

***Moringa oleifera* Lam. Leaf Extract Preserves Spatial Memory and Hippocampal Microstructure in Aluminium Chloride-Induced Neurotoxicity in Adult Wistar Rats**

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Abstract: Aluminium is a widely available and subtly consumed metal, whose actions on human health include neurotoxicity and cognitive decline, among others. It is important to protect against these aluminium effects through antioxidants, as aluminium utilises the oxidative pathway to exert its effect. Due to the abundance of antioxidants in *Moringa oleifera*, this study investigated its neuroprotective potential against aluminium chloride (AlCl₃)-induced hippocampal intoxication in Wistar rats. The phytochemical screening and toxicity (LD₅₀) of *Moringa oleifera* leaf ethanol extract (MO) were evaluated. Thirty adult male Wistar rats (150–220 g) were then assigned into six groups (n = 5): control, AlCl₃ (100 mg/kg), *Moringa oleifera* low dose (MOLD, 250 mg/kg), *Moringa oleifera* high dose (MOHD, 1,000 mg/kg), concomitant AlCl₃ + MOLD, and AlCl₃ + MOHD. These administrations were oral and lasted for 21 days. On day 22, spontaneous alternation behaviour (SAB) was tested in the T-maze, the animals sacrificed, and the brains processed for histology and immunoreactivity. The phytochemicals of MO showed flavonoids, phenols, alkaloids, saponins, and tannins as the major constituents, while its LD₅₀ was greater than 5,000 mg/kg. There was significantly (p < 0.05) less SAB, hippocampal cornu ammonis (CA) 3 chromatolysis, and decreased neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) expressions in the AlCl₃ group. Concomitant treatment with MOLD and MOHD did not significantly improve SAB, and NSE and GFAP expressions, but preserved Nissl distribution. In conclusion, the present study showed that MO showed varying effects in the hippocampus against AlCl₃ intoxication.

Keywords: Aluminium; Hippocampus; Histology; Immunohistochemistry; *Moringa oleifera*



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1. Introduction

Aluminium is ubiquitous and a known neurotoxic metal with functional and structural neuronal deficits^[1-3]. It induces oxidative stress, which is associated with alterations in diverse brain areas, irrespective of the individuals' age or species^[3-5]. The hippocampus is particularly susceptible to the effects of aluminium^[6,7]. Continuous exposure to this metal has been reported in diseased human brains, as their levels in live and autopsied brains have shown^[8-11].

Aluminium is easily and unconsciously ingested due to its presence in medicines, drinking water, and many manufactured foods and utensils^[11-13]. It has also been indicted in the aetiology of Alzheimer's disease^[8,14,15], although this is still controversial^[11,15]. The consumption of aluminium is subtle, and the risk to humans cannot be overemphasised^[3,6,10], there is a need to intentional manage its actions in the body, especially in the brain. One possible action is its chelation out from the body using continuous supplies of antioxidant-rich and free radical scavenging agents, which are replete in plants and plant-based extracts such as *Moringa oleifera* Lam^[2,16,17].

Moringa oleifera Lam. (*M. oleifera*) is a plant of the family Moringaceae, and is of particular interest in the present study due to its abundance in Nigeria and other African countries and the reported medicinal, nutritive, and antioxidant properties^[16-19]. This plant has been reported to have several health and protective benefits, making it of high economic value^[2,20,21]. Several anti-oxidant compounds, including vitamins A and C, beta-carotene, quercetin, and chlorogenic acid, have been identified in the leaf of *M. oleifera*^[18,19]. Due to its abundance, it is an important dietary vegetable, although its medicinal actions are not fully known. Exploring its antioxidant constituents with the aim of counteracting or limiting the bioavailability of heavy metals, especially aluminium, is essential since the possible mechanism of aluminium action is through the reactive oxygen species pathway^[4,5]. With its rich antioxidant contents, can the extract of *M. oleifera* leaf protect or ameliorate aluminium-induced hippocampal neurotoxicity? Although this question has already been answered to some extent by various studies, its role in hippocampal protein activities is still lagging.

This study therefore investigated the action of *M. oleifera* leaf extract on aluminium chloride-induced hippocampal protein toxicity in adult Wistar rats.

2. Materials and Methods

2.1. Animal Handling

Wistar rats and CD-1 mice were obtained from the Animal Facility of the Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria, and acclimatised for two weeks. The animals were humanely cared for in accordance with the guidelines for laboratory animal care and use^[22], and the Akwa Ibom State Ethical Committee gave the ethical approval with reference ID AK/MHS/RC/084. The rats were assigned to six groups of five animals each and were allowed standard chow and water *ad libitum*.

2.2. *M. oleifera* Extract Preparation

Fresh samples of *M. oleifera* were harvested from a local farm in Nduetong Oku within the Uyo Metropolis of Nigeria in the month of June and authenticated at the Department of Pharmacy with voucher number u.uH61/18. The leaves of *M. oleifera* were air-dried for two weeks, pulverised using a manually operated blender, and macerated in 70% ethanol for 72 hours at room temperature (26–28 °C). To eliminate the ethanol, the *M. oleifera* extract was concentrated using a rotary evaporator, and the concentrate was dried in a Plus 11 Gallenkamp oven at 45 °C. The concentrated extract was weighed and refrigerated at 4 °C until use.

