

Article

# EFFECT OF *MORINGA OLEIFERA* LAM. LEAF EXTRACT AGAINST ALUMINIUM CHLORIDE INDUCED HIPPOCAMPAL HISTOLOGY AND SERUM ENZYME ACTIVITIES IN ADULT WISTAR RATS

## *M. OLEIFERA* EXTRACT PROTECTS AGAINST ALUMINIUM CHLORIDE

**Efecto del Extracto de hoja de *Moringa Olifera* Lam. en la Histología del Hipocampo y la actividad enzimática en el suero de ratas Wistar adultas expuestas a Cloruro de Aluminio**  
Extracto de *M. oleifera* para protección del daño por Cloruro de Aluminio

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## ABSTRACT

**Introduction:** Aluminium, a ubiquitous metal implicated in some neurodegenerative diseases is linked to activation of free oxygen species. The antioxidant-rich plants, *Moringa oleifera* (MO) is reported to protect against Aluminium activities. This study investigated the actions of MO leaf extract (MOLE) against Aluminium chloride (AlCl<sub>3</sub>)- induced hippocampal cellular changes and serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) in adult Wistar rats.

**Materials and Methods:** Thirty Wistar rats weighing between 150 g and 220 g were grouped (n=5) into; 1-control (5 mL/kg distilled water), 2-AlCl<sub>3</sub> (100 mg/kg), 3-low dose MOLE (250 mg/kg), 4-high dose MOLE (1,000 mg/kg), 5-concurrent AlCl<sub>3</sub> and low dose MOLE, and 6-concurrent AlCl<sub>3</sub> and high dose MOLE. All administrations were by oral gavages for 21 days. On day 22, following deep anaesthesia and cardiac puncture, blood was obtained for serum enzyme analysis, and the brain perfusion fixed, harvested and processed for histological study.

**Results:** Results showed significantly ( $p < 0.05$ ) higher ALP level in the AlCl<sub>3</sub> group compared with the control, as well as the other test groups. However, there was no significant ( $p > 0.05$ ) AST and ALT levels. The hippocampal CA3 of the AlCl<sub>3</sub> group showed hypertrophic cells, with some of the cells having karyorrhectic features. The concurrent AlCl<sub>3</sub> and low and high doses, MOLE groups showed less of these adverse features.

**Conclusion:** These results suggest that MOLE may protect enzymatic activities against Aluminium chloride. However, its action on hippocampus is still subject to further investigation.

**Keywords:** *Moringa oleifera*; Aluminium chloride; Hippocampus; Neurotoxicity; Serum Enzyme; Histology

## 1. Introduction

The nervous system is responsible for the control or regulation of other parts of the body, where it is protected from toxins by layers of meninges and the blood-brain-barrier. These allowing only limited body and exogenous components access (Saunders *et al.*, 2016; Jacobson *et al.*, 2018; Ghannam & Al Kharazi, 2020). Nevertheless, a substance such as Aluminium still find its way into the nervous system resulting in the activation of oxygen free species and lipid peroxidation (Wang *et al.*, 2018; Krupińska, 2020). The body then activates its antioxidants, which may not be adequate to cause complete restoration of the normal milieu (Abdalla *et al.*, 2019). These more often than not cause damages, resulting in neurodegenerative disorders (Giacoppo *et al.*, 2015; Ekong *et al.*, 2017; Wang *et al.*, 2018). The resultant neurodegeneration is of significance as manifestations include widespread central nervous system damage, intellectual disability, persistent memory impairments, epilepsy and dementia among others (Exley & Vickers, 2014; Wang *et al.*, 2018). Some of the adverse actions associated with tissue injury can be detected in the serum through the enzymatic levels of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT), because they are biomarkers of tissue health status (Tang *et al.*, 2011). These enzymes have normal concentrations in the blood as 5-40 U/L for AST and 5-35 U/L for ALT, while serum ALP level is 20-140 U/L. However, when body tissue is diseased or damaged, additional enzymes are released into the blood, causing higher levels (Huang *et al.*, 2006)

