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ORIGINAL PAPER

Hesperidin, a Citrus Flavonoid, Has the Ameliorative Effects Against Experimental Autoimmune Encephalomyelitis (EAE) in a C57BL/J6 Mouse Model

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Abstract The aim of this study was determined the effects of Hesperidin (HP) on neuronal damage in brain tissue caused by Experimental allergic encephalomyelitis (EAE), an established model of multiple sclerosis in C57BL/J6 mice. To explore 40 mice were equally divided into four groups: (1) Control, (2) EAE, (3) HP, and (4) HP + EAE. 14 days after induction of EAE with MOG35-55 and pertussis toxin, the mice treated with HP at the doses of 50 mg/kg/day for 7 days subcutaneously. To our results HP treatment prevents the oxidative stress caused by EAE via a decrease in lipid peroxidations and increase in elements of the antioxidant defense systems in brain tissue. Also, EAE elevate the IL-17, express the pro-inflammatory cytokines, and caspase-3-like immunreactivity, show apoptosis, staining in EAE mice brain and increased the incidence of histopathological damage. However, immonohistochemical and histological changes were reversed with HP. Moreover, elevated TNF-α and IL-1β levels, a result of EAE, were decreased in serum and neurological deficits as clinical signs were reversed with HP treatment in EAE mice, given HP. In conclusion, HP treatment effectively prevents oxidative, immunological and histological damage in the brain caused by EAE. It was thought that the beneficial effects of HP are likely a result of its strong antioxidant and anti-inflammatory properties.

Keywords EAE · Hesperidin · C57BL/J6 · Oxidative damage · Immunological damage

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Introduction

Multiple sclerosis (MS) is one of the most common chronic inflammatory disease of the central nervous system (CNS) and experimental autoimmune encephalomyelitis (EAE) has served as the MS model that has been utilized to test novel therapies [1, 2]. MS is characterized with focal demyelinating in the white matter of the CNS and lead to apoptosis in oligodendrocytes [1, 3]. Due to damage in the brain and spinal cord, it leads to the clinical signs in patient, including physical, mental, and sometimes psychiatric problems [3]. The pathophysiology of MS is still unclear, but insights from experimental research suggested that dysfunction of blood brain barrier and migration T lymphocytes into the CNS are the hallmarks of in the pathogenesis of MS [2, 4]. In this result, demyelination and hamper nerve conduction mainly correlated with immune response (elevated cytokine level], oxidative stress (free radical formation) and apoptotic destruction [4, 5]. The studies based on EAE models [2, 4, 6] determined that the proinflammatory cytokines levels, such as IFN-gama, and TNF-alpha were increased and others such IL-4 were decreased in pathophyology of disease. Besides, several studies analyzed and showed that free radical formation, a result of mitochondrial injury in nucleotides, induced tissue injury in MS lesions [3, 7, 8]. Also, this injury can

histologically lead to apoptosis in brain tissue. Therefore, in recent years for the treatment of MS, researcher practiced the antioxidant or anti-inflammatory agents in EAE models. To date, the positive effects of many agents such as berberine, baicalein, donepezil and curcumin were investigated and it was showed that natural antioxidant therapy may be useful for MS treatment [2, 4–6].

Flavonoids such as hesperidin (HP) are a broad class of plant pigments that are structurally diverse class of polyphenolic compounds and they are strongly antioxidant compound [9]. Therefore, it was thought that flavonoids may be useful against the diseases caused the oxidative stress. HP, abundantly in *citrus* species such as lemon and orange, has been reported to possess pharmacological activities including antioxidant, analgesic, anticarcinogenic, anti-hypertensive, antiviral and anti-inflammatory [10, 11]. Oztanir et al. [12] determined that HP treatment significantly reduced lipid peroxidations and induced antioxidant systems such as glutathione and catalase against oxidative stress due to ischemia/reperfusion in brain tissue. Also HP, as daflon, is effectively used as a supplemental agent and helps to reduce edema or excess swelling [13]. Finally, it is believed that HP is a powerful radical scavenger that promotes cellular antioxidant defense system and can also traverse the brain-blood barrier [14, 15]. Therefore, it was thought that HP may prevent neurodegeneration caused by several conditions such as MS.

Because MS or EAE induces oxidative stress and inflammation in brain tissue, it was hypothesized that the antioxidative and anti-inflammatory properties of HP would ameliorate the neurological damage caused by MS in C57BL/J6 mice. Thus, the current study evaluated the oxidative stress status, immunological response and histopathological changes in the brain tissue of C57BL/J6 mice.

Materials and Methods

Reagents and Antibodies

Myelin oligodendrocyte glycoprotein ($MOG_{35–55}$) (MEVGWYRSPFSRVVHLYRNGK) and complete Freund's adjuvant containing 1 mg of heat killed Mycobacterium tuberculosis were obtained from Sigma Chemical Co. (St. Louis, MO). *Bordetella* pertussis toxin is given List biological laboratories inc. (Campell, CA). HP and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were of analytical grade or the highest grade available.

