The neuroprotective effects of dental pulp stem cells and erythropoietin in mice hippocampus after ischemia-reperfusion

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Article

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Abstract

Background

Brain strokes are the cause of death in many people, among survivors; it can cause problems such as motor and cognitive impairment. The role of the hippocampus and its damage in ischemia has been assessed by researchers. One of the treatments commonly used today by researchers in cell therapy. Therefore, this study aimed to evaluate the use of dental pulp stem cells and erythropoietin in mice hippocampus after ischemia-reperfusion.

Methods

In this study, NMRI male mice were divided into six groups. Except for the sham group, all groups group experienced ischemic hippocampus. A group received erythropoietin or dental pulp stem cells and the other group received a combination exposer of erythropoietin and DPSC, while the vehicle group received DPSC solvent and erythropoietin solvent. After eight weeks, they were subjected to a test of learning and memory by Morris water maze. Then, their brains were examined for histological assessment, and immunohistochemistry (DCX and NeuN for neurogenesis). Furthermore, VEGF was applied for angiogenesis and GFAP for gliosis examination.

Results

The behavioral function of the group receiving erythropoietin and the combined group (DPSC and erythropoietin) was better than other groups. The mean number of healthy cells in EPO, DPSC, and EPO + DPSC groups was significantly different from that of the vehicle group ($P < 0.05$). Besides, DPSC, EPO, and EPO + DPSC groups showed a significant increase in green density in comparison with the ischemia and vehicle groups ($P < 0.05$), but no difference was found between the ischemia and sham groups.

Conclusion

DPSC and erythropoietin were capable of increased neuronal function but behavioral studies revealed that outcomes of erythropoietin therapy are better than DPSC

Background

Stroke or ischemia is the leading cause of death and severe neuropathy worldwide. Brain ischemia is considered the third leading cause of death in Western countries. Ischemia results in nerve disorders such as motor disorders, sensory and vision disorders, aphasia, and neuropsychological deficits such as reduced intellectual capacity, apraxia, agnosia, spatial learning, and memory impairment (1–3).

Metabolic disorders caused by cerebral ischemia and reperfusion eventually lead to the death of neurons (including necrosis and apoptosis) (4). Certain regions of the brain and certain types of neurons are more sensitive to cerebral ischemia, including hippocampal CA1 pyramidal neurons (5, 6). Other complementary supplements are now required for the mortality of neural cells depending on the lesion area, except for conventional therapies, rehabilitation, and physiotherapy, because available strategies are not efficient enough. Therefore, to treat this common nerve disorder, the medical community needs an effective repair strategy (7–9).

Mesenchymal stem cell (MSC) transplantation using dispose of tissue-derived versus bone marrow-derived versus umbilical cord mesenchymal stem cells has improved brain function after ischemia (10). Today, postnatal human dental pulp stem cells (DPSCs) have received a lot of attention. DPSCs are stem cells derived from nerve sphincter that can be used as an appropriate cellular source without causing ethical problems in patients (12, 11). Compared with BM-MSCs, the separation of DPSCs is less invasive, has a greater ability to expand, and exhibits stronger immunosuppressive properties (13).

Various neurotrophic factors can be secreted from DPSCs, including NGF, neurotrophic 3, BDNF, and VEGF, and can increase neuronal survival, proliferation, differentiation, and migration. Also, numerous studies have shown that DPSC transplantation can increase neuroprotective effects and improve brain function after ischemia in vivo (14). On the other hand, increased expression of erythropoietin and its receptor, EPO-R, is another endogenous response of the nervous system to ischemia, which increases the tolerance to hypoxia (15). EPO is a neuroprotective medium that can cross the blood-brain barrier. In animal models, it has been shown that EPO can play a protective role in ischemic injury, cerebral and spinal cord injuries, as well as peripheral neuropathy (16, 17).

Many mechanisms of cellular protection are the beneficial effects of EPO and its variants in stroke and hypoxia-ischemic stroke models: apoptotic suppression, anti-inflammatory effects, antioxidant effects, restoration of the BBB integrity and stimulation of neurogenesis and angiogenesis (18). It seems that the combined use of dental pulp stem cells and EPO following ischemia/reperfusion injury in the hippocampus could have a higher therapeutic effect. Therefore, we decided to investigate the effects of DPSC along with erythropoietin in mice hippocampal ischemia model.

Materials And Methods

In vitro studies

Separation and isolation of stem cells

Healthy wisdom teeth in Ali Asghar and Behesht Hospitals were transferred to the cell culture laboratory of the University by soaked facial tissues. In the laboratory, the teeth were first disinfected in 70% ethanol and then washed with sterile distilled water. Then, by cutting in between the root and crown, the pulp was removed by a nerve puller 15, underneath the hood within a sterile PBS solution, followed by dividing into

smaller pieces with a surgical blade. These components were placed in a 3 mg/ml collagenase solution at 60 ° C for 60 minutes and tried to isolate the cells with an insulin syringe.

By adding about 3 ml of α-MEM solution with 20% fetal Bovine Serum, the mixture was centrifuged and the resulting precipitate was placed in a sterile PBS solution, followed by re-centrifugation. By separating the precipitate, it was pipetted and then placed in an α-MEM containing 20% fetal Bovine Serum and Pen/strep 1%. Furthermore, they were placed in a filter flask and then stored in a humidified incubator at 37 ° C with 5% CO2. On average, 1: 3 passages were performed every 5 days with EDTA- trypsin solution

The Nature Of The Cells

To verify the identity of the cells in the third passage, flow cytometry was performed for measuring the positive markers of mesenchymal cells including CD90, CD105, CD73, and CD44 as well as negative markers: hematopoietic markers (CD33 and CD31).

Stem Cell Labeling

A total of 106 cells were floated in a serum-free medium and 5μg /ml of DiI was then added to their medium and slowly pipetted. After that, the medium containing the cells and the DiI was incubated for 15 minutes. To prevent additional cytoplasmic color, 5 minutes were placed in the refrigerator. The suspension was centrifuged for 5 minutes at 1500 rpm, and the liquid was removed from the precipitate, followed by suspension by PBS. In the final stages, the procedure was followed by washing twice using PBS.

In Vivo Studies

Experimental animals

In this study, 60 NMRY mice with a weight of 30–40 g were used. They were randomly selected from the animal house of Iran University of Medical Sciences. All animals were kept in standard condition (12 hours of light and 12 hours of darkness, 24 ± 1 ° C) and had enough water and food before and after ischemia. Ethic committee of Iran University of Medical Sciences approved the experiments and all experiments were performed in accordance with relevant guidelines and regulations following the recommendations in the ARRIVE guidelines.

