontal cortex tissue.

GROUP A

Photomicrographs of the hippocampus of experimental animals administered 3ml of 0.9% NaCl solution per kg body weight as

the control [N= Neurons; G = Glia; Senile plaque in being encircled].

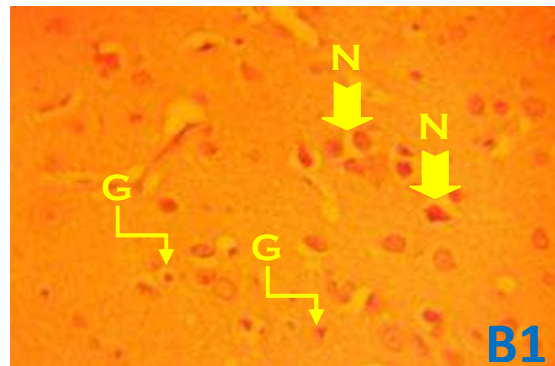
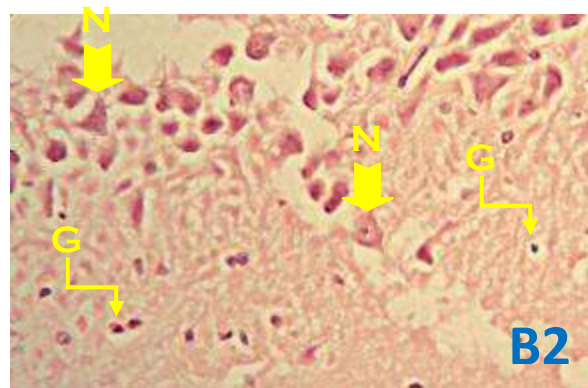


Figure B1: Histological demonstration of the frontal cortex tissue of Group B animals using the H&E staining technique [x400] showing areas of clustered neurons with heterogeneous appearance.



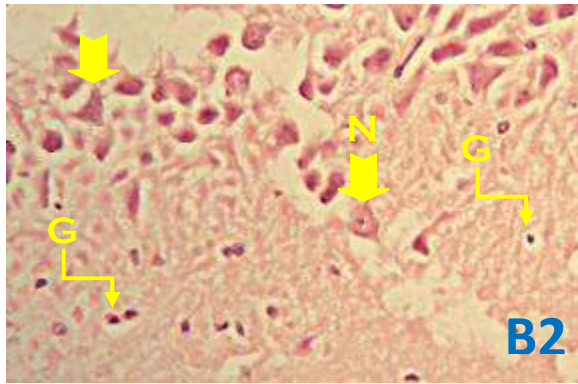


Figure B2: Histological demonstration using the Cresyl Fast Violet staining technique [x400]; showing differential pattern of distribution of neurons and glia; area of densely packed predominantly pyramidal neurons and another area of prominent glia- predominantly astrocytes.

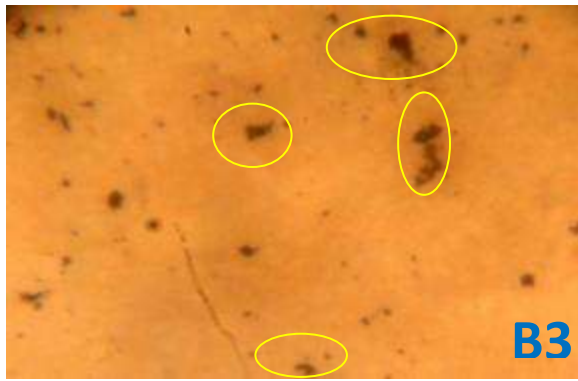


Figure B3: Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing quite numerous senile plaques of various and randomly distributed.

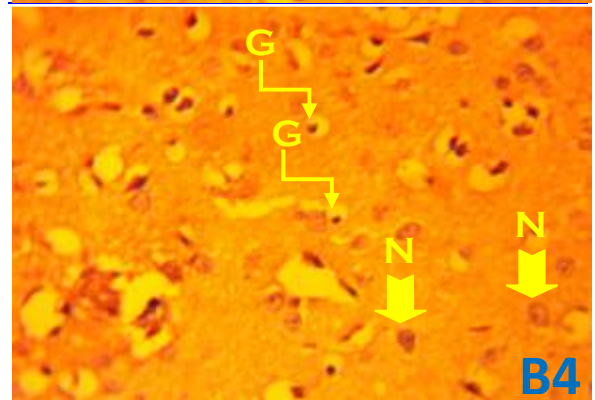
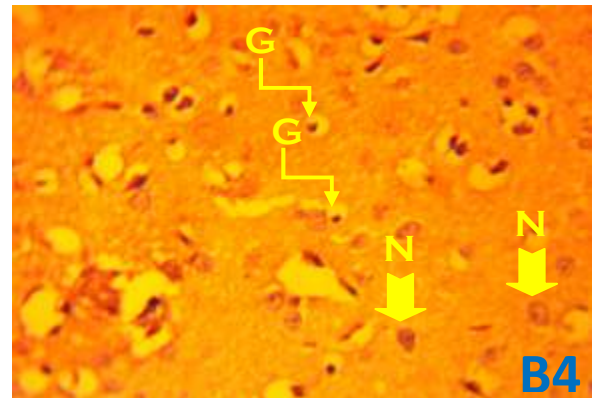
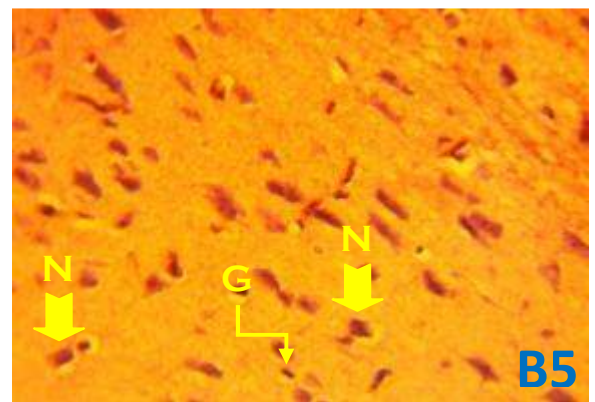