2.3. Chemical Preparation

Aluminium chloride (Sigma) was the preferred heavy metal compound over other aluminium compounds due to its mild neurotoxic effect^[6,7]. It was dissolved in distilled water, which was also the vehicle for the *Moringa oleifera* leaf extract.

2.4. Phytochemical Screening of *M. oleifera* Leaf

Phytochemical screening of *M. oleifera* leaf extract was carried out in the Department of Biochemistry laboratory of the institution. The plant extract was screened qualitatively (using colour reaction) and quantitatively, for the presence of phenols, flavonoids, tannins, steroids, saponins, and alkaloids. As illustrated by Santhi and Sengottuvel^[23]. Alkaloids were screened using Mayer's and tannic acid tests; flavonoids were screened using lead acetate and H₂SO₄ tests; phenols were screened using ferric chloride and lead acetate

tests; terpenoids were screened using Salkowski's test; saponins were screened using Froth's test; amino acids were screened using Ninhydrin and Xanthropic tests; proteins were screened using Biuret's test; and sugar was screened using Benedict's and Fehling's tests. Tannins, steroids, and anthroquinones were also screened following standard procedures^[24]. Quantitatively, the alkaloids^[25], flavonoids^[26], phenols, saponins, and tannin contents of *M. oleifera* leaves were estimated^[23,27].

2.5. Evaluation of Acute Oral Toxicity of *M. oleifera* Leaf

The acute oral toxicity of the leaf extract was evaluated using the "limit-dose," which is also known as the "up-and-down" method^[28,29]. With this method, 20 CD-1 mice (23–24 g) were randomly assigned into five groups (n = 4). The mice were fasted overnight prior to dosing on each occasion, but were allowed access to water *ad libitum*. Mice from the first group were dosed orally with 1,000 mg body weight (mg/kg) of *M. oleifera* leaf extract and allowed for 48 hours. With no sign of toxicity among the mice, this process was repeated for groups 2–5, administering, respectively, 2,000, 3,000, 4,000, and 5,000 mg/kg body weight of *M. oleifera* extract. Since there was no mortality or adverse behavioural changes at 5,000 mg/kg body weight, the dosing was discontinued. The mice were nevertheless observed for 14 days to monitor any delayed toxic effects.

2.6. Animal Treatment

The thirty male Wistar rats (150–220 g) were assigned into six groups (n = 5). Group 1 was the control and administered distilled water (5 mL/kg), while group 2 was administered 100 mg/kg aluminium chloride based on Ekong *et al.*^[2]. Taking the estimated LD₅₀ of 5,000 mg/kg, 5% (low dose) and 10% (high dose) of the LD₅₀ amounted, respectively, to 250 mg/kg of *M. oleifera* and 1,000 mg/kg. Groups 3 and 4 were respectively administered 250 mg/kg and 500 mg/kg of *M. oleifera*, while groups 5 and 6 were administered concomitant 100 mg/kg aluminium chloride and 250 mg/kg *M. oleifera*, and 100 mg/kg aluminium chloride and 1,000 mg/kg *M. oleifera*. The administrations were orally, and once daily for 21 days.

2.7. Spontaneous Alternation Behaviour Test

Twenty-four hours after the last administration (day

22), spontaneous alternation behaviour was tested in the T-maze. Briefly, rats were placed at the base of the T-maze and allowed to explore the apparatus for 60 seconds while their choice of goal arms was recorded. If the rat did not choose any arm, it was scored zero. Five trials were performed for each rat, and between trials, the maze was cleaned with 70% alcohol. Their percentage alternations were thereafter calculated^[30].

2.8. Animal Sacrifice and Tissue Processing

The rats were anaesthetized with 50 mg/kg ketamine hydrochloride (Rotex Medica, Germany) intraperitoneally and sacrificed through intracardiac perfusion, initially with phosphate-buffered saline, then subsequently with 10% buffered formalin. Immediately after, the brains were collected and post-fixed in 10% buffered formalin for 48 hours, trimmed appropriately, and processed for histological and immunohistochemical studies. Tissue blocks were sectioned at 10 µm and mounted on slides. Serial sections were then deparaffinized and taken to the water.

A set of sections were stained in 0.1% cresyl violet for 15 minutes and rinsed in tap water to remove excess. The sections were then dehydrated in ascending grades of alcohol at 2 minutes each, cleared in two changes of xylene, and mounted in dibutylphthalate polystyrene xylene (DPX)^[29]. Antigen retrieval was performed on sections for immunolabelling; sections were incubated in citric acid solution (pH 6.0) in a microwave at 100 W for 5 minutes. Thereafter, they were equilibrated by gently displacing hot citric acid with distilled water. The sections were pre-treated with 3% hydrogen peroxide for 10 minutes, and pre-incubated in normal rabbit serum. They were then incubated either with anti-neuron-specific enolase (NSE) or glial fibrillary acidic protein (GFAP) for an hour at room temperature (27 °C) in a humidified chamber, and then incubated with biotinylated anti-immunoglobulin-G secondary antibodies for 30 minutes. They were then incubated in the avidin and biotin complex for 30 minutes, and diaminobenzidine (DAB) was used as a chromogen for 5 minutes. Sections were washed with distilled water, counter-stained for 2 minutes in haematoxylin, washed, dehydrated, cleared, and mounted in DPX.

2.9. Photomicrography and Cell Count

Cover-slipped tissue sections were viewed under the

light microscope, and photomicrographs were obtained using the microscope camera linked to a computer. The cell count of tissue sections was performed by the same experimenter blinded to the experimental groupings. Photomicrographs were uploaded to the Image J software, thresholded, and manually counted.

