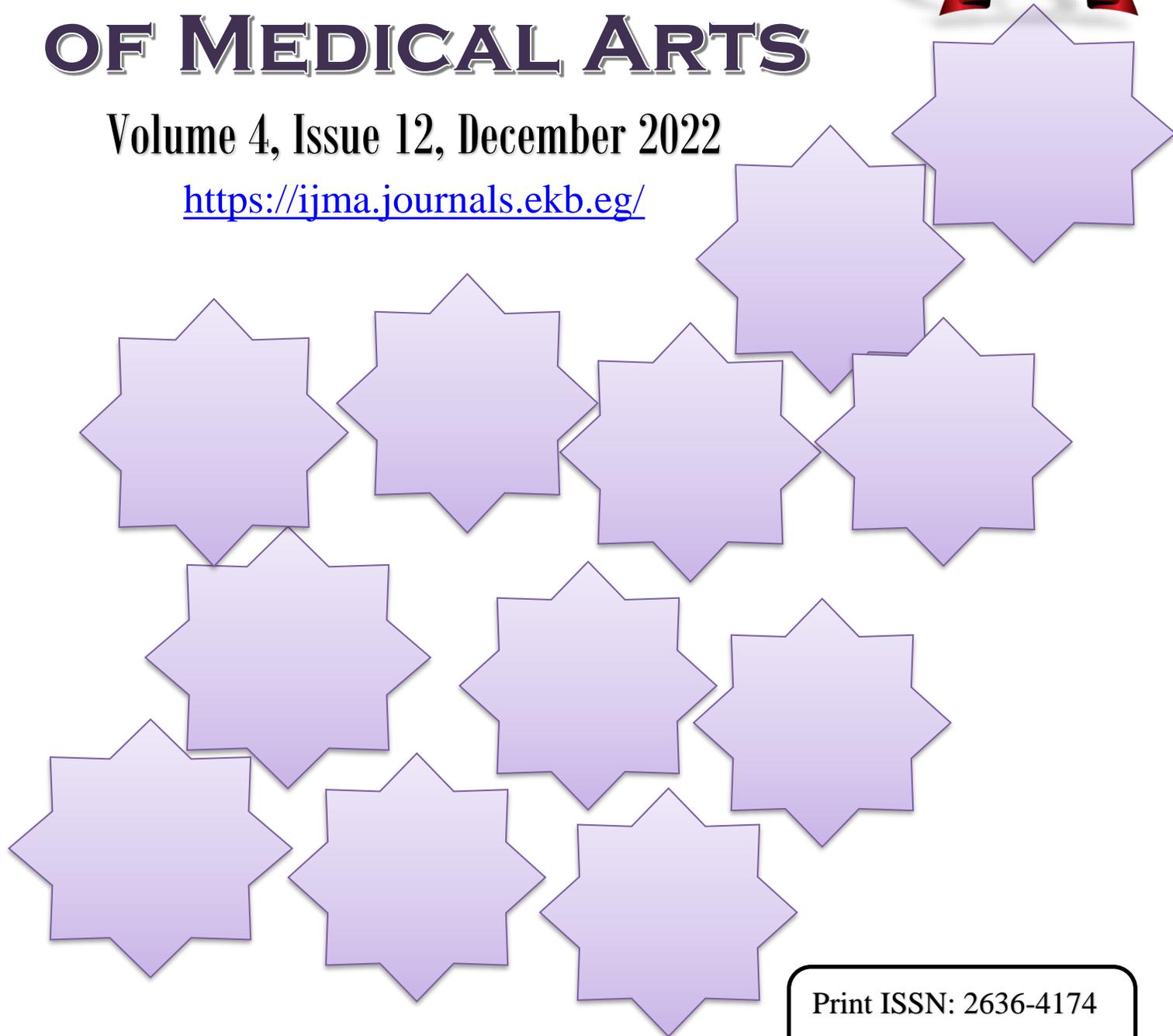


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Original Article

Cell-Free Therapy as A New Treatment Strategy in An Albino Rat Model of Cerebral Ischemia [Histological Studies]

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ABSTRACT

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Background: The therapeutic efficacy of stem cell therapy in many diseased organs based greatly on their paracrine effect. The neural stem cells are thought to have a role in improving the neurological functional, recovery, and reduction of cerebral infarction caused by cerebral ischemia/reperfusion [I/R] injury in rats.

Objective: This study was conducted to look at the histological alterations in the rat cerebral cortex after ischemia-reperfusion [I/R] injury and to assess any potential neuroprotective effects of neural stem cells conditioned media [NSC-CM] on those changes.

Material and Methods: Thirty adult male albino rats weighing about 150 - 250 gm each were used in the present work. The animals were equally divided into 3 groups [10 rats each]. Group A was subdivided into negative control group, A1, [5 rats], not subjected to any intervention, and the sham-control group, A2 [5 rats], the animal of which were subjected to a midline cervical incision with exposure of both common carotid arteries followed by closure of the incision. Group B included rats subjected to brain [I/R] to induce transient global brain ischemia. Group C included rats subjected to I/R injury [as group B] and received 1.5 ml NSC-CM injected slowly through the tail vein at 3 h, 24 h, and 48 h after ischemia onset. Animals were anaesthetized using ether inhalation, the cerebrum was dissected and fixed in 10% neutral buffered formalin and specimens from them were taken and processed for examination by light microscopes.

Results: NSC-CM significantly ameliorated neurological defects by reducing cerebral infarct volume, significant inhibition of cell apoptosis in the ischemic cerebral hemisphere

Conclusion: NSC-CM might be an alternative and effective therapeutic intervention for ischemic stroke.

Keywords: Apoptosis; Cerebrum; I/R; NSC-CM; Rat.



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INTRODUCTION

Ischemic stroke ranks second worldwide in terms of mortality and disability [1]. Tissue plasminogen activator [tPA] is the only effective treatment. The limited clinical use of tPA was due to its 4.5-hour treatment window and substantial risk of intracerebral haemorrhage [2]. Despite the fact that all neuroprotective treatments decreased neuronal cell death and infarct size in cell culture and animal models of stroke, many were ineffective or had unfavourable side effects in clinical trials. As a result, novel treatments for ischemic stroke are needed [3].

