ORIGINAL ARTICLE

Effects of grape seed extract supplementation on inflammatory biomarkers, oxidative stress, clinical symptoms, and quality of life in patients with migraine: A double-blinded randomized placebo-controlled clinical trial

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Background: The current study was conducted to assess the effect of grape seed extract (GSE) supplementation on inflammatory biomarkers, oxidative stress, clinical symptoms, and quality of life in migraine patients. **Materials and Methods:** In this randomized double-blinded controlled clinical trial, 50 patients with migraine were randomly allocated to receive either 200 mg/day GSE supplement ($n = 25$) or placebo ($n = 25$) for 8 weeks. Severity, frequency and duration of migraine attacks, headache daily result (HDR), quality of life, migraine disability, mental health, anthropometric indices, blood pressure, and serum levels of calcitonin gene-related peptide (CGRP), vascular cell adhesion molecules-1, total antioxidant capacity, and malondialdehyde were measured at baseline and end of the trial. **Results:** Based on the within-group comparison, patients in the GSE group had a significant reduction in severity, frequency and duration of migraine attacks, HDR, migraine disability, systolic blood pressure, and serum levels of CGRP. GSE group also had better scores in the migraine-specific quality of life questionnaire and mental health questionnaire. When we performed the analysis using the univariate analysis of variance, the effect of GSE on serum CGRP levels (−0.07 ± 0.03 in the GSE group vs. 0.07 ± 0.03 in the placebo group, $P = 0.003$) remained significant. **Conclusion:** This study provides evidence supporting the beneficial effects of GSE supplement on the serum levels of CGRP.
Trial registration: IRCT20121216011763N56.

Key words: Grape seed extract, migraine disorders, oxidative stress

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INTRODUCTION

Migraine is one of the most common neurological disorders characterized by moderate to severe, pulsating, and unilateral headaches and is usually accompanied by nausea, vomiting, photophobia, and phonophobia. It is one of the most common causes of disability in people under 50 years and affects 15% of people worldwide and 14% of adult Iranians. Migraine symptoms have a negative impact on the patients' quality of life.[1]

Although the physiopathology of migraine is not fully understood, evidence suggests that mitochondrial dysfunction followed by elevated levels of reactive oxygen species plays an important role in migraine

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pain. Accordingly, previous studies have shown higher levels of oxidative stress in migraine patients than healthy individuals.[2] In addition to oxidative stress, migraine is associated with high levels of inflammatory biomarkers such as calcitonin gene-related peptide (CGRP). CGRP is a migraine-specific inflammatory biomarker that is positively associated with migraine symptoms. Inflammatory cytokines are vasodilators that can induce the expression of vascular cell adhesion molecules (VCAM). Elevated levels of VCAM are associated with activation of microglia which causes neuropathic pains.[3] Therefore, intervention with anti-inflammatory or antioxidant supplements may have beneficial effects on migraine symptoms through reducing oxidative stress and mentioned inflammatory biomarkers. Previous studies have shown that the administration of isoflavones, Vitamin E, and coenzyme Q10 (Co-Q10) improves the clinical symptoms of migraine patients.[4-6]

Previous evidence has shown that grape seed extract (GSE) may have beneficial effects on oxidative stress and inflammation. GSE contains fiber (35%), fat (13%), protein (11%), water (7%), various vitamins and minerals (3%), and polyphenolic compounds. The main polyphenolic compounds of GSE are proanthocyanidins, which have antioxidant, anti-inflammatory, antihypertensive, antithrombotic, and cholesterol-lowering effects.[7] Therefore, GSE may improve migraine symptoms due to its antioxidant and anti-inflammatory properties. In an experimental study, GSE supplementation inhibited trigeminal pain signaling in an injury-free model of migraine-like pain.[8] In addition, the results of one study on primary trigeminal ganglion cultures showed that GSE would be neuroprotective by suppressing neuronal and glial excitability in the trigeminal ganglion.[9] Despite the previous evidence, no human study has examined the efficacy of GSE supplementation on inflammatory markers, oxidative stress, and clinical symptoms of migraine patients. Therefore, the current study was conducted to assess the effect of GSE supplementation on inflammatory biomarkers, oxidative stress, clinical symptoms, and quality of life in patients with migraine.

