Adipose-derived mesenchymal stem cells rescue rat hippocampal cells from aluminum oxide nanoparticle-induced apoptosis via regulation of P53, Aβ, SOX2, OCT4, and CYP2E1

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1. Introduction

Aluminum oxide nanoparticles (Al2O3-NPs) are used in many different clinical and industrial products; they have the potential to cause toxicity [1,2]. Such small particles can be taken up by cells and infiltrate the blood and lymph to induce injury [3]. An examination directed at the brains of different animals indicated that Al2O3-NPs induced oxidative stress and dysfunction of antioxidant enzyme-mediated defenses [4,5]. Furthermore, NPs caused severe damage to tissues, such as those of the liver, kidney, and the immune system [6], which stimulated the expression of pro-inflammatory cytokines and reactive oxygen species (ROS), as well as the mutation of DNA [7].

The use of nanotechnology for the treatment and control of biological systems generates potential toxicity in humans [8]. It is also noted that the toxicity is different in various body organs due to the accumulation of NPs in the tissues of such organs [9]. Moreover, the toxicity can even cross the blood–brain barrier (BBB) [10]. Al2O3-NPs in various rodents can cause neurotoxicity via cytotoxic, genotoxic, and inflammatory effects in the brain [11]. The main target by which Al2O3-NPs cause injury in the central nervous system is the brain. Al2O3-NPs deposited in different brain regions cause neurotoxicity and histopathological and ultrastructural damage in rats. Moreover, they decreased the viability of cells, caused mitochondrial dysfunction, inhibited cell cycle, and induced apoptosis in in vitro studies [12]. Al2O3 NPs have cytotoxic and genotoxic effects on CHO-K1 cells, as
well as concentration-dependent inhibition of cell division in UMR106 cells [13,14]. Furthermore, Al₂O₃ NPs exposed to pulmonary artery endothelial cells and human umbilical vein endothelial cells increased mRNA protein expression of a molecule, likely due to the generation of reactive oxygen species (ROS) and activation of redox-sensitive signaling pathways, which could be linked to cardiovascular health risks [15].

Mesenchymal stem cells (MSCs) are somewhat multipotent and undifferentiated stem cells. They can be isolated from numerous tissues, including the bone marrow, fat tissue, cord blood, and amniotic layer [16]. MSCs are attractive for use in clinical treatments, as they can be effectively isolated from almost all adult tissues [17] and have been shown to be safe and non-tumorigenic [18-20]. MSCs derived from the adipose tissue are desirable for clinical use as they are easy to extract and abundantly available [21]. AD-MSCs can differentiate into myocytes, hepatocytes, neural cells, osteocytes, chondrocytes, adipocytes, and epithelial cells of the lung, kidney, and skin [22-24]. In animal models, AD-MSCs have been utilized to alleviate hemorrhagic stroke [25] and spinal cord injury, reduce inflammation and neurodegeneration, enhance motor skills, and lower the immune system response [26]. The ability of AD-MSCs to repair tissues via their regenerative properties could restore damaged neural tissues, infected lung tissues, cystic fibrosis lung tissues [27], and wounds [28].

According to the immunomodulatory properties of MSCs, they may repair tissues and reduce oxidative stress via the expression of cytokines, chemokines, apoptosis inducers, and antitumor particles [29,30]. MSCs produce various trophic and developmental factors, influencing the neurogenesis, synaptogenesis, and astrocytosis factors [24]. Consequently, applications to protect tissues from the harmful effect of ROS have focused on stem cells [31]. In animal models, the effect of MSCs on organs, tissues, and the regulation of inflammation have been demonstrated [32-34]. Our current study illustrates the acute toxicity of Al₂O₃ NPs on the Al level, gene expressions of Sox2 and Oct4, and their effect on the brain. Also, the essential and vital pathways by which stem cells can treat damage or apoptosis induced by Al₂O₃-NPs in the hippocampal region of the rat brain.

