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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)*.