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Experimental Research Article

Intranasal administration of human adipose-derived stem cell-conditioned media ameliorates cognitive performance in a rat model of Alzheimer's disease

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ABSTRACT

Introduction: Alzheimer's disease (AD) is an age-related disorder, characterized by the gradual loss of memory and cognitive function owing to neuronal damage and brain shrinkage. This study aimed to investigate how intranasal injection of human adipose-derived stem cell-conditioned media (hADSC-CM) ameliorates cognitive performance and affects the level of estrogen receptor beta (Erβ) in the hippocampus of rats in an AD model.

Methods: A total of 32 male rats were divided into four groups, including the control, AD model, hADSC-CM, and vehicle groups. The Morris water maze was used to assess the animals' behavioral changes. Moreover, Nissl and Thioflavin-S staining were performed to evaluate the histology of the hippocampus. Immunohistochemistry was also carried out to evaluate the expression level of Erβ.

Results: The intranasal injection of hADSC-CM improved the rats' cognitive performance by reducing the number of dark cells and beta-amyloid plaques in the hippocampus in the AD model. Besides, the intranasal injection of hADSC-CM increased the level of Erβ in this model.

Conclusion: The present findings indicated that the intranasal injection of hADSC-CM ameliorated cognitive function. Amyloid plaques and dark cells also diminished in the CA1 area of the hippocampus. Moreover, the expression level of ERβ increased. It can be concluded that hADSC-CM has significant treatment benefits for AD in rats.

Keywords:
Alzheimer's Disease
Estrogen receptors
Human adipose-drive stem
Cell therapy
Cognitive performance

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Introduction

Late-onset Alzheimer's disease (AD) is an age-related disorder, characterized by the progressive loss of memory and cognitive function owing to neuronal damage and brain atrophy. It has been identified as one of the major contributors to dementia. Age is typically a significant risk factor for AD. Evidence suggests that after the age of 65, this disease occurs more frequently every five years. The accumulation of amyloid plaques, which are caused by cellular amyloid-beta (Aβ) deposits, is one of the neuropathological alterations that occur in the brains of AD patients. Another neuropathological change associated with AD is the presence of intracellular clumps of hyperphosphorylated tau protein (Castellani et al., 2010; John and Reddy 2021; Tahami Monfared et al., 2022).

The cellular and molecular features of AD emerge decades before clinical symptoms, such as cognitive impairment become apparent. The progressive accumulation of amyloid plaques and hyperphosphorylated tau proteins in the brain leads to cognitive and behavioral problems. Over the past 50 years, research has focused on understanding the link between cognitive and behavioral signs of dementia and AD. The loss of synapses in the affected areas of the brain is the main cause of cognitive impairment in AD patients (Bernabeu-Zornoza et al., 2019; Bondi et al., 2017; Guardia de Souza et al., 2020; Nobakht et al., 2011)

After menopause, estrogen levels decrease, and its neuroprotective benefits also diminish, which is associated with a higher prevalence of AD in women than in males. In other words, the neuroprotective effect of estrogen against Aβ toxicity is diminished. Estrogen lowers the level of Aβ plaques under normal conditions before menopause (Cheng et al., 2021). Besides reducing Aβ plaques, it decelerates tau protein hyperphosphorylation. Previous studies suggest that estrogen targets Aβ and tau concurrently, which may be a distinct advantage for AD treatment (Bean et al., 2014; Merlo et al., 2017; Ratnakumar et al., 2019). Estrogen exerts neuroprotective effects via estrogen receptors, particularly alpha and beta receptors (ERα and ERβ). Both ERα and ERβ are found in the hippocampus, frontal cortex, and amygdala, which are primarily affected in AD. The activation of ERα and ERβ exerts neuroprotective effects against toxicity associated with Aβ accumulation, which is important in the prevention and treatment of AD. According to certain research, ERβ plays a greater role than ERα in mediating the effects of estrogen on brain development and numerous neuroprotective processes of the brain, including neural plasticity. Therefore, ERβ-specific agonists may be an appropriate target in the development of AD medications (Honma et al., 2017; Lee et al., 2014; Paterni et al., 2014).

Stem cell therapy has shown promise as a treatment for AD, but challenges such as low cell survival and potential tumorigenesis limit its effectiveness. The use of stem cell-derived conditioned media (CM) may be a viable approach to overcome these limitations. In several disorders, neurotrophic and anti-inflammatory substances that are produced by the CM of mesenchymal stem cells (MSCs), facilitate regeneration and decrease inflammation. The injection of MSC-CM containing cell-secreted products may be a novel treatment for disorders, such as spinal cord injury, neurodegenerative diseases, myocardial infarction, and stroke. The stem cells from human exfoliated deciduous teeth-conditioned medium (SHED-CM) can have various neuroprotective effects against cognitive impairments via paracrine pathways. In the central nervous system (CNS), paracrine pathways boost endogenous activity, limit neuroinflammation, and induce neuroregeneration (de Cara et al., 2019; Mita et al., 2015; Sagaradze et al., 2019). Considering the important role of estrogen mentioned above, the main aim of this study was to investigate the effect of intranasal injection of hADSC-CM on the expression of Erβ in the rat hippocampus of the AD model.

Material and Methods

Thirty-two adult male Wistar rats (weighing between 280 and 320 g) were housed in hygienic cages and kept in a clean well-ventilated space. Rats were kept under controlled conditions with a 12/12 light/dark cycle and unlimited access to food and water. Before any experiments, they were allowed to acclimatize to their surroundings for a week. Only male rats were selected to prevent the effects of the female hormone cycle. All operations were carried out in accordance with the National Institutes of Health (NIH) guidelines. This study was approved by the Ethics and Research Committee of the Iran University of Medical Sciences (IR IR.IUMS.FMD.REC.1400.374).

