AJMB
Avicenna Journal of Medical Biochemistry
Avicenna J Med Biochem 2025;13(2):81-88. doi:10.34172/ajmb.2667
http://ajmb.umsha.ac.ir

Original Article

Therapeutic and Preventive Effects of Saffron on Modulation of GFAP and Olig2 Gene Expression in Demyelinated Rats

Leila Khaledi[1], Mahnaz Taherianfard[1*], Mehran Ghaemi[2], Azizollah Khodakaram Tafti[3], Hamid Reza Moradi[1], Maryam Asadian Ghahfarokhi[1]

[1]Department of Basic Science, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University, Mashhad, Iran
[3]Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz Iran

Article history:
Received: February 9, 2025
Revised: October 18, 2025
Accepted: October 31, 2025
ePublished: December 31, 2025

***Corresponding author:**
Mahnaz Taherianfard,
Email: mtaherian2025@gmail.com

Abstract

Introduction: Multiple sclerosis (MS) is a condition characterized by the loss of myelin. This study investigated the impact of saffron on the expression of glial fibrillary acidic protein (GFAP) and oligodendrocyte transcription factor (Olig2) genes, as well as its effect on the pathological changes in rats with demyelination induced by cuprizone (CPZ).

Methods: A total of 60 male Sprague-Dawley rats (weighing 250 ± 20 g) were randomly assigned to 4 groups: sham, MS control, saffron treatment, and saffron prevention. Demyelination was induced via oral gavage with a suspension of carboxymethyl cellulose (1%) combined with CPZ (0.6%). Blood-brain barrier (BBB) disruption was assessed by measuring Evans blue extravasation using UV-Vis spectrophotometry. The gene expression levels of GFAP and Olig2 were analyzed using real-time polymerase chain reaction (RT-PCR). Statistical analysis was carried out using one-way analysis of variance (ANOVA), with a significance level of $P<0.05$.

Results: Saffron extract significantly ($P<0.001$) reduced demyelination and pathological changes in CPZ-treated rats and alleviated BBB disruption ($P<0.01$). Furthermore, saffron extract notably ($P<0.05$) regulated the expression of Olig2 and GFAP in the hippocampus of CPZ-demyelinated rats. The Olig2 and GFAP gene expression levels in the MS control group was significantly lower than in other groups.

Conclusion: The findings of this study suggest that saffron has the potential to improve pathological changes and modulate the expression of Olig2 and GFAP genes in the hippocampus of CPZ-induced demyelinated rats.

Keywords: Saffron, Cuprizone, Demyelination, GFAP, Olig2

Please cite this article as follows: Khaledi L, Taherianfard M, Ghaemi M, Khodakaram Tafti A, Moradi HR, Asadian Ghahfarokhi M. Therapeutic and preventive effects of saffron on modulation of GFAP and Olig2 gene expression in demyelinated rats. Avicenna J Med Biochem 2025;13(2):81-88. doi:10.34172/ajmb.2667

Introduction

Multiple sclerosis (MS) is a prevalent neuroinflammatory condition that leads to significant clinical disability (1). Demyelination occurs when pathological events lead to the destruction of myelin or the cells that produce and maintain it, while axons remain relatively intact. This loss of myelin leads to abnormalities in nerve conduction and overall dysfunction (2).

Cuprizone (CPZ), scientifically known as bis-cyclohexanone-oxal-dihydrazone, is a copper derivative that induces apoptosis in oligodendrocytes within a few days (3). Studies have shown that the cuprizone model of toxic demyelination in the CNS has been widely used for many years to study demyelinating and remyelinating processes in the white matter, namely the corpus callosum (4). Research has also shown that demyelination and neuronal damage extend to the cerebral cortex (5).

The oligodendrocyte transcription factor (Olig2) plays a vital role in the development and function of oligodendrocytes. Olig2 promotes the differentiation of oligodendrocyte progenitor cells (OPCs) and supports remyelination (6).

Glial fibrillary acidic protein (GFAP) is an important marker for identifying astrocytes and plays a crucial role in maintaining the structural integrity of these cells. It is also a key indicator of astrogliosis and astroglial damage. A decrease in GFAP expression impairs differentiation of OPC and production of oligodendrocytes. Astrocytes

© 2025 The Author(s); Published by Hamadan University of Medical Sciences. This is an open-access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

are involved in various functions in the CNS, such as neuroprotection and repair. During development, they express GFAP, and an increase in GFAP levels signifies reactive astrocytosis or astrogliosis, reflecting the extent of injury (7). Additionally, astrocytes elevate the expression of oxidative stress markers and genes related to immune responses (8). They are also crucial for maintaining the extracellular environment and supporting neurons (9).

Saffron (*Crocus sativus*) is a medicinal plant that has been used as a spice for many years. Phytochemical studies have identified 4 secondary metabolites in saffron: crocin, safranal, crocetin, and a small percentage of picrocrocin. The main active component is crocin, which possesses medicinal and neuroprotective properties (10). Additionally, other studies have reported that the glycosidic esters of crocetin can prevent demyelination and neurodegeneration. These results suggest that saffron may serve as a complementary treatment for MS by inhibiting oxidative stress and preventing leukocyte infiltration into the CNS (11).

