Golestan University of Medical Sciences

Jorjani Biomedicine Journal

Online ISSN: 2645-3509

Interactive effects of endurance training combined with saffron and crocin on the hippocampal oxidant-antioxidant system in trimethyltin-induced Alzheimer's rats

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Abstract

Background: Alzheimer's disease (AD) disrupts hippocampal oxidant-antioxidant balance. Although endurance training, saffron, and crocin have shown antioxidant benefits individually, their comparative effects in Alzheimer's models are not well established. The present study investigated changes in hippocampal oxidant-antioxidant markers in trimethyltin (TMT)-induced Alzheimer's rats following eight weeks of endurance training (ET), with or without saffron (S) and crocin (Cr) supplementation.

Methods: The current study was performed on 49 male Sprague-Dawley rats (Age: 8 weeks old, weight: ~220 g). Forty-two rats received TMT (8 mg/kg, [Intraperitoneal] IP) and were randomly assigned to six groups: (1) AD, (2) ET, (3) S, (4) Cr, (5) ET+S, and (6) ET+Cr. Seven healthy rats served as controls (HC). Training groups ran on a treadmill for eight weeks (5 sessions/week, 15-30 min/session, 15-20 m/min). S and Cr groups received daily IP injections (25 mg/kg). Forty-eight hours following the intervention, hippocampal tissue was collected to measure superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (TAC). The data were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test (SPSS software [Version 22], P-Value ≤ 0.05).

Results: Compared to the AD group, MDA levels decreased by 35-56%, and SOD levels increased by 193-257% in all the intervention groups. Moreover, TAC levels showed marked improvement, rising by 185%, 220%, 253%, 309%, and 335% in Cr, S, ET, ET+Cr, and ET+S groups, respectively (P-Value < 0.05). The aforementioned findings highlight the superior antioxidant response in the combined intervention groups, compared to the response in single treatments (P-Value < 0.05).

Conclusion: Endurance training, saffron, and crocin improved hippocampal antioxidant status in Alzheimer's rats, with combined interventions yielding superior effects.

Article Type: Research Article

Article History

Received: 4 April 2024
Received in revised form: 12 October 2024
Accepted: 12 March 2025
Available online: 30 March 2025
DOI: 10.29252/jorjanibiomedj.13.1.31

Keywords

Endurance training
Saffron
Crocin
Alzheimer's disease
Total Antioxidant Capacity

Highlights

What is current knowledge?

Alzheimer's disease is associated with an imbalance in the oxidant-antioxidant system within the hippocampus. The current data indicate that AD affects approximately 6.9 million individuals in developed countries, such as the United States, and this number is projected to rise to 13.8 million by 2060. However, providing therapeutic and supportive care solutions can reduce mortality in these patients.

What is new here?

Despite a large body of research on various therapeutic methods, researchers have not yet been able to provide a suitable solution for reducing oxidative stress in brain tissue. Although previous studies have pointed to the favorable role of exercise, medicinal herbs, and their components alone, examining their interactive effect is one of the innovations of the present study.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder with a multifactorial etiology. It imposes a substantial economic and emotional burden on individuals and societies. Current data indicate that AD affects approximately 6.9 million individuals in developed countries, such as the United States, and this number is projected to rise to 13.8 million by 2060 (1).

Alzheimer's disease is considered an irreversible brain disorder involving various pathophysiological pathways, including amyloid-beta (Aβ) accumulation, neurotrophin deficiency, and oxidative stress imbalance (2). It is believed that in AD, increased levels of reactive oxygen species (ROS), resulting from mitochondrial dysfunction and Aβ fibrillation in the dentate gyrus, lead to damage to neuronal lipids, proteins, and deoxyribonucleic acid (DNA), ultimately causing neuronal death (3). Although the antioxidant defense system is normally responsible for neutralizing free radicals, in oxidative stress-related disorders, such as AD, this system becomes impaired. Deficiencies in key antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), render the system ineffective against ROS. This imbalance results in cellular damage and contributes to memory impairment (4).

