



Conjugated Linoleic Acid-Curcumin Attenuates Cognitive Deficits and Oxidative Stress Parameters in the Ethidium Bromide-Induced Model of Demyelination

Behnaz Barzegarzadeh¹ · Homeira Hatami¹ · Gholamreza Dehghan¹ · Nazli Khajehnasiri² · Mehdi Khoobi³ · Reihaneh Sadeghian^{4,5}

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Abstract

Oxidative stress has been shown to play an important role in the pathogenesis of multiple sclerosis (MS). Curcumin (CUR), an antioxidant compound, can be a potent treatment for neurodegenerative diseases, such as MS. CUR has poor bioavailability; therefore, it is used in nanoforms to increase its bioavailability. In the present study, the effects of CUR and conjugated linoleic acid-CUR (Lino-CUR) on spatial memory and oxidative stress in a putative animal model of MS were investigated. Forty-nine adult male Wistar rats (250 ± 50 g) were randomly divided into seven groups ($n = 7$): control, sham, ethidium bromide (EB), CUR (20 and 40 $\mu\text{g}/\text{kg}$) + EB, and Lino-CUR (20 and 40 $\mu\text{g}/\text{kg}$) + EB groups. Following MS induction, the groups were treated for 5 consecutive days. Finally, spatial memory and levels of oxidative stress parameters were assessed. Treatment with CUR and Lino-CUR at two doses significantly improved spatial memory and reduced oxidative stress parameters in the experimental models of MS. Furthermore, the effects of high dose (40 $\mu\text{g}/\text{kg}$) of Lino-CUR were more remarkable. These findings suggest that the microinjection of CUR in its synthetic form Lino-CUR significantly ameliorated spatial memory, through the reduction of oxidative stress markers in the brain of studied animals as a rat model of MS.

Keywords Multiple sclerosis · Curcumin · Linoleic acid-curcumin · Spatial memory · Oxidative stress

Introduction

Multiple sclerosis (MS) is a demyelinating and inflammatory disease of the central nervous system (CNS) (Höftberger and Lassmann 2017). The etiology of the MS has not yet been well understood; however, demyelination, axonal damage, and

neuronal death have been reported in MS patients (van Meeteren et al. 2005). Cognition deficits are one of the symptoms of MS that results in the hippocampus (with a major role in learning and memory) insults (Nakatomi et al. 2002).

Oxidative stress results from an imbalance between free radical production and antioxidant defense (Liguori et al. 2018). Oxidative stress and immune-mediated inflammation are important mechanisms involved in oligodendrocyte damage, demyelination, and cognition deficits (Gilgun-Sherki et al. 2004). Previous studies have demonstrated some changes in the cerebrospinal fluid (CSF) and brain tissue levels of several antioxidants in MS patients (Hunter et al. 1985). Therefore, antioxidant therapy may help restore these deficiencies and support the antioxidant defense capacity.

Curcumin (diferuloylmethane; CUR) is derived from rhizomes of *Curcuma longa*. It is a low molecular weight antioxidant that can pass the blood-brain barrier (BBB) easily (Van Horsen et al. 2008) and in addition to its antioxidant effect, CUR has many biological effects, including neuroprotective, anti-carcinogenic, anti-inflammatory, and anti-infectious properties (Elsayed 2016).

✉ Homeira Hatami
h.hatami@tabrizu.ac.ir; Homeira.Hatami9@gmail.com

¹ Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran

² Department of Biological Sciences, Faculty of Basic Sciences, Higher Education Institute of Rab-Rashid, Tabriz, Iran

³ Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁴ Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

⁵ Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

CUR is a hydrophobic molecule; thus, it penetrates the cell membrane directly (Joe et al. 2004). Previous studies on animals and humans showed that CUR is not toxic to humans even at high doses (Commandeur and Vermeulen 1996). Despite the mentioned properties of CUR, it has a poor bioavailability (Goel et al. 2008). There are some methods to moderate CUR rapid metabolism and improve its biological half-life; for example, it is used in hybrid with some other substances (Maiti et al. 2007).

It should be noted that linoleic acid belongs to the polyunsaturated fatty acids (PUFAs) with an antioxidant effect. PUFAs have are effective in lipid peroxidation (LPO) and cognition in neurodegenerative diseases (Elharram et al. 2017). No study has yet been conducted on the effects of Lino-CUR and CUR on memory in an animal model of MS. Accordingly, this study aimed at investigating the effects of Lino-CUR, a synthetic compound of CUR, and CUR on the levels of oxidative stress parameters and spatial memory in the rat's brain as an animal model of MS.

Materials and Methods

Chemicals and Equipment

Phosphate-buffered saline (PBS) and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich Chemical Co., Ltd. (USA). CUR and thiobarbituric acid (TBA) were obtained from Merck Co. (Germany). Ethidium bromide (EB) was purchased from Sinaclon Co. (Iran). Dimethyl sulfoxide (DMSO) was prepared from Daejung Chemicals & Metals Co Ltd. (Korea).

Lino-CUR

Lino-CUR was synthesized at the Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences. The general procedure for the preparation of complex substance, Lino-CUR (4-(1Z,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-1-yl)-2-methoxy phenyl (9Z,12Z)-octadeca-9,12-dienoate), was as follows: a mixture of CUR (1 mmol), linoleic acid (1 mmol), dimethylaminopyridine (1 mmol), and 1-ethyl-3-(3-dimethylaminopropyl carbodiimide) (1 mmol) in 5 mL acetone were mixed overnight. After completion of the reaction, the mixture was evaporated and the crude product purified by a silica column chromatography (the yield was an oily product and 50%). The results of the nuclear magnetic resonance (NMR) test are shown in Figs. 1 and 2.

Animals and Treatment

Forty-nine adult male Wistar rats (250 ± 50 g) were obtained from the Pasteur Institute (Tehran, Iran). All rats were housed in standard plexiglass cages, under the controlled temperature (23 ± 2 °C) and a light-dark cycle (lights on 07:00–19:00). The animals had free access to food and water. Seven days after adaptation, the rats were randomly divided into seven groups ($n = 7$ per group): (1) control, (2) sham (DMSO/PBS 1% w/w; intracerebroventricular (ICV)): receiving 1% DMSO in PBS (4 μ L/day) bilaterally for five consecutive days; (3) EB (an animal model of MS): a single injection of 0.1% EB in saline (4 μ L bilaterally; ICV) for the induction of demyelination; (4) and (5) EB + CUR (20 and 40 μ g/kg): ICV injection of CUR for 5 consecutive days followed by receiving the same volume of EB; (6) and (7) EB + Lino-CUR (20 and 40 μ g/kg): ICV injection of Lino-CUR for 5 consecutive days followed by receiving the same volume of EB.

