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Article

Effects of Crocin on brain neurotrophins, cognition, sensory and motor dysfunction in rats demyelination with Ethidium bromide

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Abstract: Objective: The purpose of this study was to investigate the effects of Crocin on brain neuroterophins, cognition, sensory and motor dysfunction and compare to fingolimod effects in experimental model of demyelination with Ethidium Bromide EB in female Wistar rats. **Methods**: Animals were assigned in to 8 groups; Sham, Sham operated (ShOp), EAE, crocin treated (Cr5,10,20 mg/kg), Vehicle, Fingolompd (Fing) and fingolimod + crocin (Cr+Fing). Demyelination was induced by single dose injection of 10 µl of EB 0.1% into the fourth ventricle of the brain. Crocin and fingolimod were applied for 21 days, daily, oral gavage. BDNF, NGF1, nerve conduction velocities, tail flake latency, balance and behavioral variables were sampled and analyzed by paired t-test and ANOVA test with repeated post hoc measurements. **Results*:*** The results showed that crocin improves all studied factors, but remarkable imrovments were observed in dosage of 10 mg/kg. Crocin (10mg/kg) and fingolimod (1mg/kg) significantly improved cognition variables in open field test, sensory and motor nerve conduction velocity, tail flick latency and clinical signs ($p<005$). In addition, applying of crocin co-administered with fingolimod led to significant increases in all assessed factors, greater than crocin or fingolimod intervention alone ($\alpha \leq 0.001$). **Conclusion**: Based on the current findings, crocin can improve the level of brain neurotrophins, exploratory behavior and nerve conduction after demyelination as close as fingolimod results. So, crocins can be considered as a neuro supportive agent in the management of degenerative diseases maybe similar to fingolimod mechanism.

Keywords*:* Crocin; Multiple scleroses; Cognition; BDNF; NGF; Demyelination

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), and is more prevalent in women than men. MS is caused by axonal degeneration which leads to demyelinating lesions within the white matter of the brain and spinal cord [1,2] The etiology of this lesion is not characterized completely but studies have shown that inflammatory infiltrates containing few auto reactive T cells and a multitude of pathogenic non-specific mononuclear cells occur in areas of demyelination, axonal loss and severe glial scarring.[1,3] Contributing to axonal pathology, in an autoimmune mechanism T-lymphocytes specific, for myelin antigens, start an inflammatory attack causing degeneration of CNS axons, causing disseminated demyelinating lesions accompanied by axonal degeneration [2].

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MS causes various sorts of damage such as cognitive impairment which affects about half of patients with this disorder [3]. Studies have reported that learning and remembering, short term memory and spatial memory are affected by demyelination [3-5]. Neurotrophins such as Brain BDNF and NGF are polypeptides belonging to the neurotrophic factor family. In the adult CNS, neurotrophins play a protective role towards specific neuronal populations [1]. According to the findings, serum neurotrophic and neurodevelopment levels, which play a major role in cognitive function, are impaired in patients with MS. Patients with MS also experience motor and sensory impairments. Due to the destruction in the myelin sheet of the PNS neurons, the velocity of nerve impulses become slower and delivers with some delay. Studies have shown impaired motor and sensory nerve conduction velocities (NCV) in MS cases [5].

One of the natural-based sources substances that have been widely proved as an effective agent in nervous related disorders is *Crocus Satilvus* L., crocin (*Freom Saffron*). Saffron and its active components such as crocin, reveals many positive effects in the brain, including antioxidant, anti-inflammation, anticonvulsant and antitumor actions [6]. Crocin also significantly increases the CREB, p-CREB, BDNF, and VGF protein expressions in the rat hippocampus [7]. Further studies have shown that crocin and crocetin, as active ingredients of saffron, are able to affect a variety of drug defenses, such as protection against cardiovascular disease, inhibition of cancer cell proliferation, as well as gastrointestinal, neural and liver cell protection. Anti-inflammatory and analgesic actions are attributed to the antioxidant capacity of this compound [8].

2. Materials and methods

2.1. Animals

Fifty six 4–6-month-old female Wistar rats with a body weight of 250±50 g were maintained on a 12 h light/12 h dark cycle, at a temperature of 25 ◦C, with free access to food and water were used to study. In standard cages complying with standard laboratory conditions such as temperature, humidity, light cycle for one week before starting experiments. All procedures for this research were approved by AJUMS ethical committee (IR.AJUMS.REC.1395.110), in accordance with the internationally accepted principles for the care and use of laboratory animals. The timeline schema of study design is presented in figure 1.

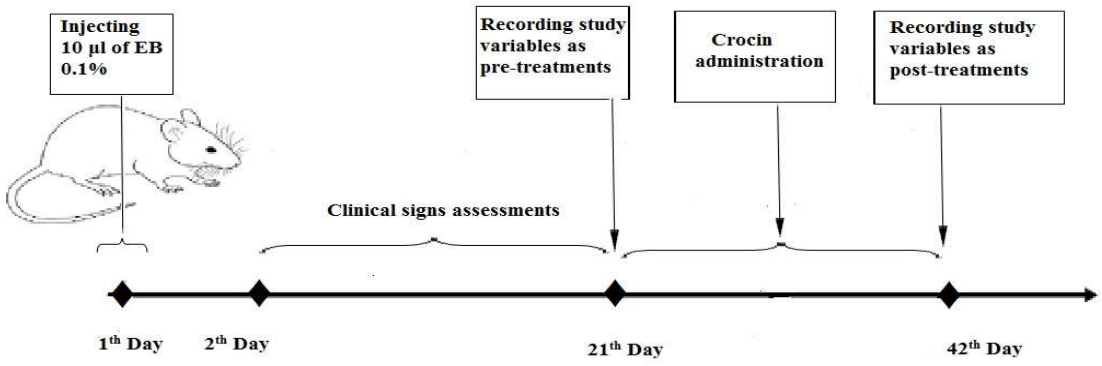

Figure 1. The timeline schema of the experiment. Rats initially immunized with injection of 10 µl of EB 0.1% and were assessed for recording clinical symptoms to day 21, then, the pre-treatment values of study variables were recorded. Crocin administration (100 mg/kg per animal, gavage) was done from day 21 to day 42. Then post-treatment values were recorded again (EB, Etidium bromide).