As the prevalence of AD and its associated mortality continues to rise, increasing attention has been directed toward identifying non-invasive therapeutic strategies. According to recent studies, regular physical activity has been recognized as a potential therapeutic approach to enhance cognitive function in aging populations and in neurodegenerative conditions (5,6). Physical exercise has also been shown to improve motor function in AD models (7) and promote the expression of certain neurotrophins (8). Previous studies have reported increases in SOD activity and improvements in short-, mid-, and long-term memory following high-intensity interval and endurance training in rats. However, no significant changes were observed in GPx and CAT levels (9). In contrast, another study reported enhanced SOD and GPx activity, reduced malondialdehyde (MDA) levels, and improved

cognitive performance in a neurotoxic model induced by stanozolol (10). These inconsistencies, particularly regarding the differential impact of exercise intensity, duration, and neurotoxic model, suggest that the effects of physical activity on the brain's oxidant-antioxidant system remain incompletely understood. Moreover, it is unclear whether exercise-induced antioxidant changes are consistent in different neurodegenerative models or influenced by specific neurotoxins, such as trimethyltin (TMT) or stanozolol. The aforementioned gaps warrant further investigation.

Given the global trend of population aging and the increasing incidence of neurological disorders, along with the side effects associated with synthetic drugs, researchers have suggested that certain herbal and antioxidant compounds might exert beneficial effects in the management of neurodegenerative diseases. One such medicinal plant is saffron, scientifically known as Crocus sativus L. (11). This plant contains a variety of bioactive constituents, including isoflavones, flavonoids, triterpenoids, quinones, phenolic acids, crocetin, crocin, safranal, and picrocrocin. Among the mentioned constituents, crocin is one of the most potent and biologically active antioxidant compounds in saffron. Due to its unique chemical structure, crocin is believed to reduce inflammatory markers, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β. Moreover, it might stimulate the transcription of antioxidant enzymes through pathways involving phosphoinositide 3-kinase (PI3K) and Akt signaling (12). The available evidence suggests that both saffron and crocin have favorable effects on cognitive performance. Saffron, in particular, has demonstrated the ability to neutralize Aβ accumulation, enhance acetylcholine function, and promote mitochondrial biogenesis in neurons (13).

Trimethyltin is a potent neurotoxic organotin compound that selectively targets the hippocampus, inducing oxidative stress, mitochondrial dysfunction, and neuronal apoptosis. Experimental studies have shown that TMT exposure leads to elevated levels of ROS, MDA, and nitric oxide, along with decreased antioxidant enzyme activity (e.g., SOD, GPx, and CAT) in the hippocampal tissue, closely mimicking the oxidative imbalance observed in human AD (14-16). These molecular alterations are accompanied by behavioral deficits, such as memory impairment, hyperactivity, and seizures, further validating TMT as a reliable model for Alzheimer-like neurodegeneration. Therefore, the use of TMT in the present study provides a mechanistically relevant platform for evaluating antioxidant interventions.

Despite previous investigations, most studies have examined saffron or crocin in isolation, without evaluating their interaction with physical exercise. Furthermore, it remains unclear whether crocin's molecular effects, such as PI3K/Akt activation, translate into additive or synergistic antioxidant responses when combined with endurance training. The recent evidence suggests that combining endurance training with antioxidant supplementation might produce synergistic effects on neuroprotective signaling pathways in Alzheimer's models, surpassing the benefits of either intervention alone (17). It remains unclear whether the effects of saffron and crocin on the nervous system are distinct or overlapping (11). Given the limited data on the individual and combined effects of exercise, saffron, and crocin on the brain's oxidant-antioxidant system, clarifying whether these interventions act independently, redundantly, or synergistically is essential for optimizing non-pharmacological strategies in AD management. Moreover, due to the growing prevalence of AD and the pressing need for simple, cost-effective therapeutic approaches, fundamental research, such as the present study, is both timely and necessary. Therefore, the current study aimed to investigate the changes in oxidant-antioxidant biomarkers in the hippocampal tissue of TMT-induced Alzheimer's rats following endurance training, saffron administration, and crocin supplementation.