The experimental procedures were confirmed by the ethics committees of the Shahrekord University of Medical Sciences and conducted following the Guide for Care and Use of Laboratory Animals (NIH Publication No.: 85-23, revised: 1985).

Surgical Procedures for ICV Injection

For ICV injection, rats were anesthetized with ketamine (100 mg/kg, intraperitoneal (i.p.)) plus xylazine (10 mg/kg, i.p.), and placed in a stereotactic apparatus (Stoelting, USA) and the scalp removed. Then, the lateral ventricle position was drilled according to the Paxinos and Watson atlas (0.6 mm posterior to the bregma, 1.6 mm lateral to the midline, and 4.0 mm below the outer surface of the skull) in both lateral ventricles. The stainless-steel cannulas were implanted through the guide cannula. The cannulas were held in position by dental cement and two stainless steel screws were inserted in the skull (Shahidi et al. 2019).

Experimental Design

Morris Water Maze Test

The Morris water maze (MWM) test is commonly used to assess spatial cognition, or more generally, learning and memory in experimental rodent models. The water maze consisted of a black circular pool with a diameter of 130 cm and a height of 50 cm filled with water (22 ± 2 °C). The maze was divided into four equal quarters and the release points were designated in each quarter as north, east, south, and west. A hidden circular platform (12 cm in diameter) was placed in the center of the southwest quadrant and

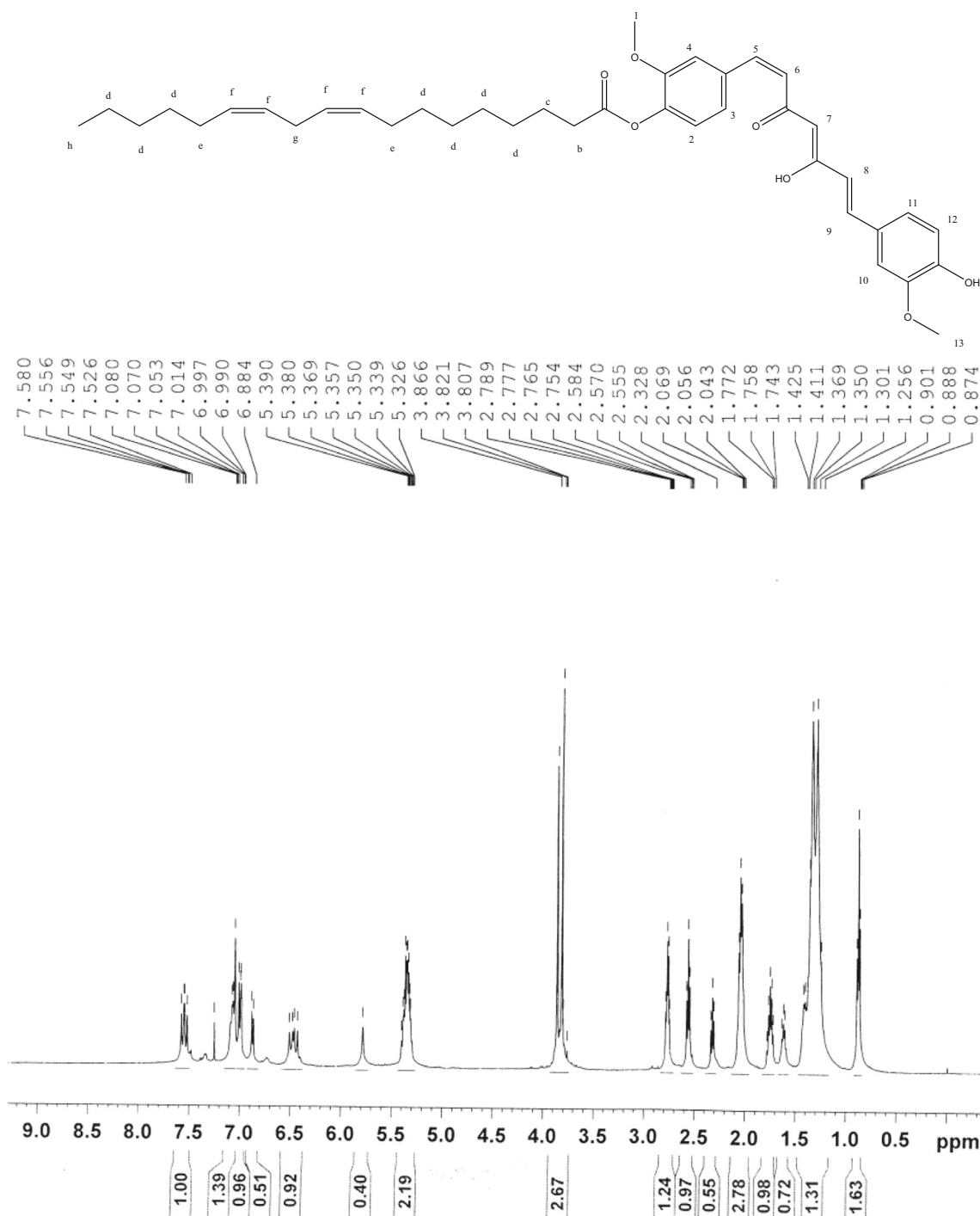


Fig. 1 Nuclear magnetic resonance (NMR) test for hydrogen atoms. ¹H NMR (CDCl₃, 500 MHz) δ : 7.55 (m, 2H, H5, 9), 7.08–6.88 (m, 6H, H2, 3, 4, 10, 12, 13 phenyl), 6.50 (m, 2H, H6, 8), 5.78 (s, 1H, H7), 5.36 (m, 4H, Hf), 3.86 and 3.82 (s, 6H, 2OMe), 2.76 (m, 2H, Hg), 2.57 (t, J =

7.0 Hz, 2H, Hb), 2.32 (t, J =7.0 Hz, 1H, Hd), 2.05 (m, 4H, He allylic), 1.75 (m, 2H, Hc), 1.62 (m, 2H, Hd), 1.42–1.25 (m, 11H, Hd), and 0.88 (t, 3H, Hh terminal)

immersed 2 cm under the water surface. The test room was lighted by a 5-lx lamp to prevent the rats from seeing the platform. The course of 5 days of training included 4 trials per day. The rats were placed in the maze facing the wall from one of the equally spaced start locations, which were randomly altered in each trial. Spatial reference cues around

the pool were maintained constantly during the training. Each trial lasted until the rat found the platform or for a maximum of 60 s. If the rat failed to find the platform, it was softly placed on the platform. At the end of each trial, the rat was allowed to rest on the platform for 20 s (Molaei et al. 2020; Morris et al. 1982).