The rats were divided into four groups, each consisting of eight rats: 1) Control group, without any injection; 2) AD model group, receiving an intraventricular injection

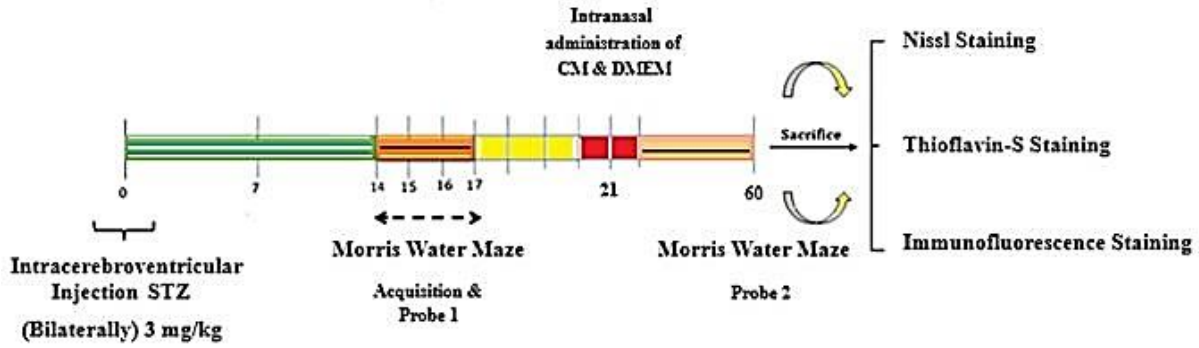

FIGURE 1. Schematic diagram of the experimental design of the study.

of streptozocin (ICV- STZ 3mg/kg); 3) Treatment group or CM group, receiving hADSC-CM after 2 weeks of receiving STZ (STZ injection + hADSC-CM); and 4) Sham or vehicle group, receiving an intraventricular injection of phosphate-buffered saline (PBS) instead of STZ and an intranasal injection of a cell culture medium instead of hADSC-CM (PBS + cell culture medium). Figure 1 shows a schematic diagram of the experimental design of the study.

Induction of AD model intraventricular injection of STZ

Alzheimer's disease model was developed via stereotaxic injection of STZ (Sigma-Aldrich, USA) into the intracerebroventricular (ICV-STZ). The rats were initially anesthetized using a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg) (Sigma-Aldrich, USA). According to a similar study (Nabavi Zadeh et al., 2023), the rats were positioned in a stereotaxic frame, and coordinates were determined relative to the bregma using a stereotaxic atlas. A single injection of STZ into the lateral ventricle (3mg/kg bilaterally 2.5µl each ventricle) was done for 5 minutes on each side (-0.8 mm posterior to the bregma, ±1.4 mm lateral to the sagittal suture, and 3.6 mm below the dura).

A single bilateral intracerebroventricular injection of streptozotocin (STZ, 3mg/kg (Sigma-Aldrich, USA) bilaterally 3µl each ventricle) was done for 3 minutes on each side. The vehicle group received 3.0 µL of PBS instead of STZ. After injection, the needle was left in place for an additional 60 seconds. Subsequently, the skin was sutured, and the animals were monitored before being returned to their home cages. Two weeks after the PBS and STZ injection in experimental groups, the animals underwent behavioral assessments.

Morris water maze (MWM) as a behavioral test

The Morris water maze is one of the most widely used tasks in the study of neural mechanisms underlying spatial learning and memory as cognitive functions. In the present study, the MWM apparatus consisted of a circular black tank (120 cm in diameter and 70 cm in height). It was placed in a dim room with many visual cues. The tank was filled with tap water to a depth of 40 cm (22±1 °C). The maze was divided into 4 equal quadrants. Within one of these quadrants, an invisible circular platform (10 cm in diameter) was submerged 1.5 cm beneath the water's surface. This platform remained stationary throughout the test period and was imperceptible to the rats. Additionally, fixed, extra-maze visual cues were positioned at various locations surrounding the maze to aid spatial navigation.

The test was conducted in two stages: stage 1 consisted of 3 days for the acquisition phase followed by 1 day for probe test (probe1), while stage 2 comprised only 1 day (probe test 2). The purpose of stage 1 was to validate the Alzheimer's model. To achieve this, a probing test was conducted after measuring the animals' performance in the acquisition phase. After Probe 1, animals were divided into control and experimental groups. Following CM injection, probe 2 was conducted one month later, and the different groups were compared.

The first and the second stages were performed two weeks and two months after the induction of the AD model (ICV- STZ), respectively. Similar to probe 1, after CM administration, probe 2 was carried out for all groups to identify the effect of CM. During the acquisition phase, the animals were placed into the water in dif-

ferent quadrants and given 90 seconds to find the hidden platform. If they failed to do so within this time, they were guided toward the platform and allowed to remain there for 30 seconds. Moreover, a probing test involving the hidden platform was performed 24 hours after the final session. The animals were released into the water to freely swim from the furthest location for 60 seconds. A charge-coupled device (CCD) camera positioned above the center of the maze automatically recorded the animals' movements using a computerized system (EthoVision, Noldus, Version 11). The acquisition phase and probe tests were conducted to assess spatial learning and memory retention, respectively. Parameters analyzed for the probe tests included the latency of the first entry to the target zone (escape latency to platform time), time spent in the target quadrant (time in the target zone), and frequency of visits to the target region.

Isolation of hADSC from the human adipose tissue

The adipose tissue was collected from patients who provided their consent and were referred to Omid Hospital in Tehran, Iran, for liposuction. The extracted fat tissue from the surface layer of the abdomen was washed four times. It was then divided into small segments and placed in a Falcon tube containing PBS. Next, collagenase 0.1% solution was added, and the mixture was incubated for 30 minutes at 37°C (95% humidity, 5% CO_2). After the incubation period, the mixture was centrifuged for five minutes at 1200 rpm. After removing the supernatant, the remaining fluid in the Falcon tube was mixed with 5 cc of Dulbecco's modified eagle medium/nutrient mixture F-12 (DMEM/F-12), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (pen/stp). Subsequently, the cells in the culture media were moved to a flask (T25) and placed in an incubator (37°C, 95% humidity, 5% CO_2). The cells at passage 4 were characterized using flow cytometry (Rehman et al., 2004).