Aluminium is ubiquitous and therefore readily available to be taken up by the body. These include through cookware, pipe-borne water supply and medication among others (Krupińska, 2020; McFarland *et al.*, 2020). The mechanism of action of Aluminium involves the activation of free oxygen species and lipid peroxidation (Krupińska, 2020). In the hippocampus, Aluminium is reported to cause pyknotic cell bodies, non-homogenous cytoplasm and karyopyknotic (Chakrabarty *et al.*, 2012; Yu *et*

*al.*, 2014). It is therefore important that intake of exogenous antioxidants are vital in protecting the body from the actions of Aluminium. Some plants are reported to be richer in antioxidant than other conventional sources. *Moringa oleifera* Lam. (*M. oleifera*) being one of such, is reported in protection against toxicants (Chu *et al.*, 2018; Villarruel-López *et al.*, 2018).

*M. oleifera* belongs to the family Moringaceae, with its leaf being the commonly utilized part of the plant. This plant contains such antioxidants as vitamins A and C, beta-carotene, quercetin and chlorogenic acid among others (López -Teros *et al.*, 2017; Vergara-Jimenez *et al.*, 2017; Lin *et al.*, 2019). The antioxidants as well as other active alkaloids, phenols and flavonoids among others make the plant important in the management of Aluminium toxicity (Vergara-Jimenez *et al.*, 2017; Falowo *et al.*, 2018). The added advantage of *M. oleifera* is its safety, as it is reportedly safe at over five thousand milligram per kilogram body weight (Ekong *et al.*, 2017). This study therefore investigated the action of *M. oleifera* leaf extract on Aluminium chloride induced hippocampal histology and serum enzyme activities in adult Wistar rat.

## 2. Materials and Methods

### *Leaf Collection, Authentication and Extract Preparation*

Fresh *M. oleifera* plant was harvested from a local farm in Nduetong Oku within Uyo Metropolis, Nigeria, and authenticated at the Department of Pharmacy, University of Uyo, Nigerian, and a specimen voucher number (u.uH61/18) was obtained from the Faculty of Pharmacy Herbarium. The *M. oleifera* leaves were then air dried under a shade for two weeks, pulverized using a manually operated blender, macerated in 70% ethanol, and kept for 72 h at room temperature. The extract was concentrated using rotary evaporator, and the concentrate dried in a plus 11 Gallenkamp oven at 45-50 °C. The dried extract was refrigerated at -4°C until use. The dried extract was dissolved in warm distilled water according to doses to be administered to the different groups of the experimental animals.

### *Animal Handling*

This research was approved by the Faculty of Basic Medical Sciences Research and Ethics Committee. Thirty adult male Wistar rats obtained from the Faculty of Basic Medical Sciences Animal House, University of Uyo, and weighing between 150 g and 220 g were used for the study. The animals were allowed to acclimatize for two weeks and were humanely cared for in accordance with the Guidelines of Laboratory Animal Care and Use (National Institute of Health, 2011). The animals were randomly selected into six groups of five animals each, and housed in well ventilated cages, under normal environmental conditions. The rats were given standard chow, and water was provided *ad libitum* throughout the course of the study.

### *Experimental Design*

The thirty Wistar rats were weighed and then randomly divided into six experimental groups of five animals each. Group 1 was the control and received distilled water (5 mL/kg), while groups 2-6 were the test groups and received respectively, Aluminium chloride alone (100 mg/kg); low dose *M. oleifera* leaf extract (MOLE, 250 mg/kg), high dose *M. oleifera* leaf extract (MOLE, 1,000 mg/kg), concurrent Aluminium chloride and low dose MOLE, and concurrent Aluminium chloride and high dose MOLE. The administration was once daily for 21 days and was by oral gavages (Table 1).