2.2. EAE induction (Immunization with ethidium bromide)

In order to induce the animal model of demyelination, direct injection of ethidium bromide into the fourth ventricle of the rat brain was performed. In the present study,

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injections of 10 µl of EB 0.1% into the fourth ventricle was used to induce demyelination.[9,10] In this method, animals are initially anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) then placed in a stereotaxic device. The body temperature was maintained at about 35.5 to 39.8. Then, by creating a longitudinal gap in the posterior part of the head, the skull surface appears with two points referring to Brigma and Lambda. After specifying fourth ventricle according to Paksinus Atlas coordination, the spot was marked and by cautious use of a dental drill, a hole was drilled into the animal's skull bilaterally to inject EB without any damage to the brain tissue. Then 10 µl (5 µl for left side, 5 µl for right side) of ethidium bromide 0.1%, diluted 10 mg/ml in phosphate buffer saline (PBS) [3,4] was then injected slowly into the fourth ventricle using a Hamilton syringe. After the solution is injected, the wound was sealed and sutured and the animal was transported to a separate cages. To determine the effects of EB, the animals were evaluated daily and demyelination symptoms were observed. The scoring of symptoms was done according to the Naghashpour et al. (2016), [9] using the 10 score system as follows: 0, no clinical disease; 0.5, partial tail paralysis; 1.0, complete tail paralysis; 1.5, complete tail paralysis and discrete hind limb weakness; 2.0, complete tail paralysis and strong hind limb weakness; 2.5, unilateral hind limb paralysis; 3, complete hind limb paralysis; 3.5, hind limb paralysis and forelimb weakness; 4.0, complete paralysis (tetraplegia); 5.0: moribund or dead (See figure 2). Then, those animal that had score 3 (not very weak to care and do experimental, not very healthy).

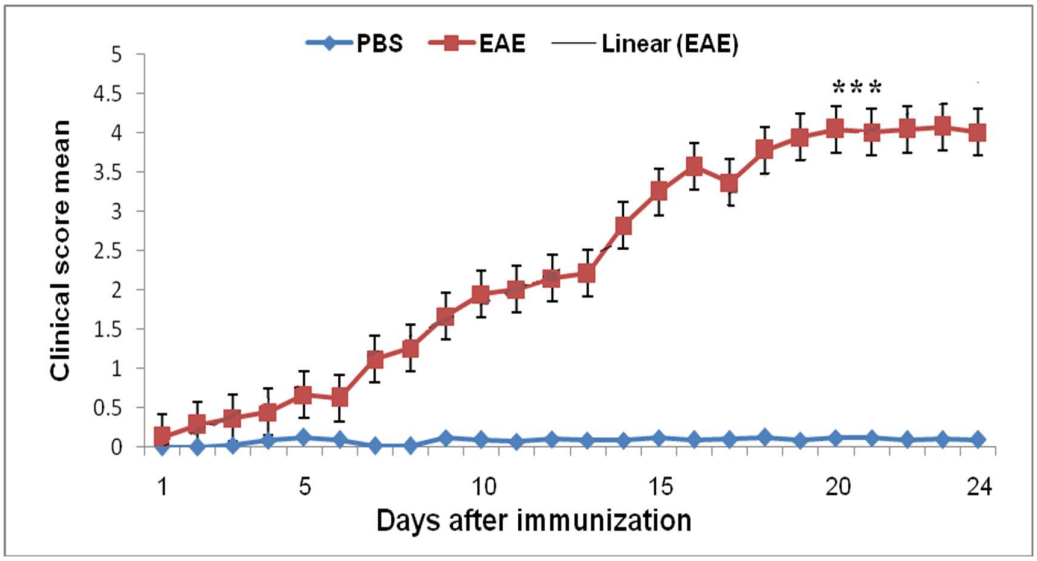

Figure 2. Effects of ethidium bromide on mean clinical scores in EAE rats. Data are presented as Mean±SEM and analyzed by Independent t-test. At the 21th day after injections, animals that injected with EB showed significant higher clinical scores compared with PBS injected group. *EAE: Experimental Autoimmune Encephalomyelitis, EB: Ethidium bromide, PBS: Phosphate buffer saline, ***: p <0.001 compared to PBS injected group (n=6).*

2.3. Sensory & motor nerve conduction velocity

For measurement of motor nerve conduction velocity (MNCV), the Power Laboratory 8 sp was used. Animals were anaesthetized at first and then MNCV was measured by stimulating the sciatic (proximal to sciatic notch) and tibial (distal to the ankle) nerves using bipolar needle (gauge) electrodes with a 3 V single stimulus. The MNCV was calculated using the following formula: MNCV = (distance between sciatic and tibial nerve stimulation point)/ (sciatic M wave latency - tibial M wave latency) [5,10].

2.4. Tail flick latency

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The latency in tail flick was assessed using tail immersion tests. Tails were immersed in cold (10 Cº) or warm (45 Cº) water and the tail flick response latency (withdrawal of the tail) was taken as the end-point response as described previously [11].

2.5. Crocin and fingolimod administration

Crocin (Cat Number, 17304) and fingolimod (FTY720, Cat Number, SML0700) were provided in the pure powder form the Sigma Aldrich Co, USA. They were diluted 1 mg/ml in PBS and crocin was administrated 5, 10 and 20 mg/kg (as it was applied in previous studies) and 1 mg/kg fingolimod of body weight for each animal [12,13]. For providing co-administration of fingolimod+ crocin dilute, 1 mg/kg fingolimod and 5 mg/kg crocin (as the lower dose) were applied and given to each animal in same time to other interventions. The intervention was given for 21 days, once a day, to each animal orally one day after EB injection. It was noted that before administration of crocin and fingolimod, the pre-treatment values of study variables were recorded. In this period, the animals were sacrificed and their brains were isolated, homogenized and maintained at -70º refrigerator for next analyzing.