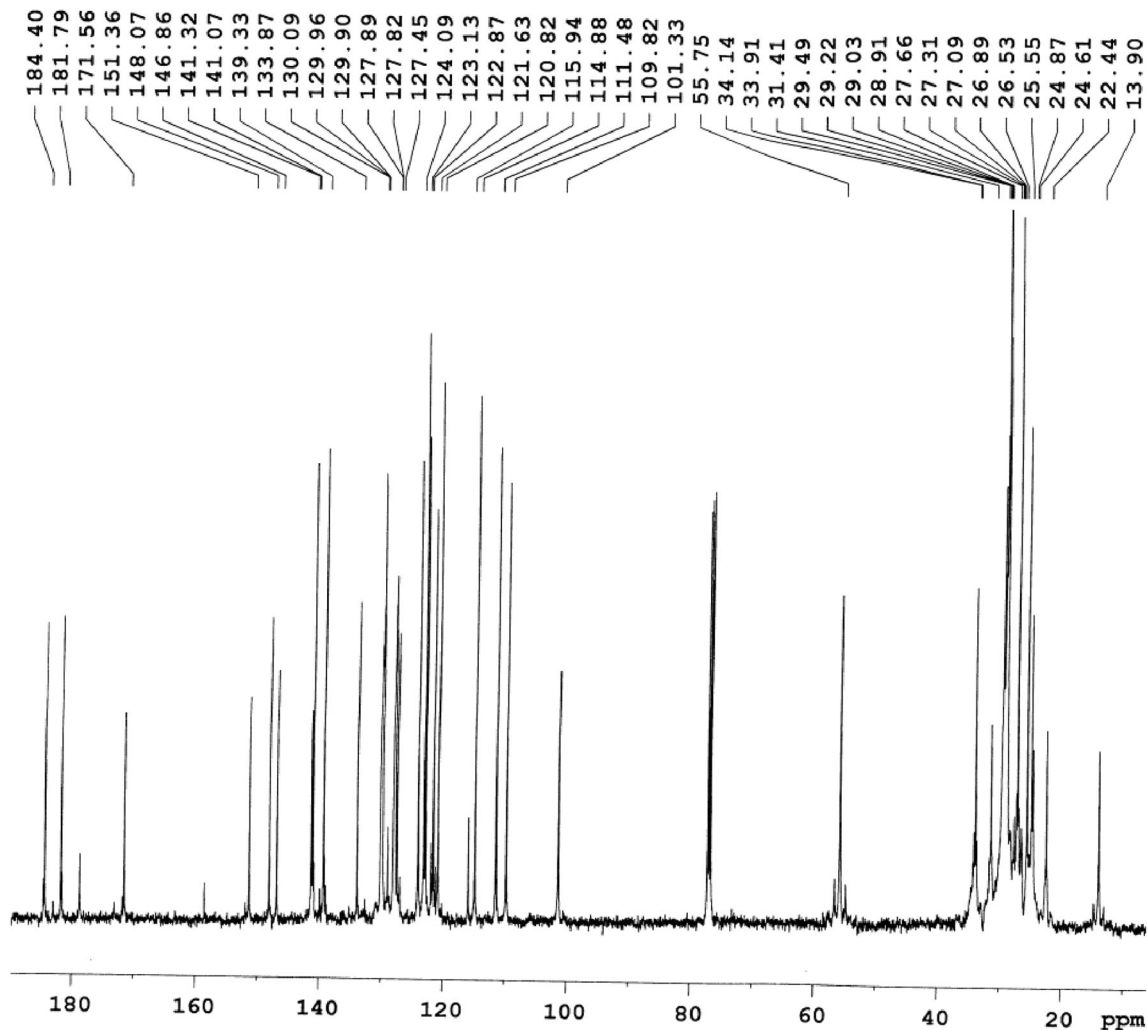


Fig. 2 Nuclear magnetic resonance (NMR) test for carbon atoms. ^{13}C NMR (CDCl_3 , 100 MHz) δ : 184.6, 181.7, 178.7, 171.5, 158.5, 151.3, 148.1, 146.8, 141.3, 441.1, 139.8, 133.8, 129.9, 128.9, 127.8, 127.4,

124.1, 123.1, 122.8, 121.6, 120.8, 115.9, 114.8, 111.4, 109.8, 101.3, 55.7, 33.9, 31.4, 29.4, 29.2, 29.1, 28.9, 27.3, 25.5, 24.8, 24.6, 22.4, and 13.9

Dissection and Preparation of Brain Homogenate

Twenty-four hours after testing days, the animals were anesthetized with an i.p. injection of chloral hydrate (400 mg/kg) (Field et al. 1993). In a deep state of anesthesia, brain tissues were dissected and frozen at -80°C and kept in a deep freezer. Frozen tissues were homogenized by 1.5 M KCl buffer. The homogenized tissues were then centrifuged for 15 min at 10,000g, and the supernatants were separated by the sampler and kept for LPO and antioxidant enzymes measurement.

Total Antioxidant Activity Assay

Total antioxidant activity (TAC) was measured by ferric reducing antioxidant power (FRAP) according to the

method by Benzie and Strain (1996). By mixing 2.5 mL of 2, 4, 6-tripyridyl-s-triazine (TPTZ) (10 mM, dissolved in 40 mM HCl) and FeCl_3 (20 mM) in 25 mL of acetate buffer, the FRAP reagent was prepared freshly (300 mM; pH: 3.6). The light blue reagent contained Fe^{3+} -TPTZ that changed to dark blue after interaction with antioxidants, which is explained by the presence of Fe^{2+} -TPTZ in the reagent. These changes were correlated with the absorbance increase as monitored at a wavelength of 593 nm. FRAP values were achieved by standard calibration curve obtained using different concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Ghadrdoust et al. 2011).