Figure B4: Histochemical demonstration of Cathepsin D expression [x400]; showing quite expression of Cathepsin D in glia and relatively low expression in neurons.



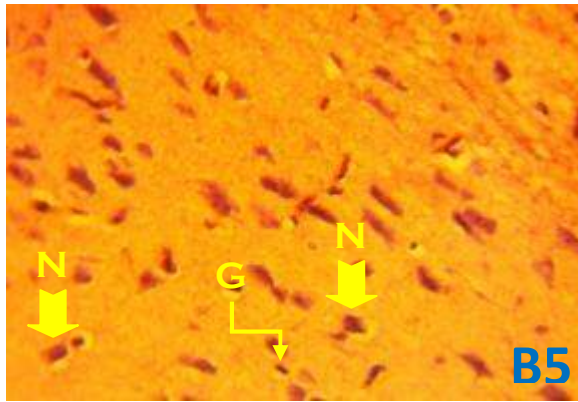


Figure B5: Histochemical demonstration of p53 expression [x400]; neurons take on unusual morphology and pattern of p53 expression in the frontal cortex tissue.

GROUP B

Photomicrographs of experimental animals administered a single dose of 2.5mg/kg body weight of $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ at the onset of experiment [N= Neurons; G = Glia; Senile plaque in being encircled]

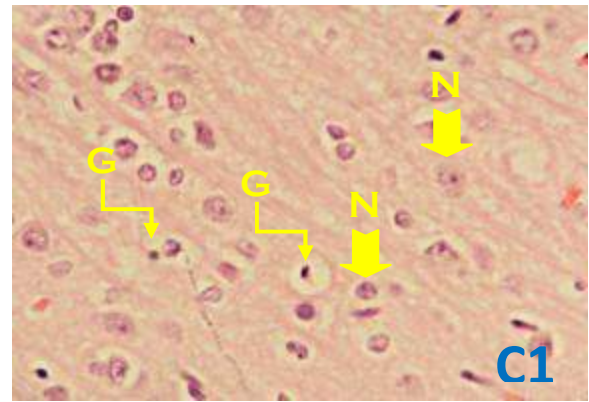
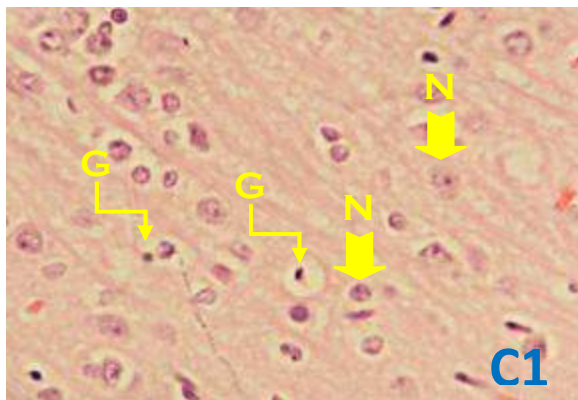


Figure C1: Histological demonstration of the frontal cortex tissue using the H&E staining technique [x400] showing normal neuron morphology and pattern of distribution; glia and neuropil.

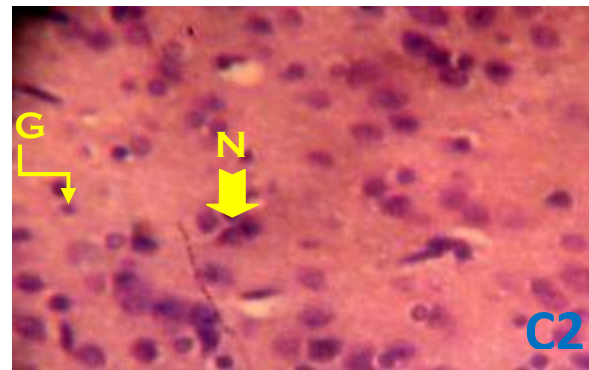


Figure C2: Histological demonstration using the Cresyl Fast Violet staining technique [x400]; showing numerous neurons as well as glia with normal morphology and normal pattern of distribution. Neuropil also appears normal.

