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#### **Original Research Article**

### Neuroprotective properties of Moringa Oleifera in cadmium and herbal alcoholic beverage induced frontal cortex damage in Wistar Rats

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#### ABSTRACT

Exposure to cadmium and consumption of herbal alcoholic beverages (HAB) is rising in Nigeria, thereby leading to the increase in the risk of neurodegenerative disorders. Preventing the damages caused by these exposures will be a valuable approach in managing neurodegenerative disorders associated with this exposure. In folkloric medicine moringa oleifera is used to manage many disease conditions especially neurodegenerative diseases. This study is therefore aimed at investigating the neuroprotective properties of Moringa Oleifera in cadmium and (HAB) induced frontal cortex damage in Wistar Rats.

Eighty Wistar Rats 73g-151g were used for the study. The rats were randomly divided into eight groups A, B1, B2, C1, C2, D, E, and F of 5 rats each. group A served as control which received 2.5mg/kgbw phosphate buffer intra-peritoneally, while group F served as Moringa-treated control and received oral administration of 2.0 mg/kgbw Moringa oleifera. Groups B1, B2, D and E were injected intra-peritoneally with 3.5mg/kgbw CdSO<sub>4</sub>.8H<sub>2</sub> Osingle dose. Group C1, C2 and D received oral administration of 0.5 ml HAB and group B2, C2and E were administered orally with 2.0mg/kgbw Moringaoleifera. The intervention lasted for four weeks and the tissues were processed histologically.

Activation of astrocytes were evident in group B1, 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 the number of swelling and pyknotic neurons and reduction of activated astrocytes

Moringa oleifera has neuroprotective properties in the frontal cortex by reducing the numbers of activated astrocytes.

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#### 1. Introduction

The Frontal Cortex is located in the anterior part of the cerebrum and extends from the superior frontal sulcus to the lateral frontal sulcus of (sylvius) and aligned to the central frontal sulcus (of Ronaldo). Histologically, it is made up of neurons and glial cells with presentation of six distinct layers (I-VI) such as: I-Molecular layer II-External granular layer, III-External pyramidal layer, IV-internal granular layer, V-internal pyramidal layer and

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VI-Multiform layer. Frontal Cortex plays a vital role in learning, personality and in information processing. 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. Cadmium can effectively pass the BBB during the developmental stage in an organism and is more toxic in newborns. Cadmium LD<sub>50</sub> of 5.7 mg/kg per body weight intraperitoneally on rats and once administered, it accumulates in different areas of the brain, induces lipid peroxidation and weakens the antioxidant defense. In battery workers Cd-induced oxidative stress was demonstrated to cause amyotrophic lateral sclerosis due

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to reduced brain SOD activity. 4

Cadmium (Cd) is a toxic metal in the environment, found in the soil, rock phosphate fertilizer and in tobacco plant. Cd is a highly accumulative toxicant with very long biological half-life.<sup>5</sup> It is not biodegradable and its levels in the environment are increasing due to industrial activities and human exposures to cadmium are inevitable.<sup>5,6</sup> Acute Cd exposure produced toxicities to the lung, liver, testes and brain, while chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis and bone fractures.<sup>7</sup> Cd is a potent carcinogen in a number of tissues of rodents and classified as a human carcinogen.<sup>8</sup> The neurotoxic effects of Cd have been reported in neonatal mouse brain.<sup>9</sup> and young rat brain.<sup>10</sup>

Cadmium produces oxidative damage to isolated rat optic nerve 11 and culture rat cortical nevrons. 12 In humans, occupational exposure to Cd is associated with nerve psychological disorders 13 and Parkinsonism has been reported in a 64 year old man exposed to Cd at a high dose. 14 Thus, accumulating evidence clearly indicates that Cd is neurotoxic in a number of settings. The mechanisms involved in neurotoxicity of Cd are poorly understood. Oxidative stress has been proposed as a mechanism for Cd toxicity in a number of tissues such as the kidney, <sup>15</sup> liver <sup>16</sup> and brain, <sup>17</sup> Due to its low permissible exposure limit (0.5  $\mu/L$  to 1.0  $\mu/L$ ) in human, over exposure may occur even in situations where trace quantities of cadmium are found. Exposures to cadmium are addressed in specific standards for the general industry, shipyard employment, construction industry and the agricultural industry. <sup>18</sup>

