Ginger Extract Reduces the Expression of IL-17 and IL-23 in the Seraand Central Nervous System of EAEMice

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ABSTRACT

Background: IL-17/IL-23 axis plays an important role in the pathogenesis of several autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS). The immunomodulatory properties of ginger are reported in previous studies. **Objective:** To evaluate the effects of ginger extract on the expression of IL-17 and IL-23 in a model of EAE. **Methods:** EAE was induced in C57BL/6 mice by immunization with myelin oligodendroglial glycoprotein and then treated with PBS or ginger extracts, from day +3 to +30. At day 31, mice were scarificed and the expression of IL-17 and IL-23 mRNA in spinal cord were determined by using real time-PCR. The serum levels of cytokines were measured by ELISA. **Results:** The mRNA expression of IL-17, IL-23 P19 and IL-23 P40 in CNS and serum levels of IL-17 and IL-23 were significantly higher in PBS-treated EAE mice than non-EAE group ($p<0.003$, $p<0.001$, $p<0.001$, $p<0.05$ and $p<0.01$, respectively). In 200 mg/kg ginger-treated EAE mice the mRNA expression of IL-17, P19 and P40 in CNS and serum IL-23 levels were significantly decreased as compared to PBS-treated EAE mice ($p<0.05$, $p<0.001$, $p<0.001$ and $p<0.05$, respectively). Moreover, 300 mg/kg ginger-treated EAE group had significantly lower expression of IL-17, P19 and P40 in CNS and lower serum IL-17 and IL-23 levels than PBS-treated EAE group ($p<0.02$, $p<0.001$, $p<0.001$, $p<0.03$ and $p<0.004$, respectively). **Conclusion:** Ginger extract reduces the expression of IL-17 and IL-23 in EAE mice. The therapeutic potential of ginger for treatment of MS could be considered in further studies.

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Keywords: Experimental Autoimmune Encephalomyelitis, Ginger, IL-17, IL-23

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INTRODUCTION

Multiple sclerosis (MS) and its animal model which is known as experimental autoimmune encephalomyelitis (EAE) are immune-mediated demyelinating diseases of the central nervous system (CNS) (1). The immune system, in particular, $CD4^+$ T-helper (Th) cells play a central role in the pathogenesis of MS and EAE diseases(2). Functionally, distinct Th cells are induced from naïve T cells upon antigenic stimulation including Th1, Th2, Th17 or regulatory T (Treg) cells which secrete distinct cytokine profiles (2). Both interferon-γ (IFN-γ)-producing Th1 cells and IL-17-producing Th17 cells are contributing in the development of MS and EAE (2,3). Treg cells related cytokines [transforming growth factor-β (TGF-β) and IL-10] have been associated with the reduction of CNS inflammation and improvement of MS and EAE symptoms (2,4). The role of Th2 cells, which produce high levels of IL-4 and IL-5 and IL-13 remain controversial (2). We have previously indicated higher concentrations of a Th17-related chemokine [C-C motif ligand 20 (CCL20)] and lower levels of a Th2/Treg-related chemokine [C-C motif ligand 22 (CCL22)] in patients with MS (5,6). Th17 cells are characterized by the secretion of several cytokines and chemokines including IL-17 (also called IL-17A), IL-17F, IL-21, IL-22, IL-23, IL-26, tumor necrosis factor-α (TNF-α), CCL20 and granulocyte monocyte-colony stimulating factor (GM-CSF), although some of these cytokines are not Th17-specific (3,7). IL-17 can influence different cells including endothelial cells, epithelial cells, fibroblasts, myeloid cells and synoviocytes(8). IL-17 induces the secretion of various inflammatory mediators including IL-8, C-X-C motif ligand 1 (CXCL1), CXCL6, IL-1β, IL-6, TNF-α, GM-CSF, macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1) and granulocyte-colony stimulating factor (G-CSF) (3,8). IL-17 is also considered as a strong inducer of neutrophil infiltration into inflammatory sites (3). Th17 cells were associated with several autoimmune and inflammatory diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), psoriasis, inflammatory bowel disease (IBD) and allergy and asthma (9).

IL-23, a member of the IL-12 family, consistsof P19 and P40 subunits, the latter being shared with IL-12. The receptor for IL-23 is also heterodimeric and consists of the IL-23R (P19-binding subunit) and the IL-12Rβ1 (P40-binding subunit) (10). IL-23 is secreted by dendritic cells (DCs) and macrophages and plays an important role in the development and full activation of Th17 cells (10). The antigenic stimulation of T cells in the presence of TGF-β and IL-6 induces the initial differentiation of naive $CD4^+$ T cells to Th17 cells (11). However, subsequent exposure to IL-23 is needed for the functional maturation and pathogenic action of Th17 cells. Indeed, TGF-β and IL-6-driven Th17 cells are inefficiently pathogenic and more exposure to IL-23 is necessary for functional maturation of Th17 cells and development of inflammatory Th17 cells (10,12). The association of the IL-23/IL-17 axis with some inflammatory diseases such as systemic lupus erythematosus (13), spondyloarthritis (14), psoriatic arthritis (15), Graves' disease (16), Crohn's disease (17), EAE and MS (18) has been reported.