MATERIALS AND METHODS

Participants

This was a randomized double-blinded, placebo-controlled, parallel clinical trial that was conducted in Isfahan, Iran, in 2023. Outpatients with migraine were recruited from the neurology clinics of Isfahan city, Iran. Migraine was confirmed by an experienced neurologist based on the third edition of the International Classification of Headache Disorders-3.[10]

Inclusion and exclusion criteria

Our inclusion criteria were willingness to participate in the study, diagnosis of migraine by a neurologist, having at least one migraine attack per month, age between 18 and 60 years, and body mass index (BMI) between 18.5 and 30 kg/m^2. We did not include patients with tension-type headache and chronic diseases such as chronic kidney diseases, cancer, and other neurological disorders. Furthermore, we did not include patients who had a history of taking any antioxidants and GSE supplements in the past 3 months, those who changed the type and dosage of medications in the past month, patients with a special diet, and those who were pregnant or lactating. Patients were excluded if they did not wish to continue the study. In addition, patients who changed the type and dosage of their medicines during the intervention and those who reported side effects related to GSE were excluded.

Out of the 83 patients who were screened for eligibility, 50 of them qualified and were the part of the study [Figure 1]. Before participating in the study, we informed all patients about the study protocol and then they were asked to sign a written informed consent form. The Ethics Committee of the Isfahan University of Medical Sciences, Isfahan, Iran has approved the protocol of the present study (ethics code: IR.MUI.RESEARCH.REC.1401.405). In addition, this clinical trial was registered in the Iranian Registry of Clinical Trials website (www.irct.ir) on March 26, 2023 (No. IRCT20121216011763N56).

Sample size calculation

Considering type I error of 5% ($\alpha = 0.05$), type II error of 20% ($\beta = 0.20$, power = 80%), and serum concentrations of CGRP as key variable,[11] We manually calculated the required sample size using the following formula:[12]

$$n = \frac{(a+b)^2 \times (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

n = sample size in each group

μ_1 = mean changes for the serum levels of CGRP in the intervention group (we considered it as − 27 ng/L based on the study of Ghorbani *et al*.[11]).

μ_2 = mean changes for the serum levels of CGRP in the control group (which was considered as 13 ng/L based on the study of Ghorbani *et al*.[11]).

S_1 = Standard deviation (SD) for the mean changes of the CGRP in the intervention group (which was considered as 59 based on the study of Ghorbani *et al*.[11]).

S_2 = SD for the mean changes of the CGRP in the control group (which was considered as 23 based on the study of Ghorbani *et al*.[11]).

a = conventional multiplier for alpha = 0.05 that was 1.96.

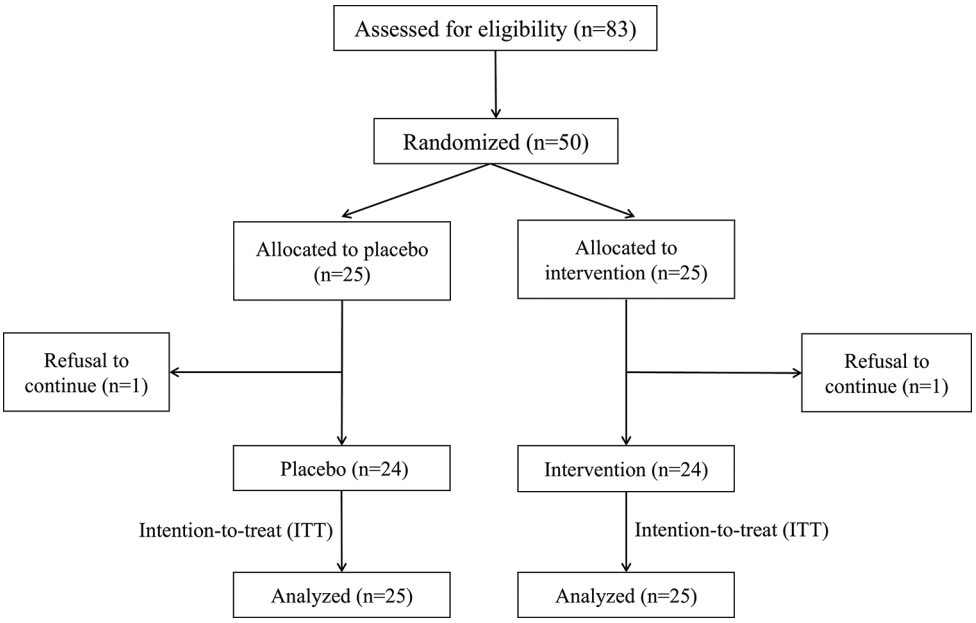

Figure 1: Flow diagram of the study

b = conventional multiplier for power = 0.80 that was 0.842.

