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Effects of Ellagic Acid on Oxidative Stress Index, Inflammatory Markers and Quality of Life in Patients With Irritable Bowel Syndrome: Randomized Double-blind Clinical Trial

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ABSTRACT

Irritable bowel syndrome (IBS) is a common disorder that affects the large intestine. Oxidative stress and inflammation play a major role in IBS. Considering the antioxidant properties of ellagic acid (EA), this study was designed to evaluate the effect of EA on oxidative stress index, inflammatory markers, and quality of life in patients with IBS. This research was conducted as a randomized, double-blind, placebo-controlled clinical trial; 44 patients with IBS were recruited. Patients who met the inclusion criteria were randomly allocated to consume a capsule containing 180 mg of EA per day (n = 22) or a placebo (n = 22) for 8 weeks. Serum levels of total antioxidant capacity (TAC), malondialdehyde (MDA), C-reactive protein (CRP), and interleukin-6 (IL-6) were measured at the beginning and the end of the study. Also, quality of life was assessed using a self-report questionnaire for IBS patients (IBS-QOL). At the end of the study, we saw a significant decrease and increase in the MDA and TAC in the intervention group, respectively ($p < 0.05$). Also, EA consumption reduced CRP and IL-6 levels, and these changes were significant in comparison with placebo group changes ($p < 0.05$). The overall score of IBS-QOL significantly decreased, and quality of life was increased ($p < 0.05$), but there were no significant changes in the placebo group. According to these findings, receiving polyphenols, such as EA, may help maintain intestinal health by modulating inflammation and oxidative stress and ultimately improving the quality of life in IBS patients.

Trial Registration: Iranian Registry of Clinical Trials Identifier: IRCT20141025019669N11

Keywords: Ellagic acid; Oxidative stress; Quality of life; Irritable bowel syndrome

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Conflict of Interest
The authors declare that they have no competing interests.

Author Contributions
Conceptualization: Khadem Haghighian H, Rashidi Nooshabadi M; Data curation: Mirzaie Z, Bastani A and Haji-Aghamohammadi AA; Formal analysis: Ahadinezhad B, Khadem Haghighian H; Funding acquisition: Khadem Haghighian H; Investigation: Mirzaie Z, Khadem Haghighian H; Methodology: Rashidi Nooshabadi M, Bastani A; Project administration: Khadem Haghighian H; Supervision: Khadem Haghighian H; Validation: Haji-Aghamohammadi AA; Writing - original draft: Mirzaie Z, Khadem Haghighian H; Writing - review & editing: Rashidi Nooshabadi M, Ahadinezhad B, Bastani A.

INTRODUCTION

Abdominal pain is one of the few clinical symptoms commonly referred to as irritable bowel syndrome (IBS) disease, which ultimately leads to a change in the function of the digestive system [1]. The prevalence rates of IBS range from 12% to 30% of the world's population [2]. Based on the investigation results conducted on IBS patients, they had a low quality of life, an average of 73 days a year. Problems such as depression, incomplete daily activities, and dietary restrictions have been reported in these patients [3]. The cost to society in terms of direct and indirect costs ranges from $15–30 billion annually [4].

Although the underlying cause of IBS is still unknown, scientific evidence suggests that it's related to increased sensitivity of the gut by factors such as food, bile acids, antibiotics, infections, sexual relations, and social and psychological incidents [5]. These changes increase the permeability of the intestine by activating local and brain immunity. The neuroendocrine responses and changes in the microbiome of the intestine ultimately leading to exacerbation of intestinal abnormality and the activation of the intestinal motion sensor, which is associated with the duration and severity of symptoms [5]. Significant changes in the inflammatory cytokines profile in these patients have been observed in scientific studies compared with healthy subjects. According to the reports of these investigations, increases in proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) occur in people with IBS [6]. In addition, anti-inflammatory factors reductions such as IL-10 has been reported in these patients [7]. Various scientific studies have confirmed the relationship between oxidative stress and inflammation. For example, the production of reactive oxygen species by resident cells such as vascular smooth muscle and endothelial cells occurs in the activity of leukocytes [8]. These oxidants are inactivated by the antioxidant defense system [9]. Polyphenols, active ingredients in fruits and vegetables, are classified among 50 antioxidant substances. Based on the results of scientific research, the protective and therapeutic properties of polyphenols have been proven in the management and remedy of IBS.

