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Paper

Moringa oleifera leaves ethanolic extract counteracts cortical neurodegeneration induced by aluminum chloride in rats

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Background: Aluminum, a well-recognized neurotoxin, is implicated in various neurodegenerative disorders. *Moringa oleifera* (*M. oleifera*), known as a miracle tree, is utilized as a functional food and nutritional supplement. This study investigates the potential preventive effects of *M. oleifera* extract on aluminum chloride (AlCl3)-induced cortical neurodegeneration in rats.
Materials and methods: Therefore, 24 adult male Wistar rats were randomly divided into four distinct groups: negative control, *M. oleifera* extract (MOE), AlCl3, and AlCl3 + MOE. Treatments were administered orally for 28 consecutive days. Cognitive performance, brain oxidative/nitrosative stress, neuroinflammation, apoptotic-cell death, and associated histopathological alterations were assessed.
Results: Our results showed that MOE improved spatial learning and memory, enhanced antioxidant superoxide dismutase enzyme activity, antagonized nitrosative stress, reduced inflammatory cytokines (tumor necrosis factor-alpha and interleukin-6), decreased caspase-3, increased Bcl-2, and facilitated repair of cortical and hippocampal structures.
Conclusions: We concluded that MOE exhibits protective effects against cortical neurodegeneration, making it a promising supplement to counteract aluminum-induced neurotoxic effects.

Key words: aluminum; hippocampus; Moringa oleifera; neurodegeneration; neurotoxicity; The Morris water maze.

Introduction

Dementia is a cognitive dysfunction that disrupts day-to-day activities and social functions. The underlying etiologies of dementia are numerous including, but not limited to, primary neurologic, neuropsychiatric as well as associations with medical disorders. Alzheimer's disease (AD) is the most prevailing type of dementia and represents a significant healthcare burden, associated with considerable disability and mortality worldwide.[1–6] Recent evidence suggests a link between the development of neurodegenerative disorders and exposure to environmental toxins.[7]

Aluminum is a widely abundant element in the Earth's crust. In the present century it is often referred to as the "aluminum age" due to its extensive use in several daily life activities. For instance, it is used as a vaccine adjuvant, in antacids, cooking wares, cosmetics, food additives, and skincare products. However, it can be found as a contaminant in many food products, including infant formula, juice, milk products, seafood, tea, and wine.

In addition, environmental sources of aluminum exposure include drinking water resulting from water treatment processes, as well as natural occurrences through the weathering of rocks and soils, or pollution-induced acid rains. Aluminum possesses excellent physical and chemical properties; thus, aluminum is extensively used in a wide range of industrial applications. Consequently, the abundance of aluminum, coupled with its extensive human use, puts individuals at a significant risk of aluminum toxicity. Aluminum is a well-recognized neurotoxin that can disrupt the blood–brain barrier and accumulate in high concentrations in brain tissues and it has been implicated in various neurodegenerative disorders. The association between the development of AD and low aluminum doses remains a matter of debate. AD has a multifactorial etiology and extremely variable presentation.[8–12]

Aluminum exposure has been shown to cause neuronal damage that mimics the clinical manifestations and pathological events that are observed in AD. The initial and dominant finding in the pathogenesis of AD is amyloid-β protein deposition in the brain, other contributing factors include dysregulated neurotransmitters, metal deposition, neuronal oxidative stress, neuroinflammation, mitochondrial dysfunction, and cholinergic neurons degeneration.[13–17]

Current treatment approaches for neurodegenerative disorders have limitations and often result in unsatisfactory clinical outcomes.[18,19] Natural phytochemicals, such as *M. oleifera* (*M.*

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oleifera), which possess neuroprotective, anti-amyloidogenic, antioxidant, and anti-inflammatory properties, have emerged as potential safe preventive options for AD.[20] *M. oleifera*, also known as a miracle tree or tree of life, is extensively used as a functional food and nutritional supplement all over the world.[21–23] Studies using animal model of AD induced by colchicine or ethylcholine aziridinium (AF64A) have demonstrated the nootropic effects of *M. oleifera*. These effects include the restoration of oxidant/antioxidant balance, increased acetylcholine concentration, and improvement of memory and cognitive impairment.[24,25] Additionally, in a rat model of aluminum-induced temporal cortical degeneration, *M. oleifera* extract administration mitigated aluminum-induced neuro-histopathological alterations in the temporal cortex.[26]

Based on these findings, we hypothesized that *M. oleifera* plant extract could exhibit neuroprotective potential in a rat model of cortical neurodegeneration induced by AlCl3. Consequently, this study was conducted to explore the possible preventive effects of MOE on AlCl3-induced rat cortical neurodegeneration. We examined its effect on cognitive performance, brain oxidative/nitrosative stress, neuroinflammation, apoptotic cell death, and the associated histopathological alterations in the brain.

Materials and methods

Preparation of *M. Oleifera* leaves ethanolic extract (MOE)

The *M. oleifera* plant was extracted under the supervision of Prof. Said Abd-Alhalim Abd-Alatty Saleh, Chairman of the Egyptian Scientific Society for Moringa, National Research Center, Dokki, Cairo, Egypt. *M. oleifera* plant leaves were extracted following the method proposed by Abdel-Daim et al.[27] The leaves were gathered, washed with distilled water, and air-dried in the shade at room temperature. Subsequently, the thoroughly dried leaves were finely powdered using a high-speed milling machine. One kg of the resulting powder was subjected to extraction in 1,000 mL of absolute ethanol for 48 h. The resulting extract underwent double filtration through a 2-μm pore size filter paper. The soaking and filtration process was repeated thrice. The resulting extract was stored in an airtight brown bottle in a refrigerator at 4 °C until its use. To ensure an alcohol-free dry substance of *Moringa* product, complete evaporation and volatilization of alcohol was applied. MOE was weighed and dissolved in distilled water to achieve a concentration of 300 mg of extract per kilogram of body weight.

Animals

Adult male Wister rats (180–230 g) were purchased from the Experimental Animal Center of Benha, Faculty of Veterinary Medicine, Benha, Egypt. The animals underwent a one-week acclimatization period before the initiation of any experimental procedures. Throughout the study, all rats had access to standard laboratory chow and water ad libitum, maintaining them under standard laboratory conditions. The study proposal received approval from the Research Ethics Committee at the Faculty of Medicine, Benha University (REC-FOMBU) in Benha, Egypt, under the approval number: Rc.36.5.2023. All procedures related to the care and handling of animals were conducted in strict adherence to the Animal Care Guidelines established by the National Institutes of Health (NIH).

Animal grouping

In the current experimental protocol, 24 adult male Wistar rats were weighed and randomly assigned to four experimental groups ($n = 6$ rats per group):

Group I (Negative control): rats left without intervention.

Group II (MOE): rats were supplemented with 300 mg/kg body weight of MOE.

Group III (AlCl3): rats were treated with 100 mg/kg body weight of AlCl3 to induce cortical neurodegeneration.