As a stem cell-based therapy for ischemic stroke, the usage of neural stem cell [NSC] therapy in particular has expanded. NSCs have been shown to hasten neurological recovery through both direct action [neuronal replacement] and indirect by its paracrine action through secreting brain-derived neurotrophic factor [BDNF], reduced inflammation, and better endoneurogenesis [4]. But the original resource, low survival, and neuronal differentiation rates [5] as well as the potential tumor formation of NSCs [6] limited their clinical application. Stem cell-based treatment is therefore inappropriate for ischemic stroke. However, in a culture-conditioned medium, NSCs produced a variety of neurotrophic factors, such as BDNF [7], glial cell line-derived neurotrophic factor [GDNF], neurotrophin-3 [NT-3] [8], and microvesicles [MVs] [9].

Neurotrophic and mitotic factors known as bFGF and EGF were very recently discovered [epidermal growth factor]. A poor functional result following an ischemic stroke is linked to low blood levels of BDNF [10], although BDNF intranasally administered provided protection from cerebral I/R harm [11]. Both GDNF and NT-3 guard against vascular and ischemic damage to the brain [12, 13]. As a kind of intercellular communication, nanosized, membrane-bound microvesicles [MVs] released by cells can transport proteins, DNA, and RNA between cells. In the culture medium, NSCs release MVs that protect nerves, have an impact on neuronal activity, and support the growth and maintenance of the nervous system [9, 14, 15].

Microvesicles derived from stem cells have been discovered to repair damaged tissues without cell replacement [16]. NSC-derived MV transplantation decreased the cerebral infarct

volume in ischemic stroke patients [17]. Injury-induced NSC-CM implantation boosted the subventricular zone NSC population and increased the production of new neurons that travelled to the wounded area in injured mouse brains [18, 19].

Because mesenchymal stem cells were in vitro converted into neural stem cell-like cells by NSC-CM, this process may promote endoneurogenesis [20]. More notably, NSC-CM reduced neuron death brought on by spinal cord damage in rats [21]. In earlier studies, we found NSC-CM dramatically reduced the rate of cell death and promoted neuronal growth [22]. Therefore, NSC-CM could be able to prevent cerebral I/R damage. This study set out to determine if intravenous treatment of NSC-CM may enhance neurological functional recovery and lessen the severity of cerebral infarction after a cerebral ischemia/reperfusion [I/R] injury in rats. Additionally, we evaluated the possible neuroprotective effect of NSC-CM on cerebral I/R injury in rats.

PATIENTS AND METHODS

NSC-Conditioned Medium Collection

Neural stem cells were isolated from the olfactory mucosa and olfactory bulbs under very restrict sterile conditions. The brain was dissected to isolate the olfactory bulb then the cribriform plate of ethmoid was dissected to collect the olfactory mucosa that covers the posterosuperior part of nasal septum and the inferior surface of the cribriform plate of ethmoid. The olfactory bulbs and olfactory mucosa were cut into small pieces then incubated in falcon tubes containing 0.1% collagenase type I [Sigma] [0.1 g collagenase type I dissolved in 100 ml phosphate buffer saline [PBS, Lonza, Verviers, Belgium] for thirty minutes at 37 °C in a shaker water bath after which the cells were washed twice more, re-suspended in complete media and seeded.

The final pellet was resuspended in 10 ml complete medium that consists of low-glucose Dulbecco's Modified Eagle's Medium with Ham's F12 [DMEM-F12] [Lonza, Verviers, Belgium] supplemented with 10% fetal bovine serum [Seralab- Brazil EU Grade] and 1% penicillin/streptomycin [Lonza, Verviers, Belgium]. The cells were seeded in 35 mm Petri-dishes at a density of 1×10^5 per dish in complete medium and were incubated in a

humidified atmosphere containing 5% CO₂, at 37 °C.

Three days after seeding, the non-adherent cells were removed and the medium was replaced with a fresh complete medium. The culture was monitored on a daily basis and the medium was changed every 3 days. After 10–14 days, when the colonies were evident, cells were passaged using 0.25% trypsin and 0.05 mmol/l EDTA [GIBCO-25300, Grand Island, NY, USA].

Cultures of rat olfactory stem cells were passaged twice a week and used at passages 1, 2, or 3. At the time of each medium change, we collected the rat NSC conditioned medium by filtering through a membrane with a pore size of 0.4 µm in diameter [Millipore, Billerica, MA, USA]. The filtered conditioned mediums were centrifuged at 1000 RPM* 10 minutes at RT. After that, we observed the medium under the microscope to make sure that there was no cell contamination. The neural stem cell conditioned medium was kept at 4° C for 7 days.

Animals

Thirty-five healthy male albino rats weighing 150–200 grams each were studied. Al-Azhar University's Faculty of Medicine's laboratory animal unit supplied them. The animals had proper ventilation, temperature, and a 12-hour light/dark cycle. Laboratory food and water were available to all animals. Five rats were used for isolation of NSCs, while the remaining animals were equally divided into 3 groups [10 rats each]; Group A was further subdivided into negative control group, A1, [5 rats], not subjected to any intervention, and the sham-control group, A2 [5 rats], each animal of which were subjected to a midline cervical incision with both common carotid arteries exposed and closed.

Brain [I/R] induced temporary global brain ischemia in Group B rats. Through a midline neck incision, "Bull dog" clamps were used to clamp both common carotid arteries for 10 minutes, then released and closed. Researchers described this procedure [23-26]. I/R-injured rats comprised Group C. [as group B]. Group C received 1.5 ml NSC-CM slowly by tail vein at 3, 24, and 48 h after ischemia onset. After ether inhalation, animals' cerebrums were dissected and preserved in 10% neutral buffered formalin, and light microscope specimens were obtained.

Light Microscopic Study [Haematoxylen and Eosin stained]

The cerebrum was dissected and fixed in 10% neutral buffered formalin to make 5 µ paraffin slices for light microscopic examination. Sections were stained with H & E stain [27] in The Histology department, Al-Azhar University.

RESULTS

Morphology of the isolated NSCs

The cultured NSCs exhibited plastic adherent affinity to the substratum of the tissue culture vessels. The adherent cells took different morphological outline during primary culture [P0] ranging from polygonal, spindle shaped, star shaped, sperm shaped and fibroblast-like. The cells displayed granular cytoplasm, many long cytoplasmic processes, and a single large vesicular nucleus with multiple nucleoli. [figure 1]. During the next passages [P1-P3] the cells appeared homogenously fibroblast-like instead of their heterogenous morphology and showed great tendency to form colonies [figure 2].

