RESEARCH PAPER

Cell-Mediated Immunomodulatory and Neuroprotective Effects of Moringa oleifera Leaf Extract Against Aluminum Chloride-Induced Hypothalamic Toxicity in Adult Male Wistar Rats.

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ABSTRACT

Background: Aluminum chloride ($AlCl_3$) is a well-established neurotoxin implicated in oxidative stress, immune dysregulation, and hypothalamic dysfunction. The search for plant-based therapeutic agents with immunomodulatory and neuroprotective properties remains critical in toxicological research.

Objective: This study evaluated the cell-mediated immunomodulatory and neuroprotective effects of Moringa oleifera leaf extract (MOLE) against $AlCl_3$-induced hypothalamic toxicity in adult male Wistar rats.

Methods: Thirty-six adult male Wistar rats (180g – 220g) were randomly assigned into six groups (n=6) and treated orally for 28 days. Aluminum chloride (100mg/kg) was administered to induce toxicity. Immunological parameters ($CD4^+$ and $CD8^+$ T-cells by flow cytometry, serum IL-2, IFN-γ and TNF-α by ELISA), delayed-type hypersensitivity (DTH) response, oxidative stress markers (MDA, SOD, CAT, GPx) in hypothalamic tissue, histopathology, immunohistochemistry, and RT-PCR gene expression analyses were performed.

Results: $AlCl_3$ exposure significantly suppressed $CD4^+$ and $CD8^+$ T-cell populations, reduced IL-2 and IFN-γ levels, elevated TNF-α ($P < 0.05$), induced oxidative stress, and caused severe hypothalamic neuronal degeneration and vacuolation. Treatment with MOLE significantly reversed these alterations in a dose-dependent manner, with the 400 mg/kg dose showing effects comparable to the Vitamin E positive control. Gene expression analysis confirmed restoration of IL-2 and IFN-γ mRNA levels. Histological and immunohistochemical examinations demonstrated marked preservation of neuronal architecture and reduced astroglia activation.

Conclusion: Moringa oleifera leaf extract exhibits potent neuroprotective and immunomodulatory effects mediated through enhancement of cell-mediated immunity and antioxidant defense systems, suggesting its potential as a therapeutic candidate against heavy metal-induced neurotoxicity.

Keyword: *Aluminum chloride; hypothalamus; cell-mediated immunity; Moringa oleifera; oxidative stress; neuroprotection; cytokines; phytotherapy.*

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Introduction

Aluminum is the third most abundant element in the Earth s crust and has no recognized physiological role in humans. However, chronic exposure to aluminum compounds, particularly aluminum chloride ($AlCl_3$), is implicated in neurotoxicity, oxidative stress, mitochondrial dysfunction, and immune dysregulation (Exley, 2020; Bondy, 2021 and Yokel, 2013). Aluminum accumulates preferentially in brain regions such as the hippocampus and hypothalamus, disrupting neuronal integrity and neuroendocrine homeostasis (Sharma et al., 2021).

The hypothalamus regulates critical functions including hormonal secretion, thermoregulation, stress responses, and immune modulation (Saper and Lowell, 2014). Damage to this region can lead to widespread physiological disturbances, including immune imbalance and increased susceptibility to neurodegenerative processes.

Oxidative stress is a central mechanism in $AlCl_3$-induced neurotoxicity, characterized by excessive reactive oxygen species (ROS) generation, lipid peroxidation (elevated MDA) and depletion of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Valko et al., 2007; Halliwell and Gutteridge, 2015).

Cell-mediated immunity (CMI), primarily driven by $CD4^+$ helper and $CD8^+$ cytotoxic T-cells, plays a pivotal role in neuroimmune regulation. Cytokines such as IL-2 and IFN-γ support T-cell proliferation and activation, while TNF-α

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promotes inflammation and apoptosis (Abbas et al., 2021 and Chaplin, 2022). Aluminum exposure disrupts T-cell function and cytokine balance, exacerbating neuroinflammation (Singh et al., 2021; Zhang et al., 2022).
Moringa oleifera (family Moringaceae) is a fast-growing medicinal plant rich in bioactive compounds including flavonoids (quercetin, kaempferol), phenolic acids (chlorogenic acid), alkaloids, saponins and glucosinolates. These phytochemicals confer potent antioxidant, anti-inflammatory and immunomodulatory properties (Leone et al., 2021; Gopalakrishnan et al., 2023; Saini et al., 2022). Preliminary phytochemical analysis of the ethanolic leaf extract in this study confirmed high total phenolic (145 180 mg GAE/g) and flavonoid (85 110 mg QE/g) contents.
While previous studies have demonstrated the protective effects of M. oleifera against various toxicities, data on its specific role in modulating cell-mediated immunity and protecting the hypothalamus against aluminum-induced damage remain limited. This study therefore evaluated the neuroprotective and immunomodulatory potential of MOLE in an experimental $AlCl_3$-induced hypothalamic toxicity model in Wistar rats.

