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Research article

Mesenchymal stem cells act as stimulators of neurogenesis and synaptic function in a rat model of Alzheimer's disease

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ABSTRACT

Background: Alzheimer's disease (AD) is one of the most common NDs leading to cognitive dysfunctions and dementia which are progressively worsen with age. Cell therapy is currently of particular interest in treatment of neurodegenerative disease (ND) such as AD. However, the effective treatment for AD is yet to be found.
Objective: In this study, the possible roles of human umbilical mesnchymal stromal cord (hUMSCs) and adipose mesenchymal stem cells (hAD-MSCs) in neurogenesis and synaptic function were investigated using a β-amyloid 1–42 (β A42)-induced AD rat model.
Methods: hUMSCs and hAD-MSCs were isolated from umbilical cord stroma and adipose tissue, respectively. The expression of Mesenchymal (CD73, CD90 and CD105) and hematopoietic (CD45 and CD133) markers of hUMSCs and hAD-MSCs were confirmed by flow cytometry. Alzheimer's rat model was created by β-amyloid 1–42 injection into the hippocampus and confirmed by Morris Water Maze and immunohistochemical staining. hUMSCs and hAD-MSCs were injected in Alzheimer's rat model, intravenously. Deposition of β-amyloid in the CA1 of hippocampus was assayed 3 months after cell administration. The expression of synaptophysin and GAP43 proteins was assessed by Western blot. Neural death was assessed by Nissl staining.
Results: The data obtained from flow cytometry showed that surface mesenchymal and hematopoteic markers of the fibroblastic like cells isolated from adipose tissue and umbilical cord were expressed highly in hUMSCs and mostly in hAD-SCs. Transplantation of MSCs reduced β-amyloid deposition in the hippocampus of the AD rats compared to the β-amyloid group. The rate of neuronal cell death in the hippocampus of the β-amyloid-treated rats was significantly increased compared to that of the control group. The percentage of apoptotic cells in this group was 72.98 ± 1.25, which was significantly increased compared to the control group. Transplantation of either hUMSCs or hAD-SCs, respectively, resulted in a significant reduction in the apoptotic rate of the neuronal cells in the hippocampus by 39.47 ± 0.01 ($p = 0.0001$) and 43.23 ± 0.577 ($p = 0.001$) compared to the β-amyloid group. MSC transplantation resulted in a significant up-regulation in the expression levels of both synaptogenic (synaptophysin) and neurogenic markers (GAP43) by 1.289 ± 0.112 ($P = 0.02$) and 1.112 ± 0.106 ($P = 0.005$) fold in the hUMSCs-treated group and 1.174 ± 0.105 ($P = 0.04$) and 0.978 ± 0.167 ($P = 0.008$) fold in the hAD-SCs-treated group, respectively.
Conclusion: Intravenous injection of hUMSCs and hAD-MSCs is a safe approach that improves synaptic function and neurogenesis via up-regulation of synaptophysin and GAP43 protein expression levels, respectively, in Alzheimer's model. Intravenous injection of both applied SCs could improve learning and cognitive impairment induced by β A42 injection.

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1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases (NDs), that leads to cognitive dysfunctions and dementia, which progressively worsen with age (Alzheimer's, 2010). Accumulation of toxic proteins, including extracellular β-amyloid (Aβ) plaques and intracellular hyperphosphorylated microtubule-stabilizing tau protein, called neurofibrillary tangles (NFTs), in various areas of brain, such as hippocampus and cortex, play important roles in the pathology of this disease. High levels or depositions of Aβ may trigger release of inflammatory cytokines or neuroinflammation from glial cells, and result in impaired synaptic plasticity, which ultimately leads to neuronal loss (Jahn, 2013; Paulson et al., 2008; Sadigh-Eteghad et al., 2015). Synaptophysin and growth-associated protein 43 (GAP-43) (also known as neuromodulin), an immature neural protein, are needed for maintenance of synaptogenesis as well as neural regeneration (Carriel et al., 2017; Meyer and Smith, 2006). The expression levels of these two genes and protein are down-regulated in AD (Bogdanovic et al., 2000; Goetzl et al., 2016). Therefore, extending the current knowledge about neurogenesis and synaptic function would help to introduce more effective therapeutic strategies targeting AD.

MSCs are pluripotent stem cells (SCs) with high capacities for self-renewal and multidirectional differentiation (Bakhtiarimoghadam et al., 2021; Najafi et al., 2020). They can be used as a reliable source of neural cells for regenerative medicine treatment. Because of the limited ability of brain self-regeneration, the conventional treatment of neurodegenerative disorders has not achieved desirable results (Yao et al., 2020). MSCs which have strong differentiation capacities have also low immunogenicity and immunomodulatory functions; and have immunoregulatory properties (Ebrahimi-Barough et al., 2015). Latest developments in the field of SC transplantation and ability of SCs to differentiate into various types of CNS neurons and glial cells, have received particular attention as a potential approach to treat various NDs, such as AD. It has been demonstrated that decreases in the amonut of Aβ plaques and NFTs result in microglial cell activation and increased neuronal survival (Hickman et al., 2018). Zhang et al. (2014) have reported that SC transplantation increased expression levels of synaptophysin and GAP-43 proteins, which are essential for neurogenesis (Zhang et al., 2014). SC therapy using MSCs is one of the therapeutic approaches treating AD. MSCs are heterogeneous multipotent populations of stromal cells that are found in several organs, including bone marrow, adipose tissue and umbilical cord. MSCs are applicable for transplantation into injured organs due to their Immunomodulatory roles (Shirian et al., 2016). Various sources of MSCs, including human umbilical cord blood, placental tissue, autologous adipose tissue, and ischemia-tolerant MSCs, are being tested in clinical trials. Accessibility, invasiveness, potential tetratomic induction, proliferation rate, cost, and efficacy should be thoroughly evaluated and compared (Fang et al., 2018). In this study, the possible roles of hUMSCs and AD-MSCs in neurogenesis and synaptic function were investigated using a β-amyloid 1–42 (β A42)-induced AD rat model. Regarding to MSCs have been shown to have potential capacity for NDs, the capacity of two sources of MSCs including hAD-MSCs and hUMSCs were investigated to find the better source for AD in clinic due to the easier isolation from each patient as autologous SCs.

2. Methods and materials

2.1. Ethic statement

All procedures were performed based on the guidelines of the ethical committee of Iran University of Medical Science.

