http://jbcp.shahed.ac.ir 10.22070/JBCP.2022.16405.1160

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol. 10, No.1, June 2022

A Semiannual Publication of
Shahed University Press

Crocin, a bioactive constituent of *Crocus sativus*, alleviates trimethyltin-induced cognitive deficits through down-regulation of hippocampal apoptosis and oxidative stress

Saeed Ghofrani[1], Mohammad-Taghi Joghataei[2,*], Siamak Afshin-Majd[3], Tourandokht Baluchnejadmojarad[4], Mehrdad Roghani[3,*]

1. Cellular and Molecular Research Center and Department of Neuroscience, School of Advanced Technology in Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of Anatomy and Neuroscience, Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Neurophysiology Research Center, Shahed University, Tehran, Iran
4. Department of Physiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Abstract

Background and Objective: Cognitive deficits are associated with neurodegenerative disorders including Alzheimer's disease (AD). Trimethyltin chloride (TMT) with potent neurotoxicity is used to induce cognitive dysfunction in rodents. Crocin is the main effective component of saffron with anti-oxidant and anti-inflammatory potential. In the present study, we investigated the effect of crocin on TMT-induced cognitive dysfunction.

Materials and Methods: TMT was i.p. administered (8 mg/kg, once) and crocin was daily given p.o. 1 h after TMT for 3 weeks at doses of 10 or 50 mg/kg. Cognitive performance was assessed in different behavioral tasks. In addition, hippocampal oxidative stress and apoptosis were measured.

Results: Treatment of TMT-challenged rats with crocin (at a dose of 50 mg/kg) prevented deficits of recognition memory in Y maze, discrimination ability in novel object discrimination (NOD) test and conditional learning and memory index in passive avoidance task. Besides, crocin significantly lowered hippocampal level of ROS and improved activity of superoxide dismutase (SOD) besides ablation of apoptotic factors including caspase 3 activity and DNA fragmentation.

Conclusion: In conclusion, crocin administration could ameliorate TMT-induced cognitive dysfunction, in part through targeting hippocampal apoptosis and oxidative stress.

Keywords: Crocin, Trimethyltin, Cognition, Neurotoxicity, Oxidative stress

1. Introduction

Neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) are characterized by gradual loss of neuronal cells, accumulation of abnormal aggregated proteins, and apoptosis with ensuing motor and cognitive deficits. AD is the most common neurodegenerative disease in the world

Revised: 19 Jan 2022, **Accepted:** 24 Aug 2022, **Available online:** 29 June 2022
Corresponding Author: Mehrdad Roghani **E-mail:** mehjour@yahoo.com

which has a complex and multifactorial etiology. Amyloid β (Aβ) and tau protein are closely involved in pathogenesis of AD. Aβ accumulation in brain tissue of AD patients induces synaptic changes and neurodegeneration with subsequent cognitive decline. Oxidative stress and apoptosis play major roles in the progression of AD. To alleviate neurodegenerative diseases such as AD, different therapeutic interventions are suggested (1-3).

Trimethyltin (TMT) is a neurotoxin and organometallic substance which induces marked neurodegeneration in the central nervous system, particularly in the hippocampus. Systemic administration of TMT in rodents induces clinical symptoms such as hyperactivity, aggressiveness, cognitive decline, and seizure-like phenotype which are also seen in AD. TMT induces neuronal cell death through inducing mitochondrial damage and oxidative stress. TMT-induced animal model is sometimes used to investigate brain dysfunction and neurodegeneration. TMT model of AD is often used for finding the potential efficacy of therapeutic candidates (4-6).

