http://jkmu.kmu.ac.ir/

Journal of Kerman University of Medical Sciences
2021; 28(3):243-251

10.22062/JKMU.2021.91664

Original Article

Crocin Administration from Childhood to Adulthood Increases Hippocampal Neurogenesis and Synaptogenesis in Male Mice

Babak Ebrahimi[1], Shayan Vafaei[2], Seyed Hamid Reza Rastegar-Moghaddam[2], Mahmoud Hosseini[3,4], Fatemeh Tajik Yabr[2], Abbas Mohammadipour[2]

1. Department of Anatomy, School of Medicine, Tehran University of medical sciences, Tehran, Iran
2. Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Applied Biomedical Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Division of Neurocognitive Sciences, Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Background: Adult hippocampal neurogenesis and synaptogenesis play a critical role in learning and memory. Crocin as a carotenoid has many neuroprotective effects but its effect on neurogenesis and synaptogenesis is unknown. In this study, the effects of crocin administration from post-lactation period to adulthood on the mice hippocampal neurogenesis and synaptogenesis were investigated.

Methods: 12 mice offspring were divided into 2 groups of control and crocin. Animals in the crocin group received 30 mg/kg of crocin from postnatal day 30 to 75 through drinking water. At the same time, the control group received drinking water without crocin. At the end of the treatment, animals were sacrificed and their brains were removed. The brains were sectioned and stained by immunohistochemical technique to evaluate the effect of crocin on hippocampal doublecortin (DCX) positive cells and synaptophysin expression.

Results: The results of the immunohistochemistry showed that the mean number of DCX^+ cells in the dentate gyrus (DG) of the crocin group was significantly higher than that in the control group. In addition, the synaptophysin expression was higher in the cornu ammonis (CA) of the hippocampus in the crocin group.

Conclusion: According to the results, consumption of crocin from childhood to adulthood may increase hippocampal neurogenesis and synaptogenesis.

Keywords: Crocin, Dentate gyrus, Hippocampus, Neurogenesis, Synaptogenesis

Citation: Ebrahimi B, Vafaei Sh, Rastegar-Moghaddam SHR, Hosseini M, Tajik Yabr F, Mohammadipour A. Crocin administration from childhood to adulthood increases hippocampal neurogenesis and synaptogenesis in male mice. *Journal of Kerman University of Medical Sciences* 2021; 28(3): 243-251. doi: 10.22062/JKMU.2021.91664

Received: 18.10. 2020
Accepted: 08.03. 2021
***Correspondence:** Abbas Mohammadipour; Email: MohammadiPA@mums.ac.ir

Published by Kerman University of Medical Sciences

Introduction

Cognitive performance in adulthood is very important and depends on many factors. One of the most important factors is the generation of new neurons in the hippocampus (1,2). It has been documented that neurogenesis occurs in mammals including humans in the dentate gyrus (DG) of the hippocampus throughout life and plays a crucial role in cognition (2-4). Along with the DG, cornu ammonis (CA) of the hippocampus that consists of CA1, CA2, and CA3 has a fundamental role in cognitive performance (5). Some important fibers connect different regions of the hippocampus with each other and their synapses are essential for cognitive performance (5). Adult hippocampal neurogenesis and synaptogenesis can be affected by various factors such as age, genetics, nutrition, stress, and toxic substances (2). The results of the previous studies have shown that one of the most important factors that affects the neurogenesis and synaptogenesis in adults is ageing. Adult hippocampal neurogenesis and synaptogenesis decreases with age as well as neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (6,7).

On the other hand, the results of the previous studies indicate the beneficial effects of nutrition on memory. Among all, carotenoids are well known. The results of Zielińska et al. showed that older people who regularly eat fruits, vegetables, and carotenoids have a higher memory performance than others (8). Crocin (active constituent of saffron) with chemical formula ($C_{44}H_{64}O_{24}$) is a carotenoid, which its protective properties are well known (9,10). Studies have shown that crocin is able to protect the neurons against oxidative stress, inflammation, diseases, and toxic materials (9-13). Crocin has also been shown to have neuroprotective effects against memory-impairing diseases such as diabetes, Alzheimer's disease, and Parkinson's disease (10,13-15).

Moreover, the results of a previous study have shown that crocin is able to improve memory and learning in ethanol-treated animals by improving the hippocampal long-term potentiation, a form of activity-dependent synaptic plasticity that plays a crucial role in learning and memory (16). In addition, crocin increases the expression of some factors that are related to neurogenesis such as neurotrophins and cAMP-response element binding protein (CREB) in the brain (17,18).