It should be noted that these health effects contribute to the treatment of the disease by preventing the inflammatory pathways' function, reducing inflammation, and improving the antioxidant status [10]. One of the strong antioxidants from the polyphenols family found in fruits and vegetables is the ellagic acid (EA) [11]. This polyphenol with a strong hydrogen bonding network can participate in many reactions [12]. EA plays an important role in reducing lipid peroxidation and inflammatory symptoms of type 1 diabetes. EA also shows anti-inflammatory and anti-proliferative effects in various types of cancer and prevents oxidative stress [13].

To date, scientific and interesting results have been reported on the effects of EA sources on reducing inflammation in the intestine. Rosillo et al. [14] investigated the EA-enriched pomegranate extract (PE) effects on the chronic Crohn's disease model in mice. Trinitrobenzenesulfonic acid was used for colonic injury through intracolonic instillation. Mice were subjected to different diets for 30 days. At the end of the study, significant decreases in myeloperoxidase activity and TNF-α levels were reported in mice fed an EA-rich diet. Also, based on the study results, a significant decrease in the expression of genes related to oxidative stress factors was observed [14]. Also, the results of Marín et al. [15] indicated that in the acute ulcerative colitis (UC) model, EA improved disease severity as well as reduced inflammatory factors (IL-6 and TNF-α). Also, in the chronic model of the disease, EA reduced intestinal inflammation and prevented the progression of the UC.

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These scientific reports indicate the beneficial effects of polyphenols on intestinal performance and their better function in health. Thus considering the beneficial effects of phenolic compounds such as EA on human health and its beneficial effects on inflammatory indicators, this study designed for the first time to evaluate the effects of EA supplementation on the index of oxidative stress, inflammatory factors, and quality of life in patients with IBS.

MATERIALS AND METHODS

Patients

This randomized, double-blinded, placebo-controlled clinical trial was done on 44 subjects aged 19–60 years old. This research was conducted at Velayat Hospital of Qazvin University of Medical Sciences, Qazvin, Iran, from January 2019 to September 2019. A total of 44 IBS patients including both genders, with normal body mass index (BMI; 19–25 kg/m^2) after a clinical examination and gastroenterologist confirmation were enrolled in the study. Patient' selection in this study was based on Rome III's diagnostic criteria for functional digestive disorders [16]. Patients with a history of abdominal surgery, gastrointestinal diseases such as celiac disease, and pregnant and lactating women, and those who have been taking supplements in the last 3 months were not included in the study. Also, having underlying illnesses like diabetes, severe psychiatric and behavioral disorders, and aspirin, warfarin, heparin, and anti-inflammatory drugs (including non-steroids, steroids, antihistamines, and mast cell stabilizers) have been other exclusion criteria. After approving approval with the ethics committee of Qazvin University of Medical Sciences, Qazvin, Iran (grant number: IR.QUMS.REC.1397.201), the protocol of the study was registered in the Iranian Registry of Clinical Trials website by the IRCT20141025019669N11 code (https://en.irct.ir/trial/35574).

Study design

All patients who met the inclusion criteria were randomly allocated to consume a capsule containing 180 mg of EA per day (n = 22) or a placebo (n = 22) for 8 weeks. Placebo capsules contained 180 mg starch that their shape, color, and size were similar to the supplement.

The supplement was purchased from Supplement Spot (Kokomo, IN, USA), and the placebo was made by the School of Pharmacy, Tabriz University of Medical Sciences (Tabriz, Iran). It should be noted that the effective dose for EA supplementation was taken from Falsaperla et al. [17]. Since oral supplementation with EA has been shown to reduce inflammation (one of the main goals of this research project), this dose was chosen as the dose of choice in this study.

