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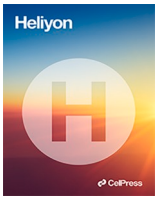

Research article

Lutein improves remyelination by reducing of neuroinflammation in C57BL/6 mouse models of multiple sclerosis

Atefeh Arab Firozjae [a], Mohammad Reza Shiran [a], Abolghasem Ajami [b], Davood Farzin [a], Mohsen Rashidi [a,c,*]

[a] Department of Pharmacology, Faculty of Medicine, Mazandaran University of Medical Science, Sari, Iran
[b] Department of Immunology, Faculty of Medicine, Mazandaran University of Medical Science, Sari, Iran
[c] The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory neurodegenerative disorder characterized by demyelination. Lutein, a xanthophyll carotenoid, has well-known antioxidant and anti-inflammatory properties. In this experiment, we aimed to investigate the neuroprotective and remyelination potential of lutein in comparison with dimethyl fumarate (DMF) as a reference drug in post-cuprizone-intoxicated C57BL/6 mice. Lutein (50, 100, and 200 mg/kg/day; p.o.) and DMF (15 mg/kg/day, i.p.) were administered either alone or in combination for three weeks at the end of the six-week cuprizone (0.2 % w/w) feeding period. At the end of the study, behavioral tests, histopathological staining, immunohistochemistry (olig2), ELISA, and real-time PCR were performed to evaluate the target parameters. Lutein treatment significantly enhanced motor functions, reversed cuprizone-induced demyelination and increased serum TAC. In addition, treatment with lutein increased the number of Olig2+ cells in the corpus callosum, reduced the IL-1β and TNF-α and increased BDNF. Lutein administration significantly increased the expression levels of genes involved in myelin production (MBP, PLP, MOG, MAG, and OLIG-1) and notably reduced GFAP expression levels. In the present study, our results showed that lutein treatment could promote remyelination and neuroprotective effects by reducing neuroinflammation and upregulating the expression of the genes involved in myelin formation These findings suggest that lutein could serve as a potential adjuvant therapy for patients with multiple sclerosis. Further clinical trials are necessary to confirm its efficacy.

1. Introduction

Multiple sclerosis (MS) is a neurodegenerative disorder characterized by demyelination caused by autoimmune responses and affects almost 2.5 million individuals globally. This condition is characterized by demyelinated lesions, oligodendrocyte death, and neuronal damage [1–3] and is the most frequent cause of non-traumatic neuronal impairment among young and middle-aged adults. Multiple sclerosis presents with a variety of symptoms, including optic neuritis, balance disorders, and mobility impairment [4–6]. Genetic predisposition, lifestyle, and viral infections contribute to the development and progression of MS [7].

* Corresponding author. Department of Pharmacology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
E-mail address: dr.mohsenrashidi@yahoo.com (M. Rashidi).

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The prominent manifestations of MS include inflammatory and demyelinating attacks. Infiltrating immune cells in the central nervous system (CNS) secret proinflammatory cytokines, including IL-1β and TNF-α, which attack protective myelin sheets and contribute to nerve cell damage [8]. In addition, oxidative injury plays a pathogenic role in MS-associated neurodegeneration and demyelination. Reactive oxygen species (ROS) can stimulate apoptotic pathways and promote the release of pro-inflammatory cytokines [9]. Chronic oxidative stress can contribute to mitochondrial damage and lead to oligodendrocytes death [10].

Current therapeutic agents for MS have limited efficacy and only can reduce its symptoms [11]. Therefore, it is required to seek for new neuroprotective medications with potent anti-inflammatory effects to alleviate MS-associated neuroinflammatory and neuronal damage. Lutein, a xanthophyll carotenoid, is found in egg yolk and dark leafy green vegetables, including kale, broccoli, spinach, and lettuce [12]. Numerous reports have indicated lutein's protective effects on age-related macular degeneration [13–17] and neurodegeneration and neuronal injury [18,19]. It has been found that lutein can promote its neuroprotective effects via suppressing the activation of NF-kB and inducing Nrf2 signaling-related antioxidant enzymes [12]. Lutein and its isomer, zeaxanthin, can cross the blood brain barrier (BBB), allowing their uptake by cerebrovascular cells [20]. Due to its neuroprotective, antioxidant, and anti-inflammatory properties, lutein may be an ideal candidate and a potential treatment for demyelinating neuronal diseases such as MS.

Several animal models have been used for MS research, including experimental autoimmune encephalomyelitis (EAE), along with neuronal toxicity models created by cuprizone, lysolecithin, and ethidium bromide [21]. The most common pre-clinical MS-like animal model is created by cuprizone-induced demyelination. Cuprizone (bis-cyclohexanone oxalyhydrazone), a widely used copper chelator, leads to rapid demyelination with high reproducibility [22] by inhibiting mitochondrial enzymes in the brain, leading to oxidative stress, oligodendrocyte apoptosis, neuroinflammation, and demyelination [1]. In this experiment, we aimed to investigate the neuroprotective potential of lutein in comparison with dimethyl fumarate (DMF), as a reference drug, and divulge the possible neuroprotective mechanisms involved by conducting behavioral, histopathological, and molecular analyses [23] in cuprizone-induced demyelination in C57BL/6 mice.

2. Materials and methods

2.1. Animals

Forty-eight male C57Bl/6 mice (20–25 g; 6–8 weeks) were bought from Royan Institute (Karaj, Iran). The mice were housed under controlled humidity and temperature (23 ± 2 °C) and normal day-night cycles.

