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# Moringa Oleifera via Antioxidants and Anti-Inflammatory Mediated Response Abates Aluminum Chloride-Induced Neurobehavioral Deficits and Hippocampal Neurodegeneration in Adult Male Wistar Rats

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## ABSTRACT

Chronic aluminum toxicity induces cellular alterations and oxidative stress thereby causing neurodegenerative disorders such as Alzheimer's disease. This study elucidated the neuroprotective efficacy of *Moringa oleifera* (MO) on aluminum-chloride ($AlCl_3$) induced Alzheimer's disease (AD) in adult Wistar rats. Forty (40) adult male Wistar rats (160±20 g) were divided into 4 groups ($n$=10). Group A received normal saline as *placebo*; Group B received 200 mg/kg body weight of $AlCl_3$ only; Group C received 100 mg/kg body weight of MO and 200 mg/kg body weight of $AlCl_3$ and Group D received 100 mg/kg body weight of MO only. All administration was done orally per day for 45 days. After the last administration, the animals underwent behavioral tests (Morris Water maze, Y-Maze, and Open field). Thereafter, animals were sacrificed by cervical dislocation and blood samples were collected to obtain serum and the brain was harvested for further analysis. The antioxidant enzymes (CAT, GSH, and SOD) levels, serum electrolyte ($Na^+$, $Ca^{2+}$, $Cu^{2+}$, and $Zn^{2+}$, except $K^+$,) and neurotransmitter levels (*dopamine, serotonin, except norepinephrine*), behavioral tests (spatial memory and learning deficit) were significantly decreased with corresponding increase in oxidative stress markers (MDA, $H_2O_2$ and NO) among $AlCl_3$ induced group. MO preserves hippocampal neurons, antioxidant levels, neurotransmitters, learning, and memory abilities induced by $AlCl_3$.

**Keywords:** Alzheimer's disease; Aluminum chloride; *Moringa oleifera*; Oxidative stress; hippocampus.

## INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive, multifactorial genetic, and environmental neurodegenerative disorder that affects more than 46 million people worldwide [1,2]. The economic impact of AD on families and society presents a major challenge to public health. Among the earliest notable symptom of AD patients are characterized by impairments in learning, memory retrieval, reasoning, communication, and one's ability to carry out daily activities [3]. Morphologically, AD is characterized by extracellular deposition of amyloid-beta (Aβ) protein combined with the formation of senile plaques, intracellular neurofibrillary tangles (NFTs), and the death of cholinergic neurons [4]. The "Aβ cascade hypothesis" assumes that excessive accumulation of Aβ in the brain is the basic pathogenetic process accountable for neuronal degenerative changes and the compromise of cognitive functions in AD [5]. Accordingly, reducing the brain Aβ burden has become a key strategy in AD therapy and prevention. Furthermore, postmortem brain surveys of patients with AD disclosed a low concentration of the neurotransmitter acetylcholine (ACh) and the enzyme choline acetyltransferase (ChAT), responsible for ACh synthesis [6]. Researchers have tried to regain the cholinergic equilibrium by inhibiting cholinesterase-mediated ACh breakdown to downturn the progression of AD and improve cognitive function [7, 8]. In AD, Aluminum (Al) is regarded as a prospective etiological factor [9, 10].

Aluminum (Al) is abundantly present in the earth's crust that is greatly linked to Alzheimer's disease (AD) etiology and pathogenesis in well-documented animal experiments and clinical research [11, 12]. Al is regarded to boost the formation and accumulation of extracellular Aß [13, 14], cholinotoxin [15] thereby causing changes in cholinergic function, a major occurrence in AD's neurochemistry [16]. Along with cognitive dysfunction, Al intoxication induces neurodegeneration and apoptotic neuronal loss [17] due to its powerful cholinotoxin [15]. Various animal studies have shown that excessive aluminum consumption can trigger neurochemical, neurobehavioral, and neuropathological brain alterations that impair rats' learning ability [18, 19].

Some natural medicinal plants have gained significant publicity as an alternative therapy for AD treatment due to their safety and effectiveness [20]. One of such medicinal plant is *Moringa oleifera* (MO), regarded as an

evergreen deciduous tree grown mainly in semi-arid, tropical and subtropical areas. The leaves of MO encompass a profile of vital trace elements and excellent sources of proteins, vitamins, beta-carotene, amino acids, and a variety of phenolics and flavonoids [21, 22]. It is regarded as one of the World's most beneficial trees, as almost every bit of the tree is useful either as food, medicinal purposes, or industrial functions [23]. The medicinal attributes of MO include antitumor, anti-epileptic, anti-diuretic, anti-inflammation, and anti-venom [24-26]. The herb is also reported to show positive anti-lead ameliorative effects and neuroprotective effects in focal cerebral ischemia [27, 28]. This study, therefore, aimed to investigate the neuroprotective and antioxidant properties of MO on aluminum-chloride ($AlCl_3$) induced behavioral impairment and neurodegeneration in adult Wistar rats.

## MATERIALS AND METHODS

***Chemicals:*** Aluminum Chloride ($AlCl_3$) was obtained from Sigma Chemical Corporation, Sigma-Aldrich, St. Louis, MO, USA. All other chemicals used in the study were of analytical grade.

**Plant collection, identification, and Extract preparation:** Fresh leaves of *Moringa oleifera* were collected from a Research farm and were identified and authenticated by Omomoh Bernand and a voucher deposited for reference purposes. The leaves were thoroughly washed and oven-dried at $37^0$ C for 48 h and pulverized into a smooth powder.

The pulverized sample (850 g) was suspended in 1000 ml of distilled water with regular agitation for 72hrs. The solution obtained was filtered and the resulting filtrate was concentrated over a water bath ($40^0$C) and yielded 463.21 g crude extract corresponding to 58.12% of the residue. The crude extract *Moringa oleifera (MO)* was kept air-tight and refrigerated before use.

