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Pre-treatment by Rosemary Extract or Cell Transplantation Improves Memory Deficits of Parkinson's Disease: When Tradition Meets the Future

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HIGHLIGHTS

- Spatial learning and memory impairment through bilateral injection of 6-OHDA (6-Hydroxydopamine) into the SNc which also leads to the destruction of neurons in the hippocampus.
- ADSCs (Adipose tissue-derived stem cells) transplantation had neuroprotective effect and recovered the memory deficit in 6-OHDA-induced rat model of Parkinson's disease.
- Rosemary extract pre-treatment compensated the memory deficit in 6OHDA-induced rat model of Parkinson's disease.

ABSTRACT: The therapeutic effect of adipose tissue-derived stem cells (ADSCs) or RE on hippocampal neurogenesis and memory in Parkinsonian rats were investigated. Male rats were lesioned by bilateral intra-nigral injections of 6-OHDA and divided into six groups: 1. Lesion 2 and 3: RE and water groups were lesioned rats pretreated with RE or water, from 2weeks before neurotoxin injection and treated once a day for 8weeks post lesion. 4&5: Cell and α -MEM (α -minimal essential médium) received intravenous injection of BrdU-labeled ADSCs or medium, respectively from 10days post lesion until 8weeks later. 6: Sham was injected by saline instead of neurotoxin. Memory was assessed using Morris water Maze (MWM), one week before and at 1, 4 and 8weeks post 6-OHDA lesion. After the last probe, the animals were sacrificed and brain tissue obtained. Paraffin sections were stained using cresyl violet, anti-BrdU (Bromodeoxyuridine / 5-bromo-2'-deoxyuridine), anti-GFAP (Glial fibrillary acidic protein) and anti-TH antibodies. There was a

significant difference of time spent in the target quadrant between groups during probe trial at 4 and 8 weeks' post-lesion. Cell and RE groups spent a significantly longer period in the target quadrant and had lower latency as compared with lesion. Treated groups have a significantly higher neuronal density in hippocampus compared to water, α -MEM and lesion groups. BrdU positive cells were presented in lesioned sites. The GFAP (Glial fibrillary acidic protein) positive cells were reduced in treated and sham groups compared to the water, α -MEM and lesion groups. Oral administration of RE (Rosemary extract) or ADSCs injection could improve memory deficit in the Parkinsonian rat by neuroprotection.

Keywords: Rosemary extract; cell transplantation; hippocampus; memory.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease and occurs in 1% of people over the age of 55. Patients suffer from motor impairment which is caused by severe lack of dopamine in the SNc and functional disruption of the basal nuclei. An important neurological feature is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) followed by the deprivation of dopamine in the striatum [1]. Recent studies have shown that 48% of patients with PD had memory loss, one of the first symptoms, and 48% of them had a dementia after 15 years [2]. There is no definite evidence about the probability of the disease. Probably, aging and genetics as well as oxidative stress, protein accumulation, mitochondrial dysfunction, external toxicity, inflammatory immune responses and environmental factors such as physical trauma and infection are involved in the onset of the disease and deterioration of SNc [3]. Loss of dopamine in the hippocampus affects spatial learning and memory. The effect of dopamine on memory is thought to play an important role in synaptic plasticity and memory mechanisms of the hippocampus [4]. In addition to pharmacological treatments, medicinal plants and cell therapy are the most important ways to treat PD symptoms. The long term use of L-Dopa (a precursor of dopamine) has been associated with dyskinesia and reduction of efficacy, which requires an increase in the amount of drug to reduce the complications of the disease. Rosemarinus officinalis leaves extract (RE) is one of the medicinal herbs with therapeutic potential to control the PD symptoms [5]. RE contains phenolic compounds that have antioxidant, anti-allergic, anti-inflammatory and anti-apoptotic effects [6]. It has protective effects on the 6-OHDA and MPTP-induced oxidative stress and apoptosis in vitro, and neuroprotective effect on dopaminergic neurons in PD [7]. Ito and colleagues also reported in 2008 that rosmarinic acid (one of the compounds of the RE), like an antidepressant, stimulates the proliferation of neurons in the dentate gyrus of the hippocampus. They also revealed the anti-apoptotic effect of rosmarinic acid on astrocytes [8]. It has been observed that carnosic acid regulate autophagy and attenuate 6-OHDA-induced neurotoxicity in vitro [9]. Transplantation of dopaminergic cells into striatum is a laboratory method that have overcome the drug treatments and is used in the improvement of PD syndrome by providing an unlimited source of dopamine synthesis through the transplanted cells [10]. The use of embryonic stem cells has many limitations, while adult stem cells override these constraints. Adult stem cells are undifferentiated cells of the body and replaced lost cells to treat neurodegenerative diseases [11]. Mesenchymal stem cells (MSCs) are a type of adult stem cells. These cells are easily accessible and can be isolated from adipose tissue and bone marrow [12]. Adipose tissue-derived stem cells (ADSCs) have the ability to differentiate into different cell types of the body. These cells survive and migrate to the lesion site and differentiate into neurons after transplantation [13]. It was also shown that ADSCs can repair the damaged tissues by secreting cytokines and growth factors through paracrine pathways [14]. Transplanted ADSCs have the ability to secrete glial-derived neurotrophic factor (GDNF) and increase the neuronal viability of the hippocampus [15].

In this study, we compared the therapeutic efficacy of RE and ADSC on the memory impairment of PD. Since the hippocampus receives dopaminergic input from the SNc [16], the destruction of dopaminergic neurons and subsequent dopamine depletion of the hippocampus can lead to memory deficits [17]. Released dopamine from SNc affects the neurogenesis in the hippocampal dentate gyrus and regulates memory formation, due to the relationship between neurogenesis and memory formation [18]. In this study, bilateral injection of 6-OHDA into the SNc of male rats caused memory impairment similar to that seen in PD. Then, the neuroprotective effect of intravenous transplantation of ADSCs or oral administration of RE on the hippocampus and memory improvements were investigated.

