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RESEACH ARTICLE

Ameliorative Effects of Aqueous Leaves Extract of *Moringa Oleifera* on Mercury-Induced Toxicity in Cerebral Cortex of Adults Wistar Rats

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Abstract

Background: Mercuric chloride toxicity poses a significant global health challenge due to its severe adverse effects on various body tissues. This study aimed to investigate the ameliorative potential of Moringa oleifera leaf extract on the cerebral cortex of adult Wistar rats exposed to mercuric chloride. **Methods:** Twenty-five adult Wistar rats (n=25) were randomly divided into five groups (n=5 per group). Group A (control) received distilled water. Group B received mercuric chloride (0.5 mg/kg). Group C received Moringa extract only. Group D received concurrent mercuric chloride (0.5 mg/kg) and Moringa extract (500 mg/kg). Group E received concurrent mercuric chloride (0.5 mg/kg) and Moringa extract (750 mg/kg). All treatments were administered orally for 21 days. The cerebral cortex was harvested, and histological analysis was done using H&E staining. **Results:** The mercuric chloride-treated group (Group B) exhibited significant neuronal loss, characterized by numerous vacuolations in the cerebral cortex photomicrographs. Concurrent administration of Moringa leaf extract (Groups D and E) minimized the number of vacuolations in a dose-dependent manner. **Conclusion:** Moringa oleifera leaf extract demonstrated an ameliorative effect against mercuric chloride-induced toxicity in the cerebral cortex of adult Wistar rats.

Keywords: Cerebral Cortex, *Moringa Oleifera,* Neuronal loss, Mercury.

Introduction

Human and animal populations interact with their environment on a daily basis and as such are exposed to a wide range of chemicals and heavy metals. These interactions with the environment occur through food, air and water (Burger *et al*., 2011; Burger & Gochfield, 2011). Mercury occurs in the environment owing to natural processes such as degassing from earth crust, emissions from volcanoes, evaporation from water bodies and anthropogenic processes from coal-fires, power stations, residential heating systems and waste incinerators (Burger & Gochfield, 2011). Mercury toxicity can be present as result of mining of mercury, gold, copper, zinc, lead and silver (ATDSR, 1999). There is a growing awareness of the effects of heavy metals such as mercury on the brain and nervous system because of its capability to cross the blood brain barrier and accumulate in the brain with associated damage (Langford & Ferner, 1999; Valko *et al*., 2005). *Moringa oleifera* is the most widely cultivated species of the genus Moringa which is the only genus in the family Moringaceae. Its English names include Moringa, drumstick tree, horseradish and ben oil tree or benzoil tree (Olson, 2010). It is a fast-growing drought-resistant tree native to the Southern Foothills of the Himalayas in Northwestern India, Nigeria, and widely cultivated in tropical and sub-tropical areas (Parotta, 1993). It is valued worldwide for its ability to treat over 300

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diseases (Fahey, 2005). Every part of *Moringa oleifera* is a storehouse of important nutrients and anti-nutrients. The leaves of *M. oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper (Kasolo *et al*., 2010). It is rich in vitamins like beta-carotene of vitamin A and phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids (Mbikay, 2012). It is also rich in reducing sugars as well as anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate (Mbikay, 2012). Cerebral Cortex was reported to be highly vulnerable to mercury intoxication as demonstrated in rodent experiments (Xu *et al*., 2012; Owoeye & farombi, 2015; Owoeye & Ojora, 2015; Owoeye *et al*., 2017). Thus, since the cerebral cortex is responsible for regulating complex activities of cognition (and primary sensory functions), and its structurally rich in lipid with poor anti-oxidant handling capacity relative to other tissues, it is more susceptible to damage by reactive oxygen species (ROS) (Snell, 2011). Damage of neurotoxicants may affect the histology and physiology of brain tissue and the changes may be assessed (Ebokaiwe *et al*., 2009). The impetus for the current work stems from earlier studies on the ameliorative effects of aqueous leaf extract of *Moringa oleifera* on heavy metals like aluminium chloride, lead, and cobalt chloride on some part of the brain like Hippocampus, Frontal Cortex, and Cerebral cortex (Garman, 2011; Owoeye & Ojora, 2015; Owoeye *et al*., 2017). There is dearth of literature on the ameliorative effects of aqueous leaf extract of *Moringa oleifera* on mercury induced toxicity on the cerebral cortex of Adults Wistar Rats. The aim of this study was to evaluate the ameliorative effect of aqueous leaf extract of *Moringa oleifera* on mercury induced toxicity on the histology cerebral cortex of Adults Wistar rats.