Light microscopic examination [Hematoxylen and Eosin stained]

Group A [control group]

Light microscopic examination of H&E-stained sections of the rat cerebrum of both control groups [A1 & A2] showed normal histological structure. The cerebral cortex was composed of organized and regularly arranged six layers of gray matter. These layers were arranged from outer to inner into; molecular, external granular, external pyramidal, internal granular, internal pyramidal and multiform layers [figure 3]. The external granular layer was formed mainly of small granular cells giving this layer a granular appearance, while the external pyramidal layer had pyramidal cells of varying sizes, together with scattered granular cells. The internal granular layer had numerous small granular cells, while the internal pyramidal layer was formed of huge pyramidal [Betz] cells and scattered granular ones. On the other hand, the neuropil was intact [figure 4]. The granular cells possessed round cell bodies and huge rounded open face nuclei in contrast to the pyramidal cells' lengthy apical dendrites, basophilic cytoplasm, and massive nuclei [figure 5].

Group B [one week after I/R]

The cerebral cortex revealed many apoptotic cells with small eccentric nuclei, little acidophilic cytoplasm and surrounded by empty spaces mostly in the middle three layers. Some shrunken pyramidal cells with darkly stained nuclei were demonstrated obviously in the internal granular layer of cerebral cortex. On the other hand, the neuropil was vacuolated and

dilated blood vessels with wide perivascular [figures 6-8].

Group C [One week after I/R and NCS-CM treatment]

The histological architecture of cerebral cortex appeared more or less similar to the control group except for few apoptotic cells in the granular layer [figures 9, 10].

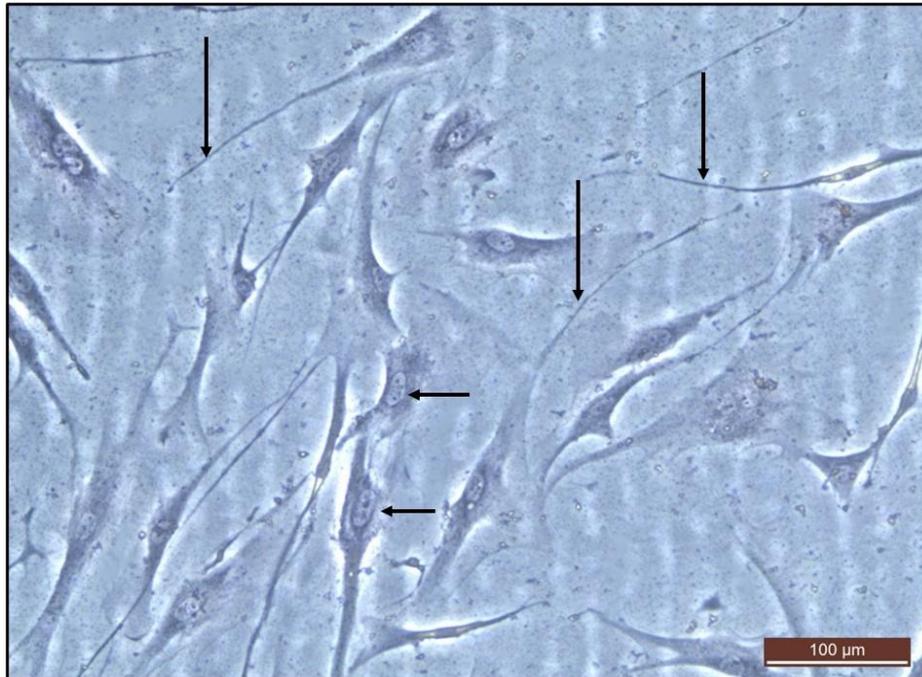


Figure [1]: A phase contrast photomicrograph of NSCs during P0 showing cells with different morphological character, very long cytoplasmic processes [Vertical arrow], granular cytoplasm, large vesicular nucleus with many nucleoli [Horizontal arrow]

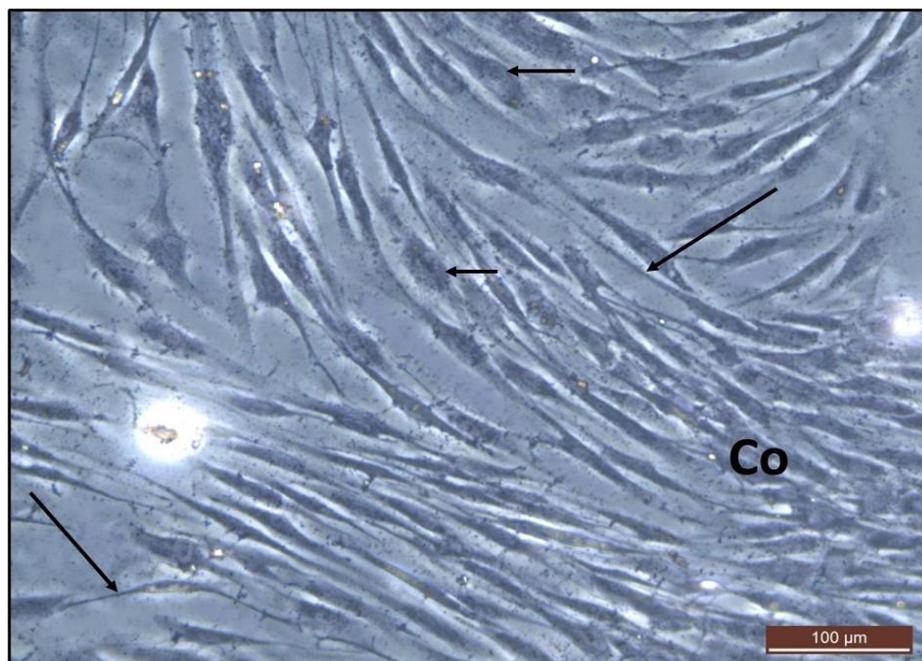


Figure [2]: A phase contrast photomicrograph of NSCs during P2 showing that the cells were homogenously fibroblast-like with long cytoplasmic processes [Long arrow], large vesicular nucleus with multiple nucleoli [Short arrow]. Large colonies [Co] were evident. Cells showed 70% confluency