MATERIALS AND METHODS

Animals

Male wistar rats weighing 220-280 g (n: 48) were purchased from Razi Research Institute, Karaj, Iran and housed at 20-24 °C under a 12 hour light/dark cycle, with free access to food and water. All experiments were done in accordance with the National Institute of Health Guide for the care and use of laboratory animals (NIH Publication No. 23–80, revised 1996) and approved by the research ethical standards in Damghan University.

Experimental groups

Animals were single-housed and kept separate for one week. Then they were trained in MWM apparatus, as we explained it in bellow. At the end of the second week, animals were positioned in a stereotaxic frame and 6-OHDA (Sigma-Aldrich, USA, CAS NO. 636-00-0) or normal saline was injected bilaterally into the SNc at specific coordinates, depended of the group. One week after injection, the second MWM training took placed. Ten days after injection of 6-OHDA, lesioned rats in cell group (Figure 1) were transplanted intravenously by BrdU- labeled ADSCs or α -MEM. The animals were subjected to two additional MWM tests, five and nine weeks after injection of 6-OHDA or normal saline. Decapitation, histological and immunohistochemical studies were done at the final week of experiments. Animals were gavaged by water or RE from 2 weeks before, until 8 weeks after the injection of 6-OHDA or normal saline. The experimental design were showed in detail in Figure 1.

Then six experimental groups were named as follows:

Lesion: 6-OHDA (6 μ g / 2 μ L of normal saline) was injected bilaterally into the SNc.

Sham: animals which received 2 μ L of normal saline instead of neurotoxin.

RE: lesioned rats were pretreated daily with RE (50 mg/kg), from two weeks prior to neurotoxin injection up to eight weeks post-injection.

Cell: lesioned rats transplanted intravenously by BrdU (Bromodeoxyuridine / 5-bromo-2'-deoxyuridine) - labeled ADSCs (106 cells per 500 μ L of medium), from 10 days post lesion until 8 weeks later.

Water: lesioned rats were gavaged by water as RE vehicle from 14 days before until 8 weeks after the lesion.

α -MEM: lesioned rats were administered intravenously by 500 μ L of medium.

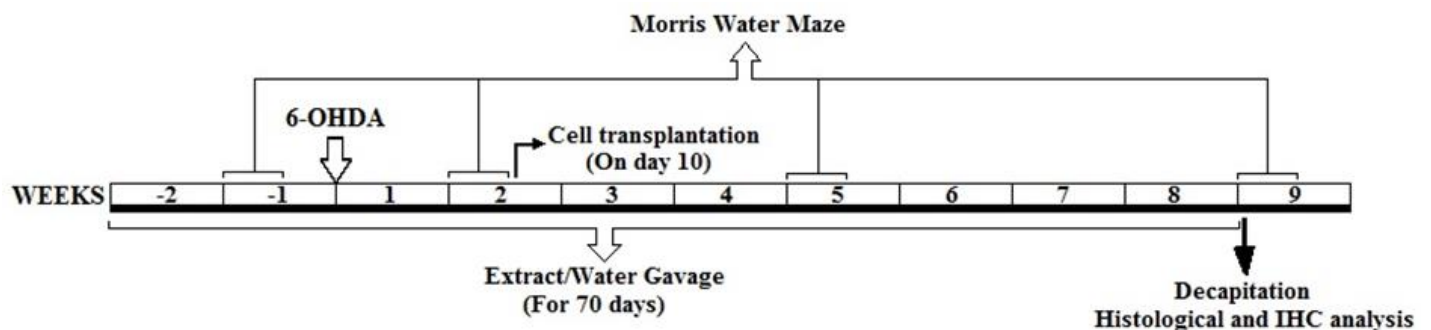


Figure 1. Experimental design. Timeline showing the behavioral and histological studies along with treatments.

Preparation of memory impairment model using 6-OHDA

Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and placed on a stereotaxic frame (Stoelting, 1886, USA). A total of six μ g 6-OHDA hydrobromide was dissolved in 2 μ L of sterile saline containing 0.2% ascorbic acid and injected bilaterally into the SNc with a Hamilton syringe (26 gauge, Hamilton, Massy, France) at a flow rate of 1 μ L/min. Stereotaxic coordinates from the bregma were: (anteroposterior -0.5 mm, mediolateral \pm 2.1 mm, and dorsoventral -7.7 mm) [19]. The syringe was left in place for 5 min after injection and then removed slowly to optimize toxin diffusion.

Morris Water Maze

The Morris water maze is an open circular pool (190 cm in diameter and 60 cm in height) that is filled approximately half-way with water (24 \pm 1 °C). A circular platform was hidden in the center of the southeast quadrant of the maze, three centimeters below the water level. In order to navigate, spatial clues in four

directions of the maze were installed on the walls of the room. Each animal was randomly placed into the water in one of four directions of north, south, east or west to find the hidden escape platform. If the animal did not find the platform within a minute, they were guided to the platform and left on the platform for 20 seconds. Training sessions continued for four days, each day four trials, and the animal's performance was recorded using a video camera connected to a computer. The memory was evaluated 24 hours after the completion of the training with a probe trial of 60 seconds. At this stage, after removing the platform, the animal was placed into the water from the opposite quadrant of the platform. Escape latency, distance moved, average speed and the time spent in the target quadrant were measured and analyzed using EthoVision XT software.

Rat ADSCs isolation and culture

The interscapular fat tissues were collected from rats (6-8 weeks age), under sterile conditions and placed in a sterilized culture dish under laminar hood. Fat tissues were digested mechanically and enzymatically with 0.075% collagenase type-I (Sigma-Aldrich; CAS NO.9001-12-1) for 1 hour at 37 °C. ADSCs were isolated, based on their capacity to adhere to the plastic flask. Isolated cells were incubated with 10% fetal bovine serum (FBS, Gibco, cat.No. 12203C) containing α -MEM (α -minimal essential medium; Gibco/BRL; cat.No. 52100-0.3), and 1% penicillin/streptomycin (Gibco; cat.No. 15140). First passage of cells was performed at 80-90% confluency. P4 cells were prepared for transplantation.