2.2. Isolation of hAD-MSCs

Human AD-MSCs were obtained from the surface layer of abdomen using liposuction procedure from individuals between the ages of 25–45 years old. The procedure was performed in a general operating room, as previously described by Dubois et al. (2008), after receiving a signed informed consent from all the participants (Dubois et al., 2008). Briefly, human AD-SCs were extracted from 250 mg adipose tissue specimens, which were washed twice with phosphate buffer saline (PBS) containing 1% penicillin-streptomycin (P/S) (Gibco, Massachusetts, USA). The samples were then incubated in 10 ml of collagenase type I (Gibco, Massachusetts, USA) in an incubator (5% CO_2, at 37 °C) for 30 min. After digesting the specimens, the samples were centrifuged for 10 min at 250–300 g, the supernatant was discarded, the palettes were resuspended in DMEM-F12 (Gibco, Massachusetts, USA) containing 10% fetal bovine serum (FBS) (Gibco, Massachusetts, USA) and 2×10^4 cells/cm^2 transferred to 25 cm^2 culture flasks. The flasks were then maintained in an incubator (5% CO_2, at 37 °C). The 70–80% confluent cells were detached with 0.25% Trypsin-EDTA (Gibco, Massachusetts, USA) followed by centrifugation at 250–300 *g* for 5 min. The cells were transferred to new flasks. After 3 passages, the cells were used for further experiments.

2.3. Isolation of hUMSCs

Fresh human umbilical cord samples were collected from full-term births at Rasoul Akram Hospital (Tehran, Iran) and were immediately transferred to the laboratory. The explant method was applied for isolation of hUMSCs. The specimens were washed using sterile PBS containing 1 % P/S and cut into 3–5 cm pieces. Vessels were removed prior to cutting the Wharton's jelly into small pieces, followed by transferring them to 25 cm^2 culture flasks containing DMEM-F12 supplemented with 10 % FBS. The culture media was changed every 2–3 days. The tissue sections were removed from the culture flasks after 10–20 days of being in culture and observing the appearance of mesenchymal cells. The cells at 70–80% of confluency were detached with 0.25 % Trypsin-EDTA and centrifuged at 250–300 *g* for 5 min. The cells were passaged three times before being used for further experiments.

2.4. Determining cell identity using flowcytometry

The cultured cells at passage 3 were evaluated for surface markers in order to determine their identity. The cells were harvested and centrifuged at 250–300 *g* for 5 min followed by resuspension of the cell pellet in PBS to the desired concentrations. A total of 10^5 cells were tested per antibody. The cells from both sources were separately incubated with monoclonal antibodies against CD-133 (#ab252553), CD-45 (#ab123522), CD-73 (#ab130451), CD-105 (#ab234265), and CD-90 (#ab23894) (Abcam, Cambridge, UK) for 20 min at room temperature in the dark. The primary antibodies were then removed and cells were washed with PBS followed by incubation with secondary antibodies which were conjugated with human FITC, PE or PerCP antibody at 4 °*C* for 30 min. Finally, the cells were fixed with 1% paraformaldehyde (PFA) solution (Sigma, Missouri, USA) and evaluated by flow cytometry (Partec PAS III Flow Cytometry System) and analyzed using Flomax software.

2.5. Animals and procedure

Forty male Wistar rats, five months old, weighing 250–320 g were used in this study. The rats, which were obtained from the Experimental and Comparative Studies Center of Iran University of Medical Sciences, were kept under the same condition of a 12:12 light: dark cycle at 22 ± 3 °C. During the experiment, rats were fed by special plates and provided with access to water. All procedures were performed based on the guidelines of the Ethical Committee of Iran University of Medical Science.

To induce AD in the rat, the animals were anaesthetized with ketamine hydrochloride (100 mg/kg) and Xylazine (2.5 mg/kg), followed by injection of 5 μL β-amyloid to the CA1 hippocampal area using a Hamilton syringe (AP: 3.8, ML: ±2.4, DV: 2.9). Animals were randomly divided into 5 groups (n = 10 per group); Control, Model (AD), Sham,

human AD-SC and human UMSC groups. Animals in the model group received intra-hippocampal injection of β-amyloid 1–42, while the sham group received aluminum hydroxide (beta-amyloid solvent). Amyloid β 1–42 powder was dissolved in deionized water to a concentration of 2 mg/mL and aggregated by incubation at 37 °C for two days before hippocampal injection. A single dose of this solution was injected. Each of the human AD-SC and human UMSC groups received 3×10^6 of the respective cell suspension in 0.5 μL PBS solution through caudal vein injection. Further investigations were performed 3 months after the initial cell injection.

2.6. Morris Water Maze (MWM)

MWM was performed to examine spatial memory. In this study, a large circular black pool (diameter: 180 cm and height: 60 cm) was filled with opaque water (22 ± 1 °C) to a depth of 35 cm. Alient external cues were placed around the pool to assist the animals in locating the hidden platform. The space around the tank or pool was characterized by 4 cardinal directions north, south, east and west, so 4 alternative positions (northwest, northeast, southwest and southeast) are provided to start measuring the Mauritius water maze. Rescue platform or hidden in this study was located 1 cm under the surface of water in the center of northwest position. The animals were tracked using a camera equipped with infrared light, which is located at a height of 180 cm and above the center of the water maze. The camera was connected to a computer and the swimming information was stored using Ethovision software version 5. The maze was placed in a room where there were various spatial signs and these signs are constant during the experiments and are visible for the animal inside the tank. The experiment lasted 5 consecutive days; each day began at 10:00 AM and ended at 12:00 PM. Training on the MVM, which consisted of 4 sessions of 4 consecutive days, initiated 4 weeks after injection of the beta amyloid. In order to track the desired parameters, namely escape latency and traveled distance, the pool was equipped with a video camera (Nikon, Melville, NY, USA).

Escape latency is the time required for each animal to reach the location of the platform. Travel distance is the length of the swimming path. On day 1 of the experiment, a visual test was performed by making the platform visible. Short-term, or working memory, was evaluated during trial days 2, 3 and 4 by submerging the platform 0.5 cm below the surface of the opaque water. The animals were given 90 s to find the location of the platform during the first 4 days of the trial. The intervals considered between the two consecutive trials and the two blocks of daily testing were 30 s and 5 min, respectively. Animals that were unable to find the platform by themselves were gently guided to it by hand.

