

Neuroprotective Effect of Nanodiamond in Alzheimer's Disease Rat Model: a Pivotal Role for Modulating NF- κ B and STAT3 Signaling

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Abstract Current therapeutic approaches of Alzheimer's disease (AD) are symptomatic and of modest efficacy, and there is no available effective cure or prevention of AD; hence, the need arise to search for neuroprotective agents to combat AD. The current study aimed at investigating the neuroprotective effect of nanodiamond (ND), adamantane-based nanoparticles, in aluminum-induced cognitive impairment in rats, an experimental model of AD. AD was induced by aluminum chloride (17 mg/kg, p.o. for 6 weeks) and confirmed by Morris water maze and Y-maze behavioral tests. Biochemical and histological analyses of the hippocampus were also performed. Aluminum-treated rats showed behavioral, biochemical, and histological changes similar to those associated with AD. ND improved learning and memory and reversed histological alterations. At the molecular levels, ND mitigated the increase of hippocampal beta-amyloid (A β ₄₂) and beta-site amyloid precursor protein cleaving enzyme-1

(BACE1) together with down-regulation of phosphorylated tau protein. It also modulated the excitatory glutamate neurotransmitter level. Furthermore, ND boosted the brain-derived neurotrophic factor (BDNF) and mitochondrial transcription factor-A (TFAM), suppressed the proinflammatory cytokine tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), and curbed oxidative stress by hampering of inducible nitric oxide synthase (iNOS). Moreover, ND augmented the hippocampal levels of phosphorylated signal transducer and activator of transcription-3 (p-STAT3) and B cell leukemia/lymphoma-2 (Bcl-2) anti-apoptotic protein while diminished nuclear factor-kappaB (NF- κ B) and caspase-3 (casp-3) expression. These findings indicate the protective effect of ND against memory deficits and AD-like pathological aberrations probably via modulating NF- κ B and STAT3 signaling, effects mediated likely by modulating N-methyl-D-aspartate (NMDA) receptors.

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Keywords Nanodiamond · Alzheimer's disease · STAT3 · Bcl2 · NF- κ B · Aluminum

Abbreviations

AD	Alzheimer's disease
A β ₄₂	Amyloid beta 1–42
AlCl ₃	Aluminum chloride
BACE1	Beta site amyloid precursor protein cleaving enzyme-1
BDNF	Brain-derived neurotrophic factor
Bcl2	B cell leukemia/lymphoma 2
Bax	Bcl-2-associated X protein
Bak	Bcl-2-antagonist/killer-1
Casp-3	Caspase-3
GSK-3	Glycogen synthase kinase-3
iNOS	Inducible nitric oxide synthase

IL-6	Interleukin-6
MEM	Memantine
MWM	Morris water maze
ND	Nanodiamond
NF- κ B	Nuclear factor kappa-B
NMDA	N-methyl-D-aspartate
p-STAT3	Phosphorylated signal transducer and activator of transcription-3
p-tau	Phosphorylated tau protein
TFAM	Mitochondrial transcription factor A
TNF- α	Tumor necrosis factor alpha
TrKB	Tyrosine receptor kinase B

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease that is characterized by progressive impairment of memory and other cognitive functions leading to complete incapacity and death [1]. The prominent morphological hallmarks of AD are extracellular deposition of amyloid- β ($A\beta$) protein as senile plaques, intraneuronal accumulation of neurofibrillary tangles formed as a result of abnormal hyperphosphorylation of cytoskeletal tau protein, and massive neuronal death [2]. The hippocampus is one of the earliest brain areas to be affected by these pathologies [3, 4]. Current therapeutic approaches of AD are symptomatic and of modest efficacy, and there is no available effective cure or prevention of AD [5].

Nanotechnology has shown promising applications in targeted drug delivery for AD, and several nanocarrier systems have been studied in recent years to increase the bioavailability and efficacy of different AD therapeutic agents [6]. Nanodiamond (ND) has emerged as a promising engineered nanomaterial for a wide range of biomedical applications due to its unique structural, chemical, and biological properties [7]; it could adsorb or conjugate with nucleic acids and drugs for gene/drug delivery [8]. ND is a member of the structural family of nanocarbons called diamondoids which are category of most promising molecular building blocks in nanomedicine [9].

Adamantane is the smallest cage structure of the diamondoids, consisting of 10 carbon atoms and 16 hydrogen atoms. Memantine is an adamantane-based neuroprotective drug that slows down progression of AD by preferentially blocking excessive stimulation of N-methyl-D-aspartate (NMDA) glutamate receptors without disrupting normal neuronal activity [10, 11]. Overstimulation of NMDA receptors plays an important role in glutamate-induced excitotoxic neuronal cell death, while normal activity of these receptors plays an essential role in the long-term potentiation which is the major physiological basis of learning and memory [12]. ND possesses an adamantane nucleus similar to that of memantine; this raises the possibility that it might have an ameliorative effect in AD via modulating NMDA receptors.

It has been demonstrated that ND undergoes systemic distribution, passes through the BBB [13], and internalized by neurons [14]. Noteworthy, ND has been shown to promote neuronal growth and differentiation of neural stem cells [15, 16]. Besides, it exhibited a protective effect against ultraviolet radiation in both cell cultures and mouse models [17]. Aluminum salts have been used extensively as food additives as well as in the treatment of drinking water, and chronic aluminum toxicity has been implicated in the development of AD [18]. Based on the present rationale, this study investigated the neuroprotective effect of ND in aluminum-induced cognitive impairment in rats, an experimental model of AD.

Materials and Methods

Drugs, Chemicals, and Kits

Nanodiamond powder was obtained from Van Moppes, Geneva, Switzerland. Memantine hydrochloride was obtained from Marcyrl Pharmaceuticals, Cairo, Egypt. Aluminum chloride was obtained from BDH, Poole, UK. Enzyme-linked immunosorbent assay (ELISA) kits were used in the present study for estimation of amyloid beta 1–42, inducible nitric oxide synthase, and nuclear factor-kappa B (EIAab Science, Wuhan, China); beta site amyloid peptide precursor cleaving enzyme 1, mitochondrial transcription factor A, and caspase-3 (CUSABIO, Wuhan, China); phosphorylated tau protein (Elabscience, Wuhan, China); tumor necrosis factor- α (IBL-America, MN, USA); interleukin-6 (RayBiotech, GA, USA); glutamate (Abnova, Taipei, Taiwan), brain-derived neurotrophic factor (Kamyia Biomedical Co, Seattle, WA, USA); phosphorylated signal transducer and activator of transcription3 (Abcam, Cambridge, UK); and B cell leukemia/lymphoma 2 protein (USCN Life Science, Wuhan, China). All other chemicals were of the highest purity and analytical grade.

