NUTRITIONAL ATTRIBUTES OF *Moringa oleifera*VIA ANTIOXIDANTS AND ANTI-INFLAMMATORY RESPONSES ABATES ALUMINUM CHLORIDE INDUCED NEUROBEHAVIORAL DEFICITS, BIOGENICAMINES IMBALANCE AND HIPPOCAMPAL NEURODEGENERATION IN ADULT MALE WISTAR RATS

ABSTRACT

Background: Chronic aluminumtoxicity induces cellular alterations and oxidative stress thereby causing neurodegenerative disorders such as Alzheimer's disease. This study elucidated the nutritional constituents of *Moringa oleifera*(MO) on aluminum-chloride ($AlCl_3$) induced AD in adult Wistar rats.

Materials and methods: Forty (40) adult male Wistar rats (160±20g) were divided into 4 groups (n=10). Group A received normal saline as *placebo*; Group B received 200mg/kg bw of $AlCl_3$ only; Group C received 100mg/kg bw of MO and200mg/kg bw of $AlCl_3$ and Group D received 100mg/kg bw of MO only. All administration was done orally per day for 45 days. After the last administration, they animals underwent behavioral tests (Morris Water maze, Y-Maze and Open field). Thereafter, animals were sacrificed via cervical dislocation andblood samples were collected to obtain serumand the brain was harvested for analysis.

Results: The antioxidant enzymes (CAT, GSH and SOD) levels, serum electrolyte (except K+) and neurotransmitter levels (except norepinephrine), behavioral tests (spatial memory and learning deficit) were significantly decreased with concomitant increase in oxidative stress markers (MDA, H_2O_2 and NO) among $AlCl_3$intoxication compared to control.

Conclusion:MO preserves hippocampal neurons, antioxidant levels, neurotransmitters, learning and memory abilitiesinduced by $AlCl_3$.

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

1. INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive, multifactorial genetic and environmental neurodegenerative disorder that affectsmore than 46 million people worldwide [1, 2]. The economic impact of AD on familiesand society presents a major challenge to public health. Among the earliestnotable symptomof AD patients are characterized by impairments in learning, memory retrieval, reasoning, communication, and one'sability to carry out daily activities [3]. Morphologically, AD is characterized byextracellular deposition of amyloid-beta (Aβ) protein combinedwith formation of senile plaques, intracellular neurofibrillarytangles (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 burdenhas become a key strategy in AD therapy and prevention.Furthermore, postmortem brain surveys of patients with AD disclosed 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 AChbreakdown to downturn the progression of AD and improve cognitive function [7, 8]. In AD, Aluminum (Al) is regarded a prospective etiological factor [9, 10]. Al is the third most abundant metal in the earth's crust and its function in AD's etiology and pathogenesis has become more perceptive, as a result of well-

documented animal experiments [11] and clinical research [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 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 includes antitumour, 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 AD in adult Wistar rats.

2. MATERIALS AND METHODS

2.1.Chemicals

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

2.2.Plant collection, identification and Extract preparation

Fresh leaves of *Moringa oleifera* was collected from Research Farm, Federal University of Technology, Akure, Nigeria and were identified and authenticated by OmomohBernand in herbarium section of Centre for Research and Development (CERAD) of the Federal University of Technology, Akure, Nigeria. FUTA0190 voucher deposited for reference purpose. The leaves were thoroughly washed and oven dried at 37^0 C for 48 h and pulverized into smooth powder.

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

2.3.Animals and experimental design

A total of Forty (40) adult male Wistar rats weighing between 140 and 180 g were purchased from the breeding colony, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria. The rats were housed in the Laboratory Animal house, Department of Human Anatomy, Federal University of Technology, Akure, Nigeria. The rats were acclimatized for 7 days; maintained under standard laboratory conditions (12- hour light/dark cycles) and had free access to a commercial pellet diet and water *ad libitium*. The animals were maintained under a controlled room temperature of 25 ± 2 ℃ and relative humidity of 60 ± 5% degree. 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 processes of protocols using the experimental animals were in accordance to the Guide for the Care and Use

of Laboratory Animals and approved by the Health Research Ethics Committee of the Federal University of Technology, Akure, Nigeria on the 19th June, 2019.

2.4.Experimental Design

The rats were divided into four groups (n = 10), labelled as groups A, B, C, and D. Group A received normal saline as *placebo*;Group B received 200mg/kg bw of $AlCl_3$ only; Group C received 100mg/kg bw of MO and 200mg/kg bw of $AlCl_3$ and Group D received 100mg/kg bw 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, illness 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 Academy of Sciences. 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 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 sacrificedthrough 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).