2.10. Statistical Analyses

One-way analyses of variance were used to compare the means for all groups for spontaneous alternation and cell count data. Thereafter, a student Newman-Keuls post-hoc test was carried out with Graphpad Prism (5.1) software. Data were presented as mean \pm standard error of mean. A probability level of $p < 0.05$ was regarded as significant.

3. Results

3.1. Phytochemicals of *M. oleifera* Leaf Ethanol Extract

The phytochemicals in the *M. oleifera* leaf showed flavonoids (4.34 g/100g) and phenols (4.10 g/100g). Others include alkaloids (2.62 g/100g), saponins (1.65 g/100g), and tannins (1.05 g/100g). Amino acids, terpenoids, anthroquinones, and sugar were in trace

amounts. However, proteins, cardenolides, and steroids were not observed (Table 1).

3.2. Median Lethal Dose of *M. oleifera* Leaf Extract

There was no mortality in any of the groups, nor was there any sign of toxicity observed with up to 5,000 mg/kg body weight of the *M. oleifera* extract 14 days later. Therefore, the process was discontinued, and the median lethal dose was estimated to be over 5,000 mg/kg body weight (Table 2).

3.3. Spontaneous Alternation Behaviour

Spontaneous alternation behaviour measures spatial memory. In the present study, there was a significant decrease ($p < 0.05$) in spontaneous alternation behaviour in the aluminium chloride group (37.50 \pm 16.14%) compared with the control (90.00 \pm 6.124%). However, there was no significant difference ($p > 0.05$) between the test groups administered 250 mg/kg of *M. oleifera* (62.50 \pm 16.14%), 1,000 mg/kg of *M. oleifera* (60.00 \pm 10.00%), aluminium chloride and 250 mg/kg of *M. oleifera* (58.33 \pm 8.33%), and aluminium chloride and 1,000 mg/kg of *M. oleifera* (50.00 \pm 10.21%) compared with the control and the aluminium chloride group (Figure 1).

Table 1: Phytochemical analyses of the *M. oleifera* leaf ethanol extract

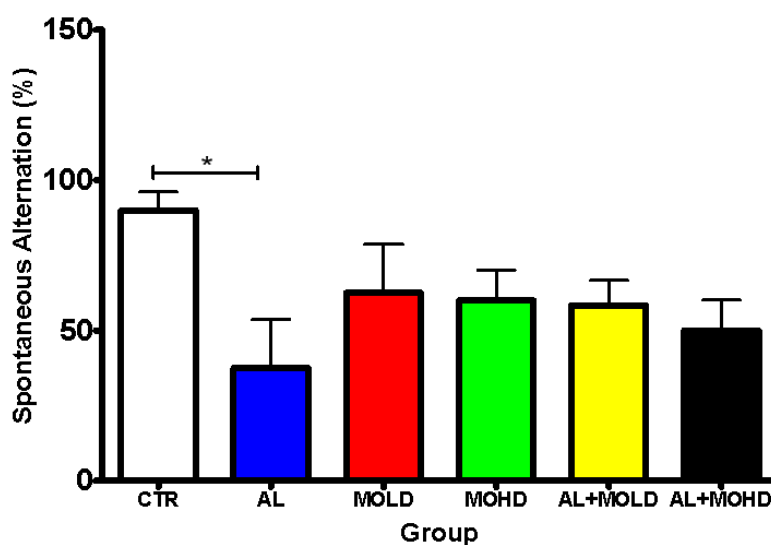
Test	Inference	Intensity	Quantity (g/100g)
Alkaloids			
(1) Meyer test	Present	++	2.62
(2) Tannic acid test	Present	+++	
Flavonoid			
(1) Lead acetate test	Present	+++	4.34
(2) H ₂ SO ₄ test	Present	+++	
Amino acid			
(1) Ninhydrin test	Present	+++	-
(2) Xanthropic test	Present	++	
Protein			
Biuret's test	Absent	-	-
Phenolics			
(1) FeCl ₃ Test	Present	+++	4.10
(2) Lead acetate test	Present	+++	
Terpenoids			
Salkowski's Test	Present	++	-
Tannins			
	Present	+++	1.05
Saponins			
Froth's Test	Present	++	1.65
Anthroquinones			
	Present	++	
Phlobatannins			
	Absent	-	
Sugar			
(1) Benedict's Test	Present	+	-
(2) Fehling's test	Present	++	
Cardenolides			
	Absent	-	-
Steroids			
	Absent	-	-

+++ = highly present; ++ = moderately present; + = lowly present; - = Absent

Table 2: Median lethal dose for the ethanol extract of *M. oleifera*

Group (n = 4)	Dosage of <i>M. oleifera</i> Leaf Extract (mg/kg)	Mice Mortality
1	1,000	0/4
2	2,000	0/4
3	3,000	0/4
4	4,000	0/4
5	5,000	0/4

LD₅₀ is over 5,000 mg/kg body weight of *M. oleifera*

**Figure 1:** Spontaneous Alternation Behaviour

* = Significantly different from CTR at $p < 0.05$
 $n = 5$; P value = 0.0694; $F = 2.470$

CTR = Control; AL = Aluminium chloride; MOLD = *M. oleifera* low dose (250 mg/kg); MOHD = *M. oleifera* high dose (1,000 mg/kg)

3.4. Nissl Substance Distribution

The cornu ammonis (CA) 3 region of the hippocampus of the control group showed Nissl substance distribution in the three layers: outer molecular, middle pyramidal, and inner polymorphic. These three layers showed well-stained Nissl substance, although the pyramidal layer contained a dense population unlike the molecular and inner polymorphic layers (**Figure 2a**).