Group IV (AlCl3 + MOE): rats were administered AlCl3 concurrently with MOE in the same manner as in group III and group II, respectively.

All treatments were orally administered once daily for consecutive 28 days. The selected regimen for AlCl3 administration was previously employed to induced cortical neurodegenerative changes in rodent models, as reported by Shunan et al., Nafea et al., and Anadozie et al.[28–30] This regimen of AlCl3 was linked to neurodegenerative features and structural aberration of brain tissues, resulting in cognitive impairment in experimental animals. Meanwhile, the selected dose of MOE exhibited neuroprotective potential against AlCl3-mediated neurotoxicity in experimental animals.[26]

Recording of rats' body weights: On day 0 and day 28 of the experiment

Calculation of brain coefficients in rats. Brain coefficient is the ratio of brain weight to body weight, expressed in g/kg.

Assessment of cognitive performance of the rats

The Morris water maze (MWM) test was used to evaluate the spatial navigation skill and memory in rats. Morris first described the water maze in 1984. The MWM test is one of the most frequently used behavioral tasks for assessing hippocampal spatial memory abnormalities.[31]

Procedures[31–33]

The animals underwent training to navigate a water maze, which comprised a circular pool measuring 180 cm in diameter and 60 cm in height. The pool was filled with water to a depth of 40 cm. The submersed platform is a cube (10 × 10 × 10 cm). The water was maintained at 28 ± 1 °C and colored black with a non-toxic food dye to conceal the platform. The pool was divided equally into 4 quadrants by 4 starting points that were equally marked on its edge as follows: North (N), South (S), East (E) and West (W). The submersed platform was consistently positioned in the middle of the SW quadrant. The rats were trained to find the submersed platform using visual cues (colored flags) placed around the room, which remained constant throughout the experiment to assist the rats in locating the hidden platform. On day 20 following drug administration, the training session commenced, consisting of 4 trials with a 10-min interval. Different starting points were used in each trial. The time it took by each rat to reach the platform was documented the initial acquisition latency (IAL), with a maximum allowed duration was set at 120 s. *This represented the Maze acquisition phase (training)*. On days 21 and 28, each rat was randomly released from any of the starting points, facing the pool wall, and tested for the retention of the previously learnt task. The latency to reach the hidden platform on both days (21 and 28) was recorded and termed as first and second retention latency (1st RL and 2nd RL), respectively. *This represented the Maze retention phase (testing for retention of the learned task)*.

Brain tissue preparation and processing

On day 29, all the animals were sacrificed. The brains were dissected, flushedd with a solution of phosphate-buffered saline mixed with 0.16 mg/mL heparin in order to eliminate any red blood cells and blood clots, then dried, and weighed. Subsequently, each brain was sagittally divided into two portions. The first portion was isolated for the processing of brain homogenates for further biochemical investigations, while the second portion was used for the histopathological and immunohistochemical examinations. The brain tissues were to obtain a homogenate with a concentration of 10% (w/v). This was achieved by homogenizing the tissues in a cold solution consisting of 50 mM Tris-HCl (pH 7.4) and 300 m*M* sucrose. The homogenates were then centrifuged at 1,000 × g for 10 min at 4 °C, and the resultant supernatants were separated for biochemical analyses.

Biochemical analyses

Determination of brain superoxide dismutase (SOD) enzyme activity and nitric oxide (NO) concentration

Both brain SOD activity and NO concentration were measured colorimetrically using commercially available assay kits (Biodiagnostic Company, Dokki, Giza, Egypt). The SOD concentrations in the supernatants of brain homogenates were assessed according to Nishikimi et al.,[34] while NO concentrations were quantified colorimetrically by assessing the nitrite in the supernatants of brain homogenates. By adding Griess reagent, nitrite is transformed into a deep purple azo product. The absorbance of this product was measured at a wavelength of 540 nm.[35]

Measurement of the proinflammatory cytokines and proapoptotic markers

Brain proinflammatory cytokines, namely tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)], along with pro apoptotic markers proapoptotic markers [Cysteinyl aspartate specific proteinase-3 (caspase-3) and B-cell lymphoma 2 (Bcl-2)] were assessed using commercially available enzyme-linked immunoassay kits (RayBiotech, USA).

Histological analyses

The second portion of each brain was fixed in 10% formalin buffer for 24 h. Following fixation, the cerebral hemisphere underwent dehydration, clearing with xylene, embedding in paraffin blocks, and processing for the preparation of serial coronal sections with a thickness of 5 μm. These sections were subjected to the followings:

1. Hematoxylin and eosin (H&E) staining to reveal the basic histological structure of the brain.[36]
2. Immunohistochemical staining for caspase-3 to detect apoptotic cell death in the brain tissues.[37] Paraffin sections (4 μm thickness) were incubated with a rabbit monoclonal caspase-3 antibody using the avidin biotin peroxidase method.

Histomorphometry assessment

The percentage of caspase-3 immunostaining in brain tissues was quantified by measuring the area. This analysis was performed in ten distinct fields on every slide for each rat, using magnifications of ×100 and ×400. Image acquisition was accomplished with the Leica ICC50W light electric microscope at the Image Analysis Unit of the Pathology Department, Faculty of Medicine, Benha University, Benha, Egypt. Histomorphometry evaluation utilized the ImageJ analyzer computer system developed by Wayne Rasband at NIH, Bethesda, Maryland, United States.

Statistics

Numerical variables were expressed as the mean ± standard deviation (SD). The normality of the numerical variables was tested using Shapiro-Wilk test, while the equality of variances was tested by Bartlett's test. Ordinary one-way analysis of variance (ANOVA) or Welch's ANOVA test was employed based on the equality of variance. All statistical tests were two-tailed, and the significance threshold was set at 5%. All statistical analyses were conducted using Graphpad Prism, Version 8.0 software (GraphPad Software, San Diego, CA, United States).

Results

Death rates

No deaths were recorded in any group of the study.

MOE increased body weight but did not alter brain coefficients of AlCl3-treated rats

AlCl3 treatment induced a statistically significant body weight loss compared with the control and MOE groups (198.2 ± 6.9 vs 244.2 ± 29.4, $P = 0.008$ and 265.8 ± 27.2 g, $P < 0.001$, respectively). While the final body weight significantly decreased following AlCl3 treatment compared with the initial body weight (198.2 ± 6.9 vs 202.5 ± 8.8 g, $P = 0.012$). In contrast, simultaneous MOE administration with AlCl3 statistically significantly increased the final body weight compared with AlCl3 treatment (241.7 ± 15.1 vs 198.2 ± 6.9 g, $P = 0.012$). Concurrent MOE administration with AlCl3 caused a statistically significant increase in the final body weight compared with the initial body weight in the rats (241.7 ± 15.1 vs 200.3 ± 6.8 g, $P = 0.002$), (Fig. 1a). The brain coefficients of rats were similarly non-significant in all groups of the study ($P > 0.05$), (Fig. 1b). The brain coefficients of rats were similarly non-significant in all groups of the study ($P > 0.05$), (Fig. 1b).