**Animals and experimental design:** A total of Forty (40) adult male Wistar rats weighing between 140 and 180 g were purchased from a breeding colony. The processes of protocols using the experimental animals were by the Guide for the Care and Use of Laboratory Animals and approved by the National Institute of Health The rats were housed in the Laboratory Animal house of the Department. The rats were maintained under the standard natural photoperiodic condition of twelve hours of light alternating with twelve hours of darkness (i.e. L: D; 12h: 12h photoperiod) at room temperature (22-26º C) and humidity of 65±5; full aeration which was enhanced by wire gauzed cage properly partitioned into four chambers and roomy enough to allow for proper ventilation and free movement within it. The floor of the cage was lined with carpet pieces and sprayed with coarse sawdust which served as a cushion. The coarse sawdust was changed every day to dispose of waste droppings and maintain proper hygiene. The rats were fed with growers marsh (pellets), purchased from a feed store-Agro feeds and flour mills, and water during the period of the experiment. The rats went through an acclimatization period of 7 days.

***Experimental Design:*** The rats were divided into four groups ( = 10), labeled as groups A, B, C, and D. Group A received normal saline as *placebo*; Group B received 200 mg/kg body weight of $AlCl_3$ only; Group C received 100 mg/kg body weight of MO and 200 mg/kg body weight of $AlCl_3$ and Group D received 100 mg/kg body weight of MO only. All administration was done via oral gavage once daily and the experiment span duration of 45 days.

All animals were observed for any anomalies, illnesses, and physical anomalies. The experimental procedures were in accordance with the provided recommendations in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institute of Health.

The rats were fed with standard rat chow and drinking water was supplied *ad libitum*. The weights of the animals were recorded at procurement, during acclimatization, at the commencement of the experiment, and weekly throughout the experimental period using a CAMRY electronic scale (EK5055, Indian). After the last administration, the animals were weighed and behavioral observations were conducted. At the end of the behavioral tests, animals were sacrificed through cervical dislocation. The rats were decapitated and blood samples were collected, centrifuged at 4°C for 10 min at 250×g, and the serum obtained was stored at -20°C until assayed The organ (Brain) was removed, cleaned and washed with saline (0.9% of sodium chloride).

**Behavioral parameters :** Before the termination of the experiment, the rats underwent neurobehavioral testing using Morris water maze and Y maze tests for spatial memory and working memory [29, 30]; and open field test for exploratory behavior and locomotor activity [31, 32].

**Morris Water Maze test:** This test was carried out to assess the spatial learning and memory of the rats. A pool of water measuring about 100 cm in diameter and 30 cm in depth was used. An escape platform about an inch deep from the surface of the water was placed in one of the quadrants outside of which was a visual cue. The animals were trained 24 hours before the actual test. During the training, each rat was placed in each of the other three quadrants for a maximum period of 60 seconds to find the escape platform at intervals of 25 minutes between quadrants until the escape latency period reduced to less than 25 seconds. During the test, the pool was colored and the animals were placed in each of the three quadrants different from the escape platform quadrant at an interval of 25 minutes between quadrants. The time is taken to find the escape platform was recorded as the escape latency period.

***Y-maze:*** This test was used to examine the working and cognitive memory of the rats. The animals were placed in a Y-maze whose arms measured 75 cm in length and 15 cm in breadth with an angle of 120° between the arms. The animals were allowed to explore the maze for 5 minutes. The manner of arm entries was recorded. A correct alternation is scored when the animal successfully explored each of the three arms of the maze per triad of exploration (e.g., XYZ, ZYX, or YZX). Once two arms were explored per triad of exploration (e.g., XYX, ZXZ, YXY), it was considered an incorrect alternation. The percentage correct alternation of each rat was estimated as a ratio of the correct alternation to the total alternation multiplied by 100.

**Open Field Test (OFT):** The open field apparatus was constructed using plywood measuring 100 cm by 100 cm and a height of 50 cm. The floor was divided into square grids each measuring 25 cm in length with a blue marker and a center square of the same length was drawn using a red marker. Each rat was picked by the tail and dropped in the center square and allowed to explore for 5 minutes while the video was captured by a camera from above the apparatus. The two behavioral patterns assessed are the number of lines crossed and rearing frequency. The number of lines crossed was the frequency with which the rat crossed one of the grid lines with all four paws; the rearing frequency was the number of times the rat stood on its hind limbs.

***Tissue collection and processing:*** After the behavioral tests were concluded, the rats were subjected to cervical dislocation, and the brain tissues were immediately excised and dissected into two hemispheres. All the right hemispheres were fixed in 4% paraformaldehyde for histological processing, while the left hemispheres were rinsed three times in 0.25 M sucrose for five minutes and stored in 30% sucrose at 4°C. Paraffin wax sections were obtained for histological analysis. The hippocampus was excised from the fixed brain and dehydrated in ascending grades of alcohol (50%, 70%, 90%, and 100%). The tissues were then cleared in xylene twice for 15 min each. Infiltration and embedding were done with paraffin wax in Leica hot air oven at 56°C with tissues eventually embedded in paraffin wax at similar orientations. Tissue sections were obtained serially using a rotary microtome (Leica RM2245) and then mounted on glass slides. Sections were taken at 30 μm for Hematoxylin and Eosin staining process using the method of Pearse [33] as modified by Fischer *et al*. [34]. The slides were analyzed using a Leica®DM5000B microscope and photographed with a Leica EC3 digital camera.

**Biochemical analysis--** ***Superoxide dismutase (SOD) assay:*** The brain tissues were placed in a 0.25 M sucrose solution and homogenized. Tissue homogenate was collected in a 5 ml sample bottle and centrifuged at 3,000 rpm for 15 minutes using a centrifuge (Model 90-1; Jiangsu Jinyi Instrument Tech, Jiangsu, China). The supernatant was collected with Pasteur pipettes into sample bottles and placed in a freezer at –4°C. SOD was using a spectrophotometric technique [35]. The reaction mixture (3 ml) contained 2.95 ml carbonate buffer, 0.02 ml of homogenate, and 0.03 ml of 2 mM SOD substrate in 0.005 N HCl, used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of the substrate, and 0.02 ml of water. The absorbance was read at a regular interval of 1 minute for 5 minutes at 480 nm. Values are expressed in U/mg of protein.

**Catalase (CAT) assay:** CAT activity was analyzed using the protocols of Clairborne [36], in a solution containing 50mM phosphate buffer, 19mM hydrogen peroxide, and tissue homogenates. The reaction was ended by the addition of a dichromate/ acetic acid solution. Values are expressed as μmole of $H_2O_2$consumed/mg protein/min.