MATERIALS

Twenty-five Wistar rats, animal wire mesh cages, saw dust for animal beddings, animal feeds (Chikun grower feeds - Kaduna) and distilled water, Whatman's filter paper #1, Weighing balance (Gallenkamp, UK).

METHODOLOGY

Experimental design: Twenty-five (25) apparently healthy adult Wistar rats weighing between 90-170g were obtained from the Department of Pharmacy Bayero University, Kano (BUK). The animals were allowed to acclimatize for two weeks at the Department of Anatomy animal house, Bayero University Kano (BUK), and were given feed and water *ad libitum*. The rats were randomly selected and assigned into five groups A, B, C, D and E with each group having 5 animals. The rats in Group A served as the control, while those in Group B, Group C, Group D, and Group E were treated with *Moringa oleifera* leaves extract and Mercury Chloride for a period of 21 days.

Extract Preparation; Fresh *Moringa oleifera* leaves were plucked from a farm along Madobi Road, Kano State. It was identified and authenticated at the Department of Plant Biology Herbarium, Bayero University Kano with accession number BUKHAN 0011.The leaves were air-dried under a shade and were allowed to dry for five days. The dried leaves were collected and grinded to a powder using a pestle and mortar. The powdered leaves were weighed using Gallenkamp (FA2104A, England) electronic weighing balance. Then 1.8 kg of the powdered leaves was soaked in 4 litres of distilled water for 72 hours at room temperature. The soaked leaves powder was first filtered using a silk material, then again re-filtered using Whatman's filter paper #1 and the residue discarded. Then the filtrate was heated at 33^0C in a water bath and was allowed to evaporate for 72hours, after fully evaporated, a dark brown sticky mass of the extract was obtained with a total yield of 3.1%. Mercuric chloride was obtained from the Department of Biochemistry at Bayero University, Kano. 0.1g of mercury chloride was dissolved in 100 ml of distilled water to form a stock solution. All the procedures and safety protocols of storing and handling toxic chemicals were observed. The LD_{50} of the *Moringa oleifera* was adopted at 300mg/kg (Mbikay, 2012), while that of the mercuric chloride was taken to be 1mg/kg (Xu *et al*., 2012).

Animal treatment: For three weeks, the body weight changes were monitored daily throughout the experimental period. All the substances were administered orally, and the study was conducted from December 2023 to January 2024. The rats in group A were given distilled water only, while the rats in group B were given 0.5mg/kg of mercuric chloride only. The rats in group C, were administered orally with 500 mg/kg body weight of the extract. The rats in Group D, were given 0.5 mg/kg of the mercuric chloride and 500 mg/kg of the extract concurrently, and the rats in Group E were given 0.5 mg/kg of the mercuric chloride and 750mg/kg of the extract. The extract and the mercury solution were administered orally to the rats, using an oro-gastric cannula on each treatment day for 21 days.

