ORIGINAL ARTICLE

Journal of Otorhinolaryngology and Facial Plastic Surgery. 2019;5(1):1-7.

DOI: https://doi.org/10.22037/ORLFPS.v5i1.27182

Neuroprotective Effects of Curcumin on the Proliferation and Viability of Neural Stem Cells against H2O2

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Article Info

Article Note:
Received: February, 2019
Accepted: April, 2019
Publish Online: September, 2019

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Keywords:
Curcumin;
H2O2;
Neural Stem Cells, NSCs;
Neuroprotection

Abstract

Background: Oxidative stress and neuroinflammation have found to be implicated in several neurodegenerative disorders (ND). Neuroprotection against oxidative agents has been proposed as a therapy on the basis that it might prevent neuroinflammation. Curcumin is an anti-oxidant with anti-inflammatory properties which has been proposed to be used as a therapeutic agent in ND.

Aim: In this study, we examined the neuroprotective effect of curcumin on neural stem cells (NSCs) exposed to H_2O_2 as an oxidative injury model.

Methods: After culturing NSCs, they were co-treated with curcumin and H_2O_2, after which their effects were tested on cell viability using MTT assay.

Results: Our results indicated that the high concentration of H2O2 significantly promoted cell death. Specifically, after using 250 µM of H2O2, the mortality increased dramatically in comparison with the control groups. On the other hand, the presence of curcumin encouraged cell survival of NSCs treated with H2O2.

Conclusion: Our result showed that curcumin has a protective effect on NSCs against H2O2 and it may ameliorate the mortality rate induced by H2O2.

Conflicts of Interest: The Authors declare no conflicts of interest.
Please cite this article as: Khoramgah MS, Peyvandi AA, Niknazar S, Darabi S, Khoshsirat S, Tahmasebinia F, Peyvandi H, Abbaszadeh HA. Neuroprotective Effects of Curcumin on the Proliferation and Viability of Neural Stem Cells against H2O2. J Otorhinolaryngol Facial Plast Surg. 2019;5(1):1-7.
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Introduction

Neural Progenitor Cells, known as Neural Stem Cells (NPCs), are multipotent cells which mainly differentiate into neurons and glial cells and make up the brain's major parts during embryonic development (1). Indeed, the development of the central nervous system (CNS) is a complicated process and the delicate regulations mechanisms are involved in the process spatiotemporally. The behavior of neuronal progenitor cells is controlled by intracellular and extracellular mechanisms and epigenetic factors as well. These factors alter the chromatin density and consequently affect the activity of the genes (2). MicroRNAs are internal regulators, diffusible signals of cell-to-cell interactions, and the interactions of cells with the extracellular matrix which are involved in the regulation of neuronal progenitors' behaviors. A major group of molecules that play a key role in regulating cell proliferation and cell differentiation are growth factors. The extracellular matrix is another factor that plays an essential role in regulating the behavior of neuronal progenitors. Changes in the membrane composition and the deletion of several genes cause corneal abnormalities and defects in cell

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migration (3). Therefore, exogenous compounds can be used to study changes in the behavioral patterns of neuronal progenitor cells as well as in neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Multiple Sclerosis (MS), and in congenital defects of CNS development (4, 5). Neuroinflammation in AD patients is characterized by an elevated level of cytokines and activated microglial cells. Use of anti-AD drugs, such as NSAIDs, offers a long-term reduction in AD risks. However, its side effects include digestive, kidney, and liver toxicity. Since curcumin has fewer side effects than these kinds of drugs, it has found to be a promising alternative (5, 6). In the brain of AD patients, a high concentration of amyloid aggregates and redox reactive metal ions has been found to be implicated in producing reactive oxygen species (ROS) and oxidative stress. Curcumin has proved to reduce oxidative stress in AD (6). Both the high and low doses of curcumin reduce the oxidized proteins and IL-12 as a pro-inflammatory cytokine in the brain. At low doses, curcumin mitigates the acidic effect of stereocritic glial markers and decreases the amyloid precursors in the nerve layers, suggesting the effectiveness of curcumin in preventing AD (4, 6). Therefore, studying the effects of curcumin on increasing the survival of the neuronal cells and also their differentiation into glial cells can probably be helpful in preventing and improving these neurological diseases. Statistical studies have indicates that the rate of neurodegenerative diseases in Indian societies is significantly lower than that among European and American societies due to the traditional use of Yellow Choba whose main ingredient is curcumin (7,8). Experiments have shown that curcumin has antioxidant, anti-inflammatory, and anti-cancer effects. It also plays a role in cell proliferation, cell differentiation, and migration. This wide spectrum of curcumin's activities is due to its interaction with intracellular signaling pathways (6). The high concentration of curcumin has a great capability to absorb and collect free radicals and ROS, while also inhibiting cancer cell development and tumor growth. On the other hand, low concentrations of curcumin activate or blocks one or more signaling pathways within the cell. In cancer cells, curcumin can inhibit signaling pathways associated with growth factors such as extracellular kinases and protein kinase C (9). Recent findings have also shown that curcumin can reduce oxidative damage as well as mental and memory defects associated with aging, and also protect hippocampal neurons from toxic stimuli and mechanical injuries [10]. In this study, we examined the protective effect of curcumin on neural stem cells (NSCs) exposed to H2O2 as an oxidative injury model.

