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The effect of ginger (*Zingiber officinale*) supplementation on clinical, biochemical, and anthropometric parameters in patients with multiple sclerosis: a double-blind randomized controlled trial†

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Introduction: different lines of evidence have shown that ginger administration may be beneficial for patients with multiple sclerosis (MS). Therefore, we aimed to investigate the effect of ginger supplementation on disability, physical and psychological quality of life (QoL), body mass index (BMI), neurofilament light chain (NfL), interlukin-17 (IL-17), matrix metalloproteinase-9 (MMP-9), and neutrophil to lymphocyte ratio (NLR) in patients with relapsing-remitting MS. *Methods*: this was a 12 week double-blind parallel randomized placebo-controlled trial with a 3 week run-in period. The treatment (n = 26) and control (n = 26) groups received 500 mg ginger and placebo (corn) supplements 3 times daily, respectively. Disability was evaluated using the Expanded Disability Status Scale (EDSS). QoL was rated using the Multiple Sclerosis Impact Scale (MSIS-29). BMI was calculated by dividing weight by height squared. Serum levels of NfL, IL-17, and MMP-9 were measured using the enzyme-linked immunosorbent assay. NLR was determined using a Sysmex XP-300™ automated hematology analyzer. All outcomes were assessed before and after the intervention and analyzed using the intention-to-treat principle. *Results*: in comparison with placebo, ginger supplementation caused a significant reduction in the EDSS (−0.54 ± 0.58 *vs.* 0.08 ± 0.23, P < 0.001), the MSIS-29 physical scale (−8.15 ± 15.75 *vs.* 4.23 ± 8.46, P = 0.001), the MSIS-29 psychological scale (−15.71 ± 19.59 *vs.* 6.68 ± 10.41, P < 0.001), NfL (−0.14 ± 0.97 *vs.* 0.38 ± 1.06 ng mL^{-1}, P = 0.049), IL-17 (−3.34 ± 4.06 *vs.* 1.77 ± 6.51 ng L^{-1}, P = 0.003), and NLR (−0.09 ± 0.53 *vs.* 0.53 ± 1.90, P = 0.038). Nevertheless, the differences in BMI and MMP-9 were not significant between the groups. *Conclusion*: ginger supplementation may be an effective adjuvant therapy for patients with relapsing-remitting MS.

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1. Introduction

Multiple sclerosis (MS) is an immune-mediated inflammatory disease of the central nervous system (CNS) that affects over 2.8 million people worldwide.[1] It is characterized by loss of the myelin sheath, degeneration of axons and oligodendrocytes, and gliosis.[2] Although the etiology of MS is poorly understood, multiple genetic and environmental factors contribute to its development. For instance, a high body mass index (BMI) significantly increased the risk of developing MS by about two-fold.[3] The most common type of MS is relapsing-remitting MS (RRMS), which is a debilitating life-long condition with the pattern of attacks of new or old symptoms (relapse) followed by a complete or partial recovery from the symptoms (remission).[2] Several disease-modifying therapies are available for RRMS, but the quality of life (QoL) of affected patients is still lower than that of patients with other chronic conditions as well as the general population. Physical disability, fatigue, depression, and anxiety are considered to be the main determinants of low QoL in patients with RRMS.[4]

It seems that some members of the interleukin (IL) group of cytokines and the matrix metalloproteinase (MMP) family are involved in the pathophysiology of MS.[5] For example, IL-17 induces the secretion of different inflammatory and oxidative

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stress biomarkers and leads to the recruitment of neutrophils to the CNS. Furthermore, IL-17 disrupts the expression of tight junction proteins and the contractility of endothelial cells and leads to the blood–brain barrier (BBB) breakdown.[6] Moreover, IL-17 functions synergistically with tumor necrosis factor-α (TNF-α) to enhance the oxidative stress-mediated apoptosis of oligodendrocytes.[7] In addition, IL-17 and TNF-α can increase the expression of MMP-9 that degrades myelin basic protein and results in myelin damage within the CNS parenchyma.[8] This cascade eventually leads to demyelination, axonal degeneration, and scarring in patients with MS.[2] Following axonal injury, neurofilament light chain (NfL), a neuron-specific cytoskeleton component that belongs to the type IV intermediate filament proteins, is released into the cerebrospinal fluid and bloodstream.[9] NfL levels have been shown to correlate with MS relapses, radiological signs of MS activity, brain atrophy, spinal cord volume loss, disability, clinical progression, and treatment efficacy.[10] It has also been reported that neutrophil to lymphocyte ratio (NLR), a biomarker of systemic inflammation and immune dysregulation, is elevated in MS patients and related to MS severity and prognosis.[11]