The Ischemia Reperfusion Injury Model And Animal Grouping

The mice were intra-peritoneal anesthetized with a combination of ketamine (70 mg /kg) and xylazine (3–5 mg/kg). After fixing the hands and feet of the animal, a 2.5-cm vertical incision in the neck midline was made. Common carotid artery was carefully removed from the vagus nerve and the internal jugular

vein and the arteries on both sides were blocked by using special micro-bulldog clamps for 20 minutes. Afterward, the clamps were opened in order to re-circulate the bloodstream (reperfusion).

The sham group was surgically treated but did not induce ischemia

Transplantation Of Dps Cells Into Hippocampus

After adjusting the Hamilton syringe on the Bregma point, the coordinates of points (-2.3 mm, ML: ± 1.3 mm, DV: +1.5 mm) were adjusted using the Paxinous and Watson Atlas for the CA1 site of the Hippocampus. A total of 1×10^5 DPS cells in a 4 µl volume of PBS were delivered at each of the target coordinates. The Injection speed was 0.25 µlit/min.

Mice successfully modeled were randomly assigned (Sarveazad, Babahajian et al. 2017) in equal numbers to five groups (n = 10 per group) as follows:

1. Ischemia-reperfusion group (I/R), in which animals underwent surgery and common carotid arteries were temporarily closed to induce ischemia.
2. Vehicle group: After induction of ischemia, they received the cell carrier material
3. EPO group, in which, animals received intraperitoneal erythropoietin (1,000 IU/kg) 24 hours after induction of ischemia, repeated one day later.
4. Stem cell group, in which, dental pulp stem cells were injected into the hippocampus of animals, 4 days after induction of ischemia.
5. EPO + Stem cell group, in which, animals received intraperitoneal erythropoietin (1,000 IU/kg) 24 hours after induction of ischemia and was repeated one day later followed by dental pulp stem cell injection into the hippocampus 4 days after induction of ischemia.

Spatial Memory

Eight weeks after the creation of an ischemia-reperfusion model, MWM was used to examine the spatial memory. This device is designed for assessing spatial and related forms of learning and includes a black cylinder with a diameter of 130 cm and a height of 60 cm, filled to a height of 27 cm with a temperature of 22 ± 2 ° C. The rescue platform is a 15 cm diameter, 1 cm below the water and in the northwest quarter. The MWM was in a room that was surrounded by outsider’s signs such as watches, posters, shelves, lights, tables, etc.

At the top of the pond, there was a camcorder that monitored the animal with infrared light while it was swimming. At first, animals were trained for 5 consecutive days, each day, the animal was thrown into the water from four different tank areas, each with a 60-second time, allowing the animal 60 seconds to find the submerged escape platform If the platform was not found, the animal was driven onto the submerged escape platform, and after 15 seconds of rest, it was thrown from the other area to the water. Swimming

time to find a hidden platform in the learning phase was an important factor in the training days. At first, the average swimming time of animals was calculated for each target group, and each training day.

After training, experiments were performed and films from the motion of the rat were displayed through a computer monitor and a special program called Ethovision (XT version 5). The assessment included measuring the speed of the rat, the distance spent in the target quadrant, and the percentage of time spent on the platform.

Nissl Staining

The number of dark and light cells was detected by histopathology and Nissl staining .Perfusion was performed for all groups of animals. After perfusion of the animal, the brain was placed in a postfix solution, preferably formalin 10%, for one week. Then, the intermediate part of the brain was subjected to tissue processing. To prepare the tissue for embedding, it was first washed with fresh water for 15 minutes and then samples were added to the processor for removing water by dehydration with a series of alcohols, 70–95% to 100%, followed by clearing and embedding with xylene and paraffin. The coronal section of samples was provided using a rotary microtome (Leica, RM2235, and Germany) with a thickness of 5 µm and placed on gelatin slides. Finally, the specimens were placed in 0.1% Cresyl fast violet acetate or Nissl Stain solution to examine the dark and light cells, and they were glued onto the slides using Entellan mounting medium. Then, the nucleus of the pyramidal neurons was counted in the form of internal-external expansion in the CA1 region, and for each section, at least four fields with a minimum of 40 microns spacing were considered. The stained slides were examined by the OLYMPUS microscope, (AX70 70) and magnification of x40. Then cell counting was done by Image J software. The pyramidal neurons in these specimens were selected at intervals of 1.7, 1.8, 0.9, and 1.2 mm according to Paxinos atlas.

Immunohistochemistry (Dup: Abstract ?)

after applying ischemia to I/R and vehicle groups, the animals were anesthetized by intraperitoneal injection of 90 units of ketamine and xylazine (1/10). After perfusion, the brain was removed and placed in a 10% formaldehyde solution for 48 hours. After performing the steps, including dehydration, clearing, and infiltration, 5 micrometers were cut from the CA1 region and placed on 0.1% gelatin slides.

In the next step, the primary antibody (abcam18723) DCX, NeuN (abcam177487) was diluted 1: 500 with PBS and placed on tissue samples. To avoid drying the surface of the tissue, a moist chamber was used. The slices were then placed at 4 ° C for 24 hours and then exposed to secondary antibody after washing.

The DAPI was used to stain the cells for 15 minutes (1 µ/ml: 1000 µ/ml PBS), which usually brings the nuclei in blue.

Statistical analysis

All information was expressed as Mean±SE. One-way, two-way ANOVA was used for statistical analysis and behavioral tests. Furthermore, One-way ANOVA was used to analyze the number of CA1 neurons. In all calculations, $P < 0.05$ was considered a significant difference.

Results

Flow cytometry

As shown in Fig. 1, a single-cell solution derived from dental pulp cell culture, positive markers including CD90, CD105 (85.43%), CD73 and CD44 (87.4%) as a marker of mesenchymal cells as well as negative markers of CD33(1.03%) and CD31 as markers of the hematopoietic cell were obtained using flow cytometry.

Mwm Behavioral Studies

Two-way ANOVA showed a significant difference between experimental groups ($P < 0.05$) and different training days ($P < 0.05$) and a significant interaction was found between training days and different groups ($P < 0.05$).

One-way analysis of variance revealed that there was a significant correlation between I/R and sham groups ($P < 0.05$), indicating the effect of the ischemic agent on animal behavior in I/R group and the animals in this group swam more time in MWM compared to the sham group. Comparison of EPO, DPSC, and EPO + DPSC groups with I/R group showed a significant relationship between swimming time for finding a hidden platform in all groups, except for the DPSC group; this means that our treatments have had a positive effect on the learning performance of the mice in the EPO and EPO + DPSC groups and they took less time to find the platform. Although the DPSC group had better learning performance than the I/R group, this difference was not significant ($P > 0.05$). A comparison of EPO and DPSC group with ischemic group demonstrated that EPO group mice had better performance. We found a better learning performance for EPO + DPSC mice as compared to EPO and DPSC groups ($P < 0.05$). Paired-samples t-test showed a significant difference between the first and fifth days of training in all groups ($P < 0.05$).

