IP Indian Journal of Neurosciences 2019;5(4):214–219

Content available at: iponlinejournal.com

IP Indian Journal of Neurosciences

Journal homepage: www.innovativepublication.com

Original Research Article

Recuperative properties of Moringa Oleifera seed oil on cadmium and alcoholic beverage induced frontal cortex damage in Wistar Rats

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ARTICLE INFO

Article history:
Received 28-10-2019
Accepted 18-11-2019
Available online 14-12-2019

Keywords:
Cadmium
frontal cortex
Moringa
Beverage
and Nissl substance

ABSTRACT

Aim: The aim of this study was to investigate the ameliorative effects by the intervention of *Moringa Oleifera* seed oil on the damage to frontal cortex of rats by cadmium and herbal alcoholic beverage.
Materials and Method: Eighty Wistar rats (73-151g,n=10)grouped as follows: A Control;B1(Cadmium) ;B2(Cadmium+Moringa seed oil) ;C1(Herbal alcoholic Beverage);C2(Herbal alcoholic Beverage +moringa seed oil);D(Cadmium +Herbal alcoholic beverage);E(Cadmium+Herbal alcoholic beverage+Moringa seed oil) and F(Moringa seed oil) for four weeks followed by histological studies.
Results: The histoarchitecture of the frontal cortex was characterized by pyknosis of nuclei which resulted in dissolution of Nissl substance. Activation of astrocytes were evidence in group B1, B2, C1, C2 and D which were exposed to cadmium and HAB while those of groups B2, C2 and E showed ameliorative effect that were evident in reduction in pyknotic neurons and reduction of activated astrocytes.
Conclusion : Based on the results, it can be deduced that *Moringa Oleifera* seed oil has neuroprotective properties.

1. Introduction

Moringa Oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia. It is known as the drumstick tree based on the appearance of its immature seed pods, the horseradish tree based on the taste of ground root preparations, and the ben oil tree from seed - derived oils.[1] The leaves, roots, seeds, bark, fruits, flowers, and stem of *M. Oleifera* have been shown to exert various pharmacological effects. Additionally, aqueous and ethanolic *M. Oleifera* seed extract (MSE) has been shown to possess various pharmacological and commercial utility, such as metal antidote, anti-oxidant, anti-asthmatic, anti-arthritic, anti-bacterial, anti-tumor, and hepatoprotective effects.[2]

Cadmium (Cd) is a heavy metal that has received considerable concern environmentally and occupationally. Cd has a long biological half-life mainly due to its low rate of excretion from the body. Thus, prolonged exposure to Cd will cause toxic effect due to its accumulation over time in a variety of tissues, including kidneys, liver, central nervous system (CNS), and peripheral neuronal systems.[3] Herbal alcoholic beverages commonly called Ogogoro, alomo bitter, Opa eyin in Nigeria. It is locally manufacture and package, it is consumed locally by the general public for sexual enhancement and as stimulants.

Various investigation has revealed the deleterious effects of high percentage of alcohol (ranging from 17% to 70%) in most of the herbal alcoholic beverages.[4] Excess consumption of alcoholic beverages has been associated with high libido causing excess sexual enhancement and over stimulation has also been linked to excess herbal alcoholic consumption. Overall effects of herbal alcoholic consumption has been revealed to cause health hazard

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https://doi.org/10.18231/j.ijn.2019.035

leading to soft tissues damage such as cardiovascular, lung, liver, kidney and brain.[4]

2. Materials and Methods

2.1. Experimental animals

A total of 80 adult wistar rats aged eight (8) weeks of both sexes weighing 73g – 151g. were used for this study, animals were bred in the animal house of the Faculty of Basic Medical Sciences, Kogi State University,Anyigba, Nigeria, the animals were maintained under standard laboratory conditions of light, temperature, humidity and ventilation. They were given rat chow and water *ad libitiu*m and the experimental animals were acclimatized for two (2) weeks before the commencement of the research work.