2.5.Behavioral parameters

Prior to the commencement 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 [31, 32].

2.5.1. 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 prior to 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 theescape 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 theescape platform quadrant at an interval of 25 minutes between quadrants. The time taken to find the escape platform was recorded as the escape latency period.

2.5.2. 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 duration of 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, ZXY, 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.

2.5.3. Open Field Test (OFT)

The open field apparatus was constructed using plywood measuring 100 cm by 100 cm and height of 50 cm. The floor was divided into square grids each measuring 25 cm in length with a

blue marker and a centre square of the same length was drawn using a red marker. Each rat was picked by the tail and dropped in the centre 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 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.

2.6.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 hemisphereswere 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 were 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 was 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 Leica®DM5000B microscope and photographed with Leica EC3 digital camera.

2.7.Biochemical analysis

2.7.1. Superoxide dismutase (SOD) assay

The brain tissues were placed in 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 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 substrate and 0.02 ml of water. The absorbance was read at regular interval of 1 minute for 5 minutes at 480 nm. Values are expressed in U/mg of protein.

2.7.2. 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 addition of dichromate/ acetic acid solution. Values are expressed as µmole of H_2O_2consumed/mg protein/min.

2.7.3. 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.

2.7.4. 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) butylatedhydroxytoluene(BHT), 10% TCA and 0.75% TBA in 0.1 mol/L of HCl. MDA was calculated by using the followingequation: R¼1.56_105 L/mol/cm, where R is the extinction coefficient. Values are expressed in nmol/mg of protein or U/mg protein.

2.7.5. 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, 100 µL of sample were incubated with 100 mL of Griessreagent (Sigma) at room temperature for 20 min. Nitrite level was determined by measuring the absorbance at 550nm using a spectrophotometer.Values are expressed in µM/g.

2.7.6. 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.

2.7.7. Brain monoamine neurotransmitters and serum electrolyte analysis

Monoamine neurotransmitter (DA, 5-HT and NE) level was estimated using 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].

2.8.Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test using Graph Prism® software. The data were reported as means ± SEM, while differences between means at $p< 0.05$ were considered significant.

3. RESULTS

3.1.Effect of treatment on behavioral parameters

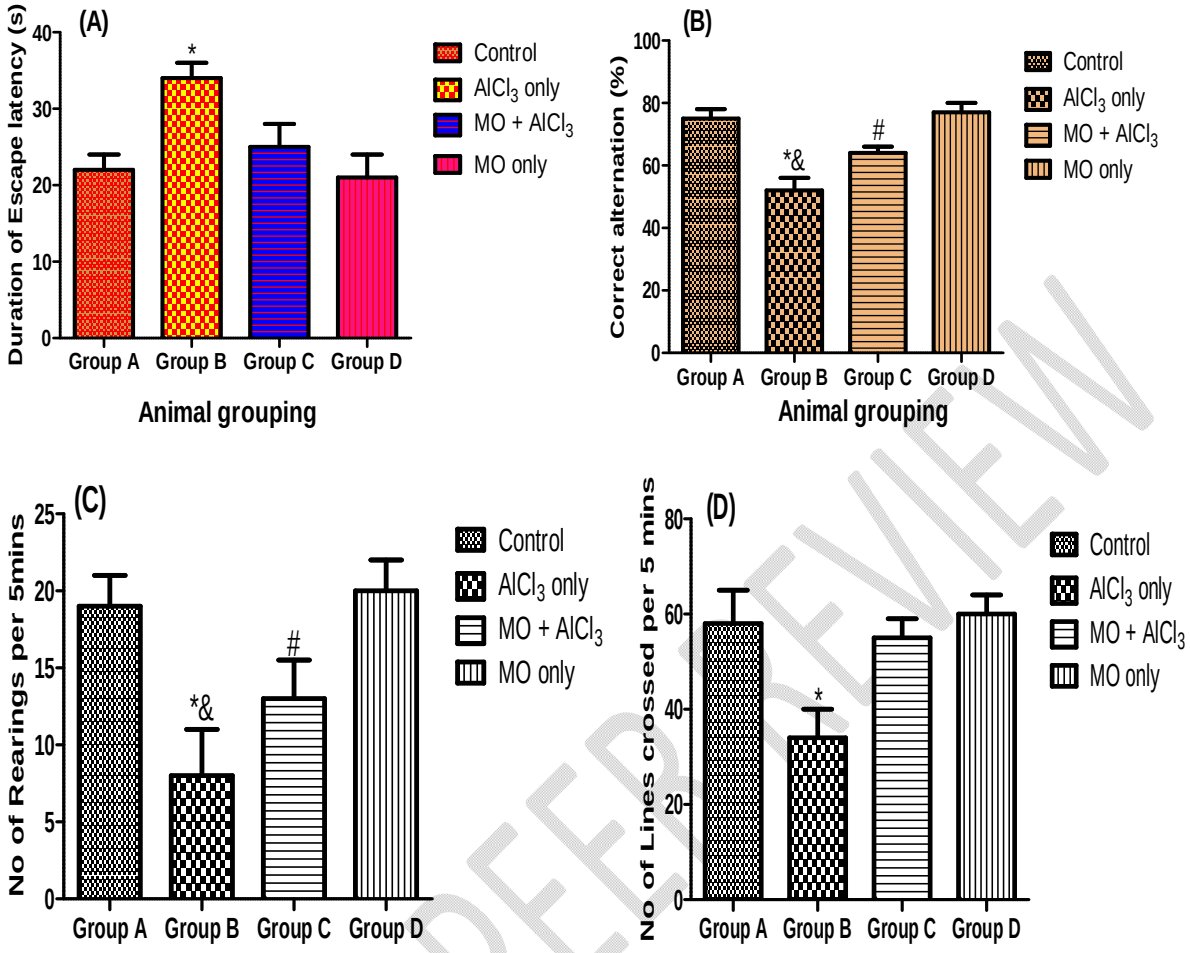
The learning and memory ability in the MWM test was determined using the duration of escape latency. The present study showed significant increase in duration of escape latency among $AlCl_3$ only group (group B)when compared to the control (group A) ($p<0.05$) (fig. 1A). However, MO and $AlCl_3$ treatment group (group C) showed a significantly decrease 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 when compared with the control (group A) (fig.1A).