Preparation of ND

Nanodiamond powder was treated with concentrated acid mixture of 68 % HNO_3 :98 % H_2SO_4 (1:9 v/v) at 100 °C for 48 h. Neutralization and washing were performed through several cycles of centrifugations (10,000 rpm for 5 min) in deionized water and ultrasonic dispersion of the pellet. The ND pellet was suspended in deionized water to form stable colloidal dispersion (after 3 months, neither sedimentation nor aggregation was observed). Size distribution was measured by dynamic light scattering at a scattering angle of 165° using a Delsa Nano C Particle Size apparatus (Beckman Coulter, Brea, CA, USA). Scattering data were collected for 70 individual measurements at a constant scattering angle and averaged for each sample. The distribution number determined for the treated ND was 15 ± 5 nm.

Animals

Adult male albino Wistar rats weighing 200–250 g were used in the present study. They were allowed acclimatization period for 2 weeks prior to testing. Animals were kept under controlled environmental conditions, room temperature (24–27 °C), constant humidity (60 ± 10 %), with alternating 12-h light and dark cycles in the animal facility of the Faculty of Pharmacy, Cairo University, Cairo, Egypt. Standard pellet diet (El-Nasr Co., AboZaabal, Egypt) and water were allowed ad libitum.

Experimental Design

Forty-nine rats were randomly divided into seven groups (seven rats/group) and were treated for 6 weeks as follows: group 1, normal group received vehicle orally (distilled water); group 2, treated orally with AlCl₃ (17 mg/kg/day) to induce AD-like pathology; group 3, injected interperitoneally (i.p.) with ND (4 mg/kg/day); group 4, treated orally with memantine (5 mg/kg/day); and groups 5, 6, and 7 were treated with AlCl₃ and either memantine, ND, or their combination, respectively. Five days before the end of the experiment, rats were trained on the Morris water maze for four consecutive days, and on the fifth day, they were subjected to the probe test as well as the Y-maze test (Fig. 1).

Behavioral Tests

Morris Water Maze Test

Spatial learning and memory were investigated using Morris water maze paradigm [19–21] in a circular pool, 150 cm in

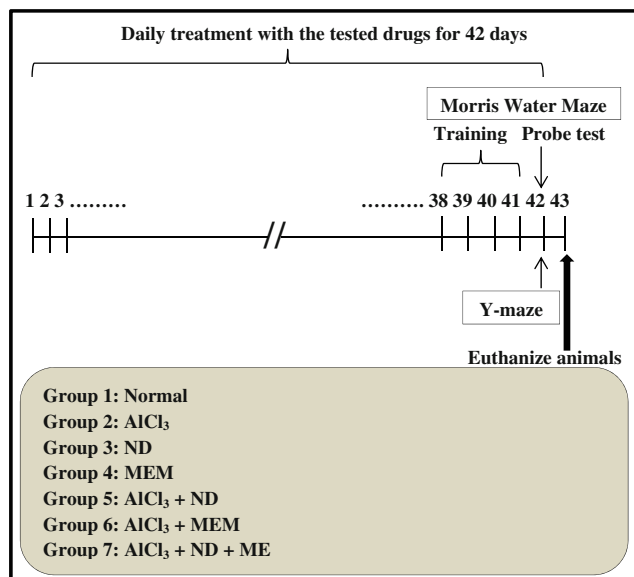


Fig. 1 Schematic representation of the experimental design. AlCl₃ aluminum chloride, ND nanodiamond, and MEM memantine

diameter and 60 cm high, filled with water (27 ± 1 °C) to a depth of 40 cm. The pool was divided into four quadrants and a movable escape platform (8-cm diameter) located in the center of a fixed quadrant. Four points equally distributed along the perimeter of the tank were served as starting locations. The apparatus was located in a room with numerous extra-maze clues, posters, and objects that remained constant throughout the experiment. Rats were allowed training sessions, each 120 s thrice a day for four consecutive days. During each acquisition trial, animals were left free to find the platform in the designated quadrant. Once the rat located the platform, it was permitted to remain on it for 10 s; while if an animal failed to reach the platform within 120 s, it was placed on the platform for 30 s. On the fifth day, a probe test (retrieval trial) was performed where the platform was removed and each rat was allowed to explore the pool for 60 s; the time spent by the animal in the target quadrant searching for the hidden platform was regarded as the index of retrieval. The passing times through the target quadrant were also recorded.

Y-Maze Spontaneous Alternation Test

The impact of different treatments on spatial recognition was further verified by assessing the spontaneous alternation behavior in Y-maze test [22, 23]. The maze was made of painted wood and consisted of three identical arms (40 cm long, 35 cm high, and 12 cm wide) positioned at equal angles and labeled A, B, and C. Rats were placed at the end of one arm and were allowed to move freely through the maze during a 5-min session. The pattern of entrance into each arm in the maze was visually monitored for every rat. Arm entry was considered to be complete when the hind paws of the rat were completely placed in the arm. Maze arms were thoroughly cleaned between tasks to remove residual odors. Alternation was defined as successive entries into the three arms on overlapping triplet set (i.e., ABC, CBA, and BCA). Total arm entries and number of alternations were recorded and used to calculate the spontaneous alternation percentage (SAP) using the following equation: SAP (%) = [(number of alternations)/(total arm entries - 2)] × 100. Same arm returns (SARs) were also recorded.

Tissue Sampling

Six weeks from the beginning of experiments and 24 h following Morris water maze probe test and Y-maze task, the rats were euthanized and the brains were quickly dissected. The hippocampi were harvested and frozen at -80 °C. The hippocampi were homogenized (10 % w/v) in phosphate buffer (pH 7.4), and the homogenates were used for the biochemical analyses. In addition, representative brains in each group were fixed in 10 % formalin for the histological examination.