A limited number of studies have examined the effects of saffron on GFAP and Olig2 in the context of MS. This study aimed to explore the expression of these genes in the hippocampus of rats with CPZ-induced demyelination. The study will also evaluate the impact of saffron on the pathological processes of demyelination.

Materials and Methods

Animals and Experimental Design

A total of 60 adult female Sprague-Dawley rats (weighing 250 ± 20 g) were obtained from the Laboratory Animal Center of Shiraz University of Medical Sciences. The rats were housed in standard conditions in a temperature-controlled room (22-24°C) with a 12-hour light/dark cycle (lights on at 06:00, off at 18:00). They had unrestricted access to food and water. All procedures involving animals were approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine at Shiraz University of Shiraz University of Medical science (IR.US.REC.1401.042).

The rats were randomly divided into 4 groups, with 15 animals per group: (1) sham: received distilled water with carboxymethyl cellulose (CMC) (1%) by gavage for 35 days; (2) MS control: treated with CPZ (0.6%) and CMC (1%) by gavage for 35 days without any additional treatment; (3) saffron treatment: received CPZ (0.6%) and CMC (1%) for 35 days, followed by saffron (60 mg/kg/day) for the next seven days; (4) saffron prevention: administered CPZ (0.6%) and CMC (1%) plus saffron (60 mg/kg/day) by gavage for 35 days.

Preparation of Saffron Extract

In the current study, high-quality saffron stigmas were collected from Qaenat (voucher number 55130). The samples were identified and authenticated by Dr. Khosravi, a professor in the Department of Biology at Shiraz University of Medical Sciences. The aqueous extract of saffron was prepared according to a previous study by Farjeh et al (12). The 100% pure crocin prepared by Sigma was diluted to different concentrations and injected into the liquid chromatography machine, and the corresponding peak was obtained. The saffron extract sample in the present study was also injected into the device at different concentrations, and the peak corresponding to each concentration was obtained. The amounts of crocin in the samples were determined by comparing the peaks of pure crocin with those from hydroalcoholic extracts. The dose of saffron extract was determined according to our previous study.

Histopathological Evaluation

At the end of the experiment, rats were euthanized by CO2 inhalation in accordance with bioethical guidelines. The brains were perfused with 20 mL of 4% formaldehyde, followed by removal of the brain for tissue processing. Tissue blocks were prepared, and 3-5 µm sections were cut using a microtome. Hematoxylin-eosin and Luxol Fast Blue staining were performed. The extent of demyelination and histopathological changes were analyzed using ImageJ software and a light microscope, respectively. A histopathology score was assigned based on the severity of tissue damage as follows: (1) low, (2) moderate, and (3) high severity.

Assessment of Evans Blue Extravasation

A 2% Evans blue solution in saline (4 mL/kg body weight) was injected into the jugular vein. After 1 hour, the rats were perfused with 50 mL of ice-cold PBS, and the brain was harvested. The hippocampus was isolated, homogenized in 1.1 mL PBS, sonicated, and centrifuged for 30 minutes at 15000 rpm at 4°C. The supernatant was collected, and 50% trichloroacetic acid was then added to each 0.5 mL aliquot and incubated overnight at 4°C, followed by centrifugation. The level of Evans blue was measured at 610 nm using a spectrophotometer (Thermo Spectronic Genesys 10 UV), and concentrations were quantified by comparison with a standard curve. Results were expressed as µg Evans blue/g tissue.

PCR Procedure: Gene Expression of Olig2 and GFAP

RNA Extraction

Total RNA was extracted using a Column RNA Isolation Kit (DENA Zist Asia, Iran). Hippocampal samples (400 µg) were homogenized in lysis buffer and centrifuged at 1500 × g for 1 minute. Then, the supernatant (600 µL) was used for RNA extraction. Finally, RNA quality and quantity were assessed using a NanoDrop spectrophotometer, and 0.5 µg of RNA from each sample was used for RT-PCR analysis.

Quantitative RT-PCR

The expression of Olig2 and GFAP was measured by real-time polymerase chain reaction (RT-PCR). GAPDH served as the normalization gene. Three primer sets were designed using Beacon Designer software (PREMIER Biosoft, USA) (Table 1). The cDNA of each

Table 1. Sequences of Primers and Probes Designed in the Study

Gene name	Forward (5`-3`)	Reverse (5`-3`)	Length	Type	Accession number	Tm
Olig2	CAGAGACCCGAGCCAA	CTTTTTCAACCTTCCGAATGTG	92	Exon-exon junction	NM_001100557.1	62.36-62.47
GFAP	AAACCGCATCACCATTCC	GCACACCTCACATCACATC	185	Intron-spanning	NM_017009.2	62.47-62.86
GAPDH	GACAGCCGCATCTTCTTG	TAACCAGGCGTCCGATAC	96	Intron-spanning	NM_017008.4	62.88- 62.93

sample was synthesized using a reverse transcription kit (M-MLV and random hexamers, DENA Zist Asia). RT-PCR was conducted with Real Q Plus 2x Master Mix Green (Ampliqon, Denmark) using a Light Cycler 96 (Roche, Germany) with the following conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 20 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 12 seconds. A melting curve analysis was also included in the program.

