

Research Article

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Protective effect of *Allium atrovioleaceum*-synthesized SeNPs on aluminum-induced brain damage in mice

<https://doi.org/10.1515/chem-2022-0245>

received October 2, 2022; accepted November 1, 2022

Abstract: This study evaluated the possible neuroprotective effect of *Allium atrovioleaceum* extract (AaE)-synthesized selenium nanoparticles (SeNPs) on aluminum (Al)-induced neurotoxicity in mice, explaining the likely mechanisms. Mice were divided into five groups: G1, control; G2, AaE group that received AaE (200 mg/kg) for 4 weeks; and groups 3, 4, and 5 received AlCl₃ (100 mg/kg) for 3 weeks. After that, G4 received AaE (200 mg/kg), and G5 received SeNPs-AaE (0.5 mg/kg) for another 1 week. Exposure to AlCl₃ boosted oxidative damage in brain tissue as evidenced by a reduction in glutathione concentrations and other antioxidant enzymes along with increased lipid peroxidation

and nitric oxide levels. There was also a rise in the concentrations of interleukin-1 β , TNF- α , and cyclooxygenase-II activities. AlCl₃-treated mice showed reduced brain-derived neurotrophic factor (BDNF) and dopamine levels, increased acetylcholinesterase (AChE) activity, and reduced Bcl-2, and Bax, and caspase-3 activities. Treatment with SeNPs-AaE significantly reduced markers of oxidative stress, inflammation, and apoptosis. In addition, in SeNPs-AaE-treated rats, levels of BDNF and dopamine were significantly increased along with a reduction in AChE as compared with the AlCl₃ group. Therefore, our results indicate that SeNPs-AaE has a potential neuroprotective effect against Al-mediated neurotoxic effects because of its powerful antioxidant, anti-inflammatory, anti-apoptotic, and neuromodulatory activities.

Keywords: aluminum, *Allium atrovioleaceum*, selenium nanoparticles, oxidative stress, apoptosis, acetylcholinesterase, BDNF

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1 Introduction

The exposure of humans to aluminum (Al) is an increasing problem, and its entry into and accumulation in the body is inevitable. Al is commonly accessible to animals and humans to the extent that toxicity can happen. Al intake can occur through inhalation of aerosols, consumption of foods, water, and pharmaceuticals, skin exposure, vaccinations, and hemodialysis machines. This metal induces oxidative damage, immunological changes, genotoxicity, inflammation, metabolic imbalance, defective iron homeostasis, apoptosis, and necrosis [1].

The mean consumption of Al was found to be about 70–140 mg per week, and despite its weak absorption (below 1%) from the gastrointestinal tract, it can accumulate with time in many tissues, including cerebral, hepatic, and renal tissues, resulting in neurotoxic and

cytotoxic effects. Therefore, it is included in the list of hazardous materials authorized by the Agency for Toxic Substances and Disease Registry (ATSDR) [2].

The brain cells are a target for Al accumulation. Al can cross the blood–brain barrier (BBB). This is considered the main route of brain Al uptake [3]. Several studies have investigated the Al concentration in cerebral tissue in Alzheimer's disease [4], multiple sclerosis, and autism [5].

Plant-derived products have been utilized as therapeutic agents with minimal adverse effects [6]. *Allium* is utilized as traditional medicine in many regions worldwide. Many studies revealed that *Allium* comprises many secondary metabolites in its bulb, flower, and leaves. *Allium* contains abundant flavonoids, saponins, saponinins, and sulfur molecules. They have many health benefits as they have many bioactivities, including antioxidant, antineoplastic, antimicrobial, and anti-inflammatory, as reviewed by Kurnia *et al.* [7].

Allium atrovioleaceum belongs to the genus *Allium* and the Liliaceae family. It is often utilized as a food additive to provide vitamins and to treat arthritis and rheumatism [8]. However, the beneficial activities of *A. atrovioleaceum* have not been studied sufficiently. In a recent study, *A. atrovioleaceum* extract remarkably improved cyclophosphamide-mediated toxicity and pathological process on testicular tissue due to its high antioxidant capacity [9].

Selenium (Se) is a micronutrient necessary for the appropriate functioning of all tissues and health body. It is a cofactor of several enzymes [e.g., glutathione (GSH) peroxidase and thioredoxin reductase]. It also has antioxidant and anti-cancer properties [10]. Organic Se exists in plant foods as selenomethionine with 90% bioavailability, while inorganic compounds (selenate and selenite) are utilized as supplementation with high bioavailability [11].

Selenium nanoparticles (SeNPs) demonstrate lower toxicity and higher biocompatibility when compared to organic or inorganic forms, attracting attention toward their application in therapy. SeNPs have been investigated as a therapy for many illnesses, such as diabetes, Alzheimer's disease [12], and inflammation-related diseases [13], such as rheumatoid arthritis. In addition, they can protect against several toxic agents, including chromium, cadmium, and chemotherapy [14]. The approaches often used for SeNPs production include the utilization of chemical compounds and biological organisms (plant extracts, fungi, or bacteria) that reduce the oxidized Se to elemental Se, green synthesis, and physical methods [15].

Plant extracts have the potential for SeNPs' biosynthesis. For instance, *Vitis vinifera* fruit extract was used to

produce elemental Se. Also, lignin could reduce and stabilize SeNPs [16]. Fruit extract of *Emblica officinalis* also has been used to produce elemental Se because of the significant content of phenolics, flavonoids, and tannin [17]. *Capsicum annuum* L. extract has the capacity to reduce Se ions into elemental form *ref.* [18]. In addition, apigenin has been used for SeNPs synthesis [19].

However, no studies have investigated the activity of green-synthesized SeNPs using *A. atrovioleaceum* extracts and their mechanisms. Therefore, the current work aimed to evaluate the likely protective effects of *A. atrovioleaceum*-synthesized SeNPs on Al-associated neurotoxicity.

2 Materials and methods

2.1 Chemicals and reagents

Al chloride, sodium selenite, and Tris-HCl underwent purchasing from Sigma Chemical Company (USA). Trichloroacetic and thiobarbituric acids were obtained from Merck (USA). In our study, the reagents utilized were of high analytical grade with purified water (ddH₂O) utilized as a solvent.