In addition, the short term memory assessment using the Y-maze test showed a significant decrease in percentage correct alternation among $AlCl_3$ only group (group B) compared to control (group A) ($p<0.05$) (fig. 1B). The treated group that received MO and $AlCl_3$ (group C) showed significant increase in percentage correct alternation when compared to the $AlCl_3$ only treated rats (group B)($p<0.05$) (fig. 1B). This revealed that MO prevents the short-term memory impairment induced by $AlCl_3$ exposure.

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 $AlCl_3$ only group (group B) relative to the control group (group A) ($p<0.05$) (fig.1C and 1D). However, the combine treatment of MO and $AlCl_3$ (group C) showed a significant increase in the number of lines crossed (exploratory drive) and rearing frequency (anxiety) when compared to$AlCl_3$ only group (group B) ($p<0.05$) (fig.1C and 1D). The number of lines crossed and the rearing frequency was similar among the MO only group (group D) and control animals (group A) (fig.1C and 1D).

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



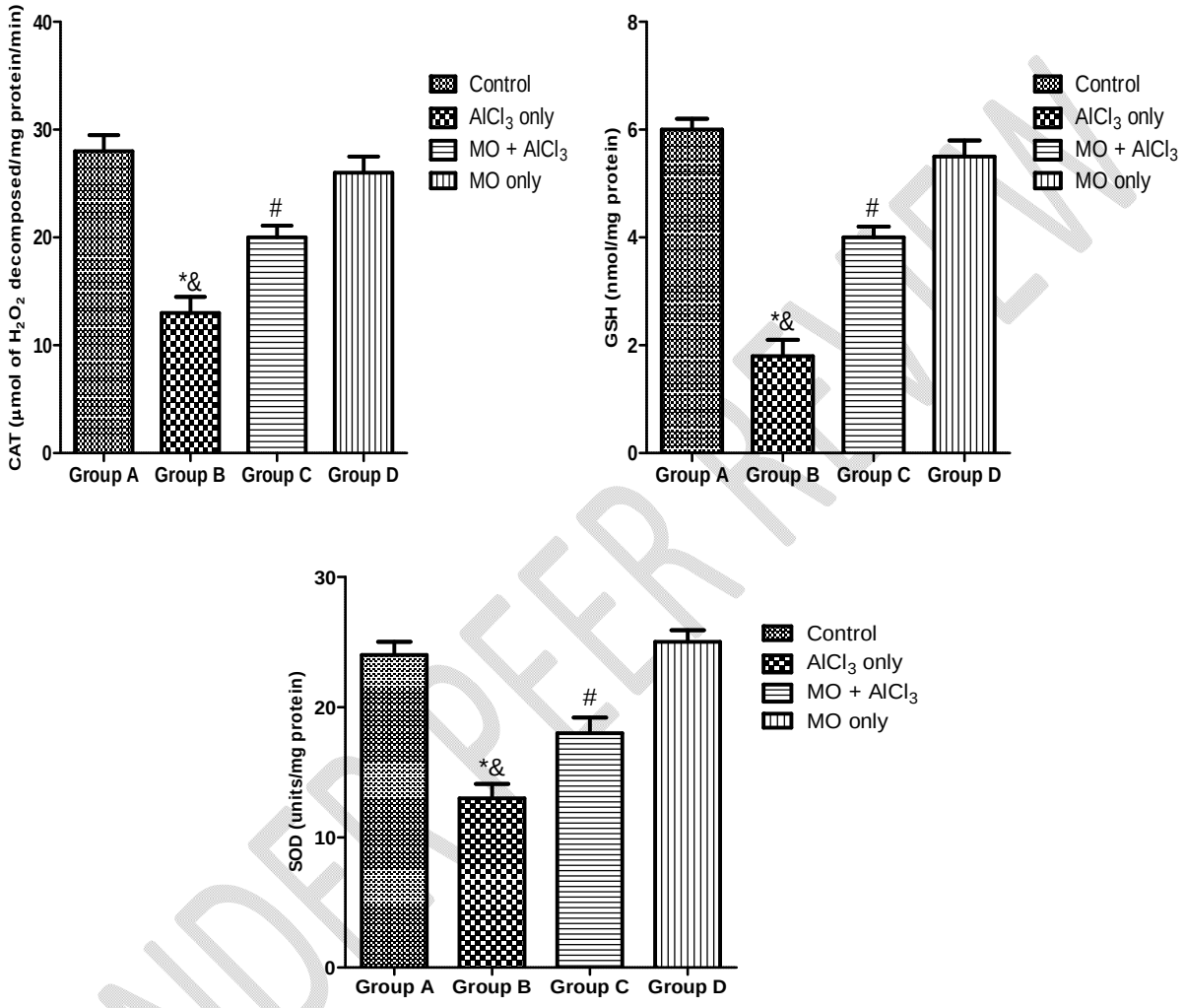
****: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.***

3.2.Effect of *Moringa oleifera*on brain antioxidant parameters (CAT, SOD, GSH)

The present results showed a significant decrease in the levels of antioxidant enzymes (CAT, SOD and GSH) in $AlCl_3$ only group (group B) when compared with the control (froup A) (p<0.05)(fig. 2). However, there was a significant increase in the levels of CAT, SOD and GSH in the group that received MO and $AlCl_3$ (group C) relative to $AlCl_3$ only group (group B) (p<0.05)(fig. 2). In addition, there was a significant decrease in brain antioxidant enzyme levels (SOD, CAT and GSH) in the group that received MO and $AlCl_3$ (Group C) in comparison with control and MO only groups (groups A and D) (p<0.05)(fig. 2). Although, the levels antioxidant

enzyme(SOD, CAT and GSH) was similar among the MO only (group D) animals and control group (group A) (fig. 2).