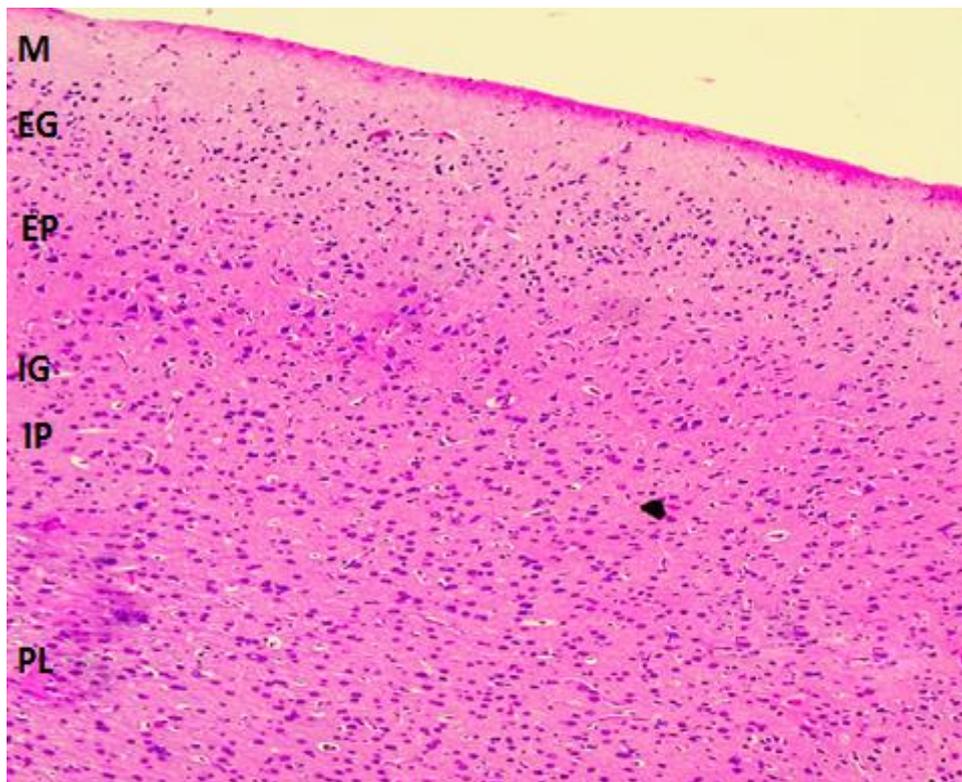


Figure [3]: Photomicrographs of sections in the cerebral cortex of frontal lobe of adult control albino rats showing: Well organized regularly arranged six layers from outer to inner surface: Molecular layer [M], external granular [EG], external pyramidal [EP], internal granular [IG], internal pyramidal [IP] and polymorphic layer [PL] [H&E x 100]

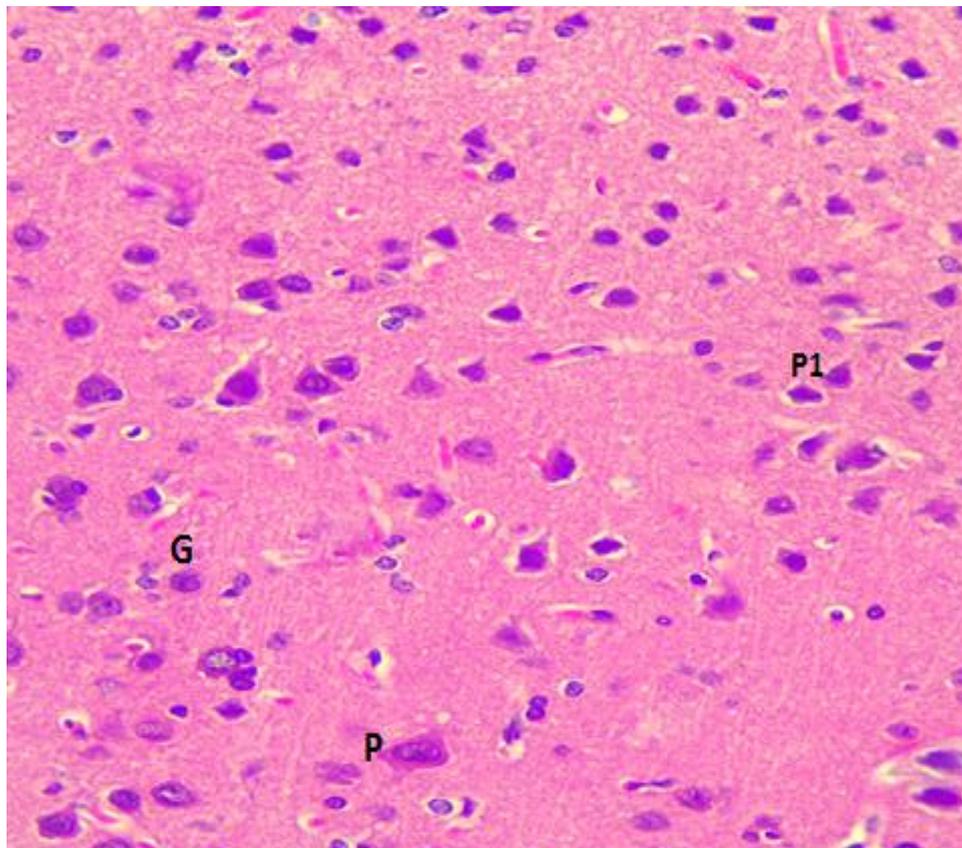


Figure [4]: Photomicrographs of sections in the cerebral cortex of frontal lobe of adult control albino rats showing: The external granular layer with Granular cells [G] and the external pyramidal layer with small [P], medium sized pyramidal cells [P1] and the neuropil was intact [H&E x 400]