Methods

Animal subjects

The present experimental study was conducted on a total of 49 male Sprague-Dawley rats with an average age of 8 weeks and a mean body weight of 220 ± 30.6 g provided from the Laboratory Animal Breeding Center of Islamic Azad University, Marvdasht Branch, Marvdasht, Iran. Upon arrival at the Exercise Physiology Laboratory of the university, the animals were housed in transparent, autoclavable polycarbonate cages (Five rats per cage) and acclimated to laboratory conditions for one week. The animal facility was maintained at an optimal ambient temperature of 22-24°C with relative humidity levels ranging from 55% to 65%. A 12-hour light/dark cycle was strictly regulated using an electronic light controller. The animals had ad libitum access to standard chow and water throughout the study. All procedures involving animals were approved by the Ethics Committee of Islamic Azad University, Lar Branch, Fars, Iran, and conducted in accordance with the ethical guidelines of Shiraz University of Medical Sciences, Shiraz, Iran (Ethics code: IR.SUMS.REC.1396.446).

Induction of Alzheimer's disease and group assignment

On the eighth day of the study, 42 out of the 49 rats received an intraperitoneal (IP) injection of trimethyltin chloride (TMT; 8 mg/kg; Sigma-Aldrich, Germany), a neurotoxic agent used to induce Alzheimer-like pathology (18). After 24 hours, the animals were observed for behavioral signs indicative of Alzheimer's induction. As previously reported, the administration of TMT is associated with distinct clinical symptoms in rats, including muscular tremors, hyperthermia, ocular and nasal hemorrhage, nausea, seizures, and tail twisting behavior. Following the confirmation of neurotoxic symptoms, the 42 TMT-treated rats were randomly assigned to six experimental groups (n = 7 per group): Alzheimer's control (AD), Saffron supplementation (S), Crocin supplementation (Cr), Endurance training (ET), Endurance training + saffron (ET+S), and Endurance training + crocin (ET+Cr). To assess the effects of disease induction on the research variables, the remaining 7 rats were assigned to a healthy control group (HC) and did not receive TMT.

Endurance training protocol

To initiate endurance training, all rats first underwent a one-week habituation phase using a five-lane motorized treadmill (Danesh Salar Iranian Co., Iran), during which they ran at a speed of 8 m/min for 5 minutes per day. Following habituation, the main training protocol was implemented over a period of eight weeks. During the first and second weeks, the rats ran on the treadmill three days per week at a speed of 15 m/min for 15 minutes per session. During the third and fourth weeks, the training duration and the speed were increased to 20 minutes and 17 m/min, respectively. During the fifth and sixth weeks, the rats exercised for 25 minutes at a speed of 19 m/min. Finally, during the seventh and eighth weeks, the training sessions lasted 30 minutes with a running speed of 20 m/min. Additionally, to ensure proper warm-up and cool-down, each session began and ended with 5 minutes of running at a speed of 8 m/min (8).

Saffron and crocin supplementation

The crocin supplement used in this study was purchased from Sigma-Aldrich (Germany, product code: 17304). Each day, 75 mg of crocin was dissolved in 4.2 cc of normal saline; subsequently, 0.3 cc of the prepared solution was intraperitoneally injected into each rat, corresponding to a dosage of 25 mg/kg per day. Similarly, for saffron extract preparation, 75 mg of saffron was ground into fine powder using a mortar and pestle each day. The powder was then dissolved in 4.2 cc of normal saline, and 0.3 cc of the resulting solution was intraperitoneally administered to each rat at a daily dose of 25 mg/kg (11). All injections were performed once daily at 2:00 p.m., approximately two hours after the completion of the exercise sessions, which were conducted from 9:00 to 10:00 a.m. Supplement administration was carried out every day throughout the intervention period to ensure consistent exposure.

Tissue dissection and sampling

Forty-eight hours after the final exercise session, all animals were anesthetized using an intraperitoneal injection of a ketamine-xylazine mixture (3:5 ratio). Upon achieving deep anesthesia and confirming the absence of pain reflexes, the skull was surgically opened, and the hippocampal tissue was carefully excised by a specialist. The harvested hippocampi were immediately stored at -80°C for subsequent biochemical analyses. All procedures were conducted in the Animal Laboratory of Islamic Azad University, Marvdasht Branch.

Biochemical measurements

The hippocampal concentrations of SOD and MDA were quantified using enzyme-linked immunosorbent assay (ELISA) kits, both manufactured by Navand Bio Lab (Iran). Total antioxidant capacity (TAC) was measured using a commercially available kit from ZellBio (Germany; Catalog No.: ZB-TAC-96A).