Antioxidant Enzyme Activity Assays

Superoxide Dismutase Assay According to the ability of the enzyme to inhibit the autoxidation of pyrogallol, superoxide

dismutase (SOD) activity in the brain homogenates was measured. Briefly, 1 mL of Tris-HCl (45 mM) buffer containing ethylenediaminetetraacetic acid was mixed with 5 μ L of homogenate supernatant and located in the spectrophotometer. The unit was autozeroed at 420 nm and 50 μ L of pyrogallol (0.2 mM) was added to the above solution and the absorbance of samples was immediately measured at 420 nm every 15 s and up to 2 min. The inhibition of pyrogallol autoxidation is proportional to the activity of SOD present in the sample (Ghasemi et al. 2019).

Catalase Assay Hydrogen peroxide (H_2O_2 ; 30 mM) was used as a substrate and 50 mM phosphate buffer (pH = 7) was used as an alternative substrate in the blank. This reaction occurred by the addition of H_2O_2 and the absorption was measured at 240 nm for 3 min (Ghasemi et al. 2019).

Designation of LPO LPO was measured based on the formation of TBA reactive substance (TBARS) from the brain homogenates. For the preparation of the TBA reagent, 1 mL of TCA (20% w/v) and TBA (0.5% w/v) was added to 1 mL of the supernatant. The dilution was heated for 15 min in boiling water. After cooling, it was centrifuged at 1000g for 10 min. The malondialdehyde (MDA) was mixed with TBA and measured with a spectrophotometer at 532 nm (Ghasemi et al. 2019).

Statistical Analysis

The data were assayed by SPSS version 16.0. MVM data were analyzed using a two-way analysis of variance (ANOVA) with days as repeated measures factor and treatments as between subjects' factor. Furthermore, one-way ANOVA was used for assessing biochemical parameters. Tukey post hoc test was used where pairwise comparisons were performed. The data are expressed as means \pm SEM. $P < 0.05$ was considered statistically significant.

Results

Effects of CUR and Lino-CUR treatment on spatial memory function

Effects on Distance Traveled

We tested all groups daily for 5 consecutive days by the MWM apparatus and the data regarding swimming speed, escape latency, and distance traveled to reach the hidden platform were analyzed. A two-way ANOVA results in terms of the distance traveled revealed the significant effects of group ($F(6, 36) = 8.692$, $P < 0.001$), day ($F(4, 24) = 124.9$, $P < 0.001$), and the interaction between group and day

($F(24, 144) = 1.541$, $P = 0.635$; Fig. 3a). In addition, the results of Tukey post hoc test showed a significant increase in distance traveled to find the hidden platform in the EB group in comparison with the control ($P < 0.001$) and sham ($P < 0.001$) groups on training days.

Also, a remarkable decrease was found in distance traveled between the CUR (20 μ g/kg) +EB group on days 2 ($P < 0.01$) and 5 ($P < 0.05$), EB + CUR (40 μ g/kg) group on days 2 ($P < 0.001$), 3 ($P < 0.001$), 4 ($P < 0.01$), and 5 ($P < 0.01$), and also EB + Lino-CUR (20 μ g/kg) ($P < 0.001$) and EB + Lino-CUR (40 μ g/kg) groups in all training days ($P < 0.001$) compared with the EB group. Moreover, there was a significant decrease in distance traveled in the EB + Lino-CUR (40 μ g/kg) group in comparison with the EB + CUR (20 μ g/kg) group on days 3 and 4 ($P < 0.05$). A significant decrease was found in distance traveled in the EB + Lino-CUR (40 μ g/kg) group in comparison with EB + Lino-CUR (20 μ g/kg) group on days 3 ($P < 0.05$) and 4 ($P < 0.05$) (Fig. 3a).

In summary, distance traveled (as a parameter to evaluate memory) showed a significant reduction in EB + CUR (40 μ g/kg), EB + Lino-CUR (20 μ g/kg), and EB + Lino-CUR (40 μ g/kg) groups in comparison to the EB group in all training days. However, it significantly decreased in the EB + Lino-CUR (40 μ g/kg) group in comparison to the EB + CUR (20 μ g/kg) group on days 3 and 4.