2.2. Induction of cuprizone-demyelination and lutein treatment

Mice received a diet containing 0.2 %(w/w) cuprizone (CPZ) (Sigma-Aldrich) mixed with standard rodent chow for 6 weeks to

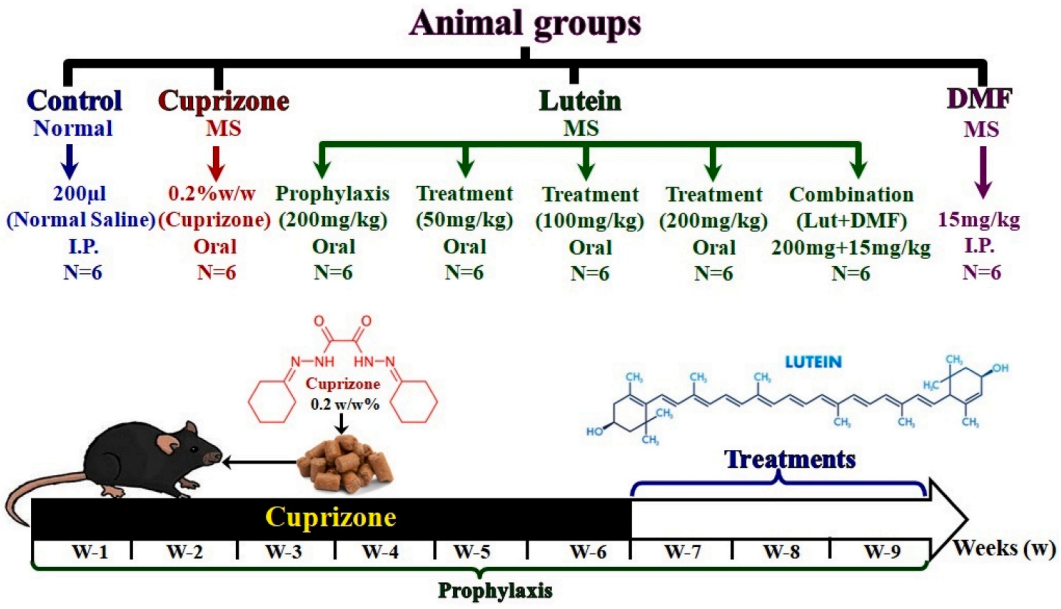


Fig. 1. Experimental design of the study. All mice, except for the control group, were fed a diet containing 0.2 % cuprizone for 6 weeks to induce demyelination, followed by a cuprizone-free diet. The mice then received treatments for an additional 3 weeks. Animals were divided into 8 groups: Control, Cuprizone (no treatment), three groups of lutein (Lut 50, 100, 200 mg/kg), Combination (Lut 200 mg/kg + DMF 15 mg/kg), DMF (15 mg/kg), and Prophylaxis group (lutein 200 mg/kg from the onset of CPZ feeding) (n = 6 per group).

induce demyelination [24]. The diet was then returned to normal for 3 further weeks. The mice were weighed every 2 weeks and at the end of the study.

The animals were randomly divided into eight different groups (n = 6 per group) (Fig. 1.).

1) **Control group**: standard rodent chow throughout the study (i.e., no CPZ).
2) **CPZ group**: 0.2 %(w/w) CPZ diet for 6 weeks and then returning to normal diet for 3 weeks (daily oral gavage of vehicle (normal saline) was given over the last 3 weeks).

3-5) **Lutein-treated groups**: CPZ-mice receiving 0.2 % CPZ diet for 6 weeks and returning to normal diet with daily oral gavage of lutein (50, 100, 200 mg/kg) for 3 weeks [25].

6) **Prophylaxis group**: CPZ-mice receiving daily oral gavage of lutein (200 mg/kg) from the day of the onset of CPZ feeding to end of the study.
7) **DMF group:** CPZ-mice receiving DMF (15 mg/kg, i.p.) as the standard treatment over the last 3 weeks [26].
8) **LUT + DMF group**: CPZ-mice receiving lutein (200 mg/kg, oral) and DMF (15 mg/kg, i.p.), as combination therapy, over the last 3 weeks.

2.3. Behavioral tests

2.3.1. Rotarod test

The Rotarod test was utilized to evaluate the motor ability and balance at the end of the experiment [27]. The mice were placed in a rotating cylinder with an accelerating speed from 4 to 40 rpm over 5 min, and the latency time to fall was counted.

2.3.2. Narrowing Beam Walking test

The Narrowing Beam Walking Test assesses locomotor performance by determining the ability of the animal to cross a narrow wooden beam [28,29]. The average time taken to traverse the beam was recorded.

2.4. Tissue collection and processing

After performing the behavioral tests, the animals were sacrificed by the intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine. Blood samples were collected, and sera were isolated. The brain was quickly removed, washed with ice-cold PBS, and dissected. Half of the brain sample was fixed in 10 % formalin for pathological processing. The entire corpus callosum of the second half of the brain was immediately frozen in liquid nitrogen and stored at −80 °C until use for qRT-PCR and ELISA assays.

2.5. Luxol fast blue (LFB) staining

This technique is a commonly used for assessing the myelination status in the CNS [30,31]. Deparaffinized samples were incubated at 60 °C with a 0.1 % LFB solution (Sigma, USA) overnight. The sections were examined under light microscopy for histological features. Demyelination status was scored according to the following metrics**:** 0 = normal myelination, 1 = mild demyelination (i.e., demyelination of 30 % of myelin tract fibers), 2 = moderate demyelination (i.e., demyelination of 60 % of myelin tract fibers), and 3 = complete demyelination [32].

2.6. Hematoxylin and eosin (H&E) staining

Hematoxylin-eosin staining was conducted to investigate tissue inflammation and the degree of the migration of immune cells to the corpus callosum region. Briefly, 5 μm thick tissue sections were prepared using a microtome and then were stained by hematoxylin and eosin (H&E) (Sigma, USA) [30].

2.7. Immunohistochemistry staining

Immunohistochemical (IHC) analysis was conducted to evaluate the expression level of Olig2 on paraffin-embedded brain tissues (2 μm). Rabbit polyclonal anti-olig2 antibodies (NBP1-28667, dilution = 1:800) were used as primary antibodies, and staining procedures were carried out following the supplier's instructions (Novus Biologicals, USA).