Natural products from traditional medicine have known as a promising choice for the treatment of neurological disorders (7). Saffron, the dried stigmas of *Crocus sativus* L. (Iridaceae), is being widely used in traditional medicine for a wide variety of neurological conditions (8). Crocin is the main effective component of saffron with a low toxicity (8). Extensive studies about its traditional use have been reported on its anti-inflammation, anti-tumor, sedation and hypnosis, anti-depression, anti-anxiety and anti-Parkinson's disease (8-10). Several papers also reported its anti-epileptic activity in experimental models (11). In addition, crocin can alleviate oxidative stress in models of brain disorders (12, 13). However, there is no evidence on beneficial effect of crocin on TMT-induced cognitive deficit. Thus, this study was conducted to evaluate whether crocin can ameliorate TMT-induced cognitive deficits in the rat and also to determine possible involvement of oxidative stress and apoptosis.

2. Materials and Methods

2.1. Experimental design

Male rats (Albino, Wistar strain) weighing 210-250 g were purchased from Shahid Beheshti University of Medical Sciences (Tehran, Iran) and kept in standard conditions with free availability of water and food. Used procedures regarding animals followed NIH guidelines for laboratory animals. Animals (n = 32) were divided into 4 experimental groups, i.e., control, TMT, crocin 10-treated TMT (TMT+crocin 10) and crocin 50-treated TMT (TMT+crocin 50). To induce a model of cognitive decline, TMT was injected once and intraperitoneally at a dose of 8 mg/kg (14). Crocin (SigmaAldrich, USA) was administered p.o. at doses of 10 or 50 mg/kg/day, 60 min after TMT injection, for 3 weeks. Behavioral tests were performed on week 3 post-TMT. All animals were killed on day 21.

2.2. Y-maze task

This test was conducted on day 15 to assess spatial recognition memory through recording spontaneous alternations (15). The used maze contained three arms with a central area and animals were tested only one time for 8 min. Alternation was as the serial entries into the arms in correct triplets (i.e. A, B, C or A, B, C and so forth).

2.3. Novel object discrimination (NOD) task

This test was conducted on day 16 to evaluate discrimination aspect of memory, as reported before (16). In this test, animals had two serial 5 min object exploration sessions with an interval of 4 h, the first session as familiarization and the second one as the choice trial. Exploration of objects was defined as sniffing, licking, or moving vibrissae. For calculation of discrimination ratio, this formula was used: (t [novel]-t [familiar])/(t [novel] +t [familiar]) multiplied by 100.

2.4. Passive avoidance test

This test was conducted during the days 17-20 according to a previous study (17) using the shuttle box device. It composed of two compartments and an electric shock was delivered by a specially-designed stimulator. On the first and second days, each animal was adapted to the environment for 5 min, on the third day, the acquisition trial was done with recording of initial latency (IL) of entrance into the dark chamber and on the fourth day, retention test was done with recording of step-through latency (STL up to a maximum of 160 s that was regarded as its cut-off).

2.5. Evaluation of hippocampal oxidative stress

After 3 weeks, hippocampal tissue (n = 7 per group) was removed with care and 5% homogenate preparation was made in cold lysis buffer and the supernatant was stored at -75°C ROS level was measured using dichlorofluorescein diacetate probe with excitation level set at 488 nm and emission level set at 525 nm (18).

Activity of supernatant superoxide dismutase (SOD enzyme) was assessed as reported before (19). In this regard, supernatant was mixed with xanthine and xanthine oxidase solution in potassium phosphate buffer (pH 7.8, 37ºC) for 40 min, and then nitroblue tetrazolium was added. Blue formazan generation and appearance was assessed at 550 nm. Bradford method

was used for supernatant determination of protein level (20).

2.6. Evaluation of apoptosis

For estimation of apoptosis, DNA fragmentation (Cell Death Detection ELISA Plus kit, Sigma-Aldrich, USA) and activity of caspase 3 (21) were determined.

2.7. Statistical analysis

All results are shown in means plus/minus S.E.M. The parametric analysis test, i.e. one-way ANOVA, was applied for data analysis and pair-wise comparison was made using Tukey post-test and with significance level at 0.05.