Methods

Bone marrow stromal cell extraction and culturing

Male Wistar rats (120±20 gr) were anesthetized with an intraperitoneal injection of 10% Chloral hydrate (0.3 ml/100 g) and then were sacrificed. Afterward, the BMSCs were extracted from rat's femurs and cultured in DMEM/F12 (Stem cell technology company, Tehran, Iran), supplemented with 10% FBS, 100 u/ml penicillin (PC), 100 mg/ml Streptomycin (SP) and 2 mM/ml L–glutamine. Finally, they were incubated within a humidified incubator with 5% v CO2 at 37˚C.

Neural stem cell differentiation

For NSC differentiation, BMSCs at the fourth passage were cultivated in 6-well culture plates using DMEM F12 medium (Gibco, Grand Island, NY, USA) supplemented with 2% B27 (Invitrogen, Germany), 20 ng/mL basic Fibroblast growth factor (bFGF; R&D Systems, Minneapolis, MN, USA), 20 ng/ml Epidermal growth factor (EGF; R&D Systems, Minneapolis, MN, USA), and 1% penicillin-streptomycin for 2 weeks.

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Determination of non-toxic dose of curcumin

The curcumin (Sigma, USA) was purchased as a powder. For determining the safe dose of curcumin, MTT assay technique was performed. Initially, 5000 NSCs cells were cultured in several plates and following 24 hours, they were treated with curcumin with different doses. In order to obtain dilutions of 3-10, 4-10, 5-10, 6-10, 7-10, 8-10 and 9-10 M, an appropriate amount of the medium without FBS was used for dissolving the powder. Each experiment of different dosage was repeated for 5 times. After 24 hours of initial treatment, the cells were re-treated to provide cells with the fresh drug. In this experiment, PC12 cells were also used as a control in a non-curcumin culture medium.

Next, for evaluating the neuroprotective effects of curcumin, two plates were prepared with similar conditions. The first plate was directly used for MTT testing after treatment with curcumin. In the second plate, following 48-hour treatment with curcumin, the cells were exposed to hydrogen peroxide (Merck, Germany) at a concentration of 125 µm for 30 minutes after which MTT test was performed. Once washed with PBS, 20 µL of MTT solution (Sigma, USA) with concentrations of l and 0.5 µM was added to each well which was kept in dark environment at 37°C for 4 hours. In the next step, the wells were drained and 200 µL of DMSO (Sigma, USA) was added. Finally, the optical absorption of the solution was measured using an ELISA device at a wavelength of 570 nm and the percentage of live cells was calculated by comparing the optical absorption of wells against the standard curve.

MTT Assay

Cell growth and survival were measured using the Sigma biochemical method (MTT). In this method, the reduction of MTT leads to the formation of crystalline Formazan by dioxygenase enzymes within the mitochondria of living cells. This method has been used as a cell survival index. Formazan insoluble crystals were dissolved in an isopropanol to form a purple solution, at which point, E cells were treated with different concentrations of curcumin (0.1, 0.5, 1 µM) for 24 hours after adding cell suspensions prepared in 24 home dishes. Afterward, the culture medium containing curcumin was removed and the cells were washed twice with PBS. Next, the cells were added to each house containing PBS and MTT (0.5 mg) for 4 hours at 37 °C. The cells were immersed in dimethyl sulfoxide (DMSO) and isopropanol (1:1 ratio) whereby purified formaldehyde was obtained. The absorbance of the final solution was measured via spectrophotometry at the wavelength of 570 nm.