Effects on Escape Latency

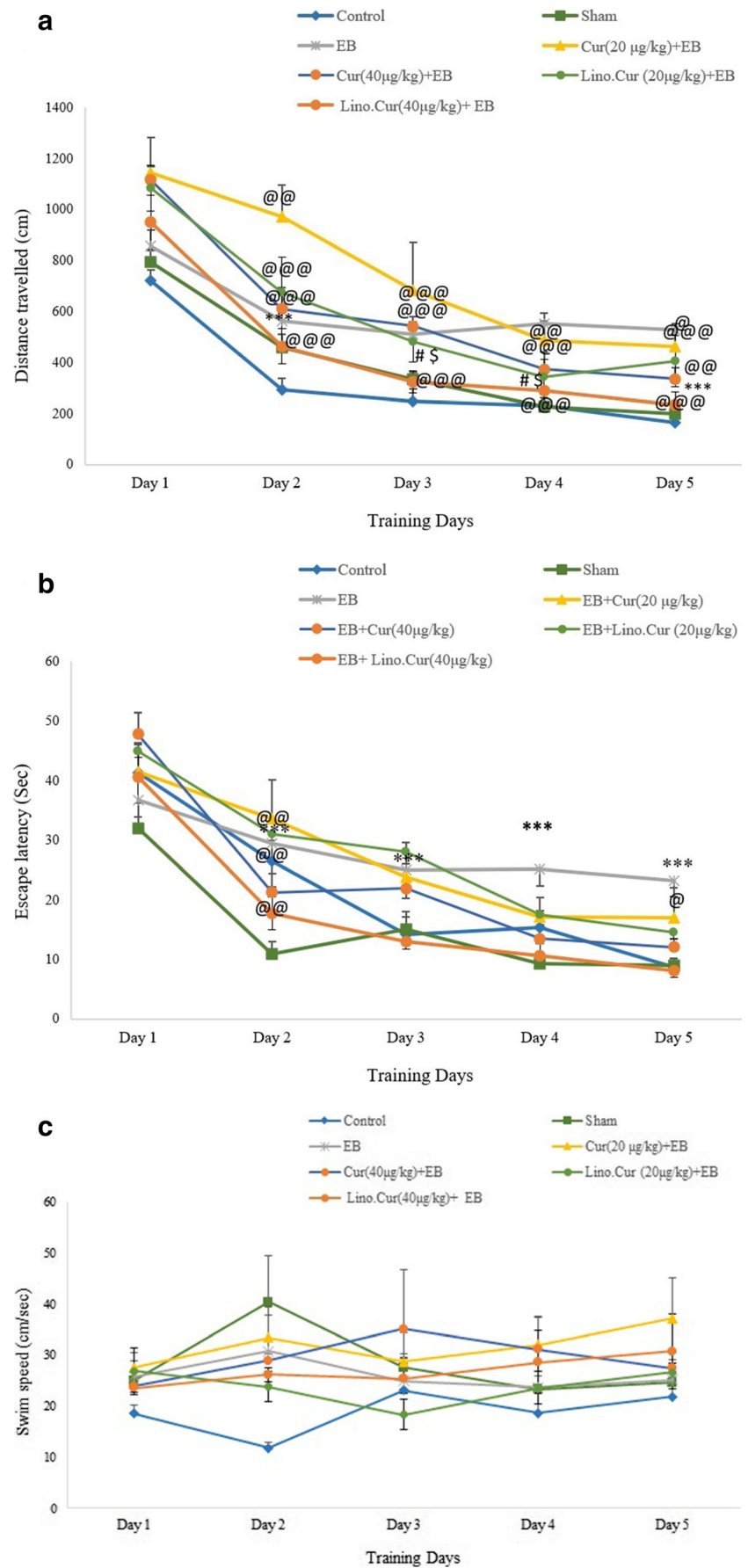
The two-way ANOVA results regarding mean escape latency showed significant effects of group ($F(6, 36) = 4.218$, $P < 0.01$), day ($F(4, 24) = 121.7$, $P < 0.001$), and the interaction between group and day ($F(24, 144) = 1.654$, $P < 0.05$) (Fig. 3b). A significant decrease was found in the EB + CUR group (20 μ g/kg) on day 5 ($P < 0.05$), EB + CUR (40 μ g/kg) ($P < 0.01$), EB + Lino-CUR (20 μ g/kg) ($P < 0.01$), and EB + Lino-CUR (40 μ g/kg) ($P < 0.01$) groups compared with the EB group on day 2 (Fig. 3b). Furthermore, there was a significant increase in escape latency to reach the hidden platform in the EB group in comparison with the control ($P < 0.001$) and sham ($P < 0.001$) groups in all training days.

Briefly, escape latency time to reach the hidden platform significantly decreased in the EB + CUR (20 μ g/kg), EB + CUR (40 μ g/kg), EB + Lino-CUR (20 μ g/kg), and EB + Lino-CUR (40 μ g/kg) groups in comparison to the EB group.

Effects on Mean Swimming Speed

Analysis of the results of mean swimming speed of rats to find the platform in different groups showed no significant effect of group ($F(6, 36) = 2.450$, $P = 0.0534$), day ($F(4, 24) = 0.9578$; $P = 0.4485$), and the interaction between group and day ($F(24, 144) = 1.483$, $P = 0.0821$) (Fig. 3c).

Fig. 3 Comparison of **a** distance travelled **b** escape latency, and **c** swimming speed in the Morris water maze test. Each column or bar represents the mean \pm SEM scores recorded in the five trials daily. “#,” “\$,” and “@” significant differences in comparison with curcumin (CUR) (20 μ g/kg) + ethidium bromide (EB), linoleic acid (Lino)-CUR (20 μ g/kg) + EB, and EB groups, respectively. * EB, CUR (20 μ g/kg) + EB, CUR (40 μ g/kg) + EB, Lino-CUR (20 μ g/kg) + EB, and Lino-CUR (40 μ g/kg) + EB groups compared with the control group



Effect of CUR and Lino-CUR on TAC in EB-Induced MS

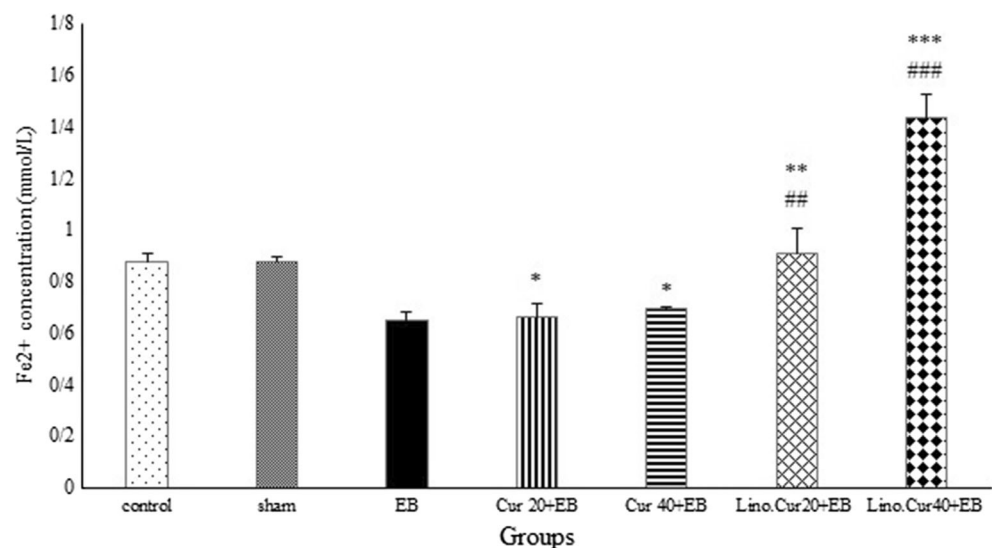
As shown in Fig. 4, compared with the control group, the level of TAC significantly decreased in the EB + CUR (20 µg/kg) ($P < 0.05$) and EB + CUR (40 µg/kg) groups ($P < 0.05$), whereas TAC increased in the EB + Lino-CUR (20 µg/kg) ($P < 0.01$) and EB + Lino-CUR (40 µg/kg) ($P < 0.001$) groups compared with the control group. Also, this parameter increased in the EB + Lino-CUR (20 µg/kg) ($P < 0.01$) and EB + Lino-CUR (40 µg/kg) ($P < 0.001$) groups compared with the EB group. TAC level showed no significant difference between the control and sham groups.