Flow cytometry

Flow cytometry was conducted to characterize hADSC using positive and negative human MSC (hADSC) markers. Following two washes with PBS, the hADSCs were trypsinized and incubated for 3-5 minutes. To neutralize trypsin activity, FBS was added to the flask. After centrifugation at 1200 rpm for five minutes, the supernatant was removed, and 5 mL of medium-free serum was added. Subsequently, the hADSCs were characterized using four positive surface markers (CD44, CD105, CD73, and CD90) and two negative markers (CD45 and CD34) (Doshmanziari et al., 2018).

CM preparation from hADSC

According to previous studies (Doshmanziari et al., 2018; Kazemiha et al., 2019; Parsaei et al., 2022), hADSC (passage 3-4) were incubated in a T25 flask (containing 5 cc of DMEM-F12 with 10% FBS and 1% PEN/Strep) until the cells reached approximately 80% confluence. At that point, the medium was replaced with serum-free DMEM/F12. After 48 hours of incubation, the CM was collected as hADSC-CM. The collected CM was then centrifuged for five minutes at 3000 rpm, passed through a 0.22-μm syringe filter, and kept at 4°C until further use.

Intranasal administration of CM and DMEM

The AD rats were anesthetized again three weeks after receiving STZ injections. The hADSC-CM was administered intranasally to the AD group for almost 10 minutes using a Hamilton microsyringe. In the vehicle group, DMEM was administered instead of CM.

Tissue preparation

Three rats were deeply anesthetized with ketamine and xylazine (100 and 15 mg/kg, respectively) and perfused with 20 mL of PBS (0.01 M, pH=7.4) followed by 250 mL of 4% paraformaldehyde in PBS (0.1 M, pH=7.4). The animals' brains were carefully removed and post-fixed in 4% paraformaldehyde in 0.1 M PBS at room temperature. Coronal slices of the brain with a thickness of 6-7 µm were extracted using the Paxinos and Watson atlas after tissue preparation (Zarbakhsh et al., 2019).

Nissl staining

In order to observe the morphological changes of the hippocampus, Nissl staining was utilized. The sections, mounted on gelatin-coated slides, were dehydrated using an ascending series of ethanol, treated with xylene for five minutes, rehydrated with a descending series of ethanol, and finally washed with distilled water. Subsequently, they were exposed to a 1% solution of Cresyl violet stain (Sigma) for three minutes, followed by 100% ethanol for five seconds, and then subjected to an ascending series of ethanol

and xylene, respectively.

Thioflavin-S staining

Thioflavin-S staining was used to evaluate Aβ plaque formation. After deparaffinization and hydration, the tissue slices were immersed in a filtered aqueous solution of 1% Thioflavin-S at room temperature for eight minutes. Thioflavin-S was kept at 4°C away from light. The segments were then rinsed twice in 80% ethanol for three minutes, in 95% ethanol for three minutes, and finally, in three exchanges of distilled water. The stained slides were protected from light as much as possible. They were finally examined under an Olympus fluorescence microscope (Doshmanziari et al., 2018).

Immunofluorescence staining

The Erβ expression in the rat hippocampus was examined via immunofluorescence labeling. The coronal brain slices were examined under a fluorescence microscope (Olympus, 40× magnification). The slides were immersed in Sigma T5912 solution (TBS 1X) inside a microwave, which was switched off once the solution reached boiling point. After 20 minutes, the samples were washed three times with PBS (five minutes of washing). The samples were then treated with H_2O_2 and methanol at a 1:9 ratio for 10 minutes. The primary antibody, mouse anti-ER monoclonal antibody (SC-390243, Santa Cruz, Germany), was diluted to a ratio of 1:100. A secondary antibody, goat polyclonal antibody against goat anti-mouse IgG (H+L), coupled to FITC (orb688924), was applied for anti-estrogen receptor (ER) detection at a dilution of 1:500. Nuclei were counterstained with DAPI (Sigma, Germany). The stained slides were stored in a light-protected environment (Doshmanziari et al., 2018).

Statistical analysis

The data were analyzed and graphs were generated using Graph Pad Prism software. Behavioral tests in groups were conducted using two-way ANOVA for the acquisition phase of the MWM, a t-test for probe 1, and one-way ANOVA for probe 2. For the comparison of the number of normal and dark neurons, Aβ plaques (with group comparisons analyzed using t-tests), and Erβ levels in the hippocampal CA1 sections (across control, AD model, vehicle, and hADSC-CM groups), one-way ANOVA tests were employed. A P-value less than 0.05 was considered statistically significant. Besides, for the MWM test, two-way ANOVA, followed by post-hoc Bonferroni's test, was used.

Results

Expression of MSC surface markers by hADSC

The hADSCs utilized for the preparation of the CM exhibited typical levels of MSC surface markers (Figures 2 & 3). Four positive surface markers, including CD44, CD105, CD73, and CD90, were used to determine the percentage of stem cells derived from human adipose tissue. Overall, 99.4% of cells expressed CD44 (Figure 2a), while 99.7% expressed CD73, CD90, and CD105 (Figures 2b, 2c, and 2d, respectively). Besides, two negative markers, CD45 and CD34, were assessed, with expression levels observed at 0.187% and 1.64%, respectively (Figures 3a and 3b, respectively).

Behavioral assessment (training phase)

MWM test

To determine the behavioral effects of STZ, spatial memory in adult animals was evaluated two weeks after the intraventricular injection. Figure 4 (a) indicates the learning results in the acquisition phase of the MWM. The distance traveled to reach the platform during the learning phase was analyzed by Two-way ANOVA and Bonferroni's post hoc (mean ± SEM). Statistical analysis revealed that the performance of rats in all groups progressively improved during the days 1 to 3 training period. Results of distance traveled to reach the platform during the learning phase indicated that compared to the icv-STZ injection group, the distance traveled in control group animals was significantly shorter from the first to 3rd days, indicating the harmful effects of STZ on the learning process (n=8; $P< 0.05$).