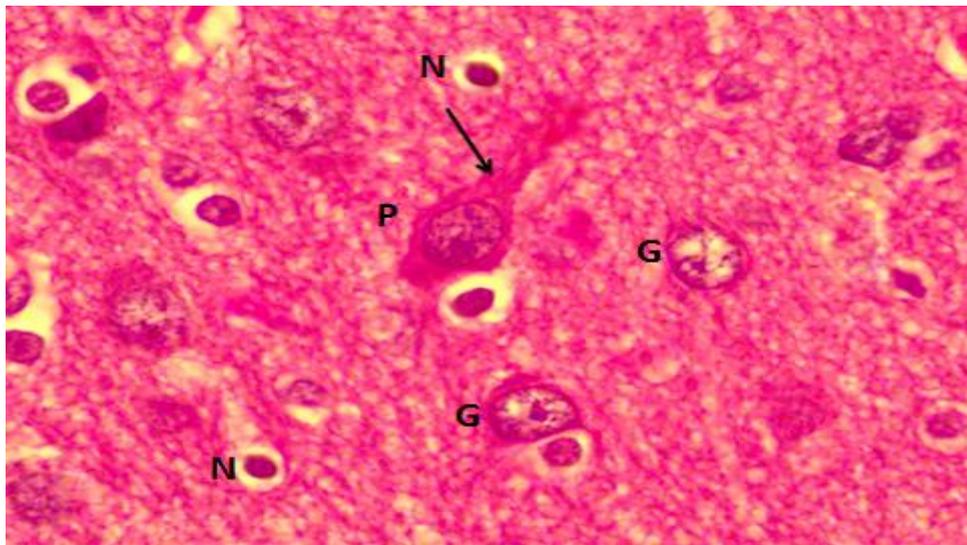
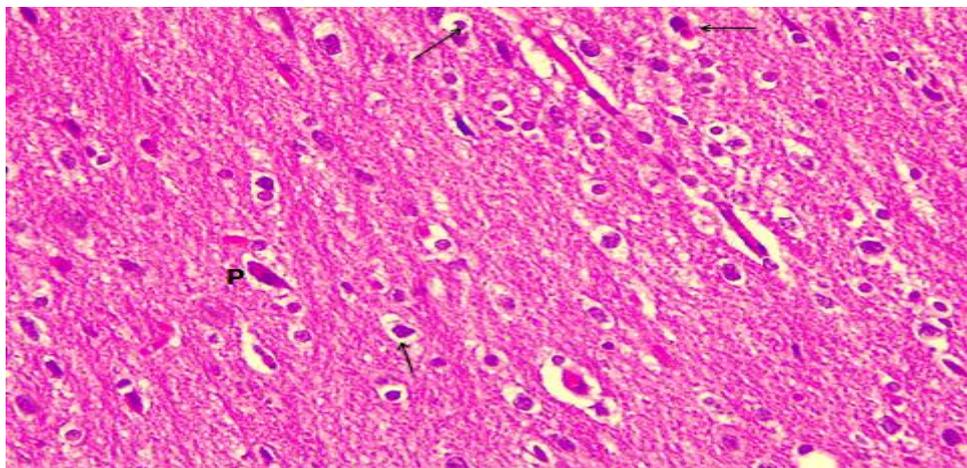


Figure [5]: A higher magnification of the internal pyramidal layer showing a large sized pyramidal cell [p] with rounded euchromatic nuclei having prominent nucleoli. The cell has long apical dendrite [arrow]. Granular cells [G] are also seen [H&E x 1000]



[Figure 6]: Photomicrographs of sections in the frontal lobe of cerebral cortex of adult albino rats one week after I/R injury showing: Many apoptotic cells having small darkly stained nuclei, little acidophilic cytoplasm [arrows] and surrounded by empty spaces are seen in the external granular layer. Shrunken pyramidal cells [p] surrounded by empty space are also demonstrated. Neuropil appears vacuolated [H&E x 400]

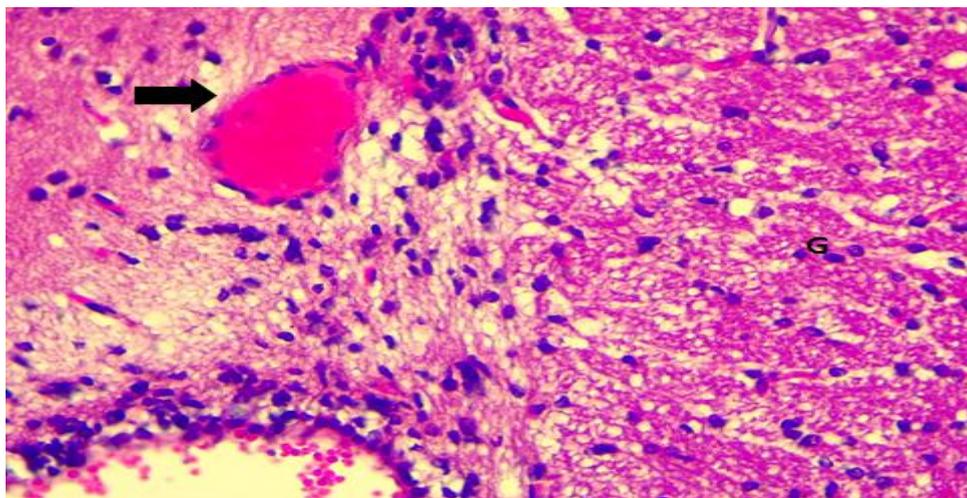


Figure [7]: Photomicrographs of sections in the frontal lobe of cerebral cortex of adult albino rats one week after I/R injury showing: The internal granular layer shows granule cells with dark nuclei and others with karyolytic nuclei [G]. the dilated and congested blood vessels [arrow heads] are seen in the surrounding neuropil [H&E x 400].