2.2 Preparation of *A. atrovioleaceum* total extract

The leaves of *A. atrovioleaceum* plants were collected from the Wadies in the western parts of the Salma Mountains to the east of Hail town, Hail region, KSA, in May 2021. A taxonomist from Ha'il University's Botany Department verified the plant's identification. After washing, the dried leaves (500 g) were extracted by maceration in methanol at ambient temperature for 2 days. In a rotary evaporator, the extract was concentrated, lyophilized, and stored for future use. The extract was diluted to 40 mg/mL by dissolving it in normal saline.

2.3 Phytochemical studies and quantitative determination of total phenolics content (TPC) and total flavonoids content (TFC) of Hail *A. atrovioleaceum*

To evaluate the active compounds in the *A. atrovioleaceum* plant, the Folin–Ciocalteu technique was used to assess TPC, and the Al chloride colorimetric test was used to evaluate TFC, as reported by Abdel Moneim [20].

2.4 Biosynthesis of SeNPs and its characterization

A volume of 5 mL of the extract solution of *A. atroviolaceum* (40 mg/mL) was mixed with 5 mL of 5 mmol/mL Na_2SeO_3 and stirred together for 24 h at 30°C. The mean size of SeNPs-*A. atroviolaceum* extract (AaE) was analyzed by a Zetasizer (Nano series, ZEN 3600, Malvern, England). Meanwhile, characterization involved Fourier transform infrared spectroscopy (FTIR) analysis to evaluate the functional groups involved in the formation of nanoparticles (PerkinElmer Spectrum 10.5.4, US).

2.5 Experimental animals and design

A total of 35 Swiss albino mice aged 6–8 weeks and weighing 22 ± 5 g were obtained from Theodor Bilharz Research Institute, Egypt. They underwent acclimatization for 7 days under room temperature, standard humidity, as well as standard light conditions. Mice received a balanced diet, with food and H_2O available ad libitum. Our experiment followed the principles of laboratory animal care from the US National Institutes of Health (8th edition). The protocol obtained its approval from Helwan University Institutional Animal Care and Use Committee (IACUC) (approval no HU2021/Z/AEO0121-02).

Animals were divided into five groups, each comprising seven animals.

Group 1: control group (Cntr), mice received 8 mL/kg of distilled water for 28 days.

Group 2: AaE group, mice received AaE (200 mg/kg BW) for 28 days (dose adjusted according to Hosseini et al. [21]).

Group 3: AlCl_3 group, mice received AlCl_3 (100 mg/kg BW) for 21 days by oral gavage (according to Singh et al. [22]) and were subsequently treated with 1 mL of distilled water for 7 days by oral gavage.

Group 4: AlCl_3 + AaE group, mice received AlCl_3 as in group 3 and were subsequently treated with AaE (200 mg/kg BW) for another 7 days by oral gavage.

Group 5: SeNPs-AaE group, mice received AlCl_3 as in group 3 and were subsequently treated with SeNPs-AaE (0.5 mg/kg BW) for another 7 days by oral gavage.

Within 24 h of the administration of the last treatment, euthanasia and scarification of mice were performed. The brain was immediately dissected on ice. After that, a part of the brain tissue samples was homogenized with 10 mM phosphate buffer and centrifuged for 12 min at 3,600 rpm. The resulting supernatant was kept at -80°C .

2.6 Measurements

2.6.1 Analysis of antioxidant markers

In the brain tissue, levels of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx), and GSH were determined according to Luck [23], Misra and Fridovich [24], Factor et al. [25], Weydert and Cullen [26], and Ellman [27], respectively.

2.6.2 Analysis of markers of oxidative damage

In brain tissues, lipid peroxidation (LPO) concentrations were quantitated according to the Ohkawa et al. [28] method, while nitric oxide (NO) concentrations were measured based on the Green et al. [29] methodology.

2.6.3 Measurement of inflammatory markers [interleukin-1 β (IL-1 β), TNF- α , and cyclooxygenase-II (Cox-II)]

ELISA kits from R&D Systems (MN, USA) were used to assess the amounts of inflammatory cytokines IL-1 β (Cat. No. RLB00), Cox-II (Cat. No. DYC4198-2), and TNF- α (Cat. No. RTA00) in the brain tissues according to the manufacturer's instructions.

2.6.4 Assessment of pro-apoptotic and anti-apoptotic proteins

The apoptosis-related factors, such as Bax (Cat. No. MBS935667), Bcl-2 (Cat. No. MBS2881713), and caspase-3 (Cat. No. MBS261814) levels, were detected in the brain tissues using rat ELISA kits (MyBioSource, SD, USA) following the manufacturer's recommendations.

2.6.5 Assessment of brain-derived neurotrophic factor (BDNF)

A part of the brain tissues was homogenized in extraction buffer (TPS, pH 8.0, Sod metavanadate 5 mM, 10% glycerol, 100 $\mu\text{g}/\text{mL}$ aprotinin, 10 mM PMSF, and 10 $\mu\text{g}/\text{mL}$ leupeptin). The homogenate was analyzed for BDNF (Cat. No. ab213899; Abcam, UK) using the ELISA technique, according to Doron et al. [30].

2.6.6 Assessment of acetylcholinesterase (AChE) activity and dopamine levels in brain tissues

AChE activity in tissue homogenates was determined as described by Auti and Kulkarni [31], while dopamine

levels were determined based on the inhibitory effect of dopamine on the oxidation of thionine by bromate in acidic media. The change in absorbance was followed spectrophotometrically at 601 nm as described by Shishebore *et al.* [32].

2.6.7 Histopathological examinations

Brain tissues were fixed in 10% neutral-buffered formalin, dehydrated, and paraffinized at room temperature for 24 h, followed by sectioning (4–5 μm). Sections were stained with hematoxylin and eosin (H&E) for light microscopy analysis.

2.6.8 Statistical analysis

Data were described as means \pm standard deviations (SDs). One-way analysis of variance and *post hoc* Duncan's test were utilized for comparing >2 groups, and then, Student's *t*-test was applied. The SPSS program (V20.0; IBM Corp, USA) was used to analyze the data. A result was considered significant when *P* value <0.05.

3 Results

3.1 Results of TFC and TPC

According to the quantitative analysis, each gram of dry weight of *A. atrovioleaceum*'s extract contains 54.70 mg quercetin equivalent of total flavonoids and 69.50 mg gallic acid equivalent of total phenols.