Probe 1

After the acquisition phase and confirmation of training, probe 1 was carried out two weeks after surgery. Figure 4 (b and c) presents the results of the probe 1 trials. The results of the t-test for evaluating the probe findings indicated that the average time spent in the target quadrant was reduced in the STZ group compared to the control group on day 4 (as the initial memory measure) (df=17, t=1.074, P=0.0170) (Figure 4b). On day 4, the frequency of crossing the hidden platform area

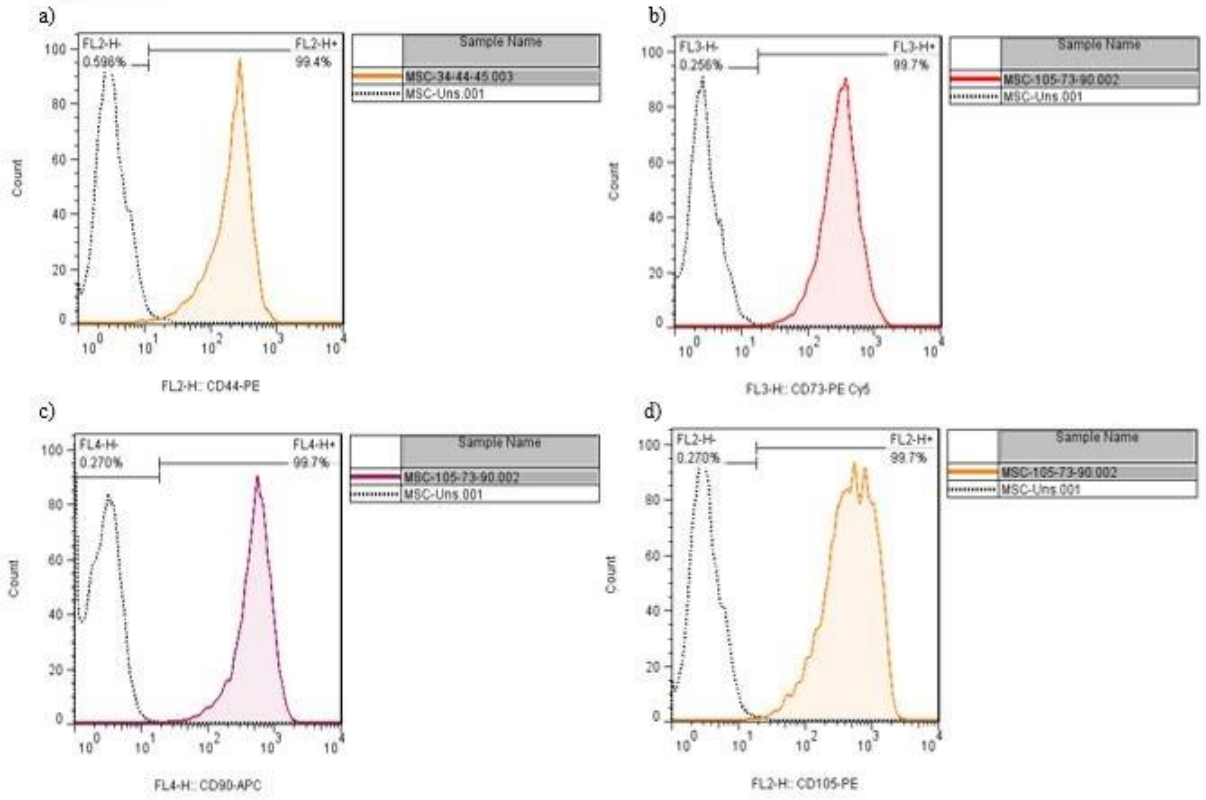

FIGURE 2. Flow cytometry histogram of hADSC. Positive antigens: 99.4% of cells expressed CD44 (a), and 99.7% of cells expressed CD73, CD90, and CD105 (Figures 2b, 2c & 2d, respectively).

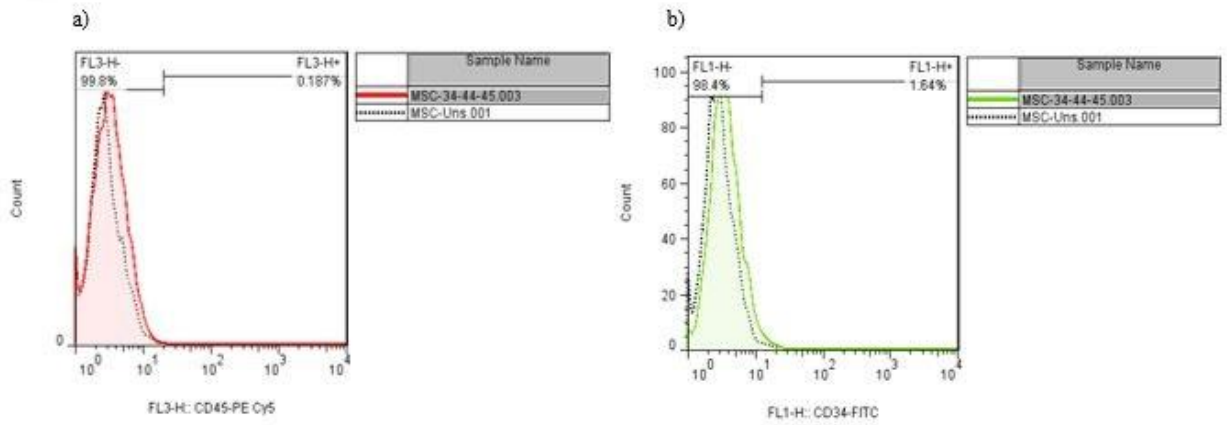

FIGURE 3. Flow cytometry histogram of hADSC. Negative antigens: 0.187% of cells expressed CD45, and 1.64% of cells expressed CD34 (Figures 3a and 3b, respectively).

