

HISTOCHEMICAL ASSESSMENT OF *MORINGA - OLEIFERA* OIL AND WALNUT OIL ON CADMIUM INDUCED LATERAL GENICULATE BODY DAMAGE IN DEVELOPING MALE WISTAR RATS (*Rattus novergicus*)

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ABSTRACT

The brain is vulnerable to oxidative damage due to its high oxygen consumption. This Study investigate the effects of cadmium on the lateral geniculate body of developing male wistar rats and ameliorative potential of *Moringa oleifera* seed oil and walnut oil extracts. Seven groups of five animals each were used in this experiment. Group A received 3ml of 0.9% normal saline; group B received 2.5mg/kg bw of 3CdSO₄.8H₂O, group C received 5mg/kg bw vitamin C & 6mg/kg bw vitamin E, group D received 5mg/kg bw vitamin C & 6mg/kg bw vitamin E + 2.5mg/kg bw Cd, group E received 2.5mg/kg bw Cd + 4mg/kg bw *Moringa oleifera* oil, group F received 2.5mg/kg bw Cd + 4mg/kg bw walnut oil, while group G received 2.5mg/kg bw Cd + 2mg/kg bw walnut + 2mg/kg bw *Moringa oleifera* oil concomitantly for 3weeks. Parameter tested includes LDH, G6PD in brain tissues, SOD and GPx enzymes in brain homogenates and serum and cresyl fast violet stain in the brain tissues. Cd administration significantly increased SOD, GPx, LDH and decreased G6PD level in brain tissue and decreased their activity in serum when compared with Group A control rats. There was marked reduction and lost in the distribution of nissl substances of the studied tissues of Cd administered animals. However, administration of vitamin C & E, walnut and *Moringa oleifera* oil restored damaged tissues. Walnut and *Moringa oleifera* seed oil therefore attenuated the oxidative damage and morphological changes induced by cadmium in the lateral geniculate body of the brain of the young male wistar rats.

Key Words: Lateral geniculate body, Antioxidant, Histochemical, Cadmium, Oxidative Damage

INTRODUCTION

Cadmium is a silver-white, blue-tinged, lustrous metal that melts at 321°C and boils at 765°C (Bertin and Averbeck 2006). This divalent element has an atomic weight of 112.4 and an atomic number of 48 (The'venod, 2009). It is insoluble in water, although its chloride and sulphate salts are freely soluble (Windholz *et al.*, 1976). The

word Cadmium is derived from "Kadmeia" the ancient name for calamine (zinc carbonate) (Nawrot *et al.*, 2008). The element Cadmium was discovered in 1817 as an impurity in Calame (Government of Canada, 1994). It is a rather rare element in nature (Cadmium mineral. greenockite) with small amounts of Cadmium king found in Zinc, Copper and Lead

ores (Islamoglu *et al.*,2006, Pietrobelli *et al.*,2009). Cadmium is generally a by-product of working with these metal ores and, therefore, its presence in the environment is essentially due to industrial waste (Wang and Gao,2009). In addition to being a by-product of Zinc and Lead mining, Cadmium is used in the Chemical industry for manufacture of such products as batteries, pigments, alloys, paints and plastics as well as in electroplating (Kurniawan *et al.*,2006, Liang *et al.*,2007). Cadmium once absorbed gets accumulated in major organs of the living system (Deepti *et al.*, 2011). Thus, the risk of chronic intoxication is the biggest problem, the chronic effects of cadmium depend on dose and on the route by which the metal enters the body and chronic inhalation causes emphysema and obstructive airways disease, before kidney damage is visible (Jahangir *et al.*, 2005). Yellow tooth discoloration, mild anemia, and disturbances in calcium metabolism, osteomalacia, and renal toxicity also are observed occasionally (Gonick, 2008). Cadmium has been shown to affect virtually every organ and system in the body in both humans and animals (Houshmandfar and Moraghebi, 2011, Sears *et al.*, 2012). Cadmium is also known to be carcinogenic, and has been linked with cancers of the lungs and prostate, pancreas and kidney (Khausakorn *et al.*, 2012). The prostate is a potential target for Cadmium carcinogenesis, although the underlying mechanisms are still unclear (Aimola *et al.*, 2012).