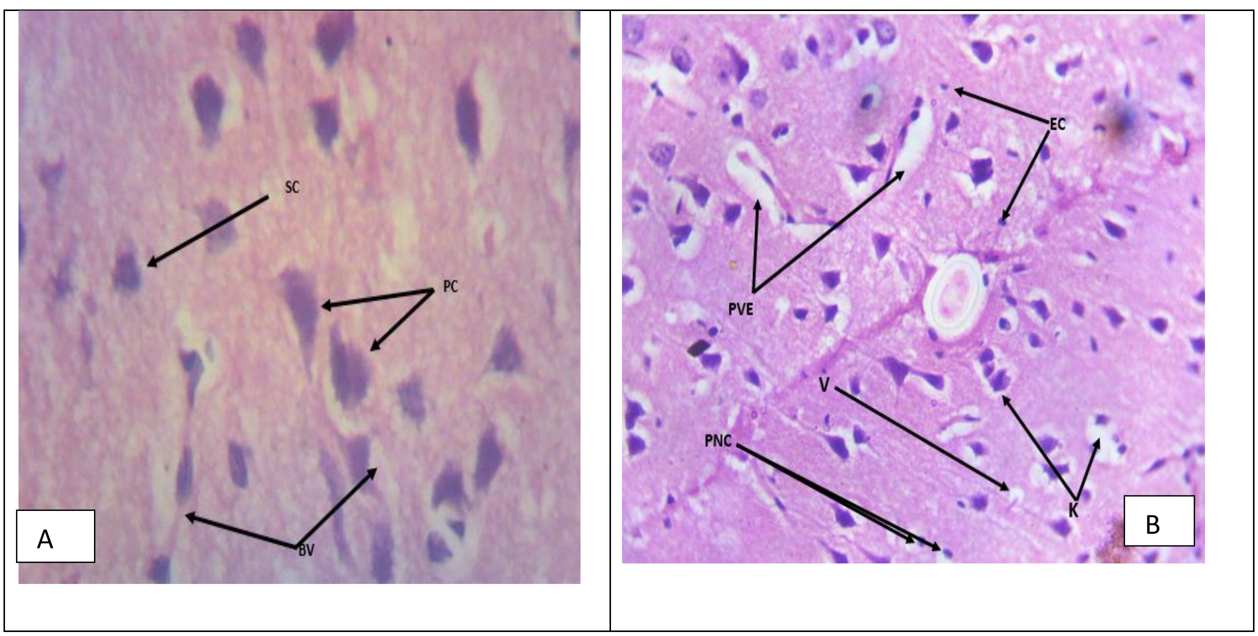
Animal Sacrifice: The animals were humanely sacrificed twenty-four hours after the last treatment through the ventral abdominal wall incision. The Wistar rats were anaesthetized using chloroform, the head decapitated and the cranium of each of the animals was opened using forceps. The brain was carefully dissected and then immediately placed in 10% formal saline for 24 hours.

Tissue processing: The Brain (cerebral cortex) tissues were processed routinely for histopathological studies using haematoxylin and eosin staining procedure for demonstrating nuclei and cytoplasm. The fixed brain (cerebral cortex) tissues were removed from the fixation medium and dehydrated using ascending grades of alcohol. Dehydrated tissues were then cleared in two changes of xylene for two hours, and then infiltrated by immersing in molten paraffin wax and allowed to solidify. The embedded tissues were blocked in a rectangular block. Rotary microtome was used in cutting the tissue sagittally at 5 µm per section. Tissues were allowed to float in a warm water bath at approximately 30ºC to help in spreading the paraffin ribbon, clean glass slides were used to pick up the tissue from the water bath. The histological slides of the cereberum were mounted on a Motic Photomicroscope fitted with a *celestron*© digital microscope imager (USA) with an inbuilt x15 objective lens. The slides were examined and photographed.

Hematoxylin* and *Eosin (H and E)* Staining Techniques*: Cerebral cortex tissues were allowed to dry by dewaxing the tissues in two changes of xylene for 3 minutes each. Descending grades of alcohol; 100%, 95%, 90% and 70% was used to hydrate the tissues for 3 minutes each, stained with Harris haematoxylin for 10 minutes, and washed with tap water to remove excess stain. The tissue slides were flooded with acid alcohol for some seconds for differentiation, washed in tap water again. Bluing was done in water, eosin. Sections were washed and used for histological observation**.**

RESULTS

The section of the cerebral cortex of rats from the control groups stained with H&E were observed to be mainly devoid of any abnormality. There was no evidence of any change in the histomorphology of the stellate cell (SC), blood vessels (BV), and pyramidal cells (PC). There were no features of congestion in the tissue sections. The entirety of the component of the brain tissue (BT) was apparently preserved as described in Plate 1A.