Therefore, regarding the neuroprotective effects of crocin and its potential to improve memory, the present study was conducted to investigate the effect of crocin on the hippocampal neurogenesis and synaptogenesis. One of the well-recognized markers in the neurogenesis is doublecortin (DCX) (3,19,20). DCX is a microtubule-associated protein, which is specifically expressed only in neuronal progenitor cells and does not exist in the mature neurons. Thus, the detection of this marker with immunohistochemistry in a region of the brain indicates the neurogenesis in that area (3,20). Therefore, in the present study, the effects of crocin on the hippocampal neurogenesis were evaluated by DCX-immunohistochemistry. In addition, the expression of synaptophysin as a synaptic molecule involved in cognitive performance was evaluated by immunohistochemistry (21-23).

Materials and Methods

Materials

The crocin was provided from the Buali Research Institute of Mashhad University of Medical Sciences, Iran. Primary antibodies including rabbit anti-DCX (ab207175) and rabbit anti-synaptophysin (ab14692) were purchased from Abcam, the USA, and the secondary antibody (DAKO En Vision + System, Peroxidase) was purchased from Dako, Denmark.

Animals and treatment groups

Adult BALB/c mice weighing 35-40 g were purchased from the animal center of Mashhad University of Medical Science and housed at 22 ± 2°C with a 12:12 h light/dark cycle in normal laboratory condition.

Male mice mate with the female mice, and after the pregnancy, female mice were kept in the separate cages. In total, 12 pregnant mice were used in this study.

Mice and their offspring were cared until 30 days after delivery. Then, one male offspring was separated from each mother, and finally, 12 male offspring were divided into two control and crocin groups (6 in each group). The crocin group received crocin daily at a dose of 30 mg/kg (10) via drinking water, and the control group received no treatment and just consumed drinking water without crocin. The duration of administration was 45 days and continued until

the 75th day after birth (postnatal day 75). All protocols in the present study were approved by the Institutional Animal Care Committee of Mashhad University of Medical Sciences (Ethical code: IR.MUMS.fm.REC.1397.17).

Sample preparation

At the end of the treatment, the animals were anesthetized by Ketamine (75 mg/kg, IP) and Xylazine (10 mg/kg, IP) (24,25), cardiac perfusion was performed, and eventually, the animals were sacrificed. Then, the brains were removed from the skull and fixed in 10% normalin for one week. Because normalin fixes the brains better than formalin, it was used as a fixative in this study. Normalin was made by combining 10 ml of formaldehyde with 90 ml of normal saline. After that, the brains were dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Using microtome, the brains were cut into 5 µm coronal serial sections5 µm-thick in coronal serial section with a 100 µ interval. The sections range from -1.5 to -2.5 to Bregma. Finally, the sections were used for immunohistochemistry (IHC) staining in order to detect doublecortin positive (DCX^+) neurons as well as synaptophysin expression in the hippocampus.

Immunohistochemistry

In the IHC staining, the procedure was performed on two consecutive days. On the first day, the sections were placed inside the xylene to remove the paraffin of the specimens. Then, they were placed in ethanol, distilled water, and PBS, respectively. Samples were exposed to 3% H_2O_2 in PBS for 15 min after heat-induced antigen retrieval. The next step was to use goat serum for 20 minutes. Finally, after washing with PBS, the samples were exposed to a primary antibody against the DCX and synaptophysin for one night (overnight). On the second day of staining, the samples were washed with PBS and exposed to secondary antibody (goat anti-rabbit IgG) for 90 min. Finally, they were exposed to DAB and placed in hematoxylin (3,10,26).

After staining, the hippocampus of all prepared slides were photographed with the Olympus imaging system.

For each slide, imaging was performed from both right and left dentate gyri and DCX^+ neurons were counted using stereological grid and the following formula:

$$N_A = \frac{\sum Q}{a/f \cdot \sum P}$$

where *NA* is the number of neurons in area, $\Sigma\bar{Q}$ is the sum of counted particles in the sections, *a/f* is the area associated with each frame, and ΣP is the sum of frame-associated points hitting the defined space (10,26,27).

Besides, image J software was used to measure average staining intensity for evaluating the

synaptophysin expression in both DG and cornu Ammonis (CA) areas of the hippocampi.

Then, average staining intensity was transformed to optical density (OD) using the following

formula for statistical analysis (28-30).

$$\text{Optical density} = \log\left(\frac{\text{Max intensity}}{\text{Mean intensity}}\right)$$

The max intensity in image J is 255.

Statistical analysis

Data were analyzed using SPSS software version 20. Data were analyzed by t-test and $P < 0.05$ was considered statistically significant. All steps of stereology and analysis of the results were performed blindly and repeated twice.