Overall, we needed a sample size of 25 persons for each group, based on the formula and considering the dropout rate of 20% in each group.

Study design and intervention

Participants were stratified according to migraine type (with aura and without aura) and BMI (18.5–24.9 and 25–30) and randomly divided into the intervention or control groups. A person who did not know the study's purpose was responsible for randomly assigning the participants. In order to distribute patients into intervention and control groups, individuals were first placed in blocks of four based on migraine type (with aura/without aura) and BMI (18.5–24.9 and 25–30). Accordingly, each person had one of the following four conditions: (1) Migraine type: With aura and BMI: 18.5–24.9, (2) Migraine type: Without aura and BMI: 18.5–24.9, (3) Migraine type: With aura and BMI: 25–30, (4) Migraine type: Without aura and BMI: 25–30. Given that the study had two intervention and control groups and the block size was chosen to be 4, we had 6 blocks: BBAA, ABBA, BABA, BAAB, ABAB, and AABB. Each block was numbered and used for 4 patients. For example, if the patient had condition one (migraine with aura and BMI 18.5–24.9 kg/m^2) and block number 2 was selected for this patient in the lottery, this patient would be placed in Group A (intervention). The second patient with a similar condition to the first patient (condition one) was also placed in block 2 and Group B (placebo). Similarly, the third and fourth patients with a similar condition to the first patient were placed in block 2 and in Groups B and A, respectively. Thus, subjects were placed in the intervention group (black GSE) and the control group (placebo). Participants in the intervention group (n = 25) received 200 mg/day black GSE (Vitagrape, Daru Pajhuh-e-Jaber) along with routine treatments for migraine. Patients in the control group (n = 25) received 200 mg/day placebo (placebo capsules containing fillers such as calcium phosphate, avicel, and gelatin) produced by Daru Pajhuh-e-Jaber along with routine treatments. Participants and the outcome assessors were blinded to treatment allocation. The packs of GSE and placebo capsules were identical in color, appearance, and taste and were given to participants at the study baseline. The duration of intervention was 8 weeks. Previous studies have shown that the daily consumption of 200 mg black GSE for 8 weeks can be effective on inflammatory markers.[13] We asked participants in both groups to return the empty packets at the end of the trial. The outcome variables were evaluated at the study baseline and end of the trial. Participants' compliance was evaluated using the following formula: (number of consumed capsules/total number of capsules) ×100. In addition, intake of supplements and placebo was monitored by phone call every week. Patients were asked not to change their dietary habits, lifestyle, physical activity, and medicines throughout the study.

To evaluate participants' dietary intakes and physical activity during the study, three 1-day dietary recalls and three 1-day physical activity recalls (including two working days and a nonworking day) were filled on the weeks of 1, 4, and 8 of intervention through telephone interview. To complete the dietary recalls, we asked participants to report dietary intakes according to household measures. Then, using available booklets, household measures were converted to grams. We considered the average of dietary

intakes in these 3 days as participants' dietary intakes during the intervention. Nutritionist IV software (based on the United States (US) National Nutrient Databank), modified for Iranian foods, was used to calculate the nutrient content of foods. Physical activity of the participants was calculated based on the following formula: (total hours of sleep in 24 h × 1 × Person's weight at baseline) + (total hours of sitting in 24 h × 1.2 × Person's weight at baseline) + (total hours of walking in 24 h × 1.4 × Person's weight at baseline) + (total hours of exercise in 24 h × 1.7 × Person's weight at baseline). In each telephone interview, possible side effects related to prescribed interventions were recorded using a structured questionnaire.

Assessment of variables

We used a standard questionnaire to gather the information on age, gender, marital status, socio-economic status, education, smoking, sleep duration, medical history, family history of migraine, alcohol use, and history of taking medications and supplements. For women, information on the menstrual cycle was collected at baseline and end of the intervention. Furthermore, at the beginning and end of the trial, we measured primary outcomes, including serum levels of total antioxidant capacity (TAC), malondialdehyde (MDA), CGRP, and VCAM-1, characteristics of migraine attacks (severity, duration, and frequency of migraine attacks), migraine disability, and secondary outcomes including quality of life, mental health, blood pressure, and anthropometric measures.