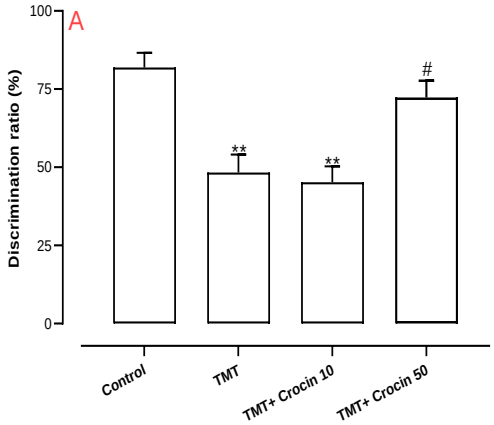
3. Results

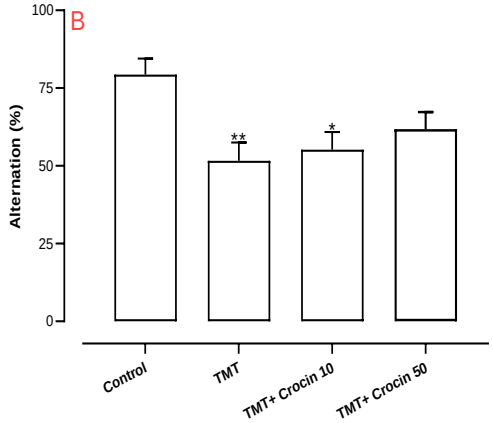
3.1. Behavioral findings

Evaluation of performance of rats in novel object discrimination (NOD) task showed a significant reduction of discrimination index in TMT group when it was compared to the control one (p value<0.01) and crocin administration at a dose of 50 mg/kg successfully prevented this reduction (p value <0.05) (Figure 1A).

Statistical analysis of data for Y-maze test showed that alternation score in untreated TMT group is significantly less as compared to the control rats (p value <0.01) and crocin treatment of TMT group at a dose of 50 mg/kg did not significantly improve this score when compared to TMT group (p value >0.05) (Figure 1B).

Figure 1C shows performance of rats in passive avoidance test as shown by STL time. In this respect, TMT group exhibited a significant deficit of retention and recall (p value <0.01) and crocin given at a dose of 50 mg/kg prevented such deficit (p value <0.05).





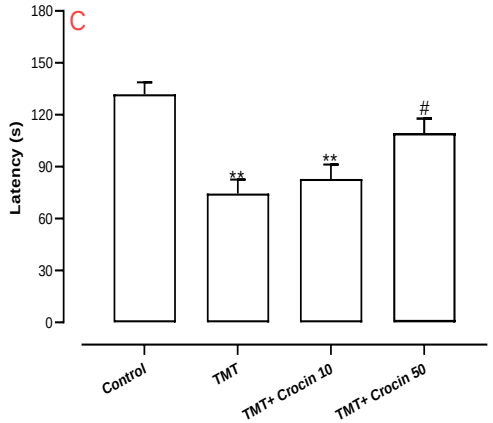


Fig 1. Findings for discrimination index in novel object discrimination task (A), alternation score in Y-maze test (B), and step-through latency (STL) in passive avoidance test (C). * p<0.05 and ** p<0.01 (as compared to the control group); # p<0.05 (as compared to the TMT group)

3.2. Biochemical findings

Figure 2 shows hippocampal levels of oxidative stress-related markers comprising ROS (A) and activity of SOD (B) in our different experimental groups. Our analysis showed that TMT group has a significantly higher quantity of ROS (p value <0.001) and significantly lower activity of SOD (p value <0.01) when compared to relevant data of the control group. Conversely, crocin administration given at a dose of 50 mg/kg/day for 3 weeks significantly improved activity of SOD (p value <0.05) and reduced quantity of ROS (p value <0.01) versus TMT group.