Recently, different lines of evidence have suggested that supplementation with ginger (*Zingiber officinale*) may be beneficial for patients with MS due to its immunomodulatory, antioxidant, anti-inflammatory, and neuroprotective properties.[12] This nutraceutical is a rich source of phytochemicals including gingerols, shogaols, paradols, gingerenone-A, zingerone, 6-dehydrogingerdione, quercetin, zingiberene, α-curcumene, α-farnesene, β-bisabolene, and β-sesquiphellandrene.[13] It has been demonstrated that ginger or its bioactive compounds can reduce IL-17 and MMP-9 expression, leukocyte infiltration, demyelination, and astrogliosis within *in vitro* and *in vivo* models.[14–17] In addition, a quasi-experimental study has indicated that ginger supplementation can decrease the serum levels of TNF-α and increase the serum levels of brain-derived neurotrophic factor, a neuroprotective protein,[18] in women with MS.[19] These interesting results encouraged us to design a randomized controlled trial and assess the effect of ginger supplementation on disability status, physical and psychological QoL, BMI, IL-17, MMP-9, NfL, and NLR in patients with RRMS.

2. Materials and methods

2.1. Trial design

Our study protocol has been previously published.[20] This study was a 12 week double-blind parallel randomized placebo-controlled trial with a 3 week run-in period. It was approved by the Medical Ethics Committee at the Isfahan University of Medical Sciences under the ethics code IR.MUI.RESEARCH.REC.1400.248. Also, it was registered at the Iranian Registry of Clinical Trials (**https://www.irct.ir**) under the registration number IRCT20180818040827N3. This trial was conducted in agreement with the Declaration of Helsinki and its later amendments.

2.2. Participants

The inclusion criteria were patients diagnosed with RRMS according to the 2017 revisions of the McDonald criteria,[21] men or non-menopausal women aged 18 to 50 years old, a score of ≤4.5 in the Expanded Disability Status Scale (EDSS),[22] no MS relapse or corticosteroid therapy for the past 3 months, no change in the type or dose of MS medications for the past 6 months, and the willingness and ability to participate in this trial. The exclusion criteria were patients with other autoimmune diseases or cancers, pregnancy, MS relapse or corticosteroid therapy during the study, changes in the type or dose of MS medications during the study, allergic reactions to ginger or placebo tablets, supplementation with antioxidants or nutrients (except vitamin D), and the consumption of <90% of ginger or placebo supplements. Eligible patients were recruited from Fars MS Association and Imam Reza (A.S) Clinic located in Shiraz, Iran. Before enrollment, oral and written informed consents were obtained from the participants. Their demographic and medical characteristics were also collected through face-to-face interviews.

2.3. Sample size

The following formula was used to calculate the required sample size:

$$n = \frac{\left(Z_1 - \frac{\alpha}{2} + Z_1 - \beta\right)^2 \left(S_1^2 + S_2^2\right)}{\left(\mu_1 - \mu_2\right)^2}.$$

Considering clinical disability (measured using EDSS) and IL-17 as the primary outcomes of the trial, 22 RRMS patients in each group and 44 in total were required. The calculations were performed based on previous studies of EDSS[23] and IL-17[24] and by assuming type I error (α) of 0.05 and type II error (β) of 0.10 (power = 90%). Taking into account a drop-out rate of ~20%, 26 RRMS patients in each group and 52 in total were recruited.

2.4. Run-in period

Before random allocation, all participants underwent a three week run-in period. During this period, RRMS subjects were requested to avoid consuming ginger and its products and to maintain their usual physical activity and dietary intake.

2.5. Randomization and blinding

The subjects were assigned to treatment (ginger) and control (placebo) groups using stratified permuted block randomization (allocation ratio = 1 : 1, block size = 4). The stratification was performed according to gender. The following website was used for the generation of a random allocation sequence: **https://www.sealedenvelope.com/simple-randomiser/v1/lists**. Ginger and placebo tablets were sealed in sequentially numbered identical bottles based on the allocation sequence. The sequence generation, allocation concealment, and assignment of participants to the groups were implemented by different trained persons. The participants, researchers, care providers,

and outcome assessors were blinded to group assignment until trial completion. It is worth mentioning that ginger and placebo tablets were exactly the same in packaging, size, shape, and color. In addition, a very small amount of ginger powder was added to bottles containing placebo tablets to give ginger odor, as previously done by Ebrahimzadeh Attari *et al.*[25] and Mahluji *et al.*[26]

2.6. Intervention

The treatment and control groups received 500 mg ginger and placebo (corn) tablets 3 times daily for 12 weeks, respectively. The subjects were instructed to orally consume each tablet along with the main meals including breakfast, lunch, and dinner. The dose and duration of supplementation were chosen based on a previous randomized controlled trial in patients with an immune-mediated inflammatory disease.[27] Both ginger and placebo tablets were provided by Dineh Iran Industries Complex, Tehran, Iran. Each 500 mg ginger tablet was standardized to contain 25 mg of gingerols. During the study, both groups were asked to maintain their usual diet and physical activity habits and to avoid consuming ginger and other products containing it.