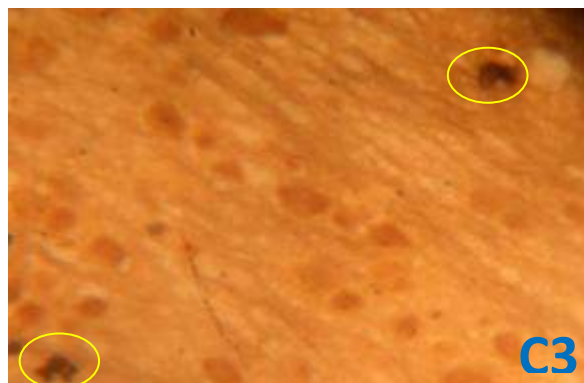


Figure C3:— Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing relatively few senile plaques.

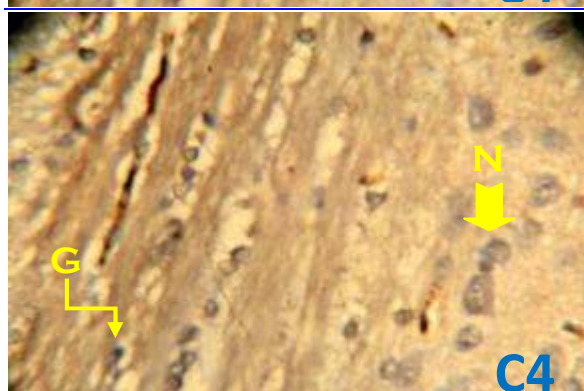
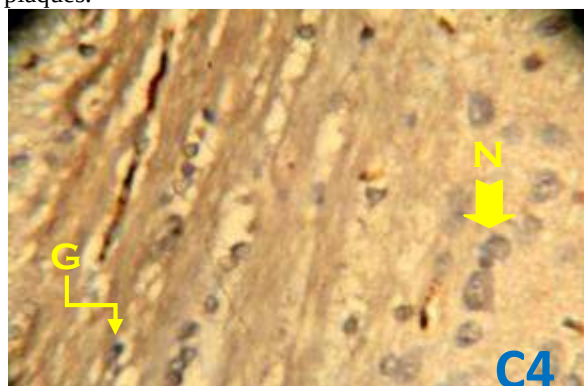


Figure C4: Histochemical demonstration of Cathepsin D expression [x400]; showing relatively

normal expression of Cathepsin D in frontal cortex tissue

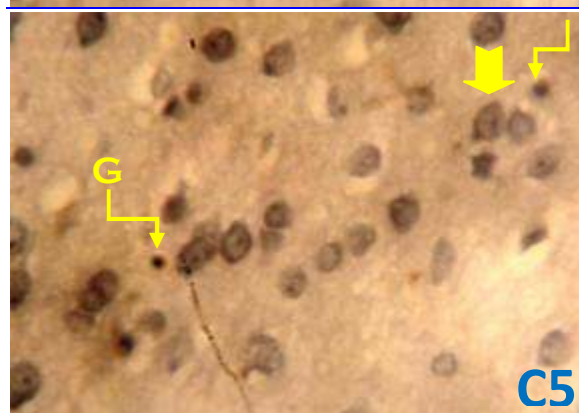
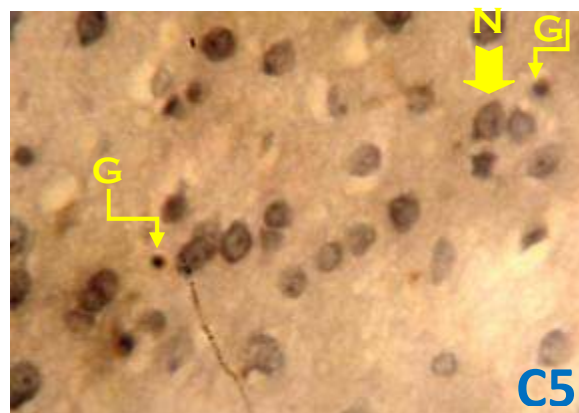


Figure C5: Histochemical demonstration of p53 expression [x400]; showing relatively moderate expression of p53 in the frontal cortex tissue.

GROUP C

Photomicrographs of the hippocampus of experimental animals administered 5mg/kg body weight Vit. C + 6mg/kg body weight Vit. E throughout the duration of experiment [N= Neurons; G = Glia; Senile plaque in being encircled]

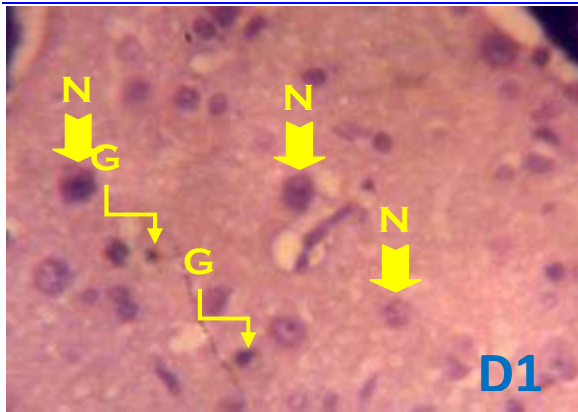
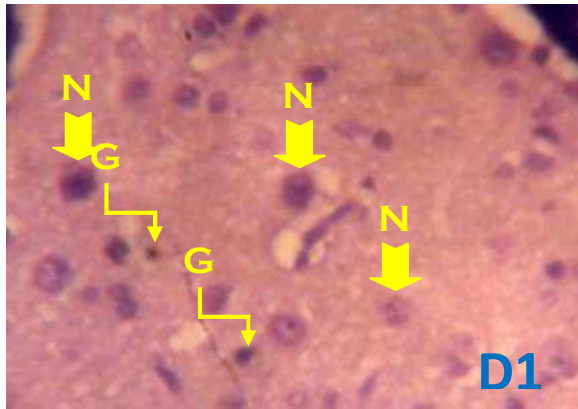