BrdU labeling and cell transplantation

The cells were incubated in medium containing 10 μ M bromodeoxyuridine (BrdU, Sigma; CAS NO.59-14-3) for 48 h and approximately labeled cells (1×10^6 / 500 μ l medium) transplanted by injection into the tail vein, at 10 days after lesion.

Rosemary extract preparation

RE containing 40% of carnosic acid (carnosic acid powder CAP25-110401) was purchased from "Hunan Geneham Biomedical Technology, China" and dissolved in distilled water.

Based on previous studies, effective dose of RE was 50 mg/kg [20-22]. Rats were gavaged orally with extract at a dose of 50 mg/kg.

Tissue preparation and immunohistochemistry

After 8 weeks, the animals were deeply anesthetized and transcardial perfusion of phosphate buffered saline (PBS) and 4% paraformaldehyde solution were carried out (100 ml of PBS followed by 200 ml of 4% Paraformaldehyde into the left ventricle). The brains were quickly extracted from the skull and fixed with 4% paraformaldehyde for 24 hours. coronal microscopic sections of 10 micron thickness were prepared at the level of + 2.8 mm relative to the bregma (within the areas of DG, CA1 and CA3 of hippocampus) and collected onto glass slides. The sections were stained with 0.75% Cresyl violet and cell counting was performed with X400 magnification. The number of neurons in the regions of DG, CA1 and CA3 were counted in five fields with an area of 400 μ m² and the means were calculated; the cell counting was repeated 7 times. Then the slides were photographed with \times 400 magnification using a digital camera (Nikon, DXM 1200, USA) equipped a fluorescent microscope (E600-Eclipse Nikon, Japan). In order to confirm the destruction of the dopaminergic neurons of the SNc, an anti-Tyrosine hydroxylase (TH) staining was performed. Some sections of SNc and hippocampus regions were boiled in citrate buffer in order to antigen retrieval and incubated with the primary antibodies: anti-TH (Anti-Tyrosine Hydroxylase, MilliporeSigma-AB152); anti-BrdU (Monoclonal anti-BrdU antibody in mouse, Sigma-Aldrich, B2531); anti-GFAP (Anti-Glial Fibrillary Acidic Protein, Sigma-Aldrich, G3893) overnight at 4 °C. Samples were incubated with the secondary anti-bodies conjugated with HRP for TH and BrdU (Abcam, ab205718) for 1 hour at 37°C, followed by incubation with 0.1% DAB (Sigma-Aldrich, D7304) substrate solution for 15 minute. For anti-GFAP immunostaining, sections were incubated for 1 hour at 4°C with fluorescein conjugated secondary antibody (Abcam, ab97029). The DAB 0.1% substrate solution was used and sections were dehydrated and mounted using DPX.

Statistical analysis

Data were analyzed by SPSS 23 software, using one-way ANOVA or Paired-sample t-test. Tukey's post hoc test was used for analyzing the data within groups. The results are indicated as mean \pm standard error of the mean (SEM) and considered as meaningful at $p \leq 0.05$.

RESULTS

Behavioral analyses

Animals of all the groups underwent a 4-day learning period one week before and one, four and eight weeks after the lesion. Paired sample t-test showed a significant difference in escape latency and distance traveled between the first and the fourth day in all the groups ($p < 0.05$), meaning that all animals were able to learn the task. One-way ANOVA showed no significant difference among the groups in escape latency, one week before the lesion, for four training days, ($F_{(5,42)} = 1.12$, $P = 0.36$), ($F_{(5,42)} = 0.36$, $P = 0.873$), ($F_{(5,42)} = 9.52$, $P = 0.733$) and ($F_{(5,42)} = 1.45$, $P = 0.213$) respectively (Figure 2.a). Also there were no significant difference among the groups in distance traveled for four training days, ($F_{(5,42)} = 1.96$, $P = 0.105$), ($F_{(5,42)} = 3.28$, $P = 0.114$), ($F_{(5,42)} = 1.17$, $P = 0.336$) and ($F_{(5,42)} = 1.05$, $P = 0.398$) respectively. In the second learning period (one week after the lesion), a significant difference in escape latency, was observed using one-way ANOVA in days 1, 2, 3 and 4; ($F_{(5,42)} = 110.31$, $P = 0.000$), ($F_{(5,42)} = 187.57$, $P = 0.000$), ($F_{(5,42)} = 93.8$, $P = 0.000$) and ($F_{(5,42)} = 143.09$, $P = 0.000$) respectively, and in distance traveled for four training days, ($F_{(5,42)} = 61.91$, $P = 0.000$), ($F_{(5,42)} = 159.5$, $P = 0.000$), ($F_{(5,42)} = 47.98$, $P = 0.000$) and ($F_{(5,42)} = 52.28$, $P = 0.000$) respectively. Tukey's post hoc test showed that animals in the lesion, water, cell and a-MEM groups had significantly higher escape latency and distance traveled in comparison with the sham and extract groups (Figure 2.b).

In the third learning periods (4 weeks after the lesion), one-way ANOVA showed a significant difference in days one through four, in escape latency ($F_{(5,42)} = 33.05$, $P = 0.000$), ($F_{(5,42)} = 92.84$, $P = 0.000$), ($F_{(5,42)} = 121.33$, $P = 0.000$) and ($F_{(5,42)} = 60.0$, $P = 0.000$) respectively, and in distance traveled ($F_{(5,42)} = 48.67$, $P = 0.000$), ($F_{(5,42)} = 65.74$, $P = 0.000$), ($F_{(5,42)} = 122.54$, $P = 0.000$) and ($F_{(5,42)} = 47.66$, $P = 0.000$) respectively. Results of the fourth learning periods (8 weeks after the lesion) were as same as third learning trials; results of escape latency respectively from first to fourth trial days were ($F_{(5,42)} = 334.47$, $P = 0.000$), ($F_{(5,42)} = 396.52$, $P = 0.000$), ($F_{(5,42)} = 300.8$, $P = 0.000$) and ($F_{(5,42)} = 569.97$, $P = 0.000$), and results of distance traveled were ($F_{(5,42)} = 188.53$, $P = 0.000$), ($F_{(5,42)} = 130.08$, $P = 0.000$), ($F_{(5,42)} = 180.48$, $P = 0.000$) and ($F_{(5,42)} = 261.41$, $P = 0.000$).