2.6. Open field test

This test is used to assess behavioral responses such as motor activity, hyperactivity and expletory behavior, as well as anxiety measurement. The sex, dimensions, shape and color of the device are different depending on the protocol. In the current protocol, the device contains a box of 40 (With 72 × 72 cm × 72 × 72 cm) wood is black in color. The device's cups are virtually unmatched by software through squares and lattice lines to 25 equal areas (Maze router). The open field area is divided into two areas of the environment and center. Usually middle 9 squares are considered as the central area and the remaining squares are considered as marginal area. The increase in time spent and the number of entry into the center is considered as a like anti-anxiety effect, as well as the center drawn squares are used to evaluate the expletory behavior. The total distance traveled and the average speed covered by animal are considered as an indicator of motor activity. After half an hour after injection, the animal is randomly placed slowly on one of the four corners of the device and then is allowed to cover the environment freely for a period of 5 minutes. During this time, the mouse's behavior is visible through an automated follow-up system. After the test, the mice were transported to their cages, and the floor and walls of the machine are chemically cleaned with 70% impregnated cotton to prevent the effects of the remaining odor from the previous animal and allowed to dry in each test [14].

2.7. Balance assessment in Rotarod test

The rotarod device was used to evaluate the balance and muscles coordination. To assess balance activity, animals was placed on a horizontal rotating spindle bar whose initial speed was 5 rpm and gradually increased to 45 rpm for 300 seconds. The time that animals could maintain balance and stay on the bar was recorded for each animal. At first, each animal was given an opportunity twice to adapt to the device, then, the animal was placed on the device three times and the average time was calculated and recorded as the balance score for each animal.

2.8. BDNF & NGF1 assay

BDNF & NGF1 and IL-10 concentrations were measured using BDNFand NGF1 ELISA Kits for rat provided from Sigma Aldrich Co. USA [15].

2.9. Statistical methods

All data are presented as Mean±SEM. The mean of data for each group in any phase was analyzed. Data were analyzed using the paired t-test for assessing the differences between pre-treatment and post-treatment values of each group and ANOVA followed

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by the LSD post hoc test for analyzing the post-treatment values of each factor between all groups. Values of $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Effects of crocin on brain neurotrophins

Figure 3 shows comparison of pre & post-treatment values of BDNF and NGF1 after 21 days. A: As shown in figure 3,A, crocin led to increase in the level of BDNF after 21 days, but only administration of crocin with dosage of 10 mg/kg showed significant increases ($p<0.01$). In addition, fingolimod and combination of crocin and filgolimod also resulted in significant increases in BDNF levels after administration ($p<0.001$). Figure 3, B shows comparison of the post-treatment concentrations of BDNF. As shown in figure 4.AB, there are significant differences in mean concentrations of BDNF between Sham group (without any intervention) and EAE group (injected by EB, treated by PBS) ($p<0.001$), between EAE group and Cr 10 (treated with Crocin 10mg/kg) ($p<0.001$), between EAE group and fing group (treated with fingolimod 1mg/kg) ($p<0.001$), between EAE group and Cr+Fing group (treated with fingolimod & Crocin 10mg/kg) ($p<0.001$). There are also significant differences between Cr+Fing and crocin groups ($p<0.01$), between Cr+Fing and fing groups ($p<0.05$).

C: As shown in figure 3,C, crocin led to increase in the level of NGF1 after 21 days, but only administration of crocin with dosage of 10 mg/kg showed significant increases ($p<0.01$). In addition, fingolimod and combination of crocin and filgolimod also resulted in significant increases in NGF1 levels after administration ($p<0.001$). Results of figure 3,D, shows that there are significant differences in mean concentrations of NGF1 between Sham Operated (injected by PBS) and EAE (injected by EB, treated by PBS) groups ($p<0.001$), between EAE and Cr 10 (treated with Crocin 10mg/kg) group ($p<0.001$), between EAE and fing groups (treated with fingolimod 1mg/kg) ($p<0.001$), between EAE group and Cr+Fing group (treated with fingolimod & Crocin 10mg/kg) ($p<0.001$). There are also significant differences between Cr+Fing and crocin groups ($p<0.05$).