**Reduced glutathione level (GSH):** GSH was assayed using the protocols of Jollow *et al.* [37] in a solution containing tissue homogenates, 4% sulfosalicylic acid, and subsequently DTNB. Values are expressed in nmol/mg of protein.

**Lipid peroxidation (LPO) level:** LPO was quantified as Malondialdehyde (MDA), using the protocols of Farombi *et al.* [38]. The reaction contained tissue homogenates, 5% (w/v) butylated hydroxytoluene (BHT), 10% TCA and 0.75% TBA in 0.1 mol/L of HCl. MDA was calculated by using the following equation: R¼1.56_105 L/mol/cm, where R is the extinction coefficient. Values are expressed in nmol/mg of protein or U/mg protein.

**Determination of Nitric oxide (NO) level (nitrite):** Nitric oxide measured as nitrite was determined using Griess reagent, according to the method of Moshage *et al.* [39]. Briefly, 1mL of the sample was incubated with 100 mL of Griess reagent (Sigma) at room temperature for 20 min. The nitrite level was determined by measuring the absorbance at 550nm using a spectrophotometer. Values are expressed in μM/g.

**Determination of Hydrogen peroxide ($H_2O_2$) level:** $H_2O_2$ level was assayed as described by the method of Aebi [40]. Values are expressed in mM/g.

**Brain monoamine neurotransmitters and serum electrolyte analysis:** Monoamine neurotransmitter (DA, 5-HT, and NE) level was estimated using the HPLC technique and the brain content of these neurotransmitters was made using the equation of Pagel *et al.* [41].

Serum electrolyte analysis ($Na^+$, $K^+$, $Ca^{2+}$, $Zn^{2+}$, and $Cu^{2+}$) concentration were estimated according to Ogunlade et al. [42].

**Statistical Analysis:** Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests using Graph Prism® software. The data were reported as means ± SEM, while

differences between means at $p< 0.05$ were considered significant.

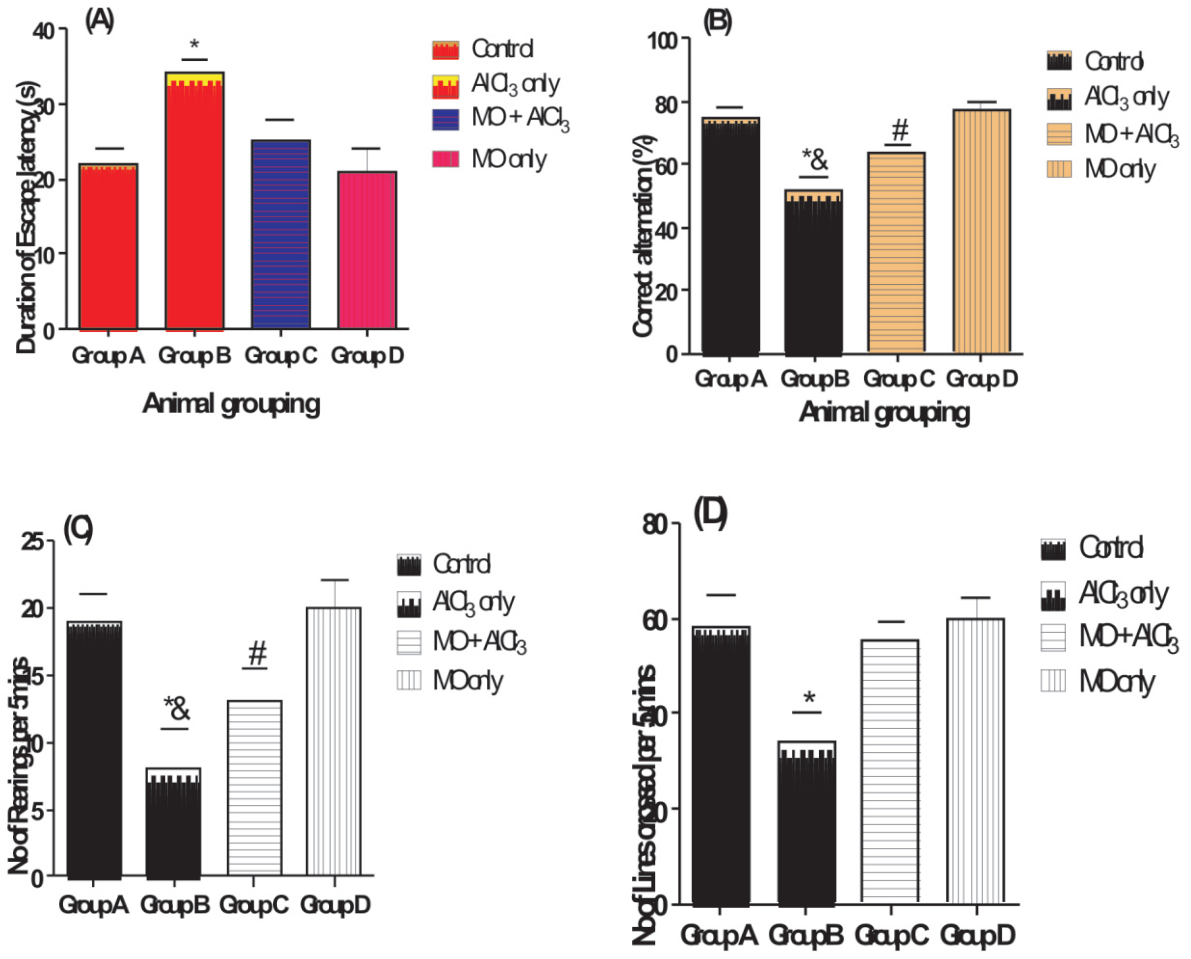
## RESULTS

Behavioral evaluation of *Moringa oleifera* responses among experimental rats

The learning and memory ability was carried out using the Morris Water Maze test. The result revealed a significant increase in the escape latency period in $AlCl_3$ exposed group (group B) relative to the control (group A) ($p<0.05$) (fig. 1A). However, MO and $AlCl_3$ treatment group (group C) showed a significant decrease in escape latency period compared with $AlCl_3$ treated group (Group B) ($p< 0.05$) (fig. 1A). The duration of escape latency of MO only group (group D) was similar relative to the control (group A) (fig.1A).