Statistical analysis

Descriptive statistics were reported as mean ± standard deviation (SD). The Shapiro-Wilk test was used to assess the normality of data distribution. One-way analysis of variance (ANOVA) was employed to determine differences among the experimental groups. Tukey's post hoc test was applied to identify the specific group differences. All statistical analyses were performed using SPSS software (Version 22). Statistical significance level was 0.05.

Results

Table 1 shows the mean and standard deviation of the study variables in the different groups. Statistical comparisons among groups were drawn using one-way ANOVA followed by Tukey's post hoc test.

Table 1. Mean ± SD of hippocampal SOD, MDA, and TAC levels in experimental groups

Groups	MAD (nmol/mg)	SOD (IU/mg)	TAC (Micromolar)
HC	0.292 ± 0.06	0.84 ± 0.07	1.37 ± 0.04
AD	0.66 ± 0.091	0.14 ± 0.017	0.34 ± 0.031
ET	0.43 ± 0.06	0.41 ± 0.06	1.20 ± 0.15
S	0.42 ± 0.05	0.50 ± 0.07	1.09 ± 0.05
Cr	0.39 ± 0.027	0.43 ± 0.07	0.97 ± 0.05
ET+S	0.29 ± 0.029	0.48 ± 0.08	1.48 ± 0.12
ET+Cr	0.38 ± 0.03	0.50 ± 0.048	1.39 ± 0.03

Abbreviations: MDA, Malondialdehyde; SOD, Superoxide Dismutase; TAC, Total Antioxidant Capacity

The results of SOD analysis are presented in Figure 1. A significant difference was observed among groups ($F = 31.90$, P-Value < 0.001, partial $\eta^2 = 0.920$). The AD group showed significantly lower SOD levels than the HC group (P-Value < 0.001, Cohen's $d = 12.03$). All intervention groups (i.e., ET, S, Cr, ET+S, and ET+Cr) exhibited significantly higher SOD levels, compared to the AD group (P-Value < 0.001), with large effect sizes (Cohen's d range: 5.03-6.00). No significant differences were observed among the intervention groups or between them and the HC group. The Alzheimer's disease (AD) group showed markedly reduced SOD activity, compared to the healthy control (HC). Endurance training (ET), saffron (S), crocin (Cr), and their combinations (ET+S and ET+Cr) increased SOD levels, compared to the AD group. The data are expressed as mean ± standard error of the mean (SEM).

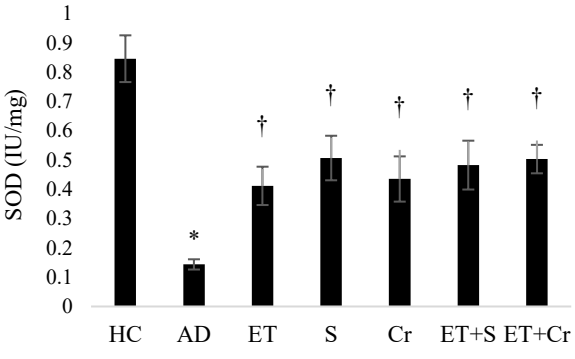

Figure 1. Superoxide Dismutase (SOD) levels in the hippocampal tissue of rats in the studied groups
* Significantly different from the HC group (P-Value ≤ 0.001)
† Significantly different from the AD group (P-Value ≤ 0.001)

As shown in Figure 2, MDA levels differed significantly among groups ($F = 15.77$, P-Value < 0.001, partial $\eta^2 = 0.844$). The AD group had significantly higher MDA levels than the HC group (P-Value < 0.001, Cohen's $d = 4.89$), whereas all intervention groups showed significantly reduced levels, compared to the AD group (P-Value < 0.001), with large effect sizes (Cohen's d range: 2.48-5.00). No significant differences were observed among the intervention groups or between them and HC. The Alzheimer's disease (AD) group showed markedly higher MDA levels, compared to the healthy control (HC) group. Endurance training (ET), saffron (S), crocin (Cr), and their combinations (ET+S and ET+Cr) significantly reduced MDA concentrations relative to the AD group, indicating improved oxidative balance. The data are expressed as mean ± standard error of the mean (SEM).