Moringa oleifera L (Moringaceae) known commonly as Ben oil tree or drumstick tree in English language, 'Okweoyibo' in Igbo, 'Zogale' or 'Gawara' or 'Habiwal' in Hausa and 'Adagbamaloye' or 'Ewe Igbale' in Yoruba grows rapidly in most regions and climatic conditions of Nigeria (Ogbunugafor *et al.*, 2011). *Moringa oleifera* is a tropical multipurpose tree that naturally grows in India, South Saharan Africa and South-America (Jahn,1988). *Moringa oleifera* originates in India, from the valleys of the southern Himalayas and today it can be found along the tropical and subtropical zones (Fuglie, 1999). *Moringa oleifera* is the most widely spread species of the plant family

Moringaceae (Abaliwano, 2008). In particular, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (Bennett *et al.*, 2003, Fahey *et al.*, 2001). It has been used for the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Ghazal *et al.*,2012, Sinha *et al.*,2012, Adegbite *et al.*,2016). All these uses have not yet been scientifically verified, but *Moringa oleifera* is considered an effective treatment for anemia, loss of appetite and it increases lactation in women (Ogbunugafor *et al.*,2012). It also combats gastric discomfort, stomach ulcers, diarrhea, dysentery, colitis and can be used as a laxative, purgative and a diuretic – to fight colds, bronchial infections, fever and head pain – rheumatic discomfort, muscular cramp, bruises and bruises – skin infections, scabies, ringworm and insect bites (Misra *et al.*,2014). In certain cases of diabetes, *Moringa oleifera* can also be used to stabilize sugar levels and can stabilize arterial tension (Divya *et al.*,2012). Most of plants uses for medicinal purposes have been correlated to their possession of antioxidant activity (Djacobou *et al.*,2014).

Black walnut, *Juglans nigra*, is an abundant tree of the eastern deciduous forest region of North America (Jelodar *et al.*,2007). The African walnut has indigenous names such as Ukpa (Igbo), Asala (Yoruba) and Hawuusa (Hausa) in Nigeria (Aladeokin and Umukoro, 2011). The walnut kernels have high oil content and were a favorite food of Amerindians (Crews *et al.*,2005). They retain their flavor when cooked and have a food value four times as great as meat (Jaradat,2005). They are very difficult to crack and the husk stains the hands a dark brown so their wild harvest today is restricted to the very dedicated (Cosmulescu *et al.*,2009). These nuts are used mainly in the candy and ice cream industries, the tree is very productive and can be grown in a variety of soils and climates (Davis *et al.*,2007). Walnuts provide a valuable timber and also a brownish-black dye that was used

by early settlers to dye hair (Bonamonte *et al.*,2001). Studies have shown that walnuts are effective in preventing gallstones (especially in women) and lower serum cholesterol in Japanese men and women (Iwamoto *et al.*,2000, SeedGuides, 2013). Melatonin which is a powerful antioxidant and also induces a good night's sleep, is present in walnuts in the bio-available form (Reiter *et al.*,2005, Rahimipanah *et al.*,2010). Walnuts are an excellent source of a proteins, fiber, B vitamins and minerals like iron, magnesium, phosphorus, copper and manganese (Sze-Tao and Sathe, 2000, Muradoglu *et al.*,2010). These nuts are helpful in easing constipation

due to their laxative effects and prevent liver damage (Hiroshi *et al.*,2008). Research indicates that walnuts may reduce the risk of Alzheimer's disease by arresting the formation of the amyloid plaques that are found in the brains of patients who suffer from this debilitating disease (Willis *et al.*, 2009).

This study investigates the effects of cadmium on the lateral geniculate body of developing male wistar rats and ameliorative potential of *Moringa oleifera* seed oil and walnut oil extracts.

MATERIALS AND METHODS

Moringa oleifera seeds were obtained from a local market in Jos, Plateau State, Nigeria and walnuts were procured from a local market in Ilorin, Kwara State, Nigeria. These seeds were then taken to Mr Jeromes a botanist in the Department of Biology, Bingham University Karu, Nasarawa State for proper identification, voucher deposited for reference purpose.

All experimental investigations were done in compliance with humane animal care as studied in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub. No NIH 86-23 (1985).

The study was carried out on thirty-five (35) young male wistar rats of body weight 80-120g. The rats were reared in the animal holdings of Bingham University Karu, Nasarawa State, Nigeria. The rats were allowed to gain maximum acclimatization before the actual commencement of the experiment. The rats were housed five in a standard laboratory cage, at a constant room temperature in a 12 hrs light: 12 hrs dark (lights on 8 a.m.–8 p.m.) cycle. They were fed with 100g standard rat diet chow daily containing proteins, carbohydrate, fats, vitamins and minerals which was purchased from Vital Feeds and Grand Cereals Ltd (Jos, Nigeria) to avoid changes in dietary compositions and weight variability and 200ml

of normal drinking tap water was provided *ad libitum*.

The thirty-five (35) young male wistar rats used in this study were randomly divided into seven (7) groups, A, B, C, D, E, F and G of five (5) animals each.

Fresh walnuts and *Moringa oleifera* seeds weighing 1500g each were oven dried at 40°C for 7days and were blended separately with nuts and seeds blender. The molten *Moringa oleifera* seeds and walnuts were then independently poured into an oil extraction machine that separated the oil from the residue at high temperature and pressure. The oil obtained was then kept at about 6°C-10°C under reduced pressure in order to maintain the constant temperature of the oil.