2.MATERIALS AND METHODS

2.1 Experimental Animals

Thirty-six healthy adult male Wistar rats (180–220 g) were procured and acclimatized for 14 days under standard laboratory conditions (temperature 22 ± 2°C, 12-h light/dark cycle, 50 60% humidity). Animals had free access to standard rodent chow and water.

2.2 Ethical Approval

All procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Technology, Owerri (Approval No: FUTO/ANAT/ETHICS/2025/017) and conducted in accordance with NIH guidelines for the care and use of laboratory animals.

2.3 Plant Material and Extract Preparation

Fresh Moringa oleifera leaves were collected from a pesticide-free farm in Imo State, Nigeria, and authenticated by a taxonomist at the Department of Botany, Federal University of Technology, Owerri. A voucher specimen (FUTO/HERB/PD/2026/043) was deposited. Leaves were washed, shade-dried and pulverized. 500g of pulverized leaf were subjected to Soxhlet extraction with 70% ethanol for 72 hours. The extract was filtered, concentrated under reduced pressure using a rotary evaporator at 40°C, and stored at 4°C. Percentage yield was calculated gravimetrically.

2.4 Phytochemical Screening

Qualitative screening for alkaloids, flavonoids, phenolic compounds, saponins, tannins, glycosides, terpenoids and glycosylates was performed using standard methods (Harborne, 1998; Trease and Evans, 2002; Anwar et al., 2007). Quantitative estimation of total phenolics and flavonoids was also conducted.

2.5 Experimental Design

Animals were randomly assigned into six groups (n=6). All treatments were administered orally for 28 consecutive days.
Group I: Control (received rat chow and water)
Group II: Were exposed to 100mg/kg $AlCl_3$
Group III: Were exposed to 100mg/kg $AlCl_3$ + 200 mg/kg MOLE
Group IV: Were exposed to 100mg/kg $AlCl_3$ + 400mg/kg MOLE
Group V: received 400 mg/kg MOLE only
Group VI: Were exposed to $AlCl_3$ + 100 mg/kg Vitamin E

2.6.Induction of Aluminum Toxicity

Aluminum chloride was dissolved in distilled water and administered orally at a dose of 100mg/kg body weight daily.

2.7 Immunological Assessments

2.7.1 Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation. Cells were stained with fluorescent anti-$CD4^+$ and anti-$CD8^+$ monoclonal antibodies and analyzed on a flow cytometer. Results are expressed as percentage positive cells.

2.8 Cytokine Quantification

Serum levels of IL-2, IFN-γ, and TNF-α were measured using commercial sandwich ELISA kits according to manufacturer instructions (absorbance at 450 nm).

2.9 Delayed-Type Hypersensitivity (DTH) Response

On day 21, rats were sensitized with 0.1mL of 10% sheep red blood cells (SRBCs) subcutaneously in the right hind paw. On day 28, a challenge dose (0.05mL of 5% SRBCs) was injected into the left hind paw. Paw thickness was measured before and 24 hours post-challenge using a digital micrometer. The difference represented the DTH response.

2.10 Oxidative Stress Biomarkers

Hypothalamic homogenates were prepared in ice-cold phosphate buffer. MDA (lipid peroxidation) was assayed by TBARS method. Activities of SOD, CAT, and GPx were determined using standard spectrophotometric kits.

2.11Histopathology

Hypothalamic tissues were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were examined under light microscopy (X100 and X400) and scored semi-quantitatively for vacuolation, neuronal loss and inflammation.