Statistical analyzes: SPSS software was used for statistical analysis. The test was conducted by One-way ANOVA and Tukey's Post Hoc. The data were expressed as Mean±SEM with significant differences observed with $P<0.05$, $P<0.01$ and $P<0.001$.

Results

Identification of BMSCs

A few hours following the initial culture of BMSCs, they began to adhere to the surface area of the flask and showed a round form. After 12 hours, they were characterized as dense fibroblast-like cell colonies.

Assessment of H2O2-induced cell death in different groups

As the cells were treated with several doses of H2O2 for 12 hours, the apoptosis rate was assessed using 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). The results revealed that elevating H2O2 concentration significantly increased cell death; particularly at the concentration of 250 µM, the mortality rate rose dramatically in comparison with the other groups. Nevertheless, there was a remarkable reduction in survival in all groups compared to the control group.

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Results of treatment by curcumin

The results of the experiments indicated that the quantity of cultivated NSC cells was altered as different concentrations of curcumin. A comparison of contrast-phase photomicrographs from different samples showed that the cell density at different viewing angles of curcumin-treated samples was different from that of the control sample in terms of quality (Fig. 1). In curcumin-treated samples at concentration of 0.1 µM, most of the plaque substrates were covered with NSC, where fewer insufficiencies were observed and colonies were formed. On the other hand, in treated samples at 0.5 µm, cell colonies with a prolonged period of time were observed on their surface. This implies that cell proliferation at a concentration of 0.5 µm is significantly increased and cellular layering can be affected by curcumin in comparison with the control group. The NSC cells that were cultivated in single-layer mode differed in light reflection; the adherent astrocytes were visible at the bottom of the container, dark, and clear colonies, and in photomicrographs.

MTT Assay results

The results of MTT biochemical tests revealed significant differences. Different concentrations of curcumin were effective in NSC cell survival. Curcumin with the 0.1 µM dose did not have a significant effect compared to the control group, while curcumin at 0.5 µm concentration induced cell growth and survival significantly ($p<0.01$**), compared to the control group. On the other hand, curcumin reduced cell growth and cell survival at a dose of 1 mM significantly ($p<0.05$) compared to the control group (Fig. 2).

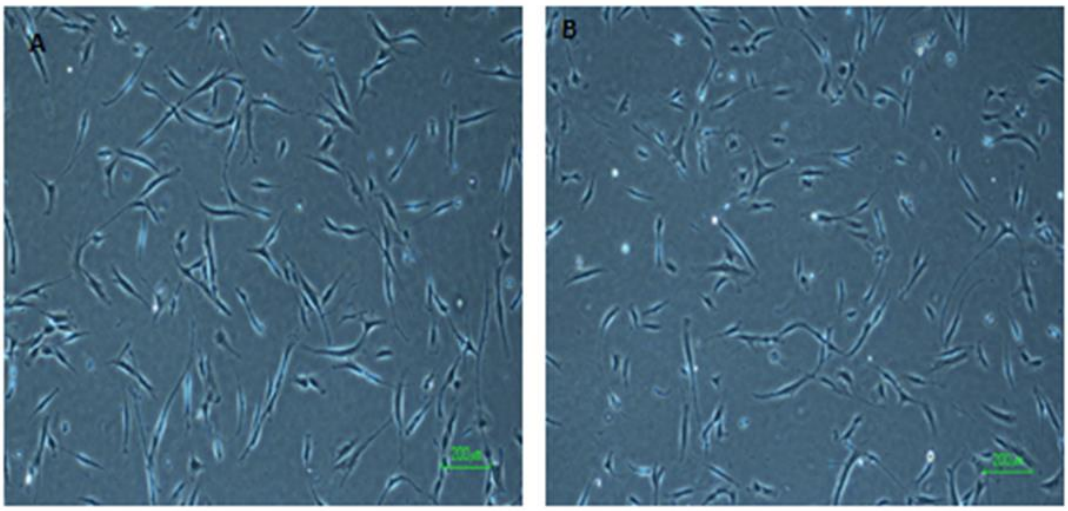

Figure 1: Effect of different concentrations of Curcumin on the morphology of NSC. (A) NSC in the affected by 350 Mm H2O2. (B) Photomicrograph of the cultivated NSC exposed to concentrations of 250 µM H2O2.