The rhizomes of the ginger (*Zingiberofficinale*) are commonly used as a flavor or food supplement. There are some antioxidants and anti-inflammatory components in ginger rhizomes (19,20). The anti-inflammatory effects of ginger and its components have been also demonstrated in patients with type 2 diabetes (21), osteoarthritis (22), rheumatoid arthritis (23,24), acute respiratory distress syndrome (25) and primary dysmenorrheal (26). The anti-inflammatory activities of ginger extract or its pungent

constituents such as gingerol and shogaol have been also demonstrated in experimental animal models such as models of airway inflammation (27), ulcerative colitis (28), neuroinflammation (29) and gastric ulcers (30).

The long-period usage of anti-inflammatory chemical medication is known to be associated with various side effects. The consuming of herbal natural products for treatment of inflammatory diseases may be more effective and have fewer side effects. We have recently reported that the ginger-treated EAE mice exhibited mild symptoms of EAE, a delay in disease onset and low infiltration of the leukocytes into the spinal cord. Moreover, treatment with ginger extract modulates the expression of IL-27 and IL-33 mRNA in EAE mice (31). The aim of the present study was to investigate the effects of ginger extract on the expression of IL-17 and IL-23 in the CNS and in the serum by using an EAE model induced by immunization with MOG in C57BL/6 mice.

MATERIALS AND METHODS

Preparation of the Ginger Extract. The ginger extract was prepared as explained previously (31). Briefly, *Zingiber officinale* (ginger) was purchased at Herbal Institute from Isfahan, Iran. The hydro-alcoholic extract of ginger was prepared by maceration method. Three kilograms fresh ginger rhizome cut into tiny pieces, air dried and ground into a fine powder using a pestle and mortar. The ginger powder was hold in a suitable container and 2000 ml ethanol 50% was added and left at room temperature for 15 hrs. Subsequently the solid phase was removed by filtration and combined extracts were concentrated at 40°C, so that the solvent was evaporated using a rotary evaporator to give an extract that was designated as an alcoholic extract. Finally, a semi-dried extract was obtained and then the appropriate amount of the originated extract was calculated and dissolved into the proportional volume of PBS. The prepared extract kept in a refrigerator until use.

Mice. Female 6 to 8 weeks old C57BL/6 mice were obtained from Pasteur Institute, Tehran, Iran. Mice were maintained in temperature-controlled conditions with a 12-hour light/12-hour dark cycle and were administered standard laboratory food and water ad libitum. All mice were housed in a room where the testing procedure was performed so as to minimize any stress response potentially induced by novel environmental cues. All experiments were conducted on-site at the Rafsanjan University of Medical Sciences and were performed in accordance with the recommendations of the Medical School Ethics Committee on Animal Experimentation.

Induction and Scoring of EAE. The EAE has been induced as explained previously (31). Briefly, the C57BL/6 mice were injected subcutaneously (s.c.) on day 0 with 400 μg of $MOG_{35–55}$ peptide emulsified in complete Freund's adjuvant containing 5 mg/ml of *M. tuberculosis* at two sites in the flank. The mice received two additional intra-peritoneal (i.p) injections of 250 ng of pertussis toxin on days 0 and 48 hours post immunization. Mice were weighed and evaluated daily for clinical symptoms of disease. The disease scored based on the following criteria: 0, asymptomatic; 1, loss of tail tone; 2, flaccid tail; 3, paralysis of one hind limb; 4, paralysis of two hind limbs; 5, forelimb and hind limb paralysis; 6, dead(32, 33). Paralyzed mice were given easy access to food and water.

Planning of Research. The mice were classified into 4 groups (5-6 mice in each) as follows: Group I (healthy control group): Mice in this group were considered as healthy

normal without EAE induction and only treated with PBS as vehicle. Group II (EAE negative control group): Mice in this group were considered as PBS-treated EAE group without receiving ginger extract. Group III (ginger-treated EAE group): The mice with EAE enrolled into this group and considered as 200 mg/kg ginger-treated group that received 200 mg/kg of ginger extract. Group IV (ginger-treated EAE group): The mice with EAE enrolled into this group and also considered as 300 mg/kg ginger-treated group that received 300 mg/kg of ginger extract.

The mice were immunized on day 0 with injection of an emulsion of MOG peptide and complete Freund's adjuvant containing *Mycobacterium tuberculosis* to induce EAE. The mice were intra-peritoneally (i.p.) administered with either vehicle (PBS) in control groups or ginger extracts (200 or 300 mg/kg BW, every other day) from day +3 to +30 in ginger treated groups. The EAE clinical scores and body weight were evaluated till day 30. At day 31 all mice were scarified, the blood samples, the spinal cords and brains were removed for more analyses.

Real-Time PCR. The mRNA expression of the IL-17 and IL-23 in the spinal cord was determined by real-time PCR. Total RNA was extracted from spinal cord using Trizol Reagent (Invitrogen, Carlsbad, CA). The purity of extracted RNA was determined by electrophoresis on an ethidium bromide pre-treated agarose gel together with measuring absorption by spectrophotometer and calculation of 260/280 ratio. The extracted RNA was converted to cDNA using a cDNA synthesis kit (Bionner, Korea) with both oligo (dT) and random hexamer primers. The process of reverse transcription was performed by using the following program: 70°C for 10 min (without reverse transcriptase enzyme), 20°C for 1 min (cooling phase), addition of reverse transcriptase enzyme, 42°C for 60 min, and the program was finished by a final step at 95°C for 10 min to terminate the reverse transcriptase activity.