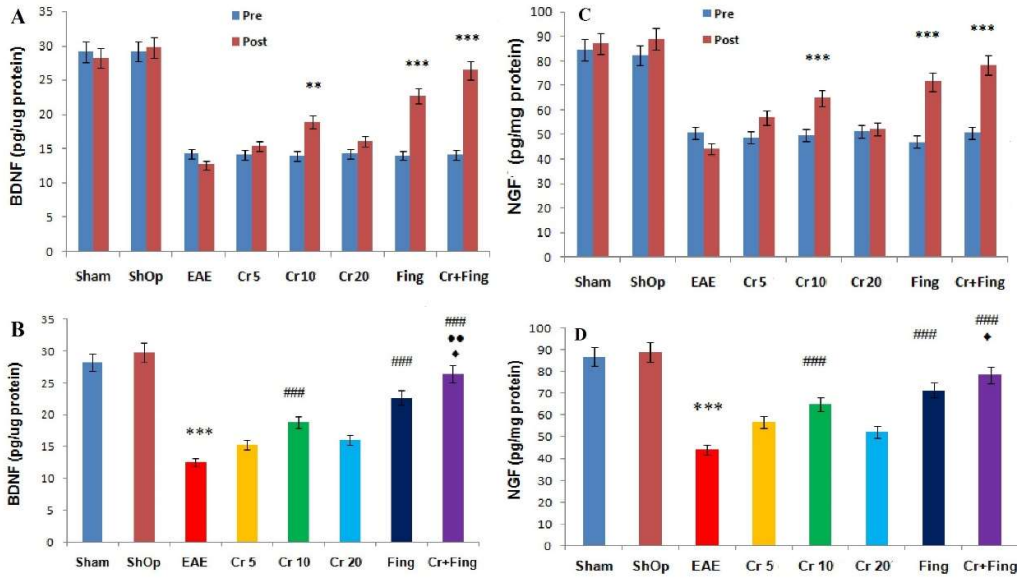

Figure 3. Effects of crocin on BDNF (A) and NGF1 (B) in whole brain tissue after 21 days with gavage administration in Wistar rats using Enzyme-Linked Immunosorbent Assay (ELISA). Data are presented as Mean±SEM and analyzed by Paired t-test. *BDNF: Brain Derived Neurotrophine Factor, NGF1: Nerve Growth Factor 1, Cr: Crocin, Fing: Fingolimod, ShOp: Sham Operated, EAE: Experimental Autoimmune Encephalomyelitis, Cr+Fing: Crocin + Fingolimod, *: p <0.05, **: p <0.01, ***: p <0.001 compared to pre-treatment values (n=7).*

3.2. Effects of crocin on sensory & motor nerve conduction velocity

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Figure 4 shows the results of ANOVAs for comparison of sensory nerve conduction velocity (SNCV) and motor nerve conduction velocity (MNCV) between all groups after 21 days administration of crocin. A: a significant reduction in SNCV was observed in the EAE (vehicle treatment) group when compared with sham and sham operated groups ($p<0.001$). Compared to the EAE (vehicle treatment), significant increases in SNCV were observed in animals that were treated by Crocin 10 & 20 mg/kg groups ($p<0.05$), Fingolimod treated group ($p<0.05$) and also in the crocin + fingolimod treated group ($p<0.001$).

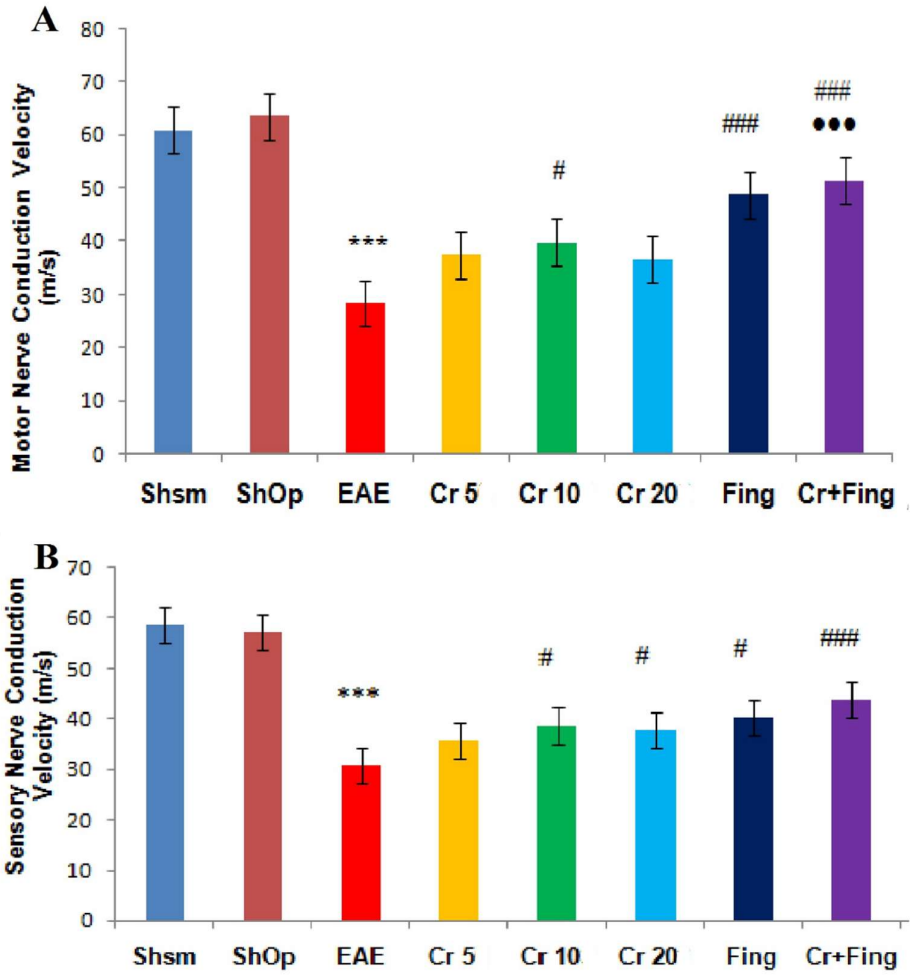

Figure 4. Effects of crocin on motor and sensory nerve conduction velocity after 21 days with gavage administration in Wistar rats. Data are presented as Mean±SEM and analyzed by ANOVA with LSD test. *Cr: Crocin, Fing: Fingolimod, ShOp: Sham Operated, EAE: Experimental Autoimmune Encephalomyelitis, Cr+Fing: Crocin + Fingolimod, ***: p <0.001 compared to Sham, #, ###: Significant difference at p<0.05, p<0.001 respectively compared to the EAE group, ●●●: Significant difference p<0.001 compared to the Cr10 group, (n=7).*

As shown in figure 4.B, compared to the sham and sham operated groups, significant reduction in MNCV was observed in the EAE (vehicle treated) group ($p<0.05$). Compared to the EAE (vehicle treated) group, significant improvements in MNCV were observed in the Crocin 10 mg/kg treated group ($p<0.001$), in the fingolimod treated group ($p<0.001$) and also in the crocin + fingolimod administration group ($p<0.001$). Furthermore, combination of the crocin + fingolimod showed significant increases in MNVC when compared to the Crocin 10 alone ($p<0.001$).