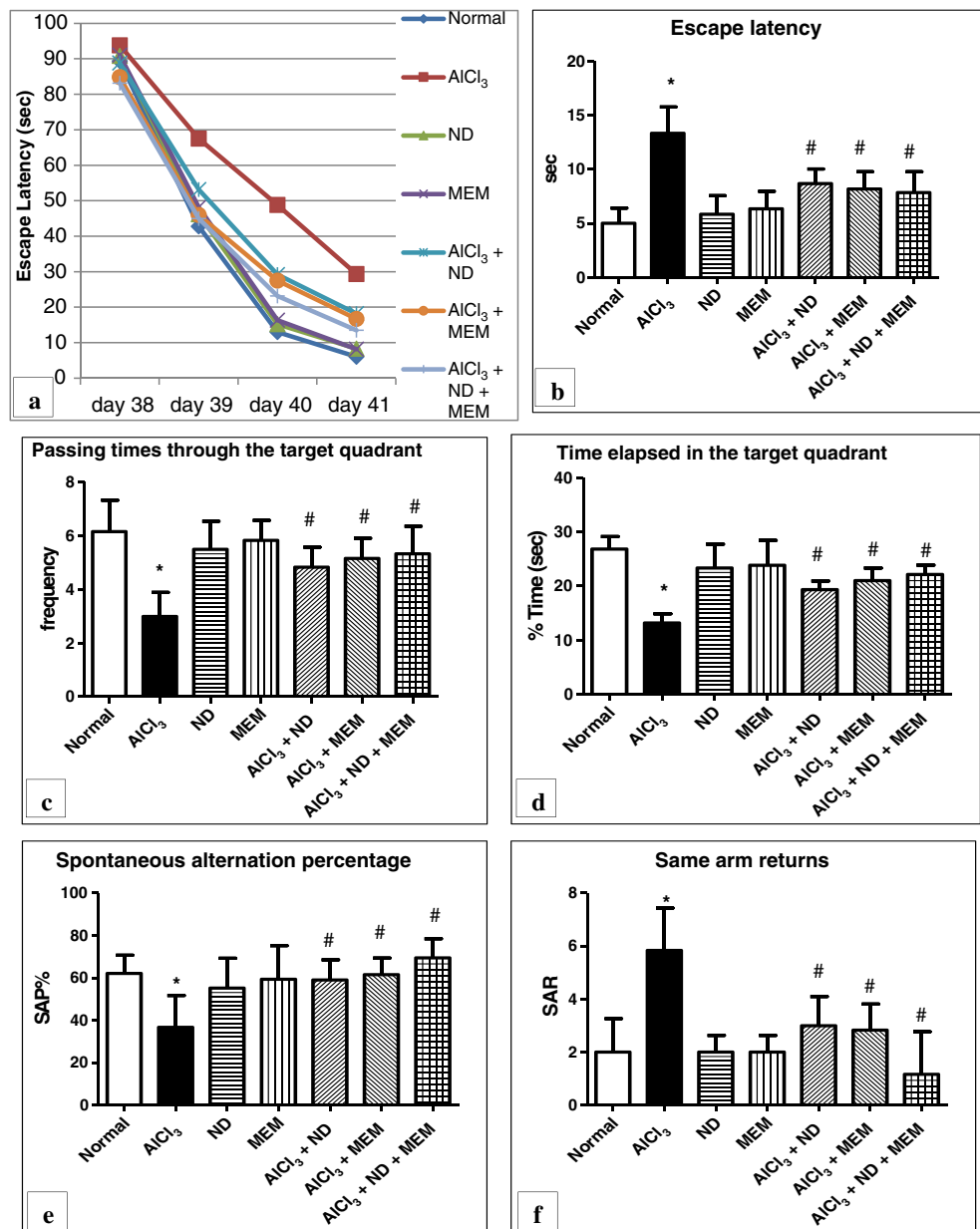
Biochemical Analysis

The hippocampal contents of beta amyloid 1–42 ($A\beta_{1-42}$), phosphorylated tau protein ($p\text{-}\tau$), beta site amyloid peptide precursor cleaving enzyme 1 (BACE1), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), brain-derived neurotrophic factor (BDNF), mitochondrial transcription factor A (TFAM), inducible nitric oxide synthase (iNOS), caspase-3, phosphorylated signal transducer and activator of transcription-3 ($p\text{-STAT3}$), B cell leukemia/lymphoma-2 (Bcl-2), and nuclear factor-kappa B (NF- κ B) were estimated using ELISA kits according to the manufacturer’s instructions.

Histopathological Examination

Whole brains of two rats, randomly selected from each group, were fixed in 10 % formalin for 24 h. The specimens were washed, dehydrated by alcohol, cleared in xylene, and embedded in paraffin at 56 °C in hot air oven for another 24 h. Sections of 3- μ m thickness were stained with hematoxylin and eosin and examined under the light microscope fitted with camera. All histopathological processing and assessment of specimens were performed by an experienced observer unaware of the identity of the sample being examined to avoid any bias.

Fig. 2 ND attenuates aluminum-induced learning and memory deficits in rats performing Morris water maze (a-d) and Y-maze (e-f) behavioral tests. In Morris water maze; **a** ND lowered the escape latency starting from the second day of the 4-days training course. The probe test (**b-d**) was carried out 24 h after the final trial with removal of the platform; **b** ND decreased the scape latency to the target quadrant, **c** ND increased the passing times through the target quadrant, **d** ND increased the time spent in the target quadrant. In Y-maze test; **e** ND increased the spontaneous alternation percentage (SAP%), **f** reduced the same arm returns (SAR). Values represent mean \pm SEM (n=7). * $p < 0.05$ compared to the normal group and # $p < 0.05$ compared to the $AlCl_3$ group. $AlCl_3$ aluminum chloride, ND nanodiamond, MEM memantine



Statistical Analysis

Comparisons between different groups were performed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer's test for multiple comparisons. Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Values were expressed as mean \pm SEM and the minimal level of significance was identified at $p < 0.05$.

Results

Behavioral Analysis

The results of the behavioral study revealed that the escape latency was increased in the aluminum-treated group as compared to control rats starting from the second day during the training course of Morris water maze test (Fig. 2a). Interestingly, ND administration afforded an improvement in reaching the platform with escape latency approaching the normal values during the third and the fourth days of training.

These actions were similar to those exerted by the reference drug memantine (MEM). Twenty-four hours after the final trial, the probe test was performed without the platform and the latency to reach the target quadrant and the time spent in the target quadrant as well as the passing times across the target quadrant were presented in Fig. 2b–d. Aluminum-treated rats spent less time in the target quadrant as compared to normal rats, whereas the treatment with ND restored these values to normal. These effects were analogous to those afforded by MEM. The same results were observed for the latency to reach the target quadrant and the passing times across the target quadrant. Aluminum significantly lowered the SAP and increased the SARs in Y-maze test as compared to the normal group, events that were reversed by ND and MEM (Fig. 2e, f).

Biochemical Analysis

The results revealed that aluminum exposure up-regulated hippocampal levels of NF- κ B by 6-fold and caspase-3 by 5.5-fold and down regulated p-STAT3 by 4.6-fold and Bcl-2 by 8.3-fold