2.12 Immunohistochemistry (IHC)

Immunoperoxidase staining for TNF-α and GFAP was performed to assess inflammation and astrocyte activation.
2.13 Gene Expression Analysis (qRT-PCR)
Total RNA was extracted from hypothalamic tissue using TRIzol reagent. cDNA was synthesized, and quantitative real-time PCR was performed for IL-2, IFN-γ, and TNF-α using SYBR Green chemistry. Expression was normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

2.14 Statistical Analysis

Data were presented as mean ± SEM. One-way ANOVA

followed by Tukey's post-hoc test was used for multiple comparisons. Statistical significance was set at $P < 0.05$. Analyses were performed using GraphPad Prism SPSS software version 20.0

3.0 RESULTS

Table 1: Effect of treatments on $CD4^+$ and $CD8^+$ T-cell populations (%)

Group	$CD4^+$	$CD8^+$
Control (feed and water only)	82.0 ± 2.5.	61.0 ± 2.0
$AlCl_3$(100mg/kg)	42.0 ± 1.8*.	31.0 ± 1.5*
$AlCl_3$ (100mg/kg) + MOLE (200mg/kg)	65.0 ± 2.1#.	48.0 ± 1.8#
$AlCl_3$ (100mg/kg) + MOLE (400mg/kg)	75.0 ± 2.0#.	58.0 ± 1.6#
MOLE (400mg/kg) only	84.5 ± 2.2	63.5 ± 1.9
$AlCl_3$ (100mg/kg) + Vit E (100mg/kg)	78.0 ± 2.3#.	59.0 ± 1.7#

(*P < 0.05 vs Control; #P < 0.05 vs $AlCl_3$)

Table 2: Serum cytokine levels (pg./mL)

Group	IL-2.	IFN-γ.	TNF-α
Control (feed and water only)	85 ± 4.	92 ± 5.	30 ± 2
$AlCl_3$(100mg/kg)	40 ± 3*.	45 ± 4*.	75 ± 6*
$AlCl_3$ (100mg/kg) + MOLE (200mg/kg).	68 ± 4#.	72 ± 5#.	45 ± 3#
$AlCl_3$ (100mg/kg) + MOLE (400mg/kg)	78 ± 3#.	88 ± 4#.	35 ± 2#
MOLE (400mg/kg) only	88 ± 4	95 ± 5	28 ± 2
$AlCl_3$ (100mg/kg) + Vit E (100mg/kg)	80 ± 4#.	90 ± 4#.	32 ± 3#

Table 3: Oxidative stress markers in hypothalamus

Parameter	Control	$AlCl_3$	$AlCl_3$ + MOLE 400.	Vit E
MDA (nmol/mg).	2.1 ± 0.2	5.8 ± 0.4*.	2.5 ± 0.3#.	2.3 ± 0.2#
SOD (U/mg).	12.5 ± 0.8.	6.2 ± 0.5*.	11.8 ± 0.7#.	12.0 ± 0.6#
CAT (U/mg).	45.2 ± 2.1	18.5 ± 1.5	38.5 ± 2.0#.	40.1 ± 1.8#
GPx (U/mg).	28.4 ± 1.5	12.3 ± 1.1	25.6 ± 1.4#.	26.8 ± 1.3#

3.1 Histological Findings:

Group A (Feed + water only)