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3.3. Effects of crocin on tail flack latency

Results of ANOVAs for comparison of tail flick latency (TFL) time between all groups after 21 days of crocin administration are shown in figure 5. A: compared to sham group, animals in EAE (vehicle treated) group showed significant reduction in the hot TFL time ($p<0.001$). In comparison with EAE group with vehicle treatment, animals showed significant improvements in the Crocin 10 mg/kg group ($p<0.001$), in the fingolimod treated group ($p<0.001$) and also in the fingolimod + crocin treated group ($p<0.001$). In addition, there were remarkable increases in the crocin + fingolimod group when compared to the Crocin 10mg/kg group ($p<0.01$) or the fingolimod treated group ($p<0.01$).

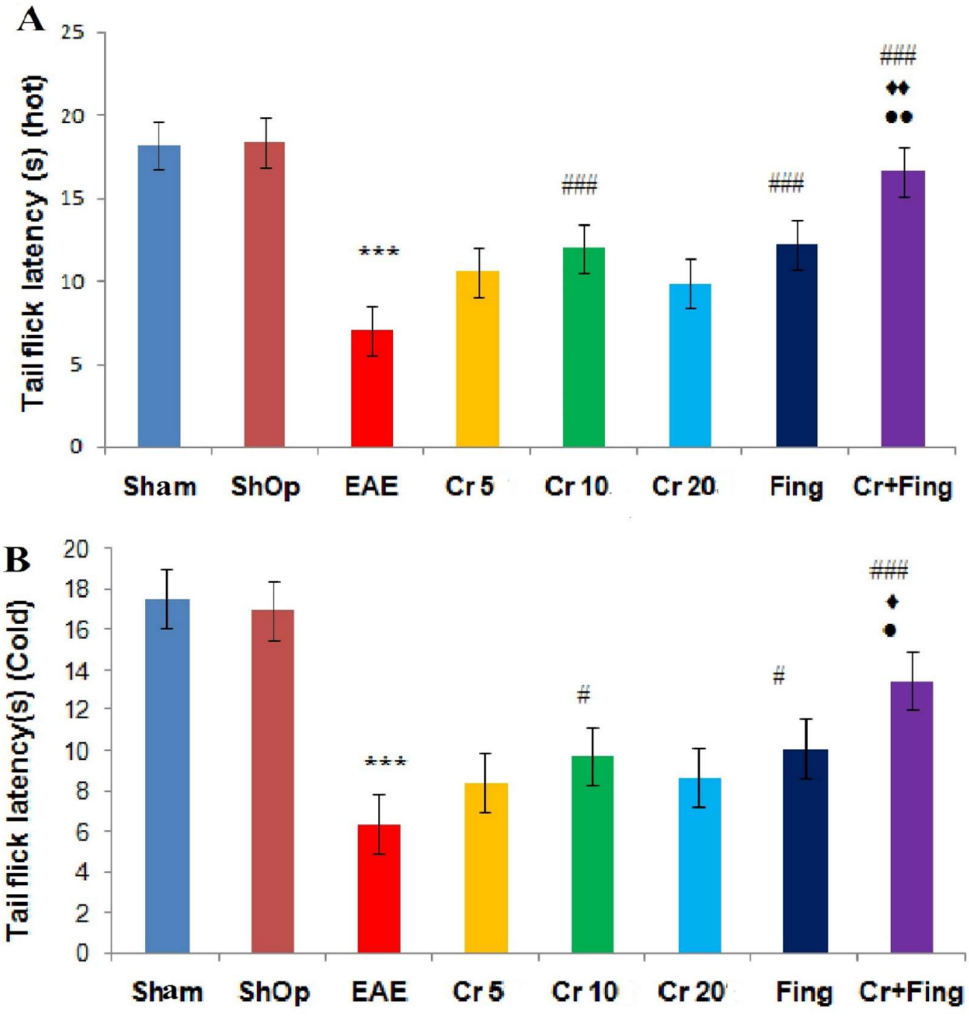

Figure 5. Effects of crocin on hot (A) and cold (B) tail flick latency (TFL) after 21 days with gavage administration in Wistar rats. Data are presented as Mean±SEM and analyzed by ANOVA with LSD test. *Cr: Crocin, Fing: Fingolimod, ShOp: Sham Operated, EAE: Experimental Autoimmune Encephalomyelitis, Cr+Fing: Crocin + Fingolimod, ***: p <0.001 compared to Sham, #, ###: Significant difference at p<0.05, p<0.001 respectively compared to the EAE group, ●, ●●: Significant difference p<0.05 and p<0.001 compared to the Cr10 group, ♦, Significant difference p<0.05 compared to the Fing group, (n=7).* .

Figure 5.B shows cold TFL time results. According to the figure 7.B, animals showed significant reduction in hot TFL time when compared to EAE (vehicle treated) group ($p<0.001$). Also, compared to EAE (vehicle treated) group, animals in Crocin 10mg/kg group, fingolimod group and crocin + fingolimod group showed significant elevation in the hot TFL time ($p<0.05$, 0.05 and 0.001 respectively). Furthermore, addition of crocin

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with fingolimod (Cr+Fing group) led to significant improvement in the hot TFL time when compared with fingolimod alone ($p<0.05$).