Herbal alcoholic beverages commonly called Ogogoro, alomo bitter, Opaeyin in Nigeria. It is locally manufacture and package; it is consumed locally by the general public for sexual enhancement and as stimulants. Various investigations have revealed the deleterious effects of high percentage of alcohol (ranging from 17% to 70%) in most of the herbal alcoholic beverages 19 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 leading to soft tissues damage such as cardiovascular, lung, liver, kidney and brain. <sup>19</sup>Moringa oleifera Lam (Moringaceae) is a highly valued plant distributed in many countries of the tropics and subtropics. It is the most widely cultivated species of the genus Moringa, which is the only genus in the family Moringaceae. 20 English common names include moringa, benzolive tree and West Indian Ben. It is also knows as drumstick tree, from the appearance of the long slender triangular seed pods, horseradish tree, from the taste of the roots which resembles horseradish or Ben oil tree.<sup>20</sup> Moringa seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non- drying oil that

resists rancidity. It has been used in salads, for fine machine lubrication and in the manufacture of perfume and hair care products. <sup>21</sup> It is an exceptionally nutritious vegetable tree with a variety of potential uses. <sup>20</sup> This tree has in recent times been advocated as an outstanding indigenous source of highly digestible Protein, Ca, Fe, Vitamin C and carotenoids suitable for utilization in many of the so called developing regions of the world where undernourishment is a major concern. <sup>21,22</sup> Moringa leaves contain more vitamin A than carrots, more calcium that milk, more iron that spinach, more vitamin C than oranges and more potassium that bananas and that the protein quality of Moringa leaves rivals that of milk and eggs. <sup>22</sup> In addition to its compelling water purifying powers and high nutritional value, *Moringa Ooleifera* is very important for its medicinal value.

Various parts of this plant such as the leaves, roots, bark, flower, seed, immature pods and fruit act as cardiac and circulatory stimulants possess antitumor, antipyretic, antiepileptic, anti-inflammation, antifungal diuretic, cholesterol lowering antioxidant, anti-diabetic, hepatopiotective, antibacterial and are being employed for the treatment of different ailments in the indigenous system. <sup>22,23</sup> However, there are few reports on the neuroprotective properties of Moringa Oleifera in Cadmium and Herbal cholic induced frontal cortex damage. This study is therefore aimed at investigating the intervention of *Moringa Ooleifera* seed extract in cadmium and herbal alcoholic beverage induced frontal-cortex damage in Wistar Rats by assessing the level of reactive astrocytes in the frontal cortex.

#### 2. Material and Methods

#### 2.1. Plant collection and extraction

Moringa Oleifera seeds were procured from Ladoke Akintola University Farm in Ogbomos ho, and identified at the Forestry Research Institute of Nigeria (FRIN), Ibadan, with a Voucher number FHI. 110266. The seeds were air-dried at room temperature and finely powdered with a blender. Two hundred grams (200g) of the pulverized plant was macerated in ethanol for 72 hours. After the extraction, the extract was sieved and filtered. The filtrate was concentrated in the oven at 40°C (Ugwu Okechukwu et al.,2013). The dried extracts were stored at 4°C until needed. Appropriate dose dilutions were made with distilled water.

#### 2.2. Determination of phytochemicals and acute toxicity

The quantitative phytochemical analyses were carried out according to the methods of Harborne (1973, 1984) and Trease and Evans (1989). Acute toxicity studies ( $LD_{50}$ ) was conducted using methods of Lorke (1989 animals were critically observed for 24 hours.

#### 2.3. Animals

Wistar Rats of both sexes weighing 73g-151g were used for this study. They were bred in the animal house of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne-Remo Campus, Nigeria, to rule out the genetic effects on the investigation and second filial generation were used for the study. The animals were maintained under standard laboratory conditions. They were given rat chow and water *ad libitium* and acclamitization was done for a period of two weeks.

#### 2.4. Ethical guidelines

All experimental investigations were done in compliance with the guideline, as stated in the "Guide to the care and use of Laboratory Animals Resources" (NRC, 1985) and in accordance with guidelines stated in IACUC and OLAW, United Kingdom.