MOE improved memory and enhanced learning skills in spatial navigation task of AlCl3-treated rats

AlCl3 treatment demonstrated a statistically significant prolongation in the initial acquisition latency to reach the platform in the pretrained rats on day 20 compared with the control and MOE groups (113 ± 5.7 vs 53.7 ± 4.1 and 42.7 ± 4.3 s, respectively, $P < 0.001$ each). Concurrent MOE administration with AlCl3 statistically significantly decreased the initial acquisition latency compared with AlCl3-treated rats (63 ± 6.4 vs 113 ± 5.7 s, $P < 0.001$). AlCl3 treatment demonstrated a statistically significant prolongation in both 1st RL (day 21) and 2nd RL (day 28) latencies to reach the hidden platform compared with the control and MOE groups [(85.3 ± 7.2 vs 18.3 ± 2.2 and 13.8 ± 2.7 s, respectively, $P < 0.001$ each) and (71.5 ± 4 vs 11.7 ± 1.9 and 10.7 ± 18 s, respectively, $P < 0.001$ each)]. Simultaneous MOE administration with AlCl3 statistically significantly decreased both 1st RL and 2nd RL latencies compared with AlCl3-treated rats [(24.7 ± 3.9 vs 85.3 ± 7.2 s) and (20 ± 3.2 vs 71.5 ± 4 s, respectively, $P < 0.001$ each)]), (Fig. 2).

MOE antagonized oxidative/nitrosative stress and neuroinflammation in the brain tissues of AlCl3-treated rats

AlCl3 treatment caused a statistically significant increase in NO concentration and a statistically significant drop in the antioxidant SOD activity in the brain homogenates compared

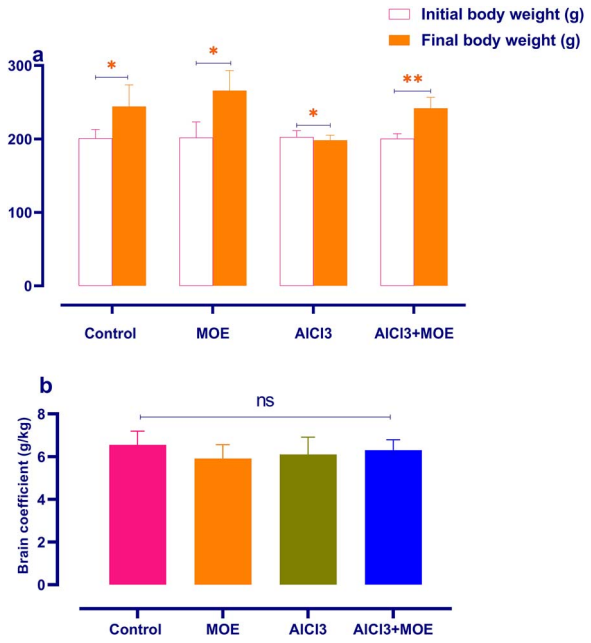

Fig. 1. Effects of MOE supplement on animals' body weight (panel a) and brain coefficient (panel b). One-way ANOVA test followed by post hoc Tukey's multiple comparisons test, at a *P*-value <0.05, based on the equality of variances. **P* < 0.05, ***P* < 0.01. Data are mean ± standard deviation, *n* = 6. Abbreviation: ns, nonsignificant; MOE, *Moringa oleifera* leaves ethanolic extract.

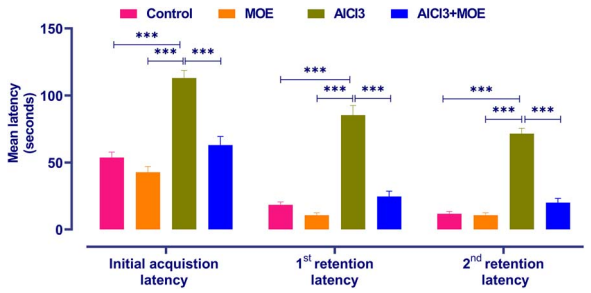

Fig. 2. Effects of MOE supplement on memory and learning skills in spatial navigation task of AlCl3-treated rats, one-way ANOVA test followed by post hoc Tukey's multiple comparisons test or Welch's ANOVA test followed by post hoc Dunnett's T3 multiple comparisons test. Data are mean ± standard deviation, *n* = 6. ****P* < 0.001. Abbreviation: MOE, *Moringa oleifera* leaves ethanolic extract.

with the control and MOE groups [(76.3 ± 7.4 vs 21.7 ± 1.1 and 22.4 ± 1.4 μmol/L, respectively, $P < 0.001$ each) and [(93.9 ± 2.8 vs 217 ± 16.5 and 222 ± 5.9 U/mg, respectively, $P < 0.001$ each)]. Additionally, concurrent MOE administration with AlCl3 statistically significantly decreased NO concentration and statistically significantly augmented SOD activity in the brain tissues compared with the AlCl3-treated group [(29.8 ± 1.1 vs 76.3 ± 7.4 μmol/L, $P < 0.001$) and [(177 ± 7.1 vs 93.9 ± 2.8 U/mg, $P < 0.001$)], (Fig. 3a and b). AlCl3 treatment caused a statistically significant increase in the brain levels of the inflammatory cytokines (IL-6 and TNF-α) compared with the control and MOE groups [(18.7 ± 0.4 vs 13.3 ± 0.6 and 13.9 ± 0.6 pg/mg, respectively, $P < 0.001$ each) and [(36 ± 1 vs 20.7 ± 0.7 and 20.8 ± 0.9 pg/mg, respectively, $P < 0.001$ each)]. Additionally, concurrent MOE administration with AlCl3 statistically significantly decreased the brain levels of the inflammatory IL-6 and TNF-α compared with the AlCl3 group [(15 ± 0.3 vs18.7 ± 0.4 μmol/L, $P < 0.001$) and [(27 ± 0.6 vs 36 ± 1 vs U/mg, $P < 0.001$)], (Fig. 3c and d).

MOE antagonized apoptotic cell-death in the brain tissues of AlCl3-treated rats

AlCl3 treatment caused a statistically significant increase in caspase-3 level and a statistically significant drop in Bcl-2 level in the brain homogenates compared with the control and MOE groups [(1.8 ± 0.14 vs 0.48 ± 0.08 and 0.52 ± 0.06 ng/mg, respectively, $P < 0.001$ each) and [(5.2 ± 0.8 vs 17 ± 0.7 and 12.9 ± 0.4 ng/mg, respectively, $P < 0.001$ each)]. Additionally, concurrent MOE administration with AlCl3 statistically significantly decreased caspase-3 level and statistically significantly increased Bcl-2 level in the brain homogenates compared with the AlCl3-treated group [(0.7 ± 0.1 vs 1.8 ± 0.14 ng/mL), $P < 0.001$) and [(14.5 ± 0.8 vs 5.2 ± 0.8 (U/mg), $P < 0.001$)], (Fig. 3e and f).