Real-time PCR was performed using a SYBR green master mix (Bionner, Korea), mixed with 200 ng of template cDNA with the suitable gene-specific primers (Table 1) in a Bio-Rad CFX96 system (Bio-Rad Company, USA) using the following program: 1 cycle of 95°C for 15 min, 40 cycles of 95°C for 15 s and 60°C for 20 s and finally 72°C for 20 s. Primers were synthesized by the Bionner Company (Korea).

Table 1. The used primers for the gene expression of IL-17 and IL-23 in the spinal cord.

Gene	Primer
IL-17	Forward: CTTGGCGCAAAAGTGAGCTCC Reverse: CTTTCCCTCCGCATTGACACA
IL-23 (P19)	Forward: ATAATGTGCCCCGTATCCAGT Reverse: CTGGCTGTTGTCCTTGAGTCC
IL-23 (P40)	Forward: GGAAGCACGGCAGCAGAATA Reverse: AACTTGAGGGAGAAGTAGGAATGG
β-Actin	Forward: AGAGGGAAATCGTGCGTGAC Reverse: CAATAGTGATGACCTGGCCGT

Real-time PCR was performed in triplicate and the β-actin gene was applied as a housekeeping gene for normalization of the amplified signals of the target genes. The quantity of cytokines mRNA in the spinal cord, expressed as units relative to the amount of β-actin mRNA. The dissociation stages, melting curves and quantitative analyses of the data were performed using CFX manager software version 1.1.308.111 (Bio-Rad, USA).

Detection of the Serum Levels of IL-17 and IL-23. At day 31, mice were bled by cardiac puncture and the sera was then isolated and stored at -20°C. Serum levels of IL-17 and IL-23 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, UK). The levels of sensitivity of the IL-17 and IL-23 kits were 4.0 and 8.0 Pg/mL, respectively.

Statistical Analysis. Data are presented as mean ± SEM. Statistical significance was determined by using ANOVA and Student's *t*-test, as appropriate. The P values of less than 0.05 were considered statistically significant. The data were analyzed by statistical SPSS software (version 18, Chicago, IL, USA).

RESULTS

The Effects of Ginger extract on the Clinical and Pathological Symptoms of EAE. The presence of EAE was approved according to the clinical signs of disease and histopathological observations of CNS(32,33). The effects of the ginger extract on the clinical and pathological symptoms of EAE were the same as we have previously reported (31). As demonstrated in Figure 1, the clinical symptoms of disease appeared in PBS-treated EAE mice earlier, at day 10, whereas 200- and 300 mg/kg ginger-treated EAE groups showed the clinical symptoms later, at days 14 and 15, respectively.

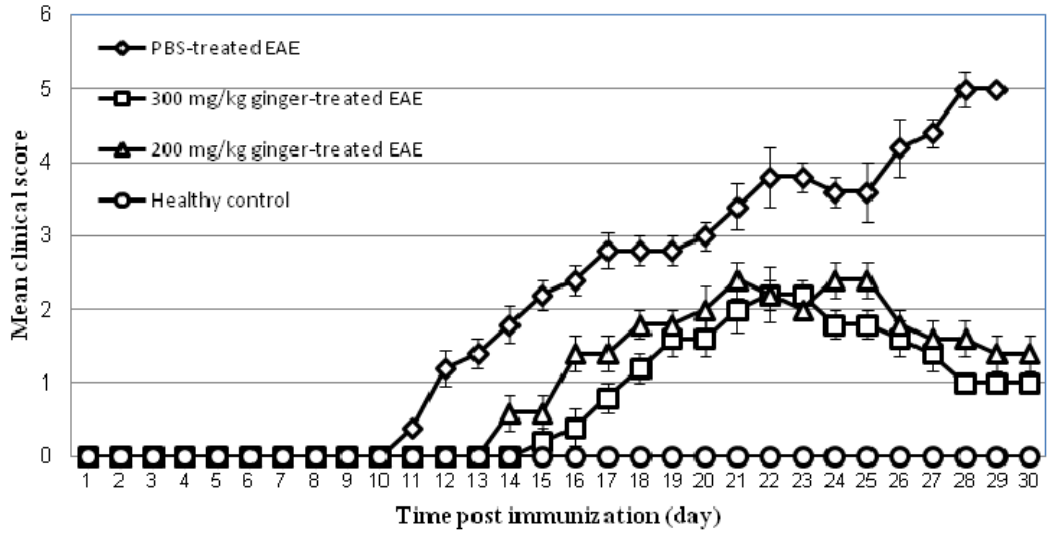

Figure 1. Comparison of the clinical scores of the EAE between ginger-treated and control groups.The maximum mean clinical score was significantly lower in 200 mg/kg and 300 mg/kg ginger-treated EAE groups as compared with PBS-treated EAE mice ($p<0.002$ and $p<0.001$, respectively)

The maximum mean clinical score (MMCS) were significantly lower in 200- and 300 mg/kg ginger-treated EAE groups in comparison with PBS-treated EAE mice ($p<0.002$ and $p<0.001$, respectively).

The Effects of Ginger Extract on the Expression of IL-17 mRNA in the Spinal Cords. The expression of IL-17 mRNA in the CNS of ginger-treated EAE mice and control groups are demonstrated in Figure 2.