Animals

The present study was approved by the Ethical Committee on Animal Research of Inonu University and carried out in accordance with The Guidelines for Animal Research from the National Institutes of Health (NIH). C57BL/J6 female mice (clean grade) weighing 18–22 g (4–6 week) were supplied by the Inonu University Laboratory Animals Research Center (Malatya, Turkey), housed in sterilized polypropylene cages, and given an ad libitum diet of standard commercial food pellets and water. All mice were kept under a 12-h light/dark cycle at an ambient temperature of 21 ± 2 °C and a humidity level of 60 ± 5 %.

Induction and Scoring of EAE

The model of EAE was based on as Fonseca-Kelly et al. [16]. Firstly, mice were anesthetized with ketamine and xylazine (Sigma) i.p. Then, they were immunized with 300 μg $MOG_{35–55}$ peptide emulsified in complete Freund's adjuvant, divided into two doses injected subcutaneously at two separate sites on the back. Control mice were injected with an equal volume of phosphate buffered saline (PBS). EAE and control mice received 200 ng pertussis toxin in 0.1 ml PBS by i.p. injection on the day of immunization (day 0) and again on day 2 (48 h later). Clinical EAE was scored daily according to previously used five point scale by Fonseca-Kelly et al. [16] and Shindler et al. [17]: no disease = 0; partial tail paralysis = 0.5; tail paralysis or waddling gait = 1.0; partial tail paralysis and waddling gait = 1.5; tail paralysis and waddling gait = 2.0; partial limb paralysis = 2.5; paralysis of one limb = 3.0; paralysis of one limb and partial paralysis of another = 3.5; paralysis of two limbs = 4.0; moribund state = 4.5; death = 5.0.

Experimental Protocol

A total of 40 animals were randomly divided into four groups (n = 10): (1) control, (2) EAE group, (3) HP group, and (4) EAE + HP. The control group was wild type without EAE induction or treatment and the mice in the control group given PBS and 0.1 % carboxymethyl cellulose (CMC) as the vehicle. In EAE and EAE + HP group immunized as described above and EAE group treated with PBS and 0.1 % CMC as the vehicle. After 14th day induction of EAE, HP was dissolved in 0.1 % CMC and administered intraperitoneally (i.p.) for seven consecutive days for mice in HP and EAE + HP groups at the doses of 100 mg/kg/day. The dose of HP was based on preliminary dose-finding experiments from our lab [12]. At the end of 7 days, all animals were sacrificed under anesthesia, and tissue and sera samples were obtained for laboratory analyses.

Biochemical Analyses

The homogenization of tissue briefly was described our previous study [18]. The levels of thiobarbituric acid

reactive substances (TBARS), total glutathione (GSH) levels and catalase (CAT), CuZn-superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined by spectrophotometric methods and these methods briefly given our previous study [18, 19].

Determination of Cytokine Levels

Cytokine production was determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to the manufacturer's instructions. TNF-α and IL-1β levels were measured using anti-rat ELISA kits from Krishgen Biosystems International (CA. 90603 USA). The plates were read at 450 nm using the CA-2000 ELISA microplate reader and washer (CIOM Medical Co., Ltd. in China). Cytokine quantities in the samples were calculated from standard curves of recombinant cytokines using a linear regression method.

Histopathological Examination

Prior to evaluation using a light microscope, the brain samples were fixed in 10 % formalin and embedded in paraffin. The paraffin-embedded specimens were cut into 5-μm thick sections, mounted on slides, and stained with hematoxylin and eosin (H&E). The tissue samples were examined using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd.; Cambridge, UK).

For immunohistochemical analysis, the sections were mounted on polylysine-coated slides. Following rehydration of the samples, they were transferred to a citrate buffer (pH 7.6) and heated in a microwave oven for 20 min, cooled for 20 min at room temperature, and then washed with PBS. The sections were immersed in 0.3 % H_2O_2 for 7 min, washed with PBS, and then incubated with a primary rabbit-polyclonal caspase-3 antibody (PA1302; Boster; Pleasanton, CA, USA) and IL-17 antibody for 2 h. The sections were then were rinsed in PBS before incubation with biotinylated goat anti-polyvalent for 10 min and then streptavidin peroxidase for 10 min at room temperature. Staining was completed after the substrate was incubated with a chromogen for 15 min and the slides were counterstained with Mayer's hematoxylin for 1 min, rinsed in tap water, and dehydrated. The caspase-3 and IL-17 kit was used according to the manufacturer's instructions, and caspase-3 and IL-17 positive cells were stained a brown color.

We examined sections about histological damage in terms of mononuclear cell infiltration, edema, necrosis and hemorrhage, neuron degeneration and plaques brain tissue for histopathological scores. Also we evaluate three different areas in each sections and 21 different areas in each group for caspase 3-like immunreactivity and IL-17 expression scores. For histological scores were made graduating of histopathological damage as absent (0), slight (1), moderate (2) and severe (3). Sections stained by immunohistochemistry for Caspase-3 and IL-17 were analysed using a semi-quantitative scoring system (0: no Caspase 3-like immunreactivity and IL-17 expression; 1: scattered Caspase 3 and IL-17 positive neurons; 2: most neurons in the tissue with Caspase 3 and IL-17 reactivity).