2.8. Determination of serum total antioxidant capacity

Serum total antioxidant capacity (TAC) was measured using the ferric ion reducing antioxidant power (FRAP) technique according to the instructions of the kit (Karmania Pars Gene Company, Iran). In this method, a distinctive colored ferrous-tripyridyl triazine complex was formed, which could be quantitatively determined spectrophotometry at 593 nm.

2.9. Quantitative real time PCR

The gene expression levels of MBP, PLP, MOG, GFAP, Olig-1, and MAG in the corpus callosum region were investigated by qRT-PCR. Total RNA was extracted from tissue samples by RNX™-PLUS kit (SinaClon Bioscience, Iran) according to the protocol of the manufacturer. Total RNA was quantified using a spectrophotometer (Nanodrop 2000, Thermo Fisher) by OD 260/280 nm. The synthesis of complementary DNA (cDNA) was achieved utilizing the Maxime RT Premix kit (iNtRON Biotechnology) according to the manufacturer's instructions. To assess the gene expression of MBP, PLP, MOG, GFAP, Olig-1, and MAG genes, qRT-PCR was performed. In brief, each reaction mixture included 7.5 μL master mix, 1 μL primer mix (forward and reverse primers), 1 μL cDNA, and 5.5 μL DEPC-treated water, resulting in a final volume of 15 μL. The thermal cycling conditions included an initial activation step at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing 55–62 °C for 10 s, and extension at 72 °C for 30 s.The primer sequences used in this study are shown in Table 1. All of the samples were assessed in duplicate and analyzed using Rotor-Gene 6000 Series Software 1.7 (Qiagen, Germany). GAPDH was utilized as the internal control gene to standardize the analysis. The results were analyzed using $2^{-\Delta\Delta CT}$ method.

2.10. Enzyme-linked immunosorbent assay

The levels of TNF-α, IL-1β, and BDNF were quantified in the brain homogenate using a sandwich ELISA technique (Elabscience, USA). The protocols used to measure these proteins followed the instructions provided by the manufacturer, and c denoted as pg/mg protein.

2.11. Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 8. Analysis of variance (ANOVA) followed by Tukey's post-hoc test was employed to assess variations between the groups. Results were expressed as mean ± SEM, and statistical significance was approved when p-value was <0.05.

3. Results

3.1. Bodyweight

The weights of the mice were measured every two weeks (Fig. 2A). At the end of the study, the mice that were given CPZ had a lower body weight compared to control mice ($p < 0.0001$) (Fig. 2B). The mice receiving prophylactic lutein therapy lost significantly less weight in comparison with control CPZ-treated animals ($p < 0.0001$).

3.2. The impact of lutein on the motor ability

The Rotarod test was used to determine the motor activity at the end of the experiment (Fig. 3A). Compared to control mice, all CPZ-treated mice displayed a reduction in rotarod times. However, lutein treatment dramatically improved rotarod performance compared to the CPZ group.

Compared to the control group, CPZ-treated mice exhibited a more prolonged time to crossing the narrow beam (Fig. 3B). Animals that received lutein post- CPZ administration demonstrated better performance compared to the CPZ-treated control group.

Table 1
Primer sequences used for amplifying target genes by qRT-PCR.

Gene	Primer sequence
MBP	F: ATGGCATCACAGAAGAGACC
	R: CATGGGAGATCCAGAGCGGC
MAG	F: CTCTATGGCACCCAGAGCCT
	R: TGTCCTTGGTGGGTCGTTTT
MOG	F: GACCTCAGCTTGGCCTGACCC
	R: TGCTGGGCTCTCCTTCCGC
PLP	F: AGCGGGTGTGTCATTGTTTGGGAA
	R: ACCATACATTCTGGCATCAGCGCA
GFAP	F: GGCGCTCAATGCTGGCTTCA
	R: TCTGCCTCCAGCCTCAGGTT
Olig-1	F: GCCCCACCAAGTACCTGTC
	R: GGAACTTGCACACCGCACA
GAPDH	F: CCTCGTCCCGTAGACAAAATG
	R: TGAAGGGGTCGTTGATGGC

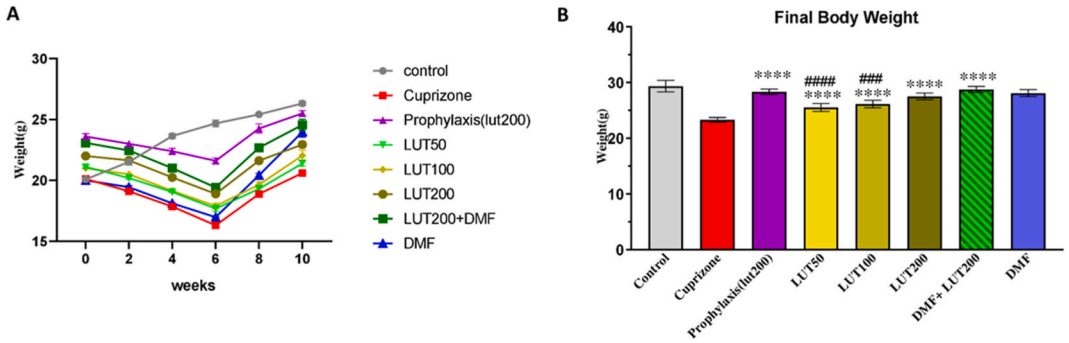


Fig. 2. Changes in mice body weights during the experiment. **(A)** Changes in body weight throughout a nine-week period. **(B)** Final body weight at the end of the study in various groups. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 vs. the Cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 significant vs. the DMF group (n = 6).