2. Materials and methods

2.1. Materials

Aluminum oxide NPs were purchased from US Research Nano materials, USA (gamma, 99+-%, average particle size 20 nm, hydrophilic, melting point determined via the high-temperature combustion method, Al₂O₃ SSA > 138 m²/g, morphology: nearly spherical, color: white). RPMI-1640 (with l-glutamine) growth medium, fetal bovine serum (FBS), and antibiotic mix were purchased from Gibco (Invitrogen, ca. USA). Collagenase Type II (Sigma-Aldrich, St. Louis, MO, USA) and mouse primary anti-CD105 and CD90.1 IgG and anti-CD45 antibodies were purchased from Thermo Fisher. Ultra-Tek polyvalent goat anti-mouse primary anti-CD105 and CD90.1 IgG and anti-CD45 antibodies were purchased from Sigma-Aldrich. SYBR green PCR Master Mix was purchased from USA.

2.2. Animal and experimental design

Adult female rats were kept at standard conditions (temperature at 23 ± 2 °C, lighting cycle 12 h light/dark; fed with standard chow and tap water) and were allowed to adapt to the laboratory housing conditions for 1 week prior to the start of the experiment.

The rats (n = 60) weighing 210 ± 50 g were divided into five groups: (I) a control group without any treatments; (II) a group receiving a daily oral dose of 9% NaCl; (III) a group receiving Al₂O₃-NPs (6 mg/kg body weight) [6] dissolved in 1 mL sodium chloride once daily for 20 days (The Al₂O₃-NPs solution was sonicated before each injection using a sonicator [35]); (IV) designated as (R), a group treated with Al₂O₃-NPs (6 mg/kg b.w.) dissolved in 9% NaCl solution once daily for 20 days, followed by a recovery period without treatment for an additional 20 days; and (V) designated as AD-MSCs-treated group, a group injected with 0.8 × 10⁵ AD-MSCs/0.5 mL phosphate-buffered saline (PBS) via the caudal vein after 20 days of oral treatment with Al₂O₃-NP. The rats were then sacrificed 5 days after MSC transplantation. The total experiment lasted 40 days.

2.2.1. Ethical approval

Adult female rats were purchased and cared for by the Assiut University Joint Animal Breeding Unit according to the National Institutes of Health guidelines for the use of experimental animals. The committee of medical ethics of the Faculty of Medicine at Assiut University reviewed and approved the research procedures employed in this study (IRB no:17900503).

2.3. Characterization of aluminum oxide nanoparticles (Al₂O₃-NPs)

2.3.1. X-ray diffraction (XRD) analysis

The crystal structures of powdered Al₂O₃-NPs were characterized by XRD by the Physics Department of Assiut University using a Philips X-ray diffractometer (Model PW 1710, Holland). The measurements were swapped from 20 = 30° to 20 = 80° using a copper X-ray tube operated at 40 kV and 40 mA, with a radiation wavelength of 1.5406 Å. The crystalline nature of the NPs was confirmed by the XRD pattern of Al₂O₃-NPs [36].

2.3.2. Transmission electron microscopy (TEM) analysis

A drop of Al₂O₃-NPs (20 nm /100 μg/L) preparation for TEM to determine the size and shape of Al₂O₃-NPs as follows. First, the powder was dissolved in H₂O and then dispersed ultrasonically. Then, the particles were deposited on a carbon-coated copper grid and dried at room temperature. The micrographs of the samples were taken using a TEM at Assiut University’s Center of Electron Microscopy, Faculty of Science.

2.3.3. Dynamic light scattering (DLS)

Mean particle size and polydispersity index (PDI) of the nanoparticles were measured at Assiut International Center of Nanoparticle (AICN), using a Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK) equipped with a backscattered light detector operating at 173°. The Zeta-potential values were measured by laser Doppler anemometry using Malvern Zetasizer Nano series ZS. All samples were diluted in distilled water and measured at 25 °C in triplicates (equilibrium time of 120 s and 15 runs). The sample volume used for all measurements was kept constant.

2.4. Isolation of AD-MSCs from rats

The solid fat from the adipose tissue of adult male rats was cut into fine pieces and then washed with sterile PBS (Lonza, Swiss). Next, the pieces were then enzymatically digested by Collagenase Type II (0.25 % in PBS in 20 % FBS) for 45 min. During the digestion incubation at 37 °C, the Falcon tubes (50 mL) were shaken every 10 min, after which the collagenase activity was halted by the addition of FBS. The cell pellet containing the AD-MSCs was reconstituted in 12 mL of culture medium after centrifugation. The suspension was filtered by cell strainer (40 μm) and plated on 10-cm culture dishes. The cells were incubated at 37 °C, 5% CO₂ for 2 weeks until the confluence reached nearly 80%. AD-MSCs were subcultured for up to three passages [37].
2.5. Characterization of AD-MSCs