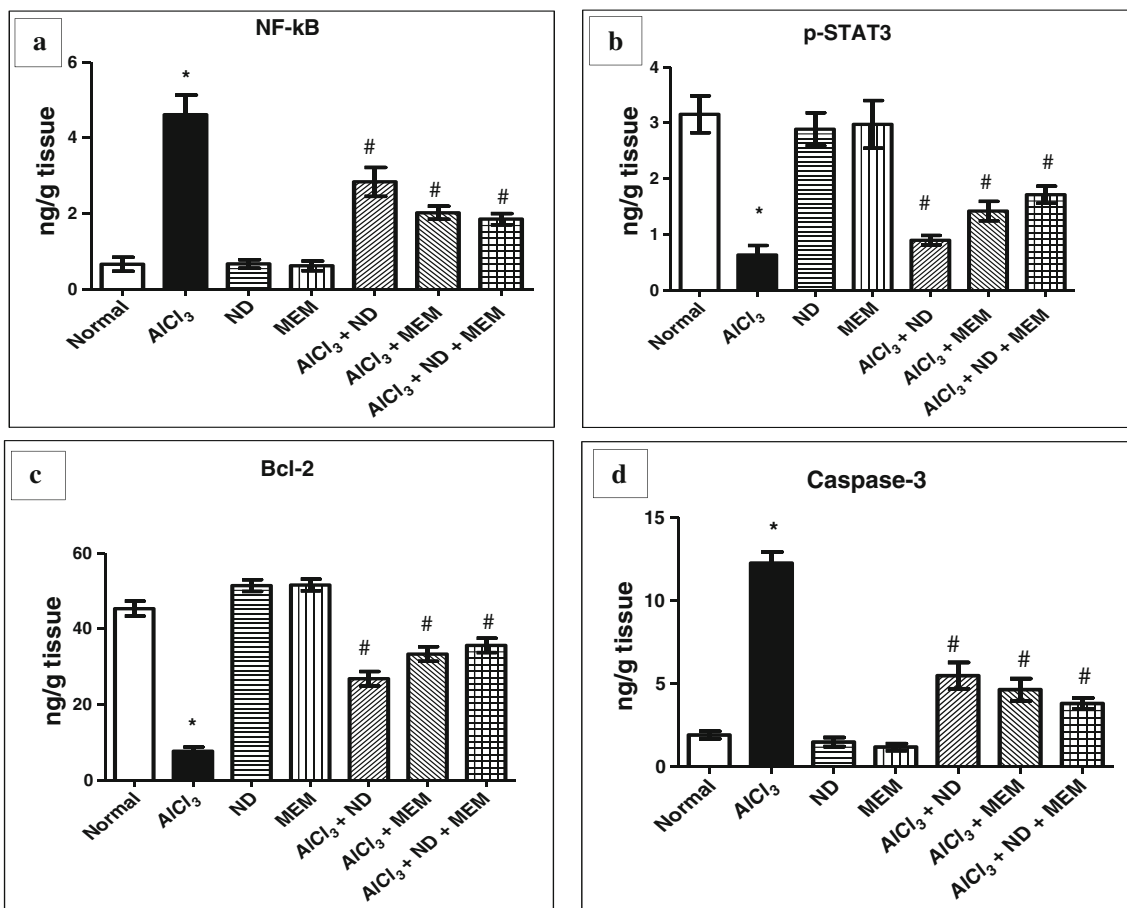


Fig. 3 ND reduces hippocampal levels of NF- κ B and caspase-3, and increases p-STAT3 and Bcl-2 in aluminum-treated rats. **a** Nuclear factor-kappa B (NF- κ B); **b** phosphorylated signal transducer and activator of transcription 3 (p-STAT3), **c** B cell leukemia/lymphoma 2

(Bcl-2), and **d** caspase-3. Values represent mean \pm SEM ($n = 7$). * $p < 0.05$ compared to the normal group and # $p < 0.05$ compared to the AlCl₃ group. AlCl₃ aluminum chloride, ND nanodiamond, and MEM memantine

compared to the normal group. However, ND reduced hippocampal levels of NF- κ B (39 %) and caspase3 (55 %) and boosted levels of p-STAT3 (42 %) and Bcl-2 (2.5-fold) as compared to aluminum chloride group, and these effects were more pronounced when it was combined with MEM. The effects of different treatments on NF- κ B, phosphorylated STAT3, Bcl-2 protein, and caspase-3 are presented in Fig. 3.

The present study also revealed that administration of aluminum increased the levels of BACE1 by 6.2-fold (Fig. 4a), A β_{42} by 4.3-fold (Fig. 4b), and the p-tau protein by 3.3-fold compared to the normal group (Fig. 4c). Interestingly, ND blunted the levels of A β_{42} (56 %), BACE1 (45 %), and p-tau (43 %) as compared to aluminum chloride group and afforded greater protection against aluminum-induced neurotoxicity when combined with MEM.

The effects of different treatments on hippocampal glutamate neurotransmitter content revealed that aluminum

administration resulted in a significant decrease in this neurotransmitter by 80 % as compared to the normal group (Fig. 5). However, ND succeeded to counteract aluminum-induced depletion of hippocampal glutamate levels (1.8-fold) as compared to the aluminum chloride group, and this improvement was more pronounced when combined with MEM.

The results also showed that aluminum administration increased the hippocampus pro-inflammatory cytokines TNF- α by 3.8-fold, (Fig. 6a), IL-6 by 4-fold (Fig. 6b), and iNOS by 3.8-fold (Fig. 6c) compared to the normal control group. ND diminished these pro-inflammatory cytokines TNF- α (39 %) and IL-6 (49 %) and reduced iNOS (52 %) as compared to the aluminum chloride group; this decrement was more pronounced when ND was combined with MEM.

On the other hand, aluminum chloride administration diminished the expression of TFAM which reached 64 % (Fig. 7a) and BDNF which reached 52 % (Fig. 7b) in the