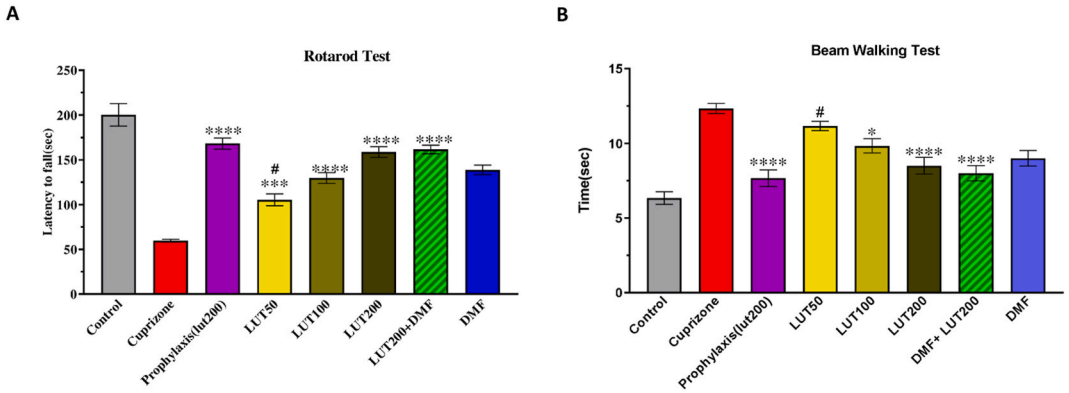


Fig. 3. (A) Rotarod test and **(B)** Beam Walking test to evaluate locomotor function. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 significant vs. the cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 significant vs. the DMF group (n = 6).

3.3. The impact of lutein on myelin status

Myelinated regions appear blue, while demyelinated regions appear white in LFB staining. As shown in Fig. 4A, the blue color intensity declined in the corpus callosum after CPZ administration compared to the standard diet control group. A remarkable decrease in the demyelination score and a notable elevation in LFB staining intensity demonstrated that lutein therapy could effectively reverse CPZ-induced demyelination (Fig. 4B).

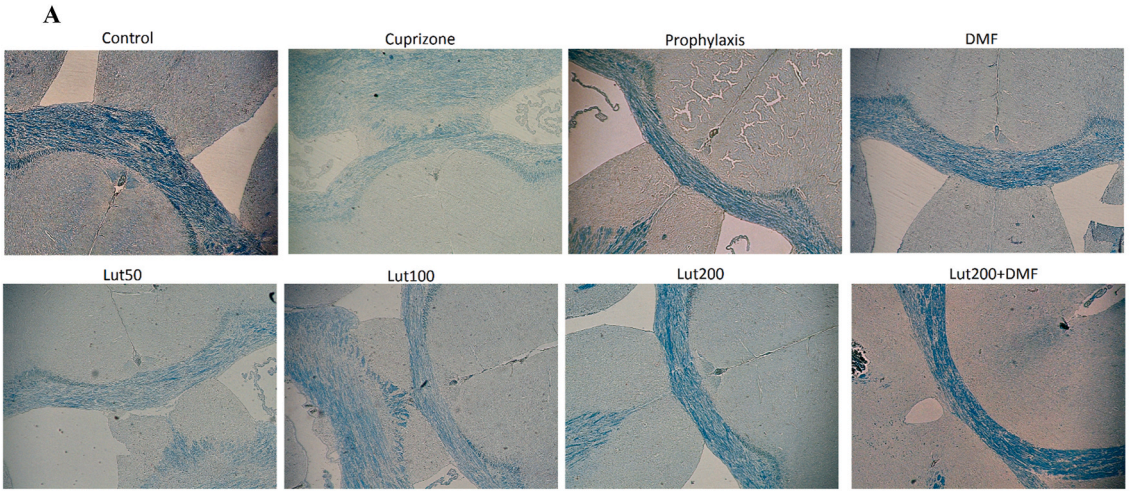
In the control group, corpus callosum showed a normal appearance in H&E staining. However, demyelinated lesions were detected in the mice fed with CPZ compared to the control group. (Fig. 4C). Lutein treatment reduced CPZ-induced detrimental effects on the corpus callosum, evidenced by near-normal histological characteristics in lutein-treated animals.

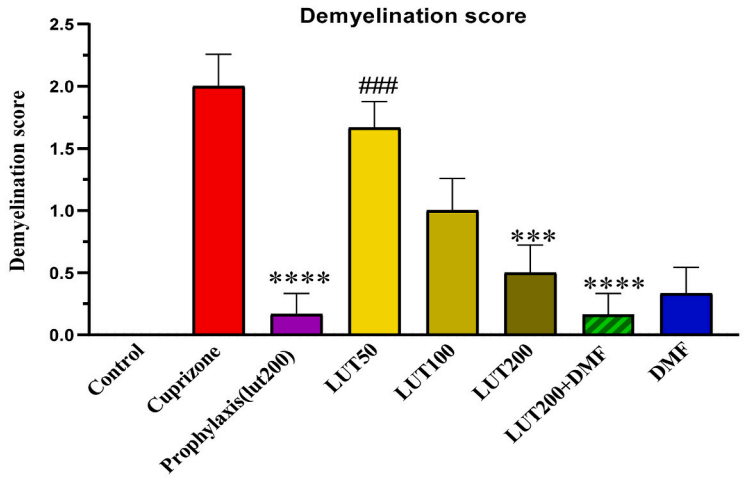
3.4. Changes in the Olig-2 marker

The immunohistochemistry staining of the Olig2 marker, was used to evaluate the frequency of oligodendrocytes in the corpus callosum region. As depicted in Fig. 5, A, Olig2-positive cells were more abundant in all CPZ-fed mice compared to control animals. The increased presence of Olig2+ cells in the CPZ-fed mice indicates a response to the demyelinating environment induced by cuprizone, which is known to promote oligodendrocyte precursor cell (OPC) activation and differentiation. The combination therapy group (Lut 200+DMF) showed a significant elevation in $Olig2^{+}$ cells within the corpus callosum region compared to CPZ-treated group ($P < 0.0001$) suggesting that lutein not only supports the survival of existing oligodendrocytes but may also promote the differentiation of OPCs into mature oligodendrocytes, thereby enhancing myelin repair mechanisms (Fig. 5B).