MOE alleviated brain histological derangements in AlCl3-treated rats

H&E-stained sections of the cerebral cortex from the control and MOE groups showed the normal six layers: layer I (molecular layer) was poorly cellular and characterized by a substantial abundance of fibers. Layer II (outer granular layer) primarily comprised clusters of cells characterized by prominent, rounded vesicular nuclei. Layer III (pyramidal layer), comprised sizable pyramidal cells exhibiting basophilic cytoplasm, prominent vesicular nuclei, and elongated apical dendrites. Additionally, glial cells with small dense nuclei were observed in this layer. Layer IV (inner granula layer) consisted of many small granule cells, layer V (inner pyramidal layer) consisted of large pyramidal cells, and layer VI (multiform layer) consisted of various variable-sized and shaped cells, (Fig. 4a–d). H&E-stained sections of the hippocampus from the control and MOE groups showed the normal three layers: the molecular showed mainly fibers and some non-pyramidal cells, the pyramidal layer showed pyramidal cells and the polymorphic layer showed cells called interneurons and many fibers, (Fig. 5a–d). In contrast, cerebral sections from the AlCl3-treated rats showed marked neural distortion and vascular congestion. In addition, apoptotic cells with neuronal shrinkage and chromatin condensation were evident. Degenerative vacuolization, wide intercellular spaces, separated layers, and inflammatory cellular aggregates in the cerebral cortex layers were observed (Fig. 6a–c). Hippocampal sections from the AlCl3-treated rats showed neuronal degeneration, inflammatory cellular aggregate, distortion of the pyramidal layer with apoptotic shrinkage cells with chromatin condensation, and considerable degenerative vacuolization, (Fig. 6d). While cerebral sections from the AlCl3 + MOE-treated rats displayed apparent improvement in the cerebral cortex structure compared with the AlCl3 group. Some areas of the brain tissues still had pathological changes such as vascular congestion, (Fig. 7a). The hippocampal sections from this group showed a nearly normal appearance of its layers. Some areas showed apoptotic cells and congested blood cells, (Fig. 7b).

MOE downregulated caspase-3 immunohistochemical expression in AlCl3-treated rats

Positive immunohisto-chemical staining of caspase-3 is appeared as brown cytoplasmic staining reflecting the degree of nuclear apoptosis. The control and MOE groups showed the normal negative reaction of brain tissue to the caspas-3 antibody, (Fig. 8a and b). While in the AlCl3 treated rats, many neurons displayed strong positive caspase-3 staining, (Fig. 8c). In the AlCl3+ MOE treated rats, a few neurons showed mild positive

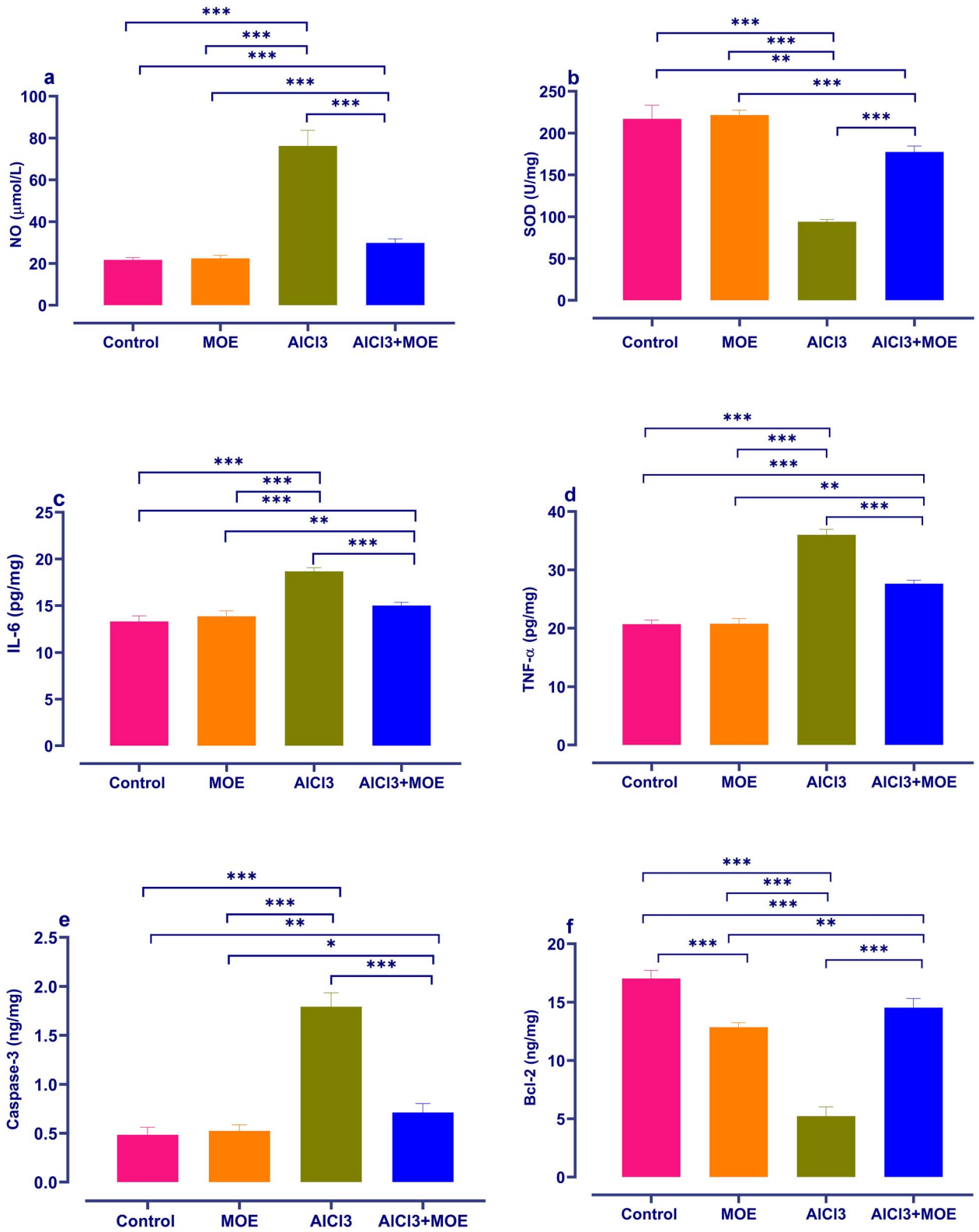

Fig. 3. Effects of MOE supplement on oxidative stress biomarkers [NO (Fig. 3a) and SOD (Fig. 3b)], inflammatory cytokine [IL-6 (Fig. 3c) and TNF-α (Fig. 3d)], and apoptosis biomarkers [caspase-3 (Fig. 3e) and Bcl-2 (Fig. 3f)] in brain homogenates of AlCl3-treated rats, one-way ANOVA test followed by post hoc Tukey's multiple comparisons test or Welch's ANOVA test followed by post hoc Dunnett's T3 multiple comparisons test. Data are mean ± standard deviation, $n = 6$. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Abbreviation: Bcl-2, B-cell lymphoma 2; Caspase-3, Cysteinyl aspartate specific proteinase-3 (IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; MOE, *Moringa oleifera* leaves ethanolic extract; NO, nitric oxide; SOD, superoxide dismutase.