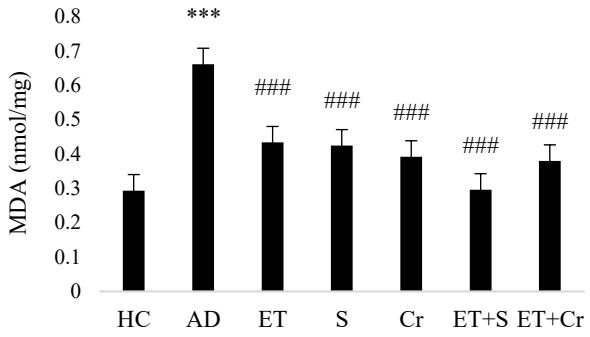

Figure 2. Malondialdehyde (MDA) levels in the hippocampal tissue of rats in the studied groups
*** Significantly different from the healthy control group (P-Value ≤ 0.001)
Significantly different from the AD group (P-Value ≤ 0.001)

The results of TAC analysis are presented in Figure 3. A significant difference was noticed among the groups ($F = 70.72$, P-Value < 0.001, partial $\eta^2 = 0.934$). The TAC levels in the AD group were significantly lower than those in the HC group (P-Value < 0.001, Cohen's $d = 28.78$), whereas all intervention groups showed significantly higher levels than the AD group (P-Value < 0.001), with very large effect sizes (Cohen's d range: 7.94-34.42). Among the interventions, ET showed higher TAC than Cr ($P = 0.018$), and ET+S had significantly higher TAC than ET (P-Value = 0.004). Additionally, ET+S and ET+Cr had significantly higher TAC than S and Cr (P-Value < 0.001), and ET+S had higher TAC than S (P-Value = 0.02). The Alzheimer's disease (AD) group exhibited a pronounced reduction in TAC, compared to the healthy control (HC) group. Endurance training (ET), saffron (S), crocin (Cr), and their combined treatments (ET+S and ET+Cr) significantly elevated TAC levels relative to the AD group. Moreover, the combined interventions (ET+S and ET+Cr) showed greater improvements, compared to saffron and crocin alone. The data are presented as mean ± standard error of the mean (SEM).

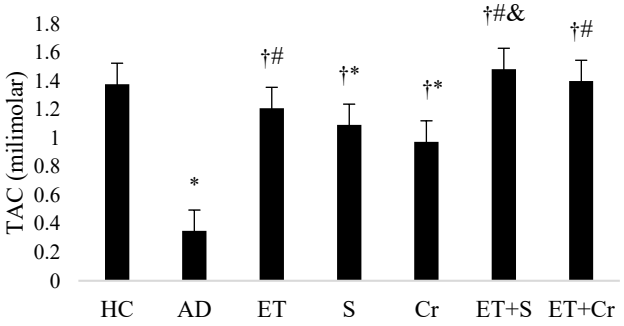

Figure 3. Total Antioxidant Capacity (TAC) levels in the hippocampal tissue of rats in the studied groups
* Significantly different from the HC group (P-Value ≤ 0.001)
† Significantly different from the AD group (P-Value ≤ 0.001)
# Significantly different from the CR (P-Value ≤ 0.001)
& Significantly different from the S group (P-Value ≤ 0.001)

Discussion

Overview of oxidative stress in Alzheimer's disease

The present study aimed to investigate changes in the oxidant-antioxidant system in the hippocampal tissue of rats with AD induced by TMT, following eight weeks of endurance training combined with saffron and crocin supplementation. The results showed that Alzheimer's induction caused a significant decrease in SOD and TAC levels in the diseased group, compared to the healthy control group, whereas the MDA level increased significantly.

Studies have demonstrated that although the mechanism of AD development is very complex, there is a close association between oxidative stress and the progression of AD (19,20). Since brain tissue is rich in lipids, it is more vulnerable to oxidative stress (19), and it has been shown that the hippocampus and cerebral cortex endure the highest levels of oxidative stress in AD (20). Although ROS plays a role in cellular signaling and defense against invading microorganisms, excessive ROS production damages cellular macromolecules, leading to apoptosis and cell death (21). Studies on mice with SOD knockout have shown that increased oxidative stress in the brain, particularly in the hippocampus, is associated with cognitive decline similar to that observed in aged mice. Similarly, the evidence has shown a reduction in endogenous antioxidant enzyme levels in the brains of preclinical animal models of AD, as well as in patients with AD (22). In oxidative stress-related diseases, including AD, the antioxidant system fails to compete with ROS due to defects in its own enzymes, such as SOD, GPx, and CAT. This failure leads to cellular damage and ultimately results in memory impairment (4).