3.5. Effect of lutein on serum TAC

As depicted in Fig. 6, CPZ administration caused a considerable reduction in TAC compared to the normal diet ($P < 0.0001$). On the other hand, lutein therapy significantly improved serum TAC. In the prophylaxis group where mice received 200 mg/kg lutein concurrently with CPZ administration, there was a notable elevation in serum TAC compared to the CPZ group ($p < 0.0001$).





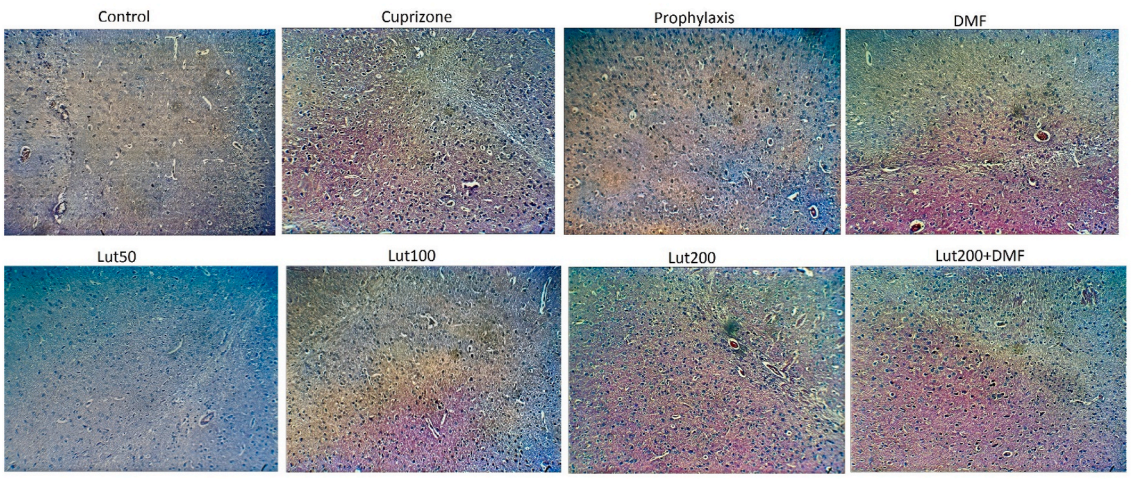


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Fig. 4. A) LFB staining of the corpus callosum in the various groups. **B)** The LFB staining of the corpus callosum in the various groups. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 significant vs. the cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001 significant vs. the DMF group (n = 6). **C)**The H&E staining of the corpus callosum in the various groups.

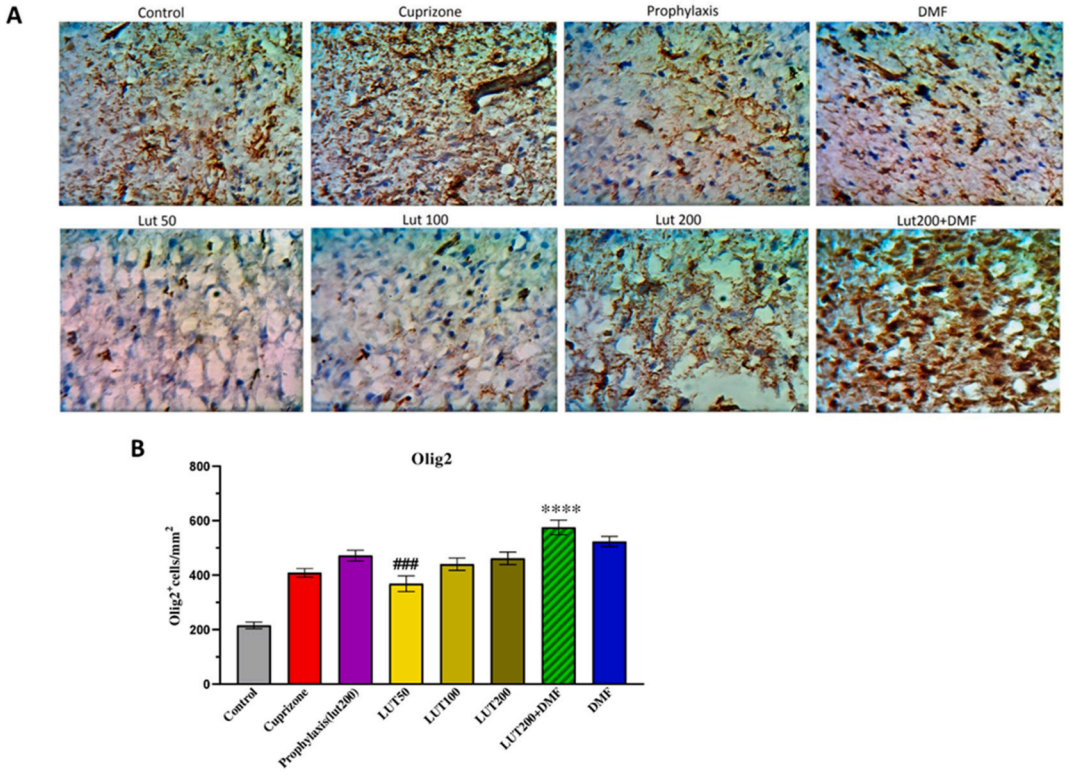

Fig. 5. A) Olig2 marker immunohistochemistry staining. **B)** Frequency of oligodendrocytes (Olig2^{+} population) in the corpus callosum region in different groups. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001significant vs. the cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 significant vs. the DMF group (n = 6).