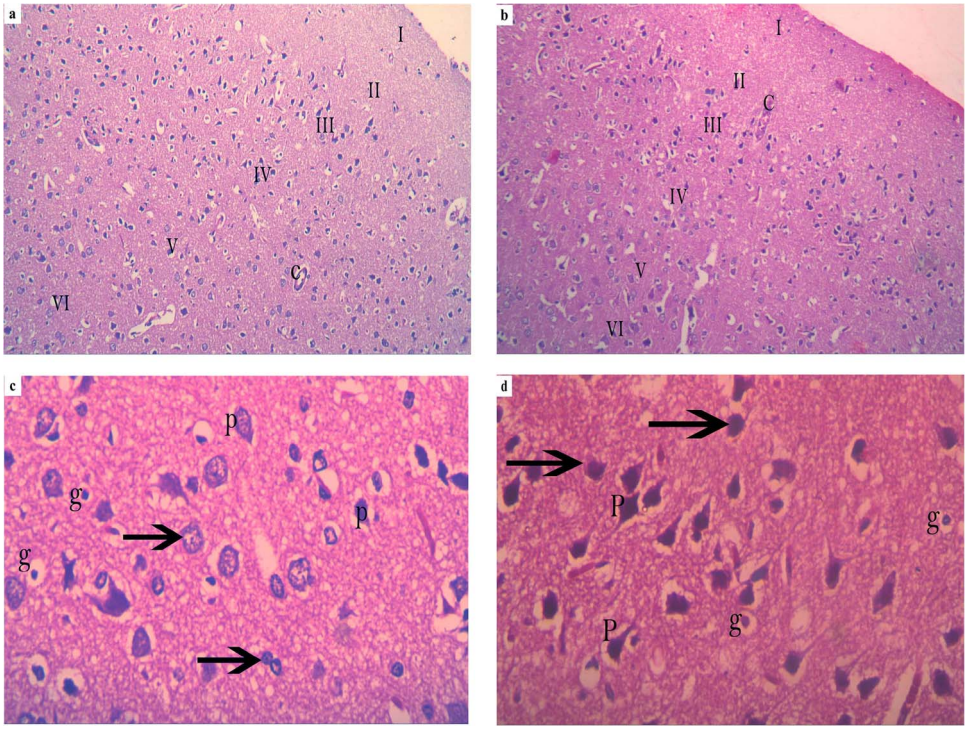

Fig. 4. a and b) The cerebral cortex of the control group and the MOE-supplemented group respectively showed normal neuronal cells in the six layers (I, II, III, IV, V, VI) of the cerebral cortex and a normal blood vessel (C). (H&E staining × 100). c and d) The cerebral cortex of the control group and the MOE-supplemented group respectively showed the deep layer containing pyramidal cells (P) with large vesicular nuclei, and long apical dendrites. Additionally, variable-sized, and shaped cells (arrows) as well as glial cells with small dens nuclei were seen (g). (H&E staining × 400).

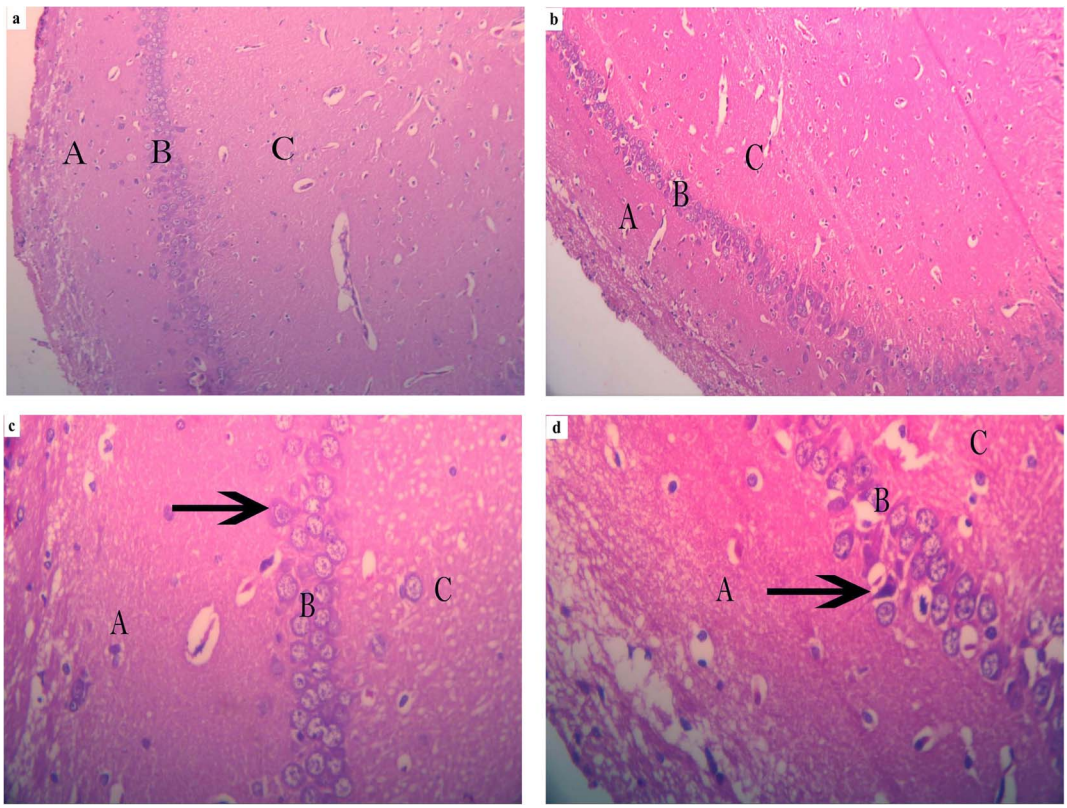

Fig. 5. a and b) The hippocampus of the control group and the MOE-supplemented group respectively showed normal structure of the hippocampus: The molecular layer (A), pyramidal layer (B) and polymorphic (C) layer. (H&E staining × 100). c and d) The hippocampus of the control group and the MOE-supplemented group respectively showed the normal structure of the hippocampus: The molecular layer (A) consisted mainly of fibers and some non-pyramidal cells, normal pyramidal layer (B) containing pyramidal cells (arrow), and polymorphic layer (C). (H&E staining × 400).