Findings of apoptosis (Figure 3) showed elevated caspase 3 activity and higher DNA fragmentation in TMT group (p<0.001) and treatment with crocin at a dose of 50 mg/kg significantly decreased both of these parameters (p<0.05 and p<0.01, respectively).

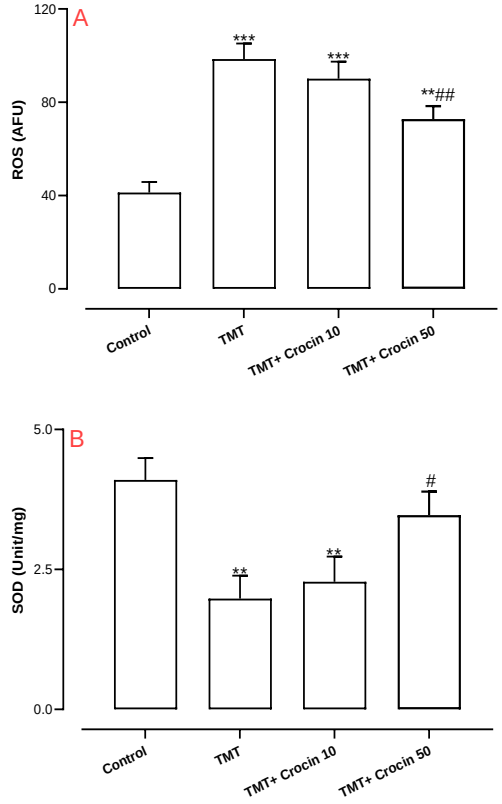


Fig 2. Findings of oxidative stress-associated factors including ROS (A) and activity of superoxide dismutase (SOD) (B) in hippocampal tissue in different groups. ** p<0.01 and *** p<0.001 (in comparison with the control group), # p <0.05 and ## pp <0.01 (in comparison with the TMT group).

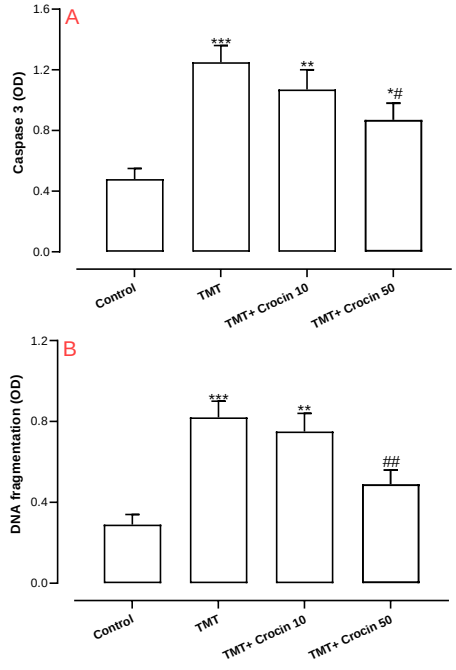


Fig 3. Hippocampal levels of apoptotic factors including caspase 3 (A) and DNA fragmentation (B) in different groups. * p < 0.05, ** p < 0.01, *** p<0.001 (versus the control group); # p < 0.05, ## p<0.01 (versus the TMT group)

4. Discussion

In this study, TMT injection caused significant cognitive deficits in our used assembly of behavioral tests including passive avoidance, novel object discrimination and Y maze tests. Such findings have also found out following TMT challenge (22-24). In contrast, crocin improved cognitive performance of rats in above-mentioned behavioral tests. Consistent with this finding, it has been shown that crocin due to its protective effect can alleviate mitochondrial damage and memory deficits induced by beta-amyloid in the rats (25) and crocin can lower spatial or aversive learning and memory impairments induced by lipopolysaccharide in rats (26). Part of beneficial effects of crocin on cognition has been attributed to its brain attenuation of neuroinflammation and oxidative stress (27, 28).