The CA3 region of the hippocampus of the test groups also showed Nissl substance distribution in the three layers. In the aluminium chloride group, some of the pyramidal cells had less Nissl staining compared with the control group (**Figure 2b**). In the low (250 mg/kg) and high (1,000 mg/kg) *M. oleifera* dose groups, the Nissl substance was well-stained in most of the pyramidal cells compared with the control (**Figures 2c and d**).

In the concomitant aluminium chloride and low (250 mg/kg) and high (1,000 mg/kg) *M. oleifera* doses, Nissl substance was well-stained throughout the layers compared with the control (**Figures 2e and f**). The population of Nissl stained cells in the aluminium chloride group was significantly lower, but significantly higher in the low (250 mg/kg) and high (1,000 mg/kg) *M. oleifera* dose groups ($p = 0.0001$, $F = 48$) compared with the control. The population of Nissl stained cells in the aluminium chloride group was significantly lower than the low (250 mg/kg) and high (1,000 mg/kg) *M. oleifera* dose groups, as well as the concomitant aluminium chloride and high (1,000 mg/kg) *M. oleifera* dose. However, the population of Nissl stained cells in the high (1,000 mg/kg) *M. oleifera* dose group was significantly higher than the low (250 mg/kg) *M. oleifera* and concomitant aluminium chloride and low (250 mg/kg) *M. oleifera* dose groups. (**Figure 2g**).