3.2 Characterization of nanoparticles' structure

The average diameter of SeNPs-AaE was 135.4 nm (Figure 1), and the average zeta potential was -18.9 mV. Furthermore, FTIR spectroscopy was used to confirm the involvement of O–H, N–H, C=O, and C–O functional groups during forming SeNPs, which were associated with bioactive molecules capping their surface. A wide peak demonstrates the O–H group at 3326.86 cm^{-1} . C–H stretch alkynes are shown by the absorption peak at 2118.89 cm^{-1} . C–O asymmetric stretch carbon compounds are accountable for the band at 1637.79 cm^{-1} . N–O stretch groups are shown by the

absorption peak at 1438.13 cm^{-1} . C=C bending for alkene groups presented by the absorption peaks at 1010.64 and 950.69 cm^{-1} . In alkyl halides, C–X stretching creates a band at 458.92 , 435.06 , 419.99 , and 410.97 cm^{-1} . This study revealed the presence of many functional groups that may be required for SeNPs-AaE reduction and stability.

3.3 Oxidative stress markers

The changes in oxidative damage biomarkers are shown in Figure 2. Mice exposed to AlCl_3 demonstrated a statistically significant elevation in LPO level and NO level in comparison with control mice and AaE-treated mice ($P < 0.05$). In mice exposed to AlCl_3 and treated with AaE or SeNPs-AaE, both LPO and NO levels showed statistically significant reduction ($P < 0.05$), with a more considerable reduction observed in the SeNPs-AaE group.

3.4 Antioxidant activity

Figure 3 demonstrates no statistically significant difference in the SOD activity between the study groups. Mice exposed to AlCl_3 demonstrated a statistically significant reduction in the CAT activity, the GPx activity, the GR activity, and the GSH level in comparison to control mice and AaE-treated mice ($P < 0.05$). Conversely, in the group of mice exposed to AlCl_3 and treated with AaE or SeNPs-AaE, there were statistically significant increases in the CAT activity, the GPx activity, the GR activity, and the GSH value as compared with the AlCl_3 group ($P < 0.05$). Notably, SeNPs-AaE was associated with an enormous rise in these antioxidant parameters (CAT activity, GPx activity, GR activity, and GSH level), meaning that it has a more potent antioxidant activity.

3.5 Inflammatory markers

As shown in Figure 4, mice exposed to AlCl_3 exhibited a significant ($P < 0.05$) rise in IL-1 β , TNF- α , and Cox-II concentrations compared to the control and AaE groups. In mice exposed to AlCl_3 and treated with AaE or SeNPs-AaE, these levels showed a statistically significant ($P < 0.05$) reduction when compared to the AlCl_3 group, and this reduction was more noticeable in the SeNPs-AaE group.

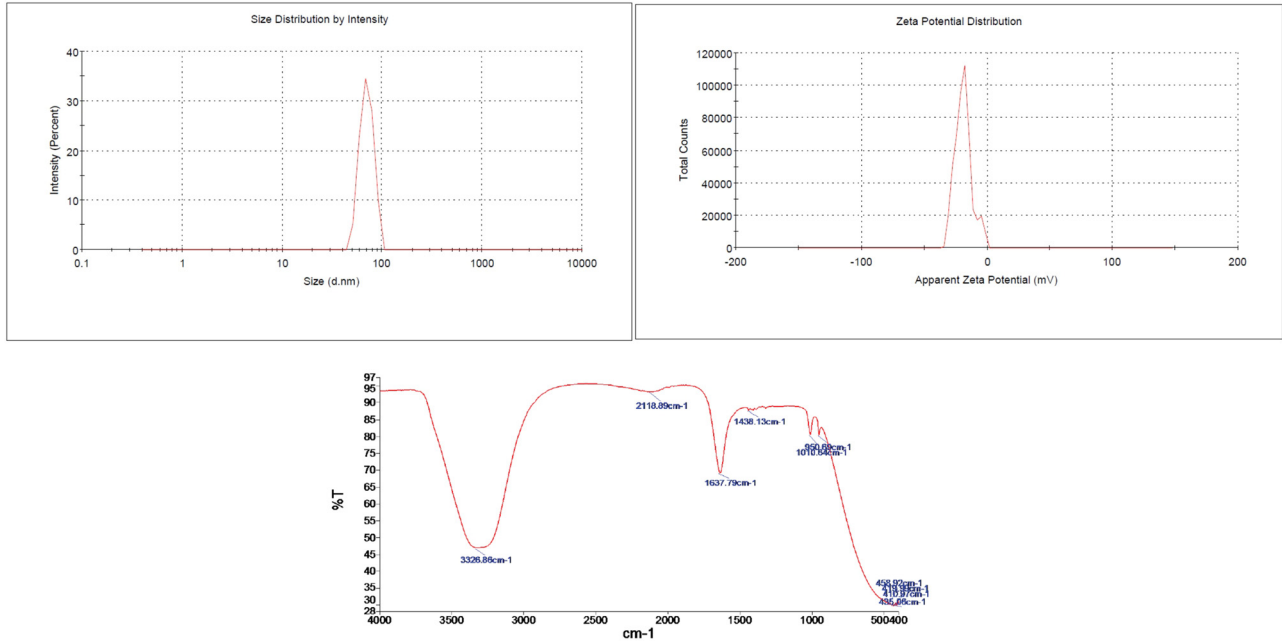


Figure 1: Characterization of *A. atrovioleaceum*-synthesized SeNPs. Upper panel: hydrodynamic diameter of SeNPs-AaE by Zetasizer. Middle panel: surface charge of SeNPs-AaE by zeta potential. Lower panel: FT-IR spectra of SeNPs-AaE.

3.6 Apoptosis markers

As shown in Figure 5, there was a notable decrease in Bcl-2 levels accompanied by a considerable increase in Bax and caspase-3 levels in the AlCl₃ group as contrasted to the control mice. When compared to the AlCl₃ group, both AaE + AlCl₃ and SeNPs-AaE + AlCl₃ groups showed a statistically significant rise in Bcl-2 levels, as well as a substantial decrease ($P < 0.05$) in apoptotic markers

(Bax and caspase-3). Furthermore, these results indicated that SeNPs-AaE has more anti-apoptotic action than AaE alone.