Biochemical assessments

At study baseline and end of the study, a 10 mL venous blood sample was taken from each patient after 12 h of fasting. Then, serum was separated from the whole blood and kept at − 80°C until further analysis. We measured the serum levels of inflammatory biomarkers including CGRP and VCAM-1 by the enzyme-linked immunosorbent assay commercial kits (Zell Bio, Germany). Previous studies have shown that CGRP increases during migraine attacks and reduces during a successful treatment.[14] In addition, previous evidence has shown a significant positive association between VCAM levels and severity of migraine attacks.[15] To evaluate the oxidative status, we measured the serum levels of TAC and MDA based on colorimetric methods (ZellBio, Germany).

Assessment of migraine attacks

At the start of the study and at the end, we determined the characteristics of migraine attacks including severity, frequency and duration of migraine attacks, and headache daily result (HDR) for each participant. To measure severity of migraine attack, we used the Visual Analog Scale. According to this scale, severity of migraine attack ranges from 0 (no pain) to 10 (the worst imaginable pain).[16] Based on the mean duration (hours) of migraine attacks, the duration of migraine attacks was determined. The frequency of migraine attacks was recorded as the number of migraine attacks per month. HDR was considered as the frequency of migraine attack × duration of migraine attack (day).

Quality of life

To evaluate the quality of life of patients, the migraine-specific quality of life (MSQ) questionnaire was completed at baseline and end of the trial. This questionnaire included 14 items and assessed patients' quality of life throughout the previous month. Each question with a six-choice answer was scored from 1 (never) to 6 (always). Then we summed all responses to calculate a total score ranging from 14 to 84. To facilitate interpretation, we transformed scores to a scale of 0–100; higher scores indicate a lower quality of life. The validity of this questionnaire has been confirmed in Iran.[17]

Migraine disability

To evaluate the unfavorable effects of migraine attacks on the patient's daily performance and well-being, we used the headache impact test (HIT-6) at the beginning and end of the trial. This questionnaire consists of 6 items. Each question has five response options: Never (6 points), rare (8 points), sometimes (10 points), very often (11 points), and always (13 points). HIT-6 score ranges from 36 to 78; higher scores show greater effects of migraine on the patient's clinical status. A score between 36 and 49 points indicates slight or no impact, 50–55 indicates intermediate impact, 56–59 indicates substantial impact, and above 60 indicates severe impact of migraine on the patient's life. The validity and reliability of this questionnaire had already been proven for the Iranian population.[18]

Mental health

To assess patients' mental health, we used a depression, anxiety, stress scale (DASS-21-items) questionnaire at baseline and end of the study. This questionnaire contains three subscales including depression, anxiety, and stress: Each subscale has seven phrases. Based on a 4-point rating scale, each phrase is scored from 0 (did not apply to me at all) to 3 (it applied to me very much or most of the time). Finally, the total score for each subscale ranges from 0 to 21. Based on the DASS guidelines, we multiplied the total score of each subscale by 2, ranging from 0 to 42; a higher score shows higher levels of depression, anxiety, and stress in patients. The validity and reliability of DASS-21 questionnaire have been previously investigated in Iran.[19]

Anthropometric measures and blood pressure

Before and after the intervention period, we measured weight at the state of minimum clothing without shoes using a digital scale to the nearest 100 g. Using a standard

stadiometer, we measured standing height without shoes to the nearest 0.5 cm. We determined BMI as weight in kilograms divided by height in meters squared. We measured systolic and diastolic blood pressures twice with an interval of 15 min at the right arm using a mercury barometer. Before blood pressure measurement, participants were asked to rest for 5 min. We considered the average of two measurements as the participants' systolic and diastolic blood pressures.

Statistical analysis

We conducted the analyses based on an intention-to-treat (ITT) approach by using the last observation carried forward protocol. In the ITT approach, we included all patients allocated to the two groups (GSE and placebo groups).[20] To check if variables follow a normal distribution, we employed the Shapiro–Wilk test. To detect the differences in qualitative variables between the intervention and control groups, we used the Chi-square test. The independent sample *t*-test was applied to examine the differences in quantitative variables between the intervention and control groups. Furthermore, we used mentioned test to compare between-group changes in outcome variables. To perform within-group comparison, the paired-sample *t*-test was applied. Univariate analysis of variance (ANOVA) was used to examine the effects of GSE consumption on outcome variables. We performed all statistical analyses using the SPSS software version 26 (SPSS, Inc. Chicago, IL, USA). $P < 0.05$ was considered significant.