Tukey's post hoc tests for third and fourth learning periods showed that sham, extract and cell groups performed significantly better ($p < 0.000$) than the water, lesion and a-MEM groups in escape latency as well as distance traveled (Figure 2.c and 2.d). At the end of third and fourth learning periods, a probe trial was taken to assess the spatial memory formation. During the 60-second probe trial, total distance traveled and time spent in the target quadrant and platform location latency were recorded. One-way ANOVA on time spent in the target quadrant, showed a significant difference among groups, both 4 and 8 weeks after the lesion ($F_{(5,42)} = 12.44$, $P = 0.000$) and ($F_{(5,42)} = 86.33$, $P = 0.000$) (Figure 2.e). ANOVA on total distance traveled, showed no significant difference during the probe trials among the groups, 4 and 8 weeks after the lesion ($F_{(5,42)} = 1.16$, $P = 0.343$) and ($F_{(5,42)} = 1.25$, $P = 0.3$). One-way ANOVA on platform location latency data indicated a significant difference among the groups ($F_{(5,42)} = 107.97$, $P = 0.000$) and ($F_{(5,42)} = 143.48$, $P = 0.000$) 4 and 8 weeks after the lesion respectively (data's not shown). Analysis followed by Tukey's post hoc test showed that cell and extract groups spent a significantly longer period in the target quadrant and had lower platform location latency as compared with lesion group, both at 4 and 8 weeks after the lesion ($p < 0.05$) (Figure 2.e).

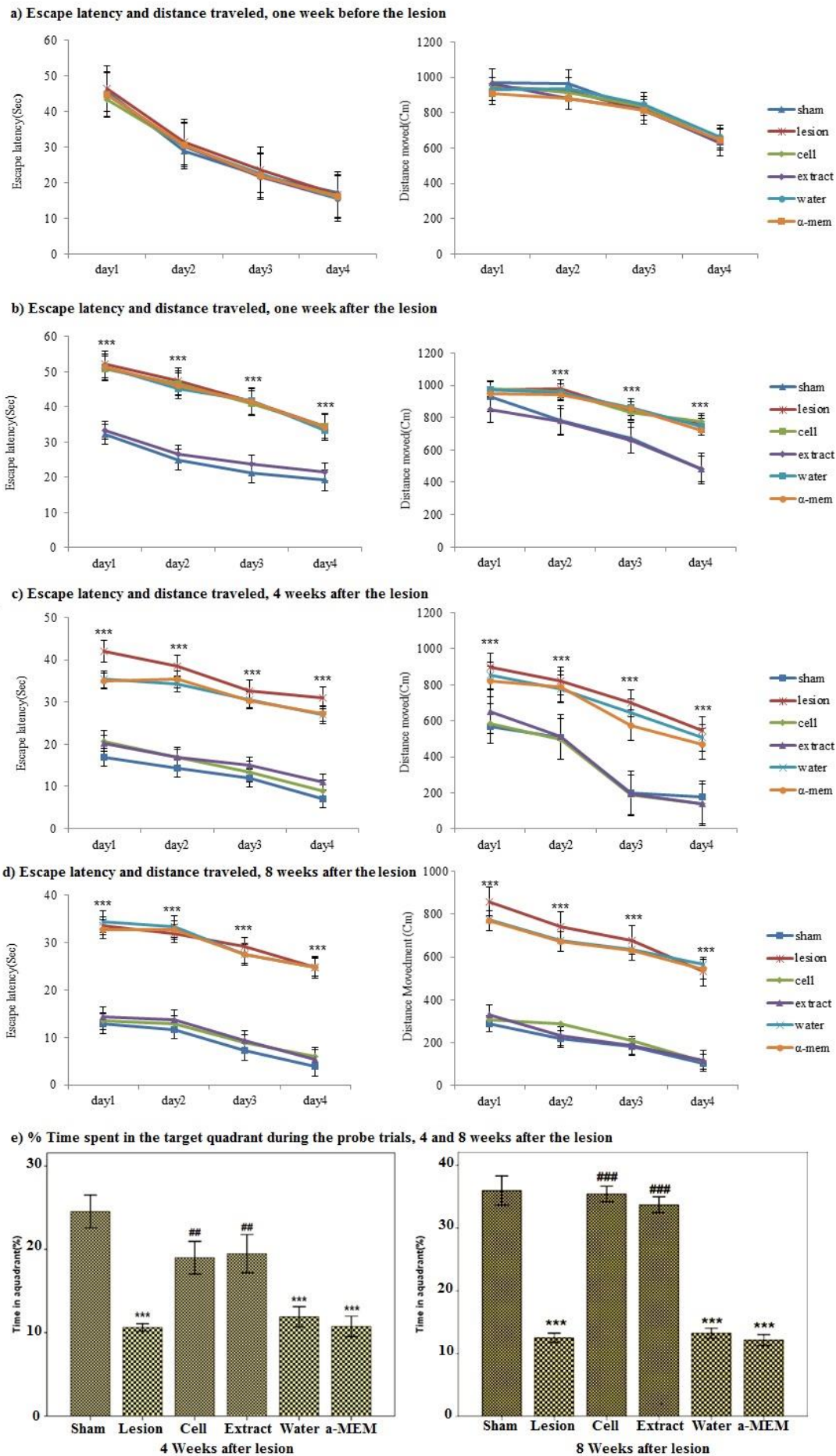


Figure 2. Morris water maze spatial learning and memory task. Escape latency and distance moved to reach the hidden platform in different training phases (a-d). Time spent in the target quadrant during the probe trials, 4 and 8

weeks after the lesion (e). *** $p < 0.000$ compared with sham. ### $p < 0.000$, ## $p < 0.00$ compared with lesion. Data are presented as mean \pm SEM.

Cresyl violet staining and Immunohistochemistry

Immunohistochemistry was used to determine TH-positive neurons in the SNc of all groups. There were reduction of TH-positive neurons in the lesion, Water and α -MEM groups compared to Sham, Cell and Extract groups, but it was decreased in the SNc of lesion group (Figures 3, A-F).