Cadmium sulphate solution was prepared by dissolving the 2.5mg of cadmium sulphate salt (CdSO₄.8H₂O) in 20ml of 0.9% w/v normal saline solution. Ascorbic acid (vitamin C) was prepared by dissolving 5mg of vitamin C in 10 ml of 0.9% w/v normal saline solution. Vitamin E (Alpha tocopherol) was prepared by dissolving 6mg of vitamin E in 20ml of olive oil.

The animals were treated as shown in the table below:

Table 1: Number of animals per group and dosage of treatment rendered

GROUPS	NUMBER OF ANIMALS	DOSAGE
A	5	3ml of 0.9% normal saline per kg bw
B	5	2.5mg/kg bw cadmium Only
C	5	5 mg/kg bw vitamin C & 6 mg/kg bw vitamin E
D	5	5 mg/kg bw vitamin C & 6 mg/kg bw vitamin E + 2.5 mg/kg bw cadmium
E	5	2.5 mg/kg bw cadmium + <i>Moringa oleifera</i> seed oil (4 mg/kg bw)
F	5	2.5 mg/kg bw cadmium + walnut oil (4 mg/kg bw)
G	5	2.5 mg/kg bw cadmium + <i>Moringa oleifera</i> seed oil + walnut oil (2 mg/kg bw each)

Animals were grouped into three control groups namely; the normal control (A), the negative control (B) and the positive control (C). Normal control group A animals received 3ml of 0.9% w/v normal saline solution orally for a period of three (3) weeks. Negative control group B animals were induced intraperitoneally with 2.5mg/kg $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ and after 72 hours of injection, the animals received 3ml of 0.9% w/v normal saline solution for 3weeks. Positive control group C animals received 5 mg/kg body weight and 6 mg/kg body weight of vitamins C and E orally respectively for 3 weeks.

Group D animals were the prophylactic group received 6 mg/kg body weight of vitamin E and 5 mg/kg body weight of vitamin C for 3weeks followed by the intraperitoneal injection of 2.5mg/kg $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ for 72hrs.

Group E, F and G animals served as the therapeutic treatment group. The animals in each group were injected intraperitoneally with 2.5 mg/kg $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$, after 72hrs of injection, the animals in group E and F received 4 mg/kg body weight of *Moringa oleifera* seed oil and 4 mg/kg body weight of walnut oil respectively for 3weeks. The animals in group G received *Moringa oleifera* seed oil and walnut oil at 2 mg/kg body weight for 3weeks.

The animals were administered orally via oral cannular with the 2 mg/kg *Moringa oleifera* seed and walnut oils, 5 mg/kg vitamin C, 6 mg/kg vitamin E and 3 ml of 0.9% w/v normal saline solution according to their body weights treated as shown in the table above for three (3) weeks. The animals were held with a glove with the left hand such that the neck region is held by the fingers to still the neck while being fed with the cannular; treatment was done every morning before the animals were fed. Twenty-four (24) hours after the last administration; the animals were sacrificed by cervical dislocation. They were laid in a supine position on the dissecting board and pinned through the fore and hind paws. A midline incision through the skin and muscles of the abdominal wall were made from the xiphisternum to the pubic symphysis, extended laterally at the lower end to achieve adequate exposure of the thoracic and abdominal cavities. The skulls of the animals were fractured open using brain forceps and the whole brain was carefully removed and weighed. The lateral geniculate body was then excised and fixed in 10% formol calcium for 72hrs. This was done for all the animals in each of the groups labelled Groups A to G. The tissues were homogenized in 0.25 M sucrose at 4°C after which it was processed for histological analysis.

At the end of the last administration (3 weeks) of treatment, blood samples were collected in 5 ml plain serum bottles via the

heart and the intraocular method using capillary tubes for enzyme analysis. Activity of Glucose-6-Phosphate Dehydrogenase in homogenate was measured using the method of Lohr and Walker (1974). The procedure is as shown in the reactive table below: Glutathione peroxidase activity in homogenate was determined using the method of Paglia and Valentine (1967) using the reagent kit made by Randox lab, Ardmore, Diamond road, Crumlin co, UK BT294QY. Enzyme activity of SOD was assayed according to the method of Misra and Fridovich (1972) using reagent kit produced by Randox lab ltd. After

the brains of the Wistar rats were harvested, they were placed in specimen bottles containing 40% formol calcium to prevent autolysis and after 24 hours, the brains were dissected to remove the Lateral Geniculate Body. The process of tissue for histological staining was used. Data for the oxidative enzyme tests were expressed as Mean \pm Standard Error of Mean (S.E.M). The data was analysed in SPSS 17.0 software (SPSS Incorporation, Chicago, USA) to determine the analysis of variance. Except where otherwise stated, $P \leq 0.05$ was taken as the level of significance.