The body weights of the animals were obtained before administration, and weekly subsequently using an electronic weighing balance. Percentage change in body weights were calculated using the equation;

$$\frac{\text{Final Weight (g)} - \text{Initial Weight (g)}}{\text{Initial Weight (g)}} \times 100$$

Twenty four hours after the last administration the rats were sacrificed following deep anaesthesia with 50 mg/kg body weight of ketamine hydrochloride (Rotex Medica, Germany) intraperitoneally. Blood for serum enzyme analysis was obtained by cardiac puncture, and the animals were sacrificed through intracardiac perfusion with 10% buffered formalin via the left ventricle of the heart. Immediately after, whole brains were collected and post-fixed in 10% buffered formalin for 48 hours, trimmed appropriately to the hippocampus region, and processed for histological studies.

**Table 1**

Schedule of administration to animals in the experimental groups

Group Treatment (n = 5)	Dosage	Duration
Distilled water (control)	5 mL/kg	3 Weeks
AlCl <sub>3</sub>	100 mg/kg b.wt	3 Weeks
MOLE (low dose)	250 mg/kg b.wt	3 Weeks
MOLE (high dose)	1,000 mg/kg b. wt	3 Weeks
AlCl <sub>3</sub> + MOLE (low dose)	100 + 250 mg/kg b.wt	3 Weeks
AlCl <sub>3</sub> + MOLE (high dose)	100 + 1,000 mg/kg b.wt	3 Weeks

AlCl<sub>3</sub> = Aluminium chloride; MOLE = *M. oleifera* leaf extract; b.wt = body weight

### 3. Tissue Processing

#### *Histological Processing*

Each brain region containing the hippocampus was processed to paraffin wax and embedded, while the tissue blocks were sectioned at 10 µm and mounted on slides. Sections were later deparaffinised and taken to water. Thereafter, stained for 15 min in haematoxylin. Excess haematoxylin was washed off and differentiated in 1% acid alcohol. The sections were washed and counterstained in eosin. Excess was washed off and the sections dehydrated through ascending grades of alcohol, cleared in xylene, and mounted in dipolycystein xylene (DPX) (Suvarna *et al.*, 2019).

Sections were viewed under the light microscope and photomicrographs were obtained using the microscope camera linked to a computer. ImageJ software was used to manually count the cells of the hippocampal *Cornu ammonis* (CA3) to determine their population.

#### *Biochemical Processing and Serum Enzyme Analysis*

Serum was separated from cells within 1 h of collection by centrifuging for 15 min. The analysis was performed using a Hitachi model 917 multichannel analyzer. Alkaline phosphatase (ALP), alanine

transaminase (ALT) and aspartate transaminase (AST) were analysed using reagent kits (Randox Laboratories, England) and according to the manufacturer's instructions.

### **Statistical Analysis**

One way analysis of variance was used to compare the means of histology cell count and serum enzyme level of all groups, and thereafter, the Newman-Keul post-hoc test was carried out, both using Graphpad prism software. Obtained data were presented as mean  $\pm$  standard error of mean. Probability level of  $p < 0.05$  was regarded as significant.

## **4. Results**

### **Effect of *M. oleifera* Leaf Extract on Body Weights**

In the course of the experiment, there was percentage body weight increase in the groups administered Aluminium chloride, low dose MOLE and the concurrent Aluminium chloride and low dose MOLE compared to the control which recorded body weight loss. Also, there was percentage body weight loss in the group administered high dose MOLE and the concurrent Aluminium chloride and high dose MOLE (Table 2).

### **Effect of *M. oleifera* Leaf Extract on Levels of ALP, AST and ALT**

There was significantly ( $p < 0.05$ ) higher ALP level in the group administered Aluminium chloride and the control, as well as the other test groups. However, there was no significant difference ( $p > 0.05$ ) in the ALP level between the other test groups and the control, and also among the other test groups (Figure 1).