Effects of endurance training

The results of the present study showed that endurance training increased SOD and TAC levels and decreased MDA levels in the hippocampal tissue of AD-afflicted rats. Researchers believe that the main mechanism by which exercise enhances antioxidant transcription is dependent on the activation of the nuclear factor erythroid-related factor 2 (NRF2). Specifically, in response to physical activity and increased ROS, NRF2 translocates to the nucleus and interacts more with the antioxidant response element (ARE). In other words, during exercise, the initial increase in PI3K leads to phosphorylation of NRF2, which causes the release of Keap1 from this protein. Subsequently, NRF2 binds more easily to its active site on DNA near the ARE. The NRF2/ARE complex then activates phase II gene expression, which is related to the transcription of antioxidant enzymes, such as SOD, GPx, and CAT (23).

Consistent with the present study, researchers showed that exercise increases SOD, brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), SIRT-1, and SIRT-3 in elderly women (24). Moreover, another study reported increased levels of Nrf2, SOD, and CAT in the hippocampus of AD rats induced by TMT following endurance training (25). In a different study, acute exercise on a treadmill elevated heat shock protein 72 (HSP-70) and TAC in the hippocampus of rats (26). Additionally, Abdi et al. showed that endurance training increased Nrf2, glutathione (GSH), and heme oxygenase-1 (HO-1) in the hippocampal tissue of AD rats (27). Although some studies have reported variable effects depending on exercise type and duration, the current study's use of an eight-week endurance protocol might have provided sufficient stimulus to activate sustained antioxidant responses. This issue might explain the consistency of the findings of the current study with NRF2-related outcomes in long-term models. Therefore, most studies indicate a positive effect of long-term exercise on the antioxidant system in neurological disorders, and the current study supports these findings.

Effects of saffron and crocin supplementation

The results of the present study showed that consumption of saffron and crocin increased SOD and TAC levels and decreased MDA levels in the hippocampal tissue of AD rats. Regarding saffron, studies have shown that this antioxidant herb can directly reduce ROS, leading to a decrease in lipid peroxidation and its marker MDA. Additionally, saffron reduces brain damage by affecting Aβ and the oxidative stress caused by it in the hippocampal tissue (28). It is also believed that saffron, due to its phenolic compounds, can enhance the non-enzymatic antioxidant system and ultimately improve TAC (29). Moreover, saffron's antioxidant properties might inhibit nuclear factor kappa-B (NF-κB) transcription factor, suppress the MAPK/JNK pathway, and inhibit cyclooxygenases and metalloproteinases in the central nervous system, thereby halting the process of inflammation and oxidation (13).

One study demonstrated that saffron consumption increased GSH and TAC and decreased MDA in the brain tissue of stroke-induced rats (30). Furthermore, crocin is reported to be a strong free radical scavenger that enhances enzymatic and non-enzymatic antioxidant capacity. Crocin intake is associated with increased transcription of antioxidants, such as SOD and GPx, and decreased MDA (31). Crocin also strengthens glutathione, ascorbic acid, and bilirubin, and ultimately enhances the antioxidant system by reducing pro-inflammatory factors and cyclooxygenase-2. In other words, crocin activates the CAMK4-PI3K/Akt-Nrf2 pathway and participates in the transcription of antioxidant enzymes through this pathway (32).

Supporting the above-mentioned finding, a review study showed that crocin activates AMP-activated protein kinase (AMPK) signaling, which subsequently improves mitochondrial biogenesis and increases antioxidant capacity (33). However, another study reported that following crocin administration, SOD, GPx, and MDA levels in the brain tissue of experimental autoimmune encephalomyelitis rats decreased (32). The discrepancy in results might be due to differences in sample populations and measurement methods. Specifically, the type of animal model (i.e., autoimmune encephalomyelitis vs. TMT-induced AD), the dosage and duration of crocin administration, and the timing of tissue sampling might influence outcomes. The present study used a well-established TMT-induced AD model and an eight-week intervention protocol, which might account for the more consistent antioxidant improvements observed. Based on the aforementioned findings, it appears that saffron, due to other compounds like safranal and quercetin, might have a greater effect on activating the non-enzymatic antioxidant system, whereas crocin directly activates the CAMK4-PI3K/Akt-Nrf2 pathway.