2.7. Outcome assessment

In this clinical trial, the primary outcomes were disability status and IL-17, and the secondary outcomes were physical and psychological QoL, BMI, MMP-9, NfL, and NLR. All outcomes were assessed at the beginning and end of the study. Possible side effects of ginger or placebo tablets, especially gastrointestinal complications, were also closely monitored during the trial period.

Disability was evaluated through physical examination by an experienced neurologist (M.P.) using the EDSS. The scores of EDSS were in the range of 0–10, and higher scores indicated greater disability in patients with MS.[22]

Physical and psychological QoL were rated using the 29-item self-report questionnaire of the Multiple Sclerosis Impact Scale (MSIS-29), which has acceptable reliability and validity in Iranian MS patients.[28] The physical and psychological scores of MSIS-29 were separately converted to a 0–100 scale, and the higher the scaled score the worse the QoL.[29]

Antecubital venous blood specimens were obtained from the participants after 12 hours of overnight fasting. The serum was promptly separated by centrifugation and stored at −80 °C until analysis. Serum levels of IL-17, MMP-9, and NfL were measured by the enzyme-linked immunosorbent assay (ELISA) technique using commercially available kits (ZellBio GmbH, Lonsee, Germany). Absolute counts of neutrophils and lymphocytes in fresh whole blood samples were also determined using a Sysmex XP-300™ automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Then, the NLR was calculated by dividing the neutrophil absolute count by the lymphocyte absolute count.

The BMI was calculated by dividing the body weight in kilograms by the height in square meters. The weight of the patients was measured to the nearest 0.1 kg in light clothing without shoes using a calibrated digital scale (Glamor BS-801, Hitachi, China). The height of the patients was measured to the nearest 0.5 cm in the upright standing position without shoes using a portable stadiometer (Seca 213, Hamburg, Germany).

2.8. Dietary intake and physical activity

Dietary intake and physical activity of the participants were assessed at the beginning and end of the trial. Dietary intakes were collected using three-day food records (two weekdays and one weekend day). Then, the energy and nutrient compositions were determined using Nutritionist IV software modified for Iranian foods (version 3.5.2, The Hearst Corporation, San Bruno, California, United States). Physical activity levels were evaluated using the short form of the International Physical Activity Questionnaire, which has acceptable reliability and validity in Iranians.[30]

2.9. Statistical analysis

Data analysis was performed according to the intention-to-treat (ITT) principle using IBM SPSS Statistics software (version 26, IBM Corporation, Armonk, New York, United States). The expectation-maximization algorithm was run to impute missing values in ITT analysis.[31] Number (percentage) was used to report qualitative variables, and mean ± standard deviation was used to express quantitative variables. The Shapiro–Wilk test and skewness and kurtosis statistics were used to check the normality of quantitative variables. Non-normally distributed data were transformed using suitable functions including logarithm, square root, or power. Between-group comparisons of demographic and medical characteristics, dietary intake, and physical activity were done using the independent *t*-test, chi-square test, or Fisher's exact test as appropriate. Within-group comparisons of all outcomes were performed using the paired *t*-test. Between-group comparisons of all outcomes were done using the analysis of covariance (ANCOVA) adjusted for baseline values and BMI. According to cumulative evidence, the ANCOVA model with baseline values as a covariate has superior efficiency and statistical properties than other conventional analytical approaches.[32] In addition, previous studies have reported that BMI is a potential confounding factor in patients with MS and can significantly affect their treatment response.[33–35] All statistical analyses of this trial were two-tailed with a significance level of 0.05.

3. Results

3.1. Participant flow and recruitment

This study was conducted in the year 2022. From a total of 196 RRMS patients assessed for eligibility, 52 met the inclusion criteria and agreed to participate in the trial. The patients were randomly assigned and received either ginger (n = 26) or placebo tablets (n = 26). In the follow-up period, one participant in the intervention group and two participants in the control group dropped out due to coronavirus disease 2019

(COVID-19), a respiratory illness caused by SARS-CoV-2.[36] Nevertheless, all 52 participants were included in the statistical analysis according to the ITT principle (Fig. 1).

3.2. Demographic and baseline characteristics

The demographic and baseline characteristics of the participants are presented in Table 1. There was no significant difference between the two groups in terms of age, gender, marital status, education level, occupation, smoking, alcohol consumption, supplementation with vitamin D, duration of RRMS, disease-modifying therapies, and baseline values of the EDSS score, MSIS-29 psychological scale, BMI, IL-17, NfL, MMP-9, and NLR. Only the MSIS-29 physical scale was significantly different between the two groups at the baseline.

3.3. Dietary intake and physical activity

The dietary intake and physical activity of the participants throughout the study are presented in Table 2. Energy, macronutrient, and micronutrient intakes were not significantly different between the intervention and control groups. Also, no significant difference was observed in physical activity levels between the groups.