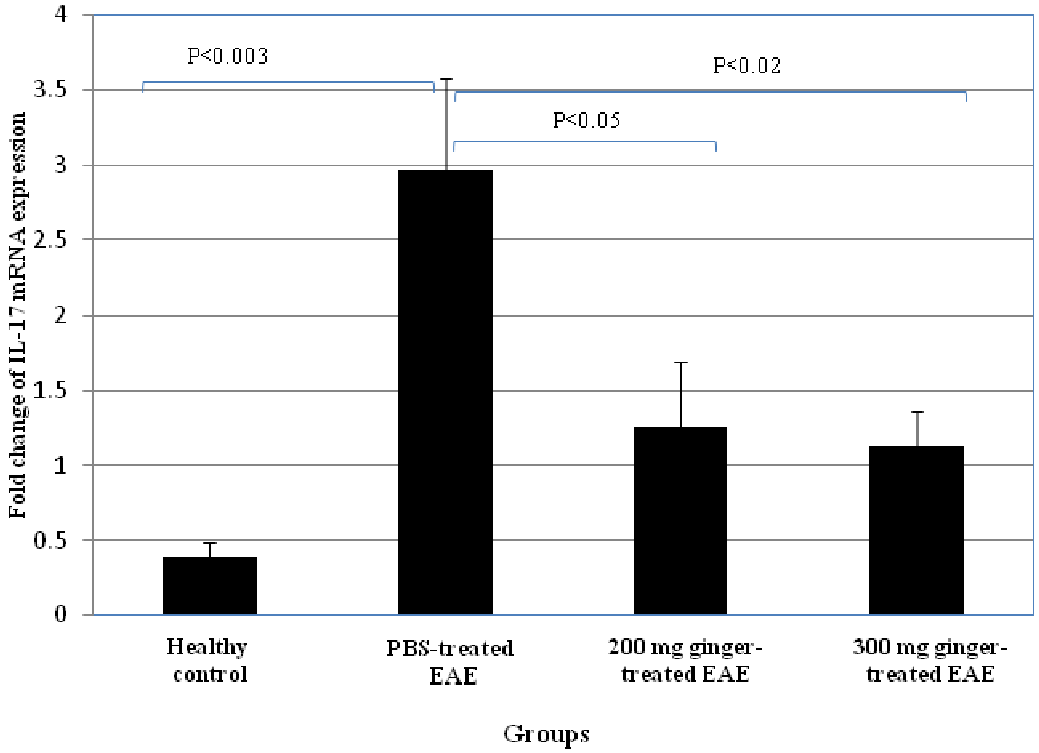

Figure 2. Comparison of the expression of IL-17 mRNA in the spinal cord between ginger-treated EAE and control groups. The expression of IL-17 mRNA significantly decreased in both 200 mg/kg and 300 mg/kg ginger-treated EAE groups as compared to PBS-treated EAE mice ($p<0.05$ and $p<0.02$, respectively).

In PBS-treated EAE mice the expression of IL-17 mRNA was significantly higher than that in healthy control group (2.97 ± 0.61 vs. 0.39 ± 0.10; $p<0.003$). The expression of IL-17 mRNA was 1.26 ± 0.43 and 1.13 ± 0.23.

for 200 mg/kg and 300 mg/kg ginger-treated EAE groups, respectively. The expression of IL-17 mRNA significantly decreased in both 200 mg/kg and 300 mg/kg ginger-treated EAE groups as compared to PBS-treated EAE mice ($p<0.05$ and $p<0.02$, respectively). No significant difference was observed between 200- and 300 mg/kg ginger-treated EAE groups regarding the IL-17 mRNA expression. However, the IL-17 mRNA expression in both 200- and 300 mg/kg ginger-treated EAE groups were significantly higher than in healthy control group ($p<0.05$).

The Effects of Ginger Extract on the Serum Levels of IL-17. Mean serum concentrations of IL-17 were 37.77 ± 18.32 Pg/mL in the healthy control group, 136.47 ± 34.43 Pg/mL in the PBS-treated EAE mice, 82.94 ± 32.66 Pg/mL in 200 mg/kg ginger-administered EAE mice and 51.83 ± 9.17 Pg/mL in 300 mg/kg ginger-treated EAE group (Figure 3).