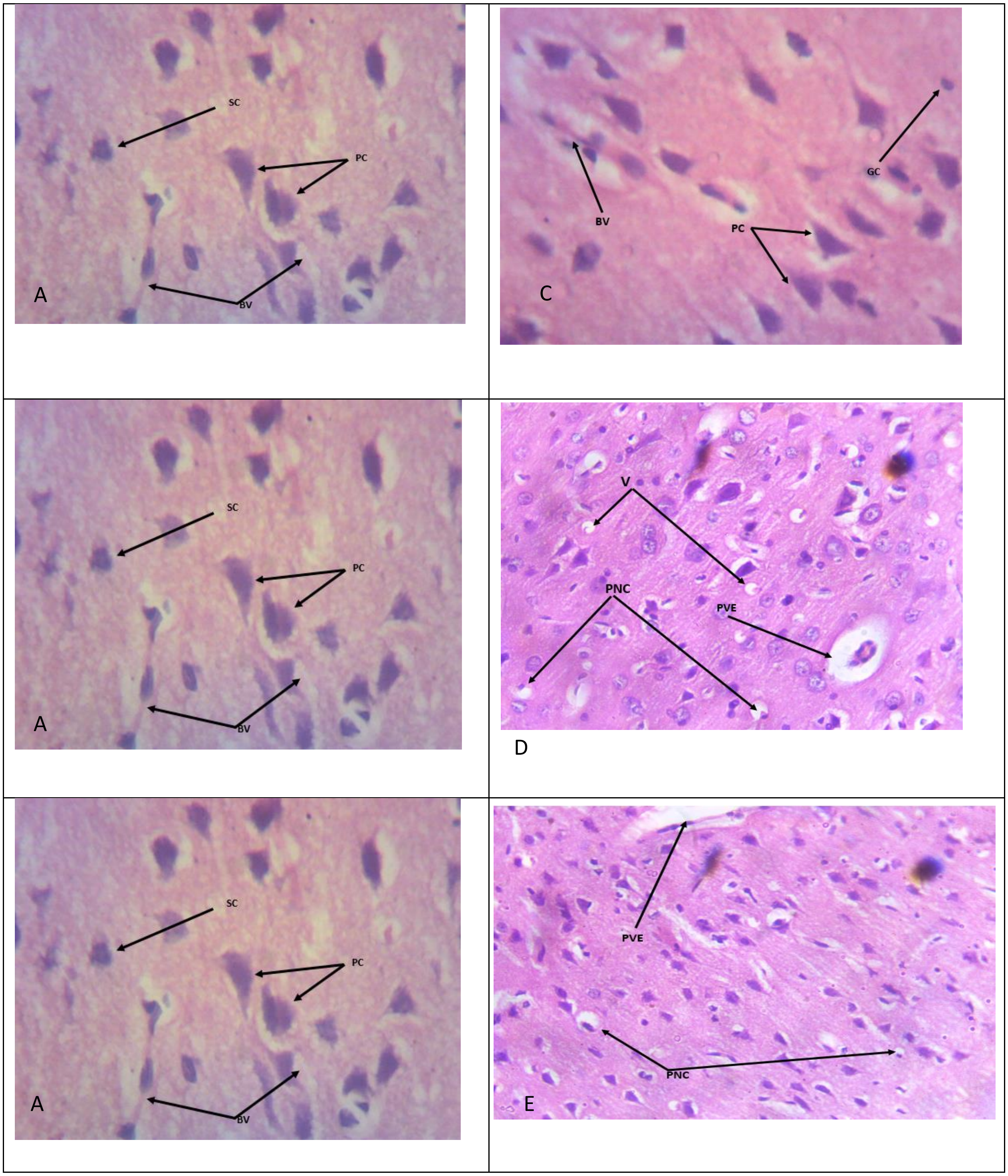


Plate 1: Cross section of Cerebral Cortex of Wistar rat from the five study groups of oral exposure to saline (control) and varying doses of *Moringa oleifera* Leaves extract and a fixed dose mercury chloride.
A. Section from control animal stained with Heamtoxylin and Eosin at (×400) showing preserved architecture with **SC:** Stellate cells; **BV:** Blood vessel; **PC:** Pyramidal Cells. B. Section from rat treated with 0.5mg/kg body weight of mercury chloride only H and E (×400) showing **PVE:** Perivascular Edema; **V:** Vacuolation, **EC:** Ependymal Cells, **PNC:** Pyknotic cells, **K:** Karyolysis. C. Section from rat treated with 500mg/kg body weight per day of the extract only. H and E (×400) showing **PC:** Pyramidal Cells; **BV:** Blood vessel; **GC:** Glial cell. D. Section from rat treated with 500mg/kg and 0.5mg/kg body weight of mercury chloride concurrently. H and E (×400). **PNC**: Pyknotic cell; **V:** Vacuolation; **PVE:** Perivascular Edema. E. Section from rat treated with 750mg/kg body weight and 0.5mg/kg body weight of mercury chloride concurrently. I.e. High dose of the extract. H and E (×400). **PVE:** Perivascular Edema; **PNC**: Pyknotic cell.