There was no significant difference ( $p > 0.05$ ) in the AST level between the test groups and the control, except in the group administered combined Aluminium chloride and high dose MOLE, which was significantly less than the control and the group administered Aluminium chloride. However, there was no significant difference ( $p > 0.05$ ) in the AST level among the other test groups (Figure 2). There was no significant difference ( $p > 0.05$ ) in the ALT level between the test groups and the control, and among the test groups (Figure 3).

### **Effect of *M. oleifera* Leaf Extract on the Histology of the Hippocampus**

The hippocampal CA3 of the control group showed its three cortical layers with no obvious histopathology; outer molecular, middle pyramidal and inner polymorphic. The molecular layer was mostly nerve processes and contained scanty cells. The pyramidal layer contained a dense population of large pyramidal shaped neurons with other small cells distributed within. The polymorphic layer contained scanty small size cells (Figure 4a).

The hippocampal CA3 of the group administered Aluminium chloride showed hypertrophic cells, with some of the cells having karyorrhectic appearance in the pyramidal layer compared with the control group (Figure 4b). The hippocampal CA3 of the group administered low dose MOLE showed slightly atrophic cells, especially in the pyramidal layers compared with the control group (Figure 4c). The hippocampal CA3 of the group administered high dose MOLE showed hypertrophic cells, especially in the pyramidal layer compared with the control (Figure 4d).

The hippocampal CA3 of the group administered Aluminium chloride and low dose MOLE showed hypertrophy of the entire cortical cells. There were some karyorrhectic features in the pyramidal layers

compared with the control (Figure 4e). The hippocampal CA3 of the group administered Aluminium chloride and high dose MOLE showed hypertrophic cortical cells with some karyorrhectic features especially in the pyramidal layer compared with the control (Figure 4f).

**Table 2**

Body weight changes of animals in the experimental groups

Groups (n = 5)	Initial body weight (g)	Final body weight (g)	Body weight change (g)	Body weight change (%)
control)	245.7 ± 20.76	238.7 ± 14.77	-7.0 ± 5.99	-2.85
AlCl <sub>3</sub>	174.7 ± 7.84**	182.0 ± 10.79**	7.3 ± 2.95	4.75
MOLE (low dose)	153.7 ± 11.61**	177.7 ± 15.10*	24.0 ± 3.49	15.69
MOLE (high dose)	148.0 ± 3.00**	143.7 ± 5.55**	-4.3 ± 2.55	-2.91
AlCl <sub>3</sub> + MOLE (low dose)	125.0 ± 7.21***	130.0 ± 4.04***	5.0 ± 3.17	4.00
AlCl <sub>3</sub> + MOLE (high dose)	161.3 ± 19.53**	158.0 ± 16.80**	3.3 ± 2.73	-2.05

Data presented as mean ± standard error of mean

\*\*\* - Significantly different at  $p < 0.001$  from the control group

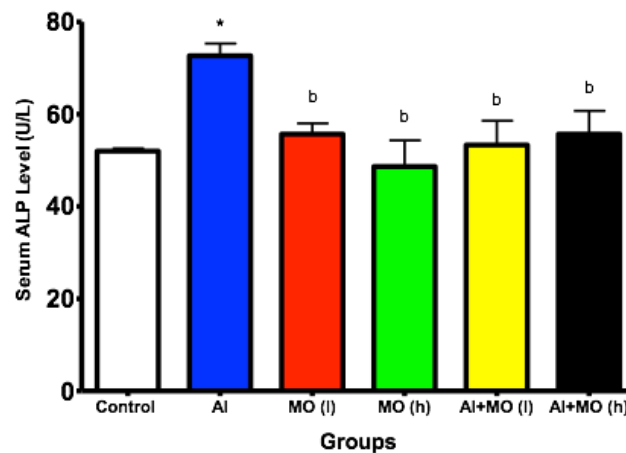
\*\* - Significantly different at  $p < 0.01$  from the control group