2.5.1. Immunocytochemistry

Paraformaldehyde (4%) was used to fix cells for 20 min at room temperature. Cells were washed in PBS 3 times for 5 min each. The cells were permeabilized using fresh 0.2% Triton X-100 in PBS for 5 min and then washed three times with PBS for 5 min each [38]. Secondary anti-polyvalent stain was also used as per the manufacturer’s protocols. First, nonspecific background staining was reduced by incubating the slides in blocking buffer for 1 h at room temperature and then washed four times with PBS. Next, the slides were incubated in primary antibodies against CD105, CD90 (2:100), and CD45 (1:100) for 1 h at room temperature and then washed four times with PBS buffer for 5 min each. Ultra-Tek anti-polyvalent stain was applied and incubated for 10 min at room temperature and then immediately counterstained and coverslipped.

2.5.2. Flow cytometry analysis for the characterization of AD-MSCs

Flow cytometry was performed by the Assiut University Faculty of Medicine. AD-MSCs were trypsinized (1% trypsin-EDTA, Sigma-Aldrich) after the third passage and then centrifuged. The cell pellets (1 μg/10⁶ cell) were suspended in 1% FBS/PBS for 30 min on ice and then incubated in fluorescein isothiocyanate (FITC)-conjugated anti-rat CD27 and CD105, or CD45 and CD34 monoclonal antibodies (BD Pharmingen, San Diego, CA, USA). Data were analyzed using the FCS Express 7 software [39,40].

2.6. Western blot analysis

Brain samples were homogenized in RIPA Lysis Buffer (1% Nonidet-P40, 1% Triton X-100, 0.5 % Na deoxycholate, 150 mM NaCl, 1 mM PMSF, 5 mM EDTA, 10 mM EGTA, 50 mM Tris–HCl, and 1% leupeptin/peptatin protease inhibitor cocktail). The protein concentration was estimated. SDS-PAGE (10 %) was utilized to resolve protein aliquots, which were transferred onto a nitrocellulose membrane. The membranes were blocked with 5% skim milk in TBS containing 0.05% Tween 20 and then incubated with primary antibodies overnight at 4 °C. Subsequently, the membranes were incubated with HRP-conjugated secondary antibodies in the blocking solution for 1 h at 24 °C. A Chemiluminescent Substrate Kit was used to visualize immunodetected bands. The anti-actin goat polyclonal antibody and Rb anti-goat HRP-conjugated antibody were used for the confirmation of equal loading. The data are expressed as mean ± SE from at least three separate experiments; the optical density of the bands was estimated as uncalibrated optical density using the ImageJ software [41].

2.7. Immunohistochemistry study

Paraffin-embedded tissues were deparaffinized in xylene, rehydrated in a series of ethanol solutions (100 % to 70 %), and submerged in water. Antigens were retrieved by boiling the slides in 1 mM EDTA, developing sections in 3% H₂O₂ for 10 min, washing with wash buffer (1X PBS) for 5 min, and then blocking each section at room temperature with 100–400 μL blocking solution for 1 h. The blocking solution was removed, and cleaved caspase 3 primary antibody was added (1:10). The antibody solution was removed, and the sections were washed for 10 min with wash buffer. Secondary antibodies were applied (1: 5000) to each incubated portion for 30 min and then removed. The sections were washed and then stained for 2–3 min with 3’, 3’-diaminobenzidine (DAB) and counterstained with hematoxylin for 2–5 min. The reaction was immediately quenched in distilled water. A light microscope was used to visualize the stained sections [42].

2.8. Quantitative real-time PCR (qRT-PCR)

RNA was extracted from brain samples using the RNA Simple Mini Kit (Invitrogen). Reverse transcription was performed using the SMART-PCR cDNA synthesis kit (Clontech Inc., Palo Alto, CA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in duplicate in 25-μL reaction mixtures containing 1-μL cDNA template, SYBR Green PCR Master Mix, and 10 pmol of each primer Master Mix. The sequences of primers are as follows: Sox2 forward, 5’-AAGGTCTGCTGGTGGTTT-3’ and reverse, 5’-ACGAAGATGGTCTTGGCAG-3’; Oct4 forward, 5’-TGTTCTCGTCATCGTCTCGG-3’ and reverse 5’-CCCGTTGTGCTGTTCAAT-3’ [43], and GAPDH forward, 5’-AATTTGGCATTGTGGAAGG-3’ and reverse, 5’-GTCCTGCTGGTGGCAGTGAT-3’. Reactions were performed in an I Cycler IQ (Bio-Rad). The level of each cDNA amplicon was standardized to that of GAPDH mRNA in the equivalent sample. The data are expressed as mean ± SE from at least three separate experiments.