RESULTS

Groups F (Cadmium + walnut oil) and G (Cadmium + walnut oil + *Moringa oleifera* seed oil) animals were observed to show a statistically significant ($p < 0.05$) increased superoxide dismutase activity in the brain tissue when compared with that of the group A (Normal control)

Groups C (Vitamin C & E), D (Vitamin C & E + cadmium), E (Cadmium + *Moringa oleifera* seed oil), F (Cadmium + walnut oil) and G (Cadmium + walnut oil + *Moringa oleifera* seed oil) animals were observed to show significant ($p < 0.05$) decreased in superoxide dismutase activity in the serum when compared with that of the control group A.

Groups B (Cadmium only), C (Vitamin C & E), D (Vitamin C & E + cadmium), E (Cadmium + *Moringa oleifera* seed oil), F (Cadmium + walnut oil) and G (Cadmium + walnut oil + *Moringa oleifera* seed oil) animals were observed to show significant ($p < 0.05$) increased in glutathione peroxidase activity in the brain tissue when compared with that of the group A.

Groups B (Cadmium only), C (Vitamin C & E), D (Vitamin C & E + cadmium), E (Cadmium +

Moringa oleifera seed oil), F (Cadmium + walnut oil) and G (Cadmium + walnut oil + *Moringa oleifera* seed oil) animals were observed to show significant ($p < 0.05$) decreased in glutathione peroxidase activity in the serum when compared with that of the group A (Normal control).

Groups F (Cadmium + walnut oil) and G (Cadmium + walnut oil + *Moringa oleifera* seed oil) animals were observed to show a significant increased ($p < 0.05$) metabolic activity in the brain tissue when compared with that of the Group A (Normal control).

Groups B (Cadmium only), C (Vitamin C & E), D (Vitamin C & E + cadmium), F (Cadmium + walnut Oil) and G (Cadmium + walnut oil + *Moringa oleifera* seed oil) animals were observed to show a significant ($p < 0.05$) decreased metabolic activity in the brain tissue when compared with that of the Group A (Normal Control).

This histology stain is a Nissl stain that stains both neurons and glia and bonds well with acidic parts of cells such as ribosomes, nuclei and nucleoli. It stains cell bodies a blue/violet.

TABLE 2: Oxidative stress enzyme activity in the brain tissue and Serum and histochemical assay of carbohydrate metabolism in the brain tissue

Groups	Parameters					
	SOD1(U/ml)	SOD 2 (U/ml)	GPx 1 (U/L)	GPx 2 (U/L)	LDH (U/L)	G6PD (U/L)
A	604±7	281±6	3458±35	4355±30	573±7	3668±33
B	601±9	279±9	3872±40*	3689±47*	587±11	4129±34*
C	758±7	252±6*	3783±48*	3460±65*	627±7	4096±39*
D	743±6	244±5*	3659±11*	4578±12*	609±4	3322±28*
E	609±4	244±5*	3101±36*	4669±66*	602±6	3544±48
F	1419±182*	248±5*	9640±77*	4230±70	4877±47*	2761±60*
G	991±16*	112±6*	6157±57*	2220±47*	3091±35*	2368±47*

Values are expressed as mean ± SEM for n=5; *p < 0.05, significantly dissimilar from control SOD 1 - Superoxide Dismutase in the Brain Tissue, SOD 2 - Superoxide Dismutase in the Serum, GPx1 - Glutathione Peroxidase in the Brain Tissue, GPx2 - Glutathione Peroxidase in the Serum, LDH - Lactase Dehydrogenase, G6PD - Glucose-6-Phosphate Dehydrogenase

