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Crocin as a multimodal therapy for multiple sclerosis: Recent breakthroughs in immunomodulation and neuroprotection

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Keywords
Crocus Sativus; Multiple Sclerosis; Immunomodulation

Abstract

Background: Multiple sclerosis (MS) is a disabling neurological disorder characterized by the existence of demyelinating lesions and affects more than 2.5 million people globally. The current approaches for patients with MS are mainly based on ameliorating symptoms and decreasing acute attacks, and so far, they have not been successful in providing effective neurological regeneration. Recently, natural-based therapies, like crocin, in light of their pharmacological and biological benefits, have acquired much attention in the treatment of neurological diseases like MS. Hence, this study attempts to answer the question of whether crocin therapy can be a suitable approach for MS with a mechanistic insight.

Methods: In this narrative literature review, all related articles (in press and published) in English were searched and assessed using electronic databases, including Web of Science, PubMed, and Google Scholar, up to April 1, 2025. The search keywords in the title and/or abstract of articles were: "Crocin" or "Crocus Sativus" and "Multiple Sclerosis" or "MS".

Results: Experimental evidence has revealed the ability of crocin to alleviate reflexive motor behavior-related tests, cognitive indices, depressive-like behavior, tail flick latency, the conduction velocity of the motor and sensory nerve, antioxidant factors, and to attenuate inflammatory factors. Besides improving some mental factors, such as anxiety, clinical outcomes indicated that crocin could modulate oxidative stress and inflammation-associated markers.

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Conclusion: The current preclinical and clinical studies have provided convincing evidence showing that crocin therapy can be a suitable candidate for MS cases; however, larger and more in-depth clinical trials are warranted to support these results.

Introduction

Multiple sclerosis (MS) is known as a complex and multifactorial disease pertinent to the central nervous system (CNS) and is described by the presence of demyelinating lesions influencing the grey and white matter.[1,2] According to reports, this autoimmune disease presently affects over 2.5 million subjects worldwide. The prevalence of MS depends on geographical and ethnic parameters. The highest rate of prevalence has been recorded in Europe (142.81 patients per 100000 persons).[3] Several risk factors related to MS have been recognized exhibiting unrelated elements like latitude, serum levels of vitamin D, sunlight exposure, smoking, virus infection [e.g., measles and Epstein-Barr virus (EBV)], and genetics.[4,5] Neurological symptoms in MS cases are different from person to person and can encompass paresthesia, abnormal vision, focal weakness, cognitive impairment, and bowel and bladder incontinence.[6] The common management approaches for MS are concentrated on alleviating symptoms, curing acute attacks, and decreasing biological problems.[7] On the other hand, one of the main hurdles of the current therapeutic strategies for this neurological condition is insufficient neuron regeneration.[8] MS-modifying therapies, such as glatiramer acetate, interferons (IFNs), and teriflunomide, reduce the MS course by regulating or inhibiting immune system activities.[7] Unfortunately, most of these therapeutic approaches are not capable of stopping nerve tissue degeneration in the aggressive stage of MS.[8] These approaches are generally costly and may cause various side effects ranging from mild to severe stages, including allergic reactions, anaphylaxis, psychotic disorders, embryofetal toxicity, and hepatotoxicity.[9] Moreover, some of these therapeutic strategies, particularly glatiramer acetate, are not able to cross the blood-brain barrier (BBB), limiting their capacity to affect neuroinflammation and neurodegeneration directly.[10] Therefore, there is still an unmet need to introduce a suitable therapy with high effectiveness and minimal side effects. Lately, natural compounds, as affordable therapeutic choices, have received much consideration and have been introduced as a preferred option over synthetic medications for many illnesses, especially neurodegenerative disorders.[11-14] Crocin is a natural compound with neuroprotective properties whose capacities in treating neuropsychological diseases have been emphasized.[15] Crocin (crocetin di-gentiobiose ester) with the molecular formula $C_{44}H_{64}O_{24}$ is defined as a colored carotenoid deriving from the stigmas of Crocus sativus or fruits of Gardenia jasminoides. This natural compound is based on dicarboxylic acid crocetin and the disaccharide gentiobiose.[16] Multiple biological and pharmacological features have been mentioned for crocin, including anti-inflammatory, anti-oxidative, anti-neoplasm, anti-viral, cardioprotective, and hepatoprotective properties.[16-19] It is stated that crocin can effectively induce remyelination in a number of CNS-related disorders without causing serious side effects.[18,20] Besides, its capability to pass BBB can make it a suitable therapy for neurodegenerative disorders.[21]

Intriguingly, experimental and clinical evidence has shown the neuroprotective characteristics of crocin against MS through different mechanisms, like modulating the immune system function and oxidative stress, and improving parameters related to cognitive and motor functions.[22,23] Hence, in this narrative literature review, we intend to summarize and argue the potential role of crocin in MS treatment by relying on the current knowledge with a focus on novel therapeutic mechanisms, such as T helper (Th)17/regulatory T cells (Treg) regulation, neurotrophic factor promotion, the functionality of novel formulations, including nano-based formulations, as well as clinical innovations, like combination therapies (i.e., crocin + fingolimod), non-conventional biomarkers (i.e., saliva/urine oxidative markers), and cognitive outcomes. Additionally, for the first time, in this work, the limitations of each experimental and clinical work and challenges for translating experimental outcomes to clinical trials have been discussed.

Search strategy: In this narrative literature review, all documents related to crocin and MS therapy were comprehensively searched and evaluated using electronic databases, comprising Web of Science, PubMed, and Google Scholar up to April 1, 2025 (Figure 1). The keywords used in this work were: "Crocin" or "Crocus Sativus" and "Multiple Sclerosis" or "MS", which were searched in the title and/or abstract of articles.