Statistical Analysis

SPSS 13.0 (SPSS Inc.; Chicago, IL, USA) was used for all statistical analyses. For biochemical, immunological and cilinical signs values, the statistical analyses were conducted using one-way analysis of variance (ANOVA) and post hoc Tukey's Honestly Significant Differences test. The degree of significance was set at $P \leq 0.01$.

The statistical analyses for histopathological/immunohistopathological scores were made with SPSS 13.0 (SPSS Inc., Chicago, Ill., USA) or MedCalc 11.0 (Belgium) statistical programs. All data are expressed as arithmetic mean ± SE. For comparisons between groups Kruskal–Wallis and Connover tests were used. Exact p values were given where available, and $p \leq 0.0001$ was accepted as statistically significant.

Results

Clinical Signs

The clinic symtoms were given in Fig. 1. It was daily evaluated the clinical signs for the day onset the disease and the neurological symptoms firstly appeared on day 6 (0.45 ± 0.09) and peaked on day 12 (3.41 ± 0.12) and then reached the plateau value in mice. the day of disease onset and the severity of symptoms were similar in the EAE and EAE + HP groups. There were no clinical signs in HP and the control group. Both EAE and EAE + HP group reached the plateau value (3.41 ± 0.12) in terms of clinical signs. However, HP treatment significantly ($P \leq 0.01$) reduced the clinical score (2.25 ± 0.13) examined on day 21 compared with EAE group (3.46 ± 0.15).

Biochemical Results

TBARS, GSH, CAT, GPx, and SOD levels in mouse brain tissue are provided (Table 1). There was a significant increase in the level of TBARS and a significant decrease in GSH, CAT, GPx, and SOD levels in the EAE groups. The control and HP group value in terms of TBARS, SOD,

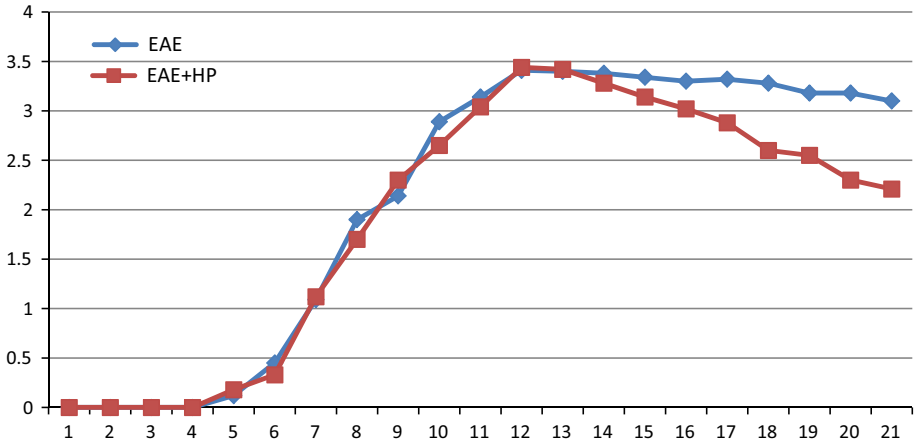

Fig. 1 The value of clinical signs in EAE and EAE + HP (*red line*) C57BlJ/6 mice. (n = 10, $P \leq 0.01$) (Color figure online)

CAT, GPx and GSH were similar. In the EAE + HP group, there was an attenuated increase in TBARS levels and diminished GSH levels and these values were closely the control group. Besides the EAE + HP group exhibited an increase in CAT, GPx, and SOD activities compared with the EAE group.

Cytokine Levels

TNF-α and IL-1β levels were given in Figs. 2 and 3 respectively. TNF-α levels were significantly increased in EAE group compared the other group. On the other hand, in EAE + HP group, TNF-α levels significantly decreased compared with EAE group. Besides, EAE lead to significantly increase in IL-1β levels compared with control and other groups. However HP treatment combined with EAE significantly decreased IL-1β level according to EAE group. Both TNF-α and IL-1β levels did not significantly change compared with a control group with the only HP treatment group.

Histopathological Results

In control (Fig. 4a) and HP (Fig. 4b) groups, cerebral cortex detected normal histological appearance. In EAE

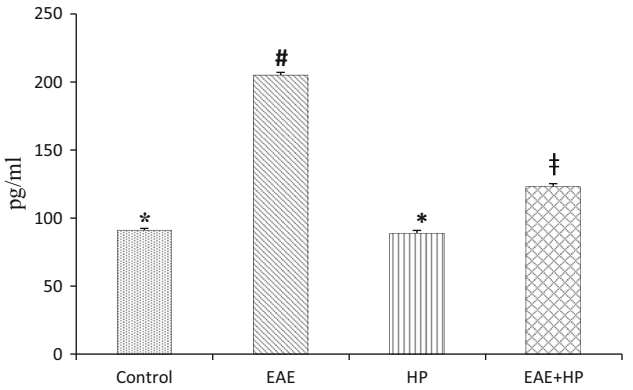

Fig. 2 Serum TNF-α levels in C57BlJ/6 mice (pg/ml ± SEM) *asterisk*, *number sign*, *not equal to* showed significant ($P < 0.01$) differences between groups

group, some histological changes were commonly observed such as mononuclear cell infiltration (Fig. 5a, b), edema (Fig. 5b), necrosis and hemorrhage (Fig. 5c), neuron degeneration (charactherized with have prominent eosinophilic cytoplasm and nuclear pyknosis; Fig. 5d) and plaques (Fig. 5e, f) brain tissue. On the other hand, histological damages such as mononuclear cell infiltration (Fig. 6a), plaques (Fig. 6b) and neuron degeneration similar to Fig. 5d (Fig. 6c) were significantly reduced in