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

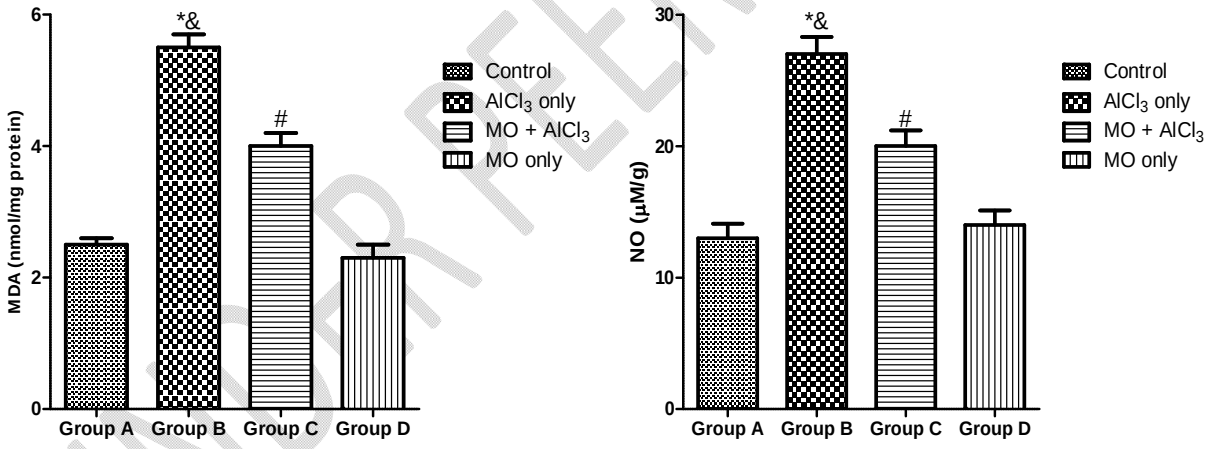


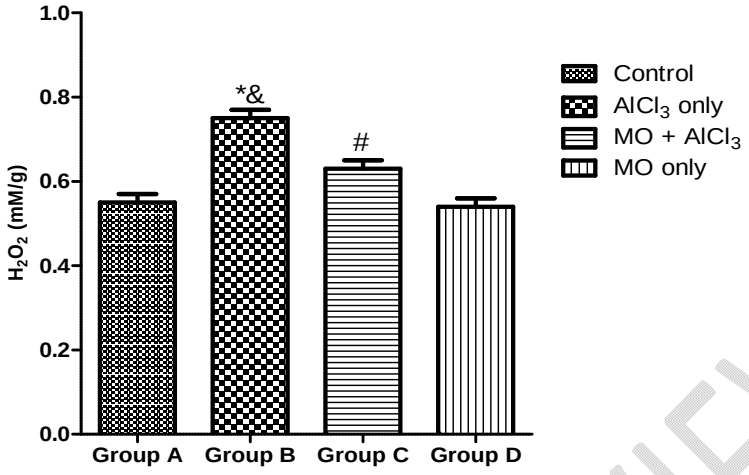
: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.

3.3.Effect of *Moringa oleifera* on brain oxidative stress markers (MDA, H_2O_2 and NO)

In the present study, there was a significant increase in the levels of MDA, H_2O_2 and NO in $AlCl_3$ only group (group B) relative to the control group (group A) (p<0.05)(fig. 3). However, there was a significant increase in the levels of oxidative stress parameters (MDA, H_2O_2 and NO) among MO and $AlCl_3$ group (group C) when compared to $AlCl_3$ only group (group B) (p<0.05) (fig. 3). Additionally, there was a significant decrease in brain oxidative stress markers (MDA, H_2O_2 and NO) in the group that received MO and $AlCl_3$ (group C) relative to the control and MO only groups (froups A and D) (p<0.05)(fig. 3). There was no significant difference in the levels of oxidative stress markers between the MO only group (group D) and control animals (group A).

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.