(frequency in the target region) was reduced in the STZ group compared to the control group (df=18, t=2.177, P=0.0430), and this decline was statistically significant (P=0.05) (Figure 3b). The present findings also revealed no significant difference between the two groups in terms of latency to initially discover the hidden platform in the target quadrant (df=10, t=0.578, P=0.5764) (Figure 4c).

Probe 2

fter probe 1 and CM administration were completed, Probe 2 was performed two months after the injection of STZ. Figure 4 depicts the results of probe 2. A one-way ANOVA examining the time spent in the target quadrant

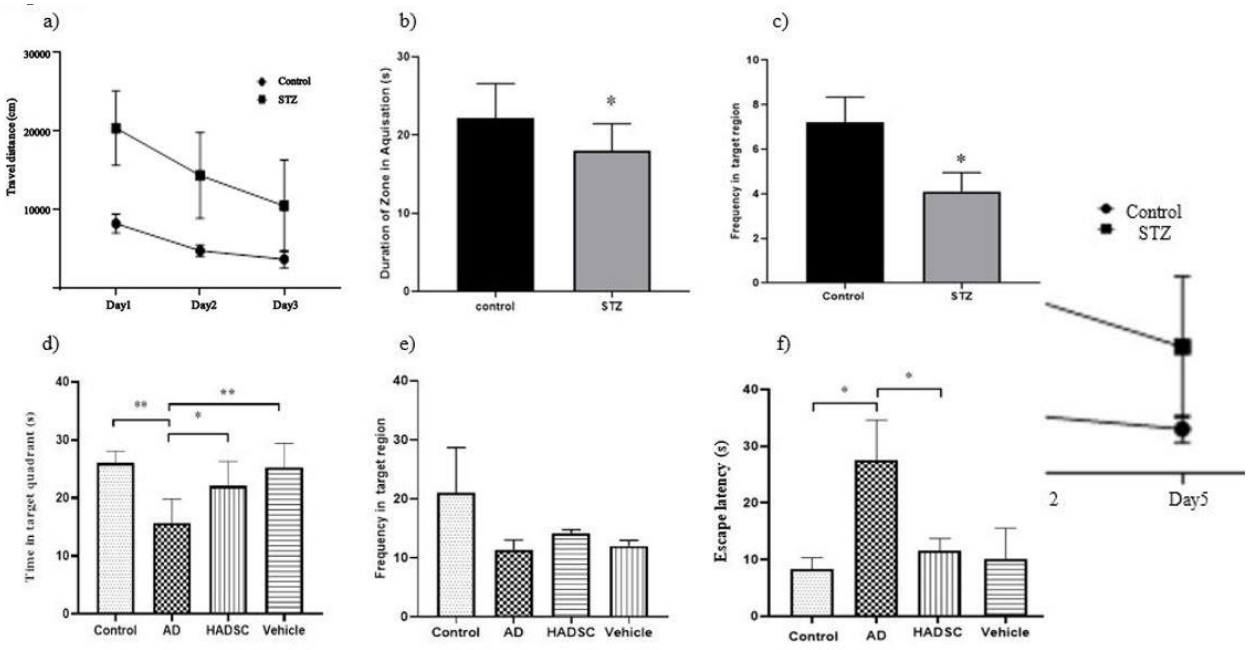

FIGURE 4. Behavioral test probe 1 and probe 2). (a) The time spent in the target quadrant in probe 1. The average time decreased in the STZ group ($P<0.05$) compared to the control group (P=0.05). (b) In probe 1, the number of passes of rats on the hidden platform decreased in the STZ group ($P< 0.05$) compared to the control group. (c) Latency to find the hidden platform in prob1, there was no significant difference between the two groups regarding latency to initially find the hidden platform in the target quadrant. (d) The time spent in the target in probe 2, There was a significant difference between the STZ and control groups regarding the time spent in the target quadrant ($P<0.01$). There was also a significant difference between the CM and STZ groups ($P<0.05$). (e) There was no significant difference between the groups regarding the number of times rats passed over the hidden platform. (f) Escape latency to initially detect the hidden platform in the target quadrant significantly increased in the STZ group ($P<0.05$) and the CM group ($P<0.05$).