Table 1 The levels of SOD, CAT, GPx, GSH and TBARS in brain tissue of C57 BL/J6 mice

	TBARS nmol/g tissue	GSH nmol/ml	CAT k/mg protein	SOD U/mg protein	GPx U/mg protein
Control	8.41 ± 0.72[a]	185.1 ± 4.18[a]	0.022 ± 0.0009[a]	23.11 ± 1.39[a]	208.7 ± 12.1[a]
EAE	12.91 ± 0.68[b]	121.4 ± 5.18[b]	0.013 ± 0.0008[b]	14.41 ± 1.20[b]	154.9 ± 18.9[b]
HP	8.68 ± 0.71[a]	190.8 ± 6.8[a]	0.021 ± 0.0011[a]	22.78 ± 2.06[a]	211.2 ± 19.2[a]
EAE + HP	10.8 ± 1.05[c]	145.3 ± 7.2[c]	0.015 ± 0.0009[c]	17.99 ± 1.38[c]	185.8 ± 16.7[c]

(Mean ± SD)

Means bearing different superscripts within same column were significantly different ($P < 0.01$)

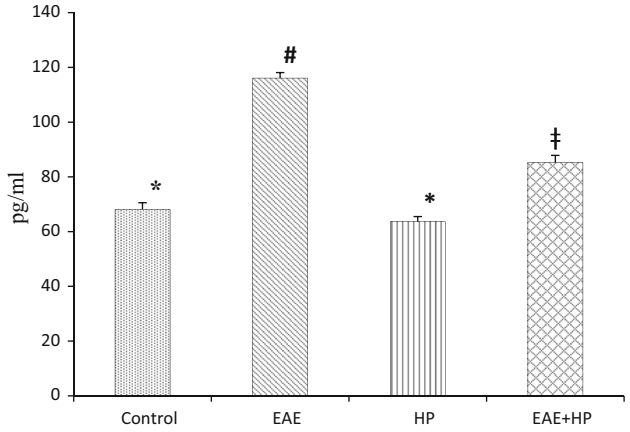

Fig. 3 Serum IL-1β levels in C57BlJ/6 mice (pg/ml ± SEM) *asterisk*, *number sign*, *not equal to* showed significant ($P < 0.01$) differences between groups

EAE + HP group and an improved histological appearance in brain tissue.

Immunohistochemically Caspase-3 positive stained cells were not observed in control (Fig. 7a) and HP (Fig. 7b) groups. The number of Caspase-3-like immunreactivity was high in EAE group (Fig. 7c). The proportion of immunohistochemically Caspase-3-like immunreactivity cells was minimal in EAE + HP group (Fig. 7d). In addition to this, in control and HP group there were no significant changes in terms of Immunohistochemically (Fig. 8a, b). Moreover, we observed IL-17 positive cells in EAE (Fig. 8c) and EAE + HP (Fig. 8d) groups with IL-17 immunohistochemical staining.

The histological and immunohistopathological scores given in Table 2. The histological damage was significantly increased in EAE group compared with others. Similarly, The stained areas of Caspase-3 and IL-17 were induced with mice brain in EAE model. There were not significant changes in control group value compared with HP group. On the other hand, HP therapy significantly reduced histological and immunohistological damages in terms of scored values.

Discussion

Multiple sclerosis, demyelating disease of the CNS, is caused severe neurological damage in young adults [20]. This damage disrupts the ability of parts of the nervous system to communicate, resulting in a wide range of signs and symptoms, including physical, mental and psychiatric problems [21, 22]. Therefore, EAE models have been developed to determine the efficacy of new therapeutic agents, such as flavonoids, for the prevention and treatment of factors associated with MS [4–7]. In this context, the efficacy of HP in ameliorating the oxidative, immunological and histological damage caused by EAE was examined in a C57BL/J6 mouse model. The present study found that treatment with HP (100 mg/kg) can protect the CNS against EAE damage via its antioxidant and anti-inflammatory properties.