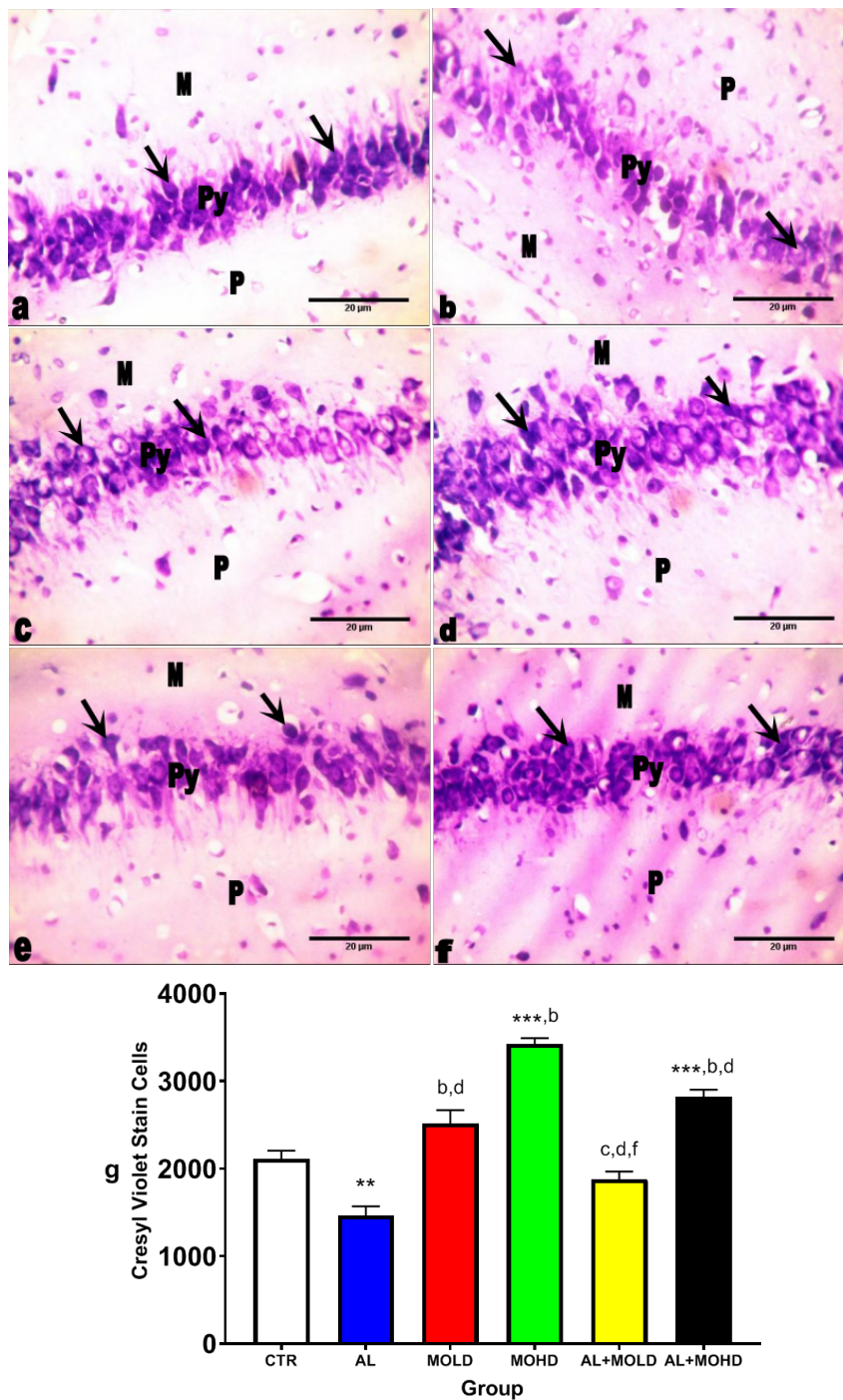


Figure 2: The Sections and Nissl Count Estimates of Hippocampal CA3. a. Control group having well-stained Nissl substance (arrows) in the three layers; b. Aluminium chloride group having less Nissl staining (arrows); c. Low dose *M. oleifera* group having well-stained Nissl substance (arrows); d. High dose *M. oleifera* group having well-stained Nissl substance (arrows); e. Aluminium chloride and low dose *M. oleifera* group having well-stained Nissl substance (arrows); f. Aluminium chloride and high dose *M. oleifera* group having well-stained Nissl substance (arrows). M = molecular layer; Py = pyramidal layer; P = polymorphic layer; Cresyl fast violet, $\times 400$; g. Nissl Count Estimate; ** = Significantly different from CTR at $p < 0.01$; *** = Significantly different from CTR at $p < 0.001$; b = Significantly different from AL at $p < 0.05$; c = Significantly different from MOLD at $p < 0.05$; d = Significantly different from MOHD at $p < 0.05$; f = Significantly different from AL+MOHD at $p < 0.05$; n = 5; P value = 0.0001; F = 48

CTR = Control; Al = Aluminium chloride; MOLD = *M. oleifera* low dose; MOHD = *M. oleifera* high dose

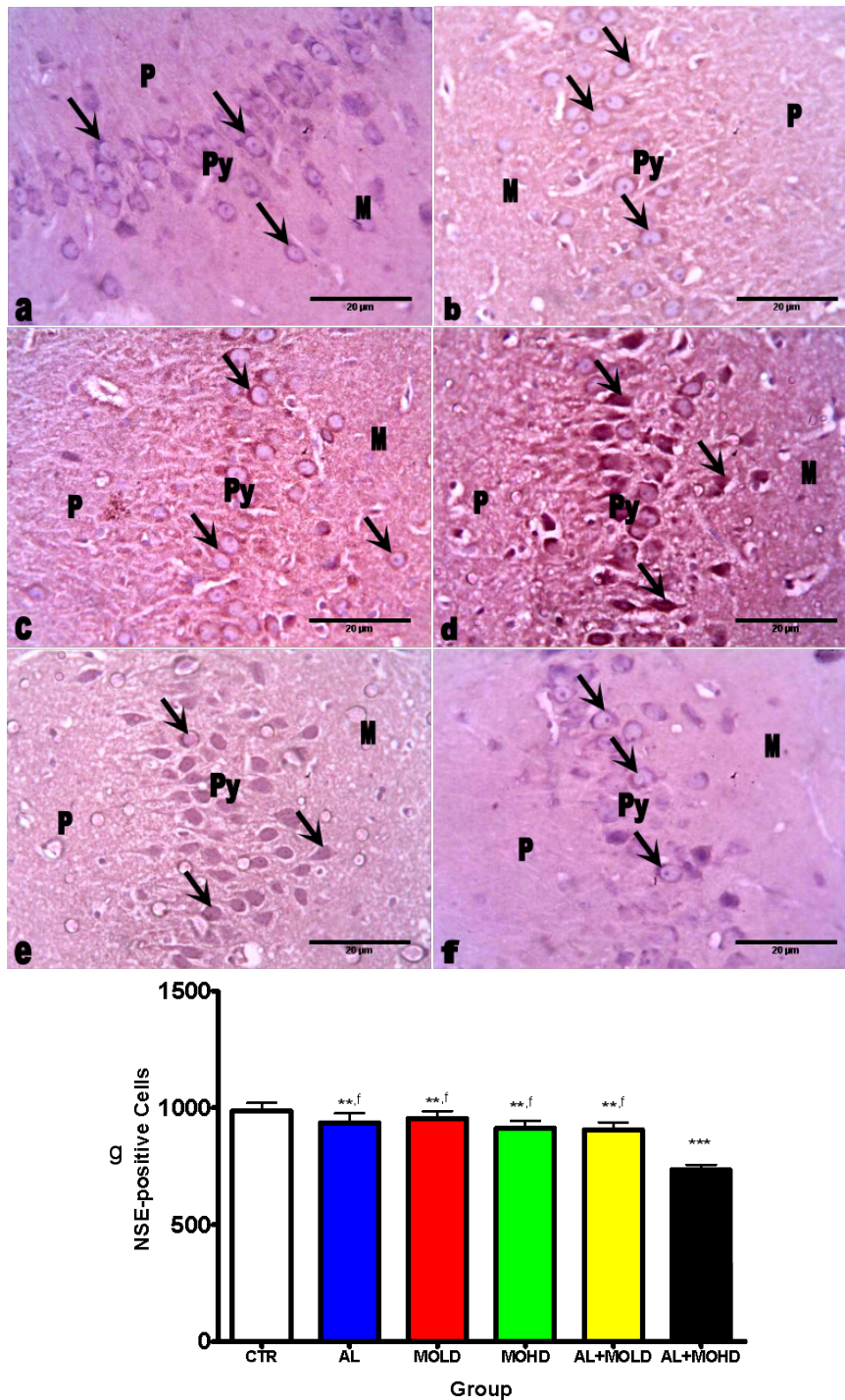


Figure 3: The Sections of the NSE-labelled Cells and Cell Count in the Hippocampal CA3.