A comparison between I/R and vehicle groups exhibited no significant difference between these groups ($P > 0.05$). Moreover, there was no significant difference between the vehicle group and the DPSC group, but the comparison of the vehicle group with EPO and EPO + DPSC groups showed a significant difference (Fig. 2A). One-way analysis of variance showed a significant difference between the groups regarding time spent in the target quadrant.

There was a significant difference in EPO, DPSC, EPO + DPSC, and sham groups as treatment groups when compared with I/R group ($P < 0.05$). This means that animals in EPO, DPSC, and EPO + DPSC groups spent a long time in the target quadrant compared to the ischemic group. When the time spent by EPO, DPSC, and EPO + DPSC groups was compared with the vehicle group, this relationship was

significant ($P < 0.05$). On the other hand, no significant relationship was seen between vehicle and I / R groups (Fig. 2B).

Histological And Histochemical Evaluations

After staining and counting light cells in the CA1 region of the hippocampus, the mean number of light cells in the ischemic group decreased significantly as compared to the sham group ($P < 0.001$), (Fig. 3), in the three treatment groups, EPO, DPSC, and EPO + DPSC, the death rate of pyramidal neurons in the CA1 region was significantly lower and the number of euchromatin cells was significantly higher than that of the ischemic group ($P < 0.05$) (Fig. 4).

The EPO + DPSC group increased the number of euchromatin cells as compared to the ischemic group and the other two treatment groups. Furthermore, the mean number of light cells in EPO, DPSC, and EPO + DPSC groups was significantly different from that of the vehicle group ($P < 0.05$), (Fig. 5).

Immunohistochemistry

In this method, two DCX and NeuN antibodies were studied in different groups. 9 weeks after transplantation of DPSC cells to the lesion site, some of them were converted to DCX-positive cells (neurons forming) and NeuN-positive (adult neurons). In the DPSC group and the EPO + DPSC group, the dental pulp stem cells have a DiI positive marker. Furthermore, a secondary antibody, FITC conjugate was used to detect DCX and NeuN cells. Due to the lack of Cells of external origin in I/R, sham, EPO, and vehicle groups, it can be concluded that all DCX and NeuN expressing cells have an internal origin. But in DPSC and EPO + DPSC groups, some of the cells have an internal origin and some other external injection origin. Considering that the treatment protocol in DPSC, EPO, and EPO + DPSC groups improved cognitive function in the MWM, it seems that they have been able to change the cell events in the area (after the lesion), such as the formation of new neurons and their transformation into adult neurons (Fig. 6–8).

In the study of the intensity of green color for DCX / FITC + samples in different groups, the following results were obtained:

1. DPSC, EPO, and EPO + DPSC groups showed a significant increase in the density of the green color as compared to the ischemic group.
2. In groups DPSC, EPO, and EPO + DPSC, the green density increased significantly compared with the vehicle group.
3. The ischemic and sham groups did not differ much from each other.

Discussion

According to research, re-perfusion reduces the level of oxygen and intracellular ischemia, which disrupts the normal metabolism of the cell and leads to cell death if oxygen is not rapidly reached. Studies have

shown that the CA1 region of the hippocampus is one of the most sensitive parts of the brain against ischemia (19). Currently, due to extensive research on the therapeutic effects of stem cells in different areas of the body, especially neurological diseases, cell therapy can be considered as one of the main approaches for treating stroke (20).

In this study, we used dental mesenchymal stem/stromal cells for 4 days and, 24 hours (EPO) after induction of ischemia by bilateral obstruction of common carotid arteries (VO 2), and then we examined the results in three steps. First, their behavioral performance was evaluated in MWM and then Nissl staining and immunohistochemistry were performed.

Early observations suggest that cerebral ischemic injury is caused by a change in the simple biochemical and physiological communication resulting from a disruption of the blood flow. These changes: Reduction of high energy factors, lactic acidosis, the lack of blood flow due to astrocytes swelling and cerebrovascular pressure (9). In this study, we chose 2VO to create an ischemic model of the hippocampus. There is a sharp decrease in the blood flow of the Hindbrain, while in the forebrain, blood flow is sufficient by the 2VO method (21). Although the intensity of the lesion is not remarkable in the 2VO method, it also leads to cell death of about 40% in the CA1 region, resulting in less mortality than other ischemia-induced methods (22).

One of the most important disorders in the hippocampal ischemia is cell necrosis and death, followed by cognitive problems. In this study, we also observed that the ischemia and vehicle groups spent more time looking for a hidden platform in the MWM and spent less time in the target quadrant compared to the sham group. In a study on the long-term effects of common carotid artery occlusion, it was concluded that cell death will increase from the second month after the obstruction, followed by cognitive problems (23), which is in agreement with our findings. We hypothesized that the use of erythropoietin and DPSC injections accelerates the process of neurogenesis and repair. EPO exists in various tissues of the body with endogenous origin including the central nervous and is over-expressed in the brain after injuries. Following ischemia and stroke, the expression of the HIF-α transcription factor is increased, followed by an increase in the production of EPO and VEGF, which initiates the neuroprotective pathway, neurogenesis, and repair (24). In this study, following an intravenous human erythropoietin injection (1000 IU/kg) 24 hours after ischemia and its repeatability on the following day, neurogenesis in the erythropoietin recipient group showed a significant increase as compared to ischemia and vehicle groups, which was confirmed by immunohistochemistry.

A study has examined the localized ischemia of the brain (25) with the same dose of erythropoietin and two consecutive days after ischemia. A mentioned study reported a reduction in the extent of the ischemic region and an increase in the rate of improvement, which was consistent with our findings. However, another study found contradictory results, where the same dose of erythropoietin for three days after induction of localized ischemia produced limited modifications for improvement, and EPO could not increase neurogenesis in the dentate gyrus (26).

According to the results of this study, the use of DPSC cells could increase neurogenesis in ischemic cells. Since the discovery of these cells in the past decade, they have attracted the attention of many researchers, and have led to the discovery of their characteristics and their regenerative capacity In vitro and in vivo for nervous system reconstruction (30–30). 9 weeks after the DPSC transplant in the hippocampus of the ischemic mice, we observed that these cells were present in the hippocampus and cortex and continued to survive. When comparing the results of the MWM with the group of ischemia in the DPSC group, it was observed that the DPSC group received better outcomes than the ischemia group during the five days of the test period. But in the reminding phase, the DPSC recipient mice spent more time in the target quarter, which this difference was not significant.