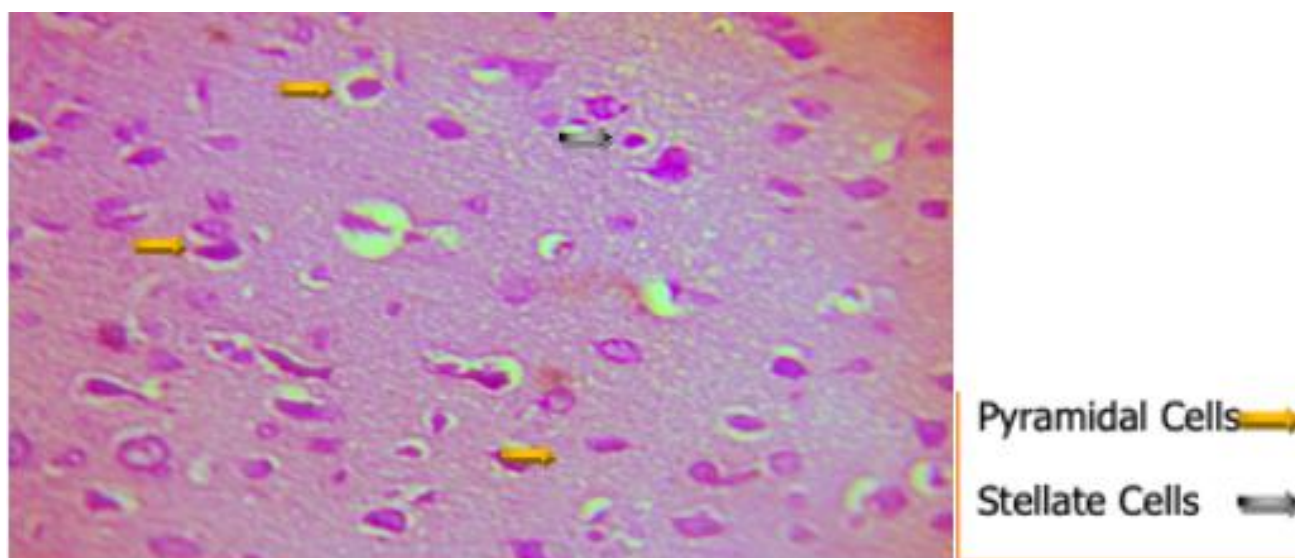


Figure 1: Group A – Normal control (Administered 3ml of 0.9% normal saline per kg bw): Micrograph of longitudinal section of the lateral geniculate body of a rat. Neuronal cell shows normal architecture with layers visibly intact and dark coloured pyramidal cells indicate presence of sufficient nissl substance. **Cresyl Fast Violet 400X**

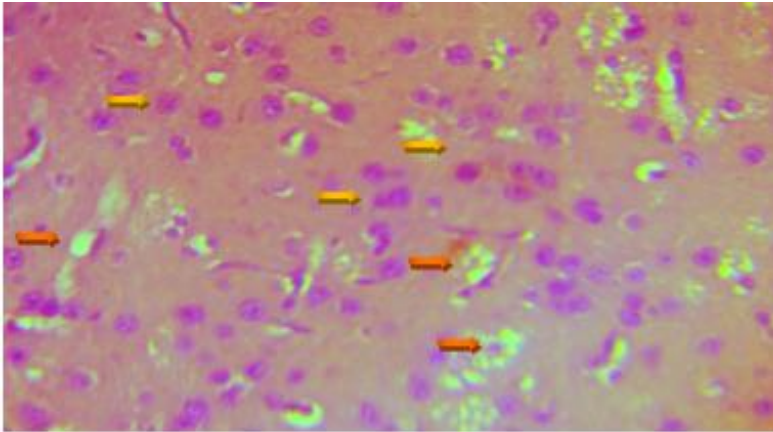


Figure 2: Group B – Negative control (Administered cadmium only): Micrograph of longitudinal section of the lateral geniculate body of a rat. Neuronal cells show signs of axonal degeneration indicating occurrence of apoptosis and light colored pyramidal cells indicate reduced nissl substance. Cresyl Fast Violet 400X

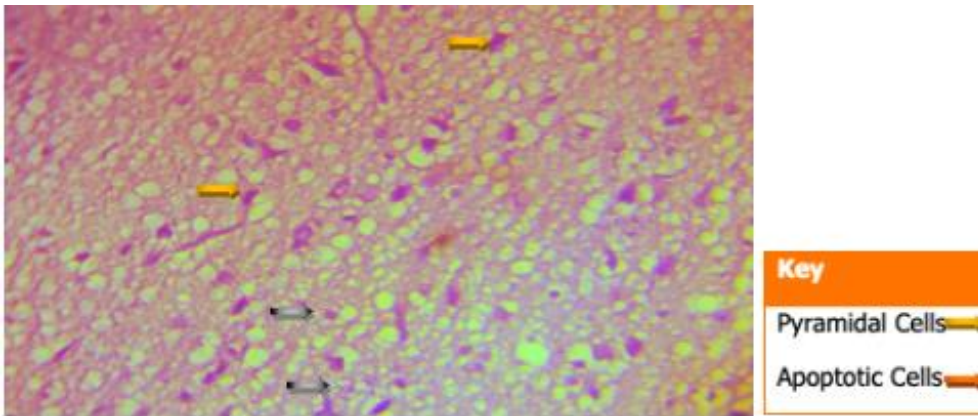


Figure 3: Group C – Positive control (Administered vitamin C & E only): Micrograph of longitudinal section of the lateral geniculate body of a rat. Neuronal cell shows normal layered architecture; mildly dark coloured pyramidal cells indicate presence of sufficient nissl substance while numerous cells indicate cell regeneration. Cresyl Fast Violet 400X