2.2. Plant collection and extraction

Moringa oleifera seeds were procured from Ladoke Akintola University Farm in Ogbomoso, Nigeria and the plant speccimen was identified with voucher number (No. FHI. 110266) assigned at Forestry Research Institute of Nigeria (FRIN), Jericho hill, Ibadan, where it was deposited, the seeds of fresh *Moringa oleifera* plant were plucked and air dried under room temperature at (29°C-35°C) for four (4) weeks, after which the seeds were pulverized into coarse form with Acrestor high speed milling machine. The coarse form (200g) was macerated in absolute ethanol. This was left to stand for 24hrs.[5] After that the extract was filter through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract were concentrated and evaporated to dryness using rotary evaporator at an optimum temperature which was between 40^0 C and 45^0 C to avoid denaturation of the active ingredients. The concentrated extract was store in the refrigerator until use[5] The percentage yield of the extract was determined by weighing the coarse *Moringa oleifera* seed before extraction and the *Moringa oleifera* ethanol seed extract after concentration and calculated using the formula.

Percentage (%) yield= Weight (g) of concentrated extract $\times$ 100

Weight of grounded Moringa Seeds

The quatitative phytochemical analyses was carried out according to the methods of Harborne (1973, 1984) and Trease and Evans (1989).

2.3. Cadmium procurement

Cadmium sulfate octahydrate ($3CdSO_4$.$8H_2$ O) with molar mass M=769.52 and net weight W=100g was purchased in June, 2012 from Guangzhou linhuada Chemical Reagent Co. Ltd., Guangdog, China,reagent Lot No: 20120524.The Cadmium stock solution was made by dissolving 11.27mg of Cadmium sulphate salt in 5.64ml of 0.9% w/v phosphate buffer at PH 7.4. The cadmium stock solution was administered intraperitoneally in doses corresponding to the weight of the rats using 1ml insulin syringes,herbal alcoholic beverage was procured locally in Nigeria.

2.4. Experimental design

The animals were randomly divided into eight (8) groups A, B1, B2, C1, C2, D, E and F of Ten (10) animals each. The control group (A) received 2.5 mg/ kgbw of phosphate buffer intraperitoneally single dose and the induced control group (B1 and B2) received 3.5 mg/ kgbw of $3CdSO_4.8H_2$ O intraperitoneally and left for 72 hours. B_1 rats were maintained under normal laboratory condition for period of four weeks and B_2 rats received 2.0 mg/ kgbw of *Moringa oleifer*a oil extract single dose daily for the period of four weeks. C_1 rats received 0.5 ml, 40% Herbal-gin via gavage, single dose daily four the period of four weeks while C_2 rats received 0.5 ml, 40% Herbal-gin and 2.0 mg/ kgbw of *Moringa oleifer*a oil extract simultaneously via gavage, single dose daily for the period of for weeks. Group D rats were injected intraperitoneally with 3.5 mg/ kgbw of Cadmium sulphate ($3CdSO_4.8H_2$ O) single dose and maintained for 72hrs[6] following oral administration of 0.5 ml, 40% Herbal-gin single dose daily for the period of four weeks. Group E animal were also injected intraperitoneally with 3.5 mg/ kgbw of Cadmium sulphate ($3CdSO_4.8H_2$ O) single dose and maintained for 72hrs[6] following oral administration of 0.5 ml, 40% Herbal-gin and 2.0 mg/ kgbw *Moringa oleifer*a seed oil extract single dose daily for the period of four weeks. Group F rats received 2.0 mg/ kgbw of *Moringa oleifer*a seed oil via gavage, single dose per day for the period of four (4) weeks.

All experimental investigations were done in compliance with the guideline, as stated in the " Guide to the care and use of Laboratory Animals Resources "[7] and in accordance with guidelines stated in IACUC and OLAW, United Kingdom as well as in compliance to the ethical committee approval of Kogi State University and Olabisi Onabanjo University, Nigeria.

2.5. Histological studies

The animals were sacrificed via cervical dislocation, the brain from all the rats were carefully excised from the skull using brain forcep, weighed and one hemisphere of the frontal cortex from all the animals were preserved in 10% formol -calcium. After 24 hours of fixation the frontal cortex of all brain tissues were routinely processed for H&E and cresyl fast violet stain, Nigeria. The brain tissues photomicrograph were captured using trioccular microscope coupled with scopeimage 9.0 (HIC) camera with specification (HDCE-10C (G012012536), made in Germany.