In the present study, the MWM test in the EPO group revealed that the mice were in both Learning and reminding phases have better outcomes than ischemia and vehicle groups. Therefore, it can be concluded that DPSC treatment of mice with hippocampal ischemic injury was capable of improving learning performance, but did not affect the memory process. In a study that was conducted using passive avoiding tests on ischemic memory of mice, mice receiving DPSC did not improve behavioral actions as compared to the vehicle group. Hence, it can be concluded that the use of EPO in treating hippocampal ischemia was more effective than DPSC. Studies have shown that DPSC cells can improve motor neurons (31) after transplantation in the spinal cord injury model and cause angiogenesis in the lower limb ischemia models (32). In various in vivo studies, functional mechanisms of treatment with DPSC cells attribute a series of factors that have paracrine effects. These factors include Chemokine stromal cell-derived factor-1 (33) and BDNF and NGF, GDNF, and VEGF (34). Additionally, assessment of the number of adult neurons in different groups using NeuN antibodies revealed that neurons in groups receiving DPSC and EPO increased significantly compared with vehicle and ischemia groups. The healthy cell count in the CA1 region by Nissl staining showed that the number of healthy cells in the sham group, EPO, DPSC and was higher when compared with the ischemia and vehicle groups, in which the two methods confirmed each other. On the other hand, MWM revealed that the groups receiving cells and the EPO had good results compared to ischemia and vehicle groups, which justify the findings of immunohistochemy and Nissl staining. According to the results of our research, despite the lack of significance, the best result was obtained by MWM in combination use of DPSC and EPO. Furthermore, more living cells in the CA1 region were observed in the DPSC + EPO group. It can be argued that all cells in the CA1 region have an endogenous origin, but they can also have an endogenous origin and injection origin in the DPSC and EPO + DPSC groups.

In the groups receiving the DPSC and EPO + DPSC, the hippocampal injected cells remain at the site of the graft and surrounding areas, such as the cortex and hippocampal dentate gyrus after 9 weeks and they were able to express DCX and NeuN by maintaining survival. This means that they also differentiated into adult neurons while producing new neurons. In both groups, both endogenous neurogenesis and adult neuronal transformation were seen in the CA1 region, which expressed DCI and NeuN as exhibited by DiI. Therefore, DPSC resulted in the production of new neurons and migration and their differentiation into adult neurons, and this improved the performance of these groups in the MWM (35, 36). Myriam Bernadine et al. reported that combination use of erythropoietin and mesenchymal stem

cells in localized ischemia has had favorable results in terms of neuronal degeneration and their transformation into neurons and glial cells in the dentate gyrus.

They conclude that erythropoietin has a synergistic effect on mesenchymal stem cells (37). By another study, erythropoietin and nerve stem cells were experimentally tested in the epilepsy model, where good results were obtained in improving symptoms and the growth of mossy fibers (38). Regarding the considerable effects of combined use of erythropoietin and stem cell types by previous studies, we also found better outcomes with their combination use. Given the risk of using stem cells and the uncertainty about their benefits, it can be concluded that the use of EPO is easier, more useful, and less risky.

Conclusion

Both the dental pulp and erythropoietin-derived stem cells can stimulate the neuronal function and convert them to adult neurons. however, behavioral studies revealed that the therapeutic effects of erythropoietin were better than DPSC. The combination effect of erythropoietin and stem cell leads to a reduction in inflammation, and the aim of this study were to reduce inflammation due to ischemia-induced so that the cell could have better homing at the lesion site and show better function.

Abbreviations

DPSC dental pulp stem cells, EPO erythropoietin, MWM Morris water maze, DCX doublecortin, VEGF Vascular endothelial growth factor, I/R group ischemia-reperfusion group, BDNF Brain-derived neurotrophic factor, NGF nerve growth factor, BM-MSCs bone marrow mesenchymal stem cells, BBB blood-brain barrier, α-MEM Alpha-Modified Eagle's Medium.

Declarations

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Authors ‘contributions

SMP wrote the main paper, MB Edited the main text and reviewed the main manuscript, MS, MK and AR conceived and designed the analysis, AJ and NA collected the data, RM contributed data and analysis tools, RA, SMP and NA performed the analysis.

Availability of data and materials

All data generated or analyzed during this study are included in this article

Ethics approval and consent to participate

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Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests .