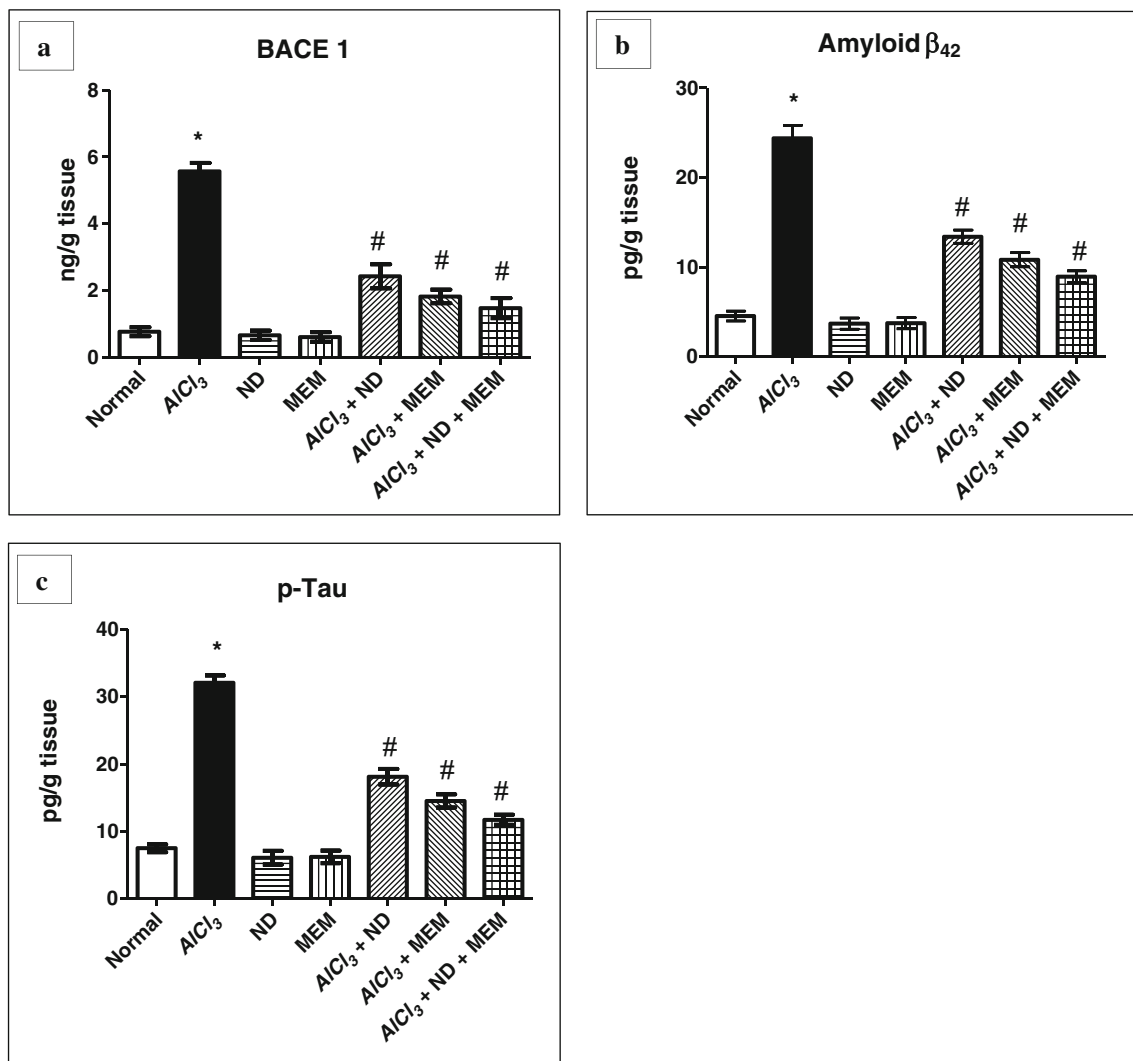


Fig. 4 ND reduces the hippocampal levels of BACE1, A β_{42} , p-tau in aluminum treated rats: **a** beta site amyloid peptide precursor cleaving enzyme 1 (BACE1); **b** amyloid beta 42 peptide (A β_{42}), **c** phosphorylated tau protein (p-tau). Values represent mean \pm SEM

(n=7). * $p < 0.05$ compared to the normal group and # $p < 0.05$ compared to the AlCl₃ group. AlCl₃ aluminum chloride, ND nanodiamond, MEM memantine

hippocampus compared to the normal control group. However, ND markedly enhanced expression of BDNF (45 %) and TFAM (54 %) as compared to the aluminum chloride group, and this enhancement was more pronounced when ND was combined with MEM.

Histological Analysis

The biochemical results of the current study were further confirmed by the histological examination of the hippocampus. The microscopic examination of the sections obtained from the normal group revealed an intact architecture of the hippocampus with normal histological appearance and distribution of the neuronal cells (Fig. 8a). On the other hand, the hippocampus of the aluminum-treated group showed degenerative changes and atrophy of the neuronal cells besides gliosis (Fig. 8b). Administration of ND or MEM to non-aluminum treated rats did not alter the normal architecture of the hippocampus (Fig. 8c, d). Moreover, ND administration to aluminum-treated rats attenuated the pathological degenerative changes in the hippocampus area (Fig. 8e). These actions were analogous to those afforded by MEM which mitigated histological alterations in the hippocampus (Fig. 8f). The same results were observed in the hippocampus of animals that received the combined treatment of ND and MEM (Fig. 8g). These observations indicate that ND mitigated the Alzheimer-like histopathological alterations in the hippocampus of aluminum-treated rats.

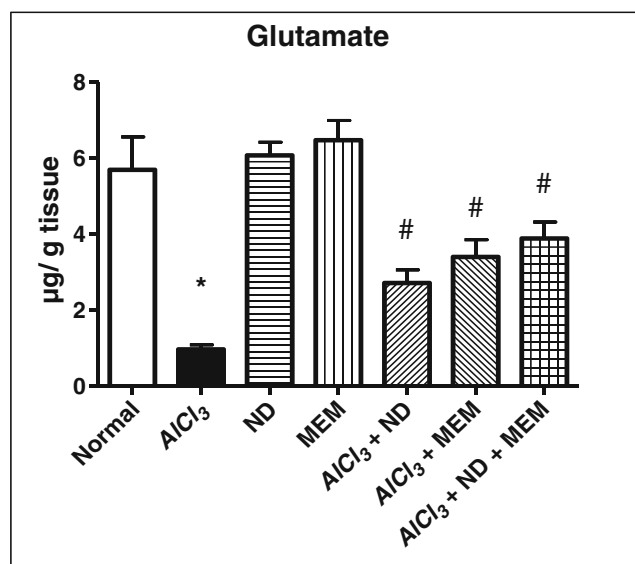


Fig. 5 ND reverses the aluminum-induced depletion of the hippocampal glutamate neurotransmitter in aluminum-treated rats. Values represent mean \pm SEM ($n=7$). * $p<0.05$ compared to the normal group and # $p<0.05$ compared to the AlCl₃ group. AlCl₃ aluminum chloride, ND nanodiamond, and MEM memantine

Discussion

The current study evaluates the neurotoxic effects of aluminum chloride administration in adult rats over a 6-week period as well as the neuroprotective effects of ND and memantine. The study highlights the impact of aluminum on memory, provides further evidence for the health hazards associated with the aluminum food additives, and describes the mechanisms by which aluminum contributes in development of AD.

This study depicts an ameliorative effect of ND on aluminum-induced learning and memory impairment in MWM and Y-maze tests which correlates to modulation of biochemical biomarkers in the hippocampus. These behavioral tasks have been ordinarily used to assess the effect of various treatments on spatial recognition learning and memory [19–23]. Previous studies indicated that high levels of aluminum in the brain can impair long-term potentiation, which is thought to be the major physiological basis of learning and memory [12, 24].

A main finding of the present work is that ND hampered NF- κ B activation and thereby alleviated the subsequent pathologic events. Up-regulation of NF- κ B in aluminum treated rats has been formerly demonstrated [25]. Activated NF- κ B translocates into the nucleus where it binds to DNA and induces expression of genes involved in inflammation including IL-8 and IL-6 [26–28]. Moreover, NF- κ B promotes BACE1 expression and impairs microglial cell-mediated phagocytosis and clearance of A β ₄₂ peptide monomers which ultimately results in aggregation into higher-order amyloid species [25, 29]. Furthermore, activated NF- κ B induces the expression of suppressor of cytokine signaling-3 (SOCS3) that acts as a negative regulator of JAK/STAT3 axis, preventing STAT3 activation [28]. Hence, NF- κ B is likely the main upstream mediator of the neuronal aberrations reported in the present study including A β deposition, diminished STAT3 phosphorylation, overexpression of pro-inflammatory cytokines, and initiation of apoptosis. Noteworthy, NF- κ B activation has been demonstrated to be mediated by glutamate NMDA ionotropic receptors and voltage-gated Ca²⁺ channels [30], and this provides a plausible evidence that ND reduced NF- κ B activation via modulating NMDA receptors.