3.4. Primary and secondary outcomes

Within- and between-group comparisons of primary and secondary outcomes during the trial are presented in Table 3. In the intervention group, EDSS, MSIS-29 physical and psychological scales, and IL-17 were significantly decreased following ginger supplementation compared with the baseline. However, in the control group, MSIS-29 physical and psychological scales were significantly increased at the end of the study compared with that of the beginning. Within-group differences for other outcomes were not significant in the intervention or control group.

In the ANCOVA model adjusted for baseline values, ginger supplementation caused a significant reduction in EDSS

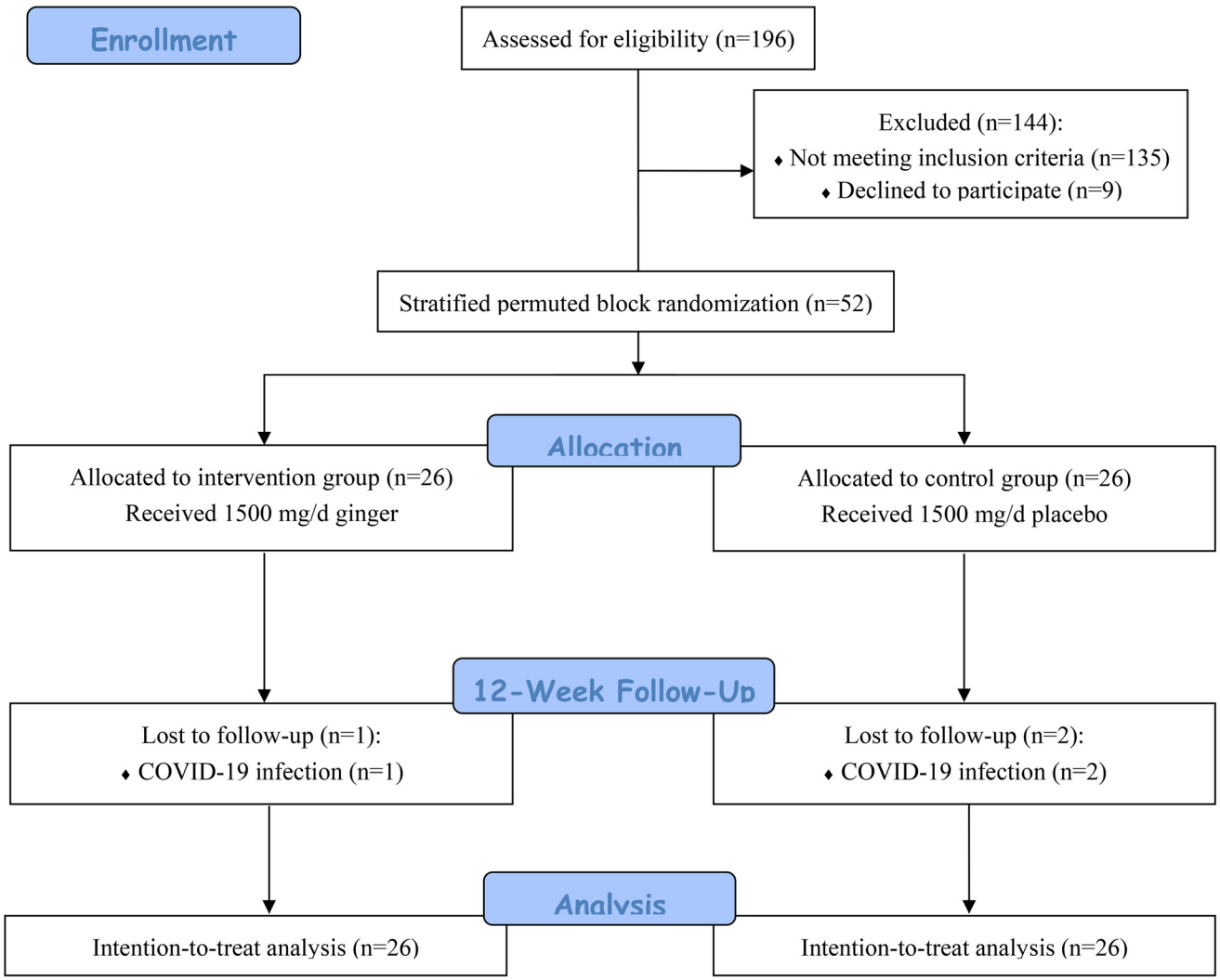

Fig. 1 The CONSORT flow diagram of the participants through the trial. CONSORT, consolidated standards of reporting trials; COVID-19, coronavirus disease 2019.