To ensure optimal efficiency, the assay was calibrated using a fivefold serial dilution of pooled RNA samples. The ΔΔCt method was used to analyze the gene expression levels, with the sham group serving as the calibrator.

Statistical Analysis

Based on a pilot study, the sample size was calculated using the formula: $n = \frac{z^2_{1-\alpha/2} \times \sigma^2}{\delta^2}$. Data analysis was performed using SPSS version 22. One-way ANOVA was used to analyze the percentage of demyelination, Evans blue extravasation, and gene expression of Olig2 and GFAP. The Mann–Whitney U test was used for histopathological analysis, and Tukey's post-hoc test was employed to identify significant differences. Statistical significance was defined as $P<0.05$. All results are expressed as mean ± SEM.

Results

The saffron extract administered at a dose of 60 mg/kg contained 4.3 mg/kg of crocin, as determined by HPLC analysis (Figure 1). Following 35 days of CPZ treatment, demyelination occurred in all groups except the sham group, which showed minimal demyelination. Both the saffron treatment and saffron prevention groups exhibited significantly less demyelination compared to the MS control group ($P<0.001$ in the corpus callosum (CC) and $P<0.01$ in the cerebellum) (Figure 2).

Hematoxylin-eosin staining revealed two types of pathological changes. First, in the MS control group, 80% of the slides displayed lymphocytic infiltration in the meninges along with marked edema and hyperemia. In contrast, these changes were observed in 61% of the slides in the saffron treatment group and 20% in the saffron prevention group (Figure 3). Second, 20% of slides from the MS control group showed gliosis and neuronal necrosis, while they were seen in 16% of the saffron treatment group and were absent in the saffron prevention group (Figure 3).

CPZ treatment led to blood-brain barrier (BBB) disruption in the hippocampus, with Evans blue leakage into the brain. The MS control group showed the highest degree of BBB damage, with significant differences ($P<0.0001$ compared to the sham group, $P<0.05$ compared to the saffron treatment, and $P<0.01$ compared to the saffron prevention group). In the saffron prevention group, the degree of BBB damage was similar to that of the sham group (Figure 4).

Gene expression analysis revealed that the expression of Olig2 was significantly reduced in the MS control compared to the sham group ($P<0.0001$), the saffron treatment group ($P<0.05$), and the saffron prevention group ($P<0.001$) (Figure 5).

Regarding GFAP gene expression, a significant decrease was observed in the MS control group ($P<0.001$) as well as in the saffron treatment and saffron prevention groups ($P<0.05$) when compared to the sham group (Figure 6).

Discussion

MS was induced by the administration of CPZ for 35 days. Toxin-induced demyelination models are particularly useful for exploring the mechanisms underlying primary myelin loss and subsequent remyelination. Oral administration of CPZ represents a well-established toxin-based model that selectively induces demyelination in specific white matter regions, particularly the corpus callosum. As a copper-chelating agent, CPZ triggers oligodendrocyte degeneration and widespread myelin depletion in several regions of the brain. It also causes mild disruption of the BBB, facilitating limited infiltration of peripheral immune cells (13). Cuprizone titration (cuprizone at different doses) results in significant demyelination. Increased activation of microglia and astrocytes was observed in regions that have undergone demyelination (14). A recent study provided new insights into the role of microglia in the processes of demyelination and remyelination in gray matter using an acute cuprizone toxicity model. The regional characteristics of demyelination and glial responses were found to be similar to those described in white matter in human MS (15).

In the present study, saffron was administered in two experimental paradigms: (1) after MS induction as a therapeutic intervention and (2) concurrently with CPZ as a preventive strategy. Histopathological assessments were performed in the corpus callosum and cerebellum, which are the primary regions affected by CPZ-induced demyelination and are commonly used to confirm the extent of myelin loss in demyelinating diseases (16). Conversely, gene expression analysis was conducted in the hippocampus, a region highly susceptible to oxidative stress and neuroinflammation during demyelinating conditions. Previous studies have shown that hippocampal astrocytes and oligodendrocyte progenitors respond dynamically to CPZ intoxication, making this