Results

The results of DCX immunohistochemistry

The analysis of IHC results showed that the mean number of doublecortin positive neurons in the dentate gyrus of crocin group was significantly higher than that in the control group ($P < 0.05$).

The mean number of these neurons in the control group was 170.33 ± 20.53 cells/mm^2 whereas

it was 227.05 ± 17.5 cells/mm^2 in the crocin group (Figures 1 and 2).

This increase in the mean number of DCX^+ neurons in the dentate gyrus, indicates an increase in neurogenesis.

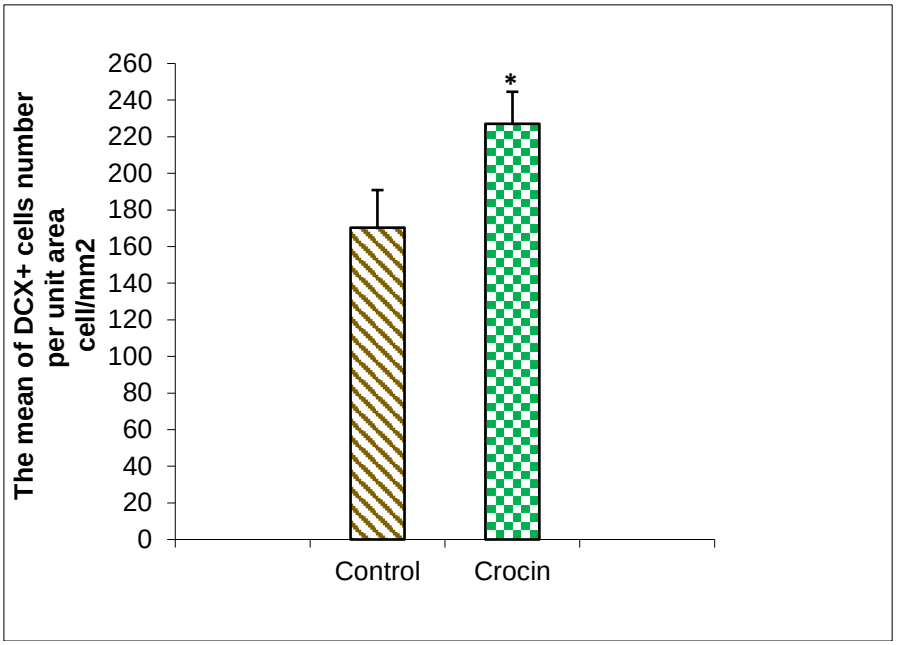

Figure 1. The comparison of the mean number of DCX+ neurons in the dentate gyrus between control and crocin groups. There is a significant difference between two groups, which indicates an increase in neurogenesis in the crocin group.
*Statistically significant at P <0.05.

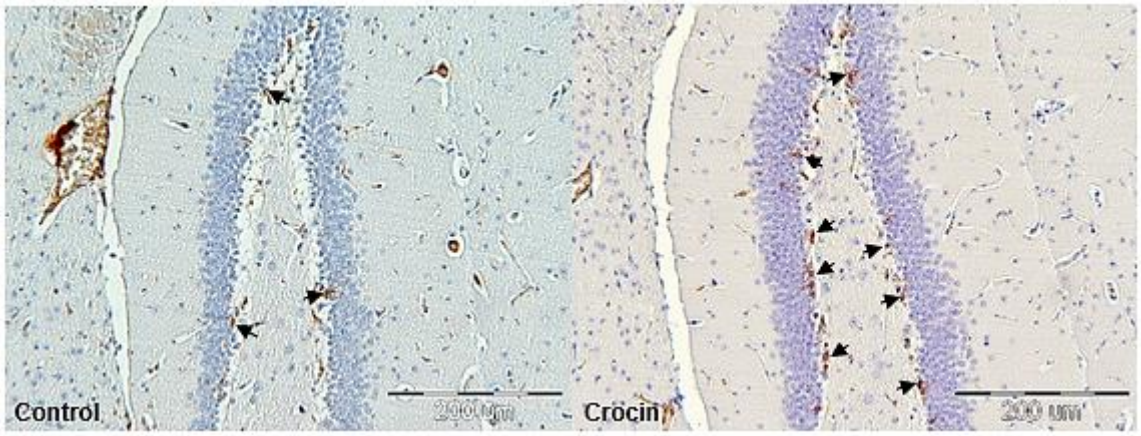

Figure 2. Microscopic image of the DG of both control and crocin groups. DCX+ cells are visible in the subgranular zone of the dentate gyri in brown (arrows). As shown in the microscopic images, the number of DCX+ neurons in the crocin group is more than that in the control group. Magnification = 200; Scale bar = 200 µm.