Figure D1: Histological demonstration of the frontal cortex tissue using the H&E staining technique [x400] showing heterogeneous neuron morphology

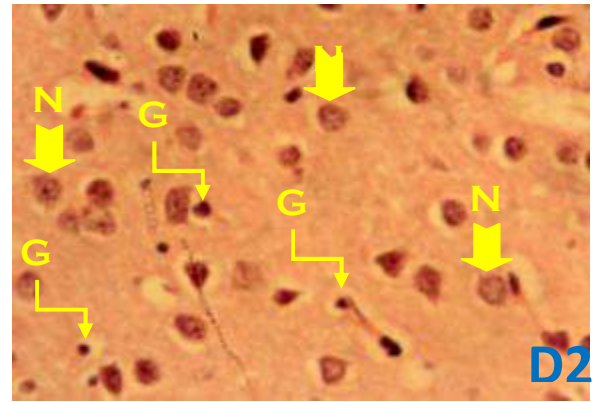
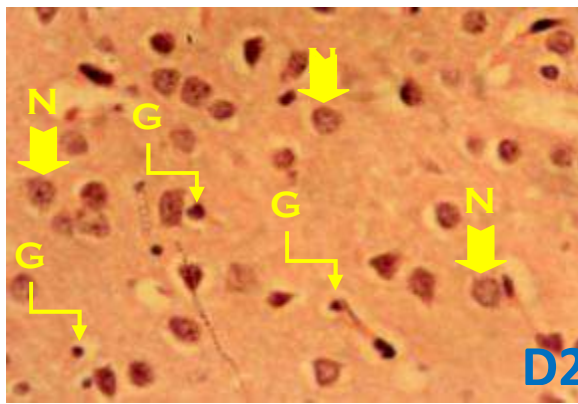


Figure D2: Histological demonstration using the Cresyl Fast Violet staining technique [x400]; showing heterogeneous neuron morphology and prominent astrocytes

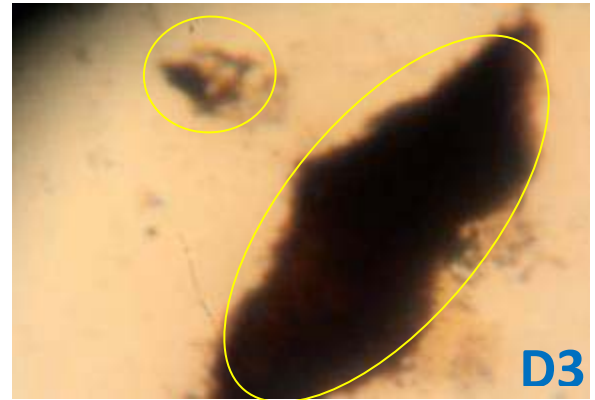


Figure D3: Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing quite large senile plaques.

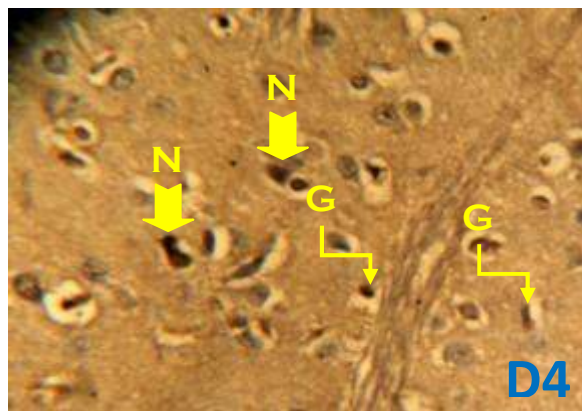


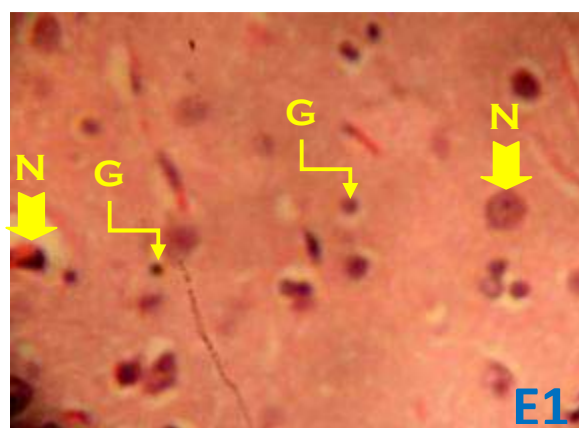
Figure D4: Histochemical demonstration of Cathepsin D expression [x400]; showing heterogeneous neuron morphology and expression of Cathepsin D.