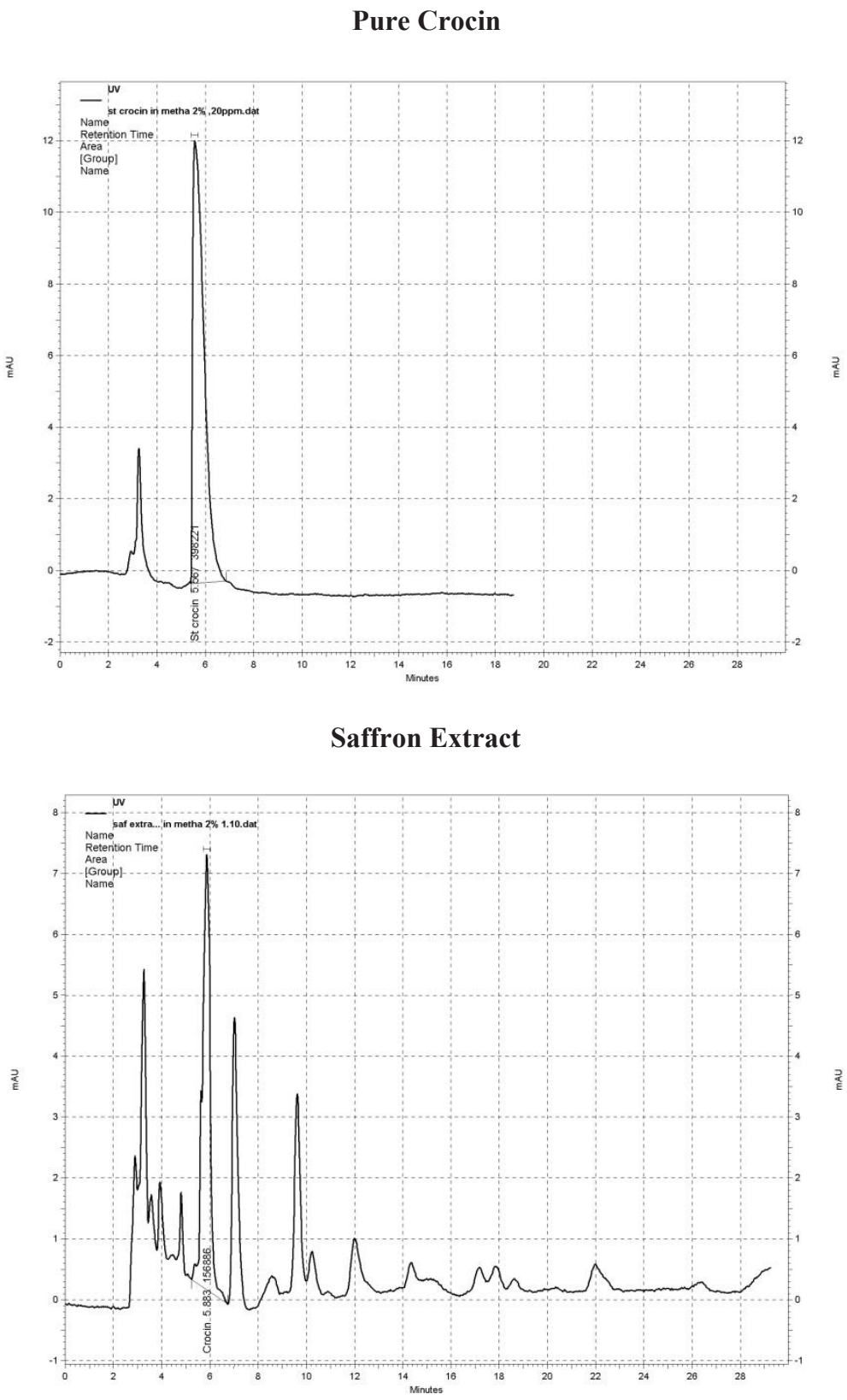
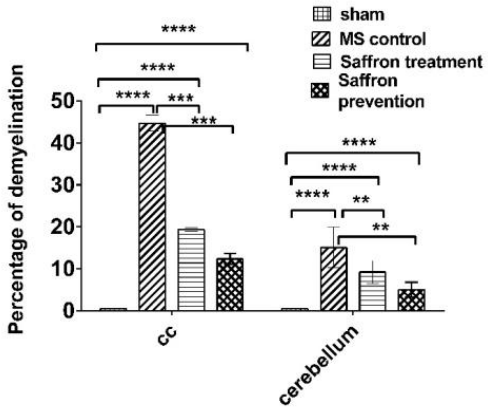


Figure 1. Comparison of HPLC Peaks of Pure Crocin and Saffron Extract at 60 mg/kg Body Weight

area particularly suitable for evaluating GFAP and Olig2 expression (17). This combined approach enabled evaluation of structural alterations in myelin-rich areas alongside molecular pathways related to neuroprotection and remyelination in saffron-treated animals.