The results of synaptophysin immunohistochemistry

The results revealed that crocin administration can increase synaptogenesis in the CA region of the hippocampus (Figures 3 and 4). The OD for synaptophysin expression in the CA was 0.266 ± 0.019 and 0.344 ± 0.013 in control and crocin groups, respectively. This difference was statistically significant ($P < 0.01$).

The mean OD for synaptophysin expression in the DG was 0.25 ± 0.017 and 0.267 ± 0.014 for control and crocin groups, respectively, and there was no significant difference between two groups (Figure 3).

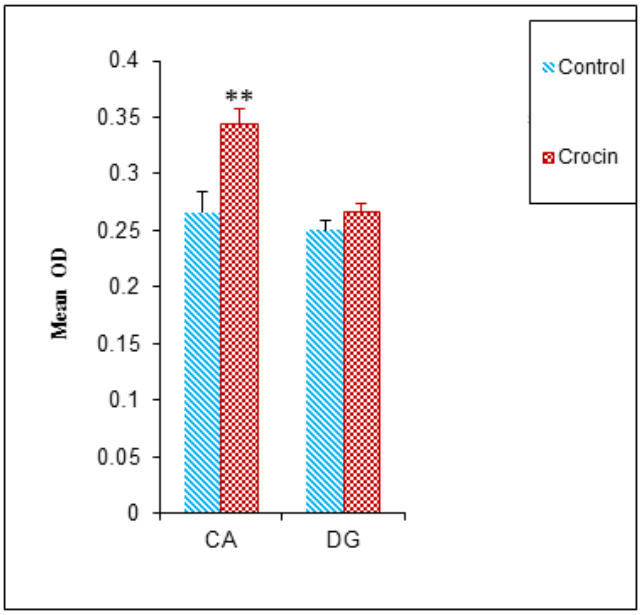

Figure 3. The comparison of the mean OD of synaptophysin expression in the hippocampus between control and crocin groups. Synaptophysin expression in the hippocampal CA region of the crocin group was significantly higher. The mean OD in the DG of the crocin group was also higher than control group but this difference was not significant.
**Statistically significant level was considered at $P < 0.01$.

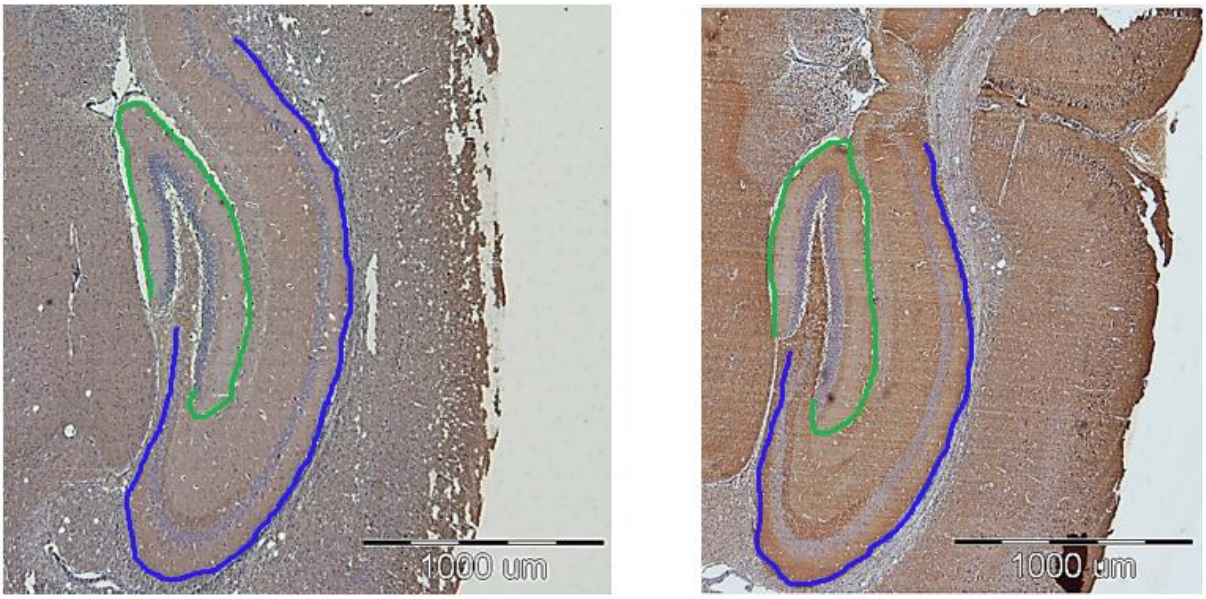

Figure 4. Microscopic image of the hippocampus in both control and crocin groups to show synaptophysin immunoreactivity. DG is marked in green and CA is marked in blue. The mean OD in the CA region of the hippocampus was significantly higher in the crocin group. Magnification = 40; Scale bar = 1000 µm.