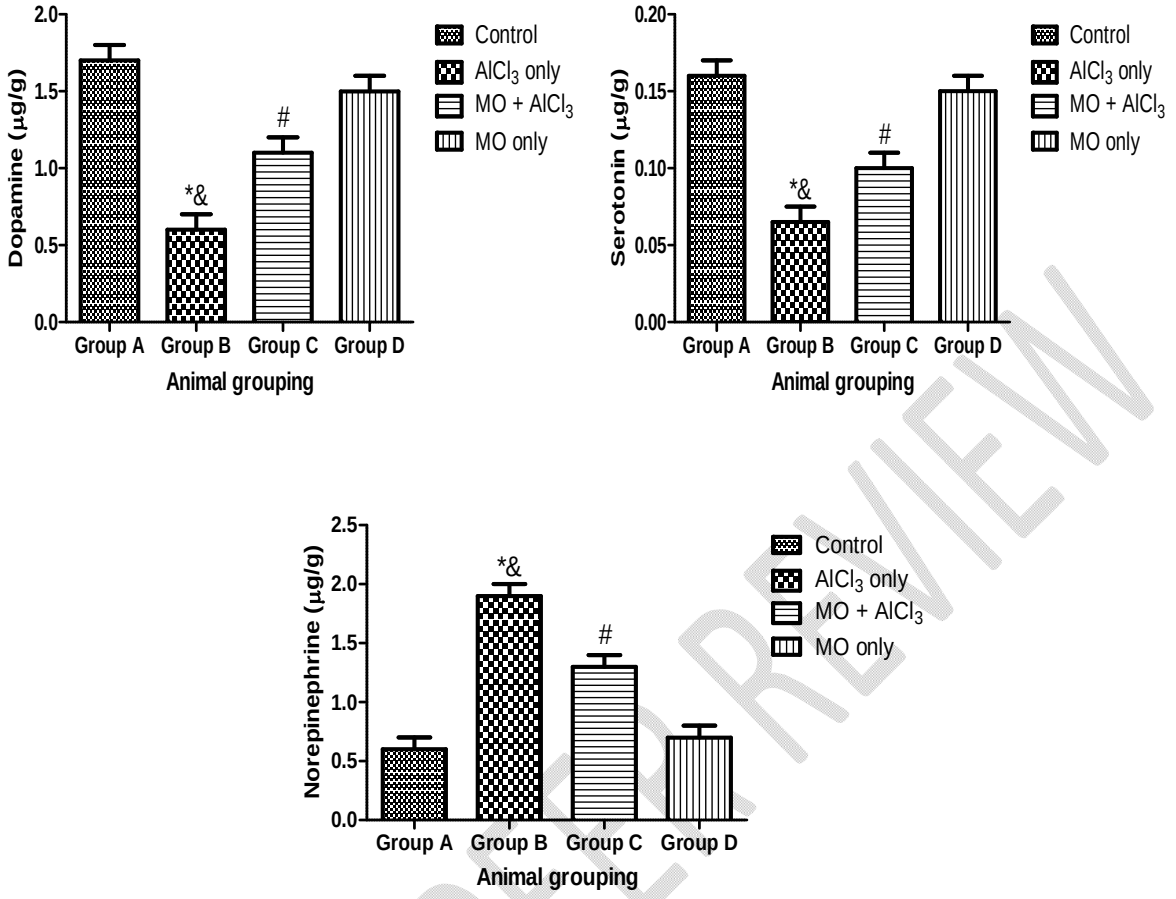


: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.

3.4.Effect of *Moringa oleifera* in brain monoamine neurotransmitters (Dopamine, Serotonin and Norepinephrine)

In this study, $AlCl_3$ only group (Group B) recorded a significant decrease in the levels of dopamine and serotonin with concomitant increase in norepinephrine level when compared with control group (group A) (p<0.05) (fig. 4). However, there was a significant decrease in dopamine and serotonin levels with a corresponding increase in norepinephrine