The administration of CPZ in this study produced several pathological alterations, including meningeal lymphocyte infiltration, edema, hyperemia, gliosis, and neuronal necrosis. Treatment with saffron extract attenuated these abnormalities. CPZ-induced neuronal damage in both young and aged rats was also associated with changes in macrophage, T-cell, and astrocyte populations

A



B C

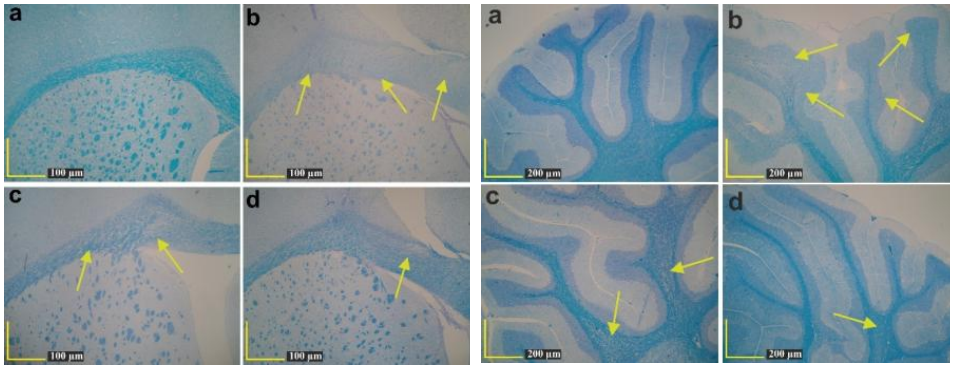


Figure 2. A, percentage of demyelination in the corpus callosum (CC) and cerebellum. Representative photomicrographs showing the effect of saffron extract on cuprizone-induced demyelination in the corpus callosum (B) and cerebellum (C). In both brain regions, intact myelin fibers appeared as intensely blue-stained areas, whereas demyelinated regions appeared pale or unstained (indicated by yellow arrows). (a) Sham group: normal architecture with dense myelin staining and well-preserved laminar organization. (b) MS control group: Extensive demyelination and loss of compact myelin structure, evident as large pale regions. (c) Saffron treatment group: partial restoration of myelin density and moderate remyelination in the affected areas. (d) Saffron prevention group: near-complete preservation of myelin integrity with minimal demyelinated zones. Luxol Fast Blue staining confirmed that saffron administration markedly reduced myelin loss in both the cerebellar and callosal white matter compared to the MS control group. Scale bars = 200 µm. The significant difference between groups was as follows: ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

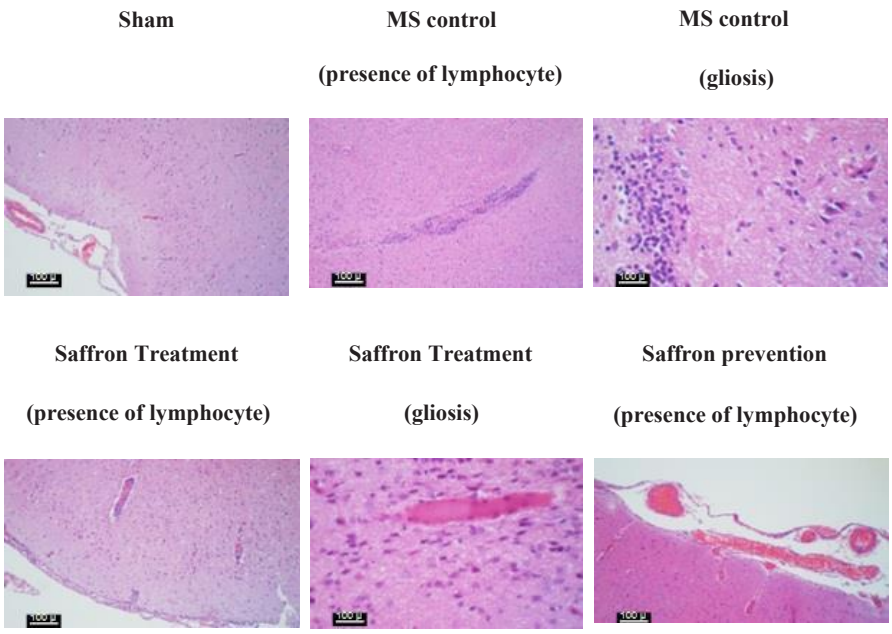


Figure 3. Microphotograph of the Brain Sections of all Groups. The section represents two pathological disorders in demyelinated rats: (1) the presence of lymphocytes and (2) gliosis

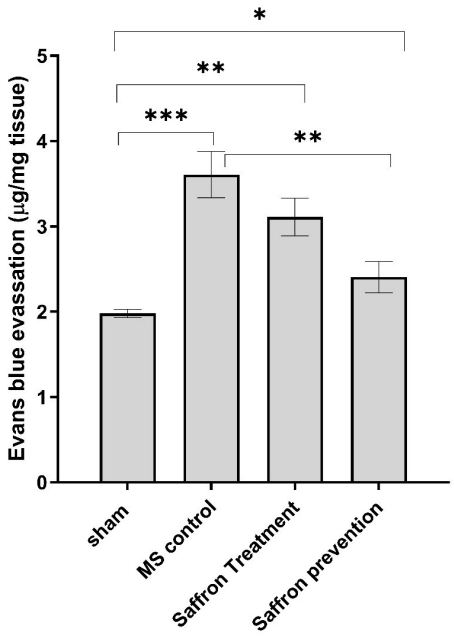


Figure 4. The Effect of Saffron on the Destruction of the BloodBrain Barrier Induced by Cuprizone in the Hippocampus. The rate of extravasation of Evans blue in the hippocampus blood vessels represents the rate of Blood Brain Barrier disruption. The significant difference between groups was as follows: * $P<0.05$, ** $P<0.01$, *** $P<0.001$