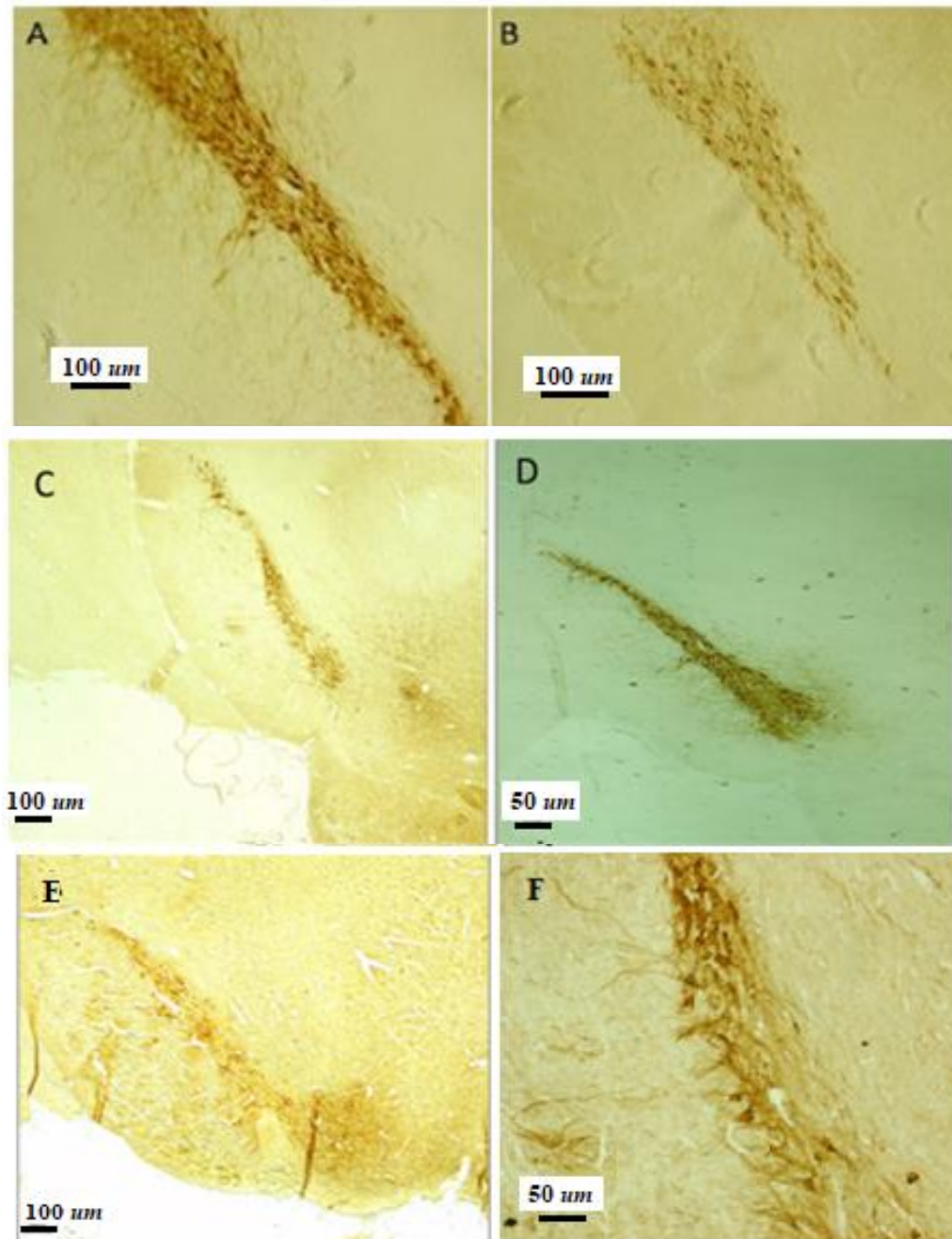


Figure 3. Immunohistochemical staining of TH-positive neurons in the SNc of Sham (A), Lesion (B), α -MEM (C), Cell (D), Water (E) and Extract (F) groups.

Cresyl violet staining showed the effect of neurotoxin 6-OHDA on neurons density in DG, CA1 and CA3 regions of the hippocampus, and also the protective effect of RE (50 mg/kg) and ADSCs transplantation (Figure 4).