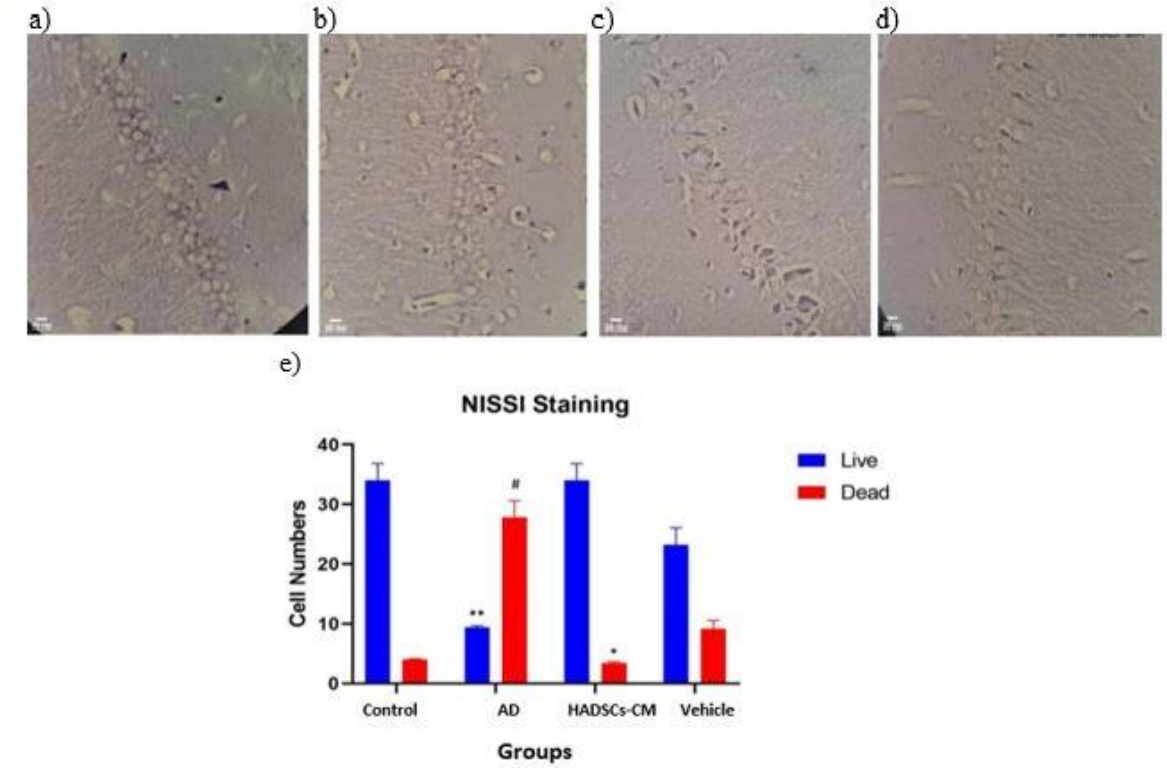

FIGURE 5. Nissl staining (CA1 region of the hippocampus). (a) In the control group and other groups, except for the AD group, cells with a round cytoplasm and clear nuclei were more visible and considered healthy. In the AD group, cells with an altered dark cytoplasm and no round shape, called dark cells, were observed. (b) The number of healthy cells in the STZ group (AD group) decreased significantly compared to the other groups; there was no significant difference between the other groups. Regarding the number of dark cells, the CM group showed a significant decrease compared to the other groups. The AD group showed a significant increase in the dark cells compared to the control group.

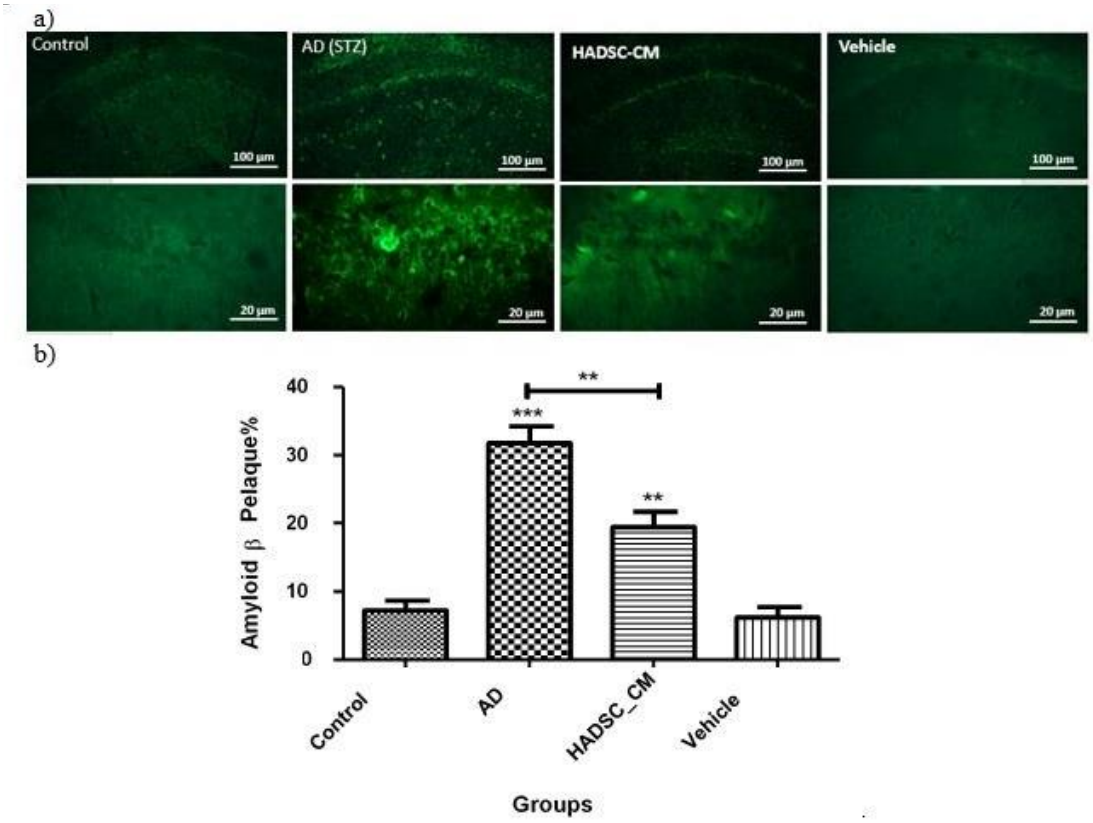

FIGURE 6. Thioflavin-S staining. (a) Thioflavin-S green reactions were completely invisible in the AD group. (b) The results showed a significant increase in Aβ plaques in the AD group compared to the control and vehicle groups. Also, there was a significant decline in Aβ plaques in the CM group compared to the AD group.

to locate the hidden platform indicated a significant difference between the STZ and control groups (P=0.01). The effect of treatment on the time spent in the target quadrant indicated a significant difference between the CM and STZ groups ($P<0.05$) (Figure 4d). The results of one-way ANOVA indicated no significant difference in the frequency of crossing the hidden platform area between the groups (Figure 4e). The results of a one-way ANOVA test on the escape latency to identify the hidden platform indicated a significant difference between the STZ and control groups ($P<0.05$). The present findings revealed a significant difference between the CM and STZ groups (a substantial decrease in latency to identify the hidden platform in the CM group) ($P<0.05$) (Figure 4f).

Nissl staining

Healthy cells were characterized by bright and clear nuclei, whereas dead (dark) cells exhibited a strong and homogeneous black color (Figure 5a). Based on the results of the ANOVA test, the number of healthy cells in the STZ group (AD group) decreased significantly compared to the other groups; nevertheless, there was no significant difference between the other groups. The CM group showed a significant reduction in the number of dark cells compared to the other groups. According to the results of the t-test, the AD group showed a significant increase in dark cells compared to the control group (Figure 5b).