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

GROUP D

Photomicrographs of the hippocampus of experimental animals administered 5mg/kg body weight Vit. C + 6mg/kg bw Vit. E + single dose 2.5mg/kg body weight 3CdSO₄.8H₂O [N= Neurons; G = Glia; Senile plaque in being encircled]



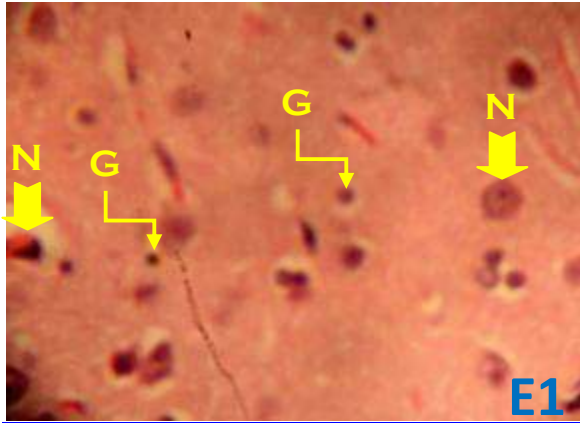


Figure E1: Histological demonstration of the hippocampus using the H&E staining technique [x400] showing neurons and glia

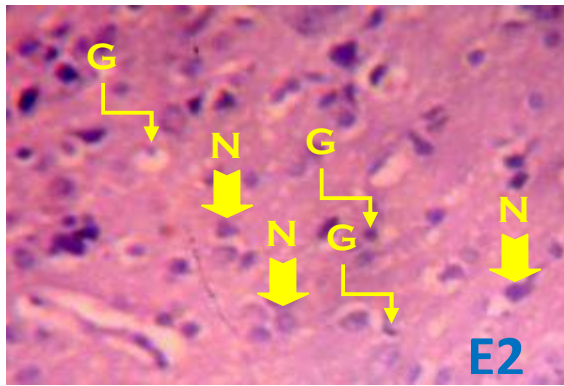


Figure E2: Histological demonstration using the Cresyl Fast Violet staining technique [x400]; showing numerous neurons as well as glia with normal morphology and normal pattern of distribution with normal neuropil

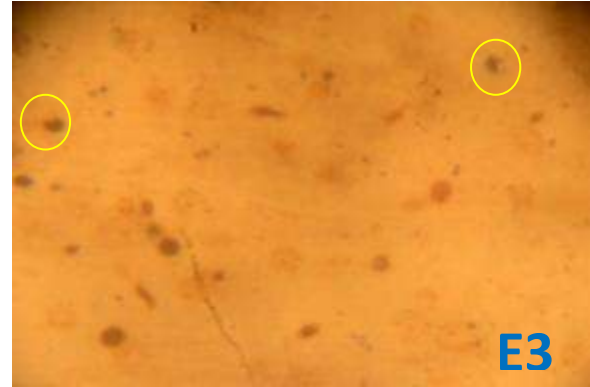


Figure E3: Histochemical demonstration of the hippocampus tissue, using the Bielschowsky staining technique [x400] showing quite few and small senile plaques





441
442

Figure E4: Histochemical demonstration of Cathepsin D expression [x400]; showing areas of enhanced expression of Cathepsin D in frontal cortex tissue



Figure E5: Histochemical demonstration of p53 expression [x400]; neurons express p53 relatively moderately

GROUP E

Photomicrographs of the hippocampus of experimental animals administered 4mg/kg body weight of *Moringa oleifera* seed oil throughout the duration of experiment

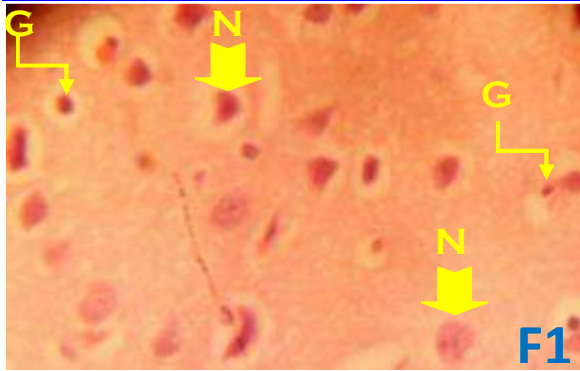
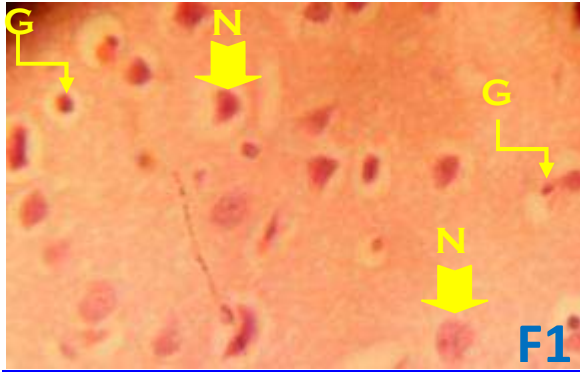


Figure F1: Histological demonstration of the frontal cortex tissue using the H&E staining technique [x400]; neurons and glia are observable

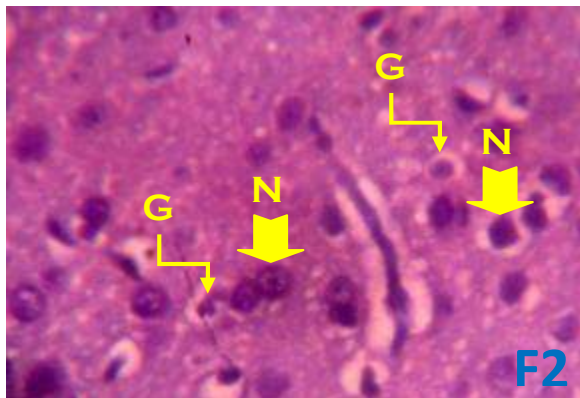


Figure F2: Histological demonstration of the frontal cortex tissue using the H&E staining technique [x400]; neurons and glia are observable