Also, the short term memory assessment using the Y-maze test showed a significant decrease in percentage correct alternation among $AlCl_3$ exposed group (group B) relative to control (group A) ($p<0.05$) (fig. 1B). The treated group that received MO and $AlCl_3$ (group C) revealed a significant increase in percentage correct alternation relative to the $AlCl_3$ intoxicated rats (group B) ($p<0.05$) (fig. 1B).

Furthermore, the assessment of exploratory drive and anxiety was determined using the open field test. The result showed a significant decrease in the number of lines crossed (exploratory drive) and rearing frequency (anxiety) among the $AlCl_3$ exposed animals (group B) in comparison to control rats (group A) ($p<0.05$) (fig.1C and 1D). However, the combination treatment of MO and $AlCl_3$ (group C) revealed a significant increase in the number of lines crossed (exploratory drive) and rearing frequency (anxiety) relative to $AlCl_3$ intoxicated group (group B) ($p<0.05$) (fig.1C and 1D).

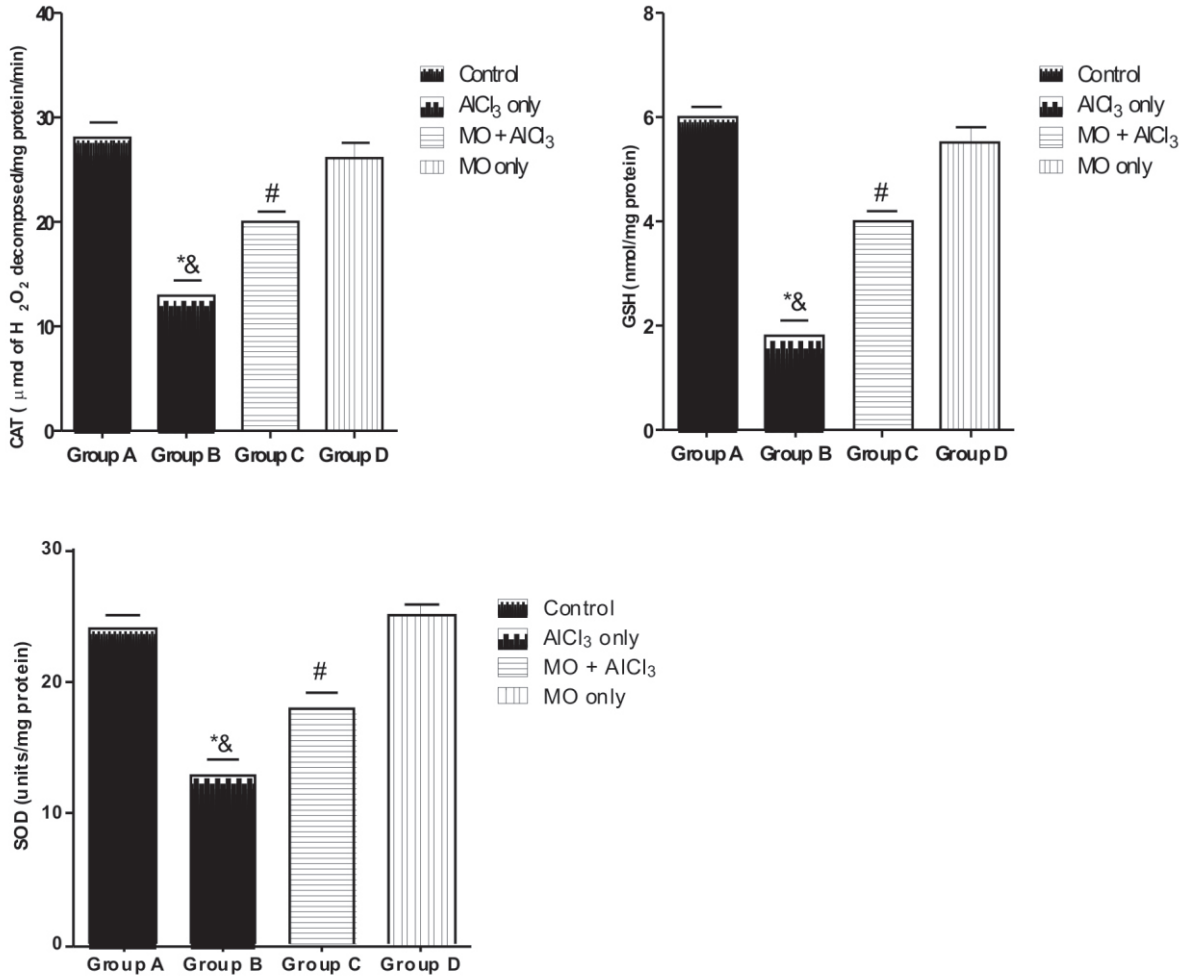



*: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.

**Figure 1:** *Moringa oleifera* response on Neurobehavioral stress tests (Morris Water Maze, Y-Maze, and Open Field tests) in Aluminum chloride-induced neurotoxicity in normal and treated rats.

*Moringa oleifera* responses on antioxidant markers (CAT, SOD, GSH) among experimental rats

There was a significant decline in antioxidant enzymes (CAT, SOD, and GSH) in $AlCl_3$ exposed group (group B) in comparison with control (group A) ($p<0.05$) (fig. 2). However, a significant increase in CAT, SOD, and GSH was observed in MO and $AlCl_3$ animals (group C) relative to $AlCl_3$ intoxicated rats (group B) ($p<0.05$) (fig. 2). Also, a significant decline in brain SOD, CAT, and GSH levels were recorded among MO and $AlCl_3$ (Group C) in comparison with control and MO only groups (groups A and D) ($p<0.05$) (fig. 2).