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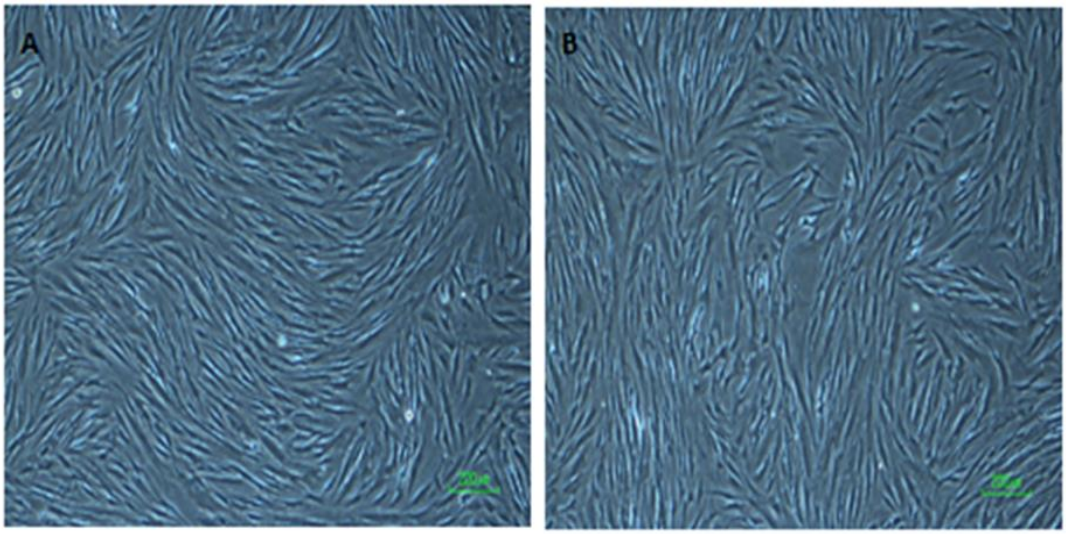

Figure 2: Effect of different concentrations of curcumin on NSC Photomicrograph of GFAP+astrocytes derived from neuronal progenitor cells cultured at concentrations of 0.1 µm (b), 0.5 µm (c), and 1 µm (d) of curcumin.

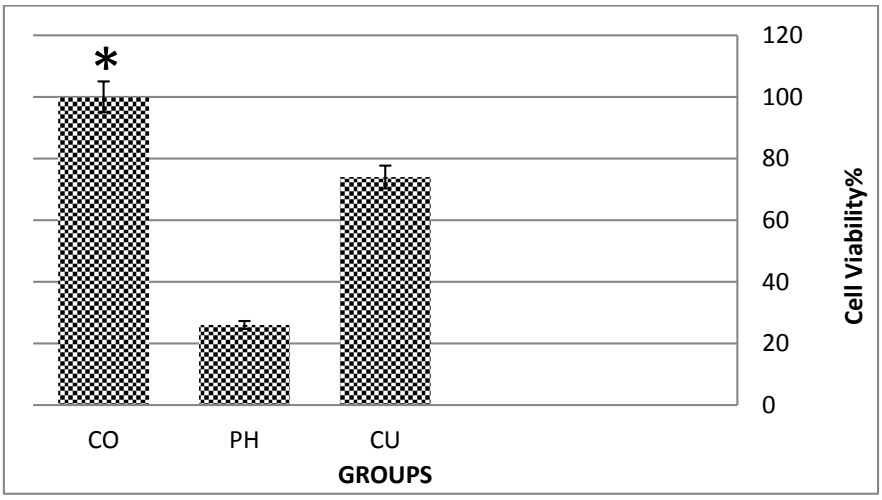

Figure 3: Effect of different concentrations of Curcumin on cell survival using MTT assay. There was a statistically significant difference between treated groups with concentrations of 0.1 and 0.5 µM of Curcumin with a control group. The data are shown as Mean±SEM; p <0.05*. (CO: control, PH: H2O2, CU: Curcumin).