level within the group that received MO and $AlCl_3$ (group C) when compared with $AlCl_3$ only group (group B) (p<0.05) (fig. 4). In addition, there was a significant decline in the levels of monoamine neurotransmitters (Dopamine, serotonin) within concomitant elevation in norepinephrine level among MO and $AlCl_3$ group (group C) relative to the control and MO only groups (groups A and D)(p<0.05) (fig. 4).

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



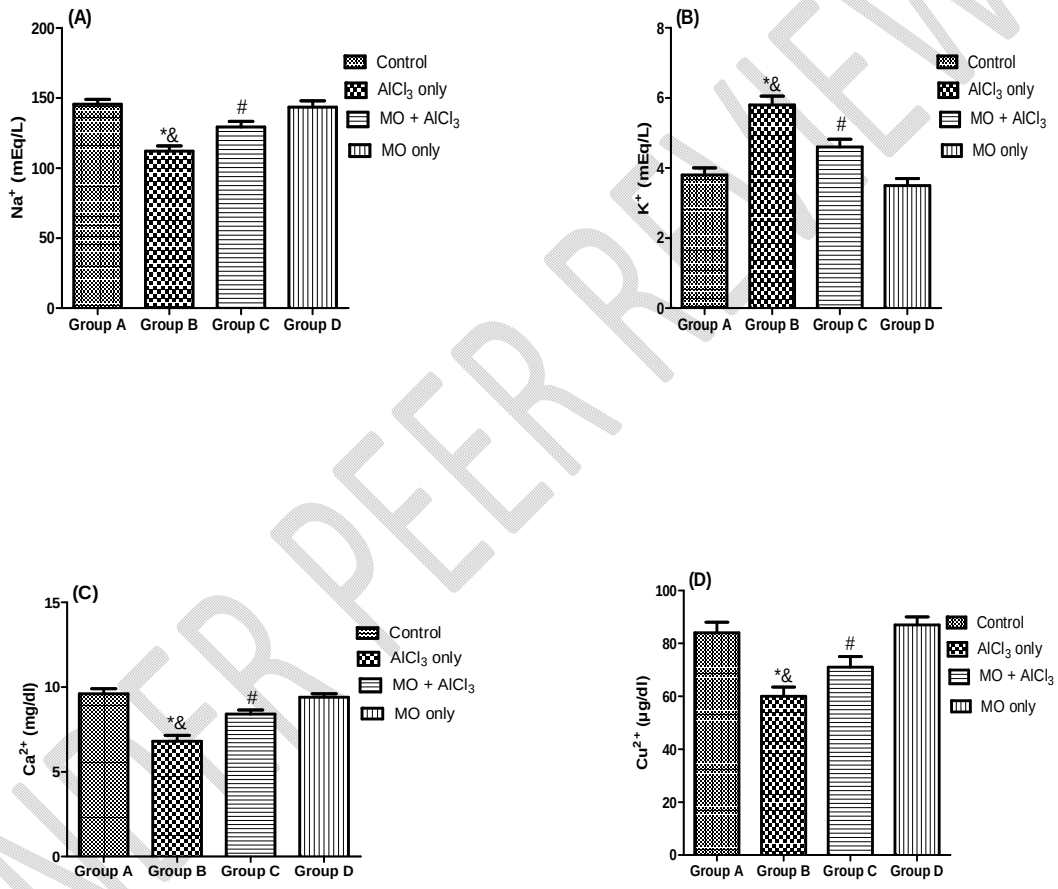
: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D.