Oxidative stress in the brains can cause irreversible injury with cell damage and subsequent cell death due to oxidation of cell components such as lipids, proteins and DNA and this condition lead to the formation of reactive oxygen species (ROS) in tissue; evidenced by elevated TBARS levels [23]. ROS can be counterbalanced by the antioxidant defense systems, both enzymatic (SOD, CAT, GPx) and non-enzymatic (GSH) Because CNS is vulnerable to oxidative stress due to its oxygen consumption, molecular antioxidant level, lipid rate of neurons or oligodentrocytes, oxidative stress may play an important role in MS [24]. In this study, the EAE lead to the inducing of lipid peroxidation caused by a significant increase in TBARS levels led to irreversible neuronal damage. Additionally, both the enzymatic (SOD, CAT, and GPx) and non-enzymatic (GSH) antioxidant defense systems were suppressed by EAE in brain tissue, as there were significant

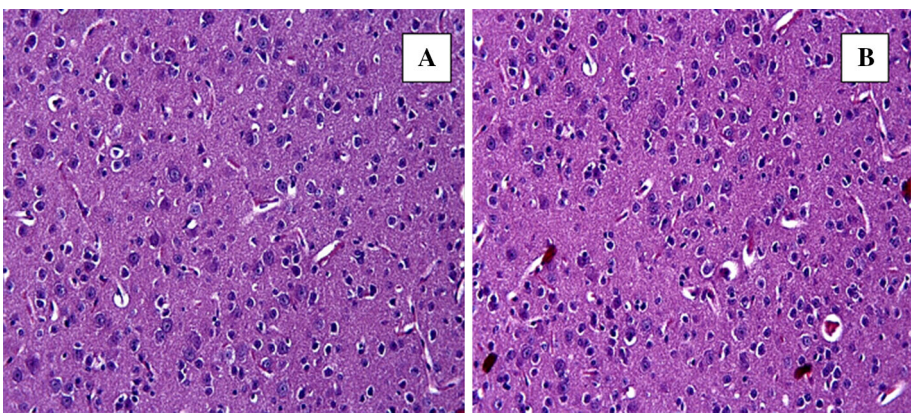

Fig. 4 In control (**a**) and HP (**b**) groups, brain tissue and neurons showed normal histological appearance. H–E;X20

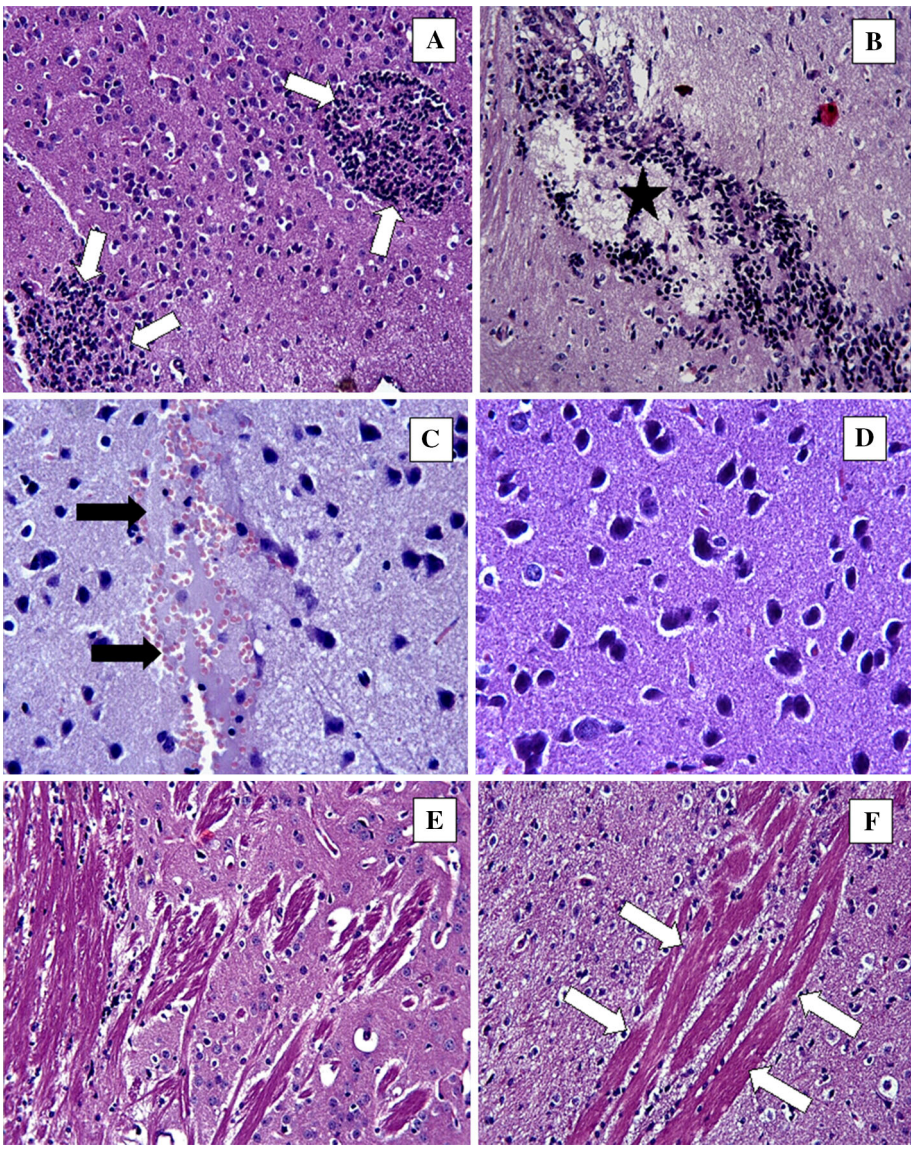

Fig. 5 **a** Mononuclear cell infiltration (*white arrows*) (H–E; ×20), **b** mononuclear cell infiltration and edema (*star*) (H–E; ×40), **c** necrosis and hemorrhage (*black arrows*) (H–E; ×40), **d** neuron degeneration (H–E; ×40) and **e**, **f** plaques were observed common in EAE group brain tissue

decreases in SOD, CAT, and GPx activities and in the levels of GSH. Similarly, Li et al. [7] demonstrated that EAE generates a significant increase in MDA levels on day 13, 20 and 30 post immunization and lead to lipid peroxidaitions in C57BL/6 J mice. Furthermore, Ljubisavljevic et al. [25] found that EAE significantly increased brain NO production and MDA level compared to the control values, whereas GSH and SOD levels was decreased in EAE rats in comparison with the control. These, and many other findings, support the present results [7, 25].