Long-term, or reference memory, was measured on day 5 of the experiment during the probe day, by removing the platform from the tank and allowing each rat to search in the area that previously contained the platform On the probe day, all the rats were placed into the pool from the west quadrant. The spent period in the target quadrant as well as the speed of each latancy animal to swim over the previous location of the platform in the pool was recorded.

2.7. Dil staining

To evaluate the cell migration toward the damage site, the injected cells were labeled using Dil staining. A total of 3×10^6 of each hUMSCs and hAD-MSCs were suspended in 1 ml PBS on 2 separate flsks and 5 μl Dil (Sigma, St. Louis, MO, USA) were then added to the each suspension. The solutions were incubated 10 min at 37 °C, and for 10 min at 4 °C to optimize staining levels. The labelled cells were washed in PBS, centrifuged and finally injected. On 3 months post-transplantation, the rats were sacrificed and their brains were harvested, fixed, and serially sectioned at 5-μm. To confirm the cell migration, the sections were examined using a fluorescence microscopy.

2.8. Immunohistochemistry

Deposition of β-amyloid in the CA1 of hippocampus was assayed 3 months after cell administration. The animals were anesthetized and perfused with 4% PFA followed by isolation of their brain which was then cut into 5-μm thick sections. Labeling of the tissue sections was performed by incubating them with anti-β-amyloid 1–42 antibody (1:100, Abcam, Cambridge, UK, #ab10148) at 4 °*C* overnight. Subsequently, the primary antibody was removed and the tissue sections were incubated with goat anti-rabbit IgG Alex flour antibody (1:200, Abcam, Cambridge, UK, #ab150077) at room temperature for 2 h. The immunostained tissue sections were observed using a Labomed fluorescent microscope equipped with an Invenio 6EIII camera (Olympus, Tokyo, Japan).

2.9. Thioflavin S staining

To detection the β-amyloid depositions in the neuronal cells, the histological sections were also stained using Thioflavin S.

2.10. Cresyl violet (Nissl) staining

The anesthetized animals were perfused with 4% PFA in 0.1 M phosphate buffer (pH 7.3), and paraffin-embedded samples were sectioned at 5 μm thicknesses (with an interval of 120 μm). The tissue sections were then deparaffinized and stained with 0.5% cresyl violet. The stained tissue sections were observed using an Olympus microscope (CX31, Tokyo, Japan) which was equipped with a digital camera (Leica, München, Germany) at 400 X magnification. Counting of a total number of 100 cells was then performed using per three high power fields. Viable cells with light cytoplasm and intact morphology stained purple with this staining, while dead cells with triangular shapes stained blue.

Three samples were selected from each group and three slices were sectioned from each sample with 5 micron interval. Finally 3 fields of each CA1 area section were selected for counting.

2.11. Western blot analysis

The rats were deeply anesthetized using intra-peritoneal administration of 40 mg/kg of ketamine and 5 mg/kg of 2% xylazine and the hippocampus samples were obtained for Western blot. Total protein was extracted from hippocampus using RIPA (Radioimmunoprecipitation) buffer containing protease cocktails and phosphatase suppressors (Sigma, Louis, MO, USA). The Bradford assay (Bio-Rad, MI, USA) was applied for evaluation of the protein concentration, and a 12% sodium dodecyl sulfate polyacrylamide gel (SDS–PAGE) was used for proteins separation.

The extracted proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad) which were primed with 5% bovine serum albumin (BSA, Sigma, Louis, MO, USA) in 100 ml TBST buffer. The non-specific binding sites were blocked by 5% nonfat dry milk followed by overnight incubation of the membranes with primary antibodies against rabbit monoclonal anti-GAP43 (Abcam, Cambridge, UK, #ab16053), rabbit monoclonal anti-synaptophysin (Abcam, Cambridge, UK, #ab16659) and rabbit monoclonal anti-GAPDH (Abcam, Cambridge, UK, #ab181602). The membranes were then washed in TBST buffer, three times for 5 min prior to being incubated with horse radish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology, CA, USA, #sc2004) at 37°Cfor 1 h.

2.12. Statistical analysis

All data were analyzed using SPSS software, version 22, and the results express as mean ± SD. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by a Tukeys post hoc test. P values $P < 0.05$ was considered significant.

3. Results

3.1. Cell morphology and identity

Both the AD-SCs and UMSCs presented a spindle shaped fibroblastic-like morphology. Human UMSCs formed colonies around the Wharton's jelly pieces, 10–20 days post-culture (Figure 1A,B). The results of flow cytometry confirmed the mesenchymal identity of the cells. CD133 and CD45 (hematopoietic markers) were expressed at low levels, on the membrane of both human AD-SCs and human UMSCs. The results indicated that 4.81% ± 0.25 and 2.58% ± 0.18 of human AD-SCs expressed CD133 and CD45, respectively. Moreover, 2.58 ± 0.05 and 2.38 ± 0.11% of human UMSCs expressed CD133 and CD45, respectively. However, larger populations of both cell sources expressed CD73, CD90 and CD105; so that, 99.8 ± 0.33, 98.5 ± 0.74 and 99.4 ± 0.41% of human UMSCs respectively expressed CD73, CD90 and CD105, while their respective expression rates in human AD-SCs were 99.4 ± 1.02, 99 ± 0.75 and 99.7 ± 0.81%. These data confirmed the mesenchymal identity of both the cell sources (Figure 2).

3.2. Behavioral assay

The travel distance was quantified in each experimental group in order to test behavioral traits. The data presented in Figure 3 indicated that the escape latency was significantly increased in the AD animals compared to the control group. Therefore, rats in the model groups spent less time in the target quadrant compared to the control group (P = 0.001). The swimming speed was not significantly different between various treatments, control and sham groups (P = 0.807). Therefore, latancy to find the platform was used as an indicator of learning during the probe test Performance, rats of the model group spent less time in the target quadrant compared to those rat in the control group (P = 0.001), while the travel distance was increased in the model group compared to that in the control group (P = 0.001). Both the human AD-SCs and UMSCs injections significantly improved learning performance in the AD-model rats. So that, time spent in the target quadrant in AD-SCs or UMSCs-treated AD rats was significantly increased (P = 0.008 and P = 0.004, respectively), while a significant decrease in the travel distance was detected in these animals (P = 0.002, and P = 0.001, respectively).

3.3. Dil staining findings

The labeled hUMSCs hAD-MSCs with Dil staining crosse BBB and migrate into the hippocampus on post-trasnplantion day 10 (Figure 4).