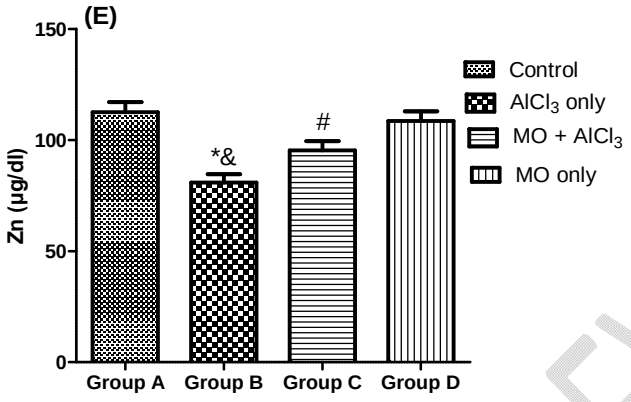
3.5.Effect of *Moringa oleifera*in serum electrolyte concentration in normal and Alzheimer's group rats

In this present study, there was a significant decrease in the concentration of Na^+, Ca^{2+}, Cu^{2+} and Zn^{2+} with concomitant increase in K^+ level within the $AlCl_3$ only group (Group B) when compared with control group (group A) (p<0.05)(fig. 5). However, a significant elevation in the concentrations of Na^+, Ca^{2+}, Cu^{2+} and Zn^{2+} with concomitant decline 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

decreaseconcentrations of Na^{+}, Ca^{2+}, Cu^{2+} and Zn^{2+} with concomitant increase in K^{+} level relative to the control and MO only groups (group A and D) (p<0.05)(fig. 5).

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.





: 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.

3.6.Effect of *Moringa oleifera* on hippocampal histomorphology against $AlCl_3$ exposure in normal and treated rats.

The photomicrograph of the $AlCl_3$only group (Group B) showed decrease thickness in the granule layer (glial layer) with decline and shrinkagein 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 decreased incidence of pathological changes 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).