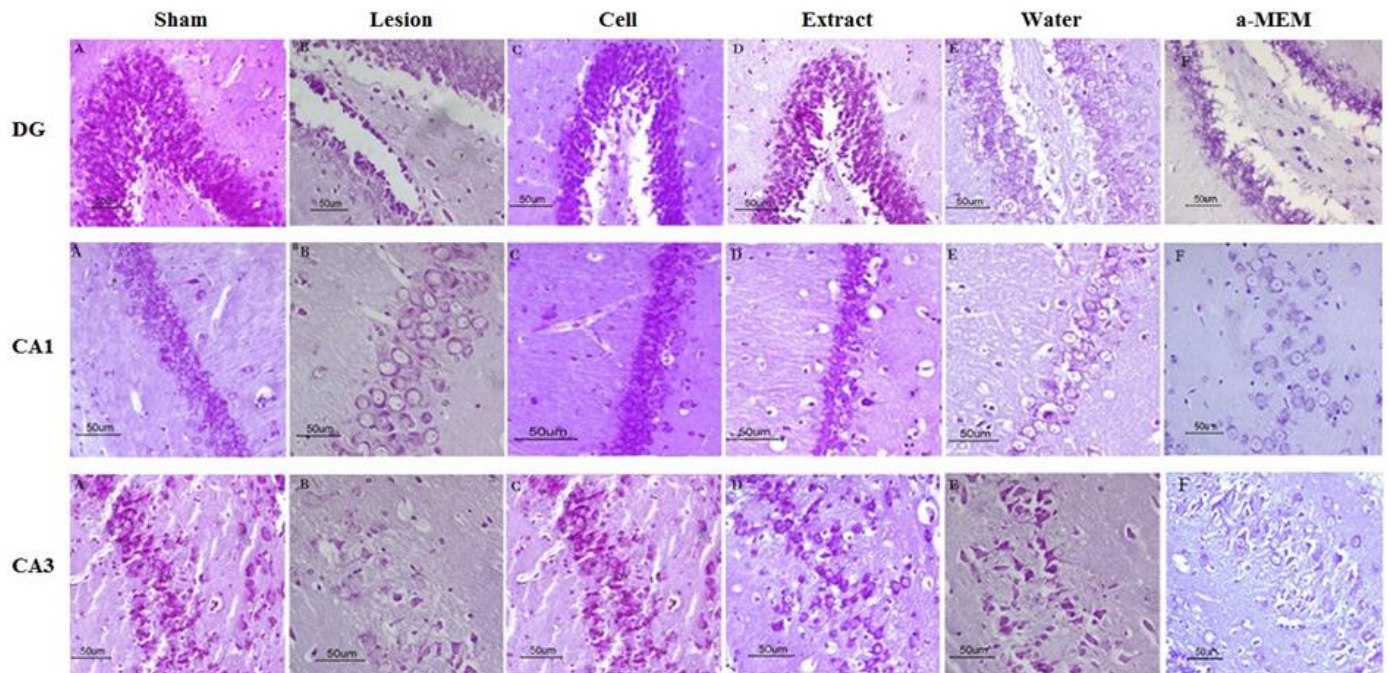


Figure 4. Cresyl violet staining of DG, CA1 and CA3 regions of the hippocampus in all groups. The neurons density of cell and extract groups were more than the other groups except the sham.

The density of neurons in the three regions of DG, CA1 and CA3 were quantitatively studied. The number of DG granular cells were significantly increased ($p < 0.000$) in the cell and extract groups, compared to lesion, water and α -MEM groups (Figure 5.a). Moreover, extract treatment and cell transplantation significantly increased ($p < 0.000$) the number of CA1 and CA3 pyramidal neurons (Figure 5. b,c).

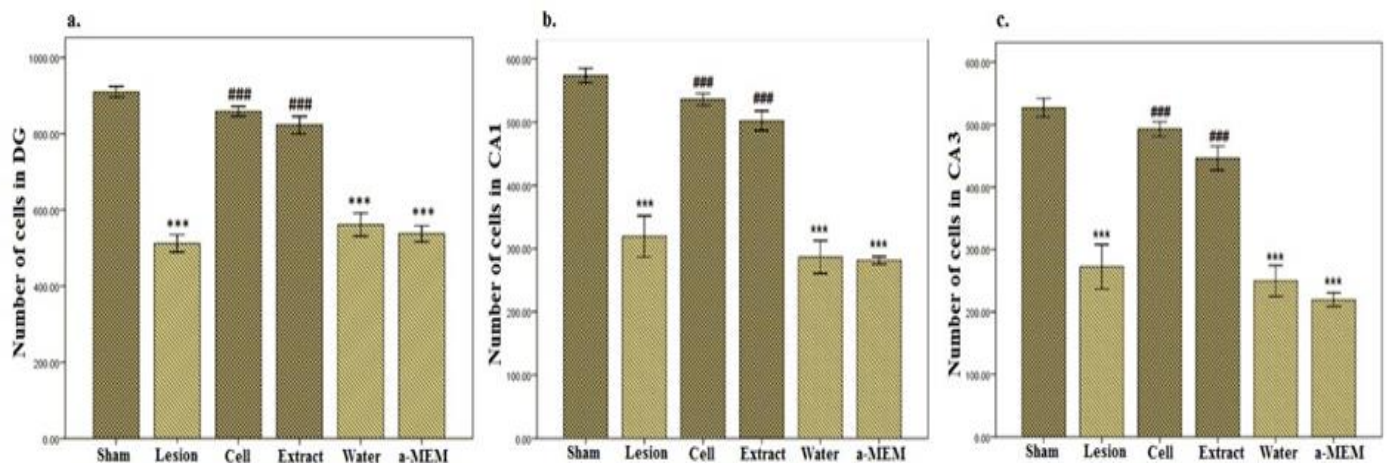


Figure 5. Cell count of Cresyl violet stained sections in DG (a), CA1 (b) and CA3 (c) of the hippocampus. The number of DG granular cells were significantly increased in the cell and extract groups, compared to lesion, water and α -MEM groups (a). Extract treatment and cell transplantation also significantly increased the number of CA1 and CA3 pyramidal neurons (b,c). *** $p < 0.000$ compared with sham. ### $p < 0.000$ compared with lesion. Data are presented as mean \pm SEM.

CD71 and CD90 (MSCs specific positive markers) positive ADSCs were shown in (Figure 6.a, b). BrdU-positive cells were also checked by anti-BrdU immunostaining, the labeled ADSCs successfully passed through blood brain barrier and successfully migrated to lesioned site (Figure 6.c).

As astrocytes activity and astrogliosis, are considered one of the main characteristics of PD, we investigated the density of astrocytes using anti-GFAP immunohistochemistry (Figures 7, a-f). GFAP positive cells in the DG (Figure 8.a) and SGZ (Figure 8.b) regions showed that the density of astrocytes in lesion, water and α -MEM increased significantly ($p < 0.000$) compared to sham, cell and extract groups.

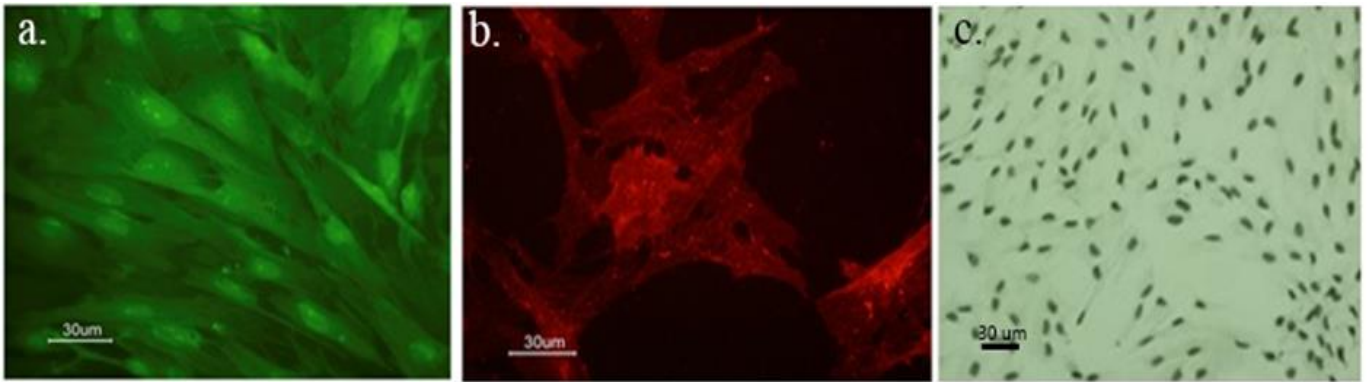


Figure 6. Cultured ADSCs expressed markers such as CD71 (a) and CD90 (b). More than 90% of the cultured cells were BrdU positive (c).