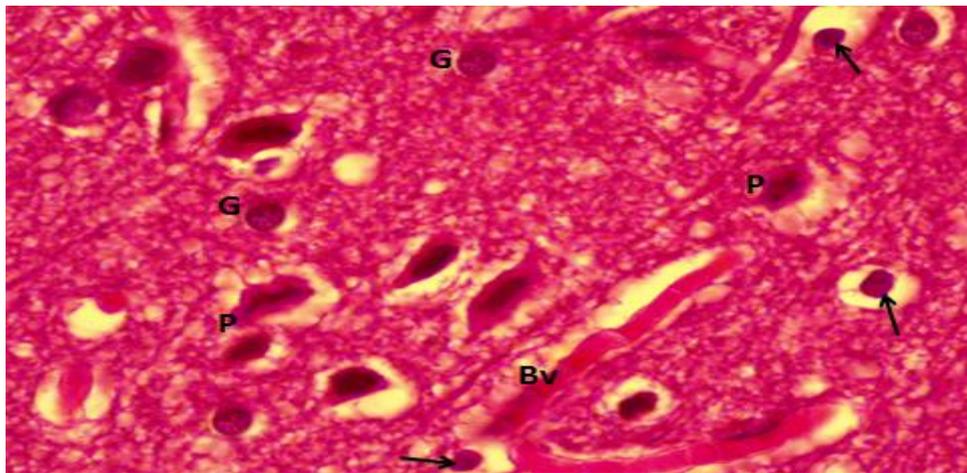


Figure [8]: Photomicrographs of sections in the frontal lobe of cerebral cortex of adult albino rats one week after I/R injury showing: many apoptotic cells having small eccentric nuclei, little acidophilic cytoplasm and surrounded by empty spaces[arrows]. Shrunken pyramidal cells with darkly stained nuclei [P] are also demonstrated. the dilated and congested blood vessels [Bv] are seen in the surrounding neuropil Few normal granular cells [G] are also seen. [H.&E × 1000]

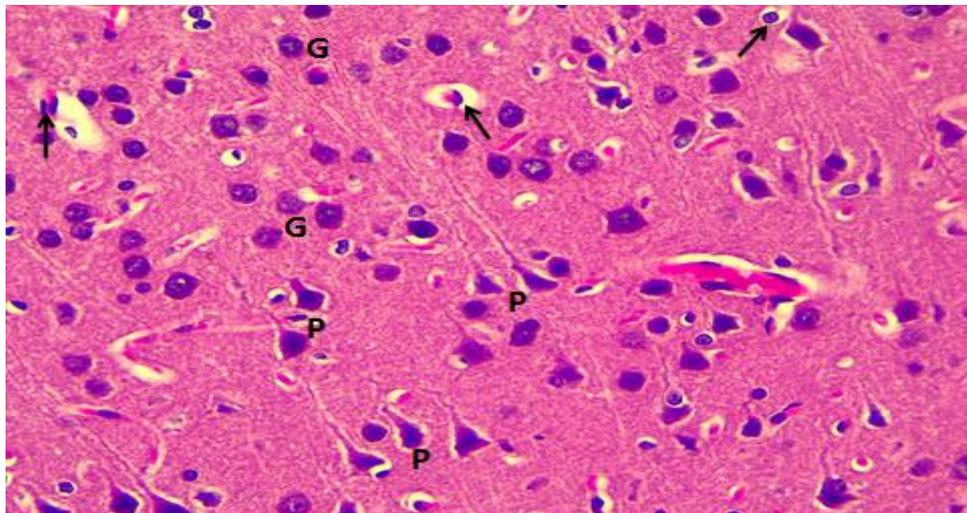


Figure [9]: Photomicrographs of sections in the frontal lobe of cerebral cortex of NSC-CM treated adult albino rats one week after I/R injury showing: Numerous normal granular cells [G] and a pyramidal cell [P] are seen in the internal granular layer, while few apoptotic cells [arrows] are also demonstrated [H&E x 400]

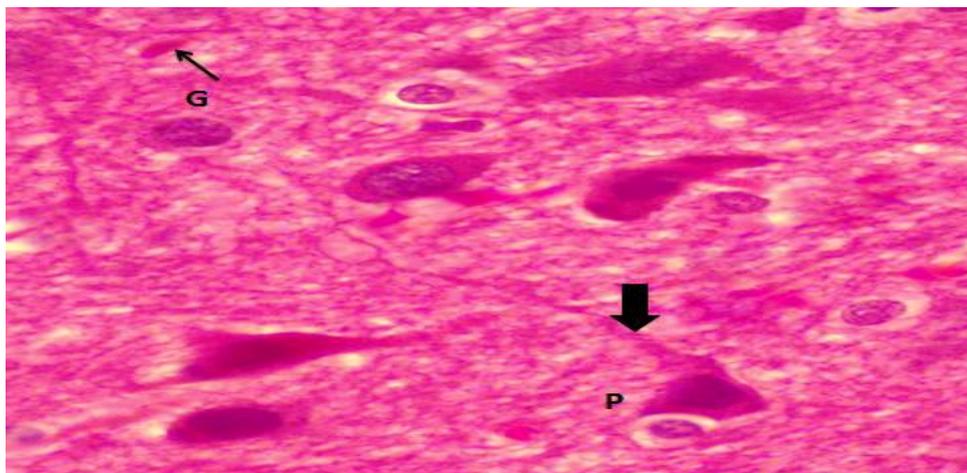


Figure [10]: Photomicrographs of sections in the frontal lobe of cerebral cortex of NSC-CM treated adult albino rats one week after I/R injury showing: Normal large pyramidal cells [P] with long apical dendrite [arrow heads] and granular cells [G] are seen in the internal pyramidal layer. A shrunken cell surrounded by empty space [arrow]is also demonstrated. [H&E x 1000]

DISCUSSION

The adult brain is extremely vulnerable to various insults. Ischemia is considered the most serious one that may result in both short and long term neurodegenerative and functional disorders [28, 29]. The current study aimed to examine the histological changes produced by transient global ischemia on the cerebral cortex of the frontal lobe of adult male albino rats. Moreover, sudden reperfusion into the brain tissue after hypoxia led to generation of more neurotoxic free radicals resulting in secondary oxidative tissue injury and delayed recovery of the neurons. Also, a sustained reduction of protein synthesis and initiation of programmed cell death occurred with depletion of cell enzymes or trophic factors essential for cell survival [30].

In the current study, to produce I/R and test the impact of the NSC-CM on brain damage brought on by temporary global ischemia, the rat model of BCCAO for 10 minutes was preferred. **Traystman et al.** [31] recommended that this model is more suited for studies on long-term survival and needs a simpler surgical setup so that reperfusion may be easily accomplished. One week after an I/R injury, the study's findings showed that there were deteriorated cells in the cerebral cortex. Additionally, the majority of the damaged neurons were located in the middle three layers of the cerebral cortex. Such finding coincides with that obtained by **Tatlisumak et al.** [32] who found that the microscopic features of cortical ischemia showed involvement generally of the middle cortical laminae; mainly layers III to V in a rat model of permanent focal ischemia. The results of this study revealed the presence of many apoptotic granular cells and shrunken necrotic pyramidal ones in the cerebral cortex one week after I/R injury. Some authors described apoptosis as a program preferentially triggered by a microcirculatory disturbance [33, 34]. Accordingly, apoptosis would significantly contribute to the neuronal damage in transient ischemia [35]. Apoptosis was reported in many studies in ischemia [36, 37].