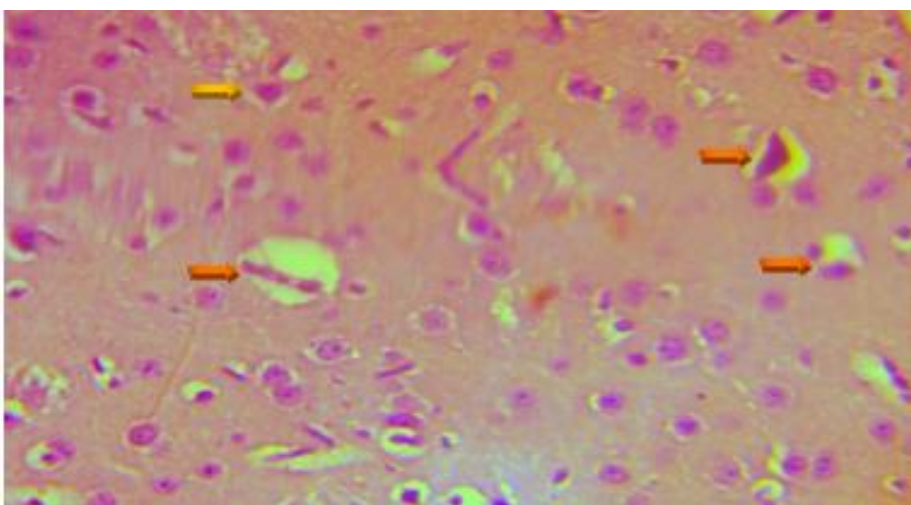


Figure 4: Group D – Positive induced (Administered vitamin C & E + cadmium): Micrograph of longitudinal section of the lateral geniculate body of a rat. Layered cell architecture intact, but a few cells show signs of axonal degeneration indicating occurrence of apoptosis and medium light coloured pyramidal cells indicate reduced nissl substance. Cresyl Fast Violet 400X

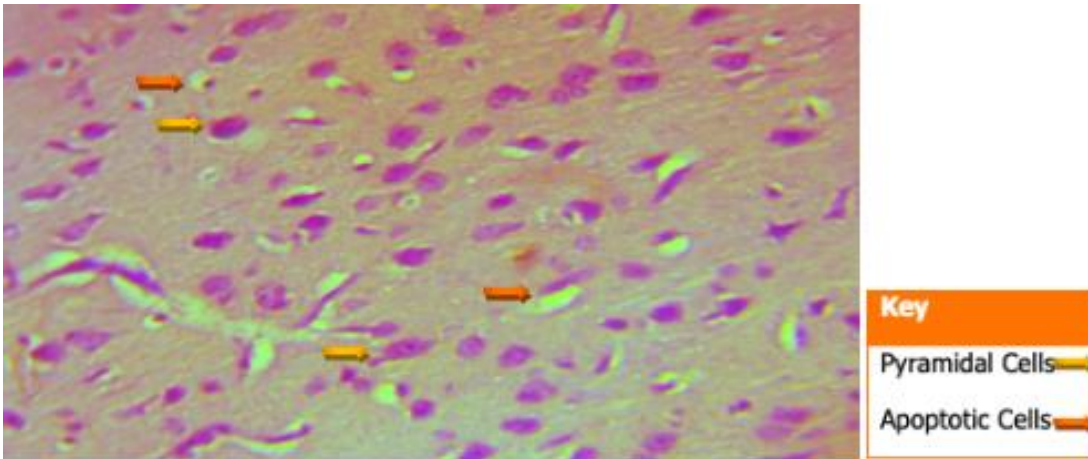


Figure 5: Group E – Administered cadmium + *Moringa oleifera* seed oil: Micrograph of longitudinal section of the lateral geniculate body of a rat. Layered cell architecture intact, but a few cells show signs of axonal degeneration indicating occurrence of apoptosis and medium light coloured pyramidal cells indicate reduced nissl substance, cell regeneration occurring. Cresyl Fast Violet 400X