Combined effects of exercise and supplementation

The results also showed that the combined consumption of saffron and crocin, along with endurance training, significantly increased SOD and TAC levels and decreased MDA levels in the hippocampal tissue of rats with AD. Moreover, TAC levels in the exercise group were significantly higher than in the crocin group, and TAC in the exercise plus saffron group was significantly higher than in the exercise-only group. Additionally, the combined saffron and crocin consumption with the endurance training group showed significantly higher TAC levels, compared to the saffron-only and crocin-only groups. It appears that exercise activates the PI3K/NRF2/ARE pathway, leading to the transcription of SOD, GPx, and CAT (33), whereas saffron reduces Aβ directly, lipid peroxidation, and strengthens the non-enzymatic antioxidant system (29). Saffron also inhibits NF-κB, the MAPK/JNK pathway, cyclooxygenases, and metalloproteinases in the central nervous system, preventing inflammation and oxidative stress (30). The main mechanism of crocin is the activation of the CAMK4-PI3K/Akt-Nrf2 pathway (31). Therefore, although the aforementioned interventions do not always act through identical pathways, they ultimately enhance each other's effects on strengthening the antioxidant system.

Consistent with the current study's findings, TAC levels in the crocin plus exercise and saffron plus exercise groups were significantly higher than those in other groups alone. Supporting this finding, one study showed that acute exercise increased MDA in the premotor cortex of the brain; nevertheless, the combination of exercise and aqueous saffron stigma extract decreased MDA levels in this brain region (28). Another meta-analysis demonstrated that saffron extract consumption increased CAT and SOD, with antioxidant improvements in supplemented groups being more prominent than in exercise groups (13). Additionally, a study showed that the administration of 50 and 100 mg/kg crocin increased SOD and GPx and reduced MDA following a fatiguing exercise session in aged rats (31).

Limitations and future directions

Given the role of exercise, crocin, and saffron in upstream signaling pathways regulating the transcription of antioxidant enzymes, the lack of direct evaluation of these molecular mechanisms represents a major limitation of the present study. Without assessing key regulators, such as NRF2, PI3K, or CAMK4, the study cannot fully elucidate the mechanistic basis of the observed antioxidant effects. Additionally, although saffron contains other bioactive compounds, such as crocetin and safranal, their effects were not investigated, which further limits the comprehensiveness of the findings. The aforementioned omissions restrict the study's potential to provide a complete picture of the involved molecular interactions. Although behavioral symptoms were used to confirm AD induction, the absence of pre-intervention cognitive assessments, such as the Morris Water Maze, also limits the precision of baseline characterization. Future studies should address the aforementioned critical gaps to strengthen mechanistic interpretation and translational relevance.

Conclusion

Based on the obtained results, exercise, crocin, saffron, and their combination improve antioxidant system function. However, the combination of exercise with crocin and exercise with saffron has a considerably more favorable effect on the antioxidant capacity in the hippocampal tissue of AD models.

Acknowledgement

The authors of this manuscript would like to express their gratitude to all those who helped in conducting this research, including the expert of the Exercise Physiology Laboratory of Islamic Azad University, Marvdasht Branch, Dr. Fatemeh Farkhaei.

Funding sources

The authors have not received any financial support for this research.

Ethical statement

All procedures involving animals were approved by the Ethics Committee of Islamic Azad University, Lar Branch, and conducted in accordance with the ethical guidelines of Shiraz University of Medical Sciences (Ethics code: IR.SUMS.REC.1396.446).

Conflicts of interest

The authors have no conflict of interest to declare.

Author contributions

In this study, Fatemeh Akbari was responsible for the implementation of the research and financial support. Mehrzad Moghaddisi was responsible for the supervision of the research and editing of the manuscript. Omidreza Salehi was responsible for the implementation and initial writing of the manuscript.

Data availability statement

Data can be provided upon request.

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Cite this article as:
Akbari F, Moghdasi M, Salehi OR. Interactive effects of endurance training combined with saffron and crocin on the hippocampal oxidant-antioxidant system in trimethyltin-induced Alzheimer's rats. *Jorjani Biomedicine Journal.* 2025;13(1):31-6. http://dx.doi.org/10.29252/jorjanibiomedj.13.1.31