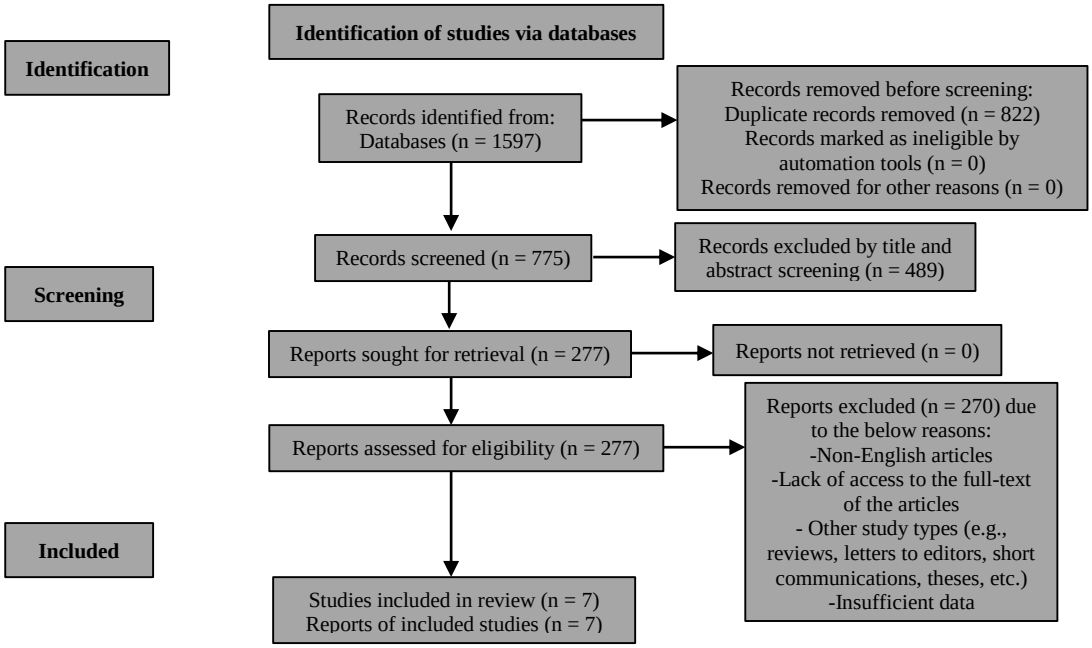

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart exhibiting the process of identification of eligible studies via databases

In-press and published articles were analyzed, and only English papers (due to resource limitations and the dominance of English-language papers in this context) were assessed. Inclusion criteria included experimental (in vitro/in vivo) and clinical articles evaluating the effects of crocin therapy for MS. Exclusion criteria included papers with no relevance to the mentioned therapy, articles with insufficient data, and gray literature (reports that were not peer-reviewed or published). The quality assessments were accomplished by two independent authors, and there was a supervising author for checking the included final articles and settling any disputes between authors.

Limitations: This work is a narrative literature review summarizing and discussing evidence about crocin therapy in MS according to the current preclinical and clinical evidence. This investigation was carried out qualitatively but not quantitatively as a systematic and meta-analysis review. Moreover, some related papers may have been ignored. Besides, this study does not uncover all diagnostic and therapeutic aspects of MS, as well as all possible therapeutic mechanisms of crocin in MS treatment.

MS and pathogenesis: Presently, MS pathogenesis remains a puzzle mainly because of a poor understanding of its etiology; however, some clues somewhat address its nature.[24] The current knowledge highlights the theory that inflammation-related mechanisms participate in disease initiation and development.[25,26] Demyelinating regions mainly establish perivenous and confluent focal lesions observed all over the CNS (in gray and white matter) with different degrees of reactive gliosis and axonal loss. Spatially, the lesion distribution of MS is partial to juxtacortical white matter, periventricular lesions of the thoracic and cervical spinal cord, and infratentorial areas like the pons and cerebellum.[27] Lesion pathologies of MS cases are generally heterogeneous and consist of cluster of differentiation (CD)8^+ T lymphocytes, $CD4^+$ T cells, activated macrophages, microglia, plasma cells, and B lymphocytes.[28,29] These days, published papers on MS have demonstrated the migration of autoreactive T-cells to the CNS following BBB impairment (Figure 2) and autoimmune cascade onset, resulting in the destruction of the myelin sheath and formation of sclerotic plaques and lesions.[30,31] Demolition of the myelin sheath, which has a core role in the integration and viability of axons, is considered the main cause of the development of MS.[30] Several immune factors take part in the destruction and demyelination of the CNS, especially Th1 and Th17 cells (Figure 2).[32,33] These cells secrete pro-inflammatory cytokines, such as IFN-gamma (IFN-γ), interleukin (IL)-1, tumor necrosis factor-alpha (TNF-α), and IL-17.[34] Autoantibodies are other immune agents that are detectable in MS plaques.

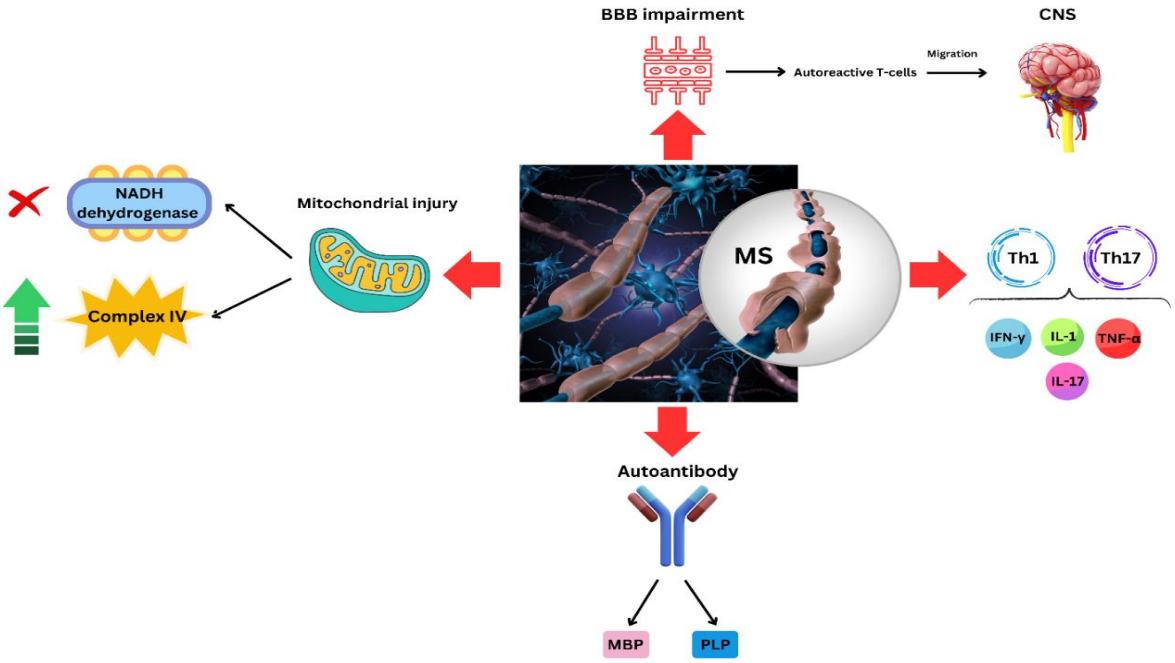

Figure 2. The representation of some pathogenic occurrences related to multiple sclerosis (MS)
CNS: Central nervous system; MS: Multiple sclerosis; BBB: Blood-brain barrier; Th1: T helper 1; Th17: T helper 17; IFN-γ: Interferon-gamma; IL-1: Interleukin-1; TNF-α: Tumor necrosis factor-alpha; IL-17: Interleukin-17; MBP: Myelin basic protein; PLP: Proteolipid protein; NADH: Nicotinamide adenine dinucleotide + hydrogen