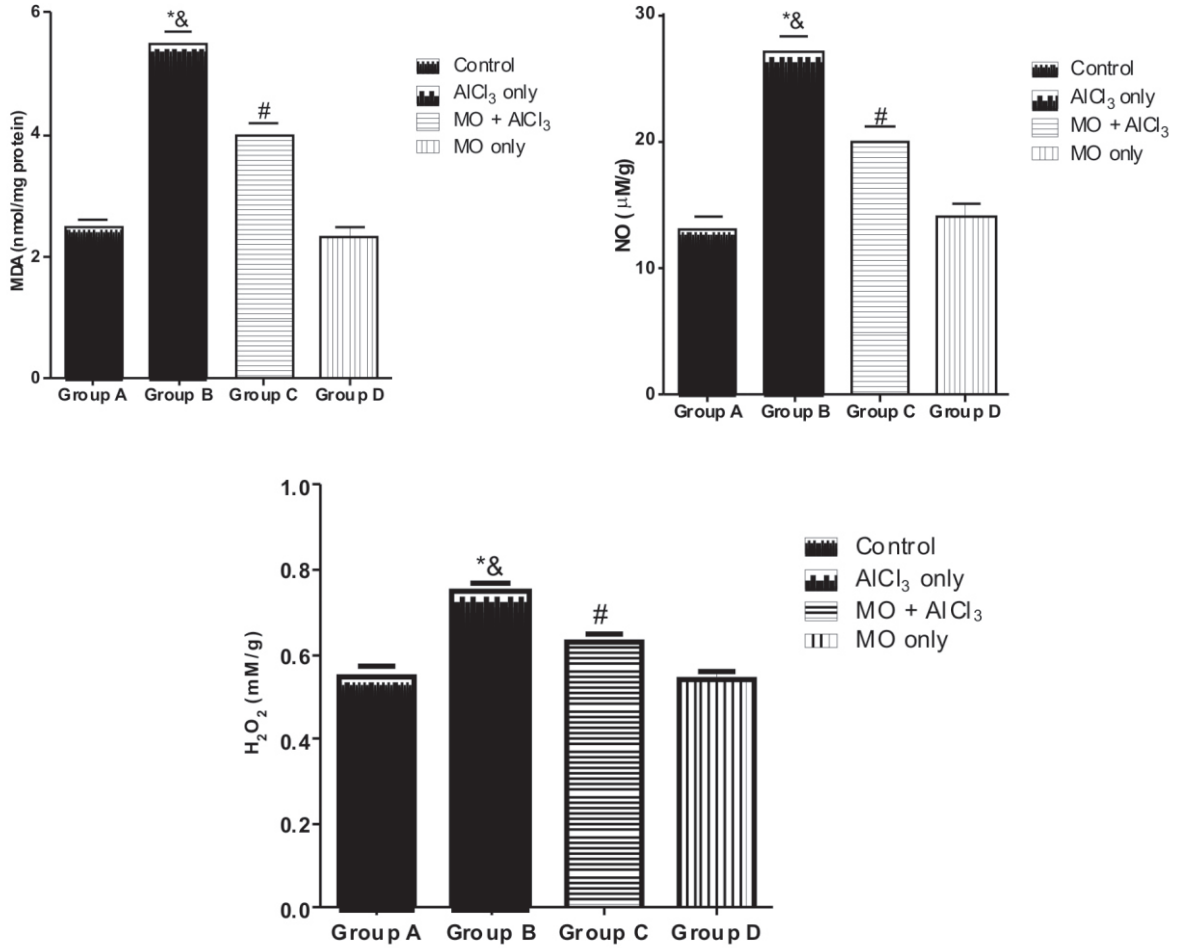



*: $p < 0.05$ as compared to group A and D; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to groups A and D.

**Figure 2:** *Moringa oleifera* response on brain Antioxidant enzymes (CAT, SOD, and GSH) in Aluminum chloride-induced neurotoxicity in normal and treated rats.

*Moringa oleifera* responses on oxidative markers (MDA, $H_2O_2$ and NO) among experimental rats

The results revealed a significant elevation in MDA, $H_2O_2$, and NO among $AlCl_3$ exposed group (group B) relative to the control (group A) ($p$<0.05)(fig. 3). However, a significant reduction in MDA, $H_2O_2$ and NO was observed among MO and $AlCl_3$ group (group C) relative to $AlCl_3$ intoxication (group B) ($p$<0.05) (fig. 3). Additionally, a significant elevation in MDA, $H_2O_2$ and NO was evident among MO and $AlCl_3$ (group C) relative to the control and MO only groups (groups A and D) ($p$<0.05) (fig. 3).

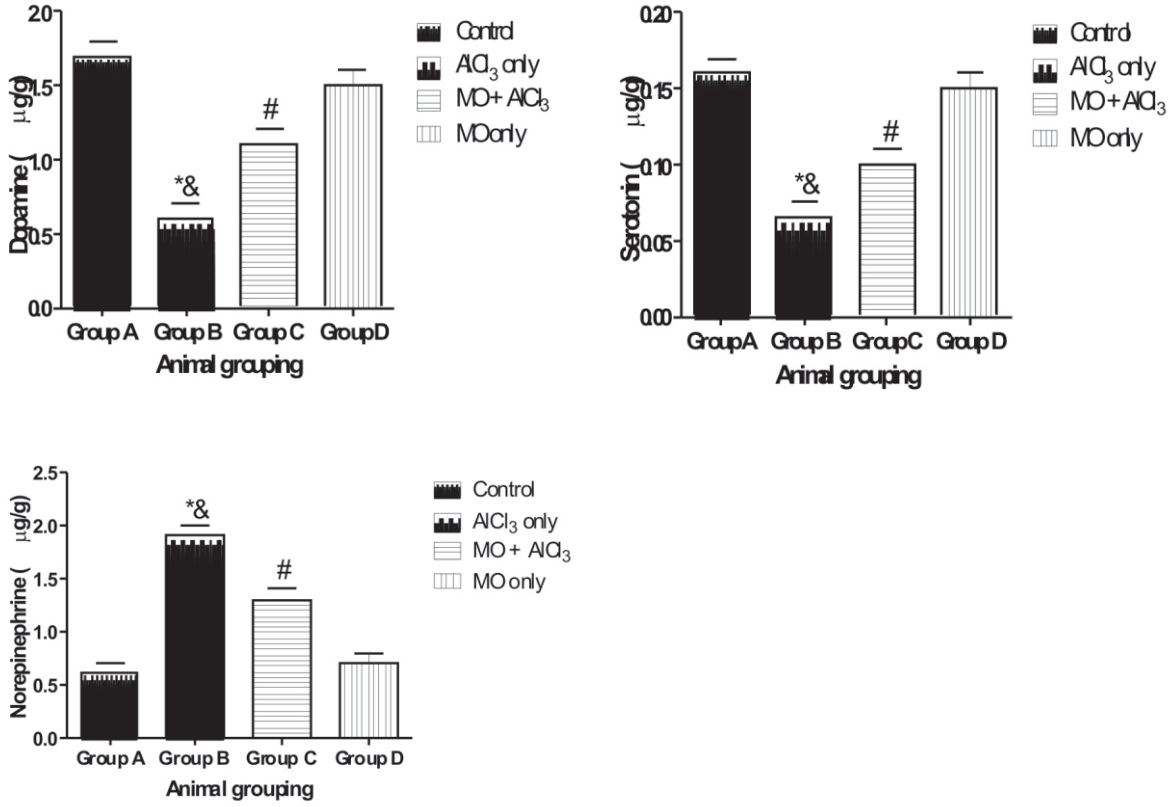



*: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.