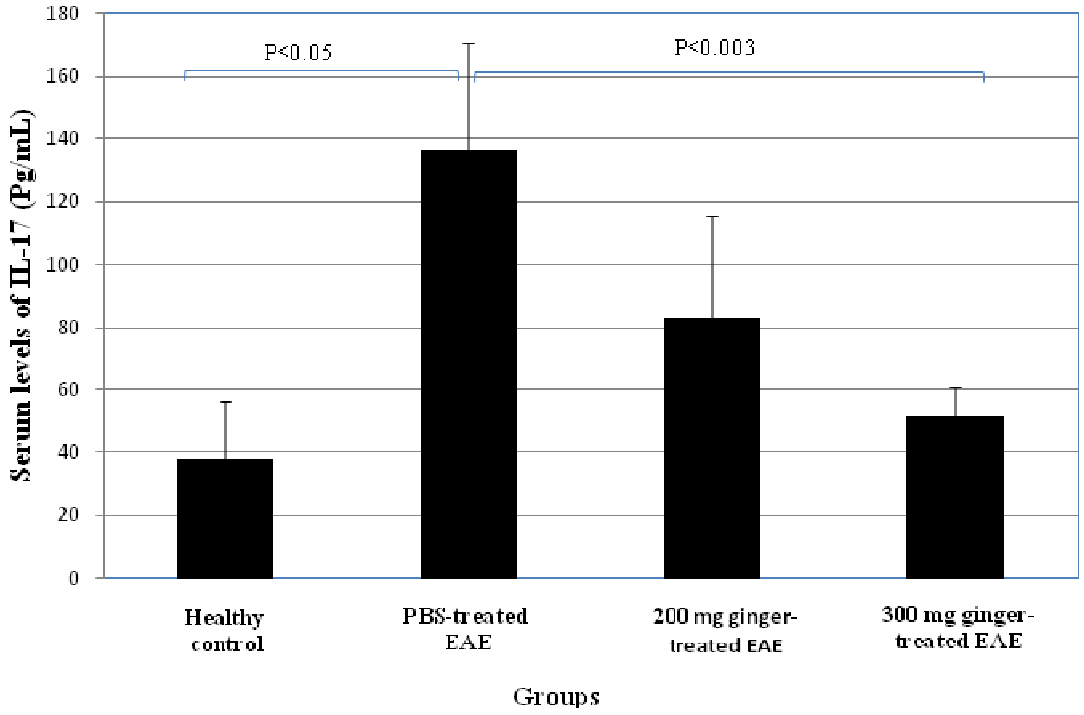

Figure 3. Comparison of the serum levels of IL-17 between ginger-treated and control groups. The mean serum levels of IL-17 in PBS-treated EAE group was significantly higher than that observed in healthy group ($p<0.05$). The mean serum levels of IL-17 in 300 mg/kg ginger-treated EAE group was significantly lower as compared to PBS-treated EAE group ($p<0.03$).

The serum concentrations of IL-17 in PBS-treated EAE mice were significantly higher as compared to healthy control group ($p<0.05$). The serum concentrations of IL-17 were significantly lower in 300 mg/kg ginger-administered EAE group in comparison to PBS-treated EAE mice ($p<0.03$).The 200 mg/kg ginger-treated EAE mice also had lower serum concentrations of IL-17 than PBS-treated EAE mice but the difference was not statistically significant. The difference of the serum concentrations of IL-17 between 200- and 300 mg/kg ginger-treated EAE groups was not significant, although, this parameter was lower in 300 mg/kg ginger-administered EAE mice.

The Effects of Ginger Extract on the Expression of IL-23 P19 in the Spinal Cords. The expression of IL-23 P19 mRNA in the CNS of ginger-treated EAE mice and control groups are demonstrated in Figure 4. The expression of IL-23 P19 mRNA were 1.00 ± 0.46 in healthy control group, 15.88 ± 1.57 in PBS-treated EAE mice, 4.55 ± 1.03 in 200 mg/kg ginger-treated EAE mice and 4.23 ± 0.95 in 300 mg/kg ginger-treated EAE group.

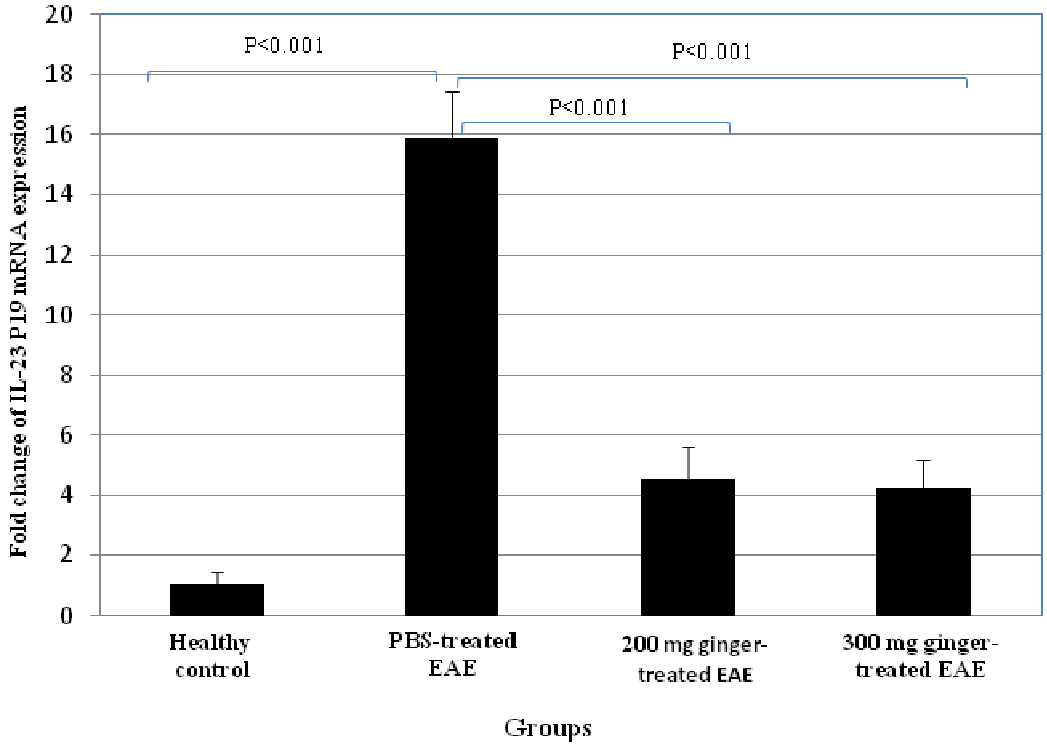

Figure 4. Comparison of the expression of IL-23 P19 mRNA in the spinal cord between ginger-treated EAE and control groups.