\* - Significantly different at  $p < 0.05$  from the control group

AlCl<sub>3</sub> = Aluminium chloride; MOLE = *M\*. oleifera* leaf extract

**Figure 1:**

Serum alkaline phosphatase levels in the experimental groups



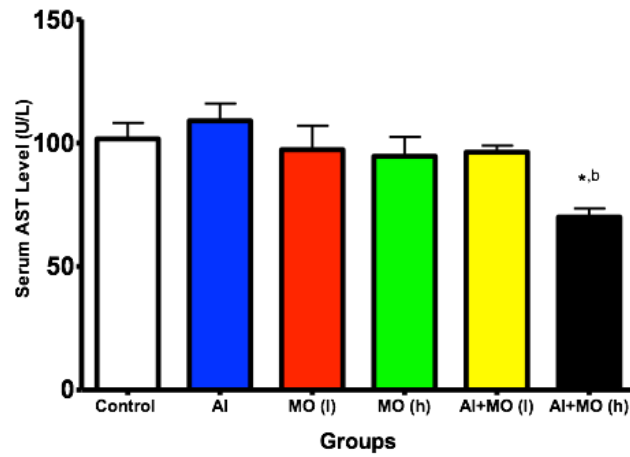
\* - Significantly different at  $p < 0.05$  from the control group

b - Significantly different at  $p < 0.05$  from Aluminium chloride group

Al = Aluminium chloride; MO = *M. oleifera*; ALP = Alkaline phosphatase

**Figure 2:**

Serum aspartate transaminase levels in the experimental groups



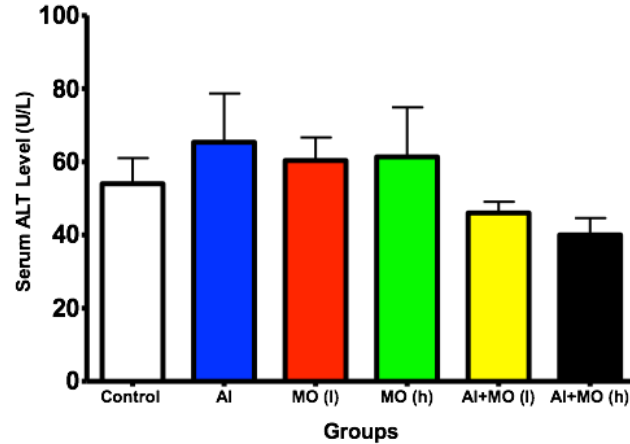
\* - Significantly different at  $p < 0.05$  from the control group