Table 1 Demographic and baseline characteristics of the RRMS patients

	Ginger (n = 26)	Placebo (n = 26)	P-Value[a]
Age (year)	36.5 ± 6.2	35.0 ± 6.9	0.41
Gender			>0.99
Male	6 (23.1)	6 (23.1)	
Female	20 (76.9)	20 (76.9)	
Marital status			0.35
Single	5 (19.2)	9 (34.6)	
Married	21 (80.8)	17 (65.4)	
Education level			0.40
≤Diploma	14 (53.8)	10 (38.5)	
>Diploma	12 (46.2)	16 (61.5)	
Occupation			0.41
Housewife	14 (53.8)	8 (30.8)	
Employee	4 (15.4)	5 (19.2)	
Freelance	5 (19.2)	9 (34.6)	
Unemployed	3 (11.5)	4 (15.4)	
Smoking habit			>0.99
Yes	5 (19.2)	6 (23.1)	
No	21 (80.8)	20 (76.9)	
Alcohol drinking			>0.99
Yes	4 (15.4)	5 (19.2)	
No	22 (84.6)	21 (80.8)	
Vitamin D supplementation			>0.99
Yes	21 (80.8)	22 (84.6)	
No	5 (19.2)	4 (15.4)	
Disease duration (year)	6.5 ± 4.6	6.4 ± 5.0	0.98
Disease-modifying therapies			
Rituximab	9 (34.6)	5 (19.2)	0.35
Teriflunomide	2 (7.7)	1 (3.8)	>0.99
Dimethyl fumarate	4 (15.4)	5 (19.2)	>0.99
Glatiramer acetate	1 (3.8)	3 (11.5)	0.61
Interferon beta	6 (23.1)	4 (15.4)	0.73
Natalizumab	1 (3.8)	1 (3.8)	>0.99
Fingolimod	1 (3.8)	5 (19.2)	0.19
Ocrelizumab	1 (3.8)	0 (0.0)	>0.99
None	1 (3.8)	2 (7.7)	>0.99
EDSS score	1.85 ± 1.21	1.50 ± 1.06	0.28
MSIS-29 PHY score	21.68 ± 19.18	12.55 ± 11.39	0.034
MSIS-29 PSY score	46.58 ± 26.81	32.59 ± 25.06	0.057
BMI (kg m^{-2})	25.33 ± 4.99	26.27 ± 6.09	0.55
IL-17 (ng L^{-1})	85.23 ± 16.52	79.30 ± 16.41	0.20
NfL (ng mL^{-1})	10.72 ± 2.06	10.58 ± 2.20	0.83
MMP-9 (ng L^{-1})	1063 ± 195	1047 ± 211	0.80
NLR	1.95 ± 0.97	2.58 ± 2.11	0.49

Note: data are expressed as mean ± standard deviation or number (percentage). Abbreviations: BMI, body mass index; EDSS, Expanded Disability Status Scale; IL-17, interleukin-17; MMP-9, matrix metalloproteinase-9; MSIS-29, Multiple Sclerosis Impact Scale-29 items; NfL, neurofilament light chain; NLR, neutrophil to lymphocyte ratio; PHY, physical; PSY, psychological; RRMS, relapsing-remitting multiple sclerosis. [a] Obtained from the independent t-test for quantitative variables and the chi-square test or Fisher's exact test for qualitative variables.

Table 2 Dietary intake and physical activity of the subjects throughout the trial

	Ginger (n = 26)	Placebo (n = 26)	P-Value[a]
Energy (kcal d^{-1})	2194 ± 499	2276 ± 695	0.63
Carbohydrate (g d^{-1})	304.2 ± 82.0	310.7 ± 99.8	0.80
Total fat (g d^{-1})	76.86 ± 21.23	82.14 ± 34.55	0.78
Protein (g d^{-1})	76.23 ± 19.34	81.00 ± 30.20	0.68
Dietary fiber (g d^{-1})	16.94 ± 5.88	18.63 ± 9.96	0.46
Saturated fatty acids (g d^{-1})	17.85 ± 5.61	17.81 ± 7.44	0.98
Cholesterol (mg d^{-1})	222.5 ± 140.6	275.9 ± 237.3	0.48
Monounsaturated fatty acids (g d^{-1})	28.58 ± 8.18	29.80 ± 9.69	0.63
Polyunsaturated fatty acids (g d^{-1})	21.57 ± 7.49	22.26 ± 10.54	0.93
Oleic acid (g d^{-1})	23.39 ± 6.86	23.92 ± 8.91	0.81
Omega-3 fatty acids (g d^{-1})	0.442 ± 0.415	0.316 ± 0.327	0.23
Omega-6 fatty acids (g d^{-1})	18.11 ± 6.61	18.38 ± 6.93	0.88
Vitamin C (mg d^{-1})	114.0 ± 87.2	101.9 ± 88.8	0.62
Vitamin B1 (mg d^{-1})	2.05 ± 0.64	1.89 ± 0.66	0.38
Vitamin B2 (mg d^{-1})	1.74 ± 0.57	1.80 ± 0.78	0.95
Vitamin B3 (mg d^{-1})	25.24 ± 6.94	25.38 ± 10.96	0.82
Vitamin B6 (mg d^{-1})	1.49 ± 0.42	1.51 ± 0.71	0.64
Vitamin B9 (μg d^{-1})	270.1 ± 107.6	315.8 ± 189.3	0.52
Vitamin B12 (μg d^{-1})	2.44 ± 1.36	2.71 ± 2.18	0.98
Vitamin A (RE per d)	452.8 ± 322.2	488.2 ± 388.8	0.76
Vitamin D (μg d^{-1})	0.811 ± 1.331	0.662 ± 1.059	0.66
Vitamin E (mg d^{-1})	4.09 ± 2.11	4.21 ± 4.82	0.31
Vitamin K (μg d^{-1})	62.43 ± 95.50	66.70 ± 57.03	0.27
Calcium (mg d^{-1})	726.5 ± 292.3	622.4 ± 246.9	0.17
Magnesium (mg d^{-1})	257.7 ± 69.2	285.9 ± 141.6	0.86
Sodium (mg d^{-1})	1724 ± 705	1801 ± 795	0.71
Iron (mg d^{-1})	15.63 ± 4.62	16.11 ± 5.46	0.74
Selenium (mg d^{-1})	0.087 ± 0.057	0.070 ± 0.045	0.25
Zinc (mg d^{-1})	8.83 ± 2.35	9.21 ± 3.39	0.64
IPAQ (MET-min per week)	963 ± 712	1013 ± 739	0.80