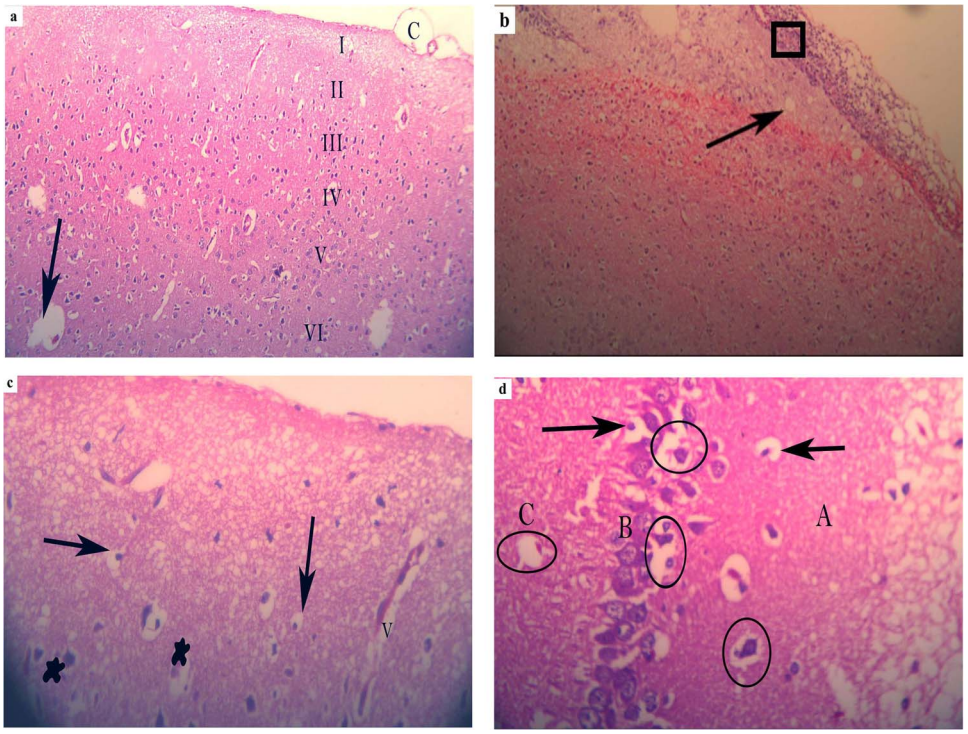

Fig. 6. a) The cerebral cortex of the AlCl3-treated group showed neuronal cells in layers I, II, III, IV, V, VI, characterized by neural disorganization and dilated congested blood vessels (C). Separation between the layers was clearly noticeable (arrow). (H&E staining × 100). b) The cerebral cortex of the AlCl3-treated group showed inflammatory cells (square) and degenerative vacuolization (arrow) within the neural cells. (H&E staining × 100). c) The cerebral cortex of the AlCl3-treated group showed apoptotic cells characterized by neuronal shrinkage, chromatin condensation (arrows), degenerative vacuolization (stars), and congested dilated blood vessels (V). (H&E staining × 400). d) The hippocampus of the AlCl3-treated group showed distorted molecular layer (A), pyramidal layer (B), and polymorphic layer (C) with numerous apoptotic shrinkage cells revealing chromatin condensation (arrows). Massive degenerative vacuolization (oval shapes) was observed (H&E staining × 400).

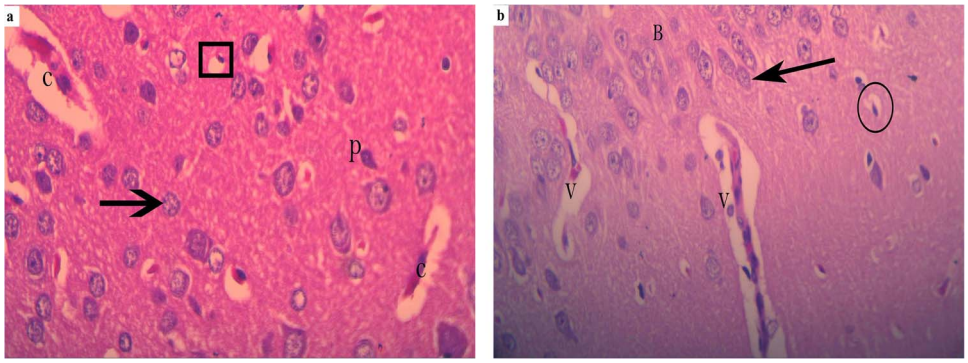

Fig. 7. a) The cerebral cortex of the MOE + AlCl3 treated group showed nearly normal cells with vesicular nuclei (arrow), normal pyramidal cells (p), and dilated congested blood vessels (C). Some cells with apoptotic changes (square) were also observed (H&E staining × 400). b) The hippocampus of the MOE + AlCl3 treated group showed nearly normal molecular layer, pyramidal layer (B) with pyramidal cells (arrow). Some cells with apoptotic changes (circle) and congested blood vessels were observed (V). (H&E × 400).

caspase-3 staining, (Fig. 8d). Histomorophometry analysis of brain homogenates showed that AlCl3 treatment caused a statistically significant increase in the mean area percent of caspase-3 immunoexpression compared with the control and MOE groups ($P < 0.001$ each). Additionally, concurrent MOE administration with AlCl3 statistically significantly decreased the mean area percent of caspase-3 immunoexpression compared with the AlCl3-treated group ($P < 0.001$), (Fig. 8e).

Discussion

Our findings elucidated the neuroprotective effects of MOE in a rat model of cortical neurodegeneration induced by AlCl3. MOE supplementation enhanced spatial learning and memory, augmented the antioxidant SOD enzyme activity, antagonized nitrosative stress, reversed both neuroinflammation and apoptotic cell-death in rats' brain, and facilitated the repair of the injured brain structures. In addition, MOE supplementation was associated with weight gain in AlCl3-treated rats.

In support of our findings, Bekhedda et al. demonstrated the anorectic effects of aluminum in female rats exposed to AlCl3 at a dose of 10 mg/kg for 15 days, as evidenced by significant body weight loss. However, the brain weight and relative brain weight of the rats did not significantly alter.[38] Consistent with our results, Zhang et al. revealed no significant changes in brain coefficients in aluminum treated rats but a significant decrease