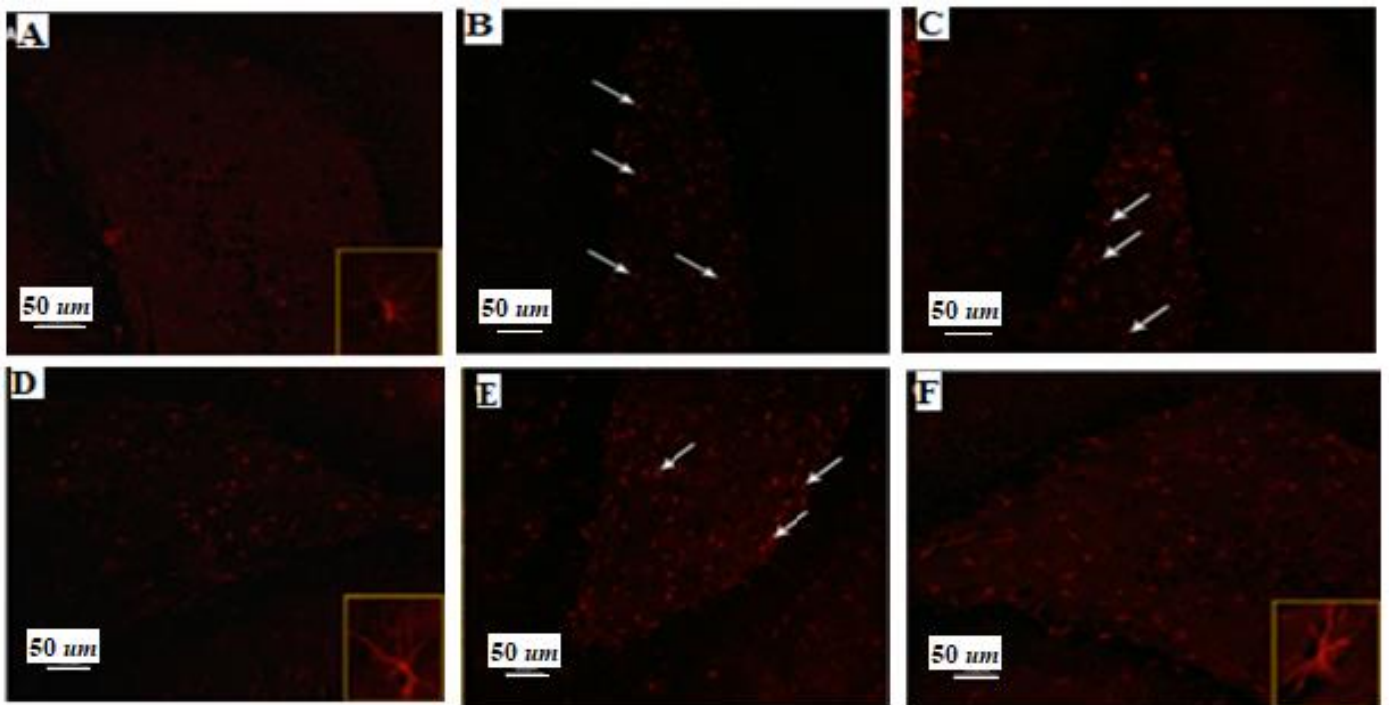


Figure 7. Immunohistochemical staining of GFAP-positive neurons in the hippocampus of Sham (A), Lesion (B), α -MEM (C), Cell (D), Water (E) and Extract (F) groups.

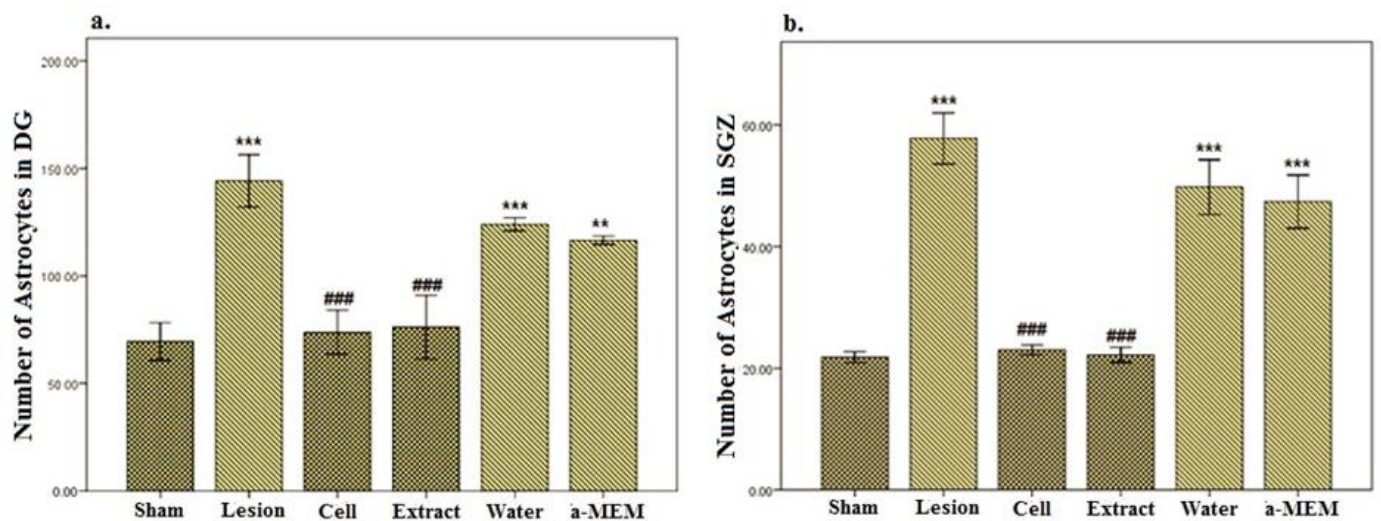


Figure 8. The number of GFAP positive astrocytes in DG (a) and SGZ (b) regions of the hippocampus. It was shown that the density of astrocytes in lesion, water and α -MEM were increased significantly compared to sham, cell and extract groups.