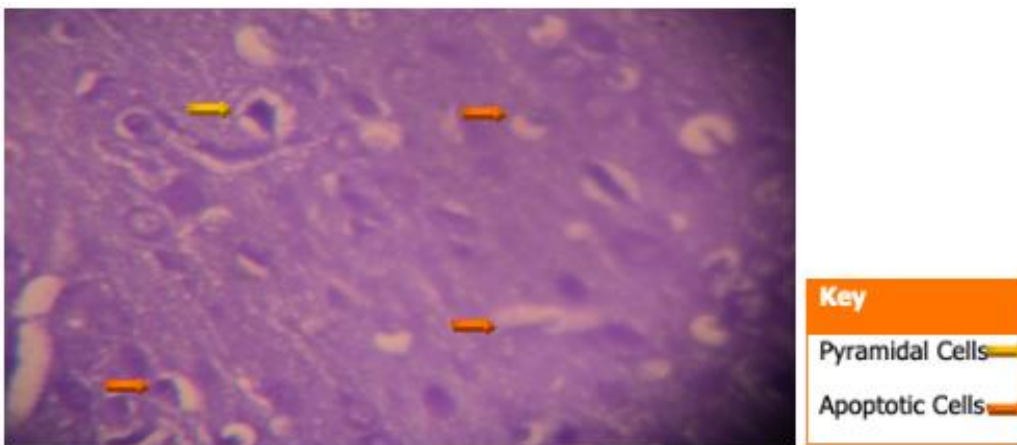


Figure 6: Group F – Administered cadmium + walnut oil: Micrograph of longitudinal section of the lateral geniculate body of a rat. Few shrunken cells show signs of axonal degeneration indicating occurrence of apoptosis and some light colored pyramidal cells indicate reduced nissl substance, cell regeneration also occurring. Cresyl Fast Violet 400X

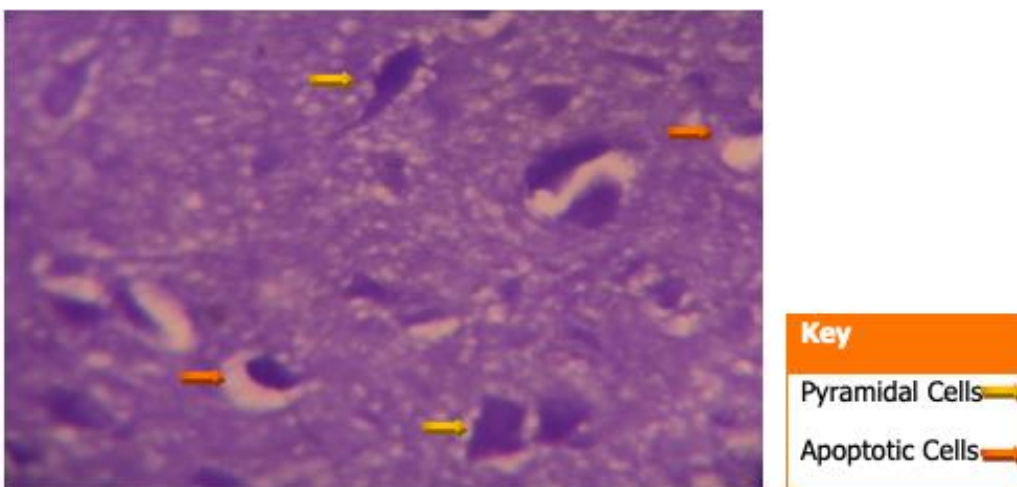


Figure 7: Group G – Cadmium + *Moringa oleifera* seed oil + walnut oil: Micrograph of longitudinal section of the lateral geniculate body of a rat. Few cells show signs of axonal degeneration indicating occurrence of apoptosis and dark coloured pyramidal cells indicate sufficient nissl substance, cell regeneration occurring. Cresyl Fast Violet 400X

DISCUSSION

Cadmium (Cd) is known to produce a variety of health hazards in humans and experimental animals due to its ability to induce severe alterations in various organs and tissues including the nervous system, following either acute or chronic exposure (Manca *et al.*, 1991). It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions (Bagchi *et al.*, 2000).

Antioxidant enzymes such as SOD and GPx are some of the primary antioxidant enzymes, which help in degrading the toxic oxidative intermediates and are considered to be the first line of cellular defense against oxidative damage (Das *et al.*, 2010). SOD is an antioxidant metallo enzyme that reduces superoxide radicals to water and molecular oxygen (Loschen *et al.*, 1974). SOD catalyzes the dismutation of superoxide radicals by scavenging free superoxide anions (O₂⁻). GPx uses GSH as a reducing substrate, and catalyzes the reduction of organic hydro peroxides (Ursini *et al.*, 1995).

In this study administration of cadmium significantly decreased superoxide dismutase (SOD1) and Glutathione Peroxidase (GPx1) activity in the brain tissue. Decreased activity of SOD reflects suppression of the scavenging ability of SOD, which may be a result of accumulation of superoxide anion radical in the brain tissue post cadmium treatment. The suppressing action of cadmium on SOD activity was also demonstrated by Ramanathan *et al.*, 2002. Walnut Oil, *Moringa oleifera* seed oil co-treatment along with cadmium normalized SOD and GPx activity in the combined treatment group in the brain tissue when compared with that of the normal control group. This shows that, the combined action of the antioxidant contents of both oils increased SOD and GPx activity in the brain tissue in order to efficiently combat and mop up the free radicals and reactive oxygen species that had been released due to the oxidative damage induced by cadmium exposure. This thus confirms the suggestion made by Law *et al.*, (2001) that antioxidants

have therapeutic importance in neurological disorders where oxidative stress is involved.