3. Results

Effects of Moringa Seed Oil (MSO), Herbal Alcoholic Beverage (HAB) on Cadmium induced Frontal cortex damage cortex neurons and glia cells with distinct PN (pyramidal neuron), normal cerebral histology

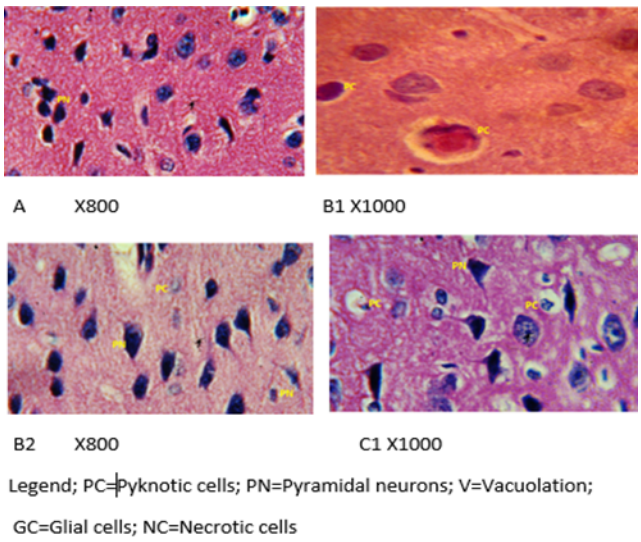

Fig. 1: Photomicrograph of a section of prefrontal cortex of Moringa and HAB treated rats showing: **A:** (control) which appears normal with intact cells and neurons; **B1:** (Cadmium only) shows swollen neurons and condensation of chromatin materials (karyolysis) and dissolution of nucleolus (chromatolysis) resulting to generation of PC (pyknotic cells); **B2:** (Cd+2mg/kg moringa) shows evidence of cell swelling, shrinkage and vacuolated neurons but, few of the neurons still retained normal shape and form; **C1:** (HAB only) shows comparably normal pyramidal cells (PC) and frontal

Assessment of Cresyl Fast Violet on the Nissl Substance in the Neurones of Frontal Cortex to evaluate site of Chromatolysis and Karyolysis in Rat Models

4. Results and Discussion

The use of plants with medicinal properties for the treatment, cure and prevention of diseases is one of the oldest medicinal methods known in history. At the beginning of the 1990s, the World Health Organization stated that 65-80% of the population of developing countries depend on medicinal plants as their only form of basic health care.[8] 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. It promotes an early oxidative stress and afterwards contributes to the development of serious pathological conditions.[9]

This investigation examined the intervention of *Moringa oleifera* seed oil extract in cadmium and herbal alcoholic

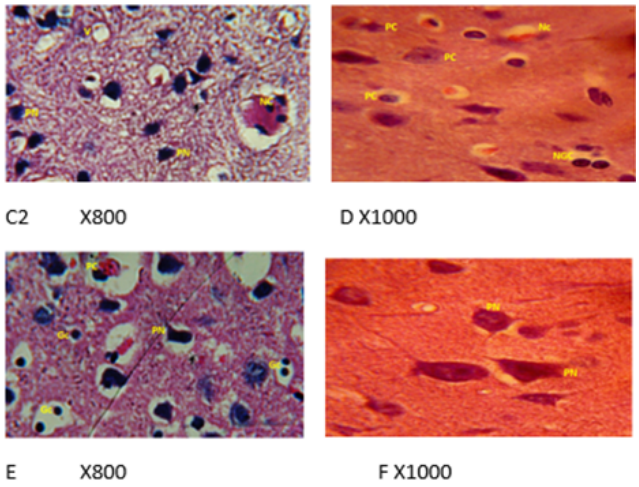

Fig. 2: Photomicrograph of a section of prefrontal cortex of Moringa and HAB treated rats showing; **C2:** (HAB+Moringa) shows evidence of cell swelling, shrinkage and vacuolated neurons but, few of the neurons still retained normal shape and form**; D:** (Cd+HAB) shows distinct cell shape and outlines with cell vacuolation (V), with neuronophagic cell (NC) which are phagocytotic in appearance; **E:** (Cd+HAB+Moringa) shows evidence of normal pyramidal neuron (PN) and increase glia cells (GC) were evidence; **F:** (Moringa only) shows prominent pyramidal cells and reappearing of neurons, with evidence of normal cells