b - Significantly different at  $p < 0.05$  from Aluminium chloride group

Al = Aluminium chloride; MO = *M. oleifera*; AST - Aspartate transaminase

**Figure 3:**

Serum alanine transaminase levels in the experimental groups

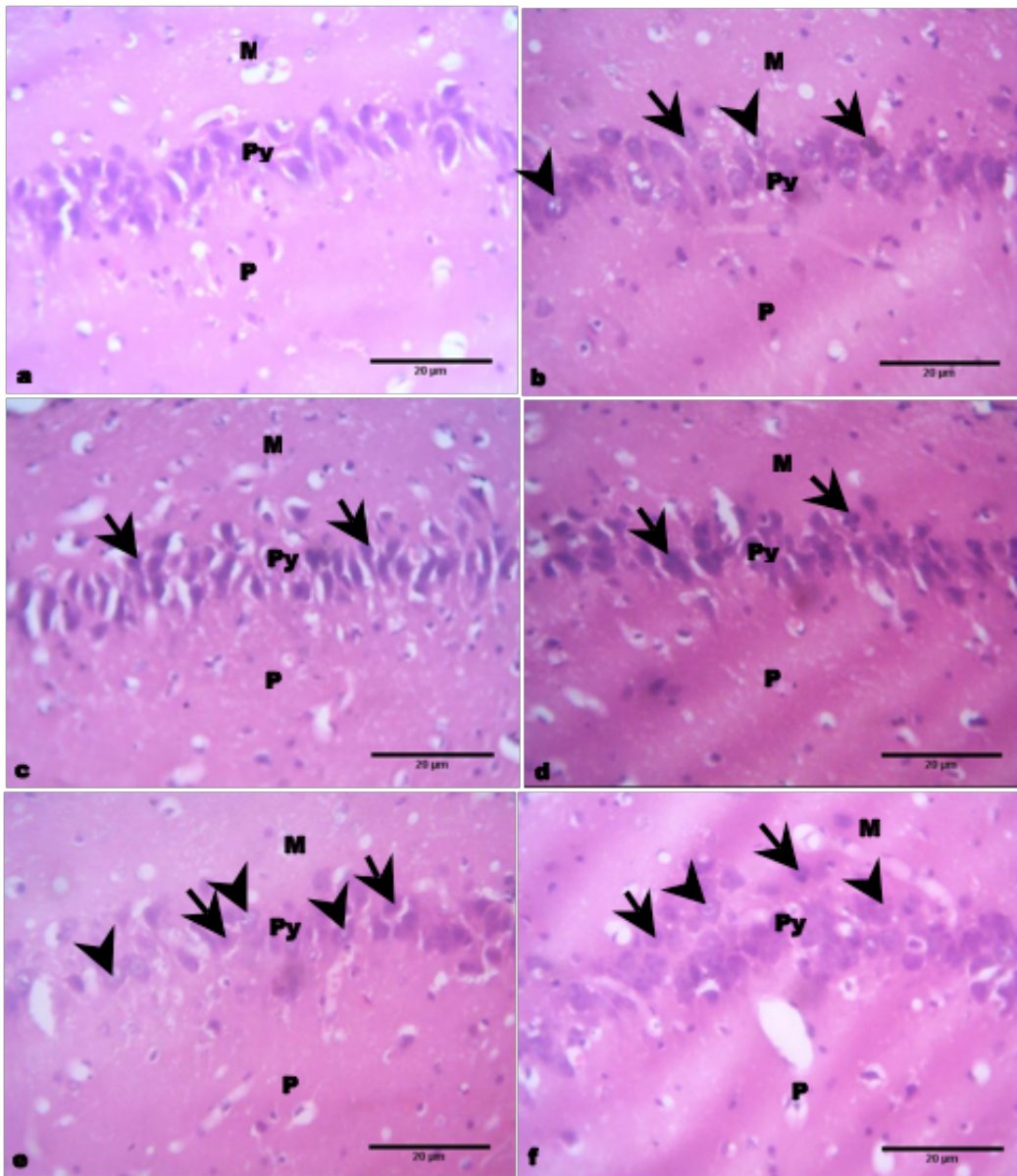


There is no significant difference ( $p > 0.05$ ) among the groups

Al = Aluminium ; MO = *M. oleifera*; ALT -= Alanine transaminase

**Figure 4:**

Histology Sections of the hippocampal CA3 region in the experimental groups



- Control group with normal histological features having three cortical layers.
- Aluminium chloride group showing hypertrophic cells (arrows), with some having karyorrhectic (arrow head) appearance.
- Low dose MOLE group having slightly atrophic cells (arrows).
- High dose MOLE group having hypertrophic cells (arrow).
- Aluminium chloride and low dose MOLE group having hypertrophy (arrow) of the entire cortical cells, with less karyorrhexis (arrow head).
- Aluminium chloride and high dose MOLE group having hypertrophic cortical cells (arrow), with less karyorrhexis (arrow head)

Outer molecular (M), middle pyramidal (Py) and inner polymorphic (P) layers (CA = *Cornu ammonis*; H&E,  $\times 400$ ).