Note: data are expressed as mean ± standard deviation. Abbreviations: IPAQ, international physical activity questionnaire. [a] Obtained from the independent t-test.

scores compared with the placebo (−0.54 ± 0.58 *vs.* 0.08 ± 0.23, P < 0.001). Also, significant reductions were observed in the intervention group compared with the control group for both MSIS-29 physical scores (−8.15 ± 15.75 *vs.* 4.23 ± 8.46, P = 0.001) and MSIS-29 psychological scores (−15.71 ± 19.59 *vs.* 6.68 ± 10.41, P < 0.001). Furthermore, serum concentrations of IL-17 were significantly reduced in the ginger group compared with controls (−3.34 ± 4.06 *vs.* 1.77 ± 6.51 ng L^{-1}, P = 0.003). In addition, NLR was significantly lowered after ginger supplementation (−0.09 ± 0.53 *vs.* 0.53 ± 1.90, P = 0.038). However, the differences in BMI and serum levels of MMP-9 and NfL were not significant between the groups. In the ANCOVA model adjusted for both baseline values and BMI, the results remained the same, except for NfL. Serum levels of NfL were significantly decreased in the treatment group compared with the placebo group (−0.14 ± 0.97 *vs.* 0.38 ± 1.06 ng mL^{-1}, P = 0.049).

3.5. Side effects

In this trial, two patients in the intervention group (7.7%) and three patients in the control group (11.5%) reported minor side effects. In the ginger group, one participant experienced heartburn (3.8%), and another participant reported abdominal pain (3.8%). In the placebo group, two patients experienced heartburn (7.7%), and one participant reported headache (3.8%).

4. Discussion

The results of this randomized placebo-controlled trial showed that a 12 week supplementation with 1500 mg d^{-1} ginger

Table 3 Within- and between-group comparisons of clinical, anthropometric, and biochemical parameters during the 12 week intervention

	Ginger (n = 26)				Placebo (n = 26)					
	Baseline	Endpoint	Change	P-Value[a]	Baseline	Endpoint	Change	P-Value[a]	P-Value[b]	P-Value[c]
EDSS score	1.85 ± 1.21	1.31 ± 1.30	−0.54 ± 0.58	<0.001	1.50 ± 1.06	1.58 ± 1.02	0.08 ± 0.23	0.10	<0.001	<0.001
MSIS-29 PHY score	21.68 ± 19.18	13.53 ± 12.69	−8.15 ± 15.75	0.001	12.55 ± 11.39	16.78 ± 15.27	4.23 ± 8.46	0.025	0.001	0.001
MSIS-29 PSY score	46.58 ± 26.81	30.88 ± 27.14	−15.71 ± 19.59	<0.001	32.59 ± 25.06	39.27 ± 29.18	6.68 ± 10.41	0.003	<0.001	<0.001
BMI (kg m^{-2})	25.33 ± 4.99	25.38 ± 4.60	0.05 ± 0.73	0.73	26.27 ± 6.09	26.38 ± 6.02	0.11 ± 0.53	0.29	0.54	0.54
IL-17 (ng L^{-1})	85.23 ± 16.52	81.89 ± 15.03	−3.34 ± 4.06	<0.001	79.30 ± 16.41	81.07 ± 16.69	1.77 ± 6.51	0.18	0.003	0.004
NfL (ng mL^{-1})	10.72 ± 2.06	10.58 ± 1.96	−0.14 ± 0.97	0.42	10.58 ± 2.20	10.96 ± 2.22	0.38 ± 1.06	0.13	0.083	0.049
MMP-9 (ng L^{-1})	1063 ± 195	1055 ± 182	−8 ± 97	0.59	1047 ± 211	1078 ± 201	31 ± 106	0.23	0.20	0.14
NLR	1.95 ± 0.97	1.86 ± 1.22	−0.09 ± 0.53	0.080	2.58 ± 2.11	3.11 ± 2.98	0.53 ± 1.90	0.19	0.038	0.049