Discussion

Based on our findings, Curcumin seems to enhance cell survival and growth of neuronal cells. However, different concentrations of curcumin have sometimes dual effects on the growth and proliferation as well as survival of cells derived from NSCs (9, 13). In a few similar studies, the effects of time-dependent curcumin have been investigated where with prolongation of treatment duration, the effect of curcumin on the proliferation also increased (10). It is believed that CNS neuroinflammatory diseases are associated with astrocyte and microglial activation. In neuroinflammatory disorders such as AD, amyloid-beta plaques are surrounded by astrocyte glycosides, leading to the neurodegeneration and neural apoptosis (14). Lim et al (14) also demonstrated the effect of curcumin on improving the process of neural repair and neurogenesis in memory-impairment of AD. Based on several studies, curcumin has positive effects on reducing the neurodegeneration by inhibiting the

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proliferation of glia and NSCs. In in-vivo experiments on AD transgenic mice, curcumin has been observed to reduce the GFAP astrocyte marker (15).

The results indicated that curcumin, as with other phytochemicals, has dose-dependent effects on the proliferation of NSC; at high doses, it is capable of reducing the proliferation and survival of NSC. The low dosage of curcumin is associated with increased cell proliferation and survival while a high dosage of curcumin could be toxic and inhibit cell growth (16). Further, it has been shown that the cultivation of NSC cells stimulates the proliferation of neuroprotective cells. In addition, in vitro curcumin, with an effective dose of 0.5 µM, increased the number of NSCs, while a high dose of 10 µm resulted in a decrease in the number of NSCs. As a result, low doses of curcumin stimulate NSC cell proliferation while high concentrations are toxic for cells (16). Dual effects of curcumin on normal cells and cells with inflammation in the nervous system are also interesting since curcumin can encourage proliferation and increase the survival of cells in healthy conditions while also preventing the death of NSC cells, but it is harmful in neuroinflammatory diseases. Therefore, in order to prevent the toxic and non-toxic effects of curcumin, in this study, very low concentrations were used.

The proliferation of NSC cells is very significant in the treatment with curcumin at 0.5 µM dose. In studies, this issue has been fully emphasized. The pathway through which curcumin promotes the proliferation of neurogenesis is likely to be the pathway of ERKs and p38 MAP kinase (10). Curcumin can enhance the proliferation of NSC by activation and phosphorylation of the above-mentioned kinases.

Curcumin increases the proliferation through effective MAP messenger pathways, such as P38 and ERK in NPCs. Consequently, curcumin is likely to affect the process of differentiation, increase the survival of these cells, and boost the cell cycle speed and proliferation of progenitor cells from the embryo's cortex. It has also been shown that curcumin has an inhibitory effect on the growth of cancer cells and inhibits their proliferation, but its precise underlying mechanisms are still unclear (16, 18). Curcumin can enhance the survival and proliferation of NSC and its derivatives.

Conclusion

To conclude, our findings suggested that curcumin has a protective effect on NSCs against H2O2.

Acknowledgments

This work was supported by Hearing Disorders Research Center of Shahid Beheshti University of Medical Sciences (Tehran, Iran). We would like to appreciate the support of the Clinical Research Development Center of Loghman Hakim Hospital (Tehran, Iran).

Conflicts of Interest

The Authors declare no conflicts of interest.

Ethics

This study was approved by the “Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran)”; Registration Code: IR.SBMU.RETECH.REC.1398.032.

Financial support

The Authors declare no financial support.

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References

1. Bhutani MK, Bishnoi M, Kulkarni SK. The anti-depressant like the effect of Curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Pharmacol Biochem Behav. 2009;92:39-43.

Journal of Otorhinolaryngology and Facial Plastic Surgery. 2019;5(1):1-7.

DOI: https://doi.org/10.22037/ORLFPS.v5i1.27182

2. Bishnoi M, Chopra K, Kulkarni SK. Protective effect of Curcumin, the active principle of turmeric (Curcuma longa) in haloperidol-induced orofacial dyskinesia and associated behavioural, biochemical and neurochemical changes in the rat brain. Pharmacol Biochem Behav. 2008;88:511-22.

3. Chen, H. Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by Curcumin. Oncogene. 1998;17:173-9.

4. Kim SJ, Son TG, Park HR, Park M, et al. Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. J Biol Chem. 2008;283:14497-505.

5. Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of Curcumin: preclinical and clinical studies. Anticancer Res. 1991;23(1A):363–98.

6. Tahmasebinia F, Pourgholaminejad A. The role of Th17 cells in auto-inflammatory neurological disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2017 Oct 3;79(Pt B):408-16.