The expression of IL-23 P19 mRNA was significantly higher in PBS-treated EAE mice in comparison to healthy control group ($p<0.001$). Both 200- and 300 mg/kg ginger-treated EAE groups had significantly lower expression of IL-23 P19 mRNA as compared to PBS-treated EAE mice ($p<0.001$). However, the expression of IL-23 P19 in 200- and 300 mg/kg ginger-treated EAE mice was significantly higher as compared with healthy control group ($p<0.01$). No significant difference was observed between 200- and 300 mg/kg ginger-treated EAE mice with respect to the expression of the IL-23 P19 mRNA.

The Effects of Ginger Extract on the Expression of IL-23 P40 in the Spinal Cords.

The expression of IL-23 P40 mRNA were 0.96 ± 0.26 in healthy control group, 32.87 ± 5.13 in PBS-treated EAE mice, 4.10 ± 1.12 in 200 mg/kg ginger-treated EAE mice and 3.23 ± 1.65 in 300 mg/kg ginger-treated EAE group (Figure 5).The expression of IL-23 P40 mRNA significantly increased in PBS-treated EAE mice in comparison with healthy control group ($p<0.001$). In both 200- and 300 mg/kg ginger-treated EAE groups the expression of IL-23 P40 was significantly lower than in PBS-treated EAE mice ($p<0.001$). No significant difference was observed between 200- and 300 mg/kg ginger-treated EAE groups with respect to the expression of the IL-23 P40 mRNA. No significant difference was also observed between 300 mg/kg ginger-treated EAE mice and healthy control group regarding the expression of the IL-23 P40 mRNA. However, the expression of IL-23 P40 in 200 mg/kg ginger-treated EAE group was significantly higher than in healthy control group ($p<0.02$).

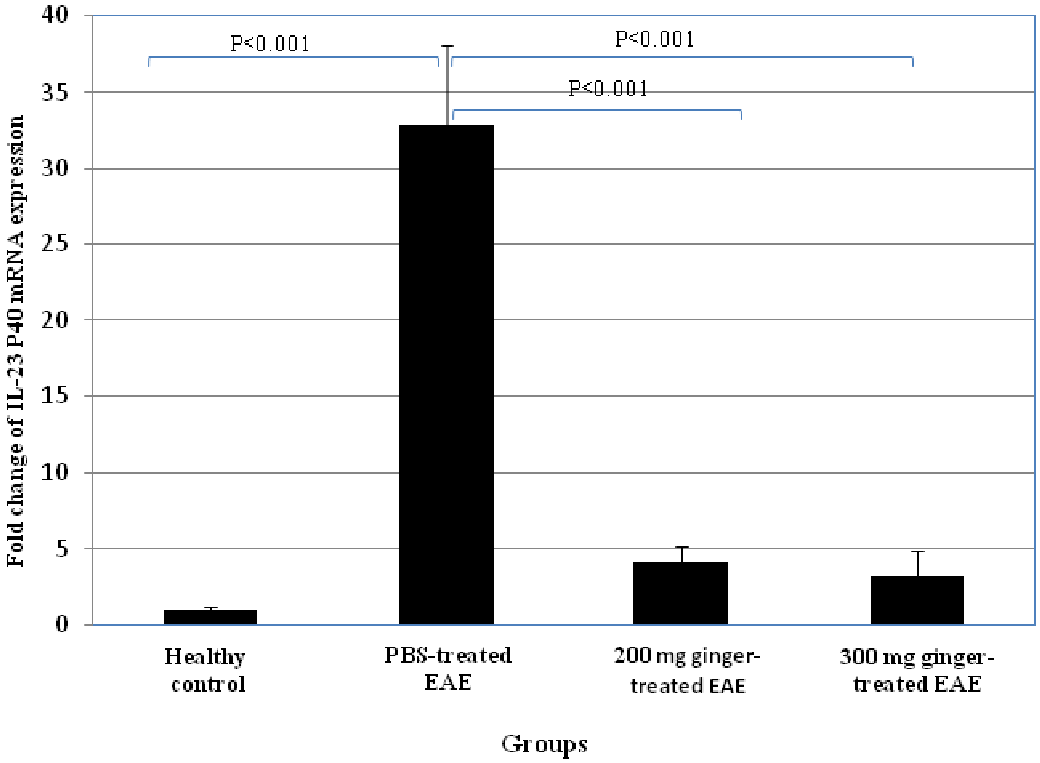

Figure 5. Comparison of the expression of IL-23 P40 mRNA in the spinal cord between ginger-treated EAE and control groups.