3.7 BDNF level

As indicated in Figure 6, animals treated with AlCl₃ exhibited a substantial decrease in BDNF levels when

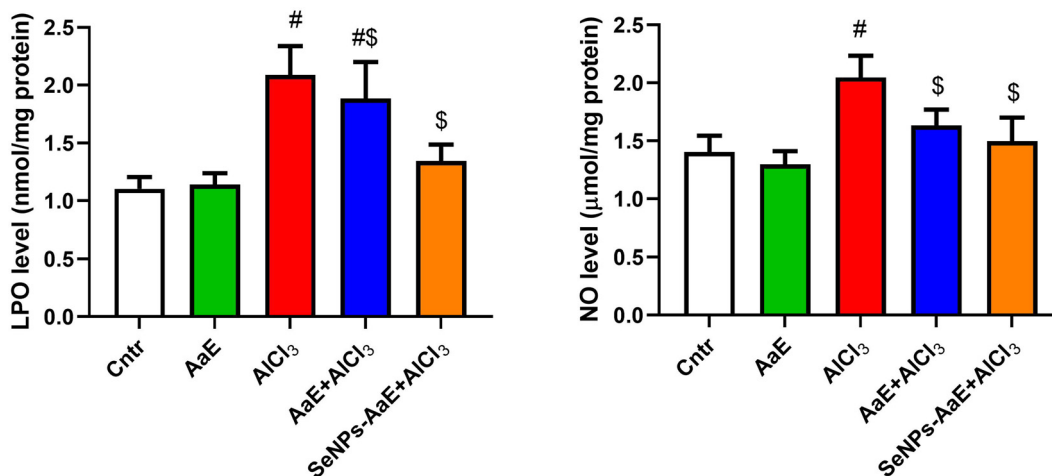


Figure 2: Effect of treatment with *A. atrovioleaceum*-synthesized SeNPs on the biomarkers of oxidative damage, LPO level, and NO level in brain tissue of mice exposed to AlCl₃. Data are represented as mean + SD. # $P < 0.05$, as compared to control mice; \$ $P < 0.05$, as compared to AlCl₃-treated mice. AlCl₃: aluminum chloride; SeNPs-AaE: selenium nanoparticles green synthesized using *A. atrovioleaceum*.

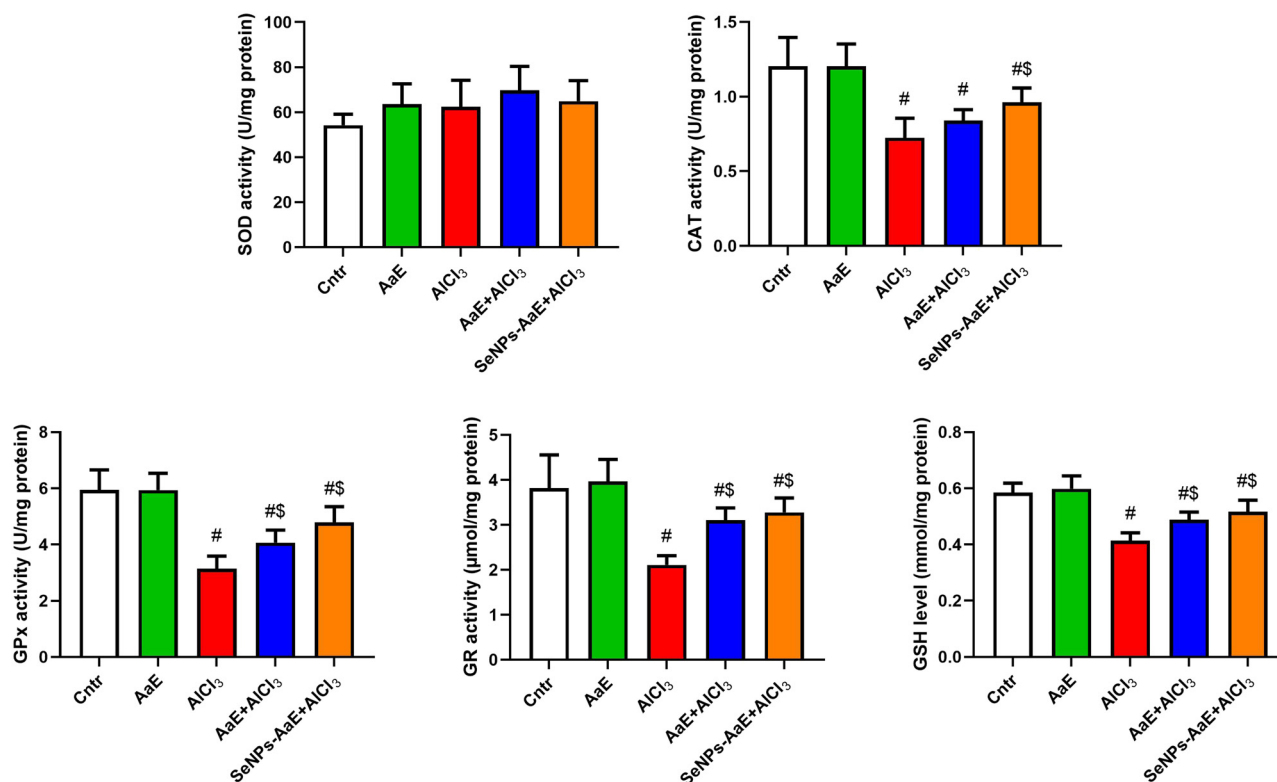


Figure 3: Effect of treatment with *A. atroviolaceum*-synthesized SeNPs on the antioxidant activities of SOD, CAT, GPx, GR, and GSH in brain tissue of mice exposed to aluminum. Data are represented as mean + SD. # $P < 0.05$, as compared to control mice; \$ $P < 0.05$, as compared to AlCl₃-treated mice. AlCl₃: aluminum chloride; SeNPs-AaE: selenium nanoparticles green synthesized using *A. atroviolaceum*.

compared to the control group ($P < 0.05$). However, compared to the AlCl₃ group, administration of SeNPs-AaE resulted in a significant ($P < 0.05$) increase in BDNF concentration. Meanwhile, no substantial change was found between the AaE + AlCl₃ and AlCl₃ groups.