- a. Control group having neuron specific enolase (NSE) positive neurons (arrows) in the three layers; molecular, pyramidal and polymorphic.
- b. Aluminium chloride group having less NSE-positive expression (arrows).
- c. Low dose *M. oleifera* group having NSE expression (arrows).
- d. High dose *M. oleifera* group having deep NSE expression (arrows).
- e. Aluminium chloride and low dose *M. oleifera* group having less NSE expression (arrow).
- f. Aluminium chloride and high dose *M. oleifera* group having less NSE expression (arrow). M = molecular layer; Py = pyramidal layer; P = polymorphic layer; NSE, $\times 400$.
- g. NSE-labelled cell count; *** = Significantly different from CTR at $p < 0.001$; f = Significantly different from AL+MOHD at $p < 0.05$; n = 5; P value < 0.0001; F = 67.90 CTR = Control; Al = Aluminium chloride; MOLD = *M. oleifera* low dose; MOHD = *M. oleifera* high dose

3.5. Neuron Specific Enolase Immunolabelling

The hippocampal CA3 region of the control group showed cytosolic expression of neuron-specific enolase (NSE) in the three layers (**Figure 3a**). In the aluminium chloride group, there was less NSE expression, with significantly ($p < 0.05$) fewer NSE-positive cells compared with the control (**Figures 3b and 3g**). The low-dose *M. oleifera* group showed similar NSE expression and significantly ($p < 0.05$) fewer NSE-positive cells compared with the control (**Figure 3c**).

The hippocampal CA3 region of the high-dose *M. oleifera* group showed deep NSE expression and significantly ($p < 0.05$) fewer NSE-positive cells compared with the control (**Figure 3d**). There were fewer NSE expressions and significantly ($p < 0.05$) fewer NSE-positive cells in the concomitant aluminium chloride with low and high doses of *M. oleifera* groups compared with the control (**Figures 3e and 3f**).

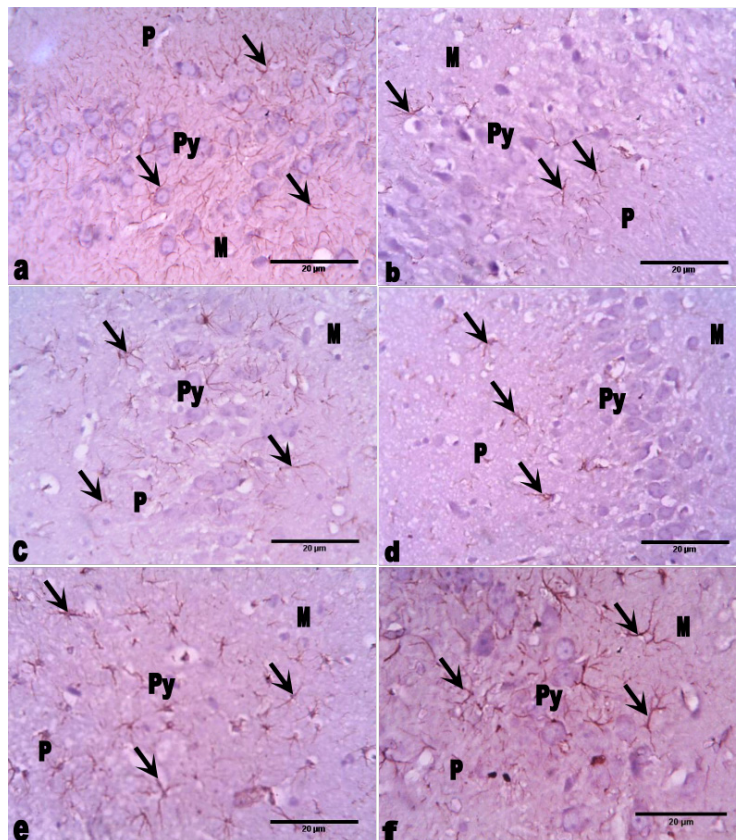
The NSE-labelled cells were not significantly different ($p > 0.05$) in the aluminium chloride group compared with the other test groups, with the exception of the concomitant aluminium chloride and high dose group (**Figure 3g**).

3.6. Glial Fibrillary Acidic Protein Immunolabelling

The CA3 region of the hippocampus of the control group showed expression of GFAP in cells of the three layers; GFAP expression was observed mostly in the processes, although some were also expressed in the soma (**Figure 4a**). In the aluminium chloride group, the GFAP-positive cells were significantly ($p < 0.05$) less compared with the control (**Figure 4b**). In the low-dose *M. oleifera* group, the GFAP-positive cells were significantly ($p < 0.05$) less compared with the control (**Figure 4c**).

The hippocampal CA3 region of the high-dose *M. oleifera* group showed the GFAP-positive cells were significantly ($p < 0.05$) less compared with the control (**Figure 4d**). In the concomitant aluminium chloride and low-dose *M. oleifera* group, the GFAP-positive cells were significantly ($p < 0.05$) less compared with the control (**Figure 4e**). In the concomitant aluminium chloride and high-dose *M. oleifera* group, there were deeper-expressing GFAP-positive cells, which were significantly ($p < 0.05$) less compared with the control (**Figure 4f**).

The GFAP-positive cells were significantly ($p < 0.05$) less in the aluminium chloride group compared to the other test groups (**Figure 4g**).