#### 2.5. Animal groupings

A total of 80 adult Wistar Rats aged eight (8) weeks of both sexes were used for this study. The animals were randomly divided into eight (8) groups A, B1, B2, C1, C2, D, E and F of 5 animals each. 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<sub>4</sub>.8H<sub>2</sub> O according to the method of Ige et al., 2010 intraperitoneally and left for 72 hours. B<sub>1</sub> rats were maintained under normal laboratory condition for period of four weeks and B2 rats received 2.0 mg/kgbw of Moringa oleifera oil extract single dose daily for the period of four weeks. C1 rats received 0.5 ml, 40% Herbal-gin via gavage, single dose daily for the period of four weeks while C2 rats received 0.5 ml, 40% Herbal-gin and 2.0 mg/kgbw of Moringa oleifera 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<sub>4</sub>.8H<sub>2</sub> O) single dose and maintained for 72hrs (Ige et al.,2010) 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<sub>4</sub>.8H<sub>2</sub> O) single dose and maintained for 72hrs (Ige et. al.,2010) following oral administration of 0.5 ml, 40% Herbal-gin and 2.0 mg/kgbw Moringa oleifera seed oil extract single dose daily for the period of four weeks. Group F rats received 2.0 mg/kgbw of Moringa oleifera seed oil via gavage, single dose per day for the period of four (4) weeks.

#### 2.6. Animal sacrifice

Twenty four hours after the last administration, all 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 histochemical stains involving toluidine blue stains and immunohistochemical stain involving GFAP.

#### 3. Results

### 3.1. The percentage yield of Moringa Oleifera seed oil extract

A total percentage of 33.1 % oil yield was obtained from 200 g of dried coarse form of the *Moringa oleifera* seed used in this study. The 33.1 % oil yield was displayed in three layers comprising of (a) the top layer presentation of light yellow colour spectrum (24.1 %), (b) middle layer of thick yellow – milky spectrum (2.2 %) and (c) the lower layer of reddish brown colour spectrum (6.8%) as shown in Table 1.

Table 1: Result of the percentage yield of Moringa Oleifera Seed

Moringa oleifera seed oil	Colour spectrum layers	Percentage yield of <i>Moring</i> a oil (%)
Top layer	Light Yellow	24.1
Middle layer	Thick Yellow-Milky (as fats)	2.2
Lower layer	Reddish Brown	6.8
Total (%)		33.1

### 3.2. Phytochemical analysis of Moringa Oleifera seed extract

The Moringa extract showed high quantities of alkaloids, carbohydrate, flavonoids, Steroids and Cardiac glycosides as shown in Table 2.

#### 3.3. Acute toxicity (LD50) in M. Oleiferaseed extract

The acute toxicity (LD<sub>50</sub>) of the *Moringa oleifera* seed oil in Wistar rats was found to be more than 1,000 mg/kg body weight of the animals and less than 5,000 mg/kg body weight.

## 3.4. Assessment of toluidine blue for astrocytes in the frontal Ccortex of adult Wistar Rats to demonstrate astrocytosis in the neuroglia

Plates 1(Control) and plate 8 (Moringa treated control) show inactivated astrocytes while plates 2 (Cd-induced group), **3** (Cd -induced treated with *M.oleifera* seed oil) 4 (HAB-induced group) 5 (HAB-induced group treated with Moringa) 6 (Cd-induced, administered HAB) and 7 (Cd-induced, administered HAB and treated with Moringa)

**Table 2:** Phytochemical Analysis of *Moringa oleifer*a Seed

 Extract

Bioactive Constituents	Moringa Seeds Extract
Alkaloids	+++
Saponins	+
Tannins	-
Flavonoids	+
Carbohydrates	++
Steroids	++
Anthraquinins	-
Cardiac	+
glycosides	

Key: - Negative, + Present, + + More Present, + + + Highly Present

Plates 1 and 8 show a preponderant of activated astrocytes by a process called astrocytosis. Viable neurons were also observed with normal distribution of Nissl substance, all at X 400.