The Effects of Ginger Extract on the Serum Levels of IL-23. The serum concentrations of IL-23 in ginger-treated EAE mice and control groups are demonstrated in Figure 6. The mean serum concentrations of IL-23 were 13.73 ± 1.44 Pg/mL in healthy control group, 25.05 ± 2.69 Pg/mL in PBS-treated EAE mice, 18.19 ± 0.89 Pg/mL in 200 mg/kg ginger-treated EAE mice and 14.09 ± 1.05 Pg/mL in 300 mg/kg ginger-treated EAE group. The serum concentrations of IL-23 were significantly higher in PBS-treated EAE mice in comparison to healthy control group ($p<0.01$). The serum concentrations of IL-23 in 200- and 300 mg/kg ginger-treated EAE groups significantly diminished as compared to PBS-treated EAE mice ($p<0.05$ and $p<0.004$, respectively). The 300 mg/kg ginger-treated EAE groups had significantly decreased serum concentrations of IL-23 than 200 mg/kg ginger-treated EAE mice ($p<0.03$). The serum concentrations of IL-23 in 200 mg/kg ginger-treated EAE group was significantly higher in comparison with normal control group ($p<0.05$).

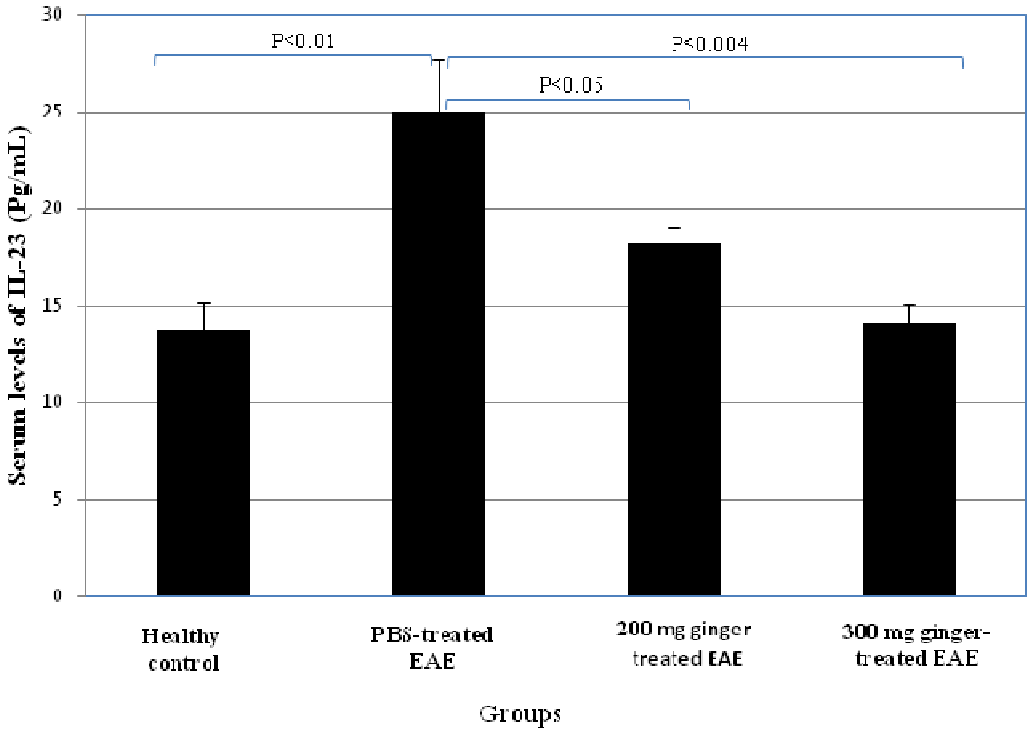

Figure 6. Comparison of the serum levels of IL-23 between ginger-treated and control groups.

DISCUSSION

The IL-23/IL-17 axis plays an important role in the pathogenesis of several inflammatory and autoimmune diseases such as systemic lupus erythematosus (13), spondyloarthritis (14), psoriatic arthritis (15), Graves' disease (16), Crohn's disease (17), EAE and MS (18).The results of the present study demonstrated that the expressions of IL-17 and IL-23 were significantly higher in the sera and spinal cords of PBS treated-EAE mice in comparison to healthy control group. These observations confirm that IL-23/IL-17 axis plays a critical role in the pathogenesis of EAE. It has been showed that IL-23 and IL-23R play an important role during the Th17 cells-related inflammatory process. IL-23 is a central cytokine inducing Th17 cells development and full activation (12). Moreover, IL-23R is expressed in macrophages infiltrating the CNS and IL-23-stimulated macrophages secrete IL-22 and IL-17 (34). IL-23 also plays a key role in the development of EAE. Mice deficient in IL-23 P19 or IL-23R are shown to be resistant to EAE development (34). Administration of monoclonal antibody specific for IL-23 P19 can also prevent EAE disease (34). Based on these results, targeting IL-23 or IL-23R could be considered as an effective therapeutic approach for treatment of EAE and MS diseases.

StimulatedTh17 cells can mediate tissue inflammation by secreting high levels of the pro-inflammatory cytokines such as IL-17 (3,10). High frequencies of autoreactive Th17 cells have been indicated in the CNS of MS patients and EAE mice (35). The high IL-17 levels in the cerebrospinal fluid (CSF) of patients with MS also represent the involvement of this cytokine in the pathogenesis of disease (36). Furthermore, RORγt (a

Th17-specific transcription factor) deficiency leads to more resistance to EAE induction (2). It has been indicated that the IL-17-deficient mice display a delayed onset, lower EAE scores and lower histological alterations with early recovery from disease (2). However, it has been reported that mice treated with monoclonal antibody against IL-17 still develop EAE with a lesser severity(34). In addition to IL-17A, Th17 cells produce other cytokines such as IL-17F, GM-CSF, IL-6, IL-21, IL-22 and TNF-α, which can play an important role in the pathogenesis of EAE and MS diseases (3,8).