Part of TMT disturbing effect on cognition is ascribed to its enhancement of oxidative stress in the brain. In this regard, TMT exposure increases brain levels of destructive ROS and perturbs the existing balance between the oxidants and antioxidants (23, 29). In this study, we showed a notable elevation of hippocampal ROS in TMT-exposed rats. In contrast, ROS quantity was significantly lower in crocin-treated TMT group at a dose of 50 mg/kg. This clearly indicates that crocin may have directly/indirectly exerted an antioxidant effect which has protected the hippocampal nerve cells against oxidative damage. Also, crocin was able to improve hippocampal activity of SOD in TMT group. In support of these findings, it has been shown that crocin can alleviate intracerebral hemorrhage-induced neuronal ferroptosis by facilitating Nrf2 nuclear translocation and amelioration of brain oxidative stress (30) and saffron extract and crocin can exert anti-inflammatory and anti-oxidative effects in a repetitive mild traumatic brain injury mouse model (31).

Neuroinflammation plays a key role in cognitive decline is Alzheimer's disease (32). TMT causes production of some inflammatory factors such as NF-kB, TNF-α and IL-1β in human dopaminergic neuroblastoma SH-SY5Y cells (29). Crocin can exert an anti-inflammatory effect through modulating NF-κB pathway and in this way is able to lower reactive gliosis and neuronal damage (12, 33).

Following TMT injection, level of apoptosis increases in the brain tissue (29, 34) which was also shown in our study by higher caspase 3 activity and elevated DNA fragmentation. Conversely, crocin was able to significantly lower both caspase 3 activity and DNA fragmentation intensity. In line with our finding, it has been shown that part of neuroprotective effect of crocin against ethanol neurotoxicity in an animal model of fetal alcohol spectrum disorders is mediated through its anti-apoptotic potential, as shown by lower caspase 3 (27) and crocin can improve memory functions in a rat model of cerebral ischemia through

reversal of hippocampal acetylcholine level and attenuation of apoptosis (35).

To conclude, our study showed that crocin can alleviate cognitive deficits following TMT challenge which is partly mediated through its direct/indirect inhibition of oxidative stress and apoptosis and this may extend our available library of therapeutic agents to reduce cognitive complications in neurotoxic situations.

Acknowledgement

This work was the results of a Ph.D. thesis project that was financially supported in part by Iran University of Medical sciences (IUMS, Tehran, Iran).

Compliance with ethical guidelines

All ethical principles and protocols were considered in this paper.

Conflict of interest

The authors declare that they have no competing interests.

Authors' contributions

All authors equally and substantially contributed in preparing all parts of the presented research.