After slowly administering 1.5 ml NSC-CM via tail vein injection at 3 h, 24 h, and 48 h after one and three hours after the removal of common carotid artery ligation, the cerebral cortex's histological architecture in the current investigation appeared to be more or less similar to that of the control group. These features were

maintained from one week up to one month after I/R. Most neurons appeared normal in the cerebral cortex except for few shrunken cells having dark nuclei surrounded by empty spaces. In the current study, in preclinical models of stroke, we found that apoptosis could be suppressed, lesion size could be decreased, and functional recovery could be aided by administering conditioned media from NSC cultures or pure media products.

In conclusion, our results demonstrated that frequent injections of NSC-CM into the tail vein greatly reduced cerebral I/R damage by preventing cell death. According to our findings, another cell-free method of treating ischemic stroke is NSC-CM.

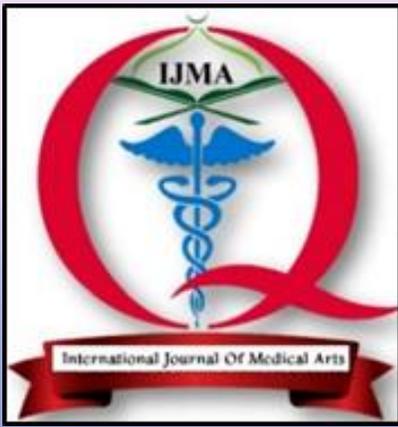
Conflict of Interest and Financial Disclosure: None.

REFERENCES

1. Fann DY, Lee SY, Manzanero S, Chunduri P, Sobey CG, Arumugam TV. Pathogenesis of acute stroke and the role of inflammasomes. *Ageing Res Rev.* 2013 Sep;12[4]:941-66. doi: 10.1016/j.arr.2013.09.004.
2. Cheng Y, Zhang J, Deng L, Johnson NR, Yu X, Zhang N, et al. Intravenously delivered neural stem cells migrate into ischemic brain, differentiate and improve functional recovery after transient ischemic stroke in adult rats. *Int J Clin Exp Pathol.* 2015 Mar 1;8[3]:2928-36. PMID: 26045801
3. Ratajczak MZ, Kucia M, Jadczyk T, Greco NJ, Wojakowski W, Tendera M, Ratajczak J. Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? *Leukemia.* 2012 Jun;26[6]:1166-73. doi: 10.1038/leu.2011.389.
4. Ryu S, Lee SH, Kim SU, Yoon BW. Human neural stem cells promote proliferation of endogenous neural stem cells and enhance angiogenesis in ischemic rat brain. *Neural Regen Res.* 2016 Feb;11[2]:298-304. doi: 10.4103/1673-5374.177739.
5. Tang YH, Ma YY, Zhang ZJ, Wang YT, Yang GY. Opportunities and challenges: stem cell-based therapy for the treatment of ischemic stroke. *CNS Neurosci Ther.* 2015 Apr;21[4]:337-47. doi: 10.1111/cns.12386.
6. Doeppner TR, Hermann DM. Stem cell-based treatments against stroke: observations from human proof-of-concept studies and considerations regarding clinical applicability.