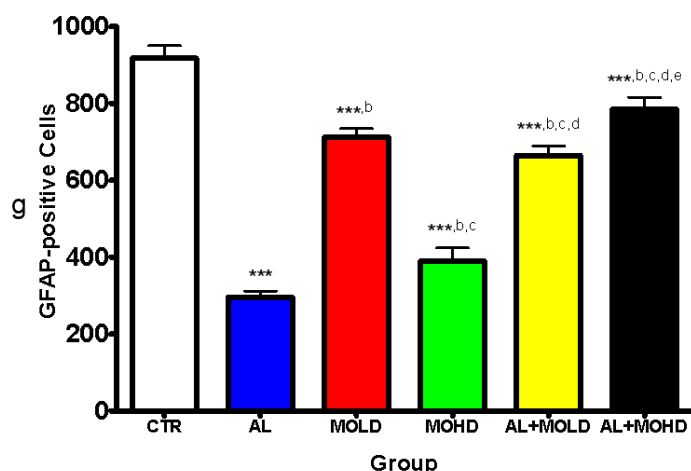


Figure 4: The Sections of GFAP labelled Cells and Cell Count in the Hippocampal CA3.

- Control group having GFAP-positive cells whose expression is mostly in the processes (arrows).
- Aluminium chloride group shows GFAP-positive cells (arrows).
- Low dose *M. oleifera* group shows GFAP-positive cells (arrows)
- High dose *M. oleifera* group shows GFAP-positive cells (arrows)
- Aluminium chloride and low dose *M. oleifera* group shows GFAP-positive cells (arrows).
- Aluminium chloride and high dose *M. oleifera* group shows GFAP-positive cells (arrow). M = molecular layer; Py = pyramidal layer; P = polymorphic layer; GFAP, $\times 400$.
- GFAP-positive cell count; *** = Significantly different from CTR at $p < 0.001$; b, c, d, e = Significantly different from AL, MOL, MOH, AL+MOL respectively, at $p < 0.05$; n = 5; P value < 0.0001 ; F = 2048; CTR = Control; Al = Aluminium chloride; MOLD = *M. oleifera* low dose; MOHD = *M. oleifera* high dose

4. Discussion

This study investigated the hippocampal neuroprotection potential of *M. oleifera* leaf extract on aluminium chloride-induced toxicity. Phytochemical screening of the *M. oleifera* leaf extract showed a moderate presence of alkaloids, amino acids, flavonoids, tannins, phenolics, saponins, terpenoids, anthroquinones, and sugar. It is believed that the various biological activities of *M. oleifera*, are influenced by their phytochemical constituents^[32, 33]. Alkaloids, anthroquinones, flavonoids, phenolics, saponins, tannins, and terpenoids protect cells against oxidative stress^[32-38]. Some other roles include free radical scavenging, metallic ion chelators, and oxidation inhibitors, among others^[37-40]. The combination of these phytochemicals and their activities in the *M. oleifera* leaf makes the plant very essential for a wide spectrum of health activities^[19, 20, 41], including nutrition^[42-44]. The present phytochemical constituents are similar to those from previous studies^[19, 43].

The oral acute lethal toxicity test of *M. oleifera* showed no mortality, even up to 5,000 mg/kg, indicating the safety of the plant for oral consumption. The present result agrees with Ekong *et al.*^[2], who reported similar oral LD₅₀ for *M. oleifera* ethanol leaf

extract.

Spontaneous alternation measures spatial memory^[30], which was affected in the present study. The spontaneous alternation behaviour was significantly decreased in the aluminium chloride group compared with the control, indicating a cognitive deficit. The present result corroborates a previous report of cognitive dysfunction and negative spatial learning and memory capacities arising from aluminium chloride^[45]. Aluminium chloride affects memory and cognition by interfering with glutamatergic, catecholaminergic, and cholinergic metabolism^[46, 47].

There was no significant spontaneous alternation behaviour between the groups administered just *M. oleifera* leaf extract and the control group, supporting the extract safety^[2, 48], which could have also related to the brain and spontaneous alternation behaviour. There was also no significant difference in the spontaneous alternation behaviour between the groups administered the concomitant aluminium chloride and *M. oleifera* leaf extract compared with the control, suggesting a protective role against aluminium chloride. *M. oleifera* is reported to protect against aluminium chloride action and alleviate learning and memory impairment^[2, 21], which the present study supports.

Nissl is the endoplasmic reticulum of neurons and a site for protein synthesis and release^[49]. Histologically, there was significantly lower Nissl number and staining in the hippocampal CA3 region of the aluminium chloride group compared with the control, indicating neuronal trauma or toxicity. In neurotoxicity, Nissl is less stained in trauma to the brain^[50], and this condition known as chromatolysis has been reported in aluminium neurotoxicity^[2,6,51,52].

The CA3 histology of the groups administered only low and high doses of *M. oleifera* showed mostly well-stained Nissl substance, and that of the high dose of *M. oleifera* was significantly more in population compared with the control. This may indicate the physiological reactivity of the Nissl substance to the *M. oleifera* extract. *M. oleifera* is reportedly safe, as already reported and in previous studies^[2]. However, this does not rule out discrete molecular changes in neurons, as reported by Abijo *et al.*^[53], which may have influenced the Nissl staining. The concomitant aluminium chloride and low and high doses of *M. oleifera* groups showed mostly well-stained Nissl substance that were significantly more in number in the concomitant aluminium chloride and high dose of *M. oleifera* group compared with the control, indicating a protective effect of *M. oleifera* to chelate the aluminium or antagonised its effect, thereby, preventing its action in this brain area. This result is in line with previous studies^[2,54].