Figure F2: Histological demonstration using the Cresyl Fast Violet staining technique [x400]; neurons and glia are observable and prominent

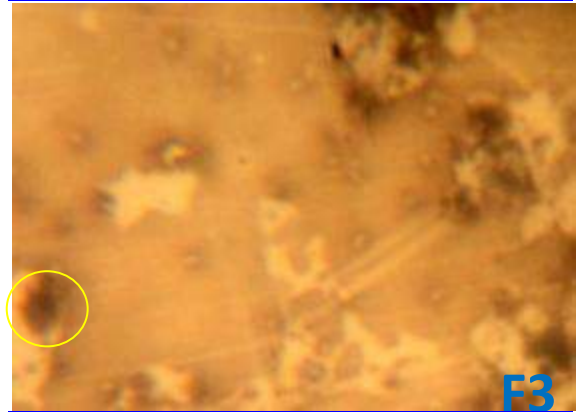
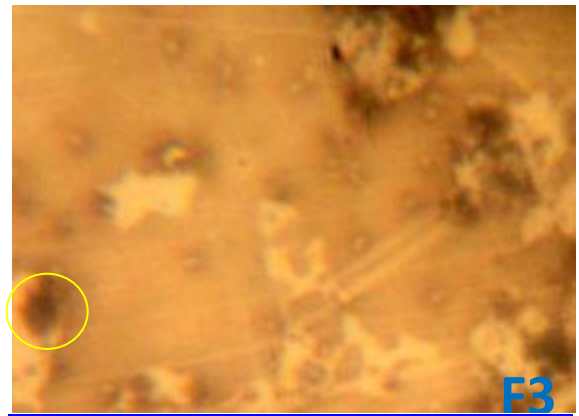


Figure F3: Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing relatively abundant senile plaques.

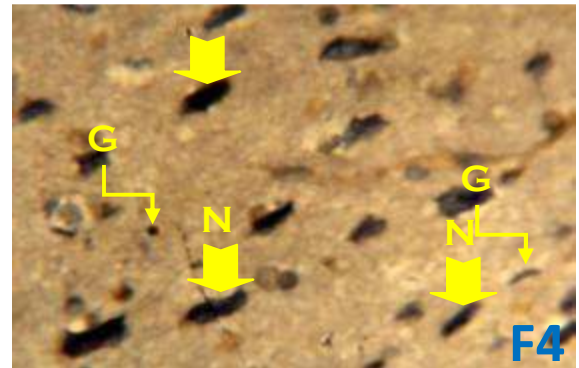


Figure F4: Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing relatively abundant senile plaques.

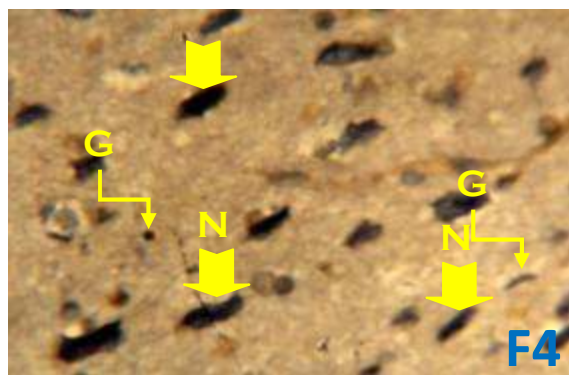


Figure F4: Histochemical demonstration of Cathepsin D expression [x400]; showing highly enhanced expression of Cathepsin D in the neurons



Figure F5: Histochemical demonstration of p53 expression [x400]; neurons p53 expression in the cells is barely observable.

GROUP F

Photomicrographs of the hippocampus of experimental animals administered 4mg/kg b.w.W of *Anacardium- occidentale* nut oil throughout the duration of experiment

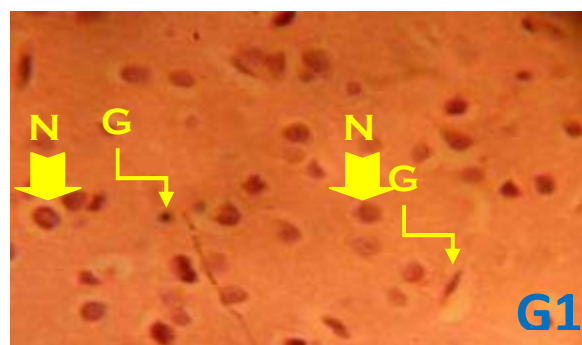


Figure G1:— Histological demonstration of the hippocampus tissue using the H&E staining technique [x400]; neurons and glia are observable and prominent