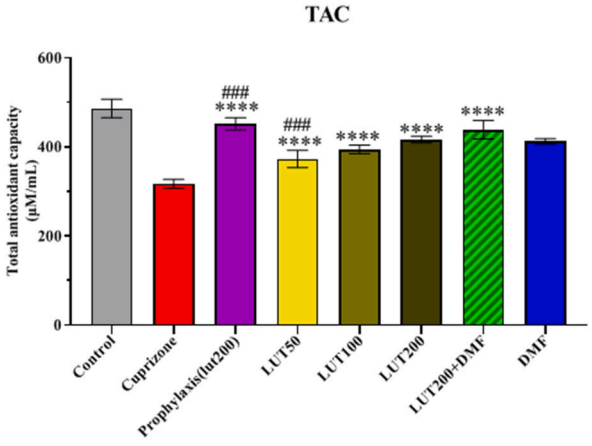

Fig. 6. Total antioxidant capacity of serum in different study groups. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 significant vs. the cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 significant vs. the DMF group (n = 6).

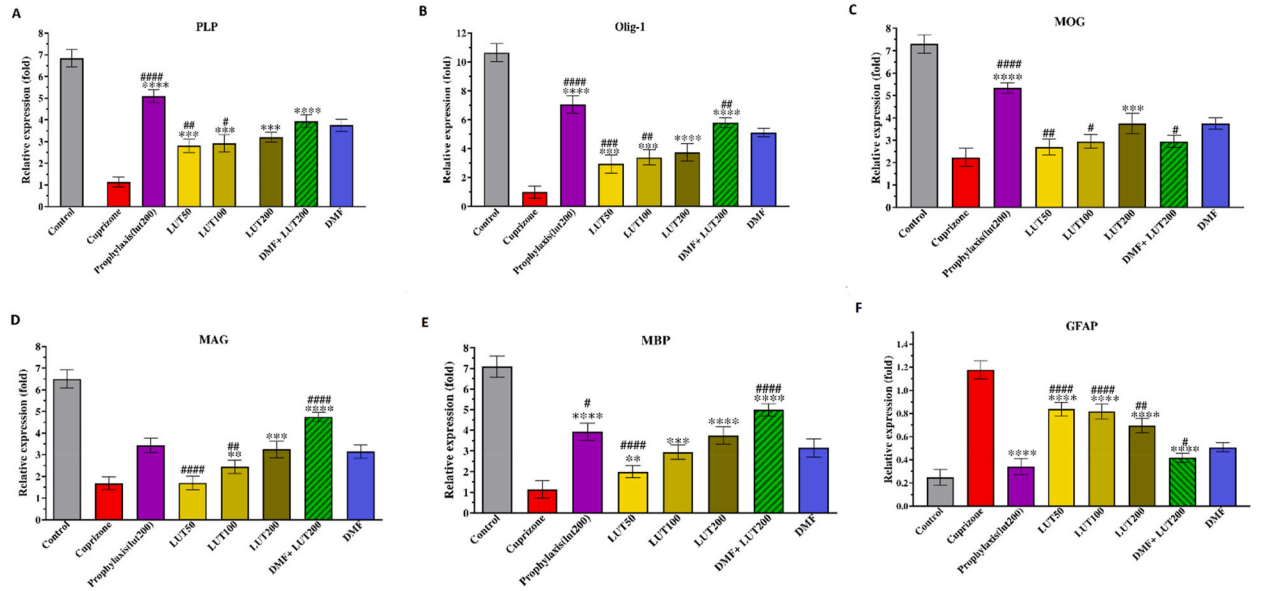

Fig. 7. The gene expression levels of PLP (**A**), Olig1 (**B**), MOG (**C**), MAG (**D**), MBP (**E**), and GFAP (**F**) assessed by qRT-PCR in various study groups. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 significant vs. the cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 significant vs. the DMF group (n = 6).

3.6. *Expression of genes involved in myelin production*

The expression levels of MBP, PLP, MOG, MAG, and OLIG-1 were significantly downregulated after CPZ administration (Fig. 7A, B, C, D and E). The expression level of GFAP, a marker of astrocytes, was upregulated after CPZ treatment. Lutein treatment significantly increased the expression levels of the genes involved in myelin production (MBP, PLP, MOG, MAG, and OLIG-1) and notably reduced the level of GFAP expression compared to the CPZ group (Fig. 7F).