Generally, TAC levels showed a significant decrease in the EB + CUR (20 µg/kg) and EB + CUR (40 µg/kg) groups in comparison to the control group, whereas it increased in the EB + Lino-CUR (20 µg/kg) and EB + Lino-CUR (40 µg/kg) groups compared with the control group.

Effect of CUR and Lino-CUR on Antioxidant Enzyme Levels in EB-Induced MS

Also, we investigated the effects of CUR and Lino-CUR administration on the levels of two antioxidant enzymes (catalase (CAT) and SOD). The levels of both enzymes were evaluated in the EB group compared with the control rats ($P < 0.05$). Also, the CAT ($P < 0.05$) and SOD ($P < 0.05$) concentrations significantly decreased in all treated groups compared with the EB group (Figs. 5 and 6). Overall, the CAT and SOD levels significantly decreased in all treated groups compared with the EB group.

Fig. 4 Effect of curcumin (CUR) and linoleic acid (Lino)-CUR treatment on ethidium bromide (EB)-induced toxicity alterations in total antioxidant activity in rat brain. Values are expressed as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ significant difference between the EB and treated groups and the control group; ## $P < 0.01$ and ### $P < 0.001$ significant difference between EB group and CUR (20 µg/kg) +EB, CUR (40 µg/kg) +EB, Lino-CUR (20 µg/kg) +EB and Lino-Cur (40 µg/kg) +EB groups



Effect of CUR and Lino-CUR on EB-Induced LPO

MDA is an indicator of lipid peroxidation (LPO) damage during EB-induced demyelination. MDA levels in the rat brain homogenates are shown in Fig. 7. MDA in the brain homogenates significantly increased (0.004 ± 0.00006) in the EB group compared with the control group (0.0014 ± 0.0001) ($P < 0.05$). MDA levels in the EB + CUR groups (20 and 40 µg/kg) significantly decreased in comparison to the EB group ($P < 0.01$). The MDA levels in the EB + Lino-CUR (20 and 40 µg/kg) group significantly decreased in comparison with the EB group ($P < 0.01$). Also, the results indicated a significant difference between the EB + Lino-Cur (40 µg/kg) and EB + CUR (20 µg/kg) groups in MDA levels ($P < 0.05$). Overall, in the EB + Lino-CUR (20 and 40 µg/kg) groups, the MDA levels significantly decreased in comparison to the EB group.

Discussion

In this study, the effects of Lino-CUR and CUR on special memory and oxidative stress in a rat model of MS were examined. The key results of the study were as follows: (1) the distance traveled to find the hidden platform and escape latency (as a parameter for spatial memory) in the EB group significantly increased in comparison with the control and sham groups; (2) conjugated Lino-CUR injection in the rats receiving EB (MS model) improved the spatial memory function compared with EB rats; (3) there was a significant decrease in the distance traveled in the EB + Lino-CUR (40 µg/kg) group in comparison with the EB + CUR (20 µg/kg) group;

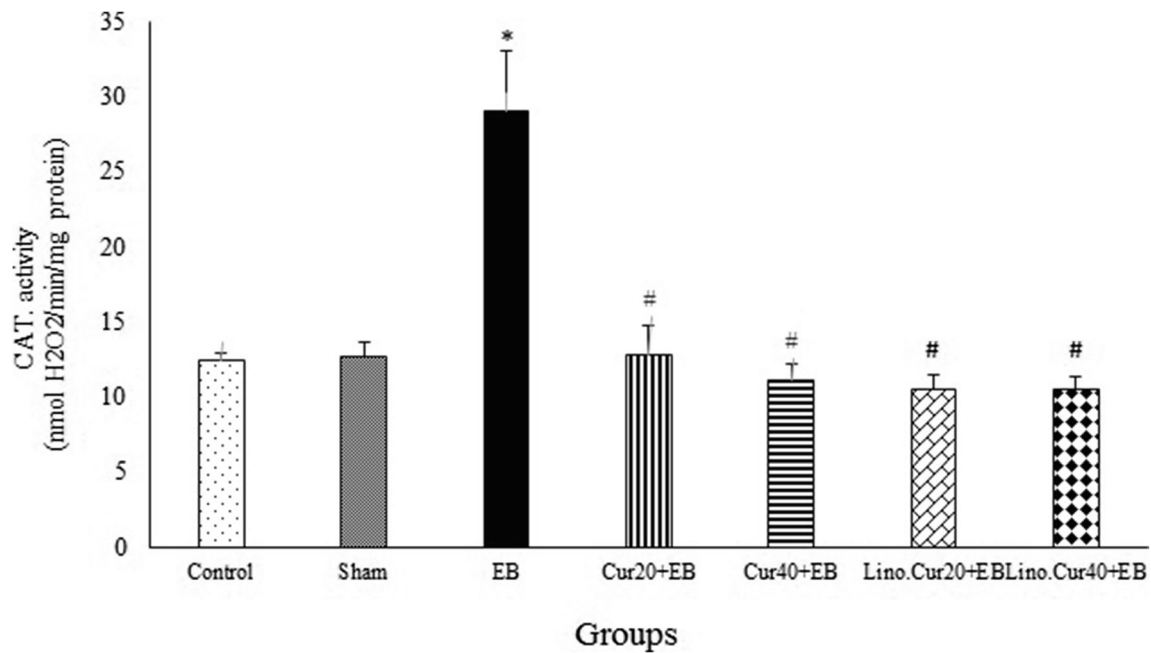


Fig. 5 Effect of curcumin (CUR) and linoleic acid (Lino)-CUR treatment on ethidium bromide (EB)-induced toxicity alterations in catalase enzyme level in rat brain. Values are expressed as mean \pm SEM. * $P < 0.05$,

significant difference between EB and treated groups and the control group; # $P < 0.05$ significant difference between treated groups and EB group

(4) the level of TAC significantly decreased in MS + CUR (20 μ g/kg) or MS + Cur (40 μ g/kg) groups compared with the control group, whereas TAC increased in MS + Lino-CUR (20 μ g/kg) or MS + Lino-cur (40 μ g/kg) rats compared with the control group; (5) the CAT and SOD concentrations significantly decreased in all treated groups in comparison to the MS group; (6) at both doses, EB + Lino-CUR (20 and 40 μ g/kg) group showed a significant decrease in the MDA levels in comparison with the EB group.