**Figure 3:** *Moringa oleifera* response on oxidative stress markers (MDA, NO, and $H_2O_2$) in Aluminum chloride-induced neurotoxicity in normal and treated rats. M

*Moringa oleifera* responses of brain monoamine neurotransmitters (Dopamine, Serotonin, and Norepinephrine) in experimental rats

$AlCl_3$ intoxicated animals (Group B) revealed a significant decline in dopamine and serotonin with a concomitant increase in norepinephrine level relative to control (group A) ($p$<0.05) (fig. 4). However, a significant decrease in dopamine and serotonin levels with concomitant elevation in norepinephrine levels were observed among MO and $AlCl_3$ (group C) relative to $AlCl_3$ exposed animals (group B) ($p$<0.05) (fig. 4). Also, a significant decline in dopamine, serotonin with concomitant elevation in norepinephrine levels were observed among MO and $AlCl_3$ (group C) relative to control and MO (groups A and D) ($p$<0.05) (fig. 4).

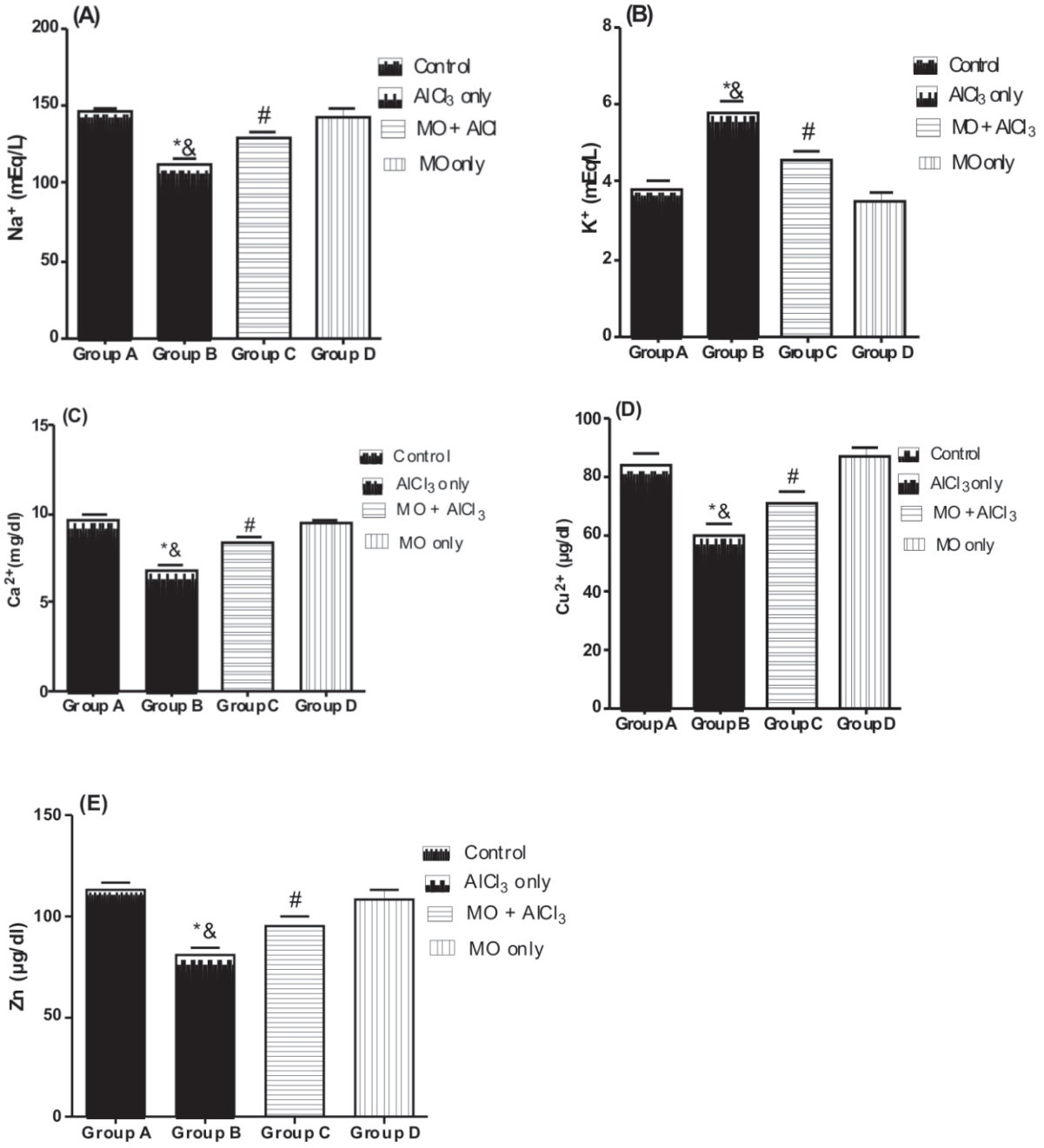



*: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.

**Figure 4:** *Moringa oleifera* response on brain monoamine neurotransmitters (dopamine, serotonin, and norepinephrine) in Aluminum chloride-induced neurotoxicity in normal and treated rats.

*Moringa oleifera* responses in serum electrolyte concentration of experimental rats

The result revealed a significant decrease in the concentration of $Na^+$, $Ca^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ with a concomitant increase in $K^+$ level among the $AlCl_3$ intoxicated animals (Group B) relative to control (group A) ($p<0.05$)(fig. 5). However, a significant increase in the concentrations of $Na^+$, $Ca^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ with a concomitant decrease in $K^+$ level among the MO and $AlCl_3$ group (group C) when compared with $AlCl_3$ only group (group B) ($p<0.05$)(fig. 5). In addition, the group that received MO and $AlCl_3$ (group C) recorded significant decrease concentrations of $Na^+$, $Ca^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ with a concomitant increase in $K^+$ level relative to the control and MO only groups (group A and D) ($p<0.05$)(fig. 5).