References

1. Devi S, Kumar V, Singh SK, Dubey AK, Kim JJ. Flavonoids: Potential Candidates for the Treatment of Neurodegenerative Disorders. Biomedicines 2021;9(2).
2. Kabir MT, Rahman MH, Shah M, Jamiruddin MR, Basak D, Al-Harrasi A, et al. Therapeutic promise of carotenoids as antioxidants and anti-inflammatory agents in neurodegenerative disorders. Biomedicine & Pharmacotherapy 2022;146:112610.
3. Singh A, Dawson TM, Kulkarni S. Neurodegenerative disorders and gut-brain interactions. The Journal of Clinical Investigation 2021;131(13).
4. Corvino V, Marchese E, Michetti F, Geloso MC. Neuroprotective strategies in hippocampal neurodegeneration induced by the neurotoxicant trimethyltin. Neurochemical Research 2013;38(2):240-53.
5. Geloso MC, Corvino V, Michetti F. Trimethyltin-induced hippocampal degeneration as a tool to investigate neurodegenerative processes. Neurochemistry International 2011;58(7):729-38.
6. Lee S, Yang M, Kim J, Kang S, Kim J, Kim JC, et al. Trimethyltin-induced hippocampal neurodegeneration: A mechanism-based review. Brain Research Bulletin 2016;125:187-99.
7. Sucher NJ, Carles MC. A pharmacological basis of herbal medicines for epilepsy. Epilepsy and Behavior 2015;52(Pt B):308-18.
8. Singh D. Neuropharmacological Aspects of Crocus sativus L.: A Review of Preclinical Studies and Ongoing Clinical Research. CNS & Neurological Disorders Drug Targets 2015;14(7):880-902.
9. Teng S, Hao J, Bi H, Li C, Zhang Y, Zhang Y, et al. The Protection of Crocin Against Ulcerative Colitis and Colorectal Cancer via Suppression of NF-κB-Mediated Inflammation. Frontiers in Pharmacology 2021;12:639458.
10. Rao SV, Muralidhara, Yenisetti SC, Rajini PS. Evidence of neuroprotective effects of saffron and crocin in a Drosophila model of parkinsonism. Neurotoxicology 2016;52:230-42.
11. Zhong K, Qian C, Lyu R, Wang X, Hu Z, Yu J, et al. Anti-Epileptic Effect of Crocin on Experimental Temporal Lobe Epilepsy in Mice. Frontiers in Pharmacology 2022;13:757729.
12. Ebrahimzadeh A, Moghadam SY, Rahimi H, Motaghinejad M, Motevalian M, Safari S, et al. Crocin acts as a neuroprotective mediator against methylphenidate‐induced neurobehavioral and neurochemical sequelae: Possible role of the CREB-BDNF signaling pathway. Acta Neurobiologiae Experimentalis (Wars) 2019;79(4):352-66.
13. Zhang X, Zhang X, Dang Z, Su S, Li Z, Lu D. Cognitive Protective Mechanism of Crocin Pretreatment in Rat Submitted to Acute High-Altitude Hypoxia Exposure. BioMed Research International 2020; 2020:3409679.
14. Corvino V, Di Maria V, Marchese E, Lattanzi W, Biamonte F, Michetti F, et al. Estrogen administration modulates hippocampal GABAergic subpopulations in the hippocampus of trimethyltin-treated rats. Frontiers in Cellular Neuroscience 2015; 9:433.
15. Kalalian-Moghaddam H, Baluchnejadmojarad T, Roghani M, Goshadrou F, Ronaghi A. Hippocampal