Oxidative stress plays an important role in MS due to cause significant cell death and neuronal damage in the brain [3]. In this context, the effects of EAE in the brain may depend primarily on increases in oxidative stress. Therefore, antioxidant agents such as HP may be useful in the brain damage caused by EAE. In this background, the findings from this study demonstrate that HP exhibits strong antioxidant activity based on the determination of TBARS, SOD, CAT, GSH, and GPx levels. It was determined that HP treatment resulted in a significant decrease in the elevated TBARS levels and a significant increase in the elements of the antioxidant defense systems that were negatively affected by EAE, including SOD, CAT, and GPx activity and GSH levels. These results indicated that HP treatment significantly reduces lipid peroxidation in the brain and reverses the oxidative damage caused by EAE in brain tissue via its antioxidant properties. To our knowledge, there are no reports investigating the beneficial effects of HP following EAE in mice. However, many investigators [12, 26, 27] examined that the effects of HP treatment have been evaluated against cerebral I/R in brain tissue. Oztanir et al. [12] determined that HP acts as a

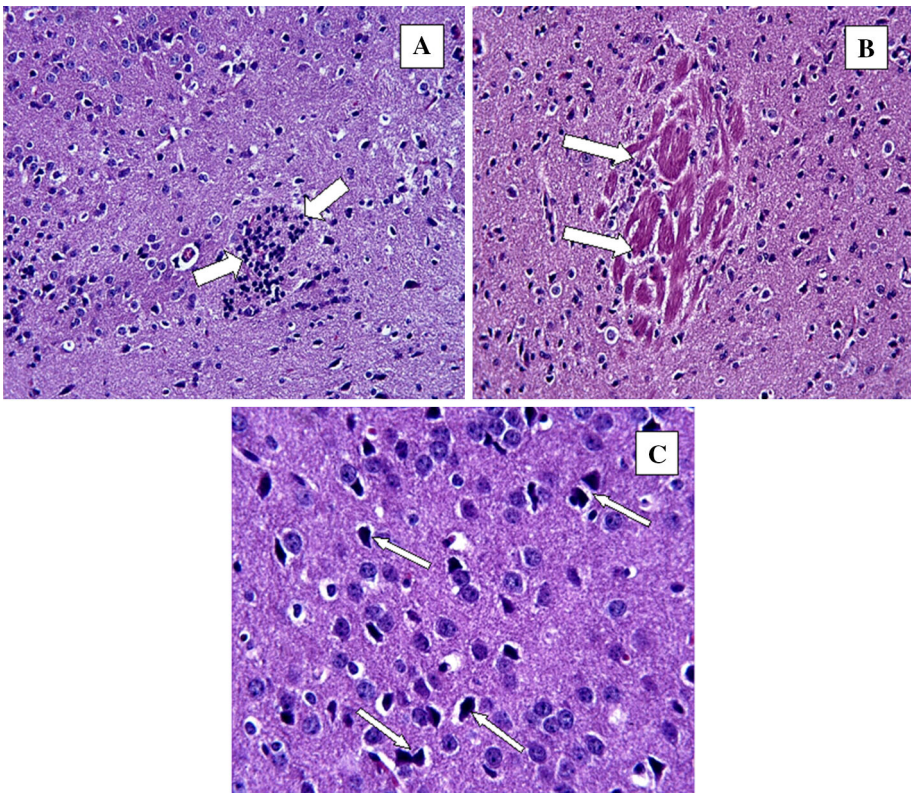

Fig. 6 The histopathological findings were significantly decreased in EAE + HP group. **a** Mononuclear cell infiltration (*white arrows*) (H–E; ×20), **b** few plaques (H–E; ×20), **c** neuron degeneration (H–E; ×40) were decreased in EAE + HP group