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Figures

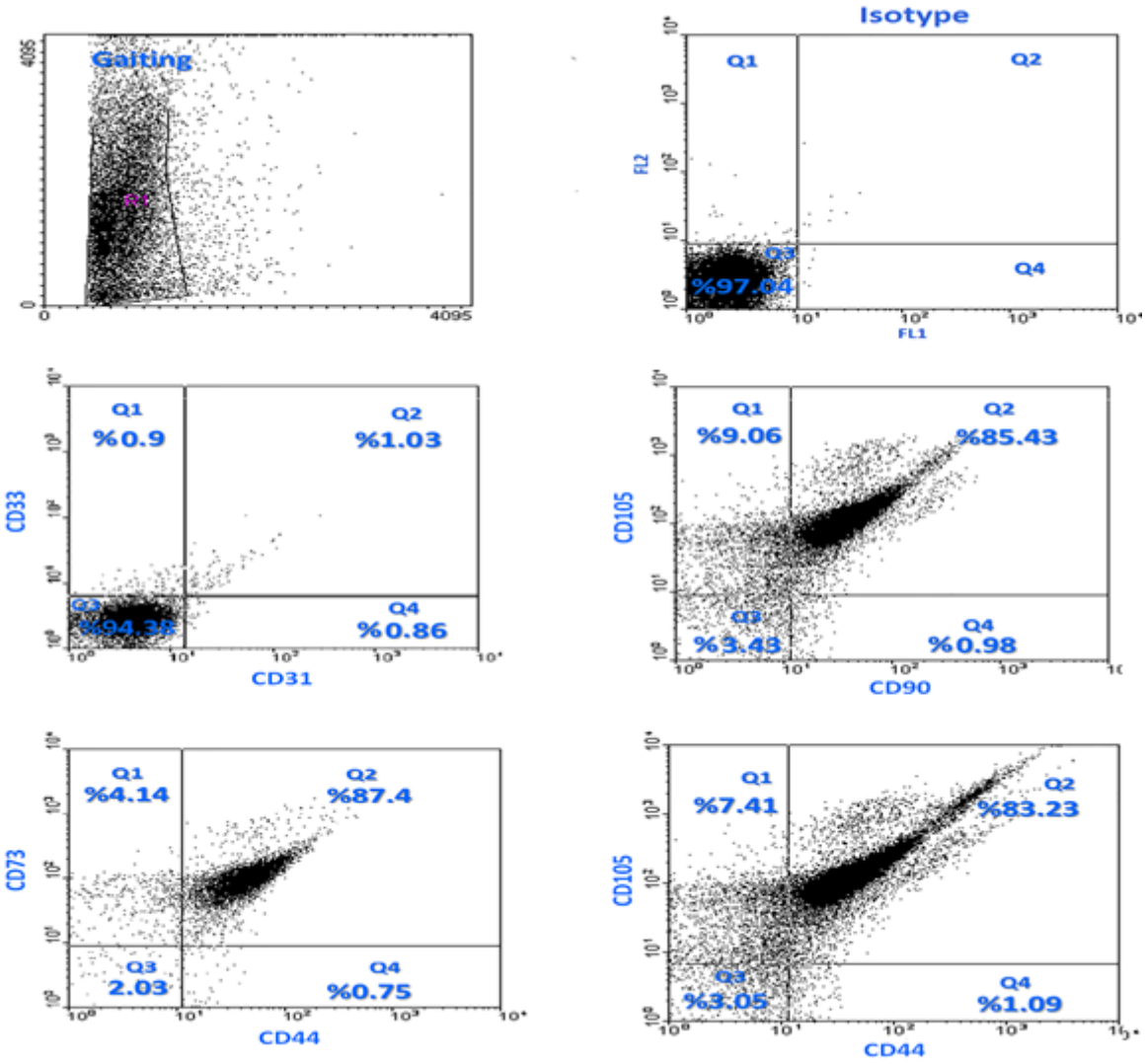
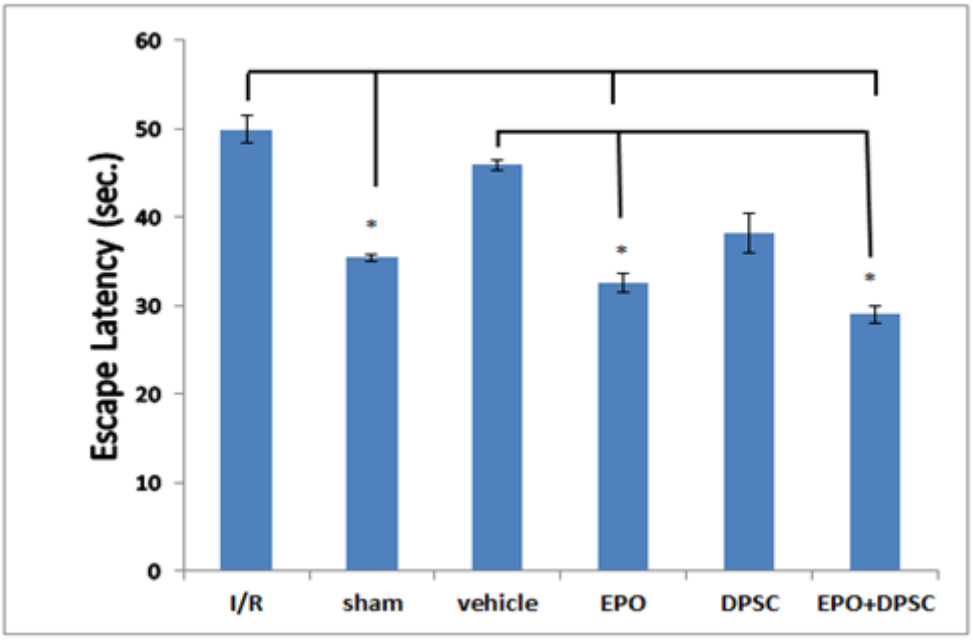


Figure 1

Flow cytometry of mesenchymal stem cells derived from dental pulp for markers, indicates positive markers including CD90, CD105 (85.43%), CD73 and CD44 (87.4%) as a marker of mesenchymal cells as well as negative markers of CD33 and CD31(1.03%). the Q2 region of the double-positive cells and the Q3 region of the double-negative cells



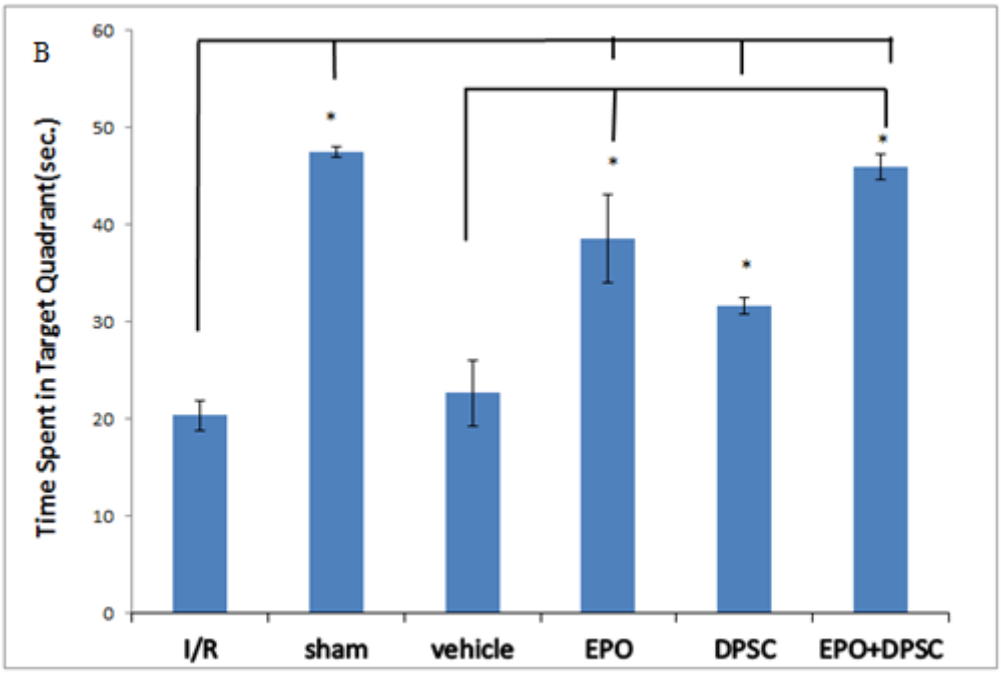


Figure 2

A. the MWM test was performed After 8 weeks and 10 animals were in each group Comparison of time spent for reaching the platform. Comparison of sham, vehicle, EPO, DPSC and EPO + DPSC groups with I/R group showed a significant relationship between DPSC and vehicle group ($P < 0.05$). In comparison with the vehicle group, EPO and EPO + DPSC groups had a significant correlation ($p < 0.05$) but this relationship was not significant between the DPSC and vehicle groups. B. Time spent in the target quadrant on the reminder day. There was a significant correlation between EPO, sham, DPSC and EPO + DPSC groups with I / R group ($P < 0.05$). EPO, DPSC and EPO + DPSC groups showed a significant correlation with vehicle group ($P < 0.05$). Values are displayed in terms of average ± standard error.