3.8 AChE activity

As indicated in Figure 7, AChE activity significantly increased in the AlCl₃ group than in the control group ($P < 0.05$). When compared to the AlCl₃ group, the SeNPs-AaE-treated mice's

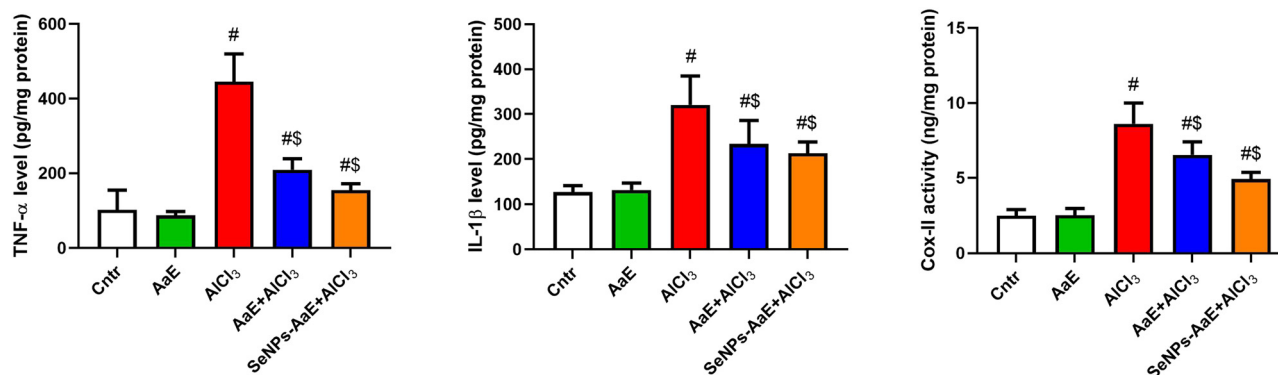


Figure 4: Effect of treatment with *A. atroviolaceum*-synthesized SeNPs on inflammatory markers, TNF-α, IL-1β, and Cox-II activity in mice exposed to AlCl₃. Data are represented as mean + SD. # $P < 0.05$, as compared to control mice; \$ $P < 0.05$, as compared to AlCl₃-treated mice. AlCl₃: aluminum chloride; SeNPs-AaE: selenium nanoparticles green synthesized using *A. atroviolaceum*.

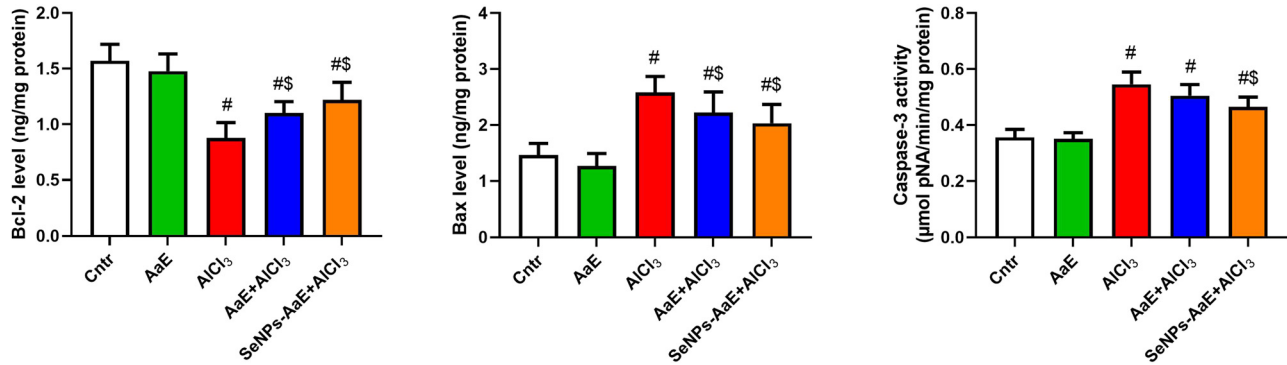


Figure 5: Effect of treatment with *A. atrovioleaceum*-synthesized SeNPs on the levels of Bcl-2, Bax, and caspase-3, in mice exposed to AlCl₃. Data are represented as mean + SD. #*P* < 0.05, as compared to the control mice; \$*P* < 0.05, as compared to AlCl₃-treated mice. AlCl₃: aluminum chloride; SeNPs-AaE: selenium nanoparticles green synthesized using *A. atrovioleaceum*.

AChE activity was considerably lower (*P* < 0.05). However, no discernible change was found between the AaE + AlCl₃ and AlCl₃ groups.

3.9 DA concentration

The changes in DA concentration are shown in Figure 8; mice exposed to AlCl₃ demonstrated a statistically significant reduction in DA level in comparison with control and AaE groups (*P* < 0.05). In mice exposed to AlCl₃ and treated with AaE or SeNPs-AaE, DA concentration demonstrated a statistically significant (*P* < 0.05) increase when compared with the AlCl₃ group; such an increase in DA concentration was more than that observed in the AaE + AlCl₃ group.

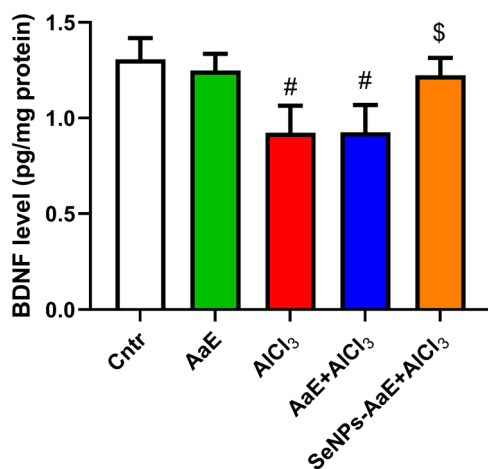


Figure 6: Effect of treatment with *A. atrovioleaceum*-synthesized SeNPs on BDNF level in mice exposed to AlCl₃. Data are represented as mean + SD. #*P* < 0.05, as compared to control mice; \$*P* < 0.05, as compared to AlCl₃-treated mice. AlCl₃: aluminum chloride; SeNPs-AaE: selenium nanoparticles green synthesized using *A. atrovioleaceum*.

3.10 Effect of SeNPs-AaE on histological alterations

As shown in Figure 9, in contrast to the AlCl₃ group (Figure 9c), which demonstrated neuronal degeneration, blood vessel congestion, inflammatory cells infiltration, necrotic and apoptotic neurons together with pyknotic nuclei in the cortical area, the control and AaE groups displayed normal brain tissue architecture (Figure 9a and b, respectively). Contrarily, AlCl₃-exposed rats treated with AaE or SeNPs-AaE exhibited nearly normal morphology (Figure 9d and e, respectively). However, some necrotic and apoptotic neurons together with pyknotic nuclei were found in the cortical tissue of brain mice.