There are shreds of evidence showing the linkage between MS signs and immunoglobulin G (IgG). Moreover, it is stated that IgG, particularly IgG against myelin basic proteins (MBP) and proteolipid proteins (PLP), is the mediator involved in disease pathogenesis.[35] It has been demonstrated that in progressive MS and relapsing-remitting MS (RRMS), microglial activation is linked with active tissue injury.[36] Microglial nodules and stimulated microglia have been detected in the white matter of subjects with progressive MS. Recently, emerging evidence has been toward the importance of mitochondrial injury in neurodegeneration and demyelination processes.[37] Proof for mitochondrial injury in the lesions of patients with MS was recognized from biochemical assessments of elevated complex IV (cytochrome c oxidase) function and disrupted nicotinamide adenine dinucleotide + hydrogen (NADH) dehydrogenase action (Figure 2) in mitochondria in the lesions of inflicted subjects and gene expression levels of motor cortex tissue.[38]

Crocin and MS

Experimental evidence: Multiple preclinical assessments have shed light on the neuroprotective action of crocin in the neuroinflammatory disease, and all of them reported positive results. Regarding this issue, Tashakori et al. set out to investigate the influences of crocin supplementation on the antioxidant defense system (ADS), depression, and reflexive motor behavior in the cuprizone (CPZ)-caused MS animal model. They co-administered CPZ and crocin (100 mg/kg orally) 3 times weekly within 5 weeks in mice with MS and showed the promoting role of crocin in the antioxidant indices [total antioxidant status (TAS), glutathione peroxidase (GPx), and superoxide dismutase (SOD)] (Figure 3) in the brain tissue and serum levels compared with the CPZ-induced MS group. In addition, the co-use of CPZ and crocin improved depressive-like behavior, as evidenced by decreasing immobility time in the tail suspension test (TST) and forced swimming test (FST) (Figure 3), compared with the CPZ-alone group. Moreover, this natural agent-based strategy could display its beneficial effects on reflexive motor behavior-related tests, including hind- and front-limb suspension (HFS), hind-limb foot angle (HLFA), grip strength (GS), negative geotaxis (vestibular function) (NG), and surface righting (SR), in comparison with the MS group. These data showed that crocin alleviated CPZ-caused demyelination and behavioral and motor disruptions, and depressive-like symptoms possibly by promoting the ADS and anti-inflammatory mechanisms.

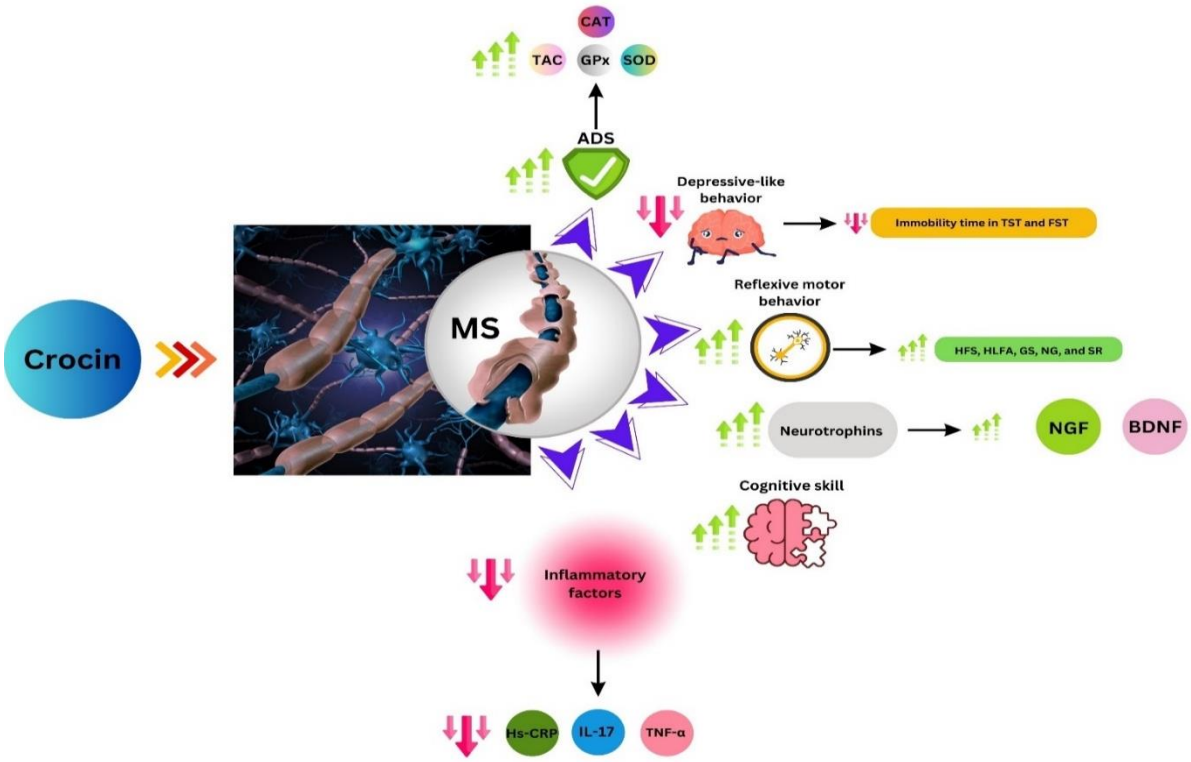

Figure 3. Therapeutic benefits of crocin therapy for multiple sclerosis (MS)
(MS: Multiple sclerosis; ADS: Antioxidant defense system; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; TAC: Total antioxidant capacity; TST: Tail suspension test; FST: Forced swimming test; HFS: Hind- and front-limb suspension; HLFA: Hind-limb foot angle; GS: Grip strength; NG: Negative geotaxis; SR: Surface righting; NGF: Nerve growth factor; BDNF: Brain-derived neurotrophic factor; Hs-CRP: High-sensitivity C-reactive protein; IL-17: Interleukin-17; TNF-α: Tumor necrosis factor-alpha)

Study design limitations of this work include small sample size (10 mice per group may decrease statistical power and raise the error risk), obscure randomization details, utilization of single dose of crocin (different doses, for instance, 50, 100, and 200 mg/kg should be tested to clarify the safety and effectiveness), the lack of a vehicle control to eliminate stress influences due to gavage procedure, and failure to assess the role of inflammatory-related factors, given that neuroinflammation is one of the main pathogenic mechanisms of MS. Besides, there are some confounding factors in this study, including stress due to handling and oral administration, diet diversity, and age and sex-related bias (assessing young male mice, restricting functionality to aged or female populations, which are associated with MS studies). However, some of these, like environmental factors (e.g., cage situations and light/dark cycles) and initial training for behavioral and rotarod tests, were controlled. On the whole, this experimental research accentuated the protective action of crocin in a CPZ-induced MS animal model.[22]