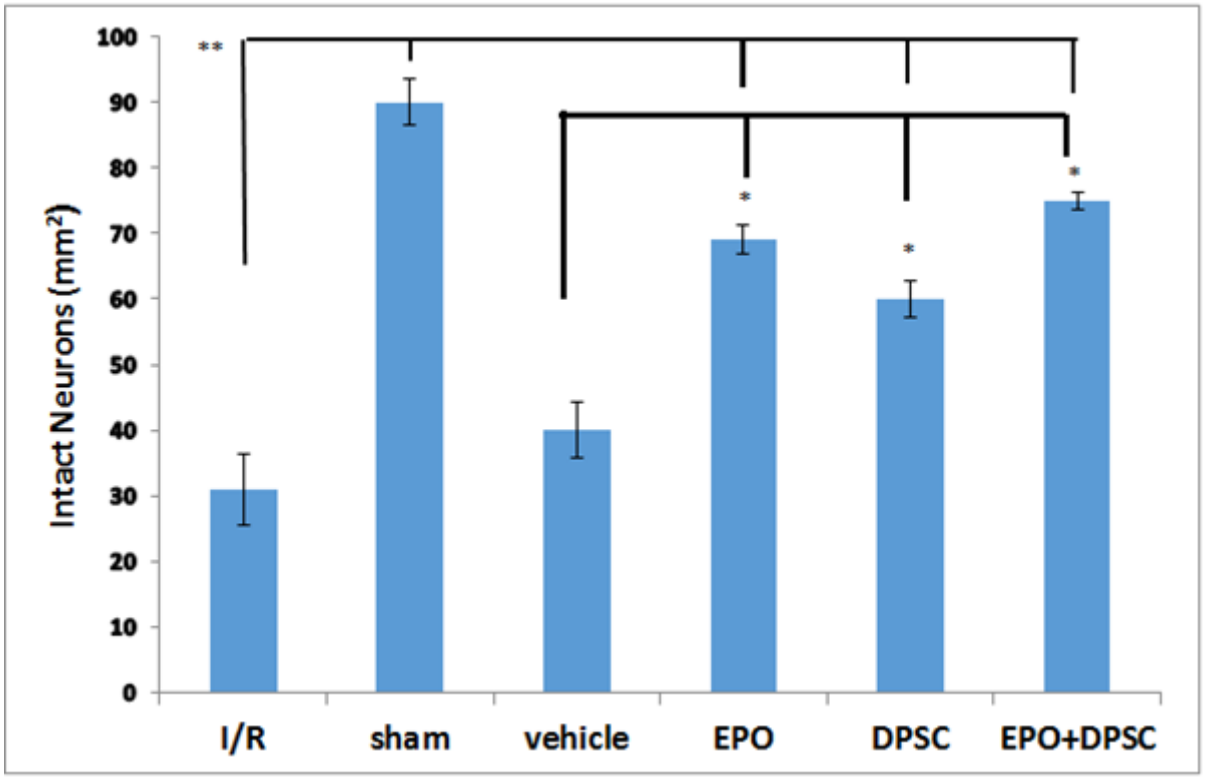


Figure 3

Cell counting in CA1. a significant correlation was found between EPO, sham, DPSC and EPO + DPSC groups (P <0.01). Meanwhile, a significant correlation of EPO, DPSC and EPO + DPSC groups with vehicle group was revealed (P <0.05). Values are displayed in terms of average ± standard error.