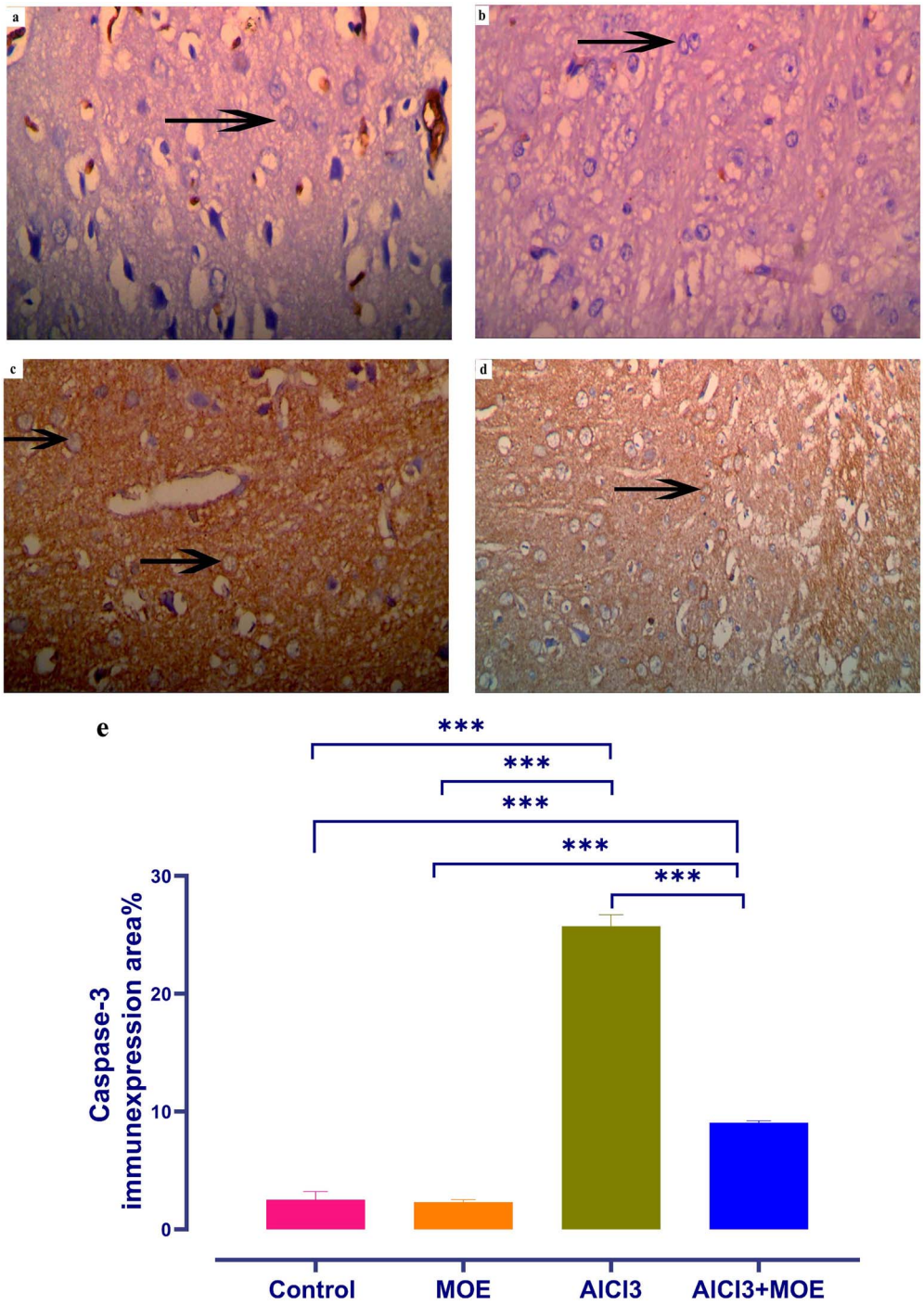

Fig. 8. a and b) The brain tissues of the control and MOE-supplemented groups respectively showed the normal negative reaction of brain tissues to the caspase-3 antibody (arrow). Caspase-3 immunostaining × 400). c) The brain tissues of the AlCl3-treated group showed most of the neurons had strong positive reaction to the caspase-3 antibody (arrows). Caspase-3 immunostaining × 400). d) The brain tissues of the AlCl3 + MOE group showed most of the neurons had mild positive reaction to the caspase-3 antibody (arrow). Caspase-3 immunostaining × 400). e) Effects of MOE supplement on caspase-3 immunoexpression area% in the brains' tissues of AlCl3-tretaed rats, one-way ANOVA test followed by post hoc Dunnett's T3 test post-hoc multiple comparisons tests. Data are mean ± standard deviation, $n = 6$. ***$P < 0.001$.

in both the hippocampal coefficient and hippocampus-to-the brain ratios, as the hippocampus is the most sensitive organ to aluminum toxicity.[39] Another study by Al-Hazm et al. observed weekly weight loss in animals exposed to AlCl3 daily for 45 days. This observation can be explained by aluminum's ability to reduce resting metabolic rate, food consumption, and absorption.[40]

In contrast, our study found that rats co-treated with MOE showed significant weight gain. *M. oleifera* plant is rich in minerals, vitamins, and other nutrients. The extract of its leaves is used to treat malnutrition and nutritional deficiencies.[41,42] In corroboration, Moringa extract significantly elicited weight gain in rats with lead poisoning and alloxan-induced diabetes.[43,44]

Animal models and cell culture studies have demonstrated that aluminum exposure leads to altered behavior and memory impairment.[10] In humans, occupational exposure to aluminum welding fumes is strongly associated with cognitive impairment,