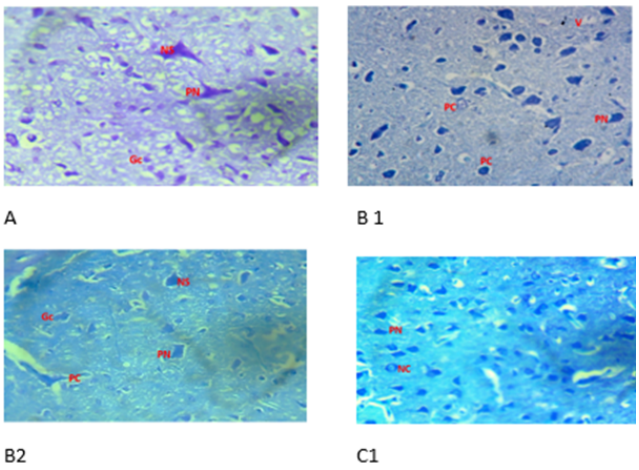

Fig. 3: Photomicrograph of a section of prefrontal cortex of Moringa and HAB treated rats showing; **A:** (control) normal distribution of Nissl substance was observed in the neurons; **B1:** (Cd only) shows less distribution of nissl body with numerous sites of dissolution of nissl substance and few number of viable neurons; **B2:** (Cd+moringa)shows low cresyl dye intensity, indicating loss of nissl body and cell vacoulation; **C1:** (HAB only) shows a reduced number of nissl body and very reduced number of neurons and the granule cells retained their normal cells outlines

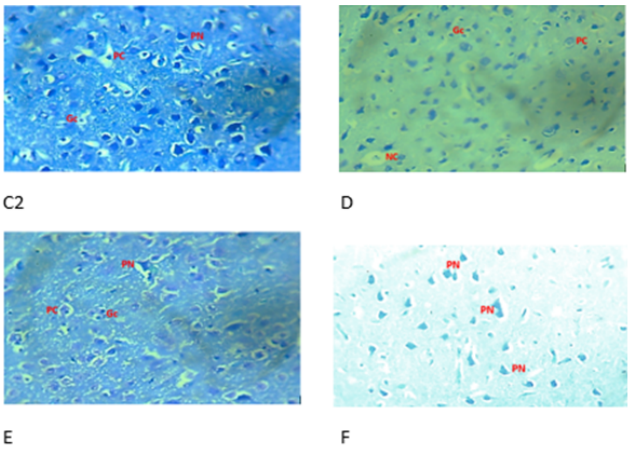

Fig. 4: Photomicrograph of a section of prefrontal cortex of Moringa and HAB treated rats showing; **C2:** (HAB+Moringa) shows a reduced number of neurons with less distribution of nissl substance, with dissolution of nissl substance (chromatolysis)**; D:** (Cd+HAB) shows a very reduced number of neurons, cell shrinkage, vacuolation with loss of nissl substance; **E:** (Cd+HAB+Moringa) shows a considerable reduced number of neurons and comparabely distribution of nissl substance was evident; **F:** (Moringa Seed Oil only) shows a comparabely normal pyramidal cells layer with normal distribution of nissl substance

beverage induced damage to the frontal cortex in Wistar Rats, which involved histological studies. Two types of cortices are recognized in the rat brain: the granular and agranular types of cortices. In the agranular type of cortex, the granular layers are not well developed. The agranular type of cortex is characteristic of the precentral gyrus and other areas in the frontal lobe. These areas give rise to large numbers of efferent fibers that are associated with motor function.[10] This normal cell arrangement and histoarchitecture is observed in the photomicrograph of the frontal cortex tissue of the control group (Group A) rats (Figure 1). The various layers of cells in the frontal cortex are seen clearly and were intact. The photomicrograph of group B1 (inFigure 1) animals induced with cadmium only was observed and it showed features of cells in the frontal cortex layers. The tissue histoarchitecture was distorted and the nuclei of most cells shrank toward the periphery of the cytoplasm while some of the neurons appeared swollen, with condensation of their chromatin materials (karyolysis). Due to this observation, it is expected that normal cell activities such as respiration and nutrient uptake are disrupted, which might cause problems in the normal body system functioning. This is confirmed by previous experiments which proved the inhibition of cell activities by cadmium.[11] Group B2 (in Figure 1) which was treated with *Moringa Oleifera* seed oil for the period of 28 days showed slight distortion and rejuvenized cells were seen with intact glia cells in the frontal-cortex tissue, this might suggests that *Moringa Oleifera* seeds oil extract have ameliorative properties against cellular damage.[12]