Discussion

The main purpose of this study was to investigate the beneficial effects of crocin on the adult hippocampal neurogenesis and synaptogenesis. The results of the present study, notably for the first time, showed that the crocin is able to increase hippocampal neurogenesis and synaptogenesis in adult brain.

In the present study, administration of crocin in the post lactation period was able to increase the number of DCX^+ neurons in adult dentate gyrus, which indicates an increase in neurogenesis. Previous studies have reported that neurogenesis rates of dentate gyrus can be affected by various factors. For example, it has been proven that neurogenesis rate can be affected by neurotrophic factors such as brain-

derived neurotrophic factor (BDNF) and neurogenesis increases by stimulating this neurotrophic factor (31). On the other hand, crocin increases the expression of neurotrophic factors, including BDNF (32). Neurotrophins have important roles in central nervous system (CNS) function. They are known as neuronal differentiation regulator and play a key role in the CNS regeneration (33).

Nerve growth factor (NGF) is another neurotrophin that along with BDNF plays an important role in neural activities. This neurotrophin role is important for neurogenesis because it regulates various stages of neuronal precursor maturation. NGF induces cell differentiation through several ways including activation of canonical NGF-TrkA-PI3K-Akt signaling axis and releasing of cyclic adenosine monophosphate (cAMP). It also increases the expression of shootin-1, which is essential for axon formation and neuron polarization (34,35).

It has been shown that anti-NGF transgenic mice has a significant reduction in neural precursors (36). Meanwhile, it has been proven that the decrease in NGF is associated with the decrease in neurogenesis in the aging brain (37). Taken together, any decrease in NGF results in neurogenesis reduction. So, it seems that crocin increases neurogenesis by increasing the neurotrophins level in the brain.

In addition, the role of CREB in neurogenesis can also be noted. CREB is present in newborn neurons of the dentate gyrus from 3 to 21 days after generation of the neuron. The results of the previous studies have shown that this factor is essential for survival of newborn neurons through various routes. For example, CREB signaling controls the expression of the paired box 6 (Pax6) and DCX in newborn neurons. It has been demonstrated that the inhibition of the CREB, leads to the loss of the Pax6 and DCX expression, which decreases the number of the newborn neurons (38). CREB increases the rate of hippocampal neurogenesis and, on the other hand, crocin has been shown to increase the amount of this factor (32). Therefore, a part of the increase in neurogenesis rate with crocin administration in the present study may be due to the increase of this marker.

Another important factor that can influence the neurogenesis of adult brain is stress (39). Stress and oxidative stress can affect the nervous system through variety of mechanisms and damage the neurons (40-42). It can also reduce the rate of neurogenesis in the dentate gyrus. On the other hand, crocin is an anti-stress and anti-oxidative stress agent (9,10,43).

In addition to the mentioned points, there are other possibilities in this regard. For example, the role of peptide orexin was also mentioned in this study. The stimulation of the hippocampus by orexin, results in an increase in the neurogenesis in the dentate gyrus (44,45). The hippocampus has a receptor for orexin A, and its neurons are strongly stimulated by this peptide (46). Therefore, crocin may also increase neurogenesis through the orexin pathway, which this is a theory and requires further studies to prove it.

In the present study, it was also found that besides neurogenesis, hippocampal synaptogenesis is also increased after 45 days crocin administration. Today, it is well documented that synaptogenesis is an integral part of the memory process and plays a vital role in learning and memory. Synaptophysin is a synaptic molecule and is the marker of synaptic density. This protein is richly expressed in the presynaptic vesicles of the axons and is the most abundant integral membrane protein of synaptic vesicles in neurons (47,48). In the previous studies, the reduction of synaptophysin has been reported in the hippocampus due to different factors such as aging (47). Therefore, it seems that crocin can support the synaptic connectivity and memory formation by preventing a decrease in synaptophysin level. The different regions of the hippocampus are connected to each other by several fibers. For example, mossy fibers connect the DG to the CA3. These fibers originate from DG and synapse with the neurons of the CA3. Some other important fibers, Schaffer collateral fibers, connect the CA3 to the CA1, which play a critical role in memory formation (5). As both DG and CA are involved in cognitive performance, in the present study, the effect of crocin on the synaptophysin expression was investigated in both DG and CA areas. Whereas, because adult hippocampal neurogenesis occurs only in the dentate gyrus (3), neurogenesis was investigated only in this area of the hippocampus. In this study, it was found that crocin administration increases the mean OD in the CA significantly, which indicates an increase in synaptogenesis in this region of the hippocampus. Whereas, no significant difference was observed in the mean OD of the DG between two groups. Its exact reason is not clear but it may be due to the abundance of synapses in the CA. Many