The current study revealed the ND induced up-regulation of phosphorylated STAT3, a key component of the cellular anti-apoptotic cascade, in the hippocampus of aluminum-treated rats. Phosphorylated STAT3 dimerizes and translocates into the nucleus where it induces transcription of target genes critical for promoting neuronal survival such as Bcl-2 [31, 32]. Moreover, phosphorylation of STAT3 has been linked with suppression of the transcription factor NF- κ B and caspase-3 [33]. Down-regulation of this essential survival protein Bcl-2 in the hippocampus of aluminum-treated rats described in this study goes in line with previous findings [34] and is likely involved in the neuronal cell death induced by aluminum.

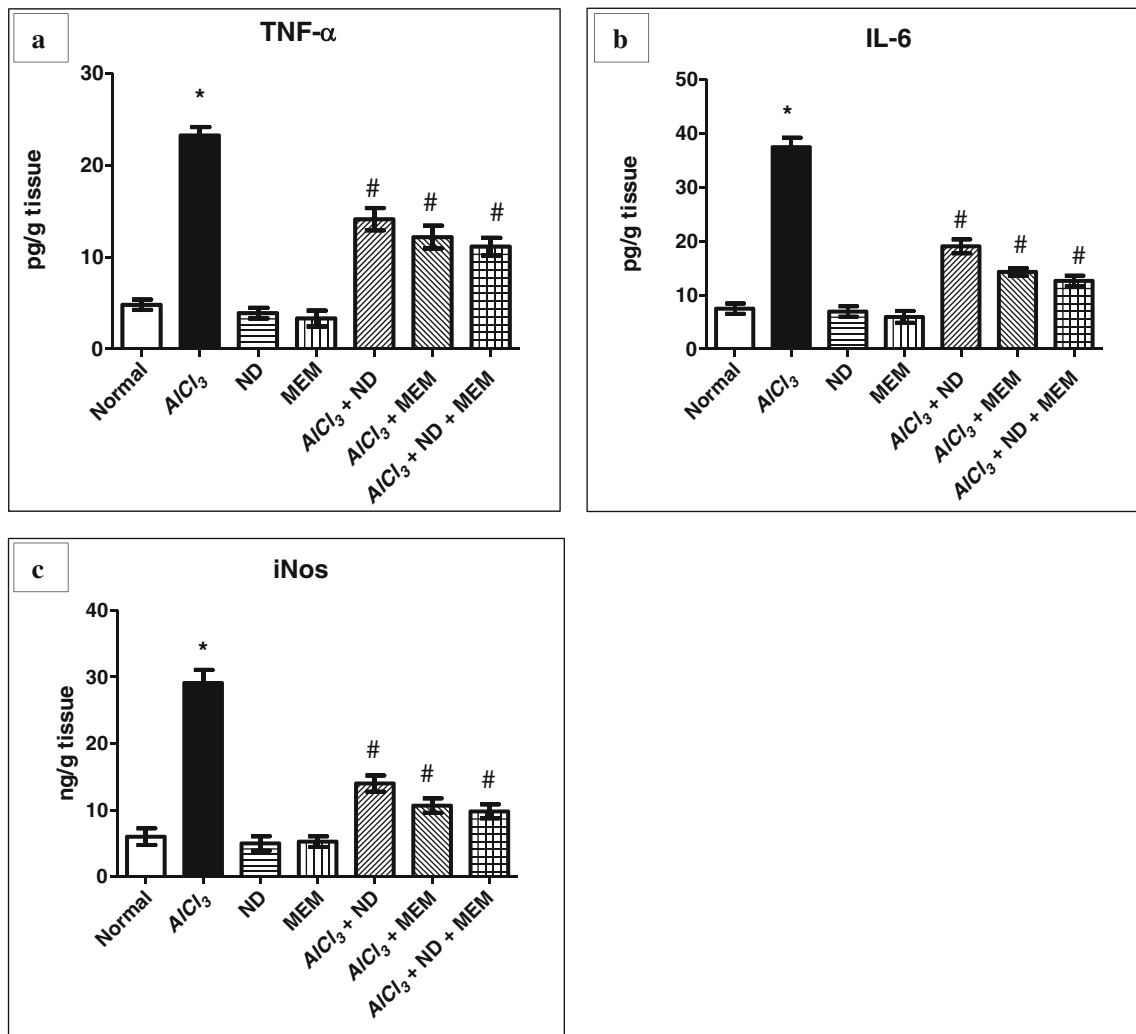


Fig. 6 ND diminishes the hippocampal levels of TNF- α , IL-6, and iNOS in aluminum-treated rats. **a** Tumor necrosis factor- α (TNF- α). **b** Interleukin-6 (IL-6). **c** Inducible nitric oxide synthase (iNOS). Values

represent mean \pm SEM ($n = 7$). * $p < 0.05$ compared to the normal group and # $p < 0.05$ compared to the AICl₃ group. AICl₃ aluminum chloride, ND nanodiamond, and MEM memantine

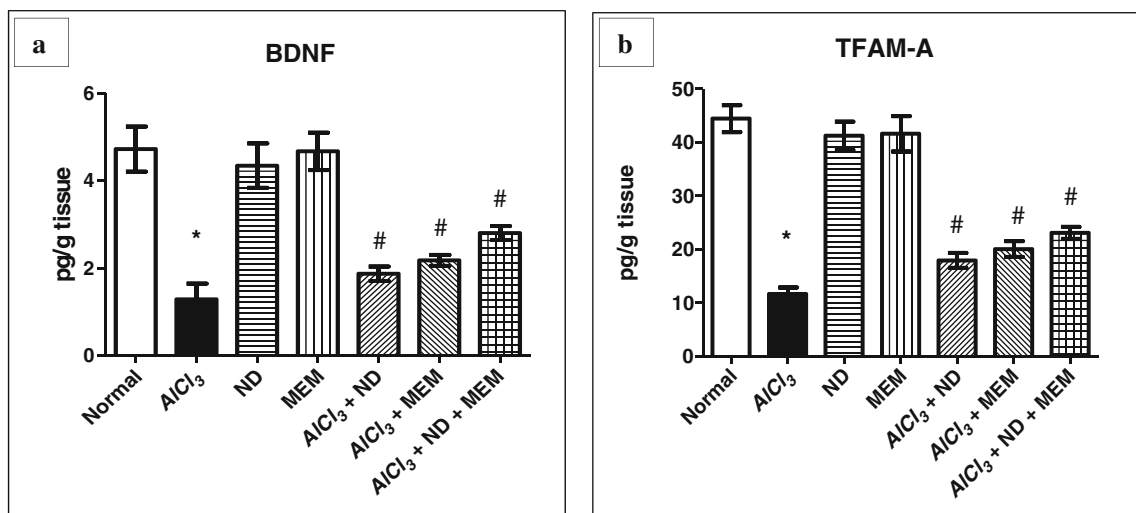


Fig. 7 ND enhances the hippocampal expression of **a** BDNF and **b** mitochondrial transcription factor A (TFAM). * $p < 0.05$ compared to the normal group and # $p < 0.05$ compared to the AICl₃ group. AICl₃ aluminum chloride, ND nanodiamond, and MEM memantine