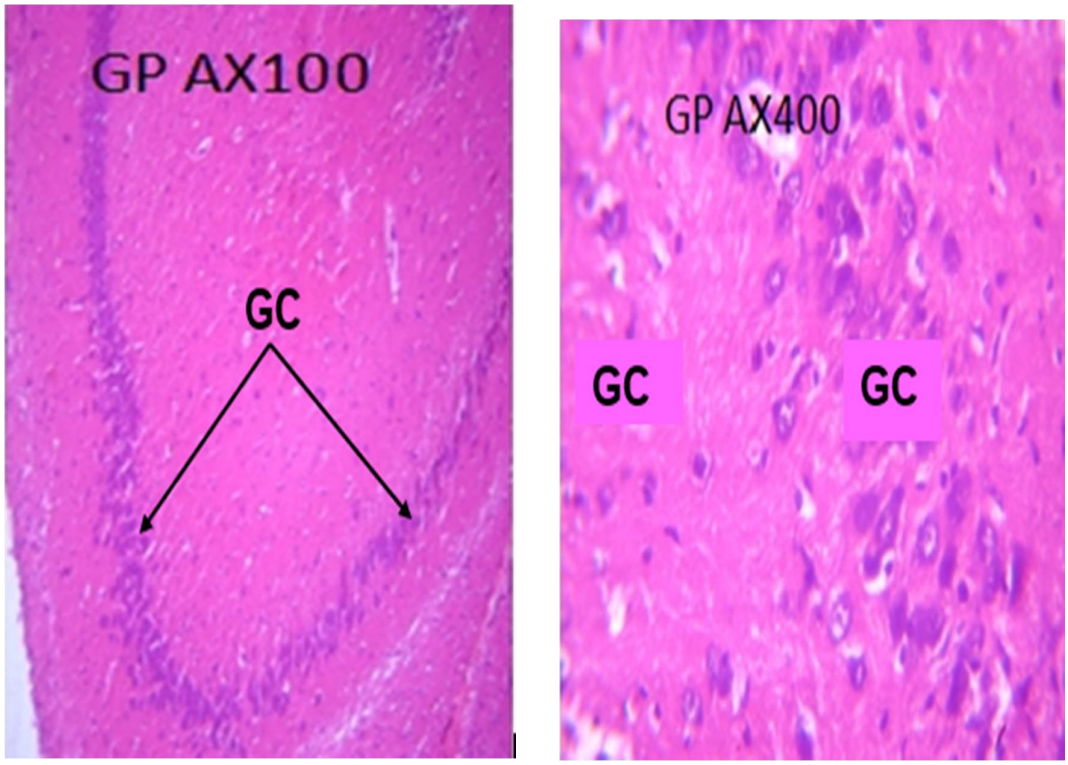

Plate 1:Photomicrograph (X100/X400 (H/E) of group A (normal control) section of hypothalamus shows active granular cells (NC)

Group B ($AlCl_3$ + Moringer oelifera (100 mg/kg)

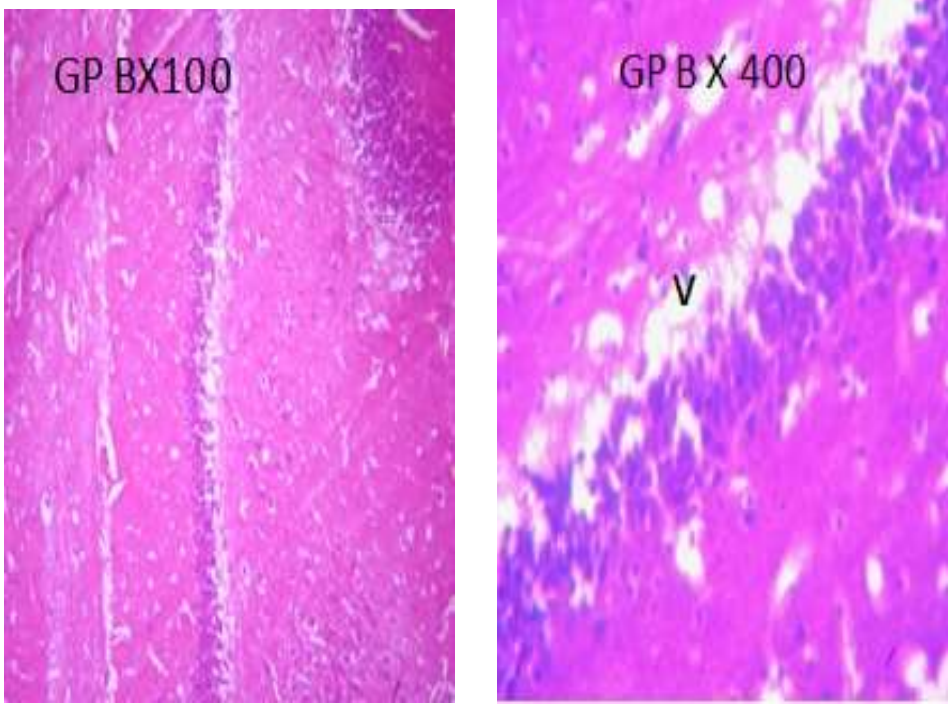

Plate 2: Photomicrograph (X100/X400(H/E) of group B (negative control) section of the hypothalamus exposed to Aluminium chloride shows moderate vacuolation (V) with loss of neuronal cells.

Group C ($AlCl_3$ + Moringer oelifera (200 mg/kg)