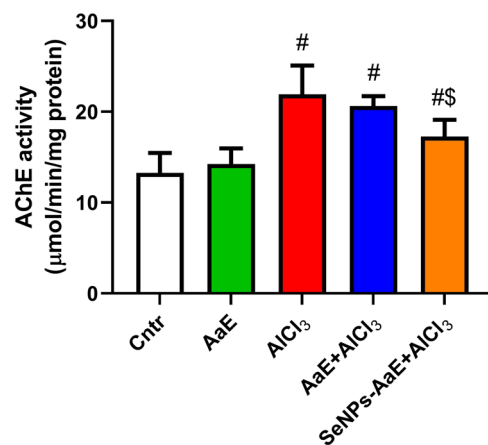


Figure 7: Effect of treatment with *A. atrovioleaceum*-synthesized SeNPs on AChE activity in mice exposed to AlCl₃. Data are represented as mean + SD. #*P* < 0.05, as compared to control mice; \$*P* < 0.05, as compared to AlCl₃-treated mice. AlCl₃: Aluminum chloride; SeNPs-AaE: Selenium nanoparticles green synthesized using *A. atrovioleaceum*.

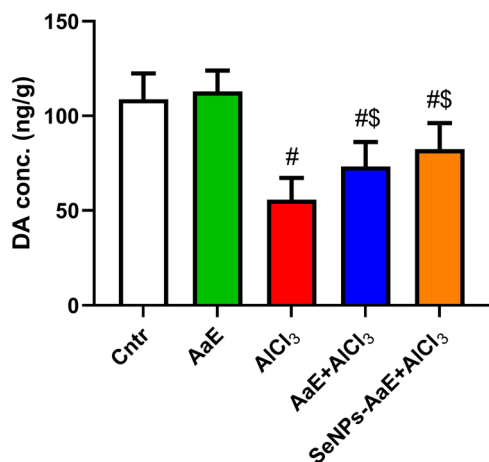


Figure 8: Effect of treatment with *A. atrovioleaceum*-synthesized SeNPs on DA concentration in mice exposed to AlCl₃. Data are represented as mean + SD. #*P* < 0.05, as compared to control mice; \$*P* < 0.05, as compared to AlCl₃-treated mice. AlCl₃: aluminum chloride; SeNPs-AaE: selenium nanoparticles green synthesized using *A. atrovioleaceum*.

4 Discussion

There is no doubt that the world is living in what is called “Al age.” The exposure of humans to Al is unavoidable and, possibly, immeasurable. The majority of humans, particularly those who live in developed countries, are at risk of chronic Al toxicity [5].

Al undergoes absorption through many routes (oral, cutaneous, and parenteral) [2] and exists in the serum and urinary samples of all populations. This metal is found naturally in the environment, food, and water. The possible Al-induced neurotoxicity suggested in humans is a serious side effect that requires protection against it [4]. Al can cross the BBB and undergoes accumulation in glial and neural cells [3]. As a result, the current work aimed at assessing the possible protective effect of *A. atrovioleaceum*-synthesized SeNPs on Al-associated neurotoxicity. Of note, the green production of SeNPs comprises the utilization of different biological agents like bacteria, fungi, plant extracts, and materials of biological origin to reduce metal salts and prepare nanoparticles [33].

Though the mechanism of Al toxicity is still not completely clarified, it is advocated that free radicals have an important role in Al-mediated neurotoxicity [2]. Indeed, the biochemical integrity of cerebral tissue is important for normal neurological function. One of the factors that contribute to the impairment of such biochemical integrity is the oxidative stress [34]. When there is an overproduction of free radicals, human body can defend itself by producing enzymatic antioxidant molecules (e.g., CAT, SOD, GR, and GPx) or non-enzymatic molecules (e.g., GSH). Al was found to significantly decrease cerebral GSH, which is produced mainly by astrocytes [35].

In the current study, exposure of mice to Al was accompanied by its diffusion in brain tissue resulting in

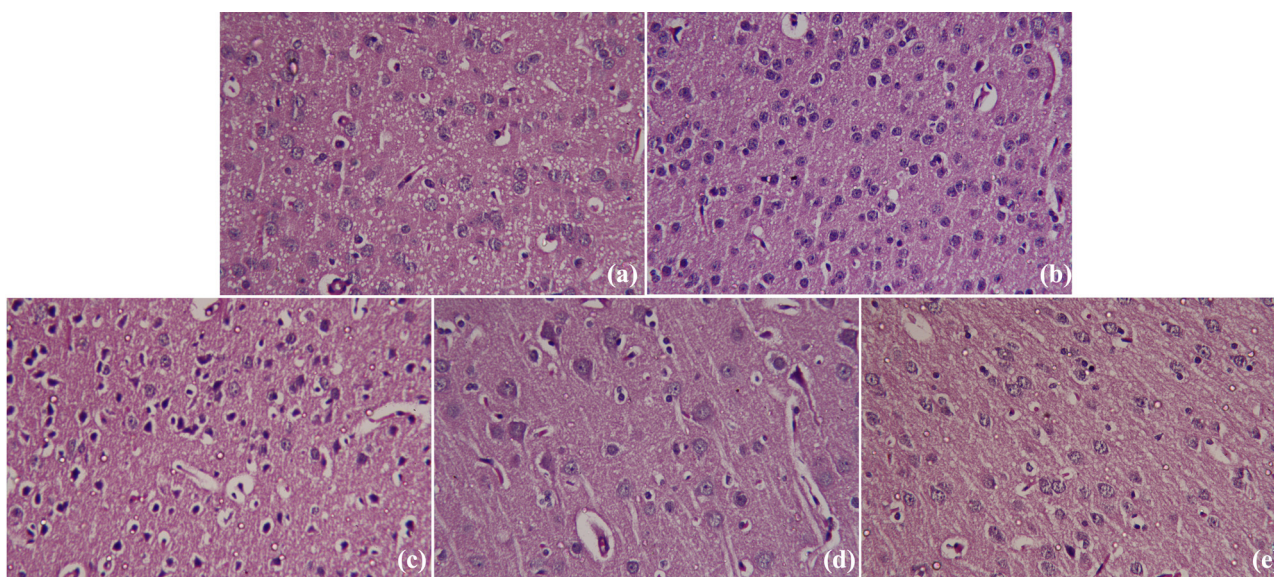


Figure 9: Histopathological examination of cortical tissue in brain of mice intoxicated with AlCl₃ and treated with AaE or SeNPs-AaE. The control (a) and AaE (b) groups showed normal brain cell morphology, whereas the AlCl₃ (c) group displayed neuronal degeneration and histological alterations. Meanwhile, treating these AlCl₃-exposed rats with AaE (d) or SeNPs-AaE (e) resulted in near-normal morphology. Magnification = 400×.

a significant reduction in antioxidant molecules, including CAT, GPx, and GR activity, as well as GSH levels, along with the considerable elevation of oxidative stress markers (LPO and NO) in comparison with the control mice. These findings highlight the role of free radicals in Al-mediated oxidative damage. The increased NO levels can be related to the capacity of Al to enhance iNOS expression [36].