Also, superoxide dismutase (SOD2) and glutathione peroxidase (GPx2) activity in the serum in groups treated with vitamin C & E, co-treated with vitamin C & E, walnut oil and *Moringa oleifera* seed oil along with cadmium were observed to show significant decreased activity in the serum when compared with that of normal control. This implied that, oxidative damage induced by cadmium on the brain tissue resulted in the migration of the SOD and GPx enzymes from the blood to the brain in order to combat and mop up the free radicals and reactive oxygen species that had been released alongside the antioxidant contents of these drugs and herbs administered thereby, leading to an evidently decreased activity of SOD and GPx in the serum. This therefore concurs with the report of Law *et al.*, 2001 that in neurological disorders where oxidative stress is involved antioxidants have therapeutic importance. These results suggest that co-treatment with walnut oil and *Moringa oleifera* seed oil, helped maintain the balance between ROS production and antioxidant defense, as can be inferred from normalized antioxidant enzyme activity values in the combined treatment group. There are reports supporting the protective role of walnut oil and *Moringa oleifera* seed oil, against oxidative stress (Emerit *et al.*, 2002).

Observation on the enzymes of carbohydrate metabolism such as lactate dehydrogenase (LDH) metabolism in the brain tissue of groups administered with cadmium and walnut oil, cadmium + walnut oil + *Moringa oleifera* seed oil animals were observed to show a significant increased metabolic activity in the brain tissue in comparison with the normal control. This shows that oxidative stress had occurred in the brain tissue due to the administration of the toxic cadmium and the antioxidant contents of both oils helped to increase the activity of the LDH enzyme in order to efficiently mop up and combat the free radicals and reactive oxygen species that had been released in the brain tissue due to

the oxidative damage. This confirms the suggestion made by Stohs and Bagchi, (1995) that cadmium is a potent cell poison and its toxicity was mediated by the oxidative damage of essential cellular macromolecules and that of Peter *et.al.*,1995 that Free radical scavengers and antioxidants, such as Glutathione, vitamin E, and vitamin C are capable of protecting against cadmium toxicity.

Also in our study Glucose-6-Phosphate Dehydrogenase (G6PD) metabolism in the brain tissue in groups treated with cadmium induced, vitamin C & E, vitamin C & E + cadmium, cadmium + walnut oil and cadmium + walnut oil + *Moringa oleifera* seed oil groups animals were observed to show a significant decreased metabolic activity in the brain tissue suggest a disruption in carbohydrate metabolism pathway and depletion of the antioxidant defence system as evident in cadmium group rats (Stohs *et al.*,2000). This also revealed occurrence of oxidative stress in the brain tissue due to the administration of the toxic cadmium however antioxidant contents of both the drugs and oils were sufficient enough to efficiently mop up and combat the free radicals and reactive oxygen species that had been released in the brain tissue due to the oxidative damage thus requiring no more help from the glutathione antioxidant usually released by G6PD following oxidative damage and concomitantly resulting in the decreased level of the G6PD enzyme in the brain tissue. This there confirms the report of Stohs and Bagchi, (1995) that cadmium is a potent cell poison and its toxicity was mediated by the oxidative damage of essential cellular macromolecules and that of Peters *et al.*, (1995) that free radical scavengers and antioxidants, such as glutathione, vitamin E, and vitamin C are capable of protecting against cadmium toxicity.

Furthermore, in the present study, administration of cadmium was observed to have induced morphopathological changes in the lateral geniculate body of the brain involving the different types of neurons (Fig.2) when compared to the normal morphology of the lateral geniculate body of the rat brains in the normal control group A (Fig. 1). The neurons were observed to have undergone either degenerative or apoptotic changes, the degenerative changes manifested by shrinkage, neurofibrillary tangles, senile plaques and deeply stained neurons in the studied regions. However, in Fig. 3-7, the neurons showed cellular regeneration after they had undergone either degenerative or apoptotic changes manifested by shrinkage, neurofibrillary tangles, senile plaques and deeply stained neurons in the studied regions. This shows that the administration of the vitamin C and E antioxidants and the antioxidant containing *Moringa oleifera* and walnut oil extracts actually helped to curb the deleterious effects of the toxic cadmium.

In conclusion, in the present investigation, the vitamin C and E antioxidant contents of *Moringa oleifera* seed oil and walnut oil helped to curb the morphopathological changes caused by the Cadmium induced damage to the lateral geniculate body of the rats' brain, maintained the activity of enzymes of Carbohydrate metabolism i.e. Lactate Dehydrogenase (LDH) and Glucose-6-Phosphate Dehydrogenase, and the activity of the oxidative stress enzymes i.e. Superoxide Dismutase (SOD) activity and Glutathione peroxidase (GPx) concentration produced in the lateral geniculate body of the brain and serum nearly at control values. Also, the combined action of the antioxidant contents of both oils evidently attenuated the oxidative damage and morphological changes induced by cadmium in the lateral geniculate body of the brain of the rats.

Conflict of interest: Authors have declared that no competing interests exist.

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