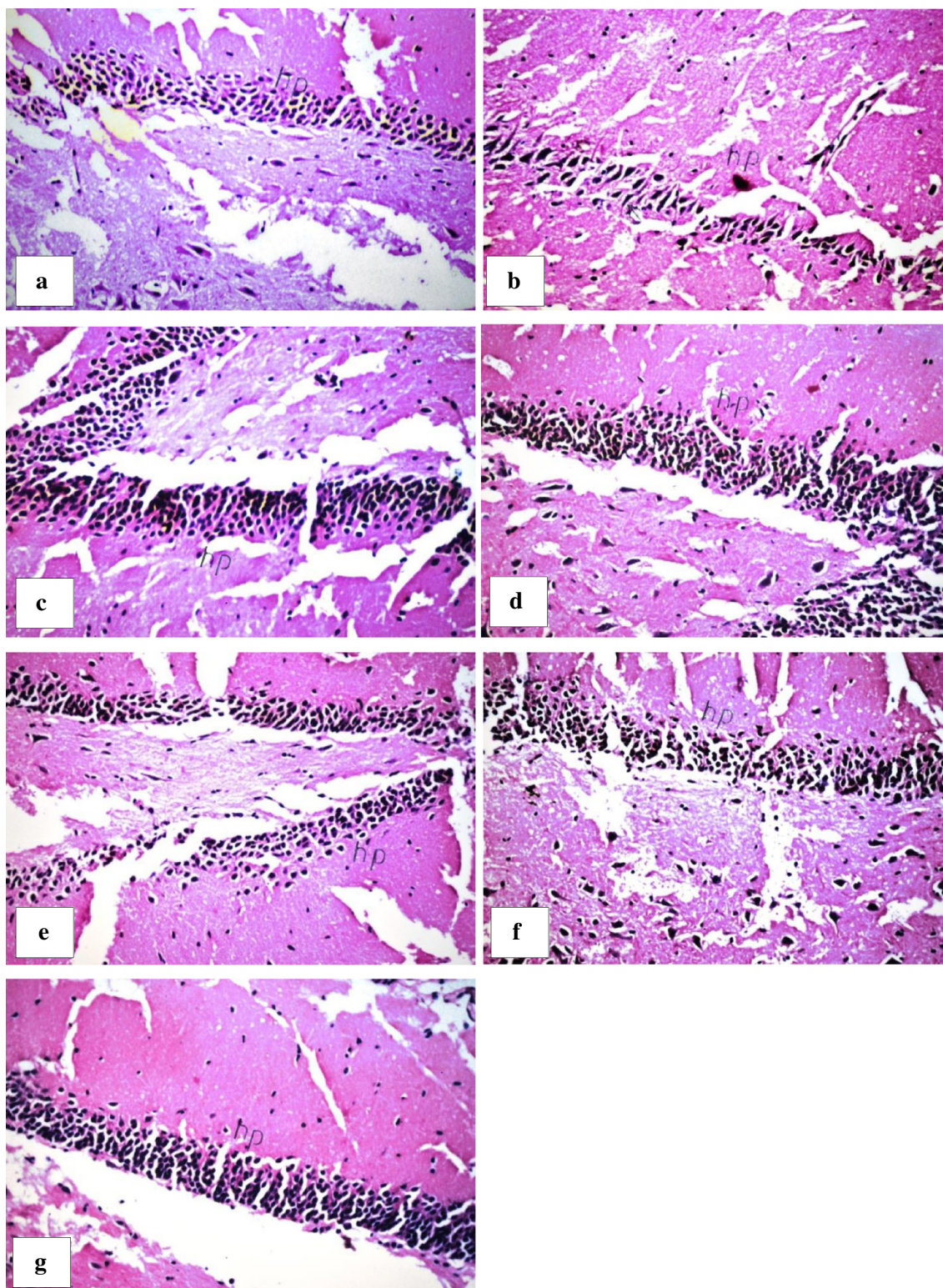


Fig. 8 ND mitigates the histopathological damage in the hippocampus of aluminum-treated rats. Representative photomicrographs (H&E $\times 400$) of sections obtained from the hippocampus harvested on the 43rd day of the study. **a** Normal group displayed intact structure of the hippocampus (hp) and normal distribution of the neuronal cells. **b** The neuronal cells in the hippocampus of aluminum-treated rats showed degeneration, atrophy, and gliosis. **c** ND group displayed normal architecture of the

hippocampus. **d** MEM group showed normal neuronal appearance in the hippocampus. **e** $AlCl_3$ + ND group revealed attenuation of the histopathological changes in the hippocampus. **f** $AlCl_3$ + MEM group displayed mitigated neuronal degeneration of the hippocampus. **g** $AlCl_3$ + ND + MEM group showed attenuated pathological changes in the hippocampus. $AlCl_3$ aluminum chloride, ND nanodiamond, and MEM memantine

Apoptosis is under heavy regulation by the two main subclasses of Bcl-2 family proteins, the anti-apoptotic members such as Bcl-2 and Bcl-xL and the pro-apoptotic members such as Bax, Bak, and BH3-domain-only proteins [35]. In response to apoptotic stimuli, BH3-domain-only proteins bind to and inactivate the anti-apoptotic Bcl-2 protein which normally inactivates the pro-apoptotic Bax/Bak proteins [36]. Activated Bax/Bak proteins oligomerize in the outer mitochondrial membrane and form pores leading to permeabilization of the mitochondrial wall with subsequent release of cytochrome c into the cytosol which triggers caspase-9 activation [37, 38] that acts on downstream targets including caspase-3 leading to apoptotic cell death [39]. Moreover, exposure to β -amyloid has been reported to be associated with activation of caspase-3 by reducing phosphorylation of STAT3, and this cascade is inhibited by activation of JAK2 [40]. Thus, aluminum-induced overexpression of caspase-3 may be attributed to blunting Bcl-2 and p-STAT3 levels. Of interest, ND administration triggered down-regulation of caspase-3 levels probably due to reinstating the Bcl2 and STAT3 signaling.

Elevated hippocampal A β levels were associated with increased expression of BACE1 in aluminum-treated rats. Indeed, A β is generated by two sequential endoproteolytic cleavages of the amyloid precursor protein by β -secretase (BACE1) and γ -secretase [41]. BACE1 cleavage is the initiating step and is a pre-requisite for A β_{42} generation, and BACE1 levels are elevated in both AD experimental models and in the AD patient brain [42–44]. Aluminum also impairs

phagocytosis of A β peptides through NF- κ B-mediated down-regulation of the sensing membrane receptor known as the triggering receptor expressed in myeloid cells 2 (TREM2) that ultimately contribute to A β_{42} peptide accumulation in the brain [45]. Downstream mechanisms of A β toxicity include phosphorylation of tau and breakdown of microtubule networks which are key underlying events for neuronal death and AD progression [46]. In the present study, augmented phosphorylation of tau protein in aluminum-induced AD rat model was elucidated. A β triggers tau phosphorylation, probably via A β -induced protease activation [47] and up-regulation of several tau-targeting kinases including glycogen synthase kinase-3 (GSK-3) [48]. Additionally, A β oligomers could be incorporated into neuronal cell membranes resulting in formation of ion channels with subsequent influx of Ca²⁺ through these amyloid channels which leads to phosphorylation of tau [49, 50]. ND reduced phosphorylated tau protein level evoked by aluminum administration. Resetting of the normal phosphorylation of tau protein is likely secondary to the suppression of A β levels.