3.4. Effects of MSC transplantation on β-amyloid expression in the hippocampus

Cellular tracing of β-amyloid protein accumulation is considered as a key indicator of AD diagnosis (Farias et al., 2011). In this study, β-amyloid was traced by IHC assay in the hippocampus. The deposition rate of β-amyloid in neural cells of the model group was 20.15% ± 0.5, which was significantly higher compared to that of the control group (P = 0.001). Transplantation of MSCs reduced β-amyloid deposition in the hippocampus of the AD rats compared to the β-amyloid group. The percentages of β-amyloid deposition in the human UMSCs and human AD-SCs groups were 12.5 ± 0.87 (P = 0.001) and 13.6 ± 0.94 (P = 0.004), respectively (Figures 5 and 6).

3.5. Effects of MSC transplantation on neuronal cell death in the hippocampus

The Nissl staining was performed to assess neuronal cell death in the hippocampus. As illustrated in Figure 7, the rate of neuronal cell death in the hippocampus of the β-amyloid-treated rats was significantly increased compared to that of the control group. The percentage of apoptotic cells in this group was 72.98 ± 1.25, which was significantly increased compared to the control group (P = 0.001). Transplantation of either hUMSCs or hAD-SCs, respectively resulted in a significant reduction in the apoptotic rate of the neuronal cells in the hippocampus by 39.47 ± 0.01 (p = 0.0001) and 43.23 ± 0.577 (P = 0.001) compared to the β-amyloid group.

3.6. Effect of MSCs transplantation improves synaptic function and neurogenesis

Protein expression levels of synaptogenesis marker, synaptophysin, and neurogenesis marker, GAP43, were evaluated by Western blot. Expression levels of both markers were considerably down-regulated in

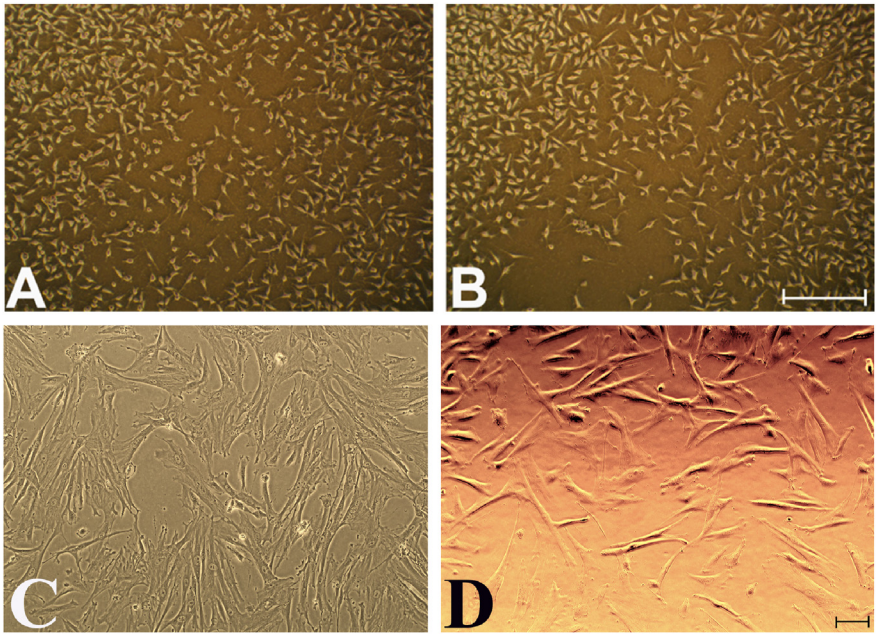

Figure 1. Morphological appearance of human UMSCs (A) and human AD-SCs (B), The third passage cells displayed typical fibroblast morphology with a spindle-shape (Scale bar+ 100 μm).

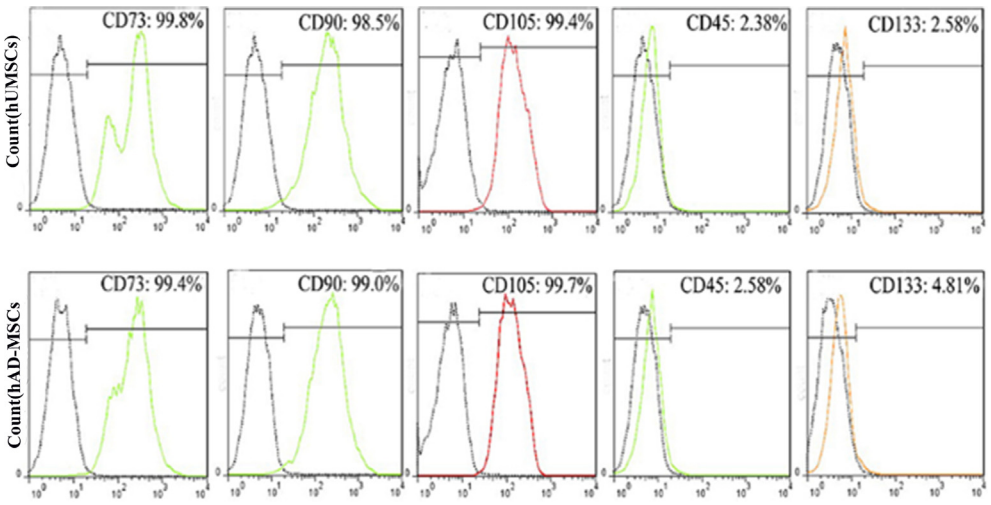

Figure 2. Immunophenotyping findings of human UMSCs and AD-SCs, flow cytometry analysis indicated that human AD-SCs and UMSCs are highly expressed MSCs markers and the expression of hematopoietic markers was observed in only a small percentage of these cells.