Fatemi et al. have evaluated the performance of crocin, fingolimod, and their combination in motor and sensory impairments, cognition, and brain neurotrophins [nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF)] in a toxic-induced demyelination model.[39] Fingolimod is a well-known synthetic disease-modifying therapy (DMT), while crocin, originating from saffron, could be a more cost-effective choice in areas where saffron is cultivated. However, large-scale production of crocin may be limited by agricultural barriers.[40,41] The MS model in this work was established by injecting ethidium bromide (EB) 0.1% (10 µl) into the brain's fourth ventricle, and crocin (5, 10, and 20 mg/kg) was administered orally for three weeks per day. The findings of this study underscore that crocin increases the levels of neurotrophins (i.e., BDNF and NGF) (Figure 3), especially at the dose of 10 mg/kg, more than fingolimod. The impact of combination therapy (crocin + fingolimod) on the levels of neurotrophins was more prominent than

single therapy with each of them. Regarding pain hypersensitivity measured by tail flick latency, it was displayed that crocin (especially at 10 mg/kg) and fingolimod significantly increased tail flick latency, and their combination exhibited the maximum effects on elevation of this variable, showing considerable reduction of pain hypersensitivity. Furthermore, in terms of motor function, the combination of these two therapeutic agents provided the best results in the balance-related test (rotarod). Besides, crocin improved the mentioned index, but its efficacy was lower than fingolimod.[39] Despite these, there were some limitations for this investigation, including a small sample size, lack of randomization, short-term intervention (three weeks of crocin therapy may not exhibit the lasting effects of this natural choice or fingolimod in chronic diseases, such as MS), and negligible information about histopathological data. In addition, heterogeneity in baseline measures (possible differences in stress responses and neurotrophin levels) and observer bias regarding behavioral tests (e.g., rotarod and open field) are a number of confounding factors present in the study that some of them by testing multiple dosages of crocin, standardization of animal housing, and using sham groups (to control solvent influences and surgical trauma) were monitored. On the whole, this research demonstrated that crocin (especially 10 mg/kg) was able to restore neurotrophin levels and promote motor and cognitive functions, imitating fingolimod's influences. Moreover, it seems that the combination of crocin and fingolimod exerts synergistic effects on the aforementioned variable.

Recently, Pazoki et al. have focused on the effects of intraperitoneal administration of various doses of crocin (minimum dose: 20 mg/kg and maximum dose: 60 mg/kg) in treating MS using the experimental autoimmune encephalomyelitis (EAE) mouse model.[42] In this experimental research, clinical symptoms, histopathology (demyelination and inflammation), T-cell proliferation, cytokine profiling [pro-inflammatory, i.e., IL-17, IL-6, IFN-γ, and TNF-α, and anti-inflammatory cytokines, i.e., transforming growth factor-beta (TGF-β), IL-10, and IL-4], and gene expression for some transcription factors regulating the differentiation of Th1, Th2, Th17, and Treg cells [e.g., forkhead box protein P3 (FoxP3), GATA-3, retinoic acid-related orphan receptor gamma t (RORγt), and T-box expressed in T cells (T-bet)] were assessed. The obtained data showed that crocin at the dose of 60 mg/kg decreased disease severity as well as demyelination and inflammation in the brain and spinal cord tissue of animal models of EAE. Crocin could also repress the production of pro-inflammatory cytokines and enhance the formation of anti-inflammatory cytokines in lymph nodes and splenocytes from an animal model of the disease. Other results attained from the quantitative polymerase chain reaction (qPCR) method indicated the capability of crocin to attenuate Th1/Th17 cells-related transcription factors (i.e., RORγt and T-bet) and promote Th2/trans-regulatory elements (Tre)-related transcription factors (i.e., FoxP3 and GATA-3). Furthermore, bromodeoxyuridine (BrdU) test performed on lymph node cells and splenocytes manifested the suppression of T cell proliferation following this natural compound-based therapy.[42] $CD4^+$ T cells exert a vital role in autoimmunity development. According to the pattern of formed cytokine and transcription factor expression, at least four main subcategories have been mentioned for these cells, including Th1, Th2, Treg, and Th17 cells.[43,44] In EAE models, it has been explained that Th1 and Th17 lineages promote autoimmunity in the CNS by increasing inflammation. In contrast, Th2 and Treg cells play a pivotal role in maintaining immune tolerance and regulating the destructive self-reactive T cells associated with MS.[45] Methodological limitations for this work include a small sample size, short-term follow-up, and the lack of a sham group without disease induction. Additionally, genetic homogeneity of animals and observer bias in the clinical scoring of paralysis are confounding factors influencing the findings of this study. However, some of these factors were controlled through standardization of animal housing and baseline disease homogenization (only animals with approved EAE induction were included).

Overall, this study confirmed that crocin could switch immune reactions from pro-inflammatory (Th1/Th17) to anti-inflammatory (Th2/Treg) profiles, attenuating CNS damage in MS.

Clinical evidence: Several clinical trials have been performed in order to demonstrate the anti-MS capacities of crocin in patients with this neurodegenerative ailment, and accordingly, promising outcomes have been published.

As an example, Ghiasian et al. have scrutinized the impacts of crocin-based therapy on deoxyribonucleic acid (DNA) damage,

inflammatory parameters, and oxidative impairments in a double-blind, randomized, and placebo-controlled study with 40 RRMS cases assigned to two groups (20 received crocin, 20 received placebo).[23] The patients were given capsulated crocin (30 mg/day) for 28 days. At the start of the study, there were no remarkable differences between the crocin and placebo groups in terms of total antioxidant capacity (TAC), malondialdehyde (MDA), total thiol group (TTG), TNF-α, IL-17, and DNA damage ($P > 0.05$).[23] Following 28 days of crocin therapy, there was a significant decrease in the levels of DNA damage, IL-17, TNF-α, and lipid peroxidation (LPO) and a dramatic elevation of TAC in the serum sample of crocin-treated cases compared with the placebo group ($P < 0.05$). However, after the mentioned treatment period, no significant differences statistically were observed between crocin and placebo groups ($P > 0.05$).[23]

The limitations of this scientific project are related to a small sample size, short period of treatment, single-center study, and placebo influences, given that the placebo group exhibited an unexpected decrease in DNA damage and LPO, showing that undetermined or psychological factors affected the outcomes.