Our findings agree with many reports stating that Al administration was associated with oxidative damage and reduced GSH and antioxidant enzymatic activity [37,38]. Such inhibition in antioxidant enzymes' activities might be because of a decreased production of such enzymes in relation to high intracellular Al concentrations and/or increased synthesis of free radicals [35].

It is obvious that oxidative damage plays a key pathophysiologic role, and it enhances brain susceptibility to damage. Mechanisms by which oxidative stress causes neuronal damage are not well-understood; however, it was advocated to increase BBB permeability and change brain morphology, resulting in neuroinflammatory process, and neuronal death [39].

Our results showed that mice exposed to AlCl₃ and treated with AaE or SeNPs-AaE showed statistically significant improvement in the oxidants/antioxidants balance as indicated by increases in the antioxidant enzymes (GR, CAT, and GPx) accompanied by reductions in the oxidative stress makers (LPO and NO). However, SeNPs-AaE resulted in a tremendous increase in the antioxidant molecules and a greater reduction in oxidative stress markers than AaE alone.

Se can quench ROS and thus inhibit oxidative stress. This comes consistence with Othman et al. [10] study that revealed a remarkable reduction in LPO and NO concentrations and a significant rise in GSH concentrations in mice treated with green-synthesized SeNPs (SeNPs-berberine). In addition, Krishnan et al. [40] revealed that green-synthesized SeNPs (from plant *Spermacoce hispida*) reduced oxidative stress (NO and LPO levels) and enhanced the endogenous antioxidants (SOD, CAT, and GPx).

SeNPs agglomeration influences their bioactivity, biocompatibility, and biological availability. Thus, their surface functionalization using a biologically active compound will not only increase its bioavailability but also can boost its therapeutic power [41]. Here comes the role of *A. atrovioleaceum*, which contains several bioactive components with antioxidants and other biologic activities [7]. This can explain the robust antioxidant activity of SeNPs that was green synthesized using *A. atrovioleaceum* extract (AaE-SeNPs).

Numerous pro-inflammatory molecules, including cytokines, interleukins, chemokines, ROS, and Cox-II, are

released from microglial cells and astrocytes. It was established that the neuroinflammatory process is a mechanism of neurotoxicity [42]. Interestingly, brain tissue is extremely susceptible to injury and inflammatory processes. IL-1 β and TNF- α are the most frequently investigated brain inflammatory markers linked to learning and memory difficulties, dementia, epilepsy, and increased susceptibility to neurotoxic substances [43].

Our results revealed a significant rise in TNF- α and IL-1 β levels in mice exposed to AlCl₃, indicating that neuroinflammation can be a potential mechanism underlying Al-induced brain injury. These findings are consistent with those of other researchers, who reported high TNF- α and IL-1 β concentrations released from microglia and astrocytes after AlCl₃ exposure, leading to neuroinflammation [44]. In addition, the overproduction of inflammatory cytokines can impair synaptic plasticity, reduce neurogenesis, and induce apoptotic processes, thus resulting in cognitive, learning, and memory defects [45].

Meanwhile, mice treated with either AaE or SeNPs-AaE after Al exposure showed a significant reduction in TNF- α and IL-1 β concentrations, signifying these molecules' anti-inflammatory effects, especially SeNPs-AaE. These results were consistent with the findings of Yuan et al. [46], who revealed that SeNPs inhibited the inflammatory response in the rat brain as indicated by reduced TNF- α and IL-1 β levels. Also, in a study by Albrakati et al. [47], prodigiosin-SeNPs' treatment reduced neuroinflammation in the hippocampus, as determined by a reduced release of pro-inflammatory markers (TNF- α , IL-1 β , and IL-6).

Cox-II is a rate-limiting enzyme that converts arachidonic acid to prostaglandin H₂, which is the precursor of numerous chemicals, including prostaglandins, prostacyclin, and thromboxanes, which are known as prostanoids and are responsible for the inflammatory response [47].

Meanwhile, mice treated with either AaE or SeNPs-AaE after Al exposure showed a significant reduction in TNF- α and IL-1 β concentrations, signifying these molecules' anti-inflammatory effects, especially SeNPs-AaE. These results were consistent with the findings of Yuan et al. [46], who revealed that SeNPs inhibited the inflammatory response in the rat brain as indicated by reduced TNF- α and IL-1 β levels. Also, in a study by Albrakati et al. [47], prodigiosin-SeNPs treatment reduced neuroinflammation in the hippocampus, as determined by a reduced release of pro-inflammatory markers (TNF- α , IL-1 β , and IL-6).

In neurodegeneration, apoptosis plays a crucial role and results in the loss of valuable neurons. This study revealed that mice treated with AlCl₃ demonstrated a

significant reduction in Bcl-2 levels accompanied by a marked increase in Bax and caspase-3 levels, indicating that AlCl₃ can harm brain tissue through AlCl₃-mediated apoptosis. In several other *in vivo* studies, AlCl₃ was found to induce apoptosis in the neuronal cells of experimental animals. Thus, apoptosis can be considered a likely mechanism of Al-induced neurotoxicity [48,49]. Meanwhile, administration of AaE and its green-synthesized SeNPs to Al-exposed animals was associated with an anti-apoptotic effect, as indicated by the significant rise in Bcl-2 levels and the significant reduction in Bax and caspase-3 levels. Contrary to our results, green-synthesized SeNPs (SeNPs-apigenin) were found to cause a substantial rise in caspase-3 and Bax, with a notable reduction in Bcl-2 [19]. Meanwhile, our results indicated that the AaE extract increased the anti-apoptotic activity when combined with SeNPs. These results were in agreement with those of Jakaria *et al.* [50], who reported that the methanolic extract of *Allium cepa* upregulated the expression of the anti-apoptotic gene (Bcl-2) in N27-A cells. Similarly, Khazaei *et al.* [6] reported that treating human breast cancer cell lines with *A. atrovioleaceum* extract decreased the expression of caspase-3. Moreover, Zhu *et al.* [51] stated that SeNPs reduced caspase-3 expression and inhibited apoptosis through the mitochondrial pathway.