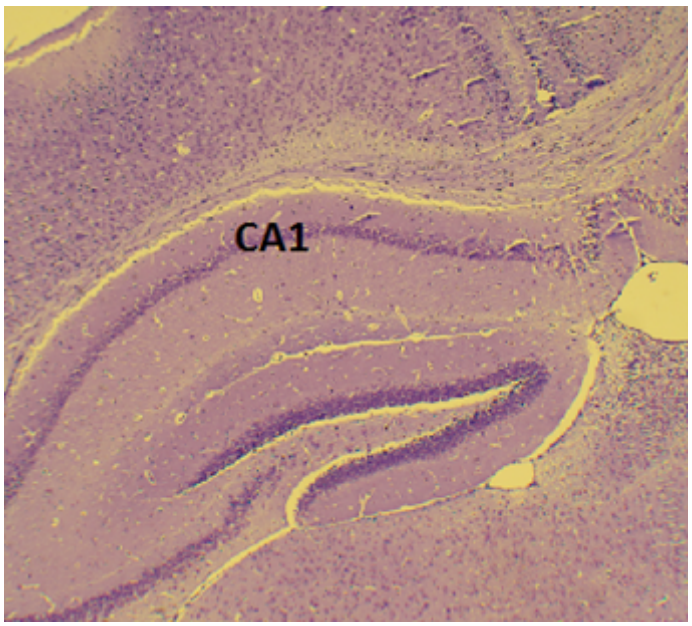


Figure 4

CA1 area of the hippocampus with a magnification of 4x

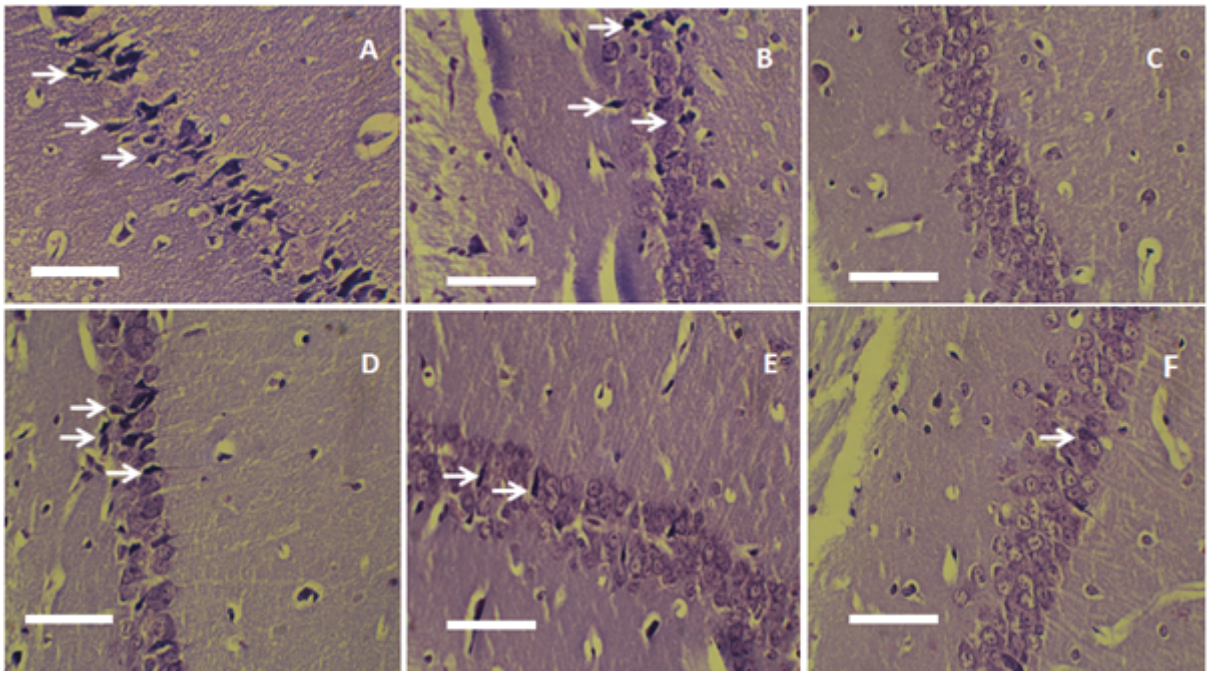


Figure 5

Cell density in the CA1 region was determined by Nissl staining. A) Group I / R, B) Vehicle group, C) Sham group, D) DPSC group, E) EPO group, F). EPO + DPSC. Arrows display Necrotic cells (40x magnification). Scale bars = 100μm

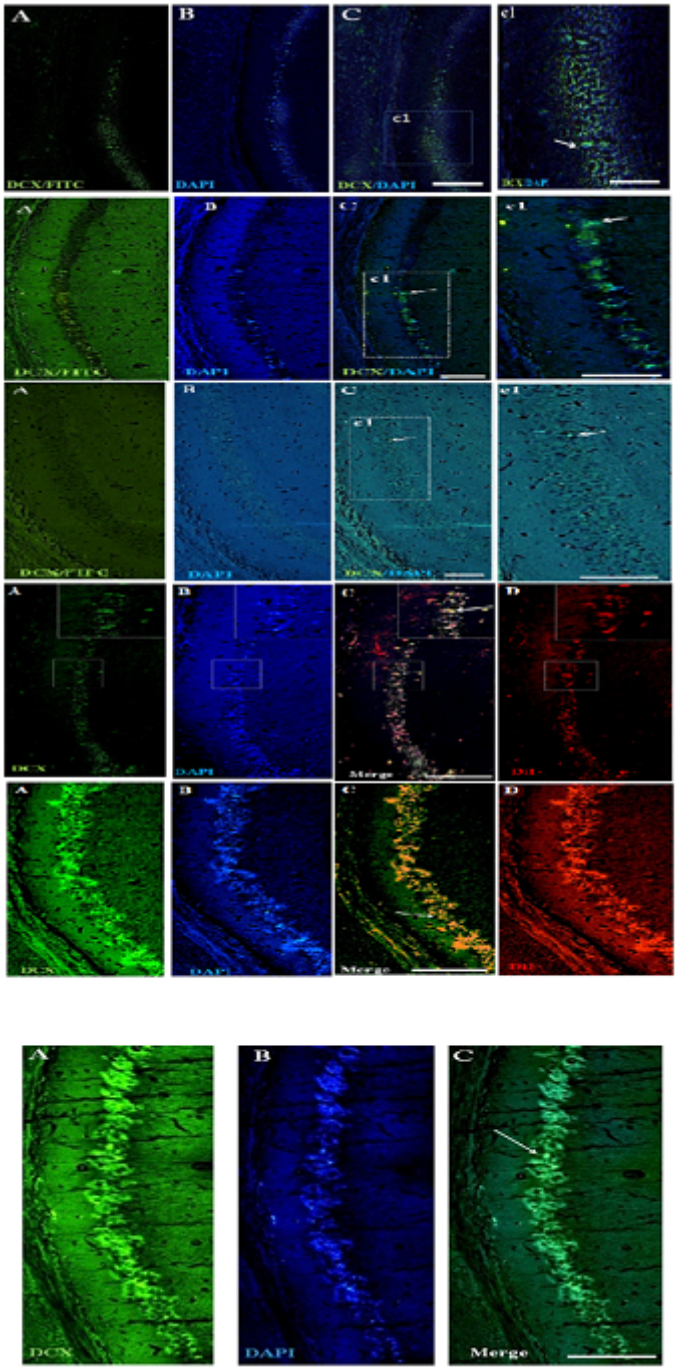
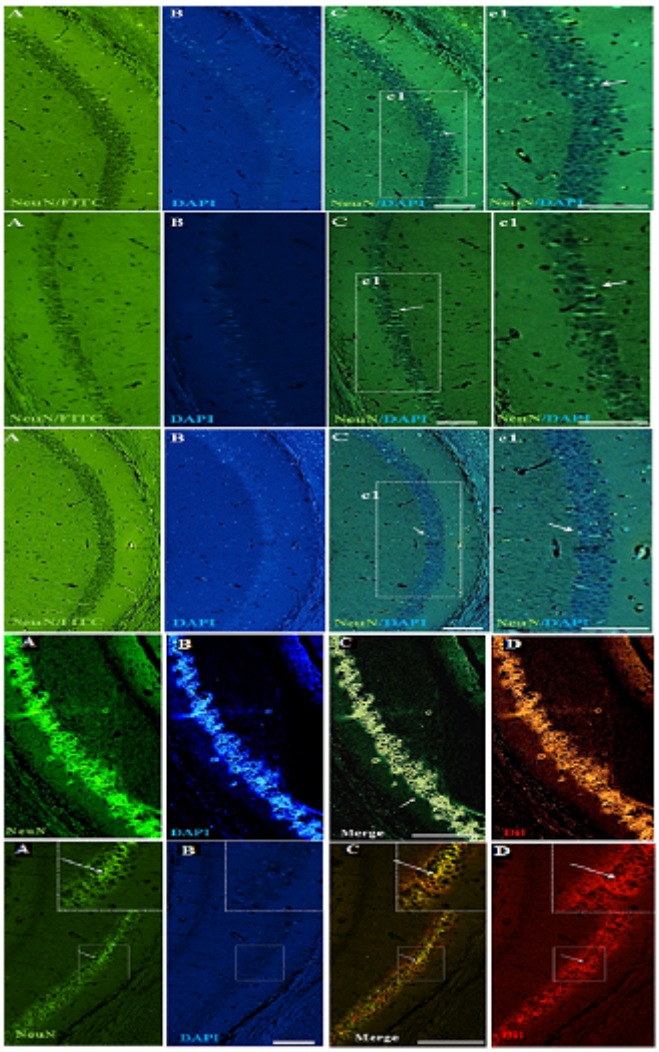