RESULTS

In the present study, 50 patients were allocated in the intervention groups. Forty-eight participants completed the trial. One patient in the treatment group and one in the placebo group withdrew before completing the trial due to personal reasons [Figure 1]. Using the ITT approach, all 50 participants were included in the final analysis. The mean compliance rate in the current study was 97 which seems to be high.

The baseline characteristics of the study participants are presented in Table 1. At the beginning of the study, patients in the intervention group had a higher frequency of migraine attacks compared to the control group. There was no statistically significant difference in terms of age, gender, marital status, education, income level, smoking, alcohol consumption, dietary supplement use, sleeping hours, disease history, weight, BMI, physical activity, family history of migraines, and taking medicine between the intervention and control groups. In terms of differences in menstrual cycle at the beginning and end of the trial, we found no significant difference between the GSE and control groups. Furthermore, we found no significant difference in dietary intakes of energy, protein, fat, carbohydrate,

Table 1: Baseline characteristics of migraine patients in the intervention and control groups

Variables	GSE (*n*=25)	Placebo (*n*=25)	*P**
Age (year)	36.28±10.52	38.24±10.68	0.52
Sex (female)	22 (88.0)	22 (88.0)	0.99
Marital status (married)	20 (80.0)	20 (80.0)	1.00
University educated)	6 (24.0)	6 (24.0)	0.99
Income (good)[a]	8 (32.0)	2 (8.00)	0.10
Smoking (current smoker)	2 (8.00)	2 (8.00)	1.00
Alcohol (alcoholic)	0	1 (4.00)	0.99
Supplement use (yes)[b]	20 (80.0)	21 (84.0)	0.99
Sleeping (≥8 h)	13 (52.0)	14 (56.0)	0.78
Disease history (yes)[c]	16 (64.0)	15 (60.0)	0.77
Weight (kg)	70.99±10.01	68.75±8.80	0.41
BMI (kg/m^2)	26.24±3.13	25.57±3.14	0.45
Physical activity (MET-h/day)	2062.31±317.11	1983.57±253.58	0.34
Family history of migraine (yes)[d]	21 (84.0)	19 (76.0)	0.48
Change in menstrual cycle (yes)	7 (28.0)	4 (16.0)	0.31
Taking medicine (yes)			
Beta blockers	19 (76.0)	22 (88.0)	0.46
Antidepressants	7 (28.0)	11 (44.0)	0.24
Anti-inflammatory	14 (56.0)	18 (72.0)	0.24
Corticosteroids	2 (8.00)	2 (8.00)	0.99
Stomach medicine	3 (12.0)	2 (8.00)	0.99
Painkillers	22 (88.0)	23 (92.0)	0.99
Hyperlipidemia	3 (12.0)	1 (4.00)	0.61
Antidiabetic	3 (12.0)	1 (4.00)	0.61
Antihypertensive	0	2 (8.00)	0.49
Anticonvulsant	21 (84.0)	23 (92.0)	0.67
Frequency of migraine attack	14.52±8.39	9.24±7.13	0.02

*Obtained from Independent sample *t*-test or Chi-square, where appropriate; [a]Income above 14 million Toman in a month; [b]Taking multivitamins, folic acid, omega-3, iron, Vitamin D, calcium, Vitamin E, and zinc supplements; [c]History of cardiovascular diseases, hypertension, diabetes mellitus, liver disease, gastrointestinal diseases, hyperlipidemia, and anemia; [d]History of migraine in father, mother, sister, brother, or children. Data are presented as mean±SD or *n* (%). BMI=Body mass index; MET-h=Metabolic equivalent task-hour; SD=Standard deviation; GSE=Grape seed extract

Vitamin C, iron, zinc, Vitamin A, Vitamin D, Vitamin E, selenium, B-vitamins, fiber, omega-3 polyunsaturated fatty acids (PUFAs), and omega-6 PUFAs throughout the trial between the two groups [Table 2].

At the end of the intervention, patients in the GSE group had a significant reduction in severity, frequency, and duration of migraine attacks, HDR, MSQ score, HIT-6 score, depression, anxiety, stress, serum levels of CGRP, and systolic blood pressure. However, when we compared these reductions with the control group, the effect of GSE on the frequency of migraine attacks (mean change: −10.84 ± 8.34 in the GSE group vs. −6.48 ± 6.46 in the control group, $P = 0.04$) and serum levels of CGRP (mean change: −0.07 ± 0.14 in the GSE vs. 0.07 ± 0.21 in the control group, $P = 0.01$) remained significant [Table 3]. When we performed the analysis using ANOVA, the effect of GSE on serum CGRP levels (−0.07 ± 0.03 in the GSE group vs. 0.07 ± 0.03 in the placebo group, $P = 0.003$) remained significant [Table 4].