3.4. Effects of crocin on locomotion and exploratory behavior

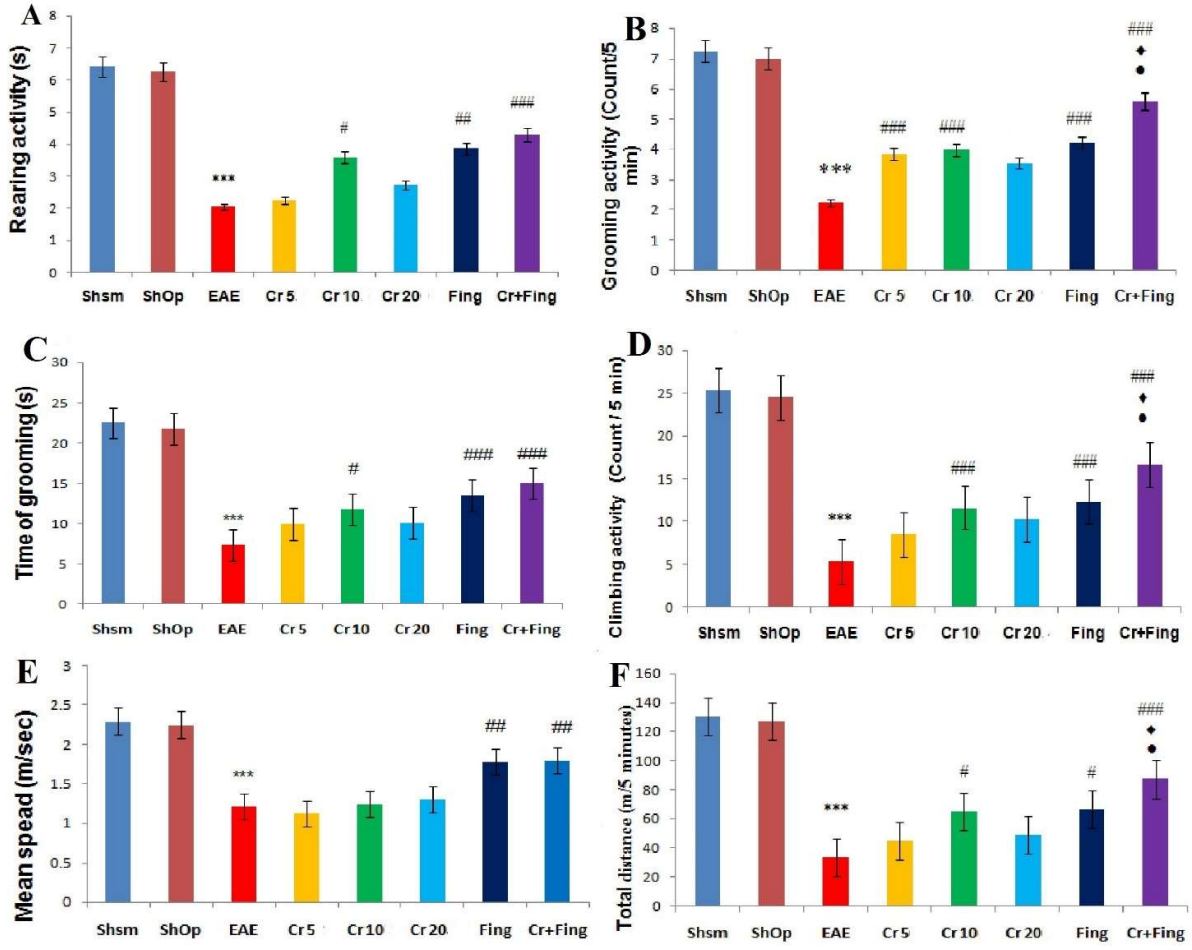

Figure 6. Effects of 21 days of crocin with gavage administration on learning and memory behaviors of animals in Open field test. A, rearing activity; B, grooming activity; C, Climbing activity; D, total distance moved; E, time of grooming; F, mean of speed in Wistar rats. Data are presented as Mean±SEM and analyzed by ANOVA with LSD test. *Cr: Crocin, Fing: Fingolimod, ShOp: Sham Operated, EAE: Experimental Autoimmune Encephalomyelitis, Cr+Fing: Crocin + Fingolimod, ***: p <0.001 compared to Sham, #, ###: Significant difference at p<0.05, p<0.001 respectively compared to the EAE group, ●: Significant difference p<0.05 compared to the Crocin10 group, ♦, Significant difference p<0.05 compared to the Fingolimod group, (n=7).*

Figure 6 shows the results of animals' locomotion and memory in open field (OP) test in 6 items; compared to sham and sham operated groups, animals in EAE (vehicle treated) group showed significant reduction in all 6 items of OP test ($p<0.001$). A, compared to EAE group with vehicle treatment, animals showed significant improvements in rearing activity in the Crocin 10 mg/kg group ($p<0.05$), in the fingolimod treated group ($p<0.01$) and also in the fingolimod + crocin treated group ($p<0.001$). B, in grooming activity factor, significant improvements were observed in crocin 5, 10 & 20 mg/kg group ($p<0.05$), in the fingolimod treated group ($p<0.001$) and also in the fingolimod + crocin treated group ($p<0.001$). Furthermore, co-administration of crocin with fingolimod led to significant better improvements when compared to fingolimod or crocin administration alone ($p<0.05$). C, in climbing activity, animals showed significant better performance in the Crocin 10

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mg/kg group (p<0.001), in the fingolimod treated group (p<0.001) and also in the fingolimod + crocin treated group (p<0.001). In addition, co-administration of crocin with fingolimod led to significant better improvements when compared to fingolimod or crocin administration alone (p<0.05).

D, in total distance covered, compared to EAE vehicle treated group, animals showed better results in the Crocin 10 mg/kg group (p<0.05), in the fingolimod treated group (p<0.05) and also in the fingolimod + crocin treated group (p<0.001). Also, co-administration of crocin with fingolimod led to significant greater covered distance when compared to fingolimod or crocin administration alone (p<0.05). E, in time of grooming factor, compared with EAE vehicle treated group, none of administrated doses of crocin lead to any significant improvements (p>0.05), however, a small increases were observed in crocin administration groups. F, in mean speed factor, also none of administrated doses of crocin lead to any significant improvements compared with EAE vehicle treated group (p>0.05).

3.5. Effects of crocin on balance status

As shown in figure 7, A, paired t-test results for comparison of pre and post-treatment scores of animals balance at post intervention phase showed that crocin improved balance scores of EAE rats, but it was no statistically significant (p>0.05).