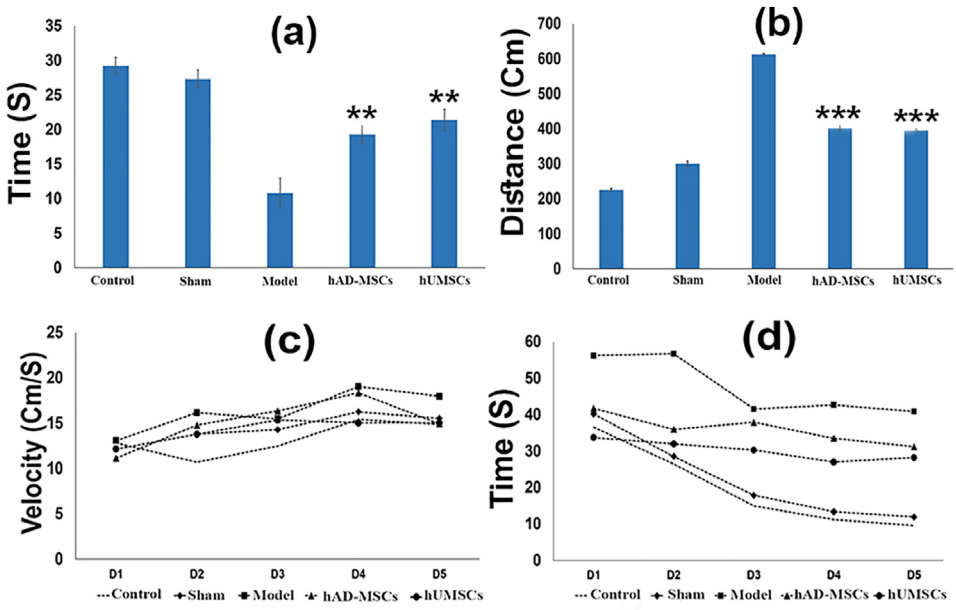

Figure 3. The effect of βA42 injections on the time spent in target quadrant (a) and distance traveled (b) in the probe test during Morris water maze testing. $p \leq 0.05$ was considered as statistically significant. Data are presented as mean ± SD. * indicates $p = 0.008$, ** indicates $p = 0.004$, # indicates $p = 0.002$, and ## indicates $p = 0.001$ (c) and (d) are related to the trials and indicate that all the animals significantly spent less time searching for the platform after 5 days of training.

the β-amyloid group compared to those in the control group. The expression levels of synaptophysin and GAP43 in this group were 0.502 ± 0.097 ($P = 0.0001$) and 0.344 ± 0.077 ($P = 0.0001$) fold, respectively, compared to those in the control group. On the other hand, MSC transplantation resulted in a significant up-regulation in the expression levels of both synaptogenic and neurogenic markers by 1.289 ± 0.112 ($P = 0.02$) and 1.112 ±, 0.106 ($p = 0.005$) fold in the hUMSCs-treated group and 1.174 ± 0.105 ($P = 0.04$) and 0.978 ± 0.167 ($P = 0.008$) fold in the hAD-SCs-treated group, respectively. These changes in the expression levels of synaptogenic and neurogenic markers in the MSC-treated groups were significant compared to those in the β-amyloid group (Figure 8) (Supplementary Figure i.e figure).

4. Discussion

The primary aim of this study was to investigate the possible role of intravenous hUMSCs and AD-MSCs transplantation in neurogenesis and synaptic function by evaluation of protein expression levels of GAP43 and synaptophysin in βA42-induced AD rat. Furthermore, the effect of hUMSCs and AD-MSCs transplantation on neuronal cell death as well as β-amyloid expression level in the hippocampus were evaluated in this study. Although administration of marketed drugs for AD, such as N-Methyl-D-Aspartate antagonists and cholinesterase inhibitors, can improve daily functions of the patients to a certain degree, they are not capable of preventing disease progression (Fang et al., 2018). SC therapy has been receiving increasing attention as a potential approach to treat various ND diseases, such as AD (Kumar et al., 2017). However, the efficacy of this method is still controversial (Gandy, 2005). To develop any SC therapeutic approach and effective cell transplantation, application of an appropriate cell type with characteristics specific to a particular disease is critical (Jindal et al., 2017). Furthermore, suitable cell for neural tissue engineering should be easily cultured and obtained, with present the capacity to differentiate into neural cells (Kumar et al., 2017). The capability of MSCs to differentiate into various types of CNS, including

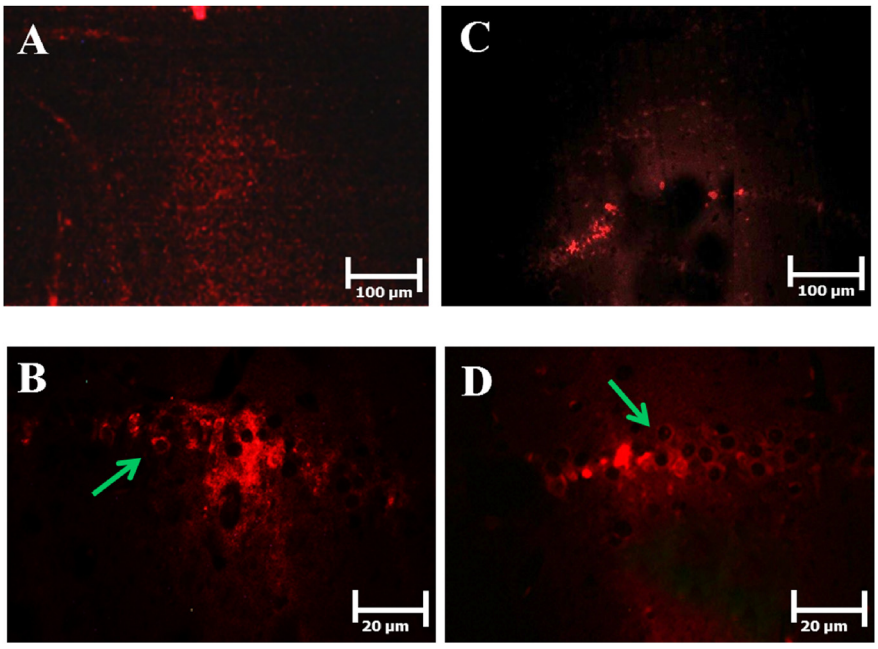

Figure 4. Dil staining, the labeled hUMSCs (A, B) and hAD-SCs (C, D). These cells crossed BBB and migrated into the hippocampus green arrow indicates the transplanted cells 100 μm (upper panel) & 400 μm (lower panel).