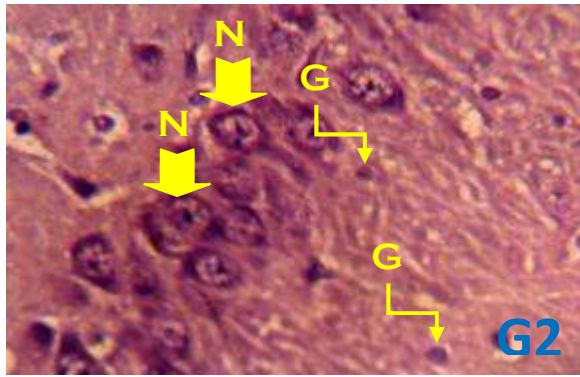


Figure G2: Histological demonstration using the Cresyl Fast Violet staining technique [x400]; showing closely packed relatively large -neurons

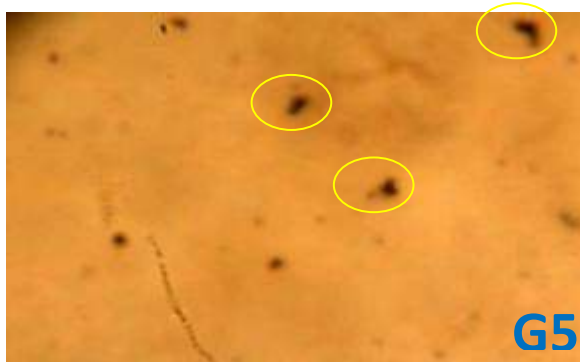


Figure G3: Histochemical demonstration of the frontal cortex tissue, using the Bielschowsky staining technique [x400] showing relatively few small-sized senile plaques.

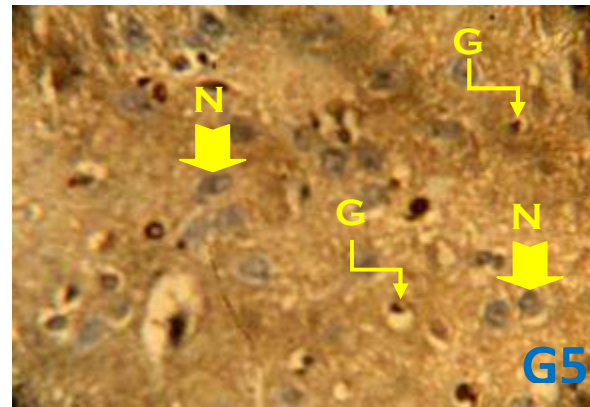
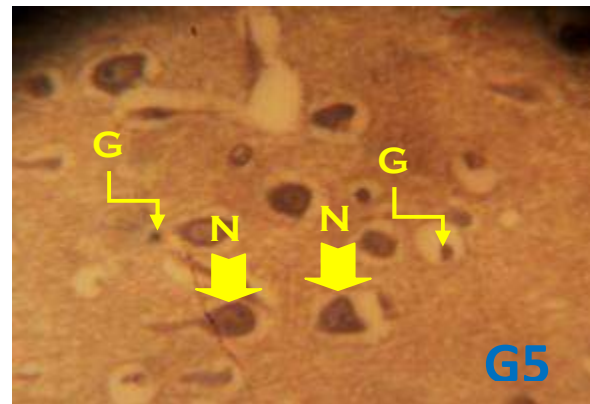


Figure G4: Histochemical demonstration of Cathepsin D expression [x400]; showing relatively enhanced expression of Cathepsin D especially in the glia



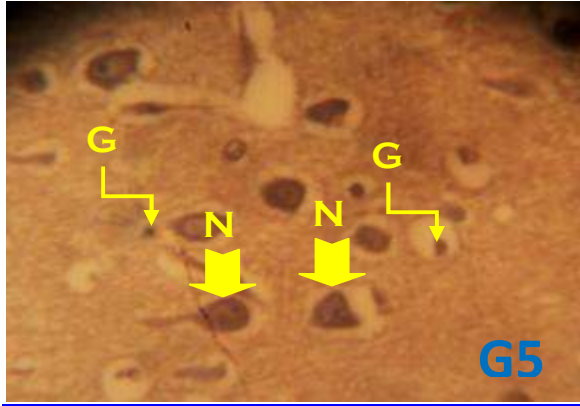


Figure G5: Histochemical demonstration of p53 expression [x400]; p53 expression in neurons and glia is barely observable.

529

GROUP G

Photomicrographs of the hippocampus of experimental animals administered 2mg/kg body weight of *Moringa oleifera* seed oil + 2mg/kg body weight of *Anacardium occidentale* nut oil throughout the duration of experiment

537

538 .

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. The histological results showed that the control group A animals have normal features of a 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 induced with only cadmium 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 damage as observed in Figure 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 $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ on the hippocampus. -This supports existing evidences of cellular destructive effects of cadmium; it has already been documented that cadmium causes cellular destruction, even cellular death at high concentration [21].

Animals in group C which were treated with 5mg/kg body weight Vitamin C and 6mg/kg body weight Vitamin E 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. 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 [22].

Animals in group D which were treated with 5mg/kg body weight Vitamin C and 6mg/kg body weight Vitamin E and single dose 2.5mg/kg body weight $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, 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 Cathepsin 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.

Animals in group E which were induced with cadmium but treated with 2mg/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. Cathepsin 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 lead to the formation of plaques is being greatly limited in this particular group. The heterogeneity of neuronal morphology is reduced [Figure E] **relative** to Group B [Figure 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 [23]. These **constituents are said** to have antioxidant effect, 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 were induced with cadmium but treated with 2mg/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 induced with cadmium and treated with *Moringa oleifera*. Neurons and glia are preserved. Cathepsin 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]. Study **showed that immature cashew** nut-shell liquid (iCNSL), a source of unsaturated long-chain phenols, and may have a potential role in protecting against oxidative damage [20].