2.9. Chromosome detection (SRY gene) by PCR

Three days after the AD-MSCs transplantation, PCR was employed to identify rat Y-chromosome-specific SRY genes from the brains of female rats. DNA was extracted from the brain using the QIAamp Tissue Kit (Qiagen, Valencia, CA, USA) as per the manufacturer’s protocols. First-strand DNA was elongated for 1 h at 42 °C. Then, DNA strand amplification was performed via 35 thermal cycles consisting of 95 °C denaturing for 30 s, 60°C annealing for 45 s, and 72 °C extension for 2 min. PCR products were then separated by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV transillumination. The sequences for the SRY gene were 5’-CATGAACG-CATTACGATGTCGTGGTC-3’ and 5’-CTGGGGAAGCAAACAGCAATCTCTT-3’ [44].

2.10. Determination of monoamine oxidase (MAO)-A and B levels and total peroxide

The activity of MAO-A and B was determined using ELISA Kit according to the manufacturer’s protocols (USA). In a microplate reader (Synergy HT; BioTek, USA), absorbance was measured using excitation within the 530/25-nm range and 590/20-nm emission detection [45]. The total peroxide content was assayed with xylenol orange as follows: first, 30 μL of tissue homogenate was incubated in 1 mL of 2.4 mg of FeSO₄. Then, 2.5 mL of distilled water, 65 μL of H₂SO₄, 22.5 mL of absolute ethanol containing 20 mg of butylhydroxytoluene, and 2 mg of xylenol orange was prepared at room temperature; tissue homogenate was reacted for 30 min [46].

2.11. Histological examination and histopathology scores

For histological and histopathological examinations, tiny pieces of brain tissue were fixed in 10 % neutral formalin (pH 7.2). Paraffin sections with a thickness of 5 μm were prepared and then stained with hematoxylin and eosin. Five parameters of brain histopathology (cytoplasmic vacuolization, region of degeneration, nuclear condensation, nuclear fragmentation, and inflammation) were scored for brain injury and histopathology [47] using the ImageJ software.

2.12. Statistical analysis

The results from all the quantitative data are indicative of at least three independent determinations. Values are expressed as mean ± SE. Student’s t-test was conducted to compare the parameters between two groups. Statistical analyses were performed using analysis of variance, and the difference was deemed significant at p < 0.001. The outcomes of the NaCl group were correspondingly similar to those of the control group; thus, only the results of the control group were shown.
3. Results

3.1. X-ray diffraction (XRD) of Al$_2$O$_3$-NPs

Five Bragg reflections corresponding to 20°(173), 38.54°(381), 45.26°(400), 67.72°(450), and 85.03°(99) were observed on sets of lattice planes, respectively. These were indexed according to the facets of the face-centered cubic (fcc) crystal structure of Al$_2$O$_3$-NPs (Research Nanomaterials USA, stock US3023) (Fig. 1a).

3.2. Transmission electron microscopy (TEM)

The diameters and frequency distributions of Al$_2$O$_3$-NPs are presented in Fig. 1b–c. The average particle size and SD were
Table 1
Dynamic light scattering measurements:

| PDI          | Average particle size by number (n.m) | Average particle size by intensity (n.m) | Formulation |
|--------------|--------------------------------------|----------------------------------------|-------------|
| 0.239 ± 0.029 | 1577 ± 120.2                         | 2257 ± 188.3                           | 1           |

Fig. 2. Detection of AD-MSCs via phase-contrast microscopy on different days and passages. (a) Day 0, (b) day 1, (c) passage (P)1, (d) P2, (e) P3 (200X), and (f) P3 (400X). AD-MSCs were characterized by expansion and morphology at different passages. The arrows indicate fibroblastic appearance.
38.31 ± 2.45 nm (n = 60) for 20 nm of Al$_2$O$_3$-NPs.

3.3. Dynamic light scattering (DLS)

The particle size in terms of intensity and numbers are shown in Table 1. Since DLS calculates the hydrodynamic diameter of the particles, the particle size measured by DLS was bigger than that measured by TEM micrographs.