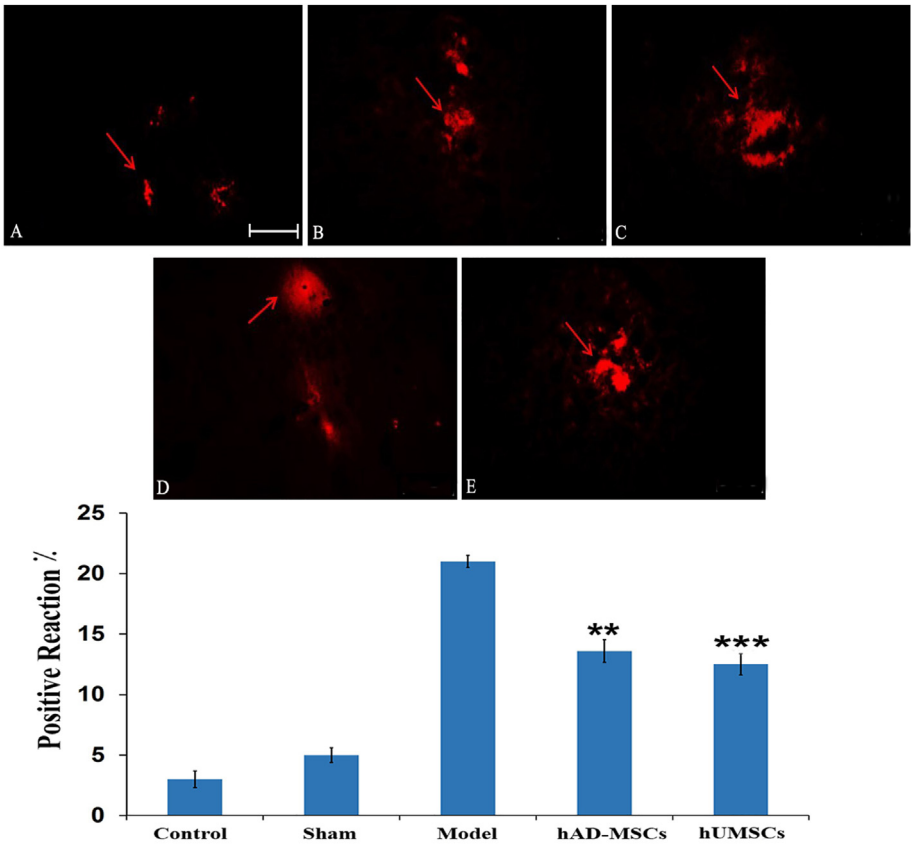

Figure 5. Immunohistochemical analysis of β-amyloid in the hippocampus. (A) Control, (B) Sham, (C) Model, (D) hAD-MSCs and (E) hUMSCs groups (red arrows indicates β-amyloid plaques). Both the hUMSCs and hAD-SCs reduced the volume of β-amyloid plaques in the hippocampus of this rat model of AD. Data are presented as mean ± SD. * indicates $p = 0.001$ and ** indicates $p = 0.004$.

neurons, glial cells, and motor-neuron-like cells, has been previously reported (Shirian et al., 2016). MSCs used in this experimental study have been recently shown to provide a promising therapeutic approach for treatment of AD (Naaldijk et al., 2017). In addition, intravenous injection of MSCs that was used in the present study has been widely shown to improve AD, experimentally (Chang et al., 2014; Cui et al., 2017).

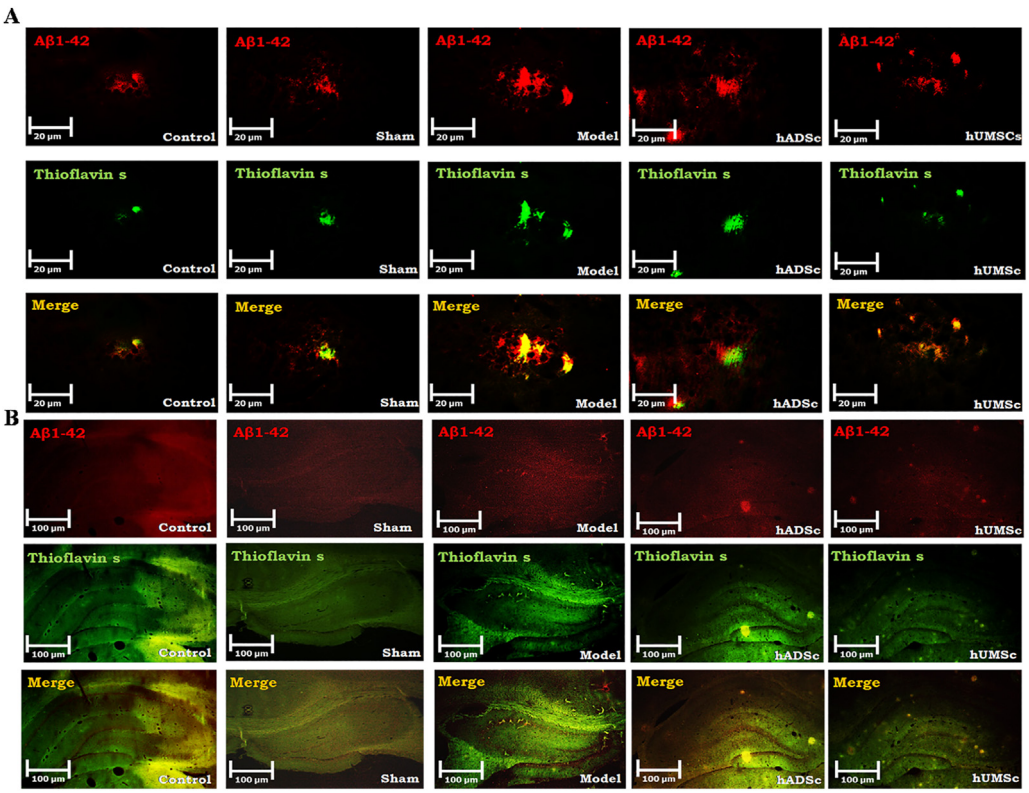

Figure 6. Immuno and Thioflavin S staining for detection of β-amyloid plaque in the CA1 area in various groups at low (A) and high (B) magnifications. The β-amyloid plaques are seen in red and green colors by immunohistochemistry and Thioflavin S staining, respectively.

Moreover, Human MSC such as hASCs have been widely used as potential candidates for cellular therapy in neurodegenerative disease (Lee et al., 2019) traumatic brain injury (Lee et al., 2018), and experimental autoimmune encephalomyelitis (Li et al., 2017). SCs obtained from several tissues, such as bone marrow and umbilical cord blood, are well characterized in terms of proliferation and differentiation properties and can be applied as an optimum source for transplantation in tissue regeneration. MSCs have been widely used in research projects since they can easily be isolated from umbilical cord and from adipose tissue AD patients using a non-invasive techniques for which ethical approval is not required for their isolation and application (Hickman et al., 2018). hUMSCs are young populations of MSCs that are isolated from umbilical cord matrix (Weiss and Troyer, 2006). Whereas, hAD-MSCs are adult stem cells which can be used for autologous transplantation.