Consistent with our hypothesis, the EB-induced MS group showed a significant impairment in spatial memory (the increase in spent time and distance traveled) compared with the sham and control rats. Patients with MS have been found with cognitive impairment (Chiaravalloti and DeLuca 2008) as well as deficits in the quality of information processing, task management, processing speed, and long-term memory (Macniven et al. 2008). Notably, visual learning, memory, and processing speed are the most important cognitive impairments in patients with MS (Benedict et al. 2006).

Fig. 6 Effect of curcumin (CUR) and linoleic acid (Lino)-CUR treatment on ethidium bromide (EB)-induced toxicity alterations in superoxide dismutase enzyme level in rat brain. Values are expressed as mean \pm SEM. * $P < 0.05$ significant difference between EB and treated groups and the control group; # $P < 0.05$ significant difference between EB and CUR 20 (μ g/kg) +EB, CUR (40 μ g/kg) + EB, Lino-CUR (20 μ g/kg) + EB, and Lino-CUR (40 μ g/kg) + EB groups

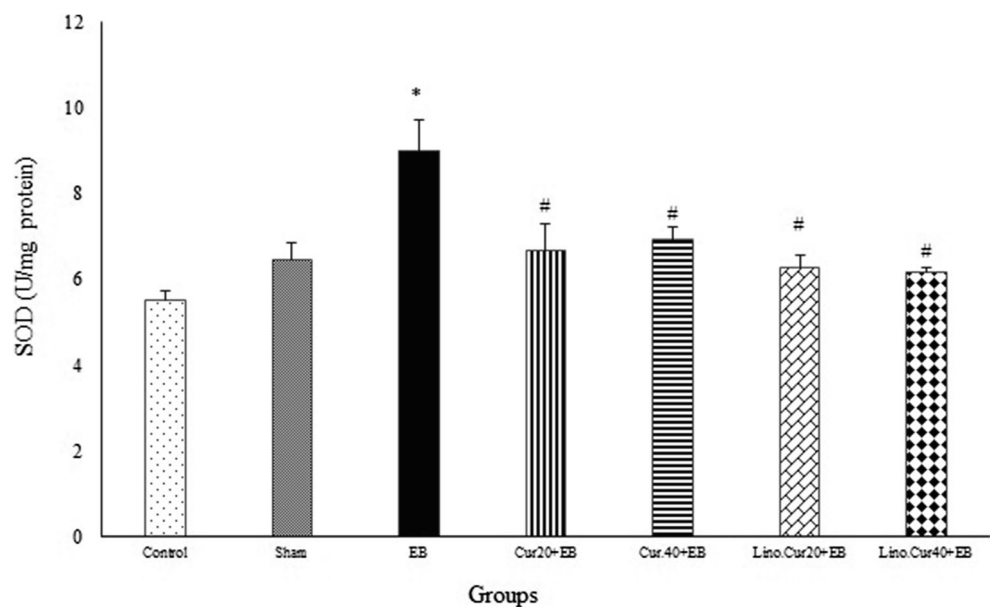
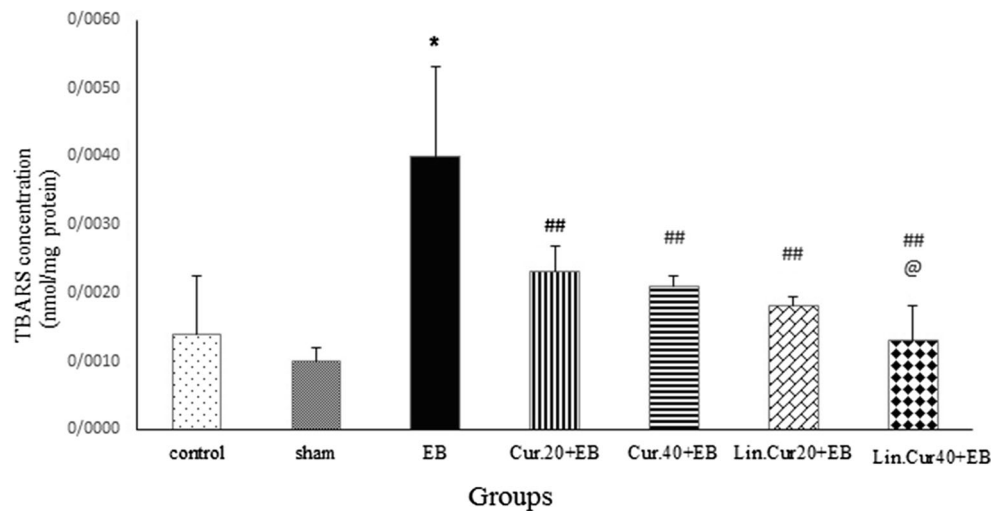


Fig. 7 Effect of curcumin (CUR) and linoleic acid (Lino)-CUR treatment on ethidium bromide (EB)-induced toxicity alterations in lipid peroxidation in the rat brain. Values are expressed as mean \pm SEM. * $P < 0.05$ significant difference between EB and treated groups and the control group; ## $P < 0.01$ significant difference between EB and treated groups; @ $P < 0.05$ significant difference between CUR (20 $\mu\text{g}/\text{kg}$) + EB, and Lino-CUR (40 $\mu\text{g}/\text{kg}$) + EB groups



Furthermore, our results highlighted the memory deficit in this disease. MS results in an alteration in the oxidant capacity of the brain, and cognition deficiency is one of the important symptoms of MS (Bagert et al. 2002). EB disturbs the balance between oxidants and antioxidant defense and causes oxidative stress in the brain (Ziehn et al. 2012). Over recent years, considerable effort has been focused on the antioxidant therapy of neurodegenerative diseases, such as MS (Bagert et al. 2002).

The present results established the effective role of antioxidants (CUR and Lino-CUR) in recovering the spatial memory of MS rats. Both doses of Lino-CUR (20 and 40 $\mu\text{g}/\text{kg}$) and the 40 $\mu\text{g}/\text{kg}$ dose of CUR in MS rats reduced spending time to find the platform compared with the MS group. The effectiveness of CUR in the improvement of spatial memory in different animal models, such as Alzheimer's disease and diabetes, has been reported, which is in line with our findings (Kuhad and Chopra 2007; Tang et al. 2009). Also, CUR improved the aluminum chloride-induced Alzheimer's disease in mice and inhibited apoptosis in cultured PC₁₂ cells by enhancing the level of Bcl2 (Pan et al. 2008).