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

631

632 Conclusion

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

642

643 References

- 644 [1] Squire LR (1992). *Memory and the hippocampus: a synthesis from findings with rats,*
645 *monkeys, and humans.* *Psychol Rev* 99(2):195-231. doi:10.1037/0033-
646 295X.99.2.195.
- 647 [2] Eichenbaum H, Cohen NJ (1993). *Memory, Amnesia, and the Hippocampal System.* MIT
648 Press: Cambridge, MA. Press.
- 649 [3] Philippe T (2007). *The Hippocampus: Neurotransmission and Plasticity in the Nervous*
650 *System.* Nova Pub[^{s1}]
- 651 [4] Hiroaki W (2007). *Neural dynamics of the cognitive map in the hippocampus.* *Cognitive*
652 *Neurodynamics* 1:2, 119-141.
- 653 [5] Annabelle C (2009). *Rewarded Outcomes Enhance Reactivation of Experience in the*
654 *Hippocampus Neuron.* 64:6, 910-921.
- 655 [6] Gross CG (1993). *Hippocampus Minor and Man's Place in Nature: A Case Study in the*
656 *Social Construction of Neuroanatomy.* *Hippocampus* 3 (4): 403–416
- 657 [7] Holleman AF, Wiberg E, Wiberg N (1985). *Cadmium.* *Lehrbuch der Anorganischen*
658 *Chemie,* 91–100. Walter de Gruyter. pp. 1056–1057
- 659 [8] Case Studies in Environmental Medicine (CSEM) (2011). *Cadmium.* Agency for Toxic
660 Substances and Disease Registry. Archived from the original on 2011-06-06. Retrieved
661 2011-05-30.[^{s2}]
- 662 [9] Pal R, Nath R, Gill KD (1993). *Influence of ethanol on cadmium accumulation and its*
663 *impact on lipid peroxidation and membrane bound functional enzymes (Na⁺, K⁺)-*
664 *ATPase and acetylcholinesterase) in various regions of adult rat brain.* *Neurochem. Int.*
665 1993, 23, 451–458.
- 666 [10] Sinha M, Manna P, Sil PC (2008). *Cadmium-induced neurological disorders:*
667 *Prophylactic role of taurine.* *J. Appl. Toxicol.* 2008, 28, 974–986.
- 668 [11] Wong KL, and Klaassen CD (1982). *Neurotoxic effects of cadmium in young rats.*
669 *Toxicol. Appl. Pharmacol.* 63, 330–337.
- 670 [12] Gutierrez-Reyes EY, Albores A, Rios C. *Increase of striatal dopamine release by*
671 *cadmium in nursing rats and its prevention by dexamethasone-induced metallothionein.*
672 *Toxicology* 1998, 131, 145–154.

- [13] Kumar R, Asic K, Agarwal K, and Seth PK (1996). *Oxidative stress mediated neurotoxicity of cadmium*. Toxicol. Lett. 89, 65–69.
- [14] Shukla GS, Srivastava RS, Chandra SV (1988). *Glutathione status and cadmium neurotoxicity— Studies in discrete brain-regions of growing-rats*. Fund Appl. Toxicol. 11, 229–235.
- [15] Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-Ngam L, Ratanachamnong P, Srisawat P, Pongrapeeporn KU (2008). *The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. leaves*. J. Ethnopharmacol. 116:439-446.
- [16] Lalas S, Tsaknis J (2002). *Extraction and identification of natural antioxidant from the seeds of the Moringa oleifera tree variety of Malawi*. JAOSC 79:677-683.
- [17] Siddhuraju P, Becker K (2003). *Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves*. J. Agric. Food Chem. 51:2144-2155.
- [18] Ogbunugafor HA, Eneh FU, Ozumba AN, Igwo-Ezikpe MN, Okpuzor J, Igwilo IO, Adenekan SO, Onyekwelu OA (2011). *Physico-chemical and antioxidant properties of Moringa oleifera seed oil*. Pak. J. Nutr. 10:409-414
- [19] Jaiswal Y, Pa T, Satish YG, Ashok V (2010). *Antioxidant Activity of Various Extracts of Leaves of Anacardium Occidentale (Cashew)*. [Research Journal of Pharmaceutical, Biological and Chemical Sciences](#). 1(4), 112-119. ~~1Department of Pharmaceutical Chemistry, C.U.Shah College of Pharmacy, S.N.D.T Women's University, Mumbai-400049, India. 2Medical Research Centre, Kasturba Health Society, Vile Parle (W), Mumbai-400 056, India.~~
- [20] Isao K, Noriyoshi M, Tae JH, Kazuo T (2005). *Antioxidant activity of anacardic acids*. Department of Environmental Science, Policy and Management, University Of California, Berkeley, Ca 94720-3112, Usa.
- [21] Akerele O (2007). *Nature Medical Bounty: don't throw it away*. World health forum 14, 390-395.
- [22] Meydani M. (1995). *Vitamin E*. Lancet; 345: 170–5.
- [23] Fuglie LJ (1999, 2000). *Moringa stem planting*.^[s3]