The obtained data from the apoptosis assay indicated that neuronal cell death or the mean percentage of apoptotic cells in the hippocampus of β-amyloid-treated rats was increased compared to the cell therapy and control groups. It has been shown that β-amyloid injection causes neurotoxicity, AD-like astrogliosis, hyperphosphorylation of tau protein and reduced memory function (Benedikz et al., 2009; Cavanaugh et al., 2014). β-amyloid triggers neuro-inflammation as well as the related processes that finally result in neural pyroptosis and apoptotic related cell death (Tan et al., 2014). Moreover, βA42 injection induces oxidative stress and glial cell activity which consequently increase neural cell death (Behl, 2005; Onyango and Khan 2006; Wyss-Coray and Mucke, 2002). MSCs secret paracrine and neurotrophic factors that prevent neural death. Neurotrophic factors that are secreted by MSCs reduce microglial and astrocyte activation and decrease oxidative stress (Lee et al., 2010). This may explain the possible mechanisms underlying neural death reduction by IV injections of hUMSCs and hAD-SCs.

Therefore, βA42 injection was used in this study to evaluate the effects of intravenous injection of hUMSCs and hAD-MSCs on synaptic and neurogenesis functions. For this purpose, β-amyloid deposition in the hippocampus was traced by IHC assay. The rate of β-amyloid deposition in neural cells of the model rats was 20.15% ± 0.5%, which indicated a significant increase compared to the control and cell transplantation groups. On the other hand, transplantation of MSCs significantly reduced β-amyloid deposition in the hippocampus of AD rats. Several factors are involved in reducing the number of βA42 plaques, including autolysosome formations, secretion of growth factors and neuro-inflammation association mechanisms (Shin et al., 2014; Yang et al., 2013). Neprylisine protease (CD10 which is expressed in MSCs) plays an important role in decomposition of b-amyloid plaques (Katsuda et al., 2013). However, expression level of CD10 in hUMSCs and hAD-MSCs is higher than that in BM-MSCs (Farias et al., 2011; Katsuda et al., 2013). In this study, no significant difference in reducing the volume of b-amyloid plaque was detected between the hAD-MSCs and hUMSCs groups.

The results of protein expression of synaptophysin and GAP43 indicated that the protein expression levels of both proteins were considerably reduced in the β-amyloid group compared to the control and both hUMSCs and hAD-MSCs groups. The mechanisms of SC delivery to the brain and migration of MSCs across the blood brain barrier (BBB) are controversial. Some studies have indicated that the intra-arterial and intravenous MSCs injections cannot across the BBB and reach the lesion site in rodent AD models. However, some studies have pointed out the ability of MSCs to migrate across the BBB (Kim et al., 2012; Ehrhart et al., 2016). Our results showed that not only hUMSCs and hADSCs are capable of migrating across the BBB and to the hippocampus in an AD rat model, but also can increase GAP-43 expression, which promote neurogenesis. Several studies have demonstrated that MSCs up-regulate the expression levels of neurogenesis markers (Ebrahimi-Barough et al., 2015; Shirian et al., 2016). GAP-43 protein plays important roles in neuronal growth and up-regulates growing axons in both the central and peripheral nervous systems during development and regeneration (Rodger and Dunlop, 2015). However, there is little evidence of synaptic maturity and neural function of MSCs-derived neurons in *in vivo* conditions (Duncan and

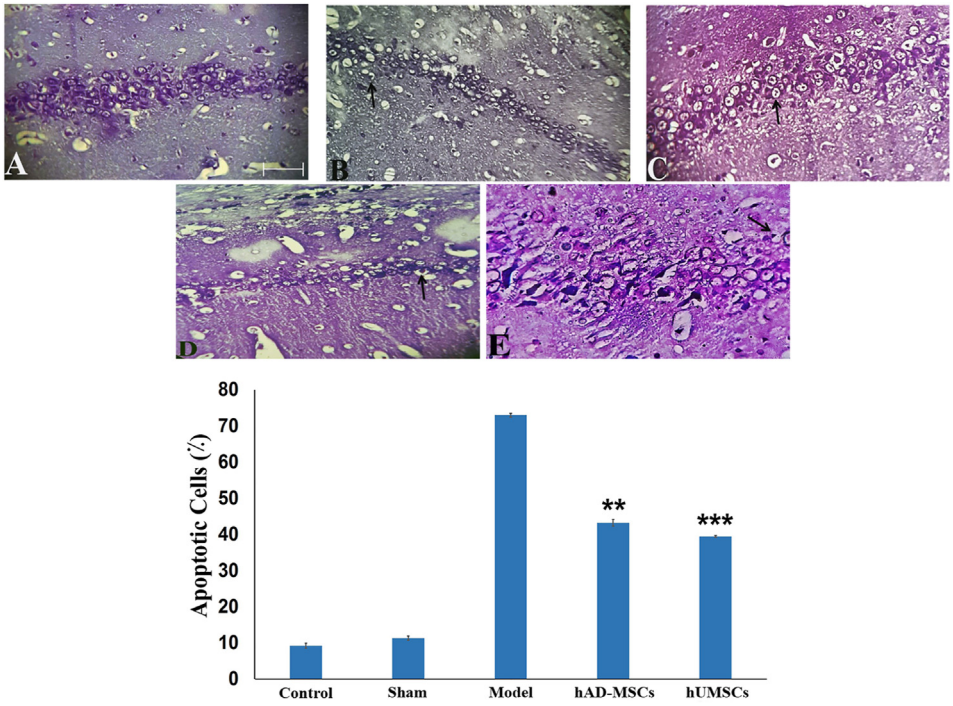

Figure 7. Nissl staining of the hippocampus. (A) Control, (B) Sham, (C) Model, (D) hAD-MSCs and (E) hUMSCs groups (Black arrows show the Nissl bodies). Photomicrographs illustrate the morphological features of the hippocampus. βA42 injection increased the number of dark neurons, and intravenous injection of hUMSCs and hAD-MSCs improved neural survival (Scale Bar 50 μm). * indicates p = 0.001, ** indicates p = 0.0001.