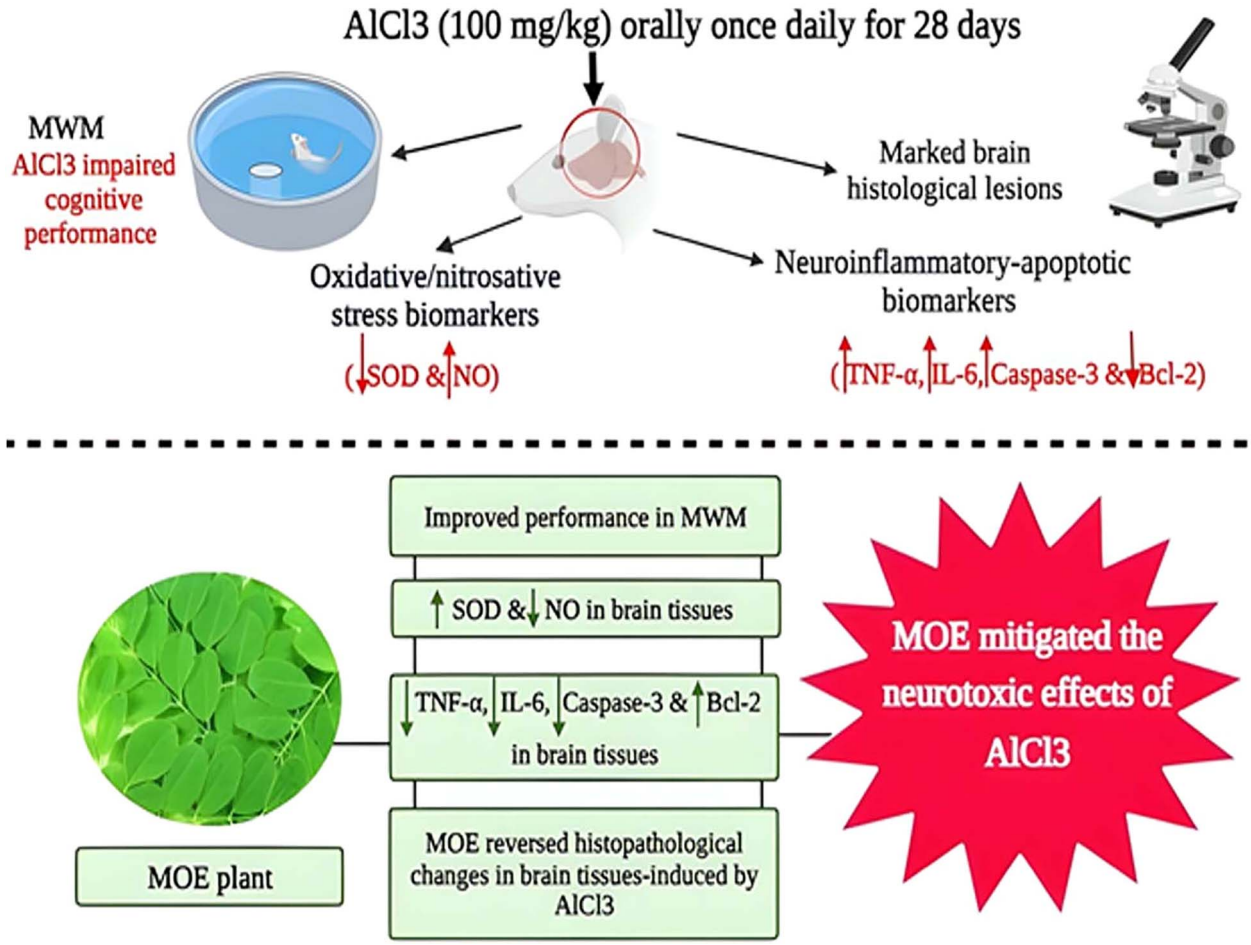

Fig. 9. Summary of the main findings of the study.

concentration difficulties, and memory problems, and abnormal changes in electroencephalogram.[45] The hippocampus and cerebral cortex are the main brain areas responsible for cognition and neurogenesis.[46] It is well-documented that aluminum exposure augments oxidative stress and neuroinflammation in the hippocampus.[47] Consistent with this, our study found that AlCl3-treated rats showed impaired memory and learning skills in the spatial navigation task of the MWM. Former studies have reported similar findings.[48–50] In addition, former studies provide strong evidence for the beneficial role of Moringa extract in mitigating memory and learning impairment induced by aging and different neurotoxins.[24,51,52]

Reactive species are key players in several pathological conditions. Excessive production of reactive species like reactive nitrogen species along with the imbalance of the body's antioxidant enzyme systems can lead to the destruction of cellular organelles, lipids, proteins, and nucleic acids. Moreover, the etiology of various diseases, including neurodegenerative disorders has been associated with oxidative damage.[53]

Although aluminum is a non-redox active element, it has a prooxidant potential in various biological systems. Aluminum accumulation leads to the generation of free radicals in the brain and interferes with free radical scavenging reactions, which could result in neurodegenerative changes comparable to those observed in AD.[54,55] It is well-recognized that aluminum can stimulate the Fenton reaction via stable aluminum-superoxide radical complexes production.[56,57] In support, a daily oral aluminum administration to rats at a dose of 100 mg/kg for 30 consecutive days caused neurotoxic effects as verified by excess NO levels and reactive oxygen species production in different brin areas such as the cerebral cortex, hippocampus, and striatum as described by Hosny and coworkers' study.[58] Furthermore, aluminum treatment resulted in a significant decline in the antioxidant SOD and catalase activity in both adult and pup rats brains.[59] NO acts as a retrograde synaptic neurotransmitter that regulates the cerebral blood flow.

NO has the ability to control protein function through post-translational modifications, specifically by engaging in S-nitrosylation of thiol amino acids. However, NO can be harmful when it reacts with the superoxide anion, forming peroxynitrite during various pathological conditions.[60,61]

Proteins, nucleic acids, lipids, biological membranes, and cellular organelles are susceptible to damage when exposed to an abundance of reactive oxygen species (ROS), which can subsequently activate cell death processes e.g. apoptosis. Apoptosis is a tightly regulated biological process that is fundamental for the development and survival of living organisms. Besides, ROS are master regulators in the progression of inflammation and tissue injury.[62,63]

Caspase-3 belongs to the cysteine proteases family that cleaves cell targets and induces cell apoptosis.[64] In contrast, B cell lymphoma 2 (Bcl-2) is an anti-apoptotic molecule that controls apoptosis, mainly by regulating the release of cytochrome *c* and other mitochondrial apoptotic events.[65,66] Prior studies clarified the proapoptotic effect of aluminum in various in vivo and in vitro models for neurotoxicity.[67–69] Supporting this, other evidence has

proposed that aluminum can induce neurotoxicity in human primary neural cells by upregulation of both the proinflammatory and pro-apoptotic genes expression.[70]

Moringa plant possesses antioxidant, antiinflammatory, and antiapoptotic potentials as it is enriched in bioactive neuroprotective phytochemical constituents, such as isothiocyanates, β-carotene, quercetin, kaempferol, ascorbic acid, flavonoids, phenolic acid, rhamnose, glycosylates, and glucomoringin. These constituents enhance the activities of brain antioxidant enzymes, improve mitochondrial functions, and maintain neurogenesis.[21,71] Accordingly, numerous prior studies have highlighted the promising effects of Moringa extract in various experimental models of xenobiotics-elicited neurotoxicity and neurodegenerative diseases.[23,26,27,72]

AlCl3 negatively alter the structure and functions of the hippocampus, including neurogenesis.[73] Accordingly, the present findings revealed that AlCl3-evoked hippocampal degenerative changes with subsequent impairment in the cognitive functions. Comparable findings reported by Aboelwafa et al. MOE treatment reversed most of the histopathological lesions elicited by AlCl3.[74] Moringa plant is well-known for its positive health effects. However, its antioxidant activity is more pronounced than that of other standard antioxidant compounds. Hence MOE ameliorating the morphological aberrations induced by AlCl3 in rats' brain mainly by its antioxidant activity.[75] Moreover, the histopathological findings strongly correlated with the biochemical results, confirming the neuroprotective potential of MOE.

Conclusion

AlCl3 significantly mediated cognitive impairment, oxidative/nitrosative stress, neuroinflammatory processes, apoptotic-cell death, and morphological brain alterations in a rat model of cortical neurodegeneration; however, MOE significantly reversed these neurotoxic effects-mediated by AlCl3, (Fig. 9). MOE could be a promising supplement for preventing the detrimental aluminum health effects, particularly its neurotoxic effects, in individuals at a high risk of aluminum exposure. Further clinical trials are warranted to validate the beneficial effects of MOE in patients with neurodegenerative disorders and ageing-related neurodegenerative disorders.

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Author contributions

R.H., A.A. and F.H. conceived, designed the experiment, and drafted the original manuscript. O.N. analyzed, interpreted the data, drafted the original draft, and wrote the final manuscript. S.M. performed histo-immuno-pathological investigation and wrote the related part. All authors contributed to drafting, and revising the article, provided the approval for the final version, and accepted the responsibility for all aspects of the work.

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