Figure 6

Cross section of the CA1 region of the hippocampus stained by the immunohistochemistry in the I/R group(first row), sham group (second group), vehicle group (third row), DPSC group (fourth row),EPO+DPSC group(fifth row), EPO (sixth row) A. DCX / FITC cells are positive B: The stained nucleus of cells with DAPI. C: Displays the merged image of DCX / DAPI. C1 The same image is merge with a larger magnification. All DCX / FITC + cells in image have an endogenous origin. D: Indicates cells labeled DiI .



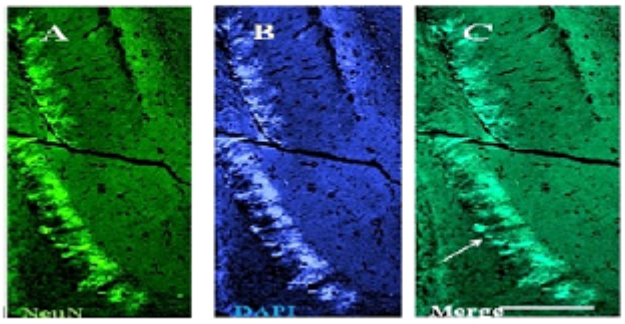


Figure 7

Cross section of the CA1 region of the hippocampus stained by the immunohistochemistry in the I/R group (first row), sham group (second group), vehicle group (third row), DPSC group (fourth row), EPO+DPSC group (fifth row), EPO (sixth row) **A**: Cells / FITCNeuN positive B: The cell's nucleus stained with DAPI. C: Displays the merged image NeuN / DAPI. C1 the same image is merged with a larger magnification. All cells with the NeuN / FITC+ have an endogenous origin. D: Indicates cells labeled DiI .

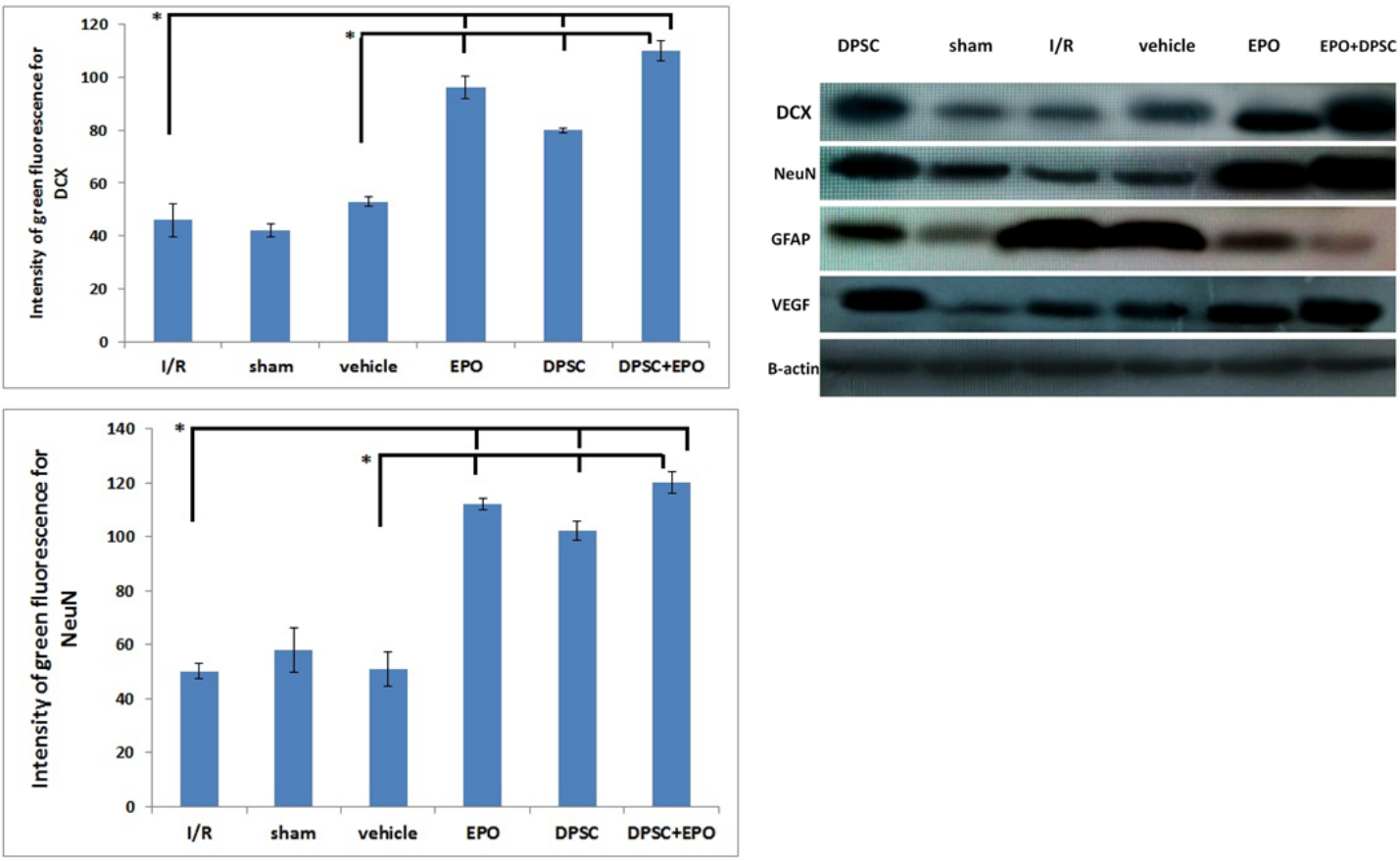


Figure 8

Evaluation of the intensity of green color of FITC/DCX + (A) and FITC/NeuN + (B) antibodies in mice hippocampus following ischemia-reperfusion in different groups using IHC technique. Comparison of green color intensity demonstrated that DCX and NeuN proteins were expressed in DPSC, EPO and EPO + DPSC groups more than I/R and vehicle groups ($P < 0.05$). Mean values ± Standard error was displayed. The grouping of blots for each protein came from different gels separated with white space.