Potential bias of the study includes selection bias (the studied population was Iranian cases, restricting ethnic diversity), performance bias (adherence to crocin administration was not reported), and possible publication bias (neutral or negative results regarding crocin therapy were not efficiently discussed, like TTG changes). Concerning confounding factors affecting outcomes of the study, lack of details about continuing or not continuing DMTs (e.g., glatiramer acetate and IFNs), affecting the results of inflammatory and oxidative markers, lack of control or report about dietary antioxidants, physical activity, and smoking, influencing oxidative stress, and disease heterogeneity due to differences in disease severity and relapse history are more critical. However, randomization and blinding, balanced demographic features, and standardized protocols for assessing biomarkers are a number of approaches of this work to control confounding factors. In short, this research accentuated the alleviating role of crocin therapy in patients with RRMS by attenuating oxidative stress (via TAC and MDA enhancement) and inflammatory mediators (i.e., IL-17 and TNF-α).

A similar study used this natural treatment with the same dose (30 mg/day for 28 days) and appraised the oxidant and antioxidant levels in the saliva and urine samples of 20 patients with MS. The evaluation of saliva samples implied that this therapy significantly decreased LPO levels and raised the function of catalase (CAT), TAC, and TTG at the end of the determined therapeutic plan ($P < 0.05$). However, there were no significant differences in terms of the variables of LPO, CAT, TAC, and TTG in the placebo group. Moreover, the assessments of urine samples indicated a remarkable reduction in the level of LPO and a considerable increment in the level of TTG and TAC following crocin prescription ($P < 0.05$). In the placebo group, TAC was significantly elevated in the urine samples of cases, but the levels of TTG and CAT did not change.[46] The limitations of this clinical investigation encompass a short period of treatment, small sample size ($n = 40$), placebo influences (unpredicted TAC elevation in the urine samples of the placebo group), and unmanaged confounding factors (e.g., lifestyle, diet, and not reporting existing therapeutic regimens related to MS). However, organized demographic features, randomization and blinding, and standardized approaches for evaluating biomarkers were helpful strategies for decreasing confounding factors. Collectively, this study emphasized the antioxidative effects of crocin in patients with MS by regulating the saliva and urine levels of oxidative and antioxidative agents.

Kouchaki et al. have noted the impacts of crocin therapy on mental health status (i.e., anxiety and depression), oxidative stress parameters [i.e., nitric oxide (NO) and MDA], and an inflammatory biomarker, high-sensitivity C-reactive protein (hs-CRP), in subjects with MS. The sample size of this research was 50 (25 cases in the placebo group and 25 cases in the crocin group). The therapeutic plan for the crocin group was accomplished by prescribing crocin at a dosage of 15 mg tablet, two times per day (30 mg total per day), for 8 weeks. The reported data from this clinical trial revealed that the administration of crocin tablets for 8 weeks significantly reduced anxiety ($P = 0.01$) and hs-CRP ($P = 0.01$) compared with the placebo group (Table 1). Despite this, the serum levels of NO and MDA did not change following administration of the mentioned therapeutic regimen.[47] Taken together, this study highlighted the suitability of crocin in MS therapy by inhibiting inflammatory mediators and promoting mental health in patients with MS.

Table 1. Experimental and clinical documents regarding the therapeutic effects of crocin on multiple sclerosis (MS)

Author	Study design (experimental vs. clinical)	Sample size	Dose/ concentration	Target	Key findings
Tashakori et al.[22]	Experimental	40 male C57BL/6 mice	100 mg/kg	TAS, GPx, and SOD	Decreasing immobility time in TST and FST ($P < 0.05$) Restoring SOD, GPx, MDA, and TAS in the serum and brain tissue ($P < 0.05$) Lowering detrimental effects related to CPZ on immobility time ($P < 0.05$)
Fatemi et al.[39]	Experimental	50 female Wistar rats	5, 10, and 20 mg/kg	NGF and BDNF	Increasing the hot TFL time in the groups receiving crocin, fingolimod + crocin, and fingolimod significantly ($P < 0.05$, $P < 0.001$, and $P < 0.05$, respectively) Elevating balance status in the crocin group not statistically significant ($P > 0.05$)
Pazoki et al.[42]	Experimental	26 mice	20 and 60 mg/kg	T cell, TNF-α, IL-6, and IL-17	Reducing paralysis severity significantly following crocin therapy compared with the control group ($P < 0.001$) Reducing inflammation and demyelination significantly following crocin therapy compared with the control group ($P < 0.001$) Repressing T cell proliferation significantly following crocin therapy compared with the control group ($P < 0.001$)
Ghiasian et al.[23]	Clinical	40 patients	30 mg/day	IL-17, TNF-α, LPO, and TAC	Decreasing LPO levels significantly following 28 days of crocin therapy compared with the placebo group ($P < 0.05$) Decreasing IL-17 and TNF-α levels significantly following 28 days of crocin therapy compared with the placebo group ($P < 0.05$) Attenuating oxidative stress-conferred DNA damage significantly following 28 days of crocin therapy compared with the placebo group ($P < 0.05$)
Ahmadi et al.[46]	Clinical	40 patients	30 mg/day	TAC, LPO, CAT, and TTG	Considerable differences in terms of LPO, TAC, and TTG markers following 28 days of crocin therapy ($P < 0.05$)
Kouchaki et al.[47]	Clinical	50 patients	30 mg/day	Hs-CRP	Decreasing hs-CRP levels significantly following crocin therapy for 8 weeks ($P = 0.01$) Reducing anxiety significantly after crocin therapy ($P = 0.01$)
Rezaeimanesh et al.[48]	Clinical	60 patients	Capsule of Cor@SeNs comprising 55 mg selenium and 5.74 mg crocin	TAC	The intervention with Cor@SeNs therapy revealed a significant and increasing effect over time on CVLT-II (effect size: 0.29, $P < 0.01$), SDMT (effect size: 0.18, $P < 0.01$), and CVLT-II-delay (effect size: 0.29, $P < 0.01$). The mentioned intervention had a significant but reducing effect on GR activity in both intervention and placebo groups (effect size: 0.20, $P < 0.01$).

GPx: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; TAC: Total antioxidant capacity; TST: Tail suspension test; FST: Forced swimming test; NGF: Nerve growth factor; BDNF: Brain-derived neurotrophic factor; Hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; IL-17: Interleukin-17; TNF-α: Tumor necrosis factor-alpha; TAS: Total antioxidant status; LPO: Lipid peroxidation; TTG: Total thiol group; GR: Glutathione reductase; CVLT-II: California Verbal Learning Test-Second Edition; Cor@SeNs: Crocin-selenium nanoparticles; DNA: Deoxyribonucleic acid; CPZ: Cuprizone; MDA: Malondialdehyde; SDMT: Symbol Digit Modalities Test; TFL: Tail flick latency

The limitations of this work include a small sample size, short periods of treatment, insufficiently evaluated biomarkers, and homogeneity of included cases. Further, one of the main biases of this work was related to the mental health outcome that was obtained self-reportedly, which could be affected by expectations or perceptions. Moreover, confounding factors comprise diverse medications, lifestyle and diet, and environmental and genetic factors. Among these, some confounding factors were monitored by randomization, double-blinding, and comparing baseline features (e.g., gender, age, and disease duration).