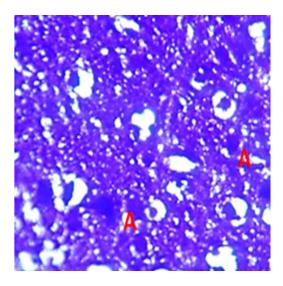


Fig. 1: Plate 1

Figures abbriviation: A: Astrocytes, N: Neuron, PN: Pyramidal Neuron, A: Astrocytes, V: Vacuolation

## 3.5. Immunohistochemical Demonstration of Glial Fibrillary Acidic Protein (GFAP) in Paraffin-Embedded Frontal Cortex Tissue Sections

Expression of glial fibirillary acidic protein (GFAP) was observed to be negative in Plates 9Control) and plate 16 (Moringa treated control) with the presentation of rosered cytoplasm and the intact blue nuclei that were evenly distributed, while while plates 10 (Cd-induced group), 11(Cd -induced treated with *M.oleifera* seed oil) 12(HAB-induced group) 1 3 (HAB-induced group treated with Moringa) 14 (Cd-induced, administered HAB) and 15(Cd-

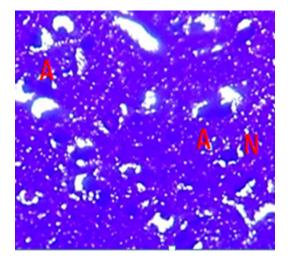
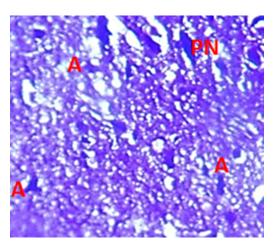


Fig. 2: Plate 2



**Fig. 3:** Plate 3

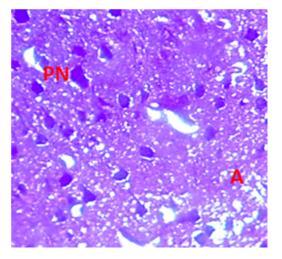
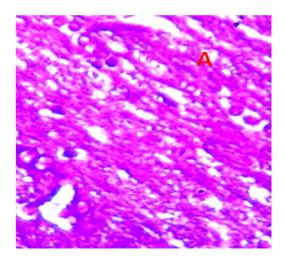
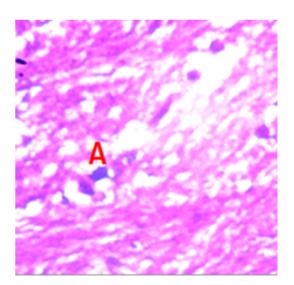


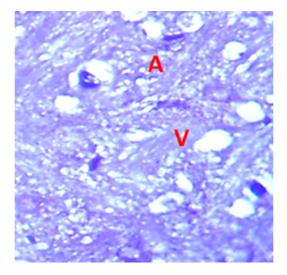
Fig. 4: Plate 4



**Fig. 5:** Plate 5



**Fig. 6:** Plate 6



**Fig. 7:** Plate 7

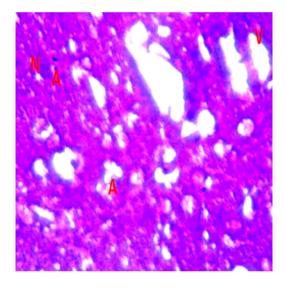


Fig. 8: Plate 8

induced, administered HAB and treated with Moringa) were positive to GFAP with the presentation of rose-red to brownish red cytoplasm with loss of nuclei in some plates such as evident in the se plates, all at X 400.

Plate 9 shows normal and numerous cerebral nuclei and a negative cytoplasm to glial fibrilliary acidic protein specific for astrocytes while plate 10 shows reduced cerebral nuclei population, positive GFAP specific for astrocytes was evident, at MAG X 400.

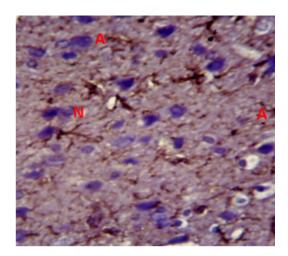
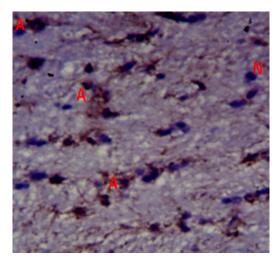
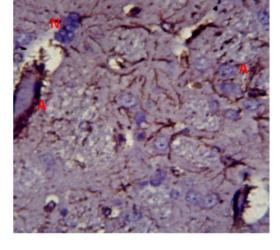