Adverse events

In the study period, participants reported no adverse effects following GSE supplementation.

DISCUSSION

Our findings showed that the supplementation with 200 mg/day GSE for 8 weeks led to a significant reduction in serum levels of CGRP. No significant effect was observed regarding the frequency, severity and duration of migraine attacks, HDR, quality of life, migraine disability, mental health, and serum levels of TAC, MDA, and VCAM-1, although the study may have been underpowered to detect it. This was the first study to examine the effect of GSE supplementation on inflammatory biomarkers, oxidative stress, clinical symptoms, and quality of life in patients with migraine.

Table 2: Dietary intakes of nutrients throughout the trial in the intervention and control groups

Variables	GSE (*n*=25)	Placebo (*n*=25)	*P**
Energy (kcal/day)	1924.08±568.84	1945.37±584.80	0.90
Protein (g/day)	76.80±38.27	66.88±19.64	0.25
Fat (g/day)	60.45±20.13	61.18±20.77	0.90
Carbohydrate (g/day)	286.87±103.66	290.55±91.85	0.89
Vitamin C (mg/day)	64.21±28.69	63.98±28.22	0.98
Iron (mg/day)	9.91±4.75	9.36±2.55	0.61
Zinc (mg/day)	7.39±2.37	7.09±1.64	0.60
Vitamin A (RE/day)	603.47±184.59	589.18±198.80	0.79
Vitamin D (μg/day)	1.96±7.45	0.74±0.75	0.42
Vitamin E (mg/day)	4.38±3.39	3.49±2.40	0.29
Selenium (mg/day)	0.10±0.32	0.22±0.56	0.33
B-vitamins (mg/day)	35.00±14.34	31.86±9.12	0.36
Fiber (g/day)	13.05±4.82	12.95±3.62	0.93
Omega-3 PUFA (g/day)	0.69±0.92	1.14±1.63	0.24
Omega-6 PUFA (g/day)	12.37±4.17	12.84±4.56	0.70

*Obtained from Independent sample *t*-test. Data are presented as mean±SD. PUFA=Polyunsaturated fatty acid; SD=Standard Deviation; RE=Retinol activity equivalents; GSE=Grape seed extract

Migraine is a neurovascular disease that is associated with high disability in affected patients.[1] It has been shown that inflammation and oxidative stress increases the severity of symptoms in these patients.[2,21] Treatment of migraine with antioxidants and anti-inflammatory agents received great attention in the recent decades.[4-6] GSE has antioxidant and anti-inflammatory properties. Nevertheless, no study has examined the effect of this supplement on clinical symptoms of migraine patients. In the current study, a significant reduction was found in serum levels of CGRP after GSE supplementation for 8 weeks. Similarly, in a double-blind placebo-controlled randomized clinical trial (RCT), daily consumption of 600 mg GSE for 28 days led to a significant decrease in high-sensitivity C-reactive protein (hs-CRP) levels in patients with type 2 diabetes mellitus.[22] In another clinical trial on overweight or obese individuals, a significant reduction was found in hs-CRP and tumor necrosis factor-α levels following GSE supplementation (300 mg/day) for 84 days.[23] In addition, similar findings were reported in a double-blind RCT on obese older adult women with 200 mg GSE supplementation.[13]

Previous evidence has reported that GSE increases the neuronal expression of glutamate decarboxylase 65 (GAD 65) and GAD 67, which are enzymes that

Table 3: The effect of grape seed extract consumption, compared with placebo, on outcome variables in migraine patients