Interestingly, a randomized, triple-blinded, placebo-controlled clinical research (2024), for the first time, recommended an alternative formulation for crocin in the treatment of MS. In this clinical project, 60 subjects with MS participated (assigned to two groups, including the intervention and placebo groups). In this work, crocin-selenium nanoparticles (Cor@SeNs), including 5.74 mg crocin and 55 mcg selenium, were administered within 12 weeks for subjects with MS, and serum levels of oxidative biomarkers [MDA, glutathione reductase (GR) activity, and TAC] and cognitive function were monitored by means of the Persian version of the Brief International Cognitive Assessment for MS (BICAMS) battery. The outcomes pointed out that there was a significant elevation in the time effects of Cor@SeNs-based therapy on the Symbol Digit Modalities Test (SDMT) ($P < 0.01$), California Verbal Learning Test-Second Edition (CVLT-II)-delay ($P < 0.01$), and the CVLT-II ($P < 0.01$) in comparison with the placebo group. Besides, the time effect of the mentioned therapeutic strategy on the function of GR in both groups was significantly decreased ($P < 0.01$). In addition, the significant promotion of TAC in the intervention group and cognitive function in both groups was reported. It is worth mentioning that the serum levels of antioxidant parameters and scores related to cognitive function in the intervention group increased compared with the placebo group; however, these differences were not statistically significant. The methodology-related limitations of this project are mainly a small sample size, a short period of therapy, homogeneity of the studied population, and placebo impacts, considering the cognitive improvement observed in both groups. The probable biases of this study can be selection bias (variations in the levels of physical activity and cognitive scores at baseline despite randomization), measurement bias (self-report assessment of cognitive function), and attrition bias (more dropouts in the placebo groups). Confounding factors encompass diverse medications, physical activity (higher baseline physical activity in the placebo group), variability in the subtypes of MS, and lifestyle factors. However, randomization and blinding, matching groups in terms of baseline characterization (e.g., gender, age, and disease duration) were a number of strategies for managing such factors.

In summing up, this study underscored that the usage of this nano-based formulation of crocin ameliorated cognitive function and TAC.[48]

The current status and future perspectives

The present preclinical data have proven the neuroprotective and anti-inflammatory potential of crocin in animal models of MS, for example, toxin-caused demyelination and EAE. As an example, in mice with EAE, crocin therapy at the dose range of 20-60 mg/kg could decrease clinical severity, demyelination, and inflammation, and orchestrate cytokine profiles and T-cell reactions. Likewise, in CPZ-established models, doses of 100 mg/kg of crocin promoted motor behavior and the capacity of the ADS. Despite these, translating these beneficial effects to human studies faces challenges. The current performed clinical trials were double and triple-blinded, randomized, and placebo-controlled trials evaluating 40-60 patients with MS and utilizing 15-30 mg/kg doses of crocin [equivalent to 187.5-375 mg/kg in animal studies (mouse species)]. One of the main challenges for translating preclinical outcomes to clinical trials is related to the heterogeneity between animal and human models of the disease. Preclinical research exploited homogeneous and induced models of the disease that followed certain features of MS without exhibiting the difficulties of disease subtypes in humans and comorbidities. On the contrary, clinical studies scrutinized heterogeneous populations of patients in terms of disease stages and concomitant therapies, which may conceal the therapeutic influences of crocin. Moreover, dissimilar to animal studies that can use high doses to attain effectiveness, human studies are dose-limited mainly due to toxicity worries, revealing a pharmacological mismatch. Diversity in measures of findings further obscures translation. Experimental evidence is chiefly noticed on mechanistic aspects, such as modulating pro-inflammatory (e.g., IL-17, IL-6,

and IFN-γ) and anti-inflammatory (e.g., TGF-β, IL-10, IL-4) cytokines and antioxidant parameters (i.e., TAS, GPx, and SOD), improving neurotrophins (BDNF) and related growth factors (NGF), promoting behavioral and motor improvements, and addressing histopathological benefits, including attenuation of demyelination and CNS inflammation. Clinical assessments are aligned with these results and have focused on patient-oriented outcomes, like serum parameters and cognitive scores. Another important issue about crocin therapy in MS in clinical assessments is to note its probable interaction with other administered drugs. Regarding this matter, there are still limited data. However, considering that crocin can modulate immune system function, it may interact with immunosuppressive drugs used in MS cases, such as mitoxantrone and azathioprine.[42,49,50] Reportedly, saffron and its compounds, such as crocin, may also inhibit key cytochrome P450 (CYP) enzymes (e.g., CYP2E1, CYP3A4, and CYP1A1/2), thereby influencing drug metabolism. This phenomenon could change drug efficacy.[51] Therefore, more clinical trials on crocin therapy for MS are required before considering it a merit treatment.

Conclusion

MS, as an autoimmune and neurological devastating condition, affects a great number of people globally, and the suggestion of an effective therapeutic strategy against it has been a puzzle. These days, harnessing natural compounds, especially natural carotenoids, has been in the spotlight of researchers to treat various diseases like MS. Among these, crocin, having considerable therapeutic and pharmacological capacities, has shown a striking role in the treatment of this neurological disorder. Crocin's mechanisms are not yet fully proven to modify MS progression; however, there is promising evidence for the possible effectiveness of crocin in patients with MS. The results of experimental investigations have accentuated this useful role by expressing the potential action of crocin in improving reflexive motor behavior-related assays (e.g., HFS, HLFA, GS, NG, and SR), depressive-like behavior, antioxidant parameters (e.g., TAS, GPx, and SOD), the release of neurotrophins (i.e., NGF and BDNF), cognitive indices, the conduction velocity of the motor and sensory nerve, and tail flick latency (Table 1). In addition to alleviating some mental health factors like anxiety, the outcomes of clinical assessments have manifested that this carotenoid agent can ameliorate oxidative stress and inflammation-related markers, as evidenced by decreasing the levels of DNA damage, IL-17, TNF-α, hs-CRP, and LPO and increasing the function of TAC, CAT, and TTG. However, there are some limitations regarding the current research, especially the small sample size, short follow-up periods, placebo influences, and homogeneity of the studied population. Hence, although there is promising evidence regarding the anti-MS potential of crocin, high-quality and more in-depth clinical trials are still required. It is suggested that larger and multicenter randomized clinical trials, assessing the long-term safety and efficacy and optimal dosing strategies, be performed to validate these findings and decipher the therapeutic capacities of crocin against MS.