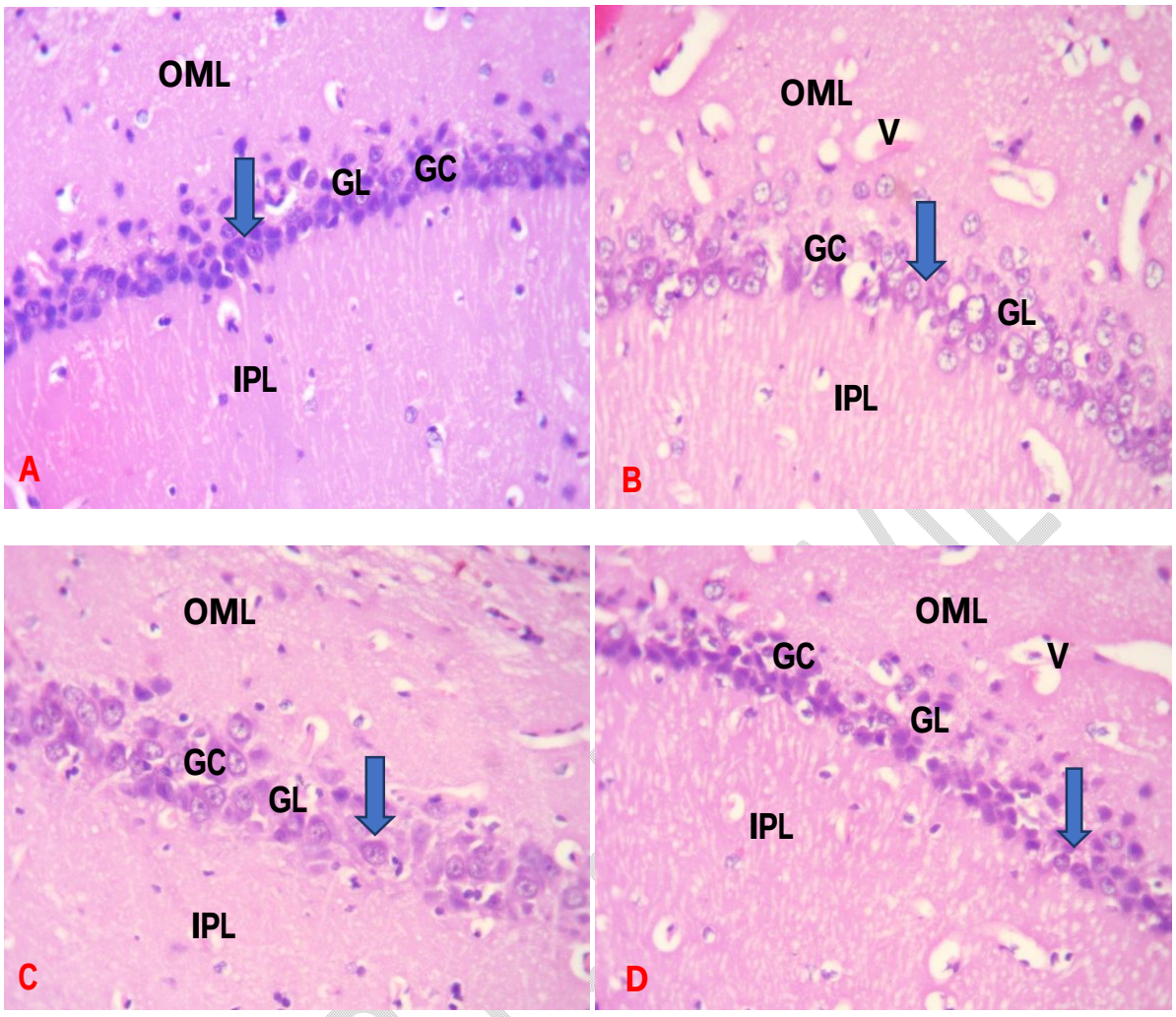


Fig. 6: A. Group A (Control): showing normal hippocampus morphology with numerous glial cells (GC) (arrow) within the glial layer (GL);
B. Group B (Lead acetate induced only group): showing few pyramidal cells with distorted glial cells (GC) (arrow) within the glial layer (GL) between the inner pyramidal 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 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: x400.

Scale bar-50µm

4. 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 disorders 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 increase 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 prevents 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, 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 challenges 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 number of line crossed and rearing frequency in the open field test with concomitant increased duration of escape latencyin the Morris water maze test when compared to the control. This observation was in agreement with previous studies after exposure of rat 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 were in agreement with previous studies that reported increased percentage alternation, decreased duration of escape latency, increased rearing frequency and number of line crossed [67-69] thereby attributing the protective role of MO against aluminum intoxicationto the numerous phytochemicals present within the plants such as flavonoids, phenols and vitamins.

Since the clinical manifestation of AD is characterized by progressive decline of cognition and behavior affecting thinking, planning, judgments and social skillsculminating into 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 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 $AlCl_3$ significantly increased oxidative stress marker (MDA) and decreased antioxidant enzymes levels (SOD, CAT, GSH) [54, 71, 72]. However, the combine administration of MO and Aluminum decreased the levels of oxidative stress and increased antioxidant enzymes 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. This findings was in accordance with previous studies that reported medicinal plants rich in flavonoids and phenols are capable of increasing the reduced antioxidants 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 alters 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 in accordance with 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 neuronal action potential [80, 81]. The present study showed that aluminum intoxication caused significant reduction in serum electrolyte levels (except potassium) when compared to the control. But the administration of MO restored the electrolytes 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 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. In accordance with 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].

5. 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 high level of polyphenols, flavonoids and other anti-oxidative compounds capable of activating cellular antioxidant system.

Ethics approval

The experimental procedures were conducted in accordance with the NIH guidelines for the care and use of laboratory animals in line with guidelines of the Department of Human Anatomy, School of health and Health Technology, Federal University of Technology, Akure, Nigeria and

the Health Research and Ethical Committee of the Federal University of Technology, Akure, Nigeria on 19th June, 2019.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.

2.

3.

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