Note: data are expressed as mean ± standard deviation. Abbreviations: BMI, body mass index; EDSS, Expanded Disability Status Scale; IL-17, interleukin-17; MMP-9, matrix metalloproteinase-9; MSIS-29, Multiple Sclerosis Impact Scale-29 items; NfL, neurofilament light chain; NLR, neutrophil to lymphocyte ratio; PHY, physical; PSY, psychological. [a] Obtained from the paired t-test. [b] Obtained from the analysis of covariance adjusted for baseline values. [c] Obtained from the analysis of covariance adjusted for baseline values and BMI.

(75 mg d^{-1} gingerols) caused significant reductions in the EDSS, MSIS-29 physical and psychological scales, IL-17, NLR, and NfL in RRMS patients. However, it had no significant effects on MMP-9 and BMI. This study also suggested that ginger had a good safety profile in patients with RRMS.

4.1. EDSS

In the present trial, ginger supplementation significantly improved EDSS scores compared with placebo. EDSS is a clinician-administered scale assessing the functional systems of the CNS and describing disease progression and disability in patients with MS.[22] Currently, EDSS is the most important endpoint in MS trials addressing the efficacy of therapeutic interventions.[37] The improvement of EDSS scores in our study (>0.5 points) seems to be both statistically and clinically significant. This is because the minimal clinically important difference (MCID), the smallest change in an outcome that is perceived by clinicians or patients as beneficial, has been recommended to be between 0.5 and 1.0 points for the EDSS.[38] Furthermore, a 0.5-point decrease or increase in the EDSS scores of 1–10 is equivalent to a step change in the neurologic impairment of MS patients.[22] Interestingly, previous studies have also confirmed the beneficial effect of ginger administration on the EDSS.[15,17,23,39,40] In MS animal models, supplementation with ginger or its bioactive compounds significantly ameliorated the clinical and pathological scores of the disease.[15,17,39] In addition, a meta-analysis of randomized controlled trials indicated that ginger supplementation significantly reduced disability in patients with osteoarthritis.[40] Furthermore, a clinical trial showed that supplementation with curcumin, which belongs to the ginger family (Zingiberaceae) and has a molecular similarity to 6-gingerol,[41] significantly decreased EDSS scores in RRMS patients.[23] The mechanism of the positive impact of ginger in MS patients has not yet been elucidated. However, it has been shown that ginger extract can prevent demyelination and improve remyelination in the corpus callosum.[42] Moreover, it has been reported that 6-gingerol can induce tolerogenic dendritic cells, promising candidates for the management of autoimmune diseases.[15]

4.2. MSIS-29

The findings of this study indicated that ginger supplementation significantly improved the physical and psychological scores of MSIS-29, a disease-specific questionnaire of QoL. The MCID for the physical component of MSIS-29 has been reported to be between 7.0 and 8.0 points.[43,44] Therefore, the improvement of physical QoL in this trial (>8.0 points) seems to be both statistically and clinically significant. To the best of our knowledge, the MCID for the psychological component of MSIS-29 has not yet been determined. Nevertheless, the improvement of the psychological subscale in this study is about twice the improvement of the physical subscale, which may imply its clinical significance. In line with our findings, previous clinical trials have shown the beneficial effect of ginger supplementation on QoL in patients with cancer or sexual dysfunction.[45,46] In addition, EDSS scores have been independently associated with QoL in MS patients, especially in patients with an EDSS score of ≤5.0.[47] Therefore, the amelioration of EDSS scores after ginger supplementation may be the reason for the betterment of physical QoL in this trial. Moreover, supplementation with ginger has been shown to reduce symptoms of anxiety and depression in human[48,49] and animal studies.[50,51] Therefore, the amelioration of psychological QoL in this study may be caused by the anti-anxiety and anti-depressant effects of ginger. These effects seem to be mediated through serotonergic pathways.[50,52]

4.3. IL-17

Our trial revealed that supplementation with ginger significantly reduced the serum levels of IL-17, an inflammatory cytokine involved in MS pathogenesis. This finding has been supported by the available literature.[14,15,39,53,54] For instance, the administration of ginger extract or 6-gingerol caused a significant decrease in the CNS expression and serum concentrations of IL-17 in animal models of MS.[14,15,39] In addition, a meta-analysis of randomized controlled trials showed anti-inflammatory effects of ginger in adult humans.[53] Also, ginger has been found to be the most potent agent against IL-17-mediated inflammation after comparing the activity of 315

natural extracts.[54] It is postulated that ginger supplementation can reduce IL-17 serum levels by suppressing the nuclear factor kappa B signaling pathway through inhibiting transforming growth factor beta-activated kinase 1.[54,55]