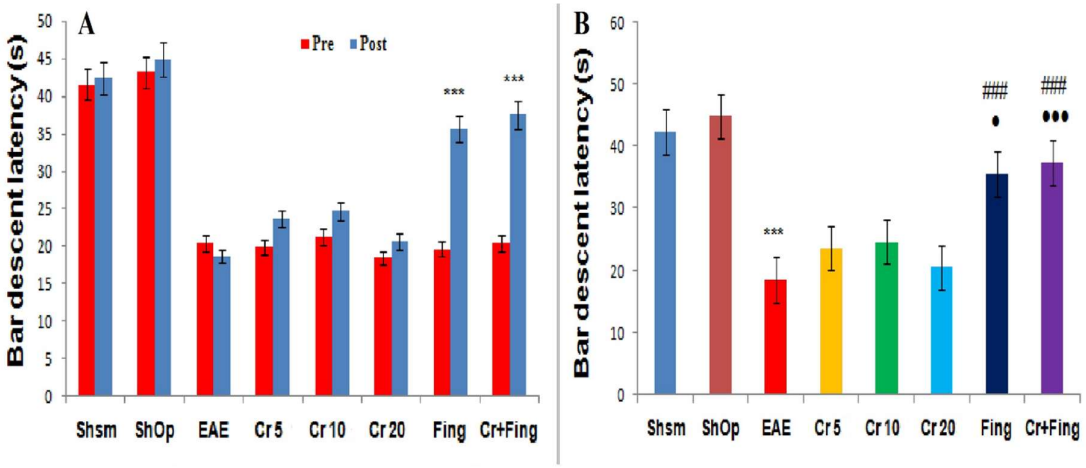

Figure 7. Effects of 21 days of crocin with gavage administration on balance status of animals in rotarod test. A, comparison of pre and post-treatment results of balance scores for each group, B, comparison of post-treatment balance scores between all groups. Data are presented as Mean±SEM and analyzed by *paired t- test (A) and* ANOVA with LSD test (B). *Cr: Crocin, Fing: Fingolimod, ShOp: Sham Operated, EAE: Experimental Autoimmune Encephalomyelitis, Cr+Fing: Crocin + Fingolimod, ***: p <0.001 compared to Sham groip, ###: Significant difference at p<0.001 compared to the EAE group, ●, ●●●: Significant difference p<0.05, p<0.001 compared to the Crocin10 group, (n=7).*

Comparison of balance scores between all groups are shown in figure 7.B. result showed that animals showed significant reduction in EAE vehicle treated group when compared to sham and sham operated groups (p<0.001). However, crocin increased balance status of the animals, but the increases were not statistically significant (p>0.05). In addition, the balance scores were increased significantly in the fingolimod treated group (p<0.01) and also in the fingolimod + crocin treated group (p<0.001). Furthermore, compared to Crocin 10, significant differences were observed in fingolimod treated group (p<0.05) and in crocin + fingolimod treated group (p<0.001).

4. Discussion

We investigated the effects of crocin (5, 10 & 20 mg/kg per animal) for 21 days after experimental autoimmune encephalomyelitis (EAE). We also administrated fingolimod

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(1 mg/kg per animal) and co-administration of crocin (10mg/kg) and fingolimod (1 mg/kg) in different groups for comparing fingolimod effects with crocin administration only. Results showed that although all applied doses of crocin led to improvements in studied variables, but the best results were observed in Crocin 10 mg/kg groups.

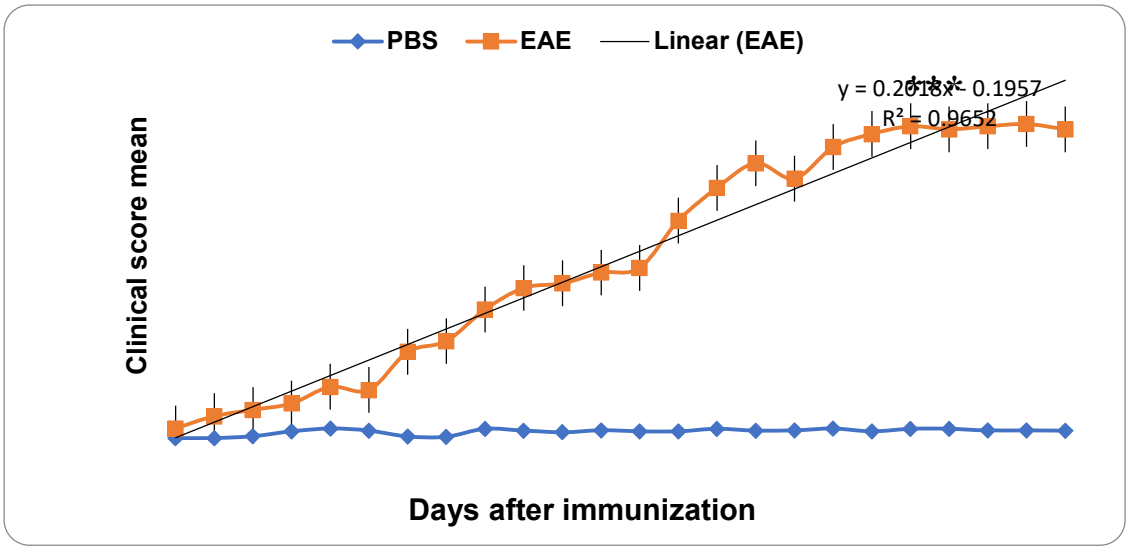

Figure 8. Effects of ethidium bromide on mean clinical scores in EAE rats. Data are presented as Mean±SEM and analyzed by Independent t-test. At the 21th day after injections, animals that injected with EB showed significant higher clinical scores compared with PBS injected group. *EAE: Experimental Autoimmune Encephalomyelitis, EB: Ethidium bromide, PBS: Phosphate buffer saline, ***: p <0.001 compared to PBS injected group (n=6).*

Regarding to the effects of crocin on neurotrophins, our findings showed that the level of brain BDNF and NGF1 increased significantly after intervention. Administration of fingolimod alone was also increased the level of brain BDNF and NGF1 significantly, but the effects were remarkable when it was administrated couple with crocin.