Outcomes	GSE (*n*=25)				Placebo (*n*=25)				*P*[b]
	Pre	Post	Change*	*P*[a]	Pre	Post	Change*	*P*[a]	
Severity of migraine attack	8.92±1.80	3.52±2.83	−5.40±2.66	<0.001	9.32±1.14	3.96±2.85	−5.36±2.64	<0.001	0.96
Frequency of migraine attack	14.52±8.39	3.68±4.63	−10.84±8.34	<0.001	9.24±7.13	2.76±3.39	−6.48±6.46	<0.001	0.04
Duration of migraine attack	10.36±4.85	1.50±2.49	−8.86±4.84	<0.001	12.24±3.79	2.98±3.81	−9.26±4.79	<0.001	0.77
HDR	160.70±132.05	5.52±10.24	−155.18±130.44	<0.001	122.68±112.58	13.80±22.62	−108.88±109.07	<0.001	0.18
MSQ score	69.38±13.06	30.57±14.50	−38.81±16.42	<0.001	68.38±9.75	33.48±17.47	−34.90±17.83	<0.001	0.42
HIT-6 score	66.60±6.22	47.12±10.56	−19.48±11.03	<0.001	65.92±3.15	49.72±10.38	−16.20±10.56	<0.001	0.29
Depression	22.00±9.49	16.32±10.16	−5.68±4.35	<0.001	21.20±8.87	15.68±8.54	−5.52±4.63	<0.001	0.90
Anxiety	15.44±8.32	11.44±7.86	−4.00±3.65	<0.001	11.52±9.38	9.12±8.35	−2.40±4.79	0.02	0.19
Stress	25.36±6.05	18.88±7.73	−6.48±4.52	<0.001	25.60±7.72	18.64±9.37	−6.96±5.54	<0.001	0.74
TAC	3.52±0.42	3.59±0.38	0.07±0.30	0.27	3.45±0.59	3.45±0.65	−0.01±0.60	0.96	0.58
MDA	2.44±0.90	2.77±1.21	0.33±1.44	0.26	2.42±0.72	2.62±1.01	0.20±1.33	0.46	0.74
CGRP	2.46±0.26	2.39±0.25	−0.07±0.14	0.02	2.44±0.33	2.51±0.26	0.07±0.21	0.10	0.01
VCAM-1	2.60±0.40	2.58±0.36	−0.02±0.28	0.75	2.77±0.41	2.74±0.36	−0.03±0.18	0.38	0.83
Weight	70.99±10.01	70.30±10.78	−0.68±2.64	0.21	68.75±8.80	66.88±8.94	−1.88±2.47	0.001	0.11
BMI	26.24±3.13	25.96±3.22	−0.28±0.98	0.16	25.57±3.14	24.85±3.11	−0.71±0.95	0.001	0.12
SBP	11.24±1.13	10.08±0.91	−1.16±1.18	<0.001	11.32±1.37	10.44±1.19	−0.88±1.13	0.001	0.40
DBP	7.80±0.91	7.52±0.77	−0.28±1.17	0.24	7.66±0.77	7.56±0.71	−0.10±1.02	0.63	0.56

*Changes obtained through this formula: Final – baseline; [a]Paired *t*-test was used to compare pre-post tests; [b]Independent sample *t*-test was used to compare groups two by two. Data are presented as mean±SD. HDR=Headache daily result; TAC=Total antioxidant capacity; MDA=Malondialdehyde; CGRP=Calcitonin gene-related peptide; VCAM-1=Vascular cell adhesion molecule-1; BMI=Body mass index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; MSQ=Migraine-specific quality of life; HIT-6=Headache impact test-6; GSE=Grape seed extract; SD=Standard deviation

Table 4: Adjusted mean changes of outcome variables throughout the trial in the grape seed extract and placebo groups*,[a]

Outcomes	GSE (*n*=25)	Placebo (*n*=25)	*P*[b]
Severity of migraine attack	−5.46±0.53	−5.31±0.53	0.84
Frequency of migraine attack	−8.67±0.80	−8.65±0.80	0.99
Duration of migraine attack	−9.65±0.64	−8.47±0.64	0.21
HDR	−136.74±3.49	−127.32±3.49	0.06
MSQ score	−38.49±3.13	−35.22±3.13	0.46
HIT-6 score	−19.26±2.09	−16.42±2.09	0.34
Depression	−5.64±0.89	−5.56±0.89	0.95
Anxiety	−3.61±0.80	−2.79±0.80	0.47
Stress	−6.48±1.02	−6.96±1.02	0.74
TAC	0.08±0.09	−0.02±0.09	0.42
MDA	0.34±0.23	0.19±0.23	0.65
CGRP	−0.07±0.03	0.07±0.03	0.003
VCAM-1	−0.04±0.04	−0.01±0.04	0.59
Weight	−0.70±0.52	−1.86±0.52	0.12
BMI	−0.27±0.20	−0.73±0.20	0.10
SBP	−1.18±0.18	−0.86±0.18	0.22
DBP	−0.21±0.15	−0.17±0.15	0.83