Thioflavin-S staining

Figure 6a presents the Thioflavin-S-related green reactions. In the AD group, this response was invisible. Figure 6b indicates coronal slices from three rats, randomly selected from five distinct CA1 areas. The color images of the sections were converted to black and white using ImageJ software, as the color intensity increased (200× magnification); the color intensity of the images was determined. White spots indicating the positive reaction of Thioflavin-S with Aβ plaques were estimated as the percentage of Aβ plaques. The results demonstrated a significant increase in the level of Aβ plaques in the AD group compared to the control and vehicle groups. There was also a significant decrease in Aβ plaques in

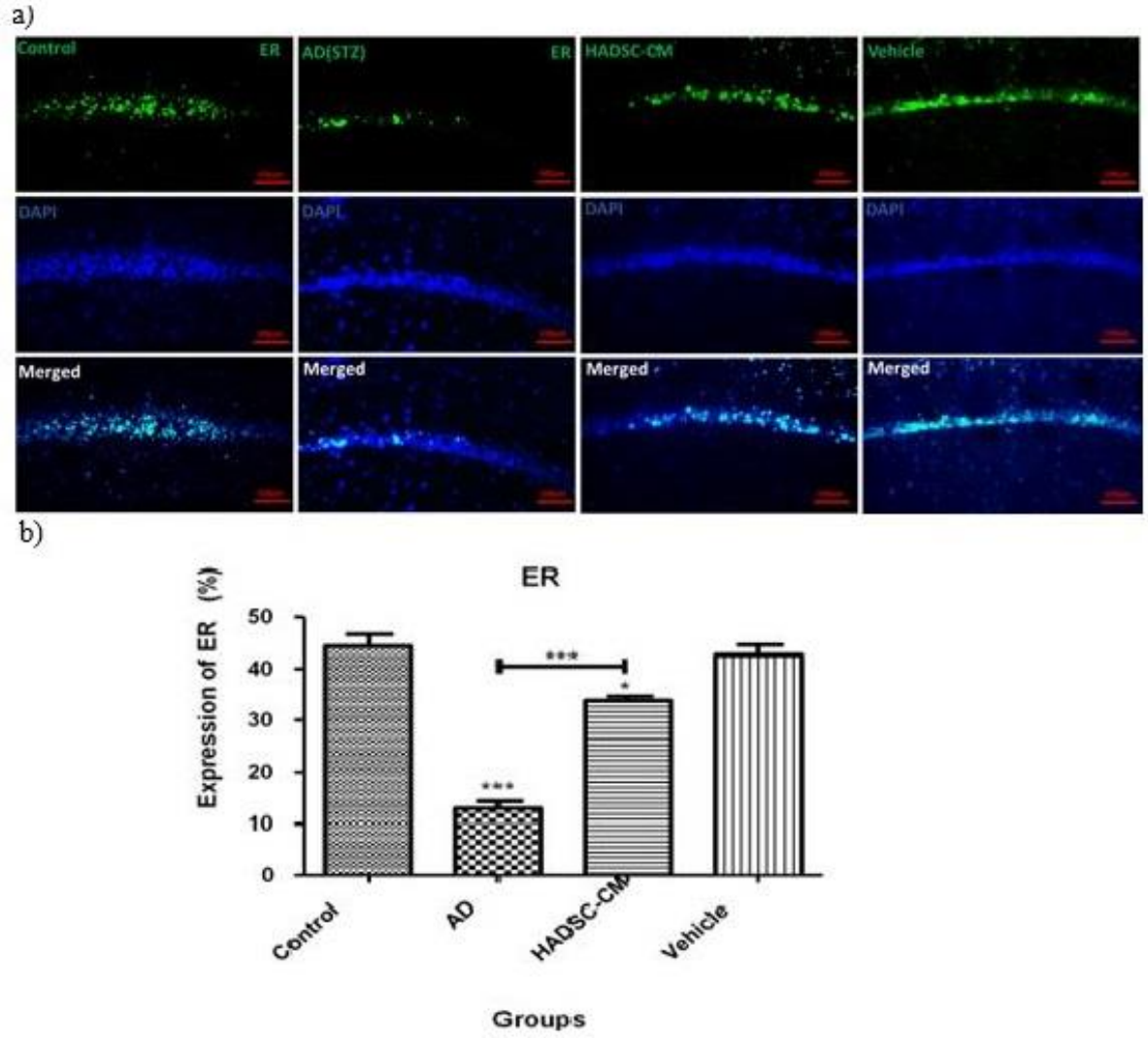

FIGURE 7. Immunofluorescence staining. (a) The immunofluorescence staining technique (CA1 region) was used to detect ERβ as a primary antibody (mouse anti-ERβ monoclonal antibody, green SC-390243, Santa Cruz, Germany) and to identify the nucleus of nerve cells (DAPI blue color) in the groups (scale bar, 100 μm). In the first row, the green fluorescent color indicates the reactions of ERβ. In the second row, the blue color shows the cell nucleus. The third row represents the merging of images in rows 1 and 2 using Photoshop. (b) Three rats in each group and five sections from each rat were used, and three different areas of the CA1 region were specified in each section (microscope lens, 20; 200× magnification). The number of cells in the CA1 region was counted in ImageJ software; the expression level of ERβ was measured using a green fluorescent dye. The ratio of fluorescent dye as positive cells to all the cells in the region was calculated and analyzed in statistical software.

the CM group compared to the AD group.

Immunofluorescence staining

According to the quantitative data, the expression level of Erβ receptors in the AD group decreased significantly compared to the control and vehicle groups. The level of ER expression in the control group was not significantly different from that of the vehicle group. Compared to the AD group, the expression level of Erβ increased significantly in the hADSC-CM group (Figures 7a & 7b). Consequently, the injection of hADSC-CM in AD rats resulted in a significant increase in the expression of ERβ in the hippocampus.