Some a few studies are available that have reported positive effects of crocin on neurotrophic factors in demyelination diseases which support our results. Farkhondeh et al. (2018) showed that crocin enhances secretion of BDNF in the hippocampus by its effect on the hippocampal electrical ignition of epilepsy [16]. Some studies have reported that long term administration of crocin significantly increases the BDNF and VGF protein expressions in the rat hippocampus as an antidepressant mediator [17,18]. In MS cases, the level of brain NGF1 decreases significantly and it can be affected by crocin which is presumed to have direct effects [19].

It has been reported that chronic administration of crocin results in increasing the level BDNF mRNA in the rat hippocampus similar to serotonergic agents [20]. These findings are also supported by Ghasemi et al. (2015) findings [21]. However, a study stated that the increase in BDNF mRNA after long term administration of crocin is not observed in rat cerebellum [17].

Our findings also showed significant improvements in exploration behavior and memory of EAE rats in open field test after long term administration of crocin. Like to the neurotrophins, administration of Crocin 10 mg/kg with fingolimod showed the best results in exploration behavior of EAE rats compared with application of crocin or fingolimod alone. In this regard, studies have shown that short or long term administration of crocin can reverse the cognitive deficits caused by neurodegenerative diseases [22-25]. Khalili & Hamzeh (2010) demonstrated that short term administration of crocin (30 mg/kg) antagonizes the passive avoidance paradigm and spatial cognition deficits in Y-

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maze task caused by Alzheimer's disease and stated its potential in the treatment of neurodegenerative diseases [14]. They stated that effectiveness of crocin is associated with mitigated cholinergic pathway and its antioxidant action.

It was reported that cognitive deficits in neurodegenerative diseases is highly related to the impairments in brain neurotrophins [19].The lower levels of neurohrophic factors in MS cases compared with normal subjects is also proven [15].

Previous studies have shown the elevation in VGF protein and mRNA levels after implication of crocin or saffron extract [20,21]. It is reported that VGF mRNA level is highly affected by BDNF and the radical role of VGF protein and mRNA in neuronal survival and plasticity, which plays a vital role in control of emotional and behavioral responses [26]. According to Deslauriers et al. (2011), neuroinflammation and endoplasmic reticulum stress are coregulated by crocin to prevent demyelination and neurodegeneration. They also stated that crocin protects oligodendrocytes exposed to cytotoxic supernatants derived from Syncytin-1expressing astrocytes. In addition, compared with healthy subjects, EAE animals shows increases in the transcript levels of the ER stress genes XBP-1/s, BiP, PERK, and CHOP in spinal cords during disease progression and most of them are suppressed by crocin on day 7 [15].

The findings of the present study also showed that motor and sensory nerve conduction velocity and tail flick latency improved by crocin co-administrated with fingolimod significantly more than other interventions. However, administration of crocin or fingolimod alone also led to significant enhancements in both MNCV and SNCV. Sarova et al. (1995) showed no relationship between NCV findings and disability among MS patients [27]; while Misawa et al. (2008) have reported that 74% of people sufferers from MS had at least one abnormality of the electrodiagnostic parameters that was not shown to be related to age, sex, and duration of the disease. However the protein compositions of peripheral and central myelin are different, but they share some similar proteins such as MBP and MAG, so, autoimmune response of body against a common antigen may cause myelin degeneration in both CNS and PNS [5]. It has been shown that peripheral blood T-cell activity in MS showed that the number of clonally expanded T-cell receptor increases accompanied by an increase in the expanded disability status scale (EDSS) which demonstrated PNS demyelination can occurs in line with CNS degeneration [28].

Our results showed increased both MNCV and SNCV of EAE animals after treatment with crocin. Decreased nerve conduction velocities are highly related to increased activity of nitric oxide putative actions at the level of EDHF synthesis release, gap junction integrity, transduction and K+-channel modulation in damaged neurons.[18] Furthermore, elevated levels of oxidants, ROs, free radicals are occurred in MS lesions that can affect the nerve conduction. Crocin is proven as potent antioxidant agent [22,29], so, the enhancements of NCV couldn be related to the anti-oxidant action of crocin by reacting with ROS, such as H2O2 and inhibited nitric oxide synthesis [16].

The clear mechanism related to the crocin effectiveness is not recognized yet, however, anti-oxidant and anti inflammatory role of crocin is considered as the main mechanism of crocin effectiveness on neurodegenerative diseases. It may play this role via binding with serum albumin, transferring to the cell and change in the spatial structure of the cell [30]. Also crocin may mediate in Ach releasing and inhabitation of Ach receptors by suppressing signaling of NF-κB/Yin and up-regulating the expression of CX3CR1 in such lesions [31]. Cholinergic anti-inflammatory pathway through reduction in lymphocyte infiltration of the CNS is also reported as related mechanism of crocin action [32]. Crocin also specifically antagonizes the inhibitory effect of ethanol on N-methyl-d-aspartate (NMDA) receptor-mediated responses in hippocampal neurons [6]. Further studies are now required to determine the clear role of the crocin on the mechanism of underlying changes in neurotrophin levels and cognition and its likely effectiveness as a treatment for MS and related disorders

4. Conclusion

Based on the current findings, administration of crocin in EB immunized rats leads to improve in brain neurothrophins levels, cognition, learning, sensory and motor impairments. Also, administration of crocin with doses 5, 10 and 20 mg/kg, the best results are contributed to the dosage of 10 mg/kg. In addition, the main intresting finding of our research was observed where co-administration of crocin with fingolimod led to better results in some variables when compared to administration of fingolimod alone. Furthermore, administration of crocin could show up great amount of the positive results of the fingolimod that is one of the main interesting findings of our research.

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