3.4. Identification and characterization of AD-MSCs

Immediately after isolation at culture day 0, AD-MSCs appeared circular and were in suspension (Fig. 2a). After 1 day of differentiation, the cells started to attach in a thin spindle shape (Fig. 2b). AD-MSCs were differentiated in different passages: in passage one (P1), some of the cells appeared spindle-shaped (Fig. 2c); in passage two (P2), the cells formed small colonies (Fig. 2d); and in passage three (P3), the cells had fibroblastic appearances (Fig. 2e, 200×, and Fig. 2f, 400×).

The immunocytochemistry of AD-MSCs at P3 showed a strong
CD45 and CD34 expression of CD27 and CD105, and positive detection of CD105 (Fig. 3). M.M. Atia and A.A.I. Alghriany demonstrated the expression of stem cell markers, especially CD27 (Fig. 4a). Contrarily, less than 1% of the cell populations showed CD45 or CD34 expression (Fig. 4b). The cell surface of at least 98% of AD-MSCs (Fig. 3a–f, 200× and 400×). The identity of AD-MSCs was confirmed by dual flow cytometry analysis at passage 3. The cell surface of at least 98% of AD-MSCs demonstrated the expression of stem cell markers, especially CD27 and CD45, which was not detected there (Fig. 3e–f, 200× and 400×). The expression of Sox2 and Oct4 by AD-MSCs. The PCR products of the SRY gene in the brain tissues of all groups are presented in Fig. 8. The SRY gene of male rats could be detected in the brain homogenates of female rats that received AD-MSCs transplantation. Lane 4 shows that AD-MSCs can migrate to the site of injury in the brain. However, no SRY gene products were detected in lanes 1, 2, and 3. Western blot detection revealed changes in protein levels in the brains of rats treated with Al₂O₃-NPs (6 mg/kg) for 20 days. The Al₂O₃-NPs and recovery rats exhibited significantly increased levels of p53, cleaved caspase-3, P450, and Aβ (117.7%, 127.5%, 190.3%, and 589.4% and 219.4%, 208.1%, 212.2%, and 381.9%, respectively) compared with the control. Also, co-treatment of Al₂O₃-NPs and AD-MSCs significantly decreased protein levels close to the levels of the control group (29.0%, 32.0%, 47.7%, and 72.3% compared with the Al₂O₃-NPs-treated rats) (Fig. 5a–e).

3.6. Immunohistochemistry (IHC) detection of cleaved caspase-3

Immunoperoxidase DAP staining was negative for immunoreactivity against cleaved caspase-3 in the control group in neurocytes and pyramidal cells in the vast majority of the hippocampus (Fig. 6a1–a2). Immune reaction against cleaved caspase-3 demonstrated a sharp increase and large homogeneous brown patches in the neurocytes of the hippocampal region in Al₂O₃-NPs-treated group (Fig. 6b1–b2, e) and the recovery group (Fig. 6c1–c2, e). Densitometry calculation revealed that the levels of cleaved caspase-3 increased by 457.1% and 762.0%, respectively, compared with the control group. In the Al₂O₃-NP + AD-MSCs group (Fig. 6d1–d2, e), no brown patches or only a few were observed in the hippocampal region, confirming the reduction of cleaved caspase-3 (67.3% compared with the Al₂O₃-NP-treated group).

3.7. Enhanced expression of Sox2 and Oct4 by AD-MSCs

The levels of Sox2 and Oct4 mRNA were quantified in the brain tissues by qRT-PCR. These levels were significantly increased by 1.0- and 0.3-fold, respectively, in the Al₂O₃-NPs + AD-MSC rats versus the Al₂O₃-NPs-treated group. Contrarily, the levels of Sox2 and Oct4 mRNA were significantly decreased in the Al₂O₃-NPs and recovery groups (0.8- and 0.5-fold and 1.07- and 0.6-fold, respectively) compared with the control group. This observation confirms the enhanced expression of Sox2 and Oct4 by AD-MSCs.

3.8. Y-chromosome-specific gene detection in the brain tissue of female rats

The PCR products of the SRY gene in the brain tissues of all groups are presented in Fig. 8. The SRY gene of male rats could be detected in the brain homogenates of female rats that received AD-MSCs transplantation. Lane 4 shows that AD-MSCs can migrate to the site of injury in the brain. However, no SRY gene products were detected in lanes 1, 2, and 3.