The possible mechanisms by which IL-17 is involved in the immunopathogenesis of MS and EAE may be through the recruitment of Neutrophils into the inflammatory sites, induction of the reactive oxygen species (ROS) in brain endothelial cells, activation of microglia cells to produce pro-inflammatory cytokines and mediators, and the stimulation of astrocytes to produce CXC chemokines(3). Elevated expression of matrix metalloproteinases (such as MMP-2, MMP3, MMP-7 and MMP-9) have also been reported in the CNS of MS patients (37). Some Th17 cells-derived cytokines (such as TNF-α) trigger the expression of matrix metalloproteinases which may have an important role in the rupture of brain blood barrier (BBB) (37).

Our results also demonstrated that the treatment of EAE mice with ginger extract decreased the expression of IL-17 and IL-23 in the CNS and serum. In both 200- and 300 mg/kg ginger-treated EAE groups the expression of IL-17 and IL-23 mRNA in the spinal cord and the serum levels of cytokines were lower as compared to PBS-treated EAE mice. The exact mechanisms by which ginger extract may influence the IL-17 and IL-23 production remains to be elucidated. IL-23 is produced by DCs and macrophages after toll like receptors (TLRs) engagement. Expression of IL-23 is further augmented by CD40 ligation (38). Within the CNS, the microglia and astrocytes act as the main producers of IL-23 (39, 40). The reducing effects of ginger on the IL-23 production may take placethrough interfering with TLRs- and/or CD40-mediated signaling pathways.

Our results also demonstrated the treatment of EAE mice with ginger extract can reduce the expression of IL-17. The ginger extract may directly and/or indirectly influence the IL-17 production. As mentioned, the initial differentiation of naive $CD4^+$ T cells to Th17 cells is dependent to the TGF-β and IL-6 (11). The antigenic stimulation of naive T cells in the presence of TGF-β and IL-6 results in the expression of the RORγt which serves as a key regulator for Th17 cells differentiation (41). IL-6 acts as a potent pro-inflammatory cytokine through the promotion of Th17 cells differentiation. It has been demonstrated that the expression of IL-6 mRNA is significantly increased in the brain during development of EAE (42). It has also been reported that when anti-IL-6R antibodies were administered shortly after MOG immunization, the development of EAE was suppressed and no Th17 cells were observed in the draining lymph nodes or in the spinal cord. However, the beneficial effects of anti-IL-6R antibodies on the disease onset and Th17 cells were disappeared when anti-IL-6R antibody administration was delayed (43). Thus, IL-6 is required for the initial differentiation of Th17 cells in the EAE model. Therefore, it is possible that the higher IL-6 production by microglia (during initial stage of EAE development) may promote the expansion of Th17 cells in the brain. The inhibitory effects of ginger and its derivatives on the IL-6 production have been demonstrated in other studies (44,45). Accordingly, the treatment with ginger extract may diminish the Th17 cells-related immune responses through inhibiting the IL-6 production.

As explained, IL-23 is a main cytokine inducing Th17 cells maintenance and full activation. Moreover, IL-1 and TNF-α promote the differentiation and activation of

Th17 cells (3,46). Within CNS, the microglia cells produce cytokines including IL-23, IL-1 and TNF-α (40). It has been demonstrated that 6-gingerol, a pungent of ginger, reduces the production of the several inflammatory medistors such as prostaglandin E2, IL-1, TNF-α and nuclear factor kappa B (NF-κB) expression in microglia (47). Accordingly, the reducing effects of ginger or its derivatives on the production of IL-23, IL-1 and TNF-α by microglia cells may result in the down-regulation of the IL-17 expression.

It has been also demonstrated that IL-27 suppresses the expression of RORγt and inhibits the differentiation of Th17 cells (48). Recently, we have indicated lower expression of IL-27 in the CNS of EAE mice (31).Treatment with ginger extract improved the expression of IL-27 mRNA in EAE mice (31). Therefore, ginger may mediate its reducing effects on IL-17 expression through the enhancing of IL-27 production, which in turn inhibits Th17 cells differentiation. Ginger may also directly influences Th17 cells through the down-regulation of the expression of RORγt, IL-17A and IL-23R. It should be noted that other proinflammatory cytokines and chemokines also play important roles in pathogenesis of EAE (3,49). Therefore, evaluation of the effects of ginger extract on the expression of other immunological parameters are important for understanding the possible molecular mechanisms by which ginger extract influence EAE pathogenesis.

In conclusion, our results showed the higher expression of IL-17 and IL-23 in the spinal cord and serum of EAE mice. Accordingly, the up-regulation of of IL-17 and IL-23 may be involved in the pathogenesis of EAE. Moreover, treatment of EAE mice with ginger extract reduces the expression of IL-17 and IL-23 in EAE mice. Further studies should be conducted to evaluate the possible therapeutic potential of ginger extract or its derivative for the treatment of EAE or MS diseases.

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