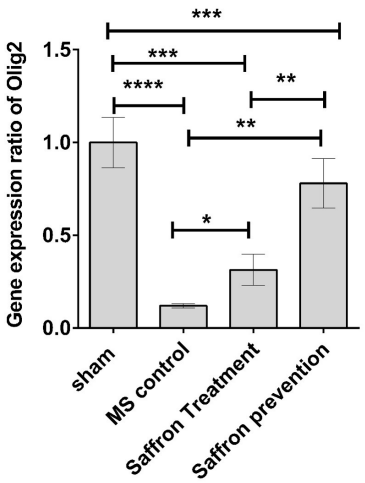


Figure 5. The Effect of Saffron on the Olig2 Gene Expression in the Hippocampus of Male Rats. The significant difference between groups was as follows: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

(18). Activated microglia produce various neurotoxic molecules, which are reduced by the neuroprotective effects of crocin and crocetin (19). Reports indicate that in the progressive stages of MS, neuronal pathology in both primary and secondary progressive MS is accompanied by inflammation in the perivascular and meningeal spaces. A positive correlation has been found between the degree of demyelination in the cortical gray matter and the density of lymphocytes in the meninges. In contrast, the density of lymphocyte infiltration in white matter did not show a correlation with demyelination (20).

In the current study, saffron extract was found to modify CPZ-induced BBB disruption. The research on cerebral ischemia shows that crocin or similar antioxidants can protect rats from cerebral ischemia by preserving the

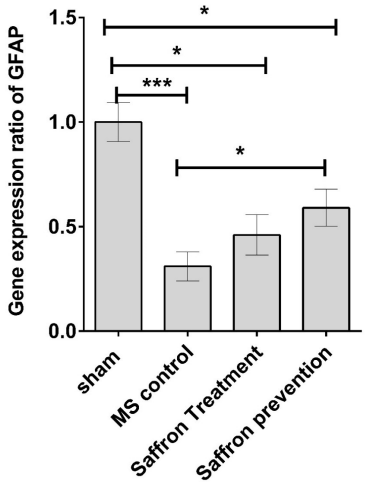


Figure 6. The Effect of Saffron on the GFAP Gene Expression Level in the Hippocampus of Male Rats. The significant difference between groups was as follows: * $P<0.05$, *** $P<0.001$

integrity of the BBB (21). Dietary supplementation with saffron extract (50 mg/kg/day) strengthened the BBB and reduced its disruption in rats, and crocin at 10 mg/kg/day produced comparable protective effects (22).

Treatment with saffron also influenced Olig2 gene expression in the hippocampus. Although Olig2 expression increased following the administration of saffron, the magnitude of increase was lower than that observed in untreated animals. Concurrent administration of saffron with CPZ appeared to enhance therapeutic responses, potentially supporting remyelination and gene regulation. Crocetin, a metabolite of crocin, has been shown to stimulate OPC differentiation by upregulating Ki67 and the transcription factor Olig2. Similarly, crocin promotes oligodendrocyte proliferation, maturation, and myelin restoration (23). Loss of Olig2-positive progenitor cells is known to impair remyelination in MS (24). Notably, saffron extract at 60 mg/kg/day significantly increased the number of Olig2-immunopositive cells (25). Olig2 is essential for oligodendrocyte lineage specification and development and has been demonstrated, both in vitro and in vivo, to enhance OPC migration, differentiation into mature oligodendrocytes, and efficient remyelination within the CNS (26).

In addition, the administration of saffron altered GFAP expression. Elevated serum GFAP levels have been proposed as a biomarker for disease progression in MS and are associated with astrocytopathy and diffuse white matter injury (27). Astrocytes constitute about one-third of CNS cells and are involved in metabolism, repair, and axon regeneration. Activated astrocytes upregulate GFAP expression (28). Astrocytes have been classified into astrocyte A1 (destructive) and astrocyte A2 (neuroprotective). Inflammation significantly increases the ratio of astrocytes A1 to A2 (29). According to an earlier report, GFAP mRNA increases 24 hours after a concussion. The use of saffron reduces the degree of concussion and decreases the extent of neuronal destruction, astrocyte and microglial activity, and the amount of GFAP (30).

However, in an immunohistochemical model, saffron at 60 mg/kg/day significantly increased GFAP gene expression, as evidenced by a greater number of GFAP-positive cells and enhanced density of GFAP protein (25).

Conclusion

The novelty of the present study lies in its examination of the effect of CPZ on gray matter. In contrast, most studies have investigated the effect of CPZ on demyelination and remyelination of white matter. On the other hand, the results of the present study highlight the importance of saffron in treating MS. Saffron improved CPZ-induced demyelination and modulated the disruption of GFAP and Olig2 gene expression in the rat hippocampus. Additionally, it reduced pathological disturbances following CPZ-induced MS.