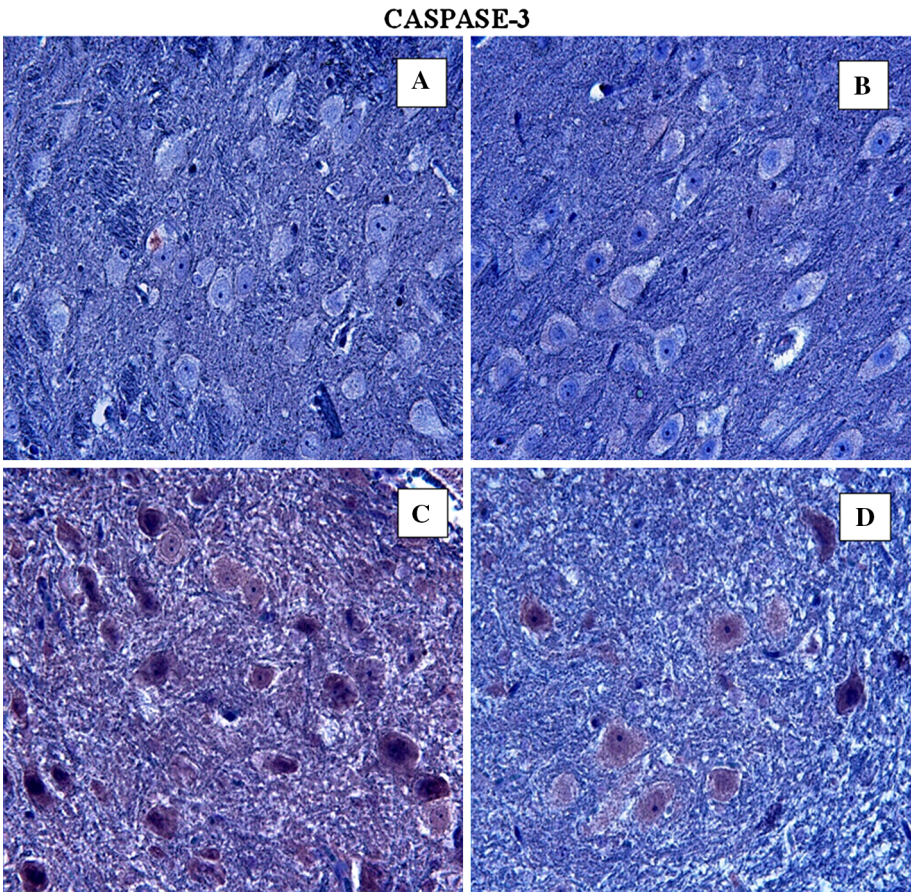

Fig. 7 Caspase- 3 immunostaining procedure in all groups. In EAE (**c**) and EAE + HP (**d**) groups, Caspase- 3 positive stained cells were observed. In EAE + HP group, positive stained cells were decreased compared with EAE group. (**a** Control, **b** HP)

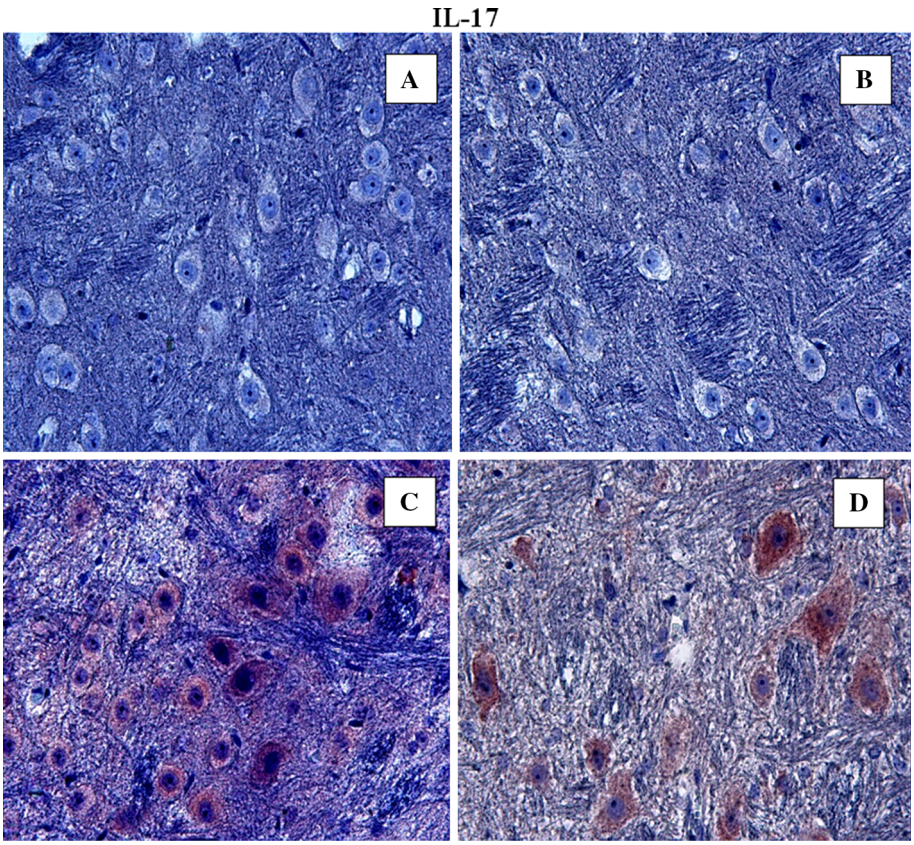

Fig. 8 IL- 17 immunostaining procedure in all groups. In EAE (**c**) and EAE + HP (**d**) groups, IL-17 positive stained cells were observed. In EAE + HP group, positive stained cells were decreased compared with EAE group. (**a** Control, **b** HP)

Table 2 The scores of histopathological and immunohistopathological data in brain tissue of C57 BL/J6 mice