The micrograph of the cross-section of the Cerebral Cortex of Wistar rats in group II that were given 0.5 mg/kg of mercury chloride only, for a period of 21 days showed changes such as perivascular edema (PVE), ependymal cells (EC), pyknotic cells (PNC), vacoulation (V), and karyolysis (K). The micrograph of the Cross Section of Cerebral Cortex of Wistar rats in Group III (C) showed certain mild cellular distortive changes in pyramidal cells (PC), glial cells (GC), and blood vessels (BV).The micrograph of the cross section of cerebral cortex in group IV (D) . Showed Perivascular Edema (PVE), Pyknotic cells (PNC), and Vacoulation (V) within the cerebral cortex.

The H&E micrograph of the Shows cross section of Cerebral cortex Micrograph group V (E) H&E (×400). The changes observed were Perivascular Edema (PVE), and Pyknotic cells (PNC).

DISCUSSION

The tissue from animals in the control illustrated neurons and pyramidal cells that were distributed in a regular predictable pattern across the layers of the cerebral cortex. Blood vessels appeared to be morphologically normal without any congestion. There was no vacoulation in the neuropil, thusruling out neuronal loss or myelin or cell processes damage. The sections from the animals exposed to mercury chloride toxicity had mild focal area of perivascular oedema (PVE), the ependymal cells appeared separated, and also had vacoulation associated with extensive damage to both neurons and glia cells. Individually, the neurons appear to be undergoing degeneration as cell bodies were poorly stained, with distorted morphology exhibiting features of acute eosinophilic neuron degeneration. Similar changes were also been reported in an previous animal study of mercury chloride toxicity which was characterised with features of neuronal toxicity including pyknosis and karyolysis of neurons [21, 12, 13, 6]. The consequence of degeneration of cerebral cortex neurons have been associated with the inability of the animal or human to perform executive functions such as self-control, planning, reasoning, attention, decision-making, judgments, overall control of motor function, and abstract thought (Kandel *et al*., 2000; Kieman & Barr, 2009). Fewer neurons, mostly pyramidal cells appear quite large and distinct within the cerebral cortex while the other morphological features appear similar to those of healthy control tissues when the Moringa extract was concomitantly administered. This finding is similar to reported positive effects of moringa extract on the cerebral cortex and suggested it could protect against devastating diseases like Alzheimer’s disease (Ganguly *et al.,* 2005). The neurons from the groups of animals treated with highest dose of the extract concurrently with mercury or with moringa extract alone were adequately distributed across the cerebral cortex and appeared apparently normal. Nevertheless, some evidence of mild focal perivascular oedema, and pyknotic pyramidal cells in both the groups treated with low and high doses of the extract (groups D and E respectively) were still identifiable even though inflammation and karyolysis were not observed in both groups unlike the tissues from mercury exposed animals. The administered moringa extract was demonstrated to be safe to the tissues section and thus the amelioration of the distortion of tissue experienced earlier with mercury can be reliably attributed to the extract.

CONCLUSION

Mercury chloride exposure in adult Wistar rat cerebral cortex produced deleterious effects that were histologically observable in form of cell death, histological disruption, perivascular oedema and vacoulations. Moringa leaf extract administration to Wistar rats was apparently safe and had no disruptive effect on the histological sections of the cerebral cortex of Wistar rats. The administration of the extract concurrently with mercury ameliorated the deleterious effect of mercury on the cerebral cortex.

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Conflict of interest: The authors declare that they have no conflict of interest

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Authors’ Contributions

MMM – Methodology (Animal treatment, histological techniques and production of micrographs), MSS –

review of literature and photomicrographs, IMB – review of literature and photomicrographs and MAS – Study design, manuscript writing and review

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