Additionally, the present study is novel due to its comprehensive approach, which investigates the effects of saffron on hippocampal pathology in demyelinated rats. It may provide valuable mechanistic insights into diseases such as MS and other demyelinating disorders. Finally, our results showed that using saffron in a preventive model is more beneficial.

Authors' Contribution

Conceptualization: Mahnaz Taherianfard.
Data Curation: Leila Khaledi.
Formal Analysis: Mahnaz Taherianfard.
Funding Acquisition: Leila Khaledi.
Investigation: Leila Khaledi, Maryam Asadian Ghahfarokhi.
Methodology: Leila Khaledi, Mehran Ghaemi, Azizollah Khodakaram Tafti, Hamid Reza Moradi, Maryam Asadian Ghahfarokhi.
Project Administration: Mahnaz Taherianfard.
Resources: Mahnaz Taherianfard.
Software: Mahnaz Taherianfard.
Supervision: Mahnaz Taherianfard.
Validation: Mahnaz Taherianfard.
Visualization: Mahnaz Taherianfard.
Writing–Original Draft: Leila Khaledi, Maryam Asadian Ghahfarokhi.
Writing–Review & Editing: Mahnaz Taherianfard, Mehran Ghaemi, Azizollah Khodakaram Tafti, Hamid Reza Moradi.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

All procedures involving animals were approved by the Ethics Committee of the SSchool of Veterinary Medicine at Shiraz University (IR.US.REC.1401.042).

Funding

This study was financially supported by Shiraz University, Shiraz, Iran (0GCB1M1755).

References

1. Kimoff RJ, Kaminska M, Trojan D. Multiple sclerosis and related disorders. Handb Clin Neurol 2022;189:177-200. doi:10.1016/b978-0-323-91532-8.00013-6
2. Höftberger R, Lassmann H. Inflammatory demyelinating diseases of the central nervous system. Handb Clin Neurol 2017;145:263-83. doi:10.1016/b978-0-12-802395-2.00019-5
3. Zhan J, Mann T, Joost S, Behrangi N, Frank M, Kipp M. The cuprizone model: dos and do nots. Cells 2020;9(4):843. doi:10.3390/cells9040843
4. Gudi V, Moharregh-Khiabani D, Skripuletz T, Koutsoudaki PN, Kotsiari A, Skuljec J, et al. Regional differences between grey and white matter in cuprizone induced demyelination. Brain Res 2009;1283:127-38. doi:10.1016/j.brainres.2009.06.005
5. Trapp BD, Vignos M, Dudman J, Chang A, Fisher E, Staugaitis SM, et al. Cortical neuronal densities and cerebral white matter demyelination in multiple sclerosis: a retrospective study. Lancet Neurol 2018;17(10):870-84. doi:10.1016/s1474-4422(18)30245-x
6. Zhang K, Chen S, Yang Q, Guo S, Chen Q, Liu Z, et al. The oligodendrocyte transcription factor 2 Olig2 regulates transcriptional repression during myelinogenesis in rodents. Nat Commun 2022;13(1):1423. doi:10.1038/s41467-022-29068-z
7. Shaygannejad A, Rafiei N, Vaheb S, Yazdan Panah M, Shaygannejad V, Mirmosayyeb O. The role of glial fibrillary acidic protein as a biomarker in multiple sclerosis and neuromyelitis optica spectrum disorder: a systematic review and meta-analysis. Medicina (Kaunas) 2024;60(7):1050. doi:10.3390/medicina60071050
8. Waller R, Woodroofe MN, Wharton SB, Ince PG, Francese S, Heath PR, et al. Gene expression profiling of the astrocyte transcriptome in multiple sclerosis normal appearing white matter reveals a neuroprotective role. J Neuroimmunol 2016;299:139-46. doi:10.1016/j.jneuroim.2016.09.010
9. Kim Y, Park J, Choi YK. The role of astrocytes in the central nervous system focused on BK channel and heme oxygenase metabolites: a review. Antioxidants (Basel) 2019;8(5):121. doi:10.3390/antiox8050121
10. Azari H, Ebrahimi S, Saeb S, Ghanbari A, Peyravian F, Mokarram P. The effect of saffron aquatic extract and crocin on the differentiation of neural stem cells into oligodendrocyte precursor cells. Shiraz E Med J 2018;19(3):e60190. doi:10.5812/semj.60190
11. José Bagur M, Alonso Salinas GL, Jiménez-Monreal AM, Chaouqi S, Llorens S, Martínez-Tomé M, et al. Saffron: an old medicinal plant and a potential novel functional food. Molecules 2017;23(1):30. doi:10.3390/molecules23010030
12. Farjah GH, Salehi S, Ansari MH, Pourheidar B. Protective effect of *Crocus sativus* L. (saffron) extract on spinal cord ischemia-reperfusion injury in rats. Iran J Basic Med Sci 2017;20(3):334-7. doi:10.22038/ijbms.2017.8364
13. Zirngibl M, Assinck P, Sizov A, Caprariello AV, Plemel JR. Oligodendrocyte death and myelin loss in the cuprizone model: an updated overview of the intrinsic and extrinsic causes of cuprizone demyelination. Mol Neurodegener 2022;17(1):34. doi:10.1186/s13024-022-00538-8
14. Wigger N, Krüger J, Vankriekelsvenne E, Kipp M. Titration of cuprizone induces reliable demyelination. Brain Res 2025;1850:149410. doi:10.1016/j.brainres.2024.149410
15. Roufagalas I, Avloniti M, Fortosi A, Xingi E, Thomaidou D, Probert L, et al. Novel cell-based analysis reveals region-dependent changes in microglial dynamics in grey matter in a cuprizone model of demyelination. Neurobiol Dis 2021;157:105449. doi:10.1016/j.nbd.2021.105449
16. Morgan ML, Teo W, Hernandez Y, Brideau C, Cummins K, Kuipers HF, et al. Cuprizone-induced demyelination in mouse brain is not due to depletion of copper. ASN Neuro 2022;14(1):17590914221126367. doi:10.1177/17590914221126367
17. Ginsberg SD, Che S. Combined histochemical staining, RNA amplification, regional, and single cell cDNA analysis within the hippocampus. Lab Invest 2004;84(8):952-62. doi:10.1038/labinvest.3700110
18. Labunets I, Rodnichenko A, Savosko S, Pivneva T. Reaction of different cell types of the brain on neurotoxin cuprizone and hormone melatonin treatment in young and aging mice. Front Cell Neurosci 2023;17:1131130. doi:10.3389/fncel.2023.1131130