4.4. NLR

As indicated, ginger supplementation significantly decreased NLR compared with placebo in patients with RRMS. It has previously been reported that MS patients have a significantly higher NLR than healthy controls.[11] NLR is an indicator of both inflammation and immunity function. As discussed in the previous subsection, ginger has anti-inflammatory effects.[53] Accordingly, it seems reasonable that NLR was reduced following supplementation with ginger. Moreover, consumption of ginger extract has been shown to significantly decrease the neutrophil count and elevate the lymphocyte count in smokers, leading to a reduction in NLR.[56] The mechanism underlying this immunomodulatory effect of ginger should be investigated in future studies.

4.5. NfL

Based on the statistical model adjusted for baseline values, ginger supplementation had no significant effect on serum levels of NfL in RRMS patients. However, based on the statistical model adjusted for baseline values and BMI, ginger supplementation significantly decreased serum concentrations of NfL. As mentioned earlier, NfL is a biomarker of neuroaxonal injury and its levels are significantly higher in patients with MS than in healthy controls.[9] Previous studies have shown that BMI has a confounding effect on blood levels of NfL. In fact, a significant negative association exists between BMI and blood concentrations of NfL.[57–60] This may be due to the increased blood volume or greater uptake of NfL by adipose tissue in people with high BMI.[58] In agreement with our results, the positive effect of ginger administration on neurofilaments has been reported in an animal study.[61] The neuroprotective effect of ginger in RRMS patients seems to be exerted through inhibiting the activity of tryptophan 2,3-dioxygenase and modulating kynurenine pathway metabolites.[62–65]

4.6. MMP-9

Supplementation with ginger had no significant effect on the serum levels of MMP-9 in RRMS patients. In contrast, multiple *in vivo* and *in vitro* studies have shown significant lowering effects of ginger or its bioactive components on MMP-9.[66–70] This contradiction may be explained by the low dose of ginger used in our study because it seems that ginger affects MMP-9 in a dose-dependent manner.[66–68] Moreover, one study has reported that 6-gingerol can reduce the activity and mRNA expression of MMP-9 but leaves its protein levels unchanged.[71] Therefore, supplementation with higher doses of ginger and assessment of MMP-9 activity and mRNA expression are recommended for future trials. In addition, the measurement of tissue inhibitor of metalloproteinase-1, a specific inhibitor of MMP-9, may help better interpret the findings.[66,68]

4.7. BMI

Ginger supplementation had no significant effect on the BMI in RRMS patients. This result is consistent with the findings of most previous trials;[26,72–74] however, a few previous studies have reported a significant lowering effect of ginger on the BMI.[25,75] This inconsistency may be due to different doses of ginger used in the studies. A recent meta-analysis of clinical trials has revealed that ginger supplementation at doses greater than 1500 mg d^{-1} can reduce the BMI.[76] The beneficial effect of ginger on the BMI may be exerted through increasing thermogenesis and lipolysis, suppression of adipogenesis, controlling appetite, and inhibition of dietary fat absorption.[77]

4.8. Safety

No serious side effects were observed in this study. A comprehensive systematic review of 109 randomized controlled trials has also shown that ginger has a good safety profile.[78] According to the Food and Drug Administration, supplementation with ginger at doses up to 4000 mg d^{-1} is generally recognized as safe.[12]

4.9. Limitations

This trial has some limitations that should be addressed in future investigations. First, the target population of our study was patients with RRMS; therefore, its results may not be generalizable to other types of MS. Second, cerebrospinal fluid samples can better reflect the levels of NfL, MMP-9, and IL-17 in MS patients; however, serum samples were collected in this trial due to practical considerations. Third, the single molecule array technique appears to be more sensitive than the ELISA method for measuring NfL.[79]

5. Conclusion

Ginger supplementation can improve the disability status and physical and psychological QoL in patients with RRMS. In addition, this inexpensive nutraceutical seems to reduce IL-17, NLR, and NfL without causing serious side effects. Therefore, supplementation with ginger may be a safe and effective adjuvant therapy for RRMS patients. More well-designed clinical trials are warranted to verify this conclusion.

Author contributions

Sahar Foshati: conceptualization, investigation, methodology, validation, visualization, data curation, formal analysis, writing – original draft. Maryam Poursadeghfard: investigation, methodology, validation, writing – review & editing. Zahra Heidari: formal analysis, writing – review & editing. Reza Amani: conceptualization, funding acquisition, methodology, project administration, supervision, writing – review & editing.

Data availability statement

The datasets supporting this article have been uploaded as part of the ESI.†

Conflicts of interest

There are no conflicts of interest to declare.

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