## 5. Discussion

This present study investigated the activity of *M. oleifera* leaf extract on Aluminium chloride induced hippocampal histology and some serum enzyme activities in adult Wistar rats. In the course of the experiment, the rats' weekly body weight were recorded, and at the end of the experiment, there was body weight gain in the Aluminium chloride, low dose *M. oleifera* and the combined Aluminium chloride and low dose *M. oleifera* groups, compared with the control. The body weight gain in the group administered Aluminium chloride alone may indicate non-adverse effect to the body metabolic processes, or a decreased catabolic compared with the anabolic process (Galgani and Ravussin, 2008). This result is at variance with Golub and Germann (2001) who reported that exposure to Aluminium chloride decreased the body weight of rats. There was body weight gain in the group administered *M. oleifera* low dose, indicating that low dose *M. oleifera* may improve anabolic processes leading to addition of adipose tissues of the rat at the given dose. It is reported that *M. oleifera* aids metabolic processes (Ferreira *et al.*, 2008), The present result agrees with Aremu *et al.* (2018) who reported that *M. oleifera* causes increase in body weight at low dose.

On the other hand, the body weight of the animals in the control group were reduced, and may be attributed to stress due to handling, as handling is reported to affect body weight negatively (Voisinet *et al.*, 1997). However, there was also body weight loss in the high dose *M. oleifera* and the combined Aluminium chloride and high dose *M. oleifera* groups. High dose *M. oleifera* contains insoluble fibre that prevents constipation and promotes a healthy digestive system (Gopalakrishnan *et al.*, 2017). This high fibre content may slow food absorption and keeps blood sugar regulated. Aside from being a plant that is super-low in calories and high in nutrients like vitamins A, C and manganese, *M. oleifera* contains an anti-oxidant called chlorogenic acid. This anti-oxidant regulates blood sugar and acts as a fat burner thereby enhancing weight loss (Atawodi *et al.*, 2003), which may have been the case in the present study, and irrespective of the presence of Aluminium chloride.

The group administered concurrent Aluminium chloride and *M. oleifera* low dose gained body weight, indicating that low *M. oleifera* dose boost body weight as previously reported (Gopalakrishnan *et al.*, 2017). This however does not preclude the activity of Aluminium chloride which may have resulted in a synergistic effect, as the group administered aluminium chloride alone gained body weight.

The enzymes; AST, ALP and ALT are serum biomarkers of tissue health status (Tang *et al.*, 2011). Increase in the levels of these enzymes is related to injury to the liver, kidney, heart, brain and other tissues (McGill, 2016). There was significantly ( $p < 0.05$ ) higher ALP level between the Aluminium chloride group and the control, indicating that Aluminium chloride may have caused tissue toxicity leading to their leakage into the serum. The present result is in line with Moshtaghie *et al.* (2006) and Akpanyung *et al.* (2018), who reported that Aluminium chloride administration increased ALP. ALP is a polyfunctional enzyme that acts as a transphorylase at alkaline pH, and plays a pivotal role in the mineralization of the skeleton of animals (Zhang *et al.*, 2011), and these may have been compromised. As there was no difference with the other test groups compared to the control, it indicates that *M. oleifera* may have counteracted the activity of the Aluminium chloride which is in line with previous a study (Akpanyung *et al.*, 2018).

In the present study, there was no significant difference ( $p > 0.05$ ) in the AST level between the test groups and the control, although the combined *M. oleifera* high dose and Aluminium chloride showed a significantly less AST level. These results indicate the non-adverse activities of the administered substances in tissues. Nevertheless, the administered Aluminium chloride showed insignificant ( $p > 0.05$ ) AST level increase, which indicates that some of the tissues may have been compromised as previously

reported (Moshtaghi *et al.*, 2006). The present results are at variance with Akpanyung *et al.* (2018), who reported that Aluminium chloride administration increased serum AST level. As AST is involved in protein and amino acid metabolism (Zhang *et al.*, 2011), this function may have been compromised.

There was no significant difference ( $p > 0.05$ ) in the ALT level between the test groups and the control, indicating non-adverse activities of the administered substances in tissues. Nevertheless, the administered Aluminium chloride showed insignificant ( $p > 0.05$ ) ALT level increase, which indicates that some of the tissues may have been compromised as previously reported (Moshtaghi *et al.*, 2006 and Akpanyung *et al.*, 2018), and in the present study. ALT is involved in protein and amino acid metabolism (Zhang *et al.*, 2011), which may have been compromised.