Neuron-specific enolase is a cytosolic protein, glycolytic isoenzyme, and marker for neuronal metabolic activity and synaptic connections. It evaluates the activity of neurons, promotes neuronal survival^[55,56], and also serves as a marker for neural dysfunction and pathology^[57]. Immunohistochemically, there was significantly less NSE expression in the hippocampal CA3 region of the aluminium chloride group, indicating its neurotoxicity. Decreased expression and number of NSE-positive cells may indicate reduced neuronal metabolic activity or damage^[58,59]. These have been reported as adaptive changes in the hippocampus known to be susceptible to neurotoxins^[51,60,61].

The low-dose *M. oleifera* group showed similar NSE-positivity, although with significantly fewer ($p < 0.05$) cells compared with the control, indicating less neuronal activity. Neuronal metabolic activity

is reflected in NSE expression^[55,56], and this may not have been affected in this group. The high dose *M. oleifera* group showed increased NSE expression, although with significantly ($p < 0.05$) fewer positive cells compared with the control, indicating increased neuronal metabolic activity. NSE is involved in neuronal repair^[56], whose pathway may have been triggered, leading to increased NSE expression. Increased neuronal metabolic activity is also reported with increased NSE expression, and this may lead to excitotoxicity.

The concomitant administration of aluminium chloride with either low- or high dose *M. oleifera* showed decreased NSE expression with significantly ($p < 0.05$) fewer positive cells compared with the control but not different from the aluminium chloride group, indicating less metabolic activity of the neurons. The lower metabolic activities may be due to the antagonising action of *M. oleifera* on aluminium chloride. This corroborates the report of Ekong *et al.*^[2].

Glial fibrillary acidic protein, an intermediate filament protein in astrocytes^[62], also serves as its marker^[63]. Immunohistochemically, there were significantly fewer ($p < 0.05$) GFAP-positive cells in the hippocampal CA3 region of the aluminium chloride group compared with the control, which may indicate neurotoxicity. Decreased GFAP-positivity has previously been reported with aluminium administration^[64], which may arise due to neurodegeneration or other detrimental conditions^[65,66]. The low and high doses of *M. oleifera* groups showed fewer GFAP-positive cells, which were also significantly different from the control group, indicating decreased GFAP activity. Decreased GFAP expression as in the present study could have resulted from loss of glial fibrillary acidic protein antigenicity^[67], which Ekong *et al.*^[2] also reported. The groups administered concomitant aluminium chloride with either low- or high dose *M. oleifera* showed significantly ($p < 0.05$) fewer GFAP-positive cells compared to the control, although significantly more than those of the aluminium chloride group. This may indicate decreased GFAP activity, not neurotoxicity. GFAP expression may be up-regulated in traumatised or injured brain^[68], but antagonistic action on this intermediate protein may have resulted in increased expression. The present result varies from other studies, which may be due to the doses of *M. oleifera* and the

target brain area^[2].

The hippocampus, a vital memory centre in the brain, is the most affected brain part in aluminium intoxication^[46]. In the present study, aluminium chloride caused cognitive decline as observed in decreased spontaneous alternation, which is probably a consequence of neuronal protein synthesis disruption and activity as reported in Nissl substance chromatolysis and decreased NSE expression. The decreased Nissl and NSE expressions arising from aluminium chloride administration could lead to neurotransmitter depletion, which may be responsible for decreased GABA, dopamine, noradrenaline, and serotonin levels reported previously^[47]. The resultant effect is memory impairment, as played out in the present study. Abnormal expression of GFAP occurs in neuroinflammation and neurodegeneration, among others^[69], which is implicated in aluminium intoxication^[46], and may consequently lead to loss of GFAP antigenicity^[67].

Moringa oleifera may have reversed the cognitive deficit by improving spontaneous alternation through the protection of Nissl, NSE, and GFAP expression. Antioxidation is reported in neuroprotection and neurotransmitter enhancement^[70,71], thus, in line with suggested mechanism for *M. oleifera* neuroprotective effect as previously reported^[16,50].

5. Conclusion

M. oleifera leaf extract contained vital nutritional and medicinal phytochemicals, and was also safe for oral consumption. The administration of aluminium chloride resulted in a decline in spontaneous alternation and hippocampal CA3 chromatolysis, which decreased NSE and GFAP expressions. However, concomitant treatment with *M. oleifera* did improve spontaneous alternation, Nissl distribution, NSE, and GFAP expressions. However, the high dose of *M. oleifera* improved Nissl distribution better.

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Compliance with Ethical Standards

Conflict of interest: There is no conflict of interests.

Ethical approval and consent to participate approval for the study was granted by the the Akwa Ibom State Ethical Committee gave the ethical approval with reference ID AK/MHS/RC/084, and all protocols involving handling and care of the animals were strictly adhered to.

Abbreviations

AlCl ₃	-	Aluminium chloride
CA3	-	Cornu ammonis
DAB	-	Diaminobenzidine
DPX	-	Dibutylphthalate polystyrene xylene
GFAP	-	Glial fibrillary acidic protein
LD ₅₀	-	Median lethal dose
MO	-	Moringa leaf extract
MOHD	-	Moringa leaf extract high dose
MOLD	-	Moringa leaf extract low dose
NSE	-	Neuron specific enolase
SAB	-	Spontaneous alternation behaviour

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