differences have recently been reported between DG and CA including differences in synaptic plasticity (49). Since CA is formed by the large pyramidal cells, the surface area of the CA is 40 times larger and needs a huge connectivity (48) and as mentioned above, this area has more synapses than DG (5, 49, 50). Another reason for this difference in OD between the DG and CA may be due to the high sensitivity of the pyramidal cells. The pyramidal cells in the CA have been shown that are more sensitive than the DG granular cells and are more affected by various internal and external factors (49).

The main limitation of the present study is that this study focused only on the hippocampal neurogenegis and synaptogenesis, and behavioral performance was not investigated in this study. Regarding the close relationship between hippocampus and cognitive performance, the authors suggest the investigation of the effects of crocin administration from childhood to adulthood on the behavioral performance such as memory and learning.

Conclusion

Regular consumption of crocin from childhood to adulthood in mice may increase neurogenesis of dentate gyrus as well as hippocampal synaptogenesis. Therefore, it may reduce the age-related learning and memory impairments, which should be investigated in the future studies.

Acknowledgements

The data acquired from this research were extracted from a research protocol (961103), which was financially supported by the Vice Chancellor for Research, Mashhad University of Medical Sciences, Mashhad.

Conflict of interests

There is no conflict of interests.

References

1. Deng W, Aimone JB, Gage FH. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat Rev Neurosci 2010; 11(5):339-50.
2. Lieberwirth C, Pan Y, Liu Y, Zhang Z, Wang Z. Hippocampal adult neurogenesis: Its regulation and potential role in spatial learning and memory. Brain Res 2016; 1644:127-40.
3. Ebrahimzadeh Bideskan A, Mohammadipour A, Fazel A, Haghir H, Rafatpanah H, Hosseini M, et al. Maternal exposure to titanium dioxide nanoparticles during pregnancy and lactation alters offspring hippocampal mRNA BAX and Bcl-2 levels, induces apoptosis and decreases neurogenesis. Exp Toxicol Pathol 2017; 69(6):329-37.
4. Mahar I, MacIsaac A, Kim JJ, Qiang C, Davoli MA, Turecki G, et al. Effects of neuregulin-1 administration on neurogenesis in the adult mouse hippocampus, and characterization of immature neurons along the septotemporal axis. Scientific Reports 2016; 6:30467.
5. Rastegar-Moghaddam SH, Mohammadipour A, Hosseini M, Bargi R, Ebrahimzadeh-Bideskan, A. Maternal exposure to atrazine induces the hippocampal cell apoptosis in mice offspring and impairs their learning and spatial memory. Toxin Reviews 2019; 38(4):298-306.
6. Kase Y, Otsu K, Shimazaki T, Okano H. Involvement of p38 in age-related decline in adult neurogenesis via modulation of wnt signaling. Stem Cell Reports 2019; 12(6):1313-28.
7. Faure A, Verret L, Bozon B, El Tannir El Tayara N, Ly M, Kober F, et al. Impaired neurogenesis, neuronal loss, and brain functional deficits in the APPxPS1-Ki mouse model of Alzheimer's disease. Neurobiol Aging 2011; 32(3):407-18.
8. Zielińska MA, Białecka A, Pietruszka B, Hamułka J. Vegetables and fruit, as a source of bioactive substances, and impact on memory and cognitive function of elderly. Postepy Hig Med Dosw (Online) 2017; 71:267-80.
9. Fani M, Mohammadipour A, Ebrahimzadeh-Bideskan A. The effect of crocin on testicular tissue and sperm parameters of mice offspring from mothers exposed to atrazine during pregnancy and lactation periods: An experimental study. Int J Reprod Biomed 2018; 16(8):519-28.
10. Haeri P, Mohammadipour A, Heidari Z, Ebrahimzadeh-Bideskan A. Neuroprotective effect of crocin on substantia nigra in MPTP-induced Parkinson's disease model of mice. Anat Sci Int 2019; 94(1):119-27.

11. Baghishani F, Mohammadipour A, Hosseinzadeh H, Hosseini M, Ebrahimzadeh-Bideskan A. The effects of tramadol administration on hippocampal cell apoptosis, learning and memory in adult rats and neuroprotective effects of crocin. Metab Brain Dis 2018; 33(3):907-16.