BDNF maintains the function and survival of neuronal cells. It controls synaptic transmission and has a central role in cognitive function and a neuroprotective effect on brain insults [52]. The current study demonstrated that mice treated with AlCl₃ had significantly lower BDNF concentrations. These findings are consistent with the previous research that found lower BDNF levels following AlCl₃ exposure [53]. In the present study, the SeNPs-AaE combination significantly counteracted these alterations in BDNF concentrations in AlCl₃-exposed animals. This improvement in BDNF levels could be attributed to the potent antioxidant activity of both Se and AaE. In this regard, earlier studies have demonstrated that Se and SeNPs could restore BDNF concentrations after neuronal injury induced experimentally in rodents [42,54]. In addition, Albrakati *et al.* [47] revealed that SeNPs' administration improves hormonal levels and modulates BDNF values in brain tissues.

Another interesting finding in the current study is that Al treatment raised the activity of AChE in brain tissues compared to the control group. Enhanced AChE activity results in acetylcholine breakdown with the subsequent depletion of its level, thus impairing the brain's cholinergic functions [55]. The increase in AChE activity after AlCl₃ exposure can be related to an allosteric interaction between Al³⁺ (as a cation) with anionic sites of

AChE, resulting in changes in the secondary structure of the brain AChE [56]. Comparable results were reported by Auti and Kulkarni [31], who revealed that Al exposure is associated with increased AChE activity in the hippocampal tissue. However, AlCl₃-exposed mice treated with SeNPs + AaE showed a significant decrease in the AChE activity when compared to the AlCl₃ group. Consistent with our results and signifying the role of SeNPs in modulating neurotransmission, Ebokaiwe *et al.* [57] observed that SeNPs reduce AChE activity in the rat brain. The authors claimed that SeNPs ameliorated neuroinflammation, improved cholinergic neurotransmission, and restored locomotor functions.

Dopamine has a significant role in several cerebral functions, such as locomotor activity, cognitive function, emotional stability, positive reinforcement, food consumption, and endocrinal regulation [58]. The toxic effects of Al on dopamine concentrations were evaluated in the present study. Where AlCl₃ administration declined the DA level, this finding suggests that Al might cause neurotoxicity, at least in part, through disruption of dopamine neurotransmission. These findings agreed with Kadhem and Enaya [59], who reported a significant reduction in dopamine levels in animals treated with Al. Werner *et al.* [60] stated that Al might inhibit the creation of tetrahydrobiopterin, which is essential in the dopamine synthesis pathway.

Meanwhile, supplementation of either AaE or SeNPs-AaE to the mice improved the level of dopamine with more improvement observed in the SeNPs-AaE group. In one study, Se administration was associated with a rise in neurotransmitter levels via monoamine oxidase inhibition [61]. Similar to our findings, Yuan *et al.* [46] reported that pre-treatment of animals with SeNPs reversed the alterations in the concentrations of neuromodulators, including DA. Similarly, Anusha *et al.* [62] concluded that apigenin (one of the flavonoids present in *A. atrovioleaceum*) modulated DA neurotransmission by increasing dopamine biosynthesis and dopamine D2 receptor expression.

In our study, the examination of H&E-stained sections revealed that Al caused significant histological changes in the brain tissues in the form of neuronal degeneration and blood vessel congestion. Moreover, the reported neuroinflammation and apoptosis in the current study was verified histologically, as infiltration by inflammatory cells and apoptosis of cerebral neurons were noticed. Consistent with our results, Said and Abd Rabo [63], in their experimental study, found that daily oral treatment of rats with AlCl₃ for 4 successive weeks caused severe congestion in the blood vessels, associated with marked karyopyknosis and degeneration of cerebral cortex neurons and the small pyramidal cells of the

hippocampus. Also, Tair et al. [64] reported neurodegeneration, gliosis, and spongiosis in the brains of $AlCl_3$ -exposed animals. Collectively, this indicates the potential neurotoxicity of Al exposure.

Notably, the histological examination revealed a neuroprotective potential of AaE or SeNPs-AaE against Al-induced neurotoxicity as demonstrated by the improvement of histological changes due to the antioxidant, anti-inflammatory, and anti-apoptotic properties of AaE or SeNPs-AaE.

5 Conclusion

Green-synthesized SeNPs using *A. atrovioleaceum* exhibited neuroprotective effects against Al-induced neurotoxicity. The mechanisms of neuroprotection may include antioxidant, anti-inflammatory, and anti-apoptotic activities, along with the modulation of AChE, BDNF, and dopamine levels.

Acknowledgments: This research was funded by Scientific Research Deanship at University of Ha'il – Saudi Arabia through project number RG-21115.

Funding information: This research was funded by Scientific Research Deanship at University of Ha'il – Saudi Arabia through project number RG-21115.

Author contributions: Project administration and resources: Mohamed S. Othman, Sofian T. Obeidat, Ghada M Aleid, and Amal H. Al-Bagawi; writing the proposal: Mohamed S. Othman and Mohga S. Abdalla; formal analysis: Sara S. Elganzoury, Mohamed S. Abdelfattah, and Ahmed E. Abdel Moneim; conceptualization: Mohamed S. Othman, Mohga S. Abdalla and Ahmed E. Abdel Moneim; methodology: Ahmed E. Abdel Moneim; writing of the article and reviewing: Mohamed M. Badawy, Alaa Fehaid, and Ola A. Habotta; funding acquisition: Mohamed S. Othman; supervision: Mohamed S. Othman, Mohga S. Abdalla, Mohamed S. Abdelfattah, and Ahmed E. Abdel Moneim. All authors have read and agreed to the published version of the article.

Conflict of interest: The authors declare no conflict of interest.

Informed consent statement: Not applicable.

Ethical approval: The principles of laboratory animal care from the US National Institutes of Health (8th edition).

The protocol obtained its approval from Helwan University Institutional Animal Care and Use Committee (IACUC) (approval no. HU2021/Z/AEO0121-02).

Data availability statement: All relevant data are within the article.

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