The real-world clinical application of crocin in this neurological disease warrants further consideration. For example, challenges related to design of suitable formulations and bioavailability (poor oral bioavailability owing to fast metabolism and low stability) as well as accessibility and affordability (given that the cost of formulation of crocin, as a saffron-derived compound, may be high) are among the main subjects that should be assumed for wide utilization of crocin in the clinical practice. Therefore, after solving these issues, it can be concluded that crocin therapy can be regarded as a possible therapeutic candidate for patients with MS.

Conflict of Interests

The authors declare no conflict of interest in this study.

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References

1. Garton T, Gadani SP, Gill AJ, Calabresi PA. Neurodegeneration and demyelination in multiple sclerosis. Neuron 2024; 112(19): 3231-51.
2. Al-Kuraishy HM, Jabir MS, Al-Gareeb AI, Saad HM, Batiha GE, Klionsky DJ. The beneficial role of autophagy in multiple sclerosis: Yes or No? Autophagy 2024; 20(2): 259-74.
3. Barrie W, Yang Y, Irving-Pease EK, Attfield KE, Scorrano G, Jensen LT, et al. Elevated genetic risk for multiple sclerosis emerged in steppe pastoralist populations. Nature 2024; 625(7994): 321-8.
4. Liu R, Du S, Zhao L, Jain S, Sahay K, Rizvanov A, et al. Autoreactive lymphocytes in multiple sclerosis: Pathogenesis and treatment target. Front

Immunol 2022; 13: 996469.
5. Maroufi H, Mortazavi SH, Sahraian MA, Eskandarieh S. Environmental risk factors of multiple sclerosis in the Middle East and North Africa region: A systematic review. Curr J Neurol 2021; 20(3): 166-84.
6. Brown SP. Diagnosis of Cervical Spinal Cord Multiple Sclerosis by a Chiropractic Physician: A Case Report. Cureus 2024; 16(6): e62618.
7. Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. Am J Med 2020; 133(12): 1380-90.e2.
8. ArefNezhad R, Motedayyen H, Mohammadi A. Therapeutic Aspects of Mesenchymal Stem Cell-Based Cell Therapy with a Focus on Human Amniotic Epithelial Cells in Multiple Sclerosis: A Mechanistic Review. Int J Stem Cells 2021; 14(3): 241-51.
9. Mirabella M, Annovazzi P, Brownlee W, Cohen JA, Kleinschnitz C, Wolf C. Treatment Challenges in Multiple Sclerosis - A Continued Role for Glatiramer Acetate? Front Neurol 2022; 13: 844873.
10. Babaesfahani A, Patel P, Bajaj T. Glatiramer. [Updated 2024 Feb 28]. In: StatPearls [Online]. Treasure Island, FL: StatPearls Publishing; 2025. Available from URL: https://www.ncbi.nlm.nih.gov/books/NBK541007/.
11. Andrade S, Nunes D, Dabur M, Ramalho MJ, Pereira MC, Loureiro JA. Therapeutic Potential of Natural Compounds in Neurodegenerative Diseases: Insights from Clinical Trials. Pharmaceutics 2023; 15(1): 212.
12. Tancreda G, Ravera S, Panfoli I. Exploring the Therapeutic Potential: Bioactive Molecules and Dietary Interventions in Multiple Sclerosis Management. Curr Issues Mol Biol 2024; 46(6): 5595-613.
13. Yazdanpanah E, Dadfar S, Shadab A, Orooji N, Nemati M, Pazoki A, et al. Berberine: A natural modulator of immune cells in multiple sclerosis. Immun Inflamm Dis 2024; 12(3): e1213.
14. Álvarez-Martínez FJ, Díaz-Puertas R, Barrajón-Catalán E, Micol V. Plant-Derived Natural Products for the Treatment of Bacterial Infections. Handb Exp Pharmacol 2025; 287: 265-93.
15. Ali A, Yu L, Kousar S, Khalid W, Maqbool Z, Aziz A, et al. Crocin: Functional characteristics, extraction, food applications and efficacy against brain related disorders. Front Nutr 2022; 9: 1009807.
16. Rekabi A, Ram A, Nazari A, Arefnezhad R, Rezaei-Tazangi F. Does crocin create new hope for the treatment of oral problems? A focus on periodontitis. Mol Biol Rep 2024; 51(1): 224.
17. Veisi A, Akbari G, Mard SA, Badfar G, Zarezade V, Mirshekar MA. Role of crocin in several cancer cell lines: An updated review. Iran J Basic Med Sci 2020; 23(1): 3-12.
18. Bastani S, Vahedian V, Rashidi M, Mir A, Mirzaei S, Alipourfard I, et al. An evaluation on potential anti-oxidant and anti-inflammatory effects of Crocin. Biomed Pharmacother 2022; 153: 113297.
19. Naimi H, Khazaei M, Sharifnia F, Hassanian SM, Sayyed-Hosseinian SH. Crocin elicits potent anti-inflammatory and fibrinolytic properties post tendon injury, A new molecule for adhesion therapy. J Tradit Complement Med 2024; 14(6): 687-96.
20. Azari H, Ebrahimi S, Saeb S, Ghanbari A, Peyravian F, Mokaram 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.
21. Su W, Wang Y, Shao S, Ye X. Crocin ameliorates neuroinflammation and cognitive impairment in mice with Alzheimer's disease by activating PI3K/AKT pathway. Brain Behav 2024; 14(5): e3503.
22. Tashakori A, Hassanpour S, Vazir B. Protective effect of crocin on cuprizone-induced model of multiple sclerosis in mice. Naunyn Schmiedebergs Arch Pharmacol 2023; 396(8): 1713-25.
23. Ghiasian M, Khamisabadi F, Kheiripour N, Karami M, Haddadi R, Ghaleiha A, et al. Effects of crocin in reducing DNA damage, inflammation, and oxidative stress in multiple sclerosis patients: A double-blind, randomized, and placebo-controlled trial. J Biochem Mol Toxicol 2019; 33(12): e22410.
24. Shah A, Panchal V, Patel K, Alimohamed Z, Kaka N, Sethi Y, et al. Pathogenesis and management of multiple sclerosis revisited. Dis Mon 2023; 69(9): 101497.
25. Lopez JA, Denkova M, Ramanathan S, Dale RC, Brilot F. Pathogenesis of autoimmune demyelination: from multiple sclerosis to neuromyelitis optica spectrum disorders and myelin oligodendrocyte glycoprotein antibody-associated disease. Clin Transl Immunology 2021; 10(7): e1316.
26. Amoriello R, Memo C, Ballerini L, Ballerini C. The brain cytokine orchestra in multiple sclerosis: from neuroinflammation to synaptopathology. Mol Brain 2024; 17(1): 4.
27. Filippi M, Preziosa P, Banwell BL, Barkhof F, Ciccarelli O, De Stefano N, et al. Assessment of lesions on magnetic resonance imaging in multiple sclerosis: practical guidelines. Brain 2019; 142(7): 1858-75.
28. Lassmann H. Multiple Sclerosis Pathology. Cold Spring Harb Perspect Med 2018; 8(3): a028936.
29. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 2000; 47(6): 707-17.
30. Jafarzadeh Bejargafshe M, Hedayati M, Zahabiasli S, Tahmasbpour E, Rahmanzadeh S, Nejad-Moghaddam A. Safety and efficacy of stem cell therapy for treatment of neural damage in patients with multiple sclerosis. Stem Cell Investig 2019; 6: 44.
31. Yousefi F, Lavi Arab F, Saeidi K, Amiri H, Mahmoudi M. Various strategies to improve efficacy of stem cell transplantation in multiple sclerosis: Focus on mesenchymal stem cells and neuroprotection. J Neuroimmunol 2019; 328: 20-34.
32. Rahmati M, Ghannadian SM, Kasiri N, Ahmadi L, Motedayyen H, Shaygannejad V, et al. Modulation of Th17 Proliferation and IL-17A Gene Expression by Acetylated Form of Apigenin in Patients with Multiple Sclerosis. Immunol Invest 2021; 50(2-3): 216-29.
33. Sedaghat N, Motedayyen H, Alsahebfosoul F, Etemadifar M, Ostadi V, Kianpour F, et al. Increased Expression of Lymphocyte Activation Gene-3 by Regulatory T Cells in Multiple Sclerosis Patients with Fingolimod Treatment. Turk J Immunol 2019; 7(1): 31.
34. Motedayyen H, Rezaei A, Zarnani AH, Tajik N. Human amniotic epithelial cells inhibit activation and pro-inflammatory cytokines production of naive CD4+ T cells from women with unexplained recurrent spontaneous abortion. Reprod Biol 2018; 18(2): 182-8.
35. McLaughlin KA, Wucherpfennig KW. B cells and autoantibodies in the pathogenesis of multiple sclerosis and related inflammatory demyelinating diseases. Adv Immunol 2008; 98: 121-49.
36. Rissanen E, Tuisku J, Vahlberg T, Sucksdorff M, Paavilainen T, Parkkola R, et al. Microglial activation, white matter tract damage, and disability in MS. Neurol Neuroimmunol Neuroinflamm 2018; 5(3): e443.
37. Datta G, Colasanti A, Rabiner EA, Gunn RN, Malik O, Ciccarelli O, et al. Neuroinflammation and its relationship to changes in brain volume and white matter lesions in multiple sclerosis. Brain 2017; 140(11): 2927-38.
38. Lassmann H, van Horssen J, Mahad D. Progressive multiple sclerosis: pathology and pathogenesis. Nat Rev Neurol 2012; 8(11): 647-56.
39. Fatemi R, Moghaddam HF, Farbod Y, Beygtashkhani R. Effects of Crocin on brain neurotrophins, cognition, balance and pain in toxic-induced demyelination model. Acta Neurol Taiwan 2024; 33(2): 48-59.
40. Hua LH, Hersh CM, Morten P, Kusel J, Lin F, Cave J, et al. The Impact of Price Reductions After Loss of Exclusivity in a Cost-Effectiveness Analysis: Fingolimod Versus Interferon Beta-1a for the Treatment of Relapsing-Remitting Multiple Sclerosis. J Manag Care Spec Pharm 2019; 25(4): 490-8.
41. Hooshyari Ardakani M, Nosengo C, Felletti S, Catani M, Cavazzini A, De Luca C, et al. Enhancing the purification of crocin-I from saffron through the combination of multicolumn countercurrent chromatography and green solvents. Anal Bioanal Chem 2024;