12. Farshid A, Tamaddonfard E. Histopathological and behavioral evaluations of the effects of crocin, safranal and insulin on diabetic peripheral neuropathy in rats. Avicenna J Phytomed 2015; 5(5):469-78.

13. Sarshoori JR, Asadi MH, Mohammadi MT. Neuroprotective effects of crocin on the histopathological alterations following brain ischemia-reperfusion injury in rat. Iran J Basic Med Sci 2014; 17(11):895-902.

14. Asadi F, Jamshidi AH, Khodagholi F, Yans A, Azimi L, Faizi M, et al. Reversal effects of crocin on amyloid β-induced memory deficit: modification of autophagy or apoptosis markers. Pharmacol Biochem Behav 2015; 139(Pt A):47-58.

15. Tamaddonfard E, Farshid AA, Asri-Rezaee S, Javadi S, Khosravi V, Rahman B, et al. Crocin improved learning and memory impairments in streptozotocin-induced diabetic rats. Iran J Basic Med Sci 2013; 16(1):91-100.

16. Abe K, Saito H. Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. Phytother Res 2000; 14(3):149-52.

17. Motaghinejad M, Safari S, Feizipour S, Sadr S. Crocin may be useful to prevent or treatment of alcohol induced neurodegeneration and neurobehavioral sequels via modulation of CREB/BDNF and Akt/GSK signaling pathway. Med Hypotheses 2019; 124:21-5.

18. Dorri SA, Hosseinzadeh H, Abnous K, Hasani FV, Robati RY, Razavi BM. Involvement of brain-derived neurotrophic factor (BDNF) on malathion induced depressive-like behavior in subacute exposure and protective effects of crocin. Iran J Basic Med Sci 2015; 18(10):958-66.

19. Seo HS, Yang M, Song MS, Kim JS, Kim SH, Kim JC, et al. Toluene inhibits hippocampal neurogenesis in adult mice. Pharmacol Biochem Behav 2010; 94(4):588-94.

20. Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, et al. Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 2005; 21(1):1-14.

21. Hai J, Su SH, Lin Q, Zhang L, Wan JF, Li H, et al. Cognitive impairment and changes of neuronal plasticity in rats of chronic cerebral hypoperfusion associated with cerebral arteriovenous malformations. Acta Neurol Belg 2010; 110(2):180-5.

22. Wang Y, Chen W, Hou B, Gao Y, Li Z, Li X, et al. Altered expression of thrombospondin-1/-2 in the cortex and synaptophysin in the hippocampus after middle cerebral artery occlusion and reperfusion. Int J Clin Exp Pathol 2018; 11(7):3267-76.

23. Robinson JL, Molina-Porcel L, Corrada MM, Raible K, Lee EB, Lee VM, et al. Perforant path synaptic loss correlates with cognitive impairment and Alzheimer's disease in the oldest-old. Brain 2014; 137(Pt 9):2578-87.

24. Bigham M, Mohammadipour A, Hosseini M, Malvandi AM, Ebrahimzadeh-Bideskan A. Neuroprotective effects of garlic extract on dopaminergic neurons of substantia nigra in a rat model of Parkinson's disease: motor and non-motor outcomes. Metab Brain Dis 2021; 36(5):927-37.

25. Yang SQ, Tian Q, Li D, He SQ, Hu M, Liu SY, et al. Leptin mediates protection of hydrogen sulfide against 6-hydroxydopamine-induced Parkinson's disease: Involving enhancement in Warburg effect. Neurochem Int 2020; 135:104692.

26. Heidari Z, Mohammadipour A, Haeri P, Ebrahimzadeh-Bideskan A. The effect of titanium dioxide nanoparticles on mice midbrain substantia nigra. Iran J Basic Med Sci 2019; 22(7):745-51.

27. Karimooy FN, Bideskan AE, Pour AM, Hoseini SM. Neurotoxic effects of stanozolol on male rats' hippocampi: does stanozolol cause apoptosis? Biomol Concepts 2019; 10(1):73-81.

28. Baradaran R, Khoshdel-Sarkarizi H, Kargozar S, Hami J, Mohammadipour A, Sadr-Nabavi A, et al. Developmental regulation and lateralisation of the α7 and α4 subunits of nicotinic acetylcholine receptors in developing rat hippocampus. Int J Dev Neurosci 2020; 80(4):303-18.

29. Abbasi F, Baradaran R, Khoshdel-Sarkarizi H, Kargozar S, Hami J. Mohammadipour A, et al. Distribution pattern of nicotinic acetylcholine receptors in developing cerebellum of rat neonates born of diabetic mothers. J Chem Neuroanat 2020; 108:101819.