The results described depletion of hippocampal glutamate neurotransmitter in response to aluminum exposure. Glutamate plays an essential role in the long-term potentiation which is thought to be the major physiological basis of learning and memory [12]. A β peptides trigger neurotoxicity partly via formation of ion channels into neuronal cell membranes with subsequent Ca²⁺ influx through these amyloid channels [49]. Calcium influx evokes excessive glutamate release resulting in

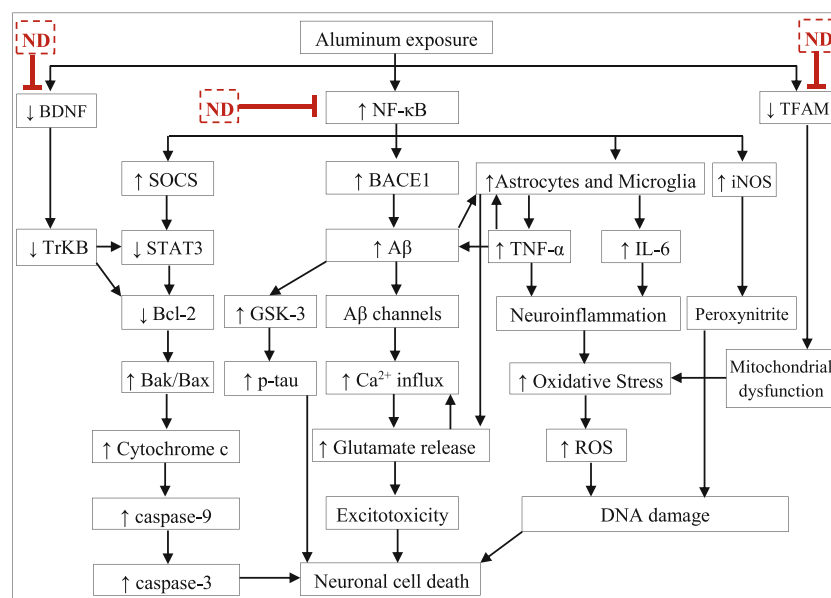


Fig. 9 Diagram depicting the alleviating actions of ND against aluminum-induced Alzheimer-like pathologic aberrations. ND attenuates NF- κ B and boosts BDNF and TFAM levels. A β_{42} amyloid beta 1–42, BACE1 beta site amyloid precursor protein cleaving enzyme-1, BDNF brain-derived neurotrophic factor, Bcl2 B cell leukemia/lymphoma 2, Bax Bcl-2-associated X protein, Bak Bcl-2-antagonist/killer-1, iNOS inducible nitric oxide synthase, GSK-3

glycogen synthase kinase-3, IL-6 interleukin-6, ND nanodiamond, NF- κ B nuclear factor kappa-B, NOS nitrogen reactive species, ROS reactive oxygen species, STAT3 signal transducer and activator of transcription 3, p-tau phosphorylated tau protein, TFAM mitochondrial transcription factor A, TNF- α tumor necrosis factor alpha, and TrKB tyrosine receptor kinase B

stimulation of cascades of events culminating in neuronal cell death [51]. Elevated TNF- α levels reported herein also could provide an additional justification where it triggers glutamate release from microglia by up-regulating glutaminase and gap junction hemichannels [52, 53]. Notably, ND reversed glutamate aberration signifying the pleiotropic actions of ND to alleviate the cognitive deficits in AD.

Inflammation, a cardinal event in AD, is triggered by aluminum administration and accumulation of the toxic A β which activates the microglia and increase the number of astrocytes in the hippocampus [54]. Generation of inflammatory cytokines including TNF- α and IL-6 in the hippocampus of aluminum-treated rats ensued in this study is probably linked to A β deposition and tau phosphorylation [55], NF- κ B activation [28], and diminished STAT3 phosphorylation [56]. Moreover, overexpression of iNOS during inflammatory response has been linked to NF- κ B activation [57]. NO elevations were triggered by iNOS promote reactions between NO and superoxide resulting in accumulation of the highly reactive oxidant peroxynitrite metabolite that leads to oxidative DNA damage and prevent protein phosphorylation, therefore disrupting signal transduction mediated by tyrosine kinases [58, 59]. In the current study, ND lowered hippocampal TNF- α and IL-6 probably via boosting STAT3 phosphorylation and halting NF- κ B which also hampered overexpression of iNOS.

BDNF affords neuroprotective actions. In particular, the hippocampus area displays the greatest abundance of BDNF which plays essential roles in neuronal proliferation and differentiation, as well as protection against variety of cellular insults [60]. BDNF promotes neuronal survival through activation of tyrosine receptor kinase B (TrkB) which induces JAK2/STAT3 signaling [61, 62]. In the present study, aluminum administration down-regulated BDNF levels in conformity with previous results [63]. Notably, ND exerted neuroprotective actions, at least partly, via elevating its hippocampal levels.

TFAM is responsible for maintenance and transcription of mitochondrial DNA and protects cells against oxidative stress [64]. It has been shown that overexpression of TFAM protects mitochondria against A β -induced oxidative damage in neurons [65], inhibits mitochondrial ROS generation, and improves mitochondrial respiratory function [66, 67]. The current results showed that while aluminum down-regulated TFAM, ND enhanced its hippocampal level. Thus, these data suggest that boosting of the hippocampal levels of TFAM, which will likely reverse the mitochondrial dysfunction, is partly implicated in ND protection against cognitive impairment (Fig. 9).

Conclusions

The study highlights, for the first time, the beneficial effects of ND against aluminum-induced AD-like pathological

perturbations. ND reversed the histopathological changes and mitigated the abnormal BACE1, A β , and p-tau overexpression. It also reversed aluminum-evoked glutamate depletion, suppressed neuronal inflammation, curbed neuronal oxidative stress, depressed caspase-3 activation, and augmented BDNF and TFAM levels. ND probably exerted protective effects by modulating NF- κ B activation which further regulates STAT3 and Bcl2 signaling, effects mediated likely by modulating NMDA receptors.

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Compliance with Ethical Standards All animal procedures were performed in accordance with the ethical procedures and policies approved by the Research Ethics Committee for Experimental and Clinical Studies of Faculty of Pharmacy, Cairo University, Egypt, and comply with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication no. 85–23, revised 1996). All efforts were exerted to minimize animal suffering.

Conflict of Interest The authors declare that there are no conflicts of interest in this work.

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