3.9. Measurements of the total peroxide levels and MAO-A and MAO-B activities

The level of the brain total peroxide and MAO-A and MAO-B activities in female rats administered Al₂O₃-NPs and recovery treatments were significantly upregulated (52.8%, 43.77%, and 136.2%, and 105.1%, 175.3%, and 211.1%, respectively) compared with the control. However, the Al₂O₃-NPs + AD-MSCs-treated group exhibited a significant downregulation of protein levels and activities by 49.1%, 48.0%, and 52.4%, respectively, compared with the Al₂O₃-NPs-treated group (Fig. 9).

3.10. Light microscopic examination

The control group exhibited proper hippocampal histochemical structure made up of a polymorphic layer, a granular layer, and a molecular layer. The granular layer mainly consists of neurons with small processes in the CA1 region, which are called small pyramidal cells (Fig. 10a), and large processes in the CA3 region, which are called large pyramidal cells (Fig. 10a). Between the pyramidal cells and in the molecular layer, there are glial cells. The hippocampus exhibited several dystrophic changes after treatment with Al₂O₃-NPs, characterized by small cell degeneration and shrinkage which surrounded by empty space, and large pyramidal cells with pycnotic and condensed nuclei in the CA1 (Fig. 10c) and CA3 (Fig. 10d) regions, respectively. In some pyramidal cells, the cytoplasm was also highly vacuolated. Between pyramidal cells and the dilatation of blood vessels in the polymorphic

Fig. 4. Flow cytometric analyses of AD-MSCs. (a) Cells were positive for the expression of CD27 and CD105, and (b) cells were negative for the expression of CD45 and CD34.
and molecular layers, several rarefied areas were observed. The recovery group demonstrated persistence of the harmful effects of Al$_2$O$_3$-NPs by a marked reduction in the granular layer thickness and a decline in the number of pyramidal cells in the granular layer in the CA1 (Fig. 10e) and CA3 (Fig. 10f) regions.

Multiple pyramidal degenerated cells were observed upon treatment with Al$_2$O$_3$-NP. Compared with those in the Al$_2$O$_3$-NPs-treated group, there was an increase in the number of rarefied areas. Stem cell treatment (Fig. 10g–h) exhibited a marked improvement in pyramidal cells, especially in small pyramidal cells. With prominent processes similar to

Fig. 5. Immunoblot and quantified densitometric analysis conducted to assess the effects of Al$_2$O$_3$-NP exposure and various treatments on the changes in the protein levels of P53, cleaved caspase-3, CYP1A1 (P450), and Aβ. The corresponding antibodies were used to assess the changes in the protein levels. Equal protein loading was confirmed by reprobing with anti-actin antibodies as a protein loading control. (a–e) Values with different letters indicate significant differences ($p < 0.001$).
those in the control group, the vast majority of the pyramidal cells demonstrated vesicular and rounded nuclei. However, a few pyramidal cells (small and large) were still reduced with condensed nuclei and dense cytoplasm. The histopathological score of the hippocampal cells was evaluated using Heijnen’s method; the highest scores were obtained in the Al$_2$O$_3$-NPs-treated and R groups. Experimental groups treated with AD-MSCs exhibited a substantial reduction in score relative to the other groups (Fig. 10 I).

4. Discussion

The present study found that AD-MSCs can rescue the hippocampus against the deleterious effects of Al$_2$O$_3$-NPs. These data are in agreement with the different reports on the role of MSCs having antioxidant, immunomodulatory, and anti-inflammatory effects [48]. It has been demonstrated that AD-MSCs promoted neuroectodermal differentiation and repair and reduced apoptotic protein levels [49,50]. MSCs also led to the downregulation of both caspase-3 mRNA expression and Bax protein expression after injection following treatment with cisplatin [24,51]. Wen et al. [52] confirmed that bone marrow MSCs also have defensive efficacy and reduced oxidation (malondialdehyde levels) and cytochrome c, eliminating neural cell damage as a result of oxidative stress reduction. These cells secreted different factors, such as nerve growth factors and brain-derived neurotrophic factor, which are major tools in the treatment of neurological damage.