synaptic plasticity restoration and anti-apoptotic effect underlie berberine improvement of learning and memory in streptozotocin-diabetic rats. European Journal of Pharmacology 2013;698(1-3):259-66.
16. Stuart SA, Robertson JD, Marrion NV, Robinson ES. Chronic pravastatin but not atorvastatin treatment impairs cognitive function in two rodent models of learning and memory. PLoS One 2013; 8(9):e75467.
17. Roghani M, Joghataie MT, Jalali MR, Baluchnejadmojarad T. Time course of changes in passive avoidance and Y-maze performance in male diabetic rats. Iranian Biomedical Journal 2006; 10(2):99-104.
18. Mohamadi-Zarch SM, Baluchnejadmojarad T, Nourabadi D, Khanizadeh AM, Roghani M. Protective effect of diosgenin on LPS/D-Gal-induced acute liver failure in C57BL/6 mice. Microbial Pathogenesis 2020; 146:104243.
19. Roghani M, Baluchnejadmojarad T. Chronic epigallocatechin-gallate improves aortic reactivity of diabetic rats: underlying mechanisms. Vascular Pharmacology 2009;51(2-3):84-9.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 1976;72:248-54.
21. Movsesyan VA, Yakovlev AG, Dabaghyan EA, Stoica BA, Faden AI. Ceramide induces neuronal apoptosis through the caspase-9/caspase-3 pathway. Biochemical and Biophysical Research Communications 2002;299(2):201-7.
22. Rostami A, Taleahmad F, Haddadzadeh-Niri N, Joneidi E, Afshin-Majd S, Baluchnejadmojarad T, et al. Sinomenine Attenuates Trimethyltin-Induced Cognitive Decline via Targeting Hippocampal Oxidative Stress and Neuroinflammation. Journal of Molecular Neuroscience 2022 in press.
23. Jeong ES, Bajgai J, You IS, Rahman MH, Fadriquela A, Sharma S, et al. Therapeutic Effects of Hydrogen Gas Inhalation on Trimethyltin-Induced Neurotoxicity and Cognitive Impairment in the C57BL/6 Mice Model. International Journal of Molecular Sciences 2021;22(24).
24. Madadi S, Katebi M, Eftekharzadeh M, Mehdipour A, Pourheydar B, Mehdizadeh M. Partial Improvement of Spatial Memory Damages by Bone Marrow Mesenchymal Stem Cells Transplantation Following Trimethyltin Chloride Administration in the Rat CA1. Basic and Clinical Neuroscience 2019;10(6):567-77.
25. Yousefsani BS, Mehri S, Pourahmad J, Hosseinzadeh H. Protective Effect of Crocin against Mitochondrial Damage and Memory Deficit Induced by Beta-amyloid in the Hippocampus of Rats. Iranian Journal of Pharmaceutical Research 2021; 20(2):79-94.
26. Azmand MJ, Rajaei Z. Effects of crocin on spatial or aversive learning and memory impairments induced by lipopolysaccharide in rats. Avicenna Journal of Phytomedicine 2021; 11(1):79-90.
27. Farhadi L, Hojati V, Khaksari M, Vaezi G. Neuroprotective Effects of Crocin Against Ethanol Neurotoxicity in the Animal Model of Fetal Alcohol Spectrum Disorders. Neurochemical Research 2022;47(4):1001-11.
28. Mohamed Mowafy S, Awad Hegazy A, D AM, Salah Abd El-Fatah S. Impact of copper oxide nanoparticles on the cerebral cortex of adult male albino rats and the potential protective role of crocin. Ultrastructural Pathology 2021; 45(4-5):307-18.
29. Song WJ, Yun JH, Jeong MS, Kim KN, Shin T, Kim HC, et al. Inhibitors of Lipoxygenase and Cyclooxygenase-2 Attenuate Trimethyltin-Induced Neurotoxicity through Regulating Oxidative Stress and Pro-Inflammatory Cytokines in Human Neuroblastoma SH-SY5Y Cells. Brain Science 2021;11(9).
30. Wang F, Li WL, Shen LJ, Jiang TT, Xia JJ, You DL, et al. Crocin Alleviates Intracerebral Hemorrhage-Induced Neuronal Ferroptosis by Facilitating Nrf2 Nuclear Translocation. Neurotoxicity Research 2022; 40(2):596-604.
31. Salem M, Shaheen M, Tabbara A, Borjac J. Saffron extract and crocin exert anti-inflammatory and anti-oxidative effects in a repetitive mild traumatic brain injury mouse model. Scientific Reports 2022;12(1):5004.
32. Ownby RL. Neuroinflammation and cognitive aging. Current Psychiatry Reports 2010; 12(1):39-45.
33. Hadipour M, Bahari Z, Afarinesh MR, Jangravi Z, Shirvani H, Meftahi GH. Administering crocin ameliorates anxiety-like behaviours and reduces the inflammatory response in amyloid-beta induced neurotoxicity in rat. Clinical and

Experimental Pharmacology & Physiology 2021; 48(6):877-89.
34. Hwang Y, Kim HC, Shin EJ. Enhanced neurogenesis is involved in neuroprotection provided by rottlerin against trimethyltin-induced delayed apoptotic neuronal damage. Life Sciences 2020; 262:118494.
35. Yuan Y, Shan X, Men W, Zhai H, Qiao X, Geng L, et al. The effect of crocin on memory, hippocampal acetylcholine level, and apoptosis in a rat model of cerebral ischemia. Biomedicine & Pharmacotherapy 2020; 130:110543.