Discussion

The results of our neurobehavioral and histological observations indicated that the administration of icv-STZ significantly impaired learning and spatial memory performance in the first phase of the MWM test. Intranasal injection of hADSC-CM to AD rats improved learning and memory disorder hallmarks while increasing Erβ expression and decreasing amyloid plaques and dark cells in the CA1 area of the hippocampus.

Previous studies have shown that estrogen protects

against AD. Reduction of estrogen may contribute to AD's destructive processes and increase the likelihood of women developing the condition after menopause. If started soon after menopause, estrogen therapy (ET) has been associated with a significantly reduced risk of AD development in women. Estrogen balances the proteolytic processing of beta-amyloid precursor protein (βAPP) and protects neurons against Aβ-induced cell death. Evidence suggests that estrogen protects neurons against the toxicity of Aβ plaques, inflammation, and oxidative stress. Numerous studies have reported that brain estrogen activity regulates Aβ and tau levels in neurons by activation of ERα, ERβ, or both (Honma et al., 2017; Merlo et al., 2017; Paterni et al., 2014; Radaghdam et al., 2021; Ratnakumar et al., 2019; Zhao et al., 2015).

The ERα and ERβ types of estrogen receptors are found in human tissues. Estrogen protects neurons by activating ERs. In the brain, ERs regulate synaptic plasticity, neuroinflammation, and neuronal differentiation and proliferation. ER is expressed in the cytoplasm of astrocytes, microglia, and neurons, particularly in the forebrain and hippocampus. According to research, the expression of ERβ decreases in the nuclei of white matter cells in the brains of AD patients.

The role of ERβ in reducing Aβ plaque deposition is critical. Besides reducing Aβ plaques, estrogen also delays tau protein hyperphosphorylation. ERβ has been shown to be more involved in the regulation of memory and learning compared to ERα. ERβ overexpression reduces Aβ deposition in the hippocampus of AD rats while improving learning and memory. The ERβ activation has been proposed as a promising treatment for AD patients. Brain ER expression differs between AD patients and healthy individuals, according to this research (Zhao et al., 2015).

The present findings showed that the number of Aβ plaques increased significantly in a rat model of AD compared to the control group, while memory and learning declined in this group according to the behavioral test. The level of Erβ expression in the AD group was much lower than that of the control group; therefore, our findings are in line with previous results. By creating an adequate neurological environment at the lesion site as a result of the release of neurotrophic and anti-inflammatory chemicals, CM may be able to heal damaged nerves. In comparison to stem cells, CM has several advantages since it is simpler to manufacture, freeze, pack, and transport. Regarding rejection issues, there are no criteria to match the receiver and the donor. In several studies, the CM of mesenchymal stem cells has been shown to enhance regeneration and reduce inflammation in various diseases (Mehrabadi et al., 2020; Sagaradze et al., 2019).

In a previous study, SHED-secreted factors (using various culture media, including M199, DMEM/Ham's F12, and SHED-CM) could maintain cell proliferation, minimize apoptosis, and enhance angiogenesis (de Cara et al., 2019). Also, MSC-CM may be useful in the prevention and treatment of retinal pathologies in various neurodegenerative diseases (Kuo et al., 2021).

Yamazaki and his colleagues reported that behavioral disorders are improved in the mouse model of AD by CM-ADSCs (Yamazaki et al., 2015). In a systematic review study, Madani Neishaboori and colleagues also noted that the use of CM-ADSCs in an AD rat model significantly improved learning and memory. Following treatment with the CM-ADSCs in the hippocampus area, there was a considerable improvement in neuronal survival rate as well as a significant reduction in Aβ plaques (Madani Neishaboori et al., 2022). Our findings are consistent with the results of these two studies.

Transforming growth factor-beta (TGF-β), interleukin-10 (IL-10), chemokine ligand 9 (CCL9), interferon-alpha (IFNα), IFNβ, nitric oxide (NO), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF) of MSC-CM may be employed as tissue regeneration and anti-inflammatory substances (Mehrabadi et al., 2019). HGF, for instance, is an antigenic, anti-apoptotic, and immunity-modifying factor. FGF2 stimulates angiogenesis and boosts stem cell survival and proliferation in vivo (Sagaradze et al., 2019). In this regard, a previous study reported that bone marrow stromal cells (BMSCs) can release estrogen (E2); the predominance of E2 secretion in BMSCs highlights their potential therapeutic use. Overall, the injection of MSC-CM with cell-secreted products is a novel treatment for neurodegenerative disorders, such as AD (Mehrabadi et al., 2019).

In line with previous research, in the current study, the effect of intranasal injection of hADSC on the expression of ERβ in the hippocampus of AD rats was investigated for the first time. To the best of our knowledge, no research has investigated the effect of hADSC-CM on the expression level of ERβ in an animal model of AD.

According to the results of the current study, STZ injection into the rats' brains resulted in cognitive deficiencies as well as an increase in amyloid plaques and dark cells in the CA1 region of the hippocampus. Additionally, a significant decrease in ER expression was seen after injecting STZ into the rats' brains. Our research showed that intranasal injection of hADSC-CM into the brains of AD rats reduced amyloid plaques and dark cells in the CA1 area of the hippocampus while improving cognitive impairments and elevating the level of ERβ expression. Therefore, it can be concluded that hADSC-CM has beneficial advantages for AD treatment.

For future studies, we suggest investigating the effect of intranasal injection of hADSC-CM on the expression of ERα in the hippocampus of male AD models.

Author Contributions

M.E and H.P: Conception and design, project administration, manuscript writing, data analysis, final approval of manuscript; N.A and M.N: Conception and design, verification of analytical methods, manuscript writing, final approval of the manuscript; S.A.SH, A.D, E.S, and J.F.B were contributed to project administration, final approval of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The authors confirm that informed written consent was obtained from all patients. The use of human material and animal tests in this study was approved by the Research Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC.1400.374), and all experimental procedures were performed in accordance with the approved guidelines of the ethical committee and ARRIVE guidelines.

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