There was a loss of cells in the tissue of animals treated with alcoholic herbal beverage rats, when compared to the control animals' cell morphology with loss in cellular integrity. This was in contrast in the induced group and the treated groups that showed ameliorative changes to the normal histology in the frontal-cortex of the animal models. Many of the metabolites from medicinal plants like *Moringa Oleifera* especially flavonoids exhibited potent antioxidant activity *in vitro* and *in vivo* according to previous studies.[13–15] The activities of these antioxidants improve cell morphology.[16,17] The photomicrograph of group C2 (Figure 1) animals' tissue showed evidence of improvement as compared to the control group. Group D animals (Figure 2) were observed to show complete loss in histoarchitecture, shrinkage, swelling and halo around the cells as seen in group B1 rats which indicate the deleterious ability of both cadmium and alcoholic herbal beverage in altering cell nuclear structures and activities.[12] Cellular damage was observed in group E (Figure 2) this shows that *Moringa oleifera* oil has a minimal ameliorative potential on frontal-cortex in combined state of both Cd and herbal alcoholic beverage. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced during the normal metabolism can damage the cells resulting in lipid peroxidation, alteration of protein and nucleic acid structures.[12,18,19]

Group F(Figure 2) showed a well-organized histoarchitecture in the frontal-cortex of the rats with intact and well-defined cell in line with normal cerebral histology as compared with control group.Cresyl fast violet demonstrates Nissl bodies which are aggregates of basophilic Rough endoplasmic reticulum, found in abundance in the neuron soma. Extensive dark purple coloration was observed in the tissue photomicrograph of the group A animals (Figure 3) indicating the presence of sufficient Nissl bodies in the cells. In a normal cell, the rough endoplasmic reticulum functions in the production and modification of proteins that are to be packaged, in addition to the production of membrane lipids and proteins.[20] The intensity of the dark purple coloration is greatly reduced which means that there is reduction of the amount of nissl bodies, dissolution of nissl substance (chromatolysis) in the cells and tissue photomicrograph of group B1(Figure 3) rats induced with cadmium. This suggests that some function of protein synthesis and packaging is lost in the cells. Heavy metals such as cadmium have been implicated in oxidative damage caused by the increase of ROS and their consequent attack on proteins, lipids, and nucleic acids, leading to failure of functioning of enzymes, distorted membrane

fluidity, and genomic damage.[21] Administration of *Moringa oleifera* seed oil to rats in group B2 (Figure 3) brought about an improvement in the intensity of the dark purple coloration signifying presence of Nissl bodies in the tissue photomicrographs of the animals was reduced but the damage was not as wide spread as in the group induced with cadmium only.

Studies have shown that *Moringa oleifera* seed has antioxidants that help to reduce oxidative stress by regulating protein biosynthesis.[17,22] This was observed in the group C1 (Figure 3) animals which demonstrated loss of Nissl bodies indicating the loss of protein synthesis with resultant cell death. Animals in group C2 (Figure 4), group D shows reduced number of neurons, cell shrinkage, vacuolation with loss of nissl substance and group E treated with *Moringa* oil showed better recuperative effects as compared with control group A rats; group F (Figure 4) rats showed similar properties as compared to control group A rats with the presence of numerous Nissl substances in their neurons, this proved the ability of Moringa Oleifera seeds to cause more production of Nissl substances in the neurons of the frontal-cortex in the experimental rat models.[18,23]

5. Conclusion

It has been found that *M. Ooleifera* seed oil has ameliorative effects on morphological damage caused by cadmium and herbal alcoholic beverage on the frontal cortex of Wistar rats. This study has given a better understanding that cadmium and herbal alcoholic beverage were capable of crossing blood brain barrier (BBB) to induce neuronal damage and immunotoxicity as evident in this study.

6. Acknowledgement

We appreciate Kogi State University Management, Tertiary Education Trust Fund and Technical Staff in the Department of Anatomy, Faculty of Basic Medical Sciences, Kogi State University and Olabisi Onabanjo University.

7. Conflict of interest

No conflict of interest among authors

8. Source of funding

None

9. Recommendation

Moringa Oleifera seed oil efficacy and medicinal importance should be advocated and more public awareness should be encouraged on the use of the plant extract, as well as implication of human exposure to heavy metals (Cd) and excessive consumption of herbal alcoholic beverages.

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Cite this article: Olusegun Dare O, Busayo E A, Samson E O. Recuperative properties of Moringa Oleifera seed oil on cadmium and alcoholic beverage induced frontal cortex damage in Wistar Rats. *Indian J Neurosci* 2019;5(4):214-219.