30. Khoshdel-Sarkarizi H, Hami J, Mohammadipour A, Sadr-Nabavi A, Mahmoudi M, Kheradmand H, et al.

Developmental regulation and lateralization of GABA receptors in the rat hippocampus. Int J Dev Neurosci 2019; 76:86-94.

31. Vilar M, Mira H. Regulation of neurogenesis by neurotrophins during adulthood: expected and unexpected roles. Front Neurosci 2016; 10:26.
32. Hassani FV, Naseri V, Razavi BM, Mehri S, Abnous K, Hosseinzadeh H. Antidepressant effects of crocin and its effects on transcript and protein levels of CREB, BDNF, and VGF in rat hippocampus. Daru 2014; 22(1):16.
33. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci 2003; 4(4):299-309.
34. Ng YP, Wu Z, Wise H, Tsim KW, Wong YH, Ip NY. Differential and synergistic effect of nerve growth factor and cAMP on the regulation of early response genes during neuronal differentiation. Neurosignals 2009; 17(2):111-20.
35. Ergin V, Erdogan M, Menese A. Regulation of shootin1 gene expression involves ngf-induced alternative splicing during neuronal differentiation of PC12 cells. Scientific Reports 2015; 5:17931.
36. Scardigli R, Capelli P, Vignone D, Brandi R, Ceci M, La F, et al. Neutralization of nerve growth factor impairs proliferation and differentiation of adult neural progenitors in the subventricular zone. Stem Cells 2014; 32(9):2516-28.
37. Colangelo AM, Follesa P, Mocchetti I. Differential induction of nerve growth factor and basic fibroblast growth factor mRNA in neonatal and aged rat brain. Brain Res Mol Brain Res 1998; 53(1-2):218-25.
38. Herold S, Jagasia R, Merz K, Wassmer K, Lie DC. CREB signalling regulates early survival, neuronal gene expression and morphological development in adult subventricular zone neurogenesis. Mol Cell Neurosci 2011; 46(1):79-88.
39. Baptista P, Andrade JP. Adult hippocampal neurogenesis: regulation and possible functional and clinical correlates. Front Neuroanat 2018; 12:44.
40. Mohammadipour A, Haghir H, Ebrahimzadeh Bideskan A. A link between nanoparticles and Parkinson's disease. Which nanoparticles are most harmful? Rev Environ Health 2020; 35(4):545-56.
41. Soleimani S, Pirmoradloo E, Farmani F, Moein S, Yousefzadi M. Antidiabetic and Antioxidant Properties of Sea Urchin Echinometra mathaei from the Persian Gulf. Journal of Kerman University of Medical Sciences. 2021; 28(1):104-115.
42. Akbari M, Dabiri S, Moeini-Aghtaei M, Nazari-Robati M. Thermostabilized chondroitinase ABC Promotes Neuroprotection after Contusion Spinal Cord Injury. Journal of Kerman University of Medical Sciences 2020; 27(5):369-379.
43. Ghadrdoost B, Vafaei AA, Rashidy-Pour A, Hajisoltani R, Bandegi AR, Motamedi F, et al. Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Eur J Pharmacol 2011; 667(1-3):222-9.
44. Ito N, Yabe T, Gamo Y, Nagai T, Oikawa T, Yamada H, et al. Icv administration of orexin-A induces an antidepressive-like effect through hippocampal cell proliferation. Neuroscience 2008; 157(4):720-32.
45. Mavanji V, Butterick TA, Duffy CM, Nixon JP, Billington CJ, Kotz CM. Orexin/hypocretin treatment restores hippocampal-dependent memory in orexin-deficient mice. Neurobiol Learn Mem 2017; 146:21-30.
46. Chen XY, Chen L, Du YF. Orexin-A increases the firing activity of hippocampal CA1 neurons through orexin-1 receptors. J Neurosci Res 2017; 95(7):1415-26.
47. Xu L, Long J, Su Z, Xu B, Lin M, Chen Y, et al. Restored presynaptic synaptophysin and cholinergic inputs contribute to the protective effects of physical running on spatial memory in aged mice. Neurobiol Dis 2019; 132:104586.
48. Nemes AD, Ayasoufi K, Ying Z, Zhou QG, Suh H, Najm IM. Growth associated protein 43 (GAP-43) as a novel target for the diagnosis, treatment and prevention of epileptogenesis. Scientific Reports 2017; 7:17702.
49. Alkadhi KA. Cellular and molecular differences between area ca1 and the dentate gyrus of the hippocampus. Mol Neurobiol 2019; 56(9):6566-80.
50. Mercer A, Thomson AM. Cornu ammonis regions-antecedents of cortical layers? Front Neuroanat 2017; 11:83.