- Front Cell Neurosci. 2014 Oct 29;8:357. doi: 10.3389/fncel.2014.00357.
7. Talaverón R, Matarredona ER, de la Cruz RR, Pastor AM. Neural progenitor cell implants modulate vascular endothelial growth factor and brain-derived neurotrophic factor expression in rat axotomized neurons. *PLoS One*. 2013;8[1]:e54519. doi: 10.1371/journal.pone.0054519.
 8. Lu P, Jones LL, Snyder EY, Tuszynski MH. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp Neurol*. 2003 Jun;181[2]:115-29. doi: 10.1016/s0014-4886[03]00037-2.
 9. Schindler SM, Little JP, Klegeris A. Microparticles: a new perspective in central nervous system disorders. *Biomed Res Int*. 2014;2014:756327. doi: 10.1155/2014/756327.
 10. Stanne TM, Åberg ND, Nilsson S, Jood K, Blomstrand C, Andreasson U, et al. Low Circulating Acute Brain-Derived Neurotrophic Factor Levels Are Associated With Poor Long-Term Functional Outcome After Ischemic Stroke. *Stroke*. 2016 Jul;47[7]:1943-5. doi: 10.1161/STROKEAHA.115.012383.
 11. Jiang Y, Wei N, Lu T, Zhu J, Xu G, Liu X. Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. *Neuroscience*. 2011 Jan 13;172:398-405. doi: 10.1016/j.neuroscience.2010.10.054.
 12. Kilic U, Kilic E, Dietz GP, Bähr M. Intravenous TAT-GDNF is protective after focal cerebral ischemia in mice. *Stroke*. 2003 May;34[5]:1304-10. doi: 10.1161/01.STR.0000066869.45310.50.
 13. Zhang J, Shi Q, Yang P, Xu X, Chen X, Qi C, et al. Neuroprotection of neurotrophin-3 against focal cerebral ischemia/reperfusion injury is regulated by hypoxia-responsive element in rats. *Neuroscience*. 2012 Oct 11;222:1-9. doi: 10.1016/j.neuroscience.2012.07.023.
 14. Krämer-Albers EM, Ping Kuo-Elsner W. Extracellular Vesicles: Goodies for the Brain? *Neuropsychopharmacology*. 2016 Jan;41[1]:371-2. doi: 10.1038/npp.2015.242.
 15. Porro C, Trotta T, Panaro MA. Microvesicles in the brain: Biomarker, messenger or mediator? *J Neuroimmunol*. 2015 Nov 15;288:70-8. doi: 10.1016/j.jneuroim.2015.09.006.
 16. Camussi G, Deregibus MC, Tetta C. Paracrine/endocrine mechanism of stem cells on kidney repair: role of microvesicle-mediated transfer of genetic information. *Curr Opin Nephrol Hypertens*. 2010 Jan;19[1]:7-12. doi: 10.1097/MNH.0b013e328332fb6f.
 17. Chiva-Blanch G, Suades R, Crespo J, Peña E, Padró T, Jiménez-Xarrié E, Martí-Fàbregas J, Badimon L. Microparticle Shedding from Neural Progenitor Cells and Vascular Compartment Cells Is Increased in Ischemic Stroke. *PLoS One*. 2016 Jan 27;11[1]:e0148176. doi: 10.1371/journal.pone.0148176.
 18. Baulch JE, Acharya MM, Allen BD, Ru N, Chmielewski NN, Martirosian V, et al. Cranial grafting of stem cell-derived microvesicles improves cognition and reduces neuropathology in the irradiated brain. *Proc Natl Acad Sci U S A*. 2016 Apr 26;113[17]:4836-41. doi: 10.1073/pnas.1521668113.
 19. Rhee YH, Yi SH, Kim JY, Chang MY, Jo AY, Kim J, et al. Neural stem cells secrete factors facilitating brain regeneration upon constitutive Raf-Erk activation. *Sci Rep*. 2016 Aug 24;6:32025. doi: 10.1038/srep32025.
 20. Ma K, Fox L, Shi G, Shen J, Liu Q, Pappas JD, Cheng J, Qu T. Generation of neural stem cell-like cells from bone marrow-derived human mesenchymal stem cells. *Neurol Res*. 2011 Dec;33[10]:1083-93. doi: 10.1179/1743132811Y.0000000053.
 21. Liang P, Liu J, Xiong J, Liu Q, Zhao J, Liang H, Zhao L, Tang H. Neural stem cell-conditioned medium protects neurons and promotes propriospinal neurons relay neural circuit reconnection after spinal cord injury. *Cell Transplant*. 2014;23 Suppl 1:S45-56. doi: 10.3727/096368914X684989.
 22. Yang H, Wang J, Sun J, Liu X, Duan WM, Qu T. A new method to effectively and rapidly generate neurons from SH-SY5Y cells. *Neurosci Lett*. 2016 Jan 1;610:43-7. doi: 10.1016/j.neulet.2015.10.047.
 23. Schwartz-Bloom RD, Miller KA, Evenson DA, Crain BJ, Nadler JV. Benzodiazepines protect hippocampal neurons from degeneration after transient cerebral ischemia: an ultrastructural study. *Neuroscience*. 2000;98[3]:471-84. doi: 10.1016/s0306-4522[00]00144-5.
 24. Moroni F, Meli E, Peruginelli F, Chiarugi A, Cozzi A, Picca R, et al. Poly[ADP-ribose] polymerase inhibitors attenuate necrotic but not apoptotic neuronal death in experimental models of cerebral ischemia. *Cell Death Differ*. 2001 Sep;8[9]:921-32. doi: 10.1038/sj.cdd.4400884.
 25. Ajao MS, Olaleye O, Ihunwo AO. Melatonin potentiates cells proliferation in the dentate gyrus following ischemic brain injury in adult rats. *J Anim Vet Adv*. 2010 Jan 1;9[11]:1633-8.
 26. Iwai M, Sato K, Kamada H, Omori N, Nagano I, Shoji M, Abe K. Temporal profile of stem cell division, migration, and differentiation from sub-ventricular zone to olfactory bulb after transient forebrain ischemia in gerbils. *J Cereb Blood Flow Metab*. 2003 Mar;23[3]:331-41. doi: 10.1097/01.WCB.0000050060.57184.E7.

27. Bancroft JD, Layton C. The hematoxylin and eosin. In: Suvarna SK, Layton C, Bancroft JD, editors, *Bancroft's theory and practice of histological techniques*, Seventh ed. Churchill Livingstone Elsevier. 2012 Oct 1;7:173-86.
28. Nakatomi H, Kuriu T, Okabe S, Yamamoto S, Hatano O, Kawahara N, *et al.* Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell*. 2002 Aug 23;110[4]:429-41. doi: 10.1016/s0092-8674[02]00862-0.
29. Tarozzi A, Angeloni C, Malaguti M, Morroni F, Hrelia S, Hrelia P. Sulforaphane as a potential protective phytochemical against neurodegenerative diseases. *Oxid Med Cell Longev*. 2013;2013:415078. doi: 10.1155/2013/415078.
30. Pei Z, Fung PC, Cheung RT. Melatonin reduces nitric oxide level during ischemia but not blood-brain barrier breakdown during reperfusion in a rat middle cerebral artery occlusion stroke model. *J Pineal Res*. 2003 Mar;34[2]:110-8. doi: 10.1034/j.1600-079x.2003.00014.x.
31. Traystman RJ. Animal models of focal and global cerebral ischemia. *ILAR J*. 2003;44[2]:85-95. doi: 10.1093/ilar.44.2.85.
32. Tatlisumak E, Inan S, Asirdizer M, Apaydin N, Hayretdag C, Kose C, Tekdemir I. Defining the macroscopic and microscopic findings of experimental focal brain ischemia in rats from a forensic scientist's point of view. *Am J Forensic Med Pathol*. 2009 Mar;30[1]:26-31. doi: 10.1097/PAF.0b013e3181873c32.
33. Andersson B, Bjelke B, Syková E. Temporal profile of ultrastructural changes in cortical neurones after a compression lesion. *Physiol Res*. 2006;55[3]:339-348. doi: 10.33549/physiolres.930723.
34. Hockenbery D. Defining apoptosis. *Am J Pathol*. 1995 Jan;146[1]:16-9. PMID: 7856725
35. Studzinski G, editor. *Apoptosis: a practical approach*. OUP Oxford; 1999 Dec 9.
36. Akerblom M, Sachdeva R, Jakobsson J. Functional Studies of microRNAs in Neural Stem Cells: Problems and Perspectives. *Front Neurosci*. 2012 Feb 7;6:14. doi: 10.3389/fnins.2012.00014.
37. Delalay C, Liu L, Lee JA, Su H, Shen F, Yang GY, *et al.* MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. *Cell Stem Cell*. 2010; 6[4]:323-35. doi: 10.1016/j.stem.2010.02.015.



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