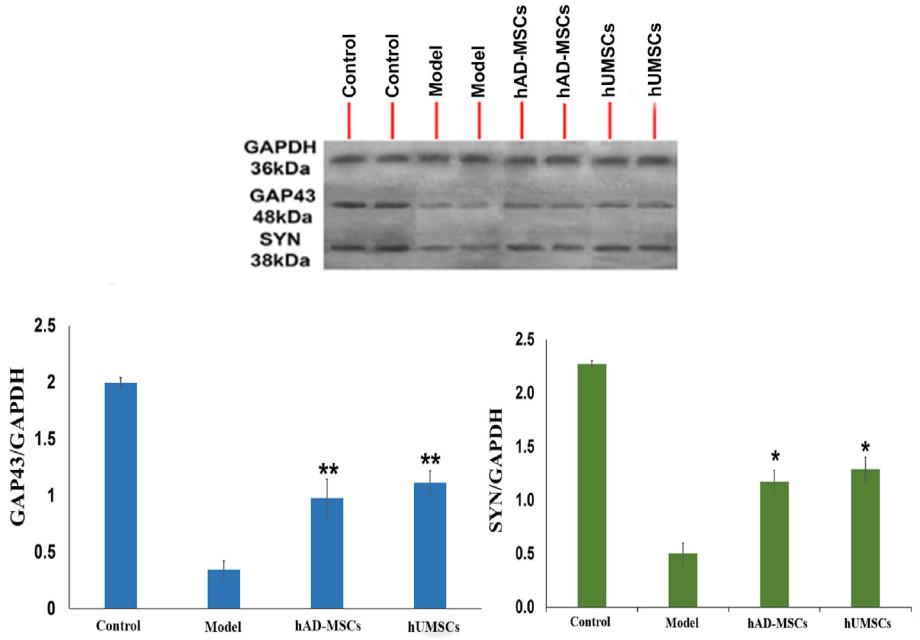

Figure 8. Western blot analysis of synaptophysin and GAP43 expression in different groups. The expression levels of synaptophysin and GAP43 in this rat model of AD were decreased. Intravenous injections of hUMSCs and hADSCs significantly increased expression levels of both proteins. (* indicates p = 0.005, ** indicates p = 0.008, # indicates p = 0.02, and ## indicates p = 0.04).

Valenzuela, 2017; Munoz et al., 2005; Teixeira et al., 2015). It is believed that the therapeutic effects of MSCs are related to their neuro-protective paracrine secretions, including BDNF, IGF-1, NGF and VEGF (Teixeira et al., 2015; Jin et al., 2002). Therefore, these secretions can stimulate proliferation, neural differentiation and formation of inner neurogenic niche. On the other hand, MSCs prevent neuro-inflammation and stimulate neurogenesis function of neural SCs in the brain (Teixeira et al., 2015).

In this study, we indicated that b-amyloid down-regulated synaptophysin expression and decreased synaptic function. Synapses are the first areas which are affected in AD and synaptic disorder causes cognitive and behavioral disturbance. Synaptophysin is a pre-synaptic protein which is

highly involved in plasticity and synaptic functions. However, the expression level of this protein is down-regulated in AD (Tampellini, 2015). Furthermore, the results of this study indicated that hUMSCs and hAD-MSCs increased the protein expression level of synaptophysin in the Alzheimer's rat model. However, there was no significant difference between the hUMSCs and hAD-SCs-treated groups in terms of protein synaptophysin expression. Therefore, both of these cell sources are capable of improving synaptic function and neurogenesis.

Memory and learning impairments are the main features of AD. Nakamura et al. (2001) have reported that βA42 injection influenced behavioral traits and resulted in learning and cognitive impairment (Nakamura et al., 2001). However, the results of MWM test indicated that the βA42-injected rats presented cognitive functional disabilities and spatial memory impairment that can validate this rat model of AD. The results of immunohistochemical evaluations demonstrated that the number of βA42 plaques in the hippocampus of rats significantly induced cognitive impairment. The travel distance was quantified in each experimental group in order to evaluate behavioral traits. A significant increase in the time of traveling was observed in the AD rats compared to the control group. Moreover, animals in model group spent less time in the target quadrant compared to control group. There was no significant difference between the treatments, control and sham groups in terms of swimming speed. Therefore, the latency to find the platform was used as an indicator of learning. We found that the time spent in the target quadrant by the model group was significantly shorter compared to the control and treatment groups. These findings indicated that both the cell sources used in this study, could reduce memory defects AD rat model.

Inflammatory responses play a critical role in AD pathogenesis (Heppner et al., 2015). Microglia is the main immune cells in the CNS that participate in neural protection and death. MSC transplantation has been shown to modulate microglial activity in the CNS to reduce Aβ via CCL5 secreted by the transplanted MSCs. *In vitro*, hippocampal neurogenesis has been dramatically increased by in the presence of MSCs. MSCs also trigger the differentiation of neural progenitor cells into mature neurons via the Wnt signaling pathway. Human MSCs have been shown to decrease the levels of Aβ42 by increasing of autophagy (Shin et al., 2014). Moreover, transplantation of hUMSCs into the hippocampi of mouse model of AD improves memory by increasing neuronal survival and reducing Aβ42 deposits. Transplantation of ADSCs improves neural function in the AD rat model.

In conclusion, intravenous injection of hUMSCs and hAD-MSCs is a safe approach that improves synaptic function and neurogenesis via up-regulation of synaptophysin and GAP43 protein expression levels, respectively, in this rat model of AD. Intravenous injections of both applied SCs can improve learning and cognitive impairment induced by β-A42 injection. Although we have found that both hUMSCs and hAD-MSCs provide a promising therapeutic approach for treatment in this rat model of AD via improvement of synaptic function and neurogenesis. It seems that hAD-SCs may be better for treating AD in the clinic, due to its greater ease of isolation and the immunological advantage of using autologous adipose-derived stem cells.

Ethical approval

All procedures were performed based on the guidelines of the ethical committee of Iran University of Medical Science.

Informed consent

Not applicable.

Declarations

Author contribution statement

Maryam Doshmanziari: Performed the experiments; Wrote the paper.

Sadegh Shirian: Analyzed and interpreted the data; Wrote the paper.

Mohammad-Reza Kouchakian, Seyedeh Farzaneh Moniri: Performed the experiments.

Saranaz Jangnoo, Niloofar Mohammadi: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Fariba Zafari: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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