CUR has strong antioxidant and anti-inflammatory effects; therefore, its effects on various neurological diseases should be considered (Bhat et al. 2019). CUR could reverse the polyglutamine (polyQ)-induced apoptosis and neuronal dysfunction in motor areas of patients with Huntington's disease (Saudou et al. 1998). Also, CUR exerts its antidepressant function by regulation of extracellular signal-regulated kinase (ERK) pathway that increases the brain-derived neurotrophic factor (BDNF) expression in the amygdala of mice (Zhang et al. 2012). It also protects dopaminergic neurons upon MPTP-induced neuronal destruction. CUR increased dopamine and tyrosine hydroxylase by inhibiting the glial fibrillary acidic protein (GFAP) and iNOS protein expression (Sharma and Nehru 2018).

CUR due to its antioxidant properties can be used as a potential therapeutic agent for treating cerebellar stroke (Tsai

et al. 2011). Previous reports have shown that CUR improves postsynaptic electrical reactivity and cell viability through the upregulation of BDNF and the reduction of inflammatory factors in the rat hippocampus (Choi et al. 2017).

Poor solubility and bioavailability of CUR remarkably affect its therapeutic application. Nanoparticle formulations have been described to improve the bioavailability of the drugs. Recently, the solid lipid-based nanoparticles of CUR were produced, which showed promoted pharmacokinetic properties. For example, the poly(lactic-co-glycolic acid) (PLGA) nanoparticles of CUR represent a ninefold increase in oral bioavailability compared with crude CUR suspension (Bhat et al. 2019).

There are some methods to moderate CUR rapid metabolism and improve its biological half-life. For example, it can be used in hybrid with some other substances (Maiti et al. 2007), like linoleic acid and forming Lino-CUR, which was applied in the present study. Moreover, our data revealed that the time spent to find the platform in the Lino-CUR group was less in comparison to the CUR group. The present study indicated that Lino-CUR-enhanced spatial memory more impressive in comparison to the CUR in an animal model of MS.

Oxidative stress has a deleterious effect on the CNS in MS. Treatment with oral and intravenous antioxidants can ameliorate the symptoms of MS disorder. Our results showed that LPO and antioxidant enzymes activity (CAT and SOD) increased and TAC (FRAP) decreased in EB-induced MS group in comparison to the control rats. Several studies have reported findings consistent or inconsistent with our results. For example, Ghaffari et al. showed that the ICV injection of EB can increase LPO and antioxidant enzyme activity and decrease TAC, which is in line with our results (Ghaffari et al. 2013). However, it has indicated that EB reduced CAT and SOD activity (Abdel-Salam et al. 2012; Spanevello et al. 2009). This result is inconsistent with the present study findings. Differences in the dose of injected EB as well as the length of the experiment can be regarded as the reasons for various

responses observed concerning antioxidant enzyme activity in the EB-induced MS model. Oxidative stress leads to the activation of an adaptive mechanism that helps to maintain the oxidative-reduction balance of the tissue and protects cells against the reactive oxygen species-mediated toxicity. Previous studies have shown that an increase in the level of antioxidant enzymes may be an adaptive response. Also, the level of antioxidant enzymes is increased in inflammation and MS (Van Horsen et al. 2008). Consequently, in the present study, such an adaptive mechanism may act as an important reason for the evaluation of antioxidant enzymes activity after the ICV injection of EB.

As previously mentioned, the application of antioxidants to treat MS using the experimental models has been widely considered. Therefore, in the present investigation, CUR and Lino-CUR were used as a treatment strategy, and based on the findings, injection of different doses of CUR and Lino-CUR reduced the activity of the antioxidant enzymes (CAT and SOD). Therefore, it can be assumed that CUR in its synthetic form was highly effective in reducing the level of antioxidant enzymes. A decline in the levels of these antioxidant enzymes in response to antioxidant therapy is because like other antioxidants, CUR and Lino-CUR decreased the reactive oxygen species, which in turn can reduce the tissue environment oxidative load resulting in a decrease in CAT and SOD levels (Van Horsen et al. 2008). Also, we found that CUR and Lino-CUR reduced LPO and increased TAC in the treated groups compared with the control rats; however, the effects of Lino-CUR was more remarkable. Zhang et al. (2017) showed that curcumin nanoparticles (cur-NPs) enhanced therapeutic effects compared with curcumin in improving neurological function. Additionally, Cur-NPs markedly suppressed oxidative stress following subarachnoid hemorrhage in rats (Zhang et al. 2017) which is consistent with the results of the present study.

Conclusion

Our findings showed that the EB-induced MS impaired spatial memory parameters. Using antioxidants (CUR and Lino-CUR) improved memory impairment in treated groups. Also, using CUR combined with Linoleic acid, as a complex substance, improved antioxidant effects of CUR, especially at higher doses of the complex (Lino-CUR, 40 µg/kg). Therefore, we provided evidence supporting the strong antioxidant effects of Lino-CUR in reducing the effect of oxidative stress on spatial memory and LPO in rat brains in the EB model of MS. It also appears that Lino-Cur is more effective in comparison to curcumin when injected centrally, whether this is due to its potency or its bioavailability needs more investigation.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Abbreviations MS, multiple sclerosis; CUR, curcumin; Lino-CUR, linoleic acid-CUR; EB, ethidium bromide; CNS, central nervous system; CSF, cerebrospinal fluid; BBB, blood-brain barrier; PUFAs, polyunsaturated fatty acids; LPO, lipid peroxidation; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; TBA, thiobarbituric acid; TAC, total antioxidant activity; DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance; ICV, intracerebroventricular; i.p., intraperitoneal; MWM, Morris water maze; FRAP, ferric reducing antioxidant power; TPTZ, 2, 4, 6-tripyridyl-s-triazine; SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; PolyQ, polyglutamine; ERK, extracellular signal-regulated kinase; BDNF, brain-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; PLGA, poly(lactic-co-glycolic acid); Cur-NPs, curcumin nanoparticles

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