*: $p < 0.05$ as compared to group A and D; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to groups A and D.

**Figure 5:** *Moringa oleifera* response on serum electrolytes ($Na^+$, $K^+$, $Ca^{2+}$, $Cu^{2+}$, and $Zn^{2+}$) in Aluminum chloride-induced neurotoxicity in normal and treated rats.

Histological observation of *Moringa oleifera* response on the hippocampus after $AlCl_3$ intoxication in experimental rats.

The histology of $AlCl_3$ only group (Group B) showed decrease in the granule layer (glial layer) with decline and shrinkage in cells that were arranged loosely, dilated blood vessels and large vacuole (distinctive attributes of hippocampal neurodegeneration) (fig. 6B) compared with the control (group A) (fig. 6A). However, the photomicrograph of the group administered with MO and $AlCl_3$ (group C) showed a decreased pathological attributes such as neuronal cell loss, vacuolation and loosely arranged degenerated cells in the hippocampus with almost normal brain histomorphology, similar to the control and MO only groups (groups A and C) (fig. 6A, 6C, and 6D respectively).

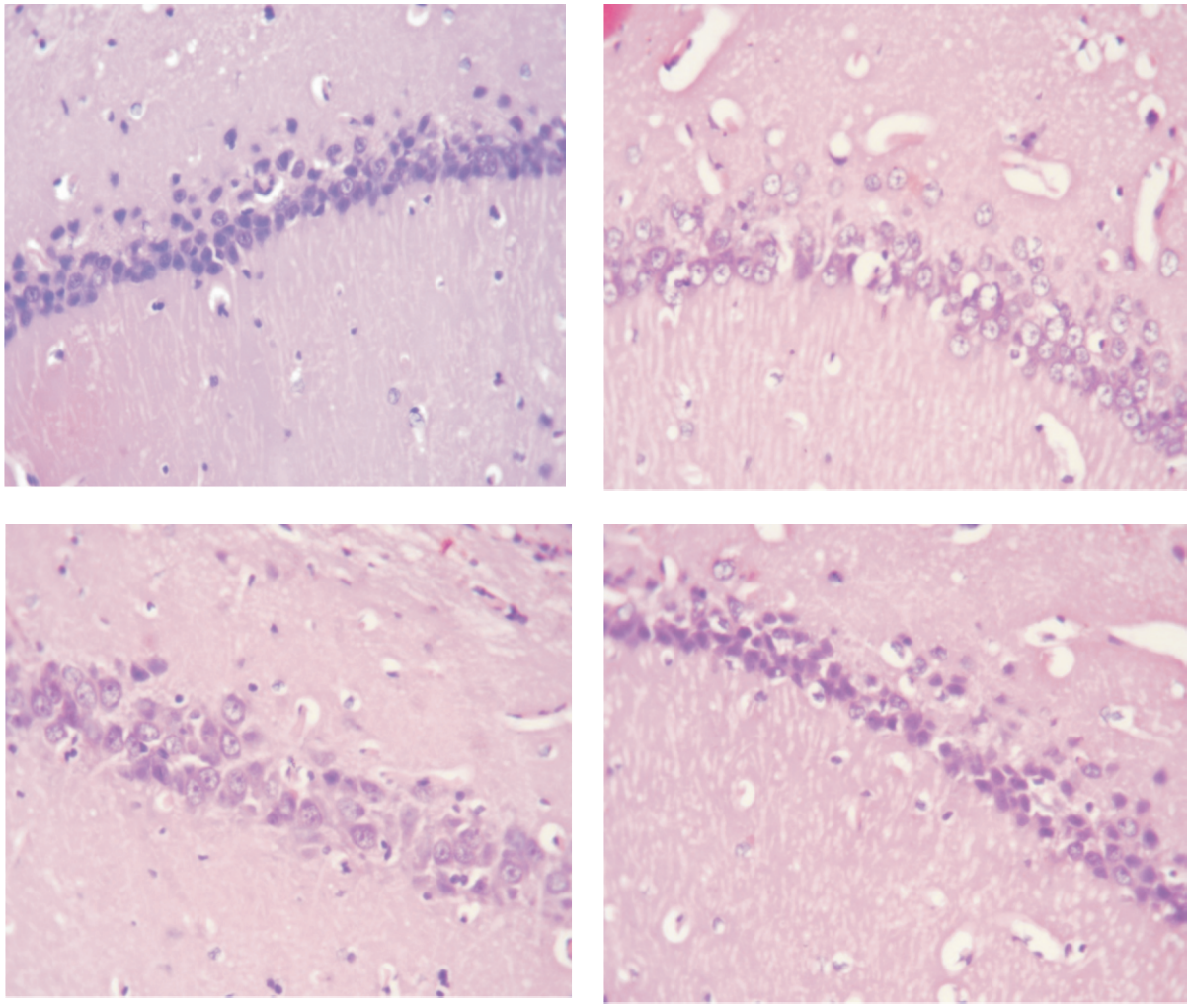


**Figure 6:** A. Group A (Control): normal hippocampus morphology with numerous glial cells (GC) (arrow) within the glial layer (GL);
B. Group B (Lead acetate-induced group): few pyramidal cells with distorted glial cells (GC) (arrow) within the glial layer (GL) between the inner pyramidal layer (IPL) and outer marginal layer (OML) and Vacuolated neuropils (V);
C. Group C (SFN and Lead acetate group): showing preserved proliferation of glial cells (GC) (arrow) within the glial layer (GL) interspersed between the inner pyramidal layer (IPL) and outer marginal layer (OML) similar to control;
D. Group D (SFN only): showing the normal orientation of glial cells (GC) within the glial layer (GL) between the inner pyramidal layer (IPL) and outer marginal layer (OML) similar to the control.