The histology of the hippocampal CA3 was investigated: The groups administered low and high doses of *M. oleifera* showed atrophic and hypertrophic features, which may indicate the presence of foreign material in the nervous tissues. Hypertrophy may be a physiological or pathological condition, and usually result from increase demand, chronic inflammatory response, hormonal dysfunctions or compensation for damage (Goldstein *et al.*, 1987). Owolabi and Ogunnaike (2014) reported that *M. oleifera* has no deleterious effect on the brain, suggesting that the present results may not be pathological. In the other hand, groups administered concurrent Aluminium chloride and *M. oleifera* low and high doses showed some karyorrhectic features in addition, indicating the inability of the given *M. oleifera* to protect or antagonize Aluminium chloride effect. This disagrees with Ekong *et al.* (2017) who reported that *M. oleifera* has an ameliorative effect on Aluminium chloride toxicity.

The hippocampal CA3 is an essential brain area for memory consolidation, which when disrupted could result in impairment leading to learning deficit. Aluminium is reported to cause cognitive impairment to this brain area, which is not surprising in the present study. However, *M. oleifera* may not have counteracted these adverse effects in the present study.

## 6. Conclusion

The given dose of Aluminium chloride in the present study did not affect body weight, but caused adverse serum levels, especially of ALP, which were counteracted by MOLE, and may have protect enzymatic activities against Aluminium chloride. However, its action on hippocampus is still subject to further investigation.

## 7. Conflicts of Interest

There is no conflict of interest

## 8. Funding of the study

Does not declare

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## RESUMEN

Introducción: El aluminio, un metal presente en diversos lugares implicado en algunas enfermedades neurodegenerativas, está relacionado con la activación de especies reactivas de oxígeno. Se informa que las plantas ricas en antioxidantes, *Moringa oleifera* (MO) protegen contra la acción del aluminio. Este estudio investigó las acciones del extracto de hoja de MO (MOLE) en los cambios celulares del hipocampo inducidos por el cloruro de aluminio (AlCl<sub>3</sub>) y los niveles séricos de fosfatasa alcalina (ALP), aspartato transaminasa (AST) y alanina transaminasa (ALT) en ratas Wistar adultas.

Materiales y métodos: SE utilizaron treinta ratas Wistar divididas en 5 grupos, los animales pesaban entre 150 gy 220 g; 1 control (5 ml / kg de agua destilada), 2-AlCl<sub>3</sub> (100 mg / kg), 3 MOLE de dosis baja (250 mg / kg), 4 MOLE de dosis alta (1000 mg / kg), 5 AlCl<sub>3</sub> concurrente y MOLE de dosis baja, y MOLE 6-concurrente y MOLE de dosis alta. Todas las administraciones fueron por sonda oral durante 21 días. El día 22, después de la anestesia profunda y la punción cardíaca, se obtuvo sangre para el análisis de las enzimas séricas y la perfusión cerebral se fijó, recogió y procesó para el estudio histológico.

Resultados: Los resultados mostraron un nivel de ALP significativamente ( $p < 0.05$ ) más alto en el grupo AlCl<sub>3</sub> en comparación con el control, así como en los otros grupos de prueba. Sin embargo, no hubo niveles significativos ( $p > 0.05$ ) de AST y ALT. El hipocampo CA3 del grupo AlCl<sub>3</sub> mostró

células hipertróficas, y algunas de las células tenían características cariorrecticas. Los grupos de  $AlCl_3$  concurrentes y dosis bajas y altas, MOLE mostraron menos de estas características adversas.

Conclusión: Estos resultados sugieren que MOLE puede proteger las actividades enzimáticas contra el cloruro de aluminio. Sin embargo, su acción sobre el hipocampo aún está sujeta a más investigaciones.

**Palabras clave:** Moringa oleifera; Cloruro de aluminio; Hipocampo; Neurotoxicidad; Enzima sérica; Histología

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