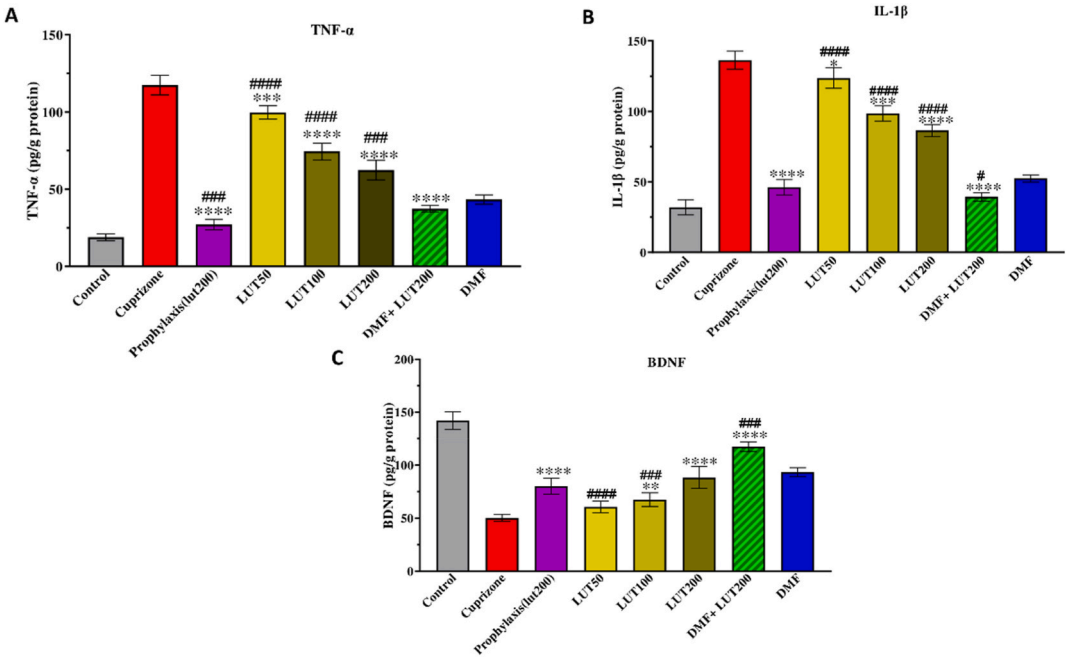

Fig. 8. The levels of inflammatory cytokines and BDNF in brain homogenate samples measured by ELISA. (**A**) TNF-α, (**B**) IL-1β, and (**C**) BDNF. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 significant vs. the cuprizone group; #P < 0.05, ##P < 0.01, ###P < 0.001, and ####P < 0.0001 significant vs. the DMF group (n = 6).

3.7. Effect of lutein on brain inflammatory markers

As shown in Fig. 8A and B, CPZ consumption for 6 weeks significantly elevated the levels of IL-1β and TNF-α in the brain homogenate compared to the control group. Conversely, treatment with various doses of lutein resulted in a significant reduction in IL-1β and TNF-α levels compared to the CPZ group.

The level of BDNF was assessed by ELISA in order to assess if this factor was involved in the neuroprotective mechanism of lutein and its protective effects on demyelination. As demonstrated in Fig. 8**(C),** CPZ administration significantly decreased BDNF level compared to the control group. On the other hand, lutein-treated mice showed an increase in BDNF level. In the lutein 200+DMF group, a considerably higher BDNF level was observed compared to the CPZ group ($p < 0.0001$).

4. Discussion

Multiple sclerosis is an inflammatory neurodegenerative disease characterized by neuronal damage. In this study the effects of lutein were assessed on neuronal myelination status and cerebral inflammation in C57BL/6 mice models of CPZ-induced MS. Lutein, as an antioxidant and anti-inflammatory compound, has been proven to have protective effects against neuronal damage in neurodegenerative disorders [12].

Our results demonstrated that mice fed with CPZ exhibited a significant reduction in their body weights throughout the study period, an observation that was in line with previous studies [30,33,34]. Luxol fast blue staining revealed an increase in myelin density in the groups receiving lutein compared to CPZ-fed mice. Our results further showed that lutein improved motor coordination, especially in mice receiving this agent in a prophylactic manner (i.e., concurrent with CPZ). Lutein therapy also increased serum TAC, highlighting its anti-oxidative effects. In addition, all CPZ-fed mice had increased levels of Olig2, a marker of oligodendrocytes, which was consistent with previous studies demonstrating that Olig2 expression was increased following cuprizone intoxication [35,36]. In addition, treatment with lutein increased the number of Olig2+ cells in the corpus callosum, suggesting that lutein could promote remyelination by oligodendrocyte maturation.

In this study, we observed that cuprizone intoxication significantly decreased the expression levels of the genes involved in myelin formation. This finding was in line with some earlier research indicating the downregulation of myelin-related genes following cuprizone intoxication [36,37]. We further found that cuprizone intoxication was associated with the significant upregulation of inflammatory markers such as TNF-α and Il-1β and the downregulation of neurotrophic factors like BDNF.

Cuprizone-induced mitochondrial dysfunction in oligodendrocytes leads to ROS accumulation, disruption of lipid and protein synthesis, and ultimately, oligodendrocyte apoptosis [38]. Consequently, agents with anti-oxidative properties can potentially protect against oligodendrocyte apoptosis and myelin loss following cuprizone intoxication. Numerous studies have confirmed the antioxidant and neuroprotective effects of lutein against oxidative and inflammation-induced neuronal impairment [18,19,39]. A study by Sasaki et al. showed that lutein, by acting as an antioxidant, could protect retinal neurons from oxidative and inflammation-induced damage in C57Bl/6 diabetic mice [40]. In line, Nataraj et al. (2016) demonstrated that lutein protected dopaminergic neurons against MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin)-induced apoptotic death and motor impairment by preventing mitochondrial injury in animal models of Parkinson disease [41]. Consistently, our results showed that lutein could improve antioxidative capacity by restoring serum TAC in cuprizone-intoxicated mice [42].

IL-1β and TNF-α, two pro-inflammatory cytokines, have been reported to increase during neuroinflammation in MS [9]. In the present study, cuprizone intoxication significantly elevated the levels of TNF-α and IL-1β. This observation was in agreement with the report of Zhu et al., noting that cuprizone intoxication increased the level of TNF-α in the corpus callosum of C57BL/6 mice [43]. On the other hand, Ghaiad et al. reported that cuprizone-intoxicated mice showed a nonsignificant elevation in TNF-α level [1]. Our findings demonstrated that lutein could effectively reverse the pro-inflammatory status nurtured by cuprizone poisoning. In the mice receiving prophylactic lutein (i.e., lutein treatment from the onset of cuprizone intoxication), a significant downregulation was noticed in TNF-α and IL-1β levels. In parallel, it has been suggested that long-term lutein treatment can effectively reduce inflammatory factors and enhance neuroprotection [44].

Dimethyl fumarate is a standard medicine used to treat MS. The biological function of DMF encompasses anti-oxidative, anti-apoptotic and immunomodulatory effects. This agent was reported to reduce IL-2 and TNF-α levels and activate the Nrf2 transcription factor [45,46]. In this study, DMF was used as a reference drug to evaluate the neuroprotective effects of lutein. Our findings indicated that combination treatment with lutein 200 mg/kg + DMF 15 mg/kg delivered more anti-inflammatory potential than DMF alone, suggesting synergism between these compounds due to their shared neuroprotective mechanisms, including anti-oxidative and anti-inflammatory effects.