	Histopathologic damage	Caspase 3-like immunreactivity	IL-17
Control	0.29 ± 0.18^{a}	0.19 ± 0.08^{a}	0.24 ± 0.10^{a}
EAE	2.71 ± 0.18^{b}	1.67 ± 0.11^{b}	1.48 ± 0.11^{b}
HP	0.57 ± 0.20^{a}	0.38 ± 0.11^{a}	0.48 ± 0.11^{a}
EAE + HP	1.86 ± 0.26^{c}	1.14 ± 0.14^{c}	1.10 ± 0.12^{c}

(Mean ± SEM)

Means bearing different superscripts within same column were significantly different ($P \leq 0.0001$)

powerful antioxidant and reduces lipid peroxidations in brain tissue through decreases TBARS levels and increases in SOD, CAT, GPx and GSH levels following cerebral ischemia in C57BL/J6 mice. Moreover, Kamisli et al. [10] demonstrated that HP prevents warm cisplatin-induced neuronal damage via its antioxidant properties in rats. Such findings are in agreement with the present findings and confirm our results. These results demonstrated that HP treatment can be protective against EAE due to its strong antioxidant properties and may prevent damage and lipid degeneration in neurons.

Recent studies [5, 16, 24] showed that EAE caused significant histopathological and immunohistopathological changes in brain and spinal cord such as apoptosis, chronic demyelination, neuronal loss and inflammation. Similarly, in the present study, it was revealed that EAE caused structural changes in brain tissue compared with the control group. Mainly, the histological damage included mononuclear cell infiltration, edema, necrosis/neuronal loss and hemorrhage, neuron degeneration and plaques in brain tissue. Additionally, EAE caused an increase in caspase 3-like immunreactivity and IL-17 positive cells, which are indicative of the apoptotic state of neurons and the rate of inflammation in cells. These results showed that EAE caused significant inflammation and apoptosis in neurons due to increase of caspase-3-like immunreactivity and IL-17 stained cells. On the other hand, we observed that HP treatment attenuates the negative histological changes caused by EAE, including a notably significant decrease in caspase 3-like immunreactivity and IL-17-stained cells.

Similarly, Resveratrol, another antioxidant agent, prevents neuronal loss, inflammation and apoptosis in mice [16]. Moreover, our previous study [12] about the protective effect of HP against global cerebral ischemia injury showed that HP treatment can be prevent the negative histological changes caused by I/R, including a notably significant decrease in caspase 3-like immunreactivity. In this context, it can be assumed that there is a correlation between the oxidative status of the brain and EAE-induced histopathological changes. For this reason, we conclude that HP may provide protective effects in EAE by inhibiting the elevation of oxidative stress status and preventing histological damage in brain tissue.

TNF-α, known as cachexin, or cachectin, is an adipokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction., the activation of variety of cell types and expression of adhesion molecules, cytokines and chemokines in the CNS were induced with TNF-α production with Th1 response [28]. Additionaly, IL-1β, a proinflammatory cytokine also known as catabolin, is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. Therefore, the TNF-α and IL-1β expression in serum parallels the disease course in EAE [28]. In this study, we determined that TNF-α and IL-1β levels significantly increase in EAE mice compared to control and other groups in serum samples. However, these elevated values were decreased with HP treatment and values were closely control levels in mice. This conclusion indicated that HP can be reversed inflammation caused by EAE in serum samples with in the body. Similarly, recent studies [28, 29] showed that EAE led to a significant increase in cytokine levels such as TNF-α and IL-1β in mice and these studies confirmed our results. As far as we are aware, there is no study about how HP treatment effect cytokine level in EAE. On the other hand, the studies about the HP effect on immune response [30, 31] showed that HP has the anti-inflammatory effect on immune system. Therefore, we thought that the beneficial effects of HP on EAE in terms of cytokine levels are based on its anti-inflammatory and antioxidant properties.

Finally, in this study, we determined that the clinical signs in mice caused by EAE were showed as neurological deficits such as partial and total tail paralysis, waddling gait, partial and total limb paralysis. On the other hand the neurological deficits were decreased with HP treatment compared with EAE group. This result showed that HP treatment ameliorates the EAE clinical signs. It was thought that these beneficial effects were correlated with the positive effects of HP in oxidative, histological/immunohistological and immunological examinations. Similarly, Paula et al. [28] and Warford et al. [29] confirmed our results and claimed that genistein, a flavonoid, and AF4, flavonoid enriched fraction treatment can be prevent clinical symptoms of EAE in mice.

Conclusion

The current study demonstrated that EAE inducted with $MOG_{35–55}$ in C57BL/J6 mice results in neurodegenerative effects associated with induces in oxidative stress, cytokine levels and histopathological changes in brain tissue and increase in clinical signs as neurological deficits. Additionally, treatment with HP (100 mg/kg/day) for seven consecutive days following after 14 days of immunizations generally reversed the potentially negative effects of EAE, likely due to its strong antioxidant, radical scavenging and anti-inflammatory properties. Therefore, based on the present results, it is proposed that HP ameliorates the neuronal damage caused by EAE in mice.

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Conflict of interest The authors have declared no conflict of interest.

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