7. Kulkami S, Dhir A, Akula KK. Potentials of Curcumin as an antidepressant. Sci World J. 2009;9:1233-41.

8. Tahmasebinia F, Emadi S. Effect of metal chelators on the aggregation of beta-amyloid peptides in the presence of copper and iron. Biometals. 2017 Apr;30(2):285-93.

9. Dhandapani KM, Mahesh VB, Brann DW. Curcumin suppresses growth and chemoresistance of human glioblastoma cells via AP-1 and NFkappaB transcription factors. J Neurochem. 2007;102(2):522–38.

10. Thiyagarajan M, Sharma SS. Neuroprotective effect of Curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Life Sci. 2004;74:969–85.

11. Cole GM, Teter B, Frautschy SA. Neuroprotective effects of Curcumin. Adv Exp Med Biol. 2007; 595: 197–212.

12. Jiang J, Wang W, Sun YJ, Hu M, et al. Neuroprotective effect of Curcumin on focal cerebral ischemic rats by preventing blood-brain barrier damage. Eur J Pharmacol. 2007;561:54–62.

13. Niknazar S, Nahavandi A, Peyvandi AA, Peyvandi H, Ahmady Roozbahany N. Hippocampal NR3C1 DNA methylation can mediate part of preconception paternal stress effects in rat offspring. Behav Brain Res. 2017;324,71-6.

14. Peyvandi AA, Ahmady Roozbahany N, Peyvandi H, Abbaszadeh HA, Majdinasab N, Faridan M, Niknazar S, 2018b. Critical role of SDF-1/CXCR4 signaling pathway in stem cell homing in the deafened rat cochlea after acoustic trauma. Neural Regen Res. 2018;13:154-60.

15. Lin JK. Molecular targets of Curcumin. Adv Exp Med Biol. 2007;595:227-43.

16. Griffin WST, Sheng JG, Royston MC, Gentleman SM, McKenzie JE, Graham DI, et al. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. Brain Pathol. 1998;8:65-72.

17. Sefati N, Abbaszadeh H, Fadaei Fathabady F, Abdolahifar MA, Khoramgah MS, Darabi S, et al. The Combined Effects of Mesenchymal Stem Cell Conditioned Media and Low-Level Laser on Stereological and Biomechanical Parameter in Hypothyroidism Rat Model. J Lasers Med Sci. 2018;9(4):243-8.

18. Mattson MP, Cheng A. Neurohormetic phytochemicals: Low dose toxins that induce adaptive neuronal stress responses. Trends Neurosci. 2006;29:632-41.

19. Chen H, Zhang ZS, Zhang YL, Zhou DY. Anticancer Res. 1999;19:3675–80.

20. Darabi S, Tiraihi T, Noori-Zadeh A, Rajaei F, Darabi L, Abbaszadeh HA. Creatine and retinoic acid effects on the induction of autophagy and differentiation of adipose tissue-derived stem cells into GABAergic-like neurons. J Babol Univ Med Sci. 2017;19(8):41-9.

21. Peyvandi AA, Abbaszadeh HA, Ahmady Roozbahany N, Pourbakht A, Khoshsirat S, Haddadzade Niri H, Peyvandi H, Niknazar S. Deferoxamine promotes mesenchymal stem cell homing in noise-induced injured cochlea through PI 3K/AKT pathway. Cell Prolif. 2018;51(2):e12434.

22. Thiyagarajan M, Sharma SS. Neuroprotective effect of Curcumin in middle cerebral artery occlusion induced focal cerebral ischemia in rats. Life Sci. 2004;74:969-85.

23. Jiang J, Wang W, Sun YJ, Hu M, Li F, Zhu DY. Neuroprotective effect of Curcumin on focal cerebral ischemic rats by preventing blood-brain barrier damage. Eur J Pharmacol. 2007;561:54-62.

24. Abbaszadeh HA, Tiraihi T, Sadeghi Y, Delshad AR, Sadeghizadeh M, Taheri T, et al. Decrease in Cavity Size and Oligodendrocyte Cell Death Using Neurosphere-Derived Oligodendrocyte-Like Cells in Spinal Cord Contusion Model. Iran Biomed J. 2018;22(4):246-57.

25. Unnikrishnan MK, Rao MN. Curcumin inhibits nitriteinduced methemoglobin formation. FEBS Lett. 1992;301:195-6.