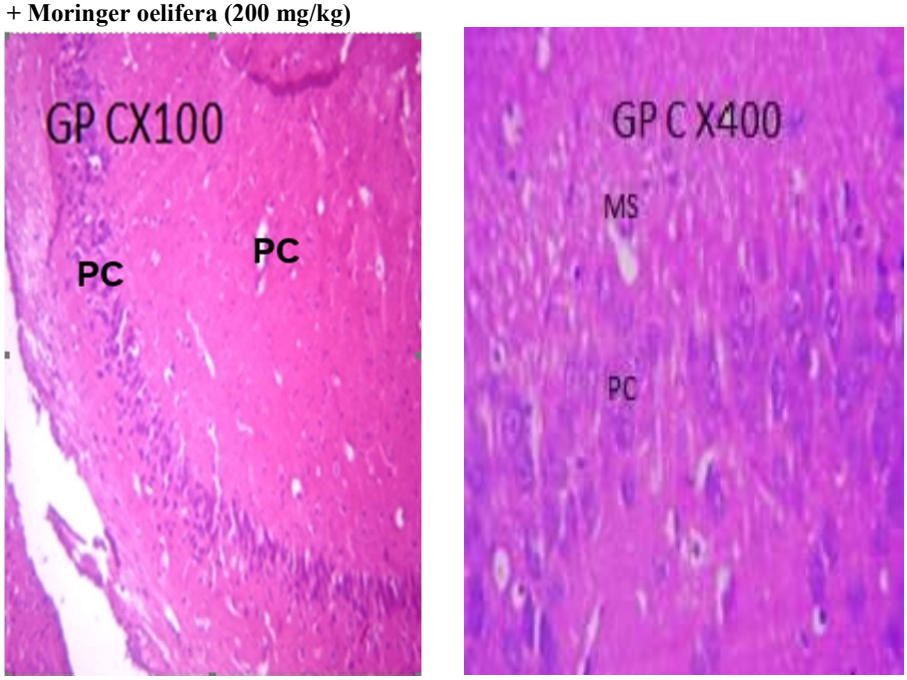

Plate 3: Photomicrograph (X100/X400(H/E) of group C section of the hypothalamus exposed to Aluminium chloride and subsequently treated with 200mg/kg of Moringer oelifera extract shows moderate healing with mild micro cystic space (MS) and well outlined Pyramidal cell (PC).

Group D ($AlCl_3$ + Moringer oelifera (400 mg/kg)

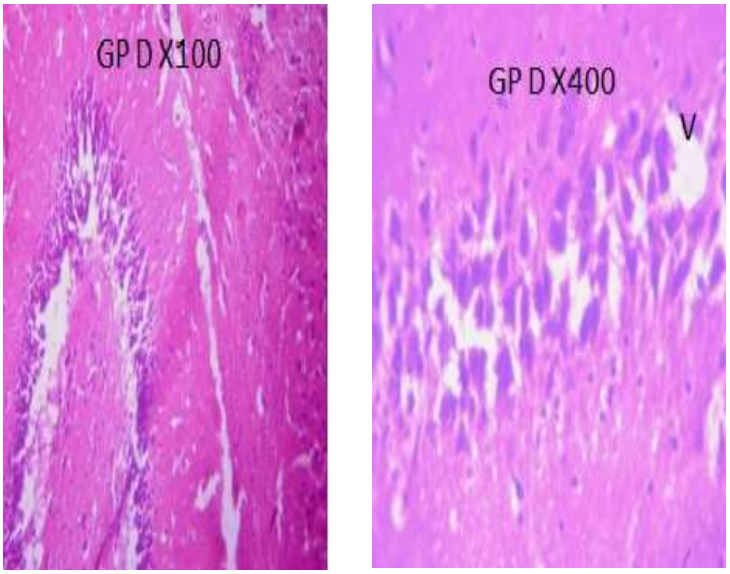

Plate 4: Photomicrograph (X100/X400(H/E) of groupD section of the hypothalamus induced with Aluminium chloride and treated with 400mg/kg of Moringer oelifera extract shows mild healing with moderate vacuolation (V) of nueronal cell cell (N).

Group E ($AlCl_3$ + Vitamin E (100 mg/kg)