BDNF has a critical role in the proliferation and differentiation of oligodendrocyte precursor cells (OPCs) and can promote the repair of myelin following demyelination [47,48]. The present study showed that cuprizone treatment significantly decreased the BDNF level in the corpus callosum compared to control, which was in line with previous studies [49,50]. Lutein therapy improved the level of BDNF in all treatment groups. A previous study reported that lutein/zeaxanthin isomers increased BDNF levels in C57BL/6 mice models of traumatic brain injury [51]. Pan et al. demonstrated that DMF treatment increased the level of BDNF in the brain tissue of a hypothyroid rat model [52]. These findings were consistent with the present finding, as we observed that combination therapy (lutein + DMF) resulted in a considerable elevation in BDNF level compared to the CPZ-intoxication group, suggesting a synergistic effect between lutein and DMF.

Demyelination is a characteristic of MS, and MBP, PLP, MAG, and MOG are the most abundant proteins in the structure of myelin. These proteins play essential roles in the structure, stability, and function of myelin [53]. Several studies have demonstrated that CPZ

intoxication reduces the expression of myelin proteins, thereby disturbing myelin compaction, leading to myelin loss [54,55]. In the present investigation, CPZ treatment was associated with a considerable reduction in the expression levels of the genes encoding myelin proteins. Compared to the CPZ group, a significant elevation in the expression of these genes was observed in CPZ-fed mice receiving lutein. The transcription factor, Olig1, plays a key role in myelogenesis and the development of oligodendrocyte precursor cells. Our results showed that the gene expression level of Olig1 was decreased after cuprizone intoxication in the corpus callosum. Prophylactic treatment with lutein significantly increased Olig1 gene expression level. These results were supported by histopathological and behavioral findings, indicating that lutein treatment could improve remyelination and motor function.

Lutein, a xanthophyll carotenoid, exhibits significant antioxidant activity by reducing oxidative stress-induced inflammation and damage to cells and tissues [12]. It suppresses the activation of inflammatory transcription factors such as NF-κB and STAT3, leading to a decrease in the expression of inflammatory cytokines (IL-1β, IL-6, MCP-1, TNF-α) and enzymes (COX-2, iNOS) [56]. Additionally, lutein activates the transcription factor Nrf2, which further reduces oxidative stress [56]. In our study, we observed that lutein treatment significantly enhanced motor functions and reversed cuprizone-induced demyelination by reduction of neuroinflammation, upregulation of myelin-related genes and an increase in the number of Olig2+ cells wich is critical for oligodendrocyte development and survival in C57BL/6 mice. These mechanisms of actions, could facilitate the repair of myelin and improve neuronal function, thereby explaining the observed behavioral and phenotypic changes in our study.

5. Conclusion

In the present study, our results showed that lutein treatment could promote remyelination and neuroprotective effects by reducing neuroinflammation and upregulating the expression of the genes involved in myelin formation. Also, lutein improved motor function in cuprizone-induced C57BL/6 mice models of MS(Fig. 9). These promising preclinical findings indicate that lutein may serve as a potential adjuvant therapy for patients with multiple sclerosis. However, to establish its safety and efficacy in humans, further clinical trials are essential.

CRediT authorship contribution statement

Atefeh Arab Firozjae: Writing – original draft, Formal analysis. **Mohammad Reza Shiran:** Methodology. **Abolghasem Ajami:** Writing – review & editing, Methodology. **Davood Farzin:** Writing – review & editing, Methodology. **Mohsen Rashidi:** Writing – review & editing, Funding acquisition, Conceptualization.

Ethics statement

All experiments were carried out in accordance with ethical guidelines of working with laboratory animals, and the protocol was approved by the Ethics Committee of Mazandaran University of Medical Science (Ethical Code: IR.MAZUMS.4.REC.1400.10302). The study was conducted in compliance with the ARRIVE guidelines for the reporting of animal studies.

Data availability statement

All datasets generated for this study are included in the article.

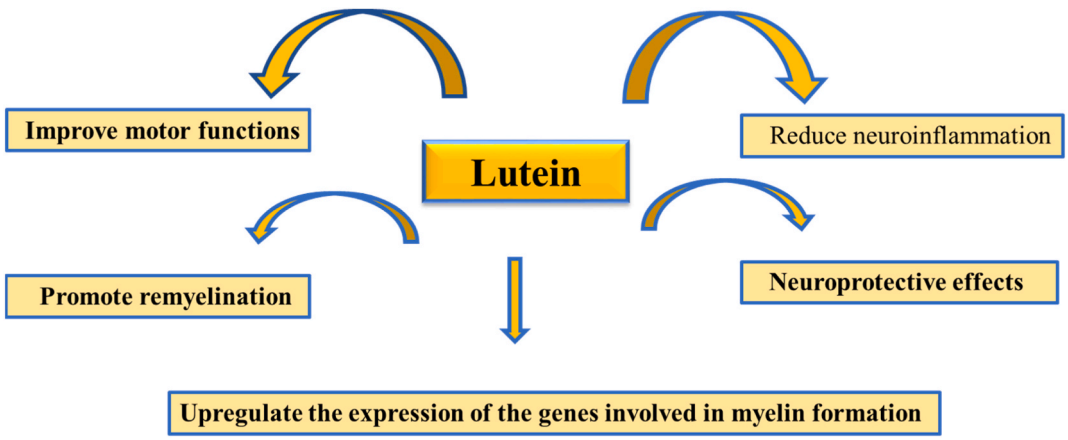

Fig. 9. The main mechanisms of action of Lutein in cuprizone demyelination model.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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