Fig. 9: Plate 9

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. <sup>24</sup>



**Fig. 10:** Plate 10



**Fig. 13:** Plate 13

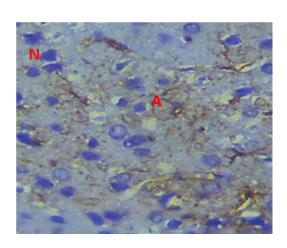


Fig. 11: Plate 11

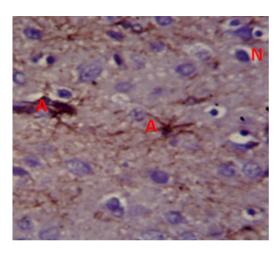


Fig. 14: Plate 14

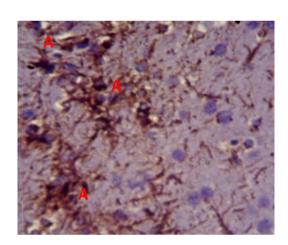
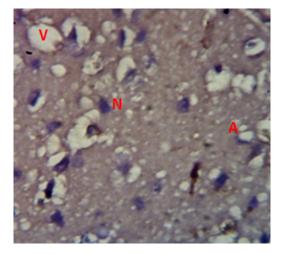


Fig. 12: Plate 12



**Fig. 15:** Plate 15

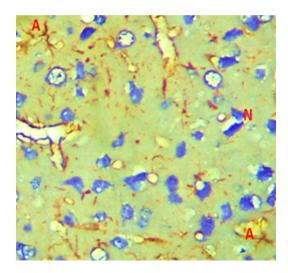


Fig. 16: Plate 16

This investigation examined the intervention of *Moringa oleifera* seed oil extract in cadmium and herbal alcoholic beverage induced damage to the frontal cortex in Wistar Rats,

In the acute toxicity test, up to a dose of 5000 mg/kg there was no death recorded in the treated animals. This shows that Moringa Oleifera is safe for use because of its wide safety margin. Alkaloids, flavonoids, saponins and terpenoids are phytochemicals revealed in Moringa Oleifera from the phytochemical screening. Pharmacological activities associated with alkaloids and flavonoids include its ability to prevent cancer, inhibit inflammation, pain and prevent free radical generation. Flavonoids have been shown to have neuroprotective properties and this might be responsible for the neuroprotective property of Moringa Oleifera in the frontal cortex observed in this study.

Astrocytes are supporting cells of the nervous tissue and are found in all brain regions. They contact blood vessels, pial surfaces and enfold synapses in their functions to maintain the concentration of ions, neurotransmitters and other metabolites within normal levels in the extracellular space. They also play a fundamental role in inducing blood brain barrier (BBB) functions in cerebral vessels (Neoplasia involving astrocytomas).

Glial fibrillary acidic protein (GFAP) is a 51,000 molecular weight intermediate filament protein expressed in normal, reactive and neoplastic cells (astrocytes, Bergman glis, ependymocytes and certain oligodendrocytes). It is absent from neurons. GFAP is also found in cells of the adenohypophysis, schwannomas, neurofibromas and pleomorphic adenomas of the salivary gland. <sup>27,28</sup>

The results of the present study in both Toluidene blue and GFAP frontal cortex tissue sections of group B1, C1 and D animals showed a preponderance of reactive astrocytes with indication of piloid gliosis (astrocytomas), it was evident that the astrocytes have undergone hypertrophy

(enlargement) and hyperplasia (proliferation) in response to a great and many pathological processes including hypoxic-ischemic damage and trauma as compared with group A (control) rats with non-reactive astrocytes. <sup>27,28</sup>

The frontal cotex brain tissue sections in group B2, group C2 and group E animals showed few reactive astrocytes and there were no or little evidence of piloid gliosis as compared with tissue section of group A rats with nonreactive astrocytes, this observation proved the recuperative evidence of Moringa oleifera seed oil in abating the free oxygen species (ROS) in the brain by cadmium and herbal alcoholic beverages as reported by Harper and Butterworth 2002. Group F rats showed evidence of non- reactive astrocytes as compared with group A animal with nonactivated astrocytes which was an evidence that Moringa oleifera seed oil is a non-toxic substance with protective functions. This study has shown that Moringa oleifera oil extract has antioxidant properties that might have ameliorated morphological damage caused by cadmium and herbal alcoholic beverages by regenerating pyramidal and neuroglial and improving distribution of Nissl bodies and reducing the level of reactive astrocytes in the frontal cortex of the treated rats. 27,28

#### 4. Acknowledgement

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#### 5. Conflict of interest

None

#### 6. Source of funding

Tertiary Education Trust Fund (TETFUND)

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