416(10): 2553-64.
42. Pazoki A, Askaripour M, Zargarani S, Yazdanpanah E, Haghmorad D. Crocin as a potential therapeutic agent for multiple sclerosis: insights from experimental autoimmune encephalomyelitis model in mice. Immunopharmacol Immunotoxicol 2025; 47(1): 131-9.
43. Sonar SA, Lal G. Differentiation and Transmigration of CD4 T Cells in Neuroinflammation and Autoimmunity. Front Immunol 2017; 8: 1695.
44. Pawlak M, Ho AW, Kuchroo VK. Cytokines and transcription factors in the differentiation of CD4(+) T helper cell subsets and induction of tissue inflammation and autoimmunity. Curr Opin Immunol 2020; 67: 57-67.
45. Mirzaei R, Zamani F, Hajibaba M, Rasouli-Saravani A, Noroozbeygi M, Gorgani M, et al. The pathogenic, therapeutic and diagnostic role of exosomal microRNA in the autoimmune diseases. J Neuroimmunol 2021; 358: 577640.
46. Ahmadi SA, Kazemi A, Sabahi M, Razipour S, Salehipour A, Ghiasian M, et al. Probable antioxidant therapy of Saffron Crocin in patients with multiple sclerosis: A randomized controlled trial. Biomedicine 2020; 40(4): 516-21.
47. Kouchaki E, Rafiei H, Ghaderi A, Azadchehr MJ, Safa F, Omidian K, et al. Effects of crocin on inflammatory biomarkers and mental health status in patients with multiple sclerosis: A randomized, double-blinded clinical trial. Mult Scler Relat Disord 2024; 83: 105454.
48. Rezaeimanesh N, Rafiee P, Saeedi R, Khosravian P, Sahraian MA, Eskandarieh S, et al. The effect of crocin-selenium nanoparticles on the cognition and oxidative stress markers of multiple sclerosis patients: a randomized triple-blinded placebo-controlled clinical trial. Biometals 2024; 37(2): 305-19.
49. Foo EC, Russell M, Lily O, Ford HL. Mitoxantrone in relapsing-remitting and rapidly progressive multiple sclerosis: Ten-year clinical outcomes post-treatment with mitoxantrone. Mult Scler Relat Disord 2020; 44: 102330.
50. Nonino F, Baldin E, Ridley B, Casetta I, Iuliano G, Filippini G. Azathioprine for people with multiple sclerosis. Cochrane Database Syst Rev 2021; 2021(7): CD015005.
51. Bathaei P, Imenshahidi M, Vahdati-Mashhadian N, Hosseinzadeh H. Effects of Crocus sativus and its active constituents on cytochrome P450: a review. Naunyn Schmiedebergs Arch Pharmacol 2025; 398(9): 11407-20.