*** $p < 0.000$, ** $p < 0.00$ compared with sham. ### $p < 0.000$ compared with lesion. Data are presented as mean \pm SEM.

DISCUSSION

In this study, spatial memory of Parkinsonian rats was evaluated using MWM. The results of the training phase, before lesion showed the ability of all animals to learn, as revealed by decrease in escape latency and distance traveled, from first to fourth day of training. All lesioned groups, except extract group, had lower learning ability than sham in training sessions, one week post lesion. By the other word, pretreatment with RE before lesion compensates the negative effects of injury on learning ability. The learning ability of cell treated rats was similar to sham group, it means that cell injection as pre-treatment with extract could improve spatial memory impairment in injured rats in the 3rd and 4th training sessions.

The process of memory retention was evaluated one day after the end of 3rd and 4th training sessions, which was performed 4 and 8 weeks after the lesion. There was no significant difference in the distance traveled between the groups, suggesting that the development of this model leads to memory impairment before motor disorder. Pre-treated animals with RE and also cell injected animals showed significant increase of time spent in target quadrant as compared with sham, in former probe tests. The results of this study clearly demonstrated the effects of cognitive improvement of RE and ADSCs in the Parkinson's model.

Rasoolijazi and coauthors reported the protective effect of carnosic acid against memory deficit in Alzheimer' disease [21]. Memory impairment of Parkinsonian rats was improved by oral administration of RE [22]. Schwerk in 2015, showed that ADSCs transplantation lead to improve cognitive performance [23].

RE has antioxidant, anti-tumor and anti-inflammatory activities. Carnosic acid, carnosol, rosemarinic acid and ursolic acid were the main polyphenolic compounds of extract. Among these compounds, carnosic acid exhibited a promoting antioxidant activity [24]. Reports also confirmed the anti-depressant, anti-allergic, anti-inflammatory, anti-tumor, and anti-apoptotic effects of this extract [25]. Lee reported the antioxidant effect of extract on dopaminergic neurons and believed that rosemarinic acid with an antioxidant effect plays a neuroprotective role on dopaminergic neurons [25]. Also, Du and colleagues in 2010 showed the neuroprotective effects of RE against 6-OHDA and MPTP neurotoxins by anti-apoptotic and antioxidant properties in vitro [26]. Many researchers have focused on the effects of the RE and its components on aging or trauma induced cognitive dysfunctions, and showed its therapeutic potentials [27-29].

Today, research on stem cells and their therapeutic use have been progressed. Mesenchymal stem cells can differentiate into many cell types including neural cells [30]. It was reported that labeled stromal cells injected into the striatum can express neuronal and glial markers and relatively improve PD symptoms. Yan et al. in 2013 showed that MSCs can inhibit inflammatory factors produced by active microglia and suppress microglial activity in vitro [31]. MSCs can also modulate the production of neurotrophic factors and subsequently, ameliorate neurological disorders [32].

In this study, we also evaluated the histological effects of RE and ADSCs on the hippocampus. There were studied in DG, CA1 and CA3 regions of the hippocampus. Cell counting showed that administration of RE at a dose of 50 mg / kg, and also ADSCs transplantation at 8 weeks after 6-OHDA-induced models, significantly increased the number of granular cells in the DG region and also, the number of pyramidal cells in CA1 and CA3 regions compared with lesion, water and α -MEM groups, while the treated groups showed no significant difference of cell density as compared to sham. The results showed that the reduction of cell density induced by the 6-OHDA lesion was completely compensated by the treatment with the extract or stem cell transplantation. Thus, ADSCs were considered to have a therapeutic effect in PD.

It was also observed that the number of GFAP positive astrocytes increased in the lesion, α -MEM and water groups compared with the sham group. Cell transplantation or RE reduced the density of GFAP positive cells as compared to the sham group. Therefore, the treatments not only improved the density of the granular and pyramidal cells of the damaged areas of the hippocampus, but also decreased the density of GFAP-positive astrocytes. Astrogliosis is an important response of astrocytes to infection, trauma, α -synuclein accumulation, ischemia, and neurodegenerative diseases [33]. Astrogliosis and microgliosis in the SNc of patients with PD are the main features of the disease [34]. Activated glial cells can play a role in the death of dopaminergic neurons by activating apoptosis through cytokines (such as TNF- α , IL- β , IL-6 and interferon gamma, and production of nitric oxide), released into the neurons and stimulate lipid peroxidation, DNA breaks and inhibition of mitochondrial metabolism [35-37]. It was suggested that inhibition of astrocytes activity during inflammatory and damaging processes can be a therapeutic way to reduce neuronal damage during PD [38]. The immunohistochemical study of anti-GFAP showed that the number of GFAP positive astrocytes decreased in the animals treated with RE or ADSCs.

CONCLUSIONS

The model used in this study was a suitable model for PD memory impairment. This model results in spatial learning and memory impairment through bilateral injection of 6-OHDA into the SNc which also leads to the destruction of neurons in the hippocampus. The Parkinsonian rats, treated with RE or ADSCs, appeared almost the same as the animals in the sham group in the behavioral, histological and immunohistochemical studies, and no significant difference was observed between the cells and RE-treated groups. The results indicated that ADSCs transplantation or RE pre-treatment had neuroprotective effect and supported the recovery of memory deficit in 6-OHDA-induced rat model of PD.

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

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