The current investigation found that Al$_2$O$_3$-NPs treatment and recovery led to the stimulation of ROS and oxidative stress. The high deposition of A$eta$ protein in the hippocampal region of the brain is an indicator of ROS. The high levels of A$eta$ protein induce apoptosis through the activation of proapoptotic pathways via cleaved caspase-3, P53, and P450. Also, the co-administration of AD-MSCs decreased apoptotic cell death by decreasing the levels of these proteins in the hippocampal region of the brain. Bodart-Santos et al. [53] used MSCs of human Wharton’s jelly to protect the hippocampal neurons in an A$eta$-exposed cell culture. These cells could prevent synapse damage caused by amyloid-$eta$ and oxidative stress. In rats, a significant change was observed in the mRNA expression of A$eta$, CYP450 enzyme CYP 1A2, and oxidative stress markers following oral exposure to a high dose of nano-copper [54]. A$eta$ accumulation induced mutation in mitochondrial DNA, which led to mitochondrial dysfunction [8,55] and initiation of
lipid peroxidation [43].

Cell apoptosis was induced via Aβ deposition in the brain, leading to cleavage of caspase-3, which is capable of cleaving genomic DNA during apoptosis [34]. Liu et al. [12] revealed that nano-Al₂O₃ reached the brain via the olfactory nerve pathway, resulting in a severe decrease in the expression of Bcl-2 and Mdm2 and stimulating P53 and P21 expression. These observations are supported by reports that nano-alumina and NPs can alter the ability of the mitochondrial

Fig. 7. (a–b) qRT-PCR and expression analysis of Sox2 and Oct4 in different treatments. Results and values were normalized to GAPDH mRNA levels.

Fig. 8. Agarose gel electrophoresis for PCR products of the SRY gene in the brain tissues of different groups. SRY-positive DNA marker in lane M; groups 1, 2, and 3 were negative for SRY, and group 4 was positive for the SRY gene in lane 4 after the female rats received AD-MSCs from male rats (GADPH served as an internal reference gene).

Fig. 9. The total peroxide levels and MAO-A and MAO-B activities in the brain homogenates of the control and treated rats. Values indicated by unlike letters were significantly different (p < 0.001).
membrane to perform its function. Also, they can trigger oxidative stress, disrupt integrity, and decrease protein secretion [56]. Al-NPs can also alter the brain membrane by reducing the integrity of lipoproteins, and partial BBR damage caused by Al accumulation causes damage to both the hippocampi of rats in vivo [5] and human hepatocellular carcinoma cells [57] and liver and kidney cells [58, 59].

Our results indicate an increase in brain Sox2 and Oct4 expression levels after the administration of AD-MSCs compared with the Al2O3-NPs-treated group nearly like control. Accordingly, other data indicated that SOX2 overexpression, which enhances OCT4 expression through genetic modification, improved brain neuronal differentiation. Sox2 and its family members activate signaling pathway molecules and pluripotent transcription factors that directly or indirectly influence neuron protection as well as control its expression to treat brain tumors in humans and mice [60].

Alshatwi et al. [61] were observed ANP toxicity in human mesenchymal stem cells (hMSCs) may be induced by an increase in oxidative stress after only 24 h of exposure and there were dose-dependent effects. In response to ANPs, the expression levels of oxidative stress-responsive enzymes CYP1A were up-regulated, and the antioxidant enzyme SOD expression was found to be significantly reduced. That confirms our result, after a 5-day of AD-MSCs injection via caudal vein they reduce the toxicity of Al2O3 NP due to its immunomodulatory response.

AD-MSCs also release different trophic factors that attenuate neuroinflammation, promote angiogenesis and neurogenesis, and reduce apoptosis. MSCs are induced to secrete anti-inflammatory factors via pro-inflammatory signals, such as lipopolysaccharide, tumor necrosis factor-α, and nitric oxide [62]. AD-MSCs are largely capable of treating hidden damage via a variety of anti-inflammatory cytokines and chemokines [26]. Most MSCs do not cross the BBB to the site of injury but instead modify periphery immunologic factors [63, 64]. For example, prostaglandin E2 serves as a potent immunomodulatory factor that is constitutively expressed via the COX-2 anti-inflammatory pathway [65]. In the injured gastric mucosa in rats, BM-MSCs and AD-MSCs have been