H and E: ×400 magnification

## DISCUSSION

Neurodegenerative disorders such as AD are progressive diseases characterized by gradual neuronal loss with several etiologies such as genetic, metabolic process, and environmental/toxic agents (neurotoxicants) [43, 44]. AD is categorized as a progressive, irreversible disorder characterized by learning and memory impairments caused by increased oxidative stress, disturbance in antioxidant enzymes levels, reduced mono anime neurotransmitters, and pathological features in hippocampal neurons [45-47]. The global detrimental influence of neurodegenerative diseases in recent years has increased the discovery of natural (herbs or plants) compounds with numerous and significant pharmacological properties as an alternative method of addressing many brain disorders since they are abundant, affordable, and reliable [48, 49]. Studies have shown that numerous plant-derived phytochemicals prevent the risk of cancer and some chronic diseases such as AD [50-52].

Since adequate learning capability is important for survival and social adaptation of humans and animals, a rapid learning process is significant for day to day activities such as escaping from predators and other environmental problems [53]. A serious loss of cognitive function such as memory impairments, attention, and problem-solving has been recognized as a major medical challenge among AD people[44, 54]. Presently in this study, aluminum intoxication causes learning and memory impairments evident by decreased correct alternation in the Y-maze test, decreased in a number of the line crossed and rearing frequency in the open field test with concomitant increased duration of escape latency in the Morris water maze test when compared to the control. This observation was in agreement with previous studies after exposure of rats and mice to $AlCl_3$and other neurotoxicants [55-61]. Additionally, previous studies have also observed that oral administrations of Al to rodents cause learning and memory deficits [62-64]. Similarly, impairment of rats' learning and memory after Al treatment in drinking water in the passive avoidance task has been reported [65, 66]. However, administration of MO with Al exposure significantly improved acquisition and retention latencies; ameliorate memory declination compared to the $AlCl_3$ only group and this was in agreement with previous studies that reported increased percentage alternation, decreased duration of escape latency, increased rearing frequency and the number of the line crossed [67-69] thereby attributing the protective role of MO against aluminum intoxication to the numerous phytochemicals present within the plants such as flavonoids, phenols, and vitamins.

Since the clinical manifestation of AD is characterized by a progressive decline of cognition and behavior affecting thinking, planning, judgments, and social skills culminating into the inability to carry out daily activities, oxidative stress has been implicated in the occurrence of AD [70]. Aluminum exposure is an established environmental risk factor in inducing oxidative stress neurological damage similar to the manifestation of AD [42, 54]. In the present result, Aluminum intoxication induced an increase in MDA, NO, and $H_2O_2$ with a concomitant decrease in SOD, CAT, and GSH indicating an increase in oxidative stress. This was in agreement with previous studies that reported the intraperitoneal injection of AlCl3 significantly increased oxidative stress marker (MDA) and decreased antioxidant enzyme levels (SOD, CAT, GSH) [54, 71, 72]. However, the combined administration of MO and Aluminum decreased the levels of oxidative stress, and increased antioxidant enzyme levels probably attributed to the strong antioxidant, anti-inflammatory, and neuroprotective activities of MO in mopping up excess ROS and free radicals that could elevate oxidative stress levels. These findings supported previous studies that reported medicinal plants rich in flavonoids and phenols are capable of increasing the reduced antioxidant levels (CAT, SOD, and GSH) and decreased the elevated oxidative stress markers (MDA) caused by toxins/ agents capable of oxidative stress-induced damages [72,73]. The protective efficacy of MO against oxidative stress-induced AD through aluminum exposure may be correlated to numerous phytochemicals present within the plants such as ascorbic acid and phenols (catechin, epicatechin, ferulic acid, ellagic acid, and myricetin) through scavenging the free radicals.

Previous research reported that decreased brain monoamine neurotransmitters were also associated with memory impairments as occurred in AD [42, 54, 75]. In this present study, aluminum intoxication significantly decreased dopamine and serotonin levels with concomitant increased in norepinephrine level when compared with the control. It was reported by previous studies that environmental neurotoxicants caused elevated neopterin levels in AD brain patients and also alter levels of brain neurotransmitters by depressing cerebrospinal fluid tetrahydrobiopterin levels required for neurotransmitters synthesis [76]. On the other hand, combine treatment of MO and Aluminum significantly elevated the decreased dopamine and serotonin levels but reduced the increased norepinephrine level which was following previous researchers that showed restorations of various neurotransmitters (such as glutamate, aspartate, gamma-aminobutyric acid, acetylcholine, glycine, dopamine, and serotonin) after administration of plants rich in flavonoids and phenolic compounds in several animal models [77-79].

The dependent on Na-K ATPase for the homeostasis of membrane electrochemical gradients is usually altered by oxidative stress thereby causing potential misfiring of the neuronal action potential [80, 81]. The present study showed that aluminum intoxication caused a significant reduction in serum electrolyte levels (except potassium) when compared to the control. But the administration of MO restored the electrolyte levels ($K^+$, $Na^+$, $Ca^{2+}$, $Zn^{2+}$,

and $CU^{2+}$) probably due to the potent antioxidants and anti-inflammatory phytochemicals that scavenge the excess free radicals.

Experimental studies have associated the degeneration of hippocampal neurons to be influenced by oxidative stress, disturbance of calcium homeostasis, vascular supply reduction, and program cell death (apoptosis) [82-86]. The present result showed that aluminum intoxication caused hippocampal neuronal degeneration, cytoplasmic vacuolization, gliosis, and pyknosis changes with reduced granular cell layer thickness compared with the control. However, the co-treatment of MO and Aluminum showed preserved histomorphology of the neuronal hippocampus indicating that MO is capable of preventing pathological hippocampal neuronal damage induced by aluminum exposure similar to the control and MO only group. Following the previous observation, the improved neuronal dysfunction within the hippocampus could be attributed to the presence of flavonoids present within MO thereby suggesting its neuroprotective and cognitive enhancements potential against oxidative stress-induced neurotoxicant [87].

## CONCLUSION

The treatment with *Moringa oleifera* possesses neuroprotective and memory-enhancing potential by suppressing oxidative stress-induced aluminum intoxication probably due to the presence of a high level of polyphenols and flavonoids such as quercetin capable of activating cellular antioxidant system.

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## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Ethical approval

The experimental procedures were in line with guidelines of the Department of Human Anatomy, School of Health and Health Technology, Federal University of Technology, Akure, Nigeria, and Research Committee of the Federal University of Technology, Akure, Nigeria.

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