*Changes obtained through this formula: Final – baseline; [a]Adjusted for baseline values of outcome variables; [b]Obtained from the univariate ANOVA. Data are presented as mean±SE. SE=Standard error; HDR=Headache daily result; TAC=Total antioxidant capacity; MDA=Malondialdehyde; CGRP=Calcitonin gene-related peptide; VCAM-1=Vascular cell adhesion molecule-1; BMI=Body mass index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; MSQ=Migraine-specific quality of life; HIT-6=Headache impact test-6; ANOVA=Analysis of variance; GSE=Grape seed extract

mediate the production of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA).[9] Furthermore, it has been shown that GSE stimulates neuronal expression of the gamma-aminobutyric acid B receptor 1 (GABAB1) subunit, which is responsible for binding GABA to receptors and forming a functional complex with the gamma-aminobutyric acid B receptor 2 subunit. The GSE-mediated increase in the functional expression of the GABAB receptor through upregulation of GABAB1 in trigeminal neurons can directly oppose the cellular effects of stimulatory agents such as nitric oxide and cytokines that facilitate CGRP release.[9] Moreover, in a preclinical model of episodic migraine, GSE supplementation inhibited trigeminal pain signaling through activation of endocannabinoid receptors and suppression of CGRP expression centrally.[8]

In this study, frequency, severity, and duration of migraine attacks were not affected by GSE supplementation. Based on our literature search, we found that other antioxidants have similar effects on migraine symptoms. In a RCT, no significant reduction was seen in frequency, severity and duration of migraine attacks following curcumin supplementation (80 mg/day) for 2 months.[24] In another clinical trial, daily consumption of 150 mg resveratrol for 3 months did not improve the severity of migraine attacks.[25]

In the current study, we found no significant change in antioxidant indices and serum levels of VCAM-1 following GSE supplementation. Contrary to our findings, in a randomized controlled trial, alpha-lipoic acid supplementation (600 mg/day) for 12 weeks showed a significant reduction in serum levels of VCAM-1 and MDA in patients with episodic migraine. However, in that RCT, no statistically significant change in serum levels of TAC was reported.[26,27] Another RCT showed that supplementation with Co-Q10 (400 mg/day) led to a significant decrease in MDA levels, with no effect on TAC levels.[6] The difference between our findings and those obtained from the previous studies might be due to the fact that the sample size in our study was calculated based on the CGRP variable. Therefore, this sample size might be inadequate to find the true effect of GSE supplementation on MDA and VCAM-1 levels.

In this study, GSE supplementation had no significant effect on quality of life, migraine disability, and mental health in migraine patients. This might be explained by the lack of GSE effect on the frequency, severity, and duration of migraine attacks. Similarly, a double-blinded RCT showed that receiving 50 mg/day of soy isoflavones for 8 weeks did not improve psychological disorders in women with migraine.[4] Furthermore, in another clinical trial, quality of life and migraine disability were not affected by resveratrol supplementation (150 mg/day) for 3 months in women with migraine headaches related to the menstrual cycle.[25]

Our study had some strengths. This was the first clinical trial that examined the effect of GSE supplementation on inflammatory biomarkers, oxidative stress, clinical symptoms, and quality of life in migraine patients. Furthermore, most participants completed the trial and we had little dropout. Moreover, adherence to the prescribed interventions was high in the current study. Despite these strengths, our study had some limitations. The sample size of this study was small. This made us unable to do subgroup analyses based on gender and other important variables. Furthermore, the small sample size likely led to an underpowered analysis for several outcomes (e.g. TAC, MDA, VCAM-1, and quality of life). In the present study, we tested serum samples (preintervention and postintervention) simultaneously. This could have influenced our findings. In addition, because of low financial resources, we could not measure an appropriate blood biomarker to evaluate accurate compliance to the intervention. Furthermore, we used dietary recall, instead of food record, to evaluate the dietary intakes of participants. Recall bias is a major limitation of food recall that can affect the accuracy of dietary data.

In conclusion, we found that GSE supplementation significantly reduced serum levels of CGRP in migraine

patients. However, characteristics of migraine headaches including frequency, severity, and duration of migraine attacks and HDR as well as quality of life, migraine disability, mental health, and serum levels of TAC, MDA, and VCAM-1 were not affected. Regarding nonsignificant findings, it should be considered that the current study is likely underpowered for these endpoints. Future studies with higher sample sizes are needed to examine the different doses of GSE on different types of migraine.

Ethical approval and consent to participate

Informed written consent was signed by all the participants. This study was approved by the Ethical Committee of the Isfahan University of Medical Sciences, Isfahan, Iran.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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