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**Fig. 10.** Photomicrographs in the CA1 region (a, c, e, and g) and the CA3 region (b, d, f, and h) of the hippocampus proper. (a) The control group, showing polymorphic layer (PL), granular layer (G), molecular layer (M), small pyramidal cells (↑), neural processes (△), and glial cells (white arrow). (b) The control group, showing large pyramidal cells (↑), large neural processes (△), and glial cells (white arrow). (c–d) The Al2O3-NPs-treated group showing degenerated and shrunken pyramidal cells surrounded by spaces (↑), vacuolated cytoplasm (white arrow), rarefied areas (△), and dilated blood vessels (asterisk). (e–f) The recovery group showing degenerated pyramidal cells (↑) and many rarefied areas (△). (g–h) The Al2O3-NP + AD-MSCs group showing pyramidal cells with vesicular nuclei and prominent processes (↑) and a few shrunken pyramidal cells with condensed nuclei (△). (H&E stain, scale bar =50 μm). (i) The histopathology score of the liver was measured as defined in the methods section. The results are presented as the mean standard error of at least three separate experiments. The letters of the columns are significantly different (p < 0.001).
identified by the SRY gene [66], which is in agreement with our results that some AD-MSCs will move to the site of brain injury after BBR damage.

Another explanation was observed the role of AD-MSCs to reduce the effect of AlO3-NP may be due to the reduction of microglia activation. Microglia are the brain's resident immune cells and play an important role in neuroinflammation and, as a consequence of injury, undergo phenotypic transformation and activation [67]. Microglia activation is associated with chronic neuronal inflammation following brain injury. Activated microglia secrete IL-1α and TNF-α after injury, which stimulate neurotoxic reactive astrocytes, restricting neuron survival, outgrowth, and synaptogenesis [68]. M1 phenotype microglia release pro-inflammatory cytokines and oxidative mediators, whereas M2 phenotype microglia release anti-inflammatory cytokines and neurotrophic factors [69]. Ruppert et al. [70] found that AD-MSCs effectively reduced the number of M1 microglia 3 days after injury. However, the percentage of M2 microglia was increased 14 days after injury, indicating an anti-inflammatory change.

Recently, chronic exposure to ethanol in rats was demonstrated to increase MAO-A and MAO-B protein activities that stimulated the apoptotic cascade in renal epithelial cells. These cells were the key source of ROS, H2O2 generation, and mitochondrial cytochrome c release [71]. Total peroxide is also a possible destructive ROS marker and has been shown to be generated in pathological conditions at high concentrations [72]. Our findings are in line with those of previous researchers in terms of the elevation of these proteins and their activities due to AlO3-NP exposure. However, after AD-MSCs co-administration, a return of total peroxide levels & MAO-A & MAO-B activities nearly to normal state was observed. All of our AlO3-NP exposure outcomes were supported by histopathological changes in the hippocampal neurons. Other findings in various tissues also supported our observations, whereas acute doses of Al-NPs and AlCl3 have been demonstrated to cause histopathological alterations in the liver, brain, and kidney [58, 73]. The restoration of the cerebral cortex structure and hippocampal cells in aluminum-administered rats to the normal state has also been demonstrated due to the endogenous nerve growth factors and immunomodulatory influenced by BM-MSCs [23,74].

5. Conclusions

Our findings indicate that exposure to and recovery from AlO3-NPs induced toxicity in the hippocampal region of the brain. AlO3-NPs were responsible for the induction of apoptosis via two pathways: stimulation of oxidative stress and increase in Aβ protein levels. The high level of Aβ induces apoptosis directly by activating proapoptotic pathways via cleaved caspase-3, PS3, and P450 and indirectly by increasing the levels of total peroxide and the activities of MAO-A and MAO-B as well as decreasing the expression of Sox2 and Oct4 proteins. The co-administration of AD-MSCs reduced toxicity induced by aluminum oxide nanoparticles and restored the expression of the above proteins to their normal state by regulating the levels of Sox2 and Oct4.

Author contributions

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Conception and designed the work, supplied materials, Conceptualization, Methodology, isolated AD-MSCs, Software, Data curation, Writing- Original draft preparation, Supervision, Reviewing, analyzed and interpreted data, final approval of the version to be published.

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Visualization, Investigation, Assisted with experiments, Validation, Writing, Reviewing, supplied materials, contributed to the study design, isolated AD-MSCs, and wrote histopathology and reference portions of the manuscript.

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Declaration of Competing Interest

The authors declare no competing financial interests.

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