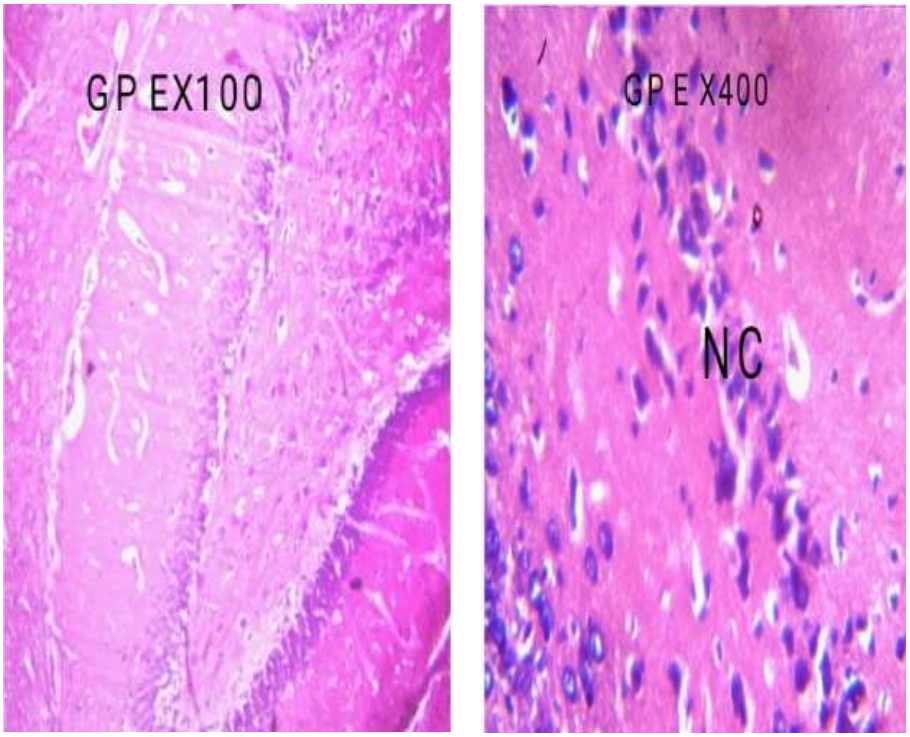

Plate 5:Photomicrograph (X100/X400(H/E) of group E section of the hypothalamus exposed to Aluminium chloride and subsequently treated with 100mg/kg of Vitamin E (Tocopherol) shows moderate healing with active neuronal cell (NC).

3.2 Oxidative Stress Markers

$AlCl_3$ elevated MDA and depleted SOD, CAT and GPx activities. MOLE 400 mg/kg significantly reversed these changes, comparable to Vitamin E (Table 3).

3.3 Delayed-Type Hypersensitivity Response

$AlCl_3$ suppressed DTH response (reduced paw edema). MOLE, particularly at the higher dose, significantly

enhanced the response, indicating restored CMI.

3.4 Histopathological and Immunohistochemical Findings

Control: Normal neuronal architecture with active granular cells.

$AlCl_3$: Moderate to severe vacuolation, neuronal loss, pyknosis, and inflammation.

MOLE-treated (esp. 400 mg/kg): Dose-dependent preservation of neurons, reduced vacuolation, and restoration of normal cytoarchitecture (comparable to Vitamin E).

IHC: Increased TNF-α and GFAP expression in $AlCl_3$ group; marked reduction in MOLE-treated groups.

3.5 Gene Expression

qRT-PCR results corroborated ELISA data, showing restored IL-2 and IFN-γ mRNA and reduced TNF-α mRNA in MOLE-treated groups.

4.0 DISCUSSION

This study demonstrates that MOLE effectively mitigates $AlCl_3$-induced hypothalamic toxicity through multifaceted mechanisms involving antioxidant activity, immune restoration and anti-inflammatory effects. The observed elevation in MDA and depletion of antioxidant enzymes in the $AlCl_3$ group align with established mechanisms of aluminum neurotoxicity (Valko et al., 2007). Phytochemicals in MOLE (quercetin, kaempferol, chlorogenic acid) act as potent ROS scavengers and metal chelators, explaining the restoration of redox balance.

Immunomodulation was evidenced by recovery of $CD4^+/CD8^+$ populations, Th1 cytokines, and DTH response. Suppression of TNF-α and GFAP indicates reduced neuroinflammation and astrogliosis. These findings are consistent with the known immunomodulatory properties of M. oleifera (Gopalakrishnan et al., 2023).

Histological and molecular data provide strong correlative evidence of neuroprotection. The 400 mg/kg dose consistently showed superior efficacy, comparable to the standard antioxidant Vitamin E.

4.1Limitations of the study

These includes the use of only male rats and a single toxicity model. Future studies should explore molecular pathways (e.g., Nrf2, NF-κB), female animals and translational potential.

5.0 Conclusion

Ethanolic leaf-extract of Moringa oleifera provides significant protection against $AlCl_3$-induced hypothalamic toxicity in Wistar rats by attenuating oxidative stress, restoring cell-mediated immunity, suppressing inflammation and preserving neuronal integrity. These results highlight MOLE as a promising natural therapeutic agent for mitigating heavy metal-induced neurotoxicity and warrant further preclinical and clinical investigations.

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Conflict of Interest

The authors guarantee responsibility for all the data published in this manuscript. The authors confirm the absence of a conflict of interest and the absence of their financial interest in conducting this research and articulating this manuscript. This manuscript is extracted and written from an original research work and has never been published, neither is it under consideration for publication elsewhere.

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