19. Nam KN, Park YM, Jung HJ, Lee JY, Min BD, Park SU, et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. Eur J Pharmacol 2010;648(1-3):110-6. doi:10.1016/j.ejphar.2010.09.003
20. Zuroff LR, Benjamins JA, Bar-Or A, Lisak RP. Inflammatory mechanisms underlying cortical injury in progressive multiple sclerosis. Neuroimmunol Neuroinflamm 2021;8:111-33. doi:10.20517/2347-8659.2020.35
21. Shahbaz K, Chang D, Zhou X, Low M, Seto SW, Li CG. Crocins for ischemic stroke: a review of current evidence. Front Pharmacol 2022;13:825842. doi:10.3389/fphar.2022.825842
22. Batarseh YS, Bharate SS, Kumar V, Kumar A, Vishwakarma RA, Bharate SB, et al. *Crocus sativus* extract tightens the blood-brain barrier, reduces amyloid β load and related toxicity in 5XFAD mice. ACS Chem Neurosci 2017;8(8):1756-66. doi:10.1021/acschemneuro.7b00101
23. Zang C, Liu H, Ju C, Yuan F, Ning J, Shang M, et al. *Gardenia jasminoides* J. Ellis extract alleviated white matter damage through promoting the differentiation of oligodendrocyte precursor cells via suppressing neuroinflammation. Food Funct 2022;13(4):2131-41. doi:10.1039/d1fo02127c
24. Kuhlmann T, Miron V, Cui Q, Wegner C, Antel J, Brück W. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain 2008;131(Pt 7):1749-58. doi:10.1093/brain/awn096
25. Javid Z, Taherianfard M, Sajedianfard J, Moradi HR. Effects of saffron hydro-alcoholic extract on the hippocampal oligodendrocyte transcription factor 2 and glial fibrillary acidic protein distribution in female demyelinated rats. Iran J Physiol Pharmacol 2023;7:19-31.
26. Wegener A, Deboux C, Bachelin C, Frah M, Kerninon C, Seilhean D, et al. Gain of Olig2 function in oligodendrocyte progenitors promotes remyelination. Brain 2015;138(Pt 1):120-35. doi:10.1093/brain/awu375
27. Saraste M, Bezukladova S, Matilainen M, Sucksdorff M, Kuhle J, Leppert D, et al. Increased serum glial fibrillary acidic protein associates with microstructural white matter damage in multiple sclerosis: GFAP and DTI. Mult Scler Relat Disord 2021;50:102810. doi:10.1016/j.msard.2021.102810
28. Zhong K, Wang RX, Qian XD, Yu P, Zhu XY, Zhang Q, et al. Neuroprotective effects of saffron on the late cerebral ischemia injury through inhibiting astrogliosis and glial scar formation in rats. Biomed Pharmacother 2020;126:110041. doi:10.1016/j.biopha.2020.110041
29. Li T, Liu T, Chen X, Li L, Feng M, Zhang Y, et al. Microglia induce the transformation of A1/A2 reactive astrocytes via the CXCR7/PI3K/Akt pathway in chronic post-surgical pain. J Neuroinflammation 2020;17(1):211. doi:10.1186/s12974-020-01891-5
30. Shaheen MJ, Bekdash AM, Itani HA, Borjac JM. Saffron extract attenuates neuroinflammation in rmTBI mouse model by suppressing NLRP3 inflammasome activation via SIRT1. PLoS One 2021;16(9):e0257211. doi:10.1371/journal.pone.0257211