The pharmacological remedy was similar in the 2 groups. All patients were advised not to alter their diet and physical activity habits during the study. The allocation sequence was blinded by using a table of random numbers. The patients were divided into 2 groups by randomized block allocation according to BMI. In this study, the patient, researcher, and specialist physician were blind to supplements and placebo. The capsules were packaged by a third party outside the study and grouped with codes A and B so that the researcher was unaware of the contents of the capsule. After allocation, the participants were referred to the lab for blood sample testing the next day. Anthropometric measurements, clinical history, and demographic data of each individual were evaluated. Quality of life was assessed using a self-report questionnaire for IBS patients (IBS-QOL). IBS-QOL has 34 items about physical and psychosocial function due to IBS. This questionnaire includes a 5-point Likert response scale: not at all, slightly, moderately, quite a lot, and extremely. All questions of this

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questionnaire are divided into 8 subscales, including dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual, and relationships. The minimum score for this test is 34 and a maximum of 170. The higher the individual's score, the lower the quality of life [18]. Height and weight were measured at the beginning and the end of the study using digital scales and stadiometers. BMI was calculated by dividing the weight in kilograms by height in meters squared. Three-day food recalls were used to assess dietary intake, and Nutritionist IV program (First Databank Inc., San Bruno, CA, USA) modified for Iranian food composition was used to estimate participants' dietary intake. Also, to evaluate the physical activity, we used the International Physical Activity Questionnaire (IPAQ). Data from the IPAQ were converted to metabolic equivalent minutes/week using existing guidelines [19].

Laboratory methods

After 10–12 hours of overnight fasting, blood samples were collected from patients. Each sample contains 10 mL of blood. A temperature of −20°C was used to freeze the serums, and then samples were stored at −80°C for future laboratory evaluations. Serum levels of total antioxidant capacity (TAC) were measured by a spectrophotometric method using Randox TAS (Randox Laboratories, Crumlin, UK) by an autoanalyzer (Model Alcyon 300; Abbott, Abbott Park, IL, USA). Serum malondialdehyde (MDA) levels were measured by thiobarbituric acid method. Inflammatory factors, including IL-6 and C-reactive protein (CRP) concentration, were measured by ELISA kit (Koma Biotech Inc., Seoul, Korea) and immune turbid metric assay (Pars Azmoon kit; Pars Azmoon Inc., Tehran, Iran), respectively.

Sample size calculation

We used the MDA factor before and after the intervention to determine the sample size used in Hosseini et al.'s study [20]. Therefore, if the mean and standard deviation of the MDA before and after the supplementation was 3/3 ± 1 and 2/1 ± 0/7, it was calculated as 18 people for each group. Considering the drop out in participants during the study, 22 people were considered for each group.

$$N = [(Z1 - \alpha/2 + Z1 - \beta)^2 (SD1^2 + SD2^2)]/\Delta^2$$

Statistical analysis

Statistical analyses were conducted using SPSS version 20 (IBM Corp., Armonk, NY, USA). All data were presented as mean ± standard deviation and were checked for normality by the Kolmogorov-Smirnov test. Due to the normal distribution of variables, the paired sample t-test and the independent sample t-test were applied to analyze differences in variables within and between groups, respectively. The $p < 0.05$ was considered statistically significant.

RESULTS

Total of 44 patients with IBS were recruited for the study. Twenty-two patients completed the study in the intervention group. One patient from the placebo group dropped out for personal reasons (**Figure 1**). Patient compliance in this study was 97.72%. The final analysis was done on the subjects who finished the study. The patient's demographic and baseline characteristics are presented in **Table 1**. The mean age of all participants was 34.91 ± 5.34 years. There was no significant difference in age between the intervention and placebo groups (34.55 ± 4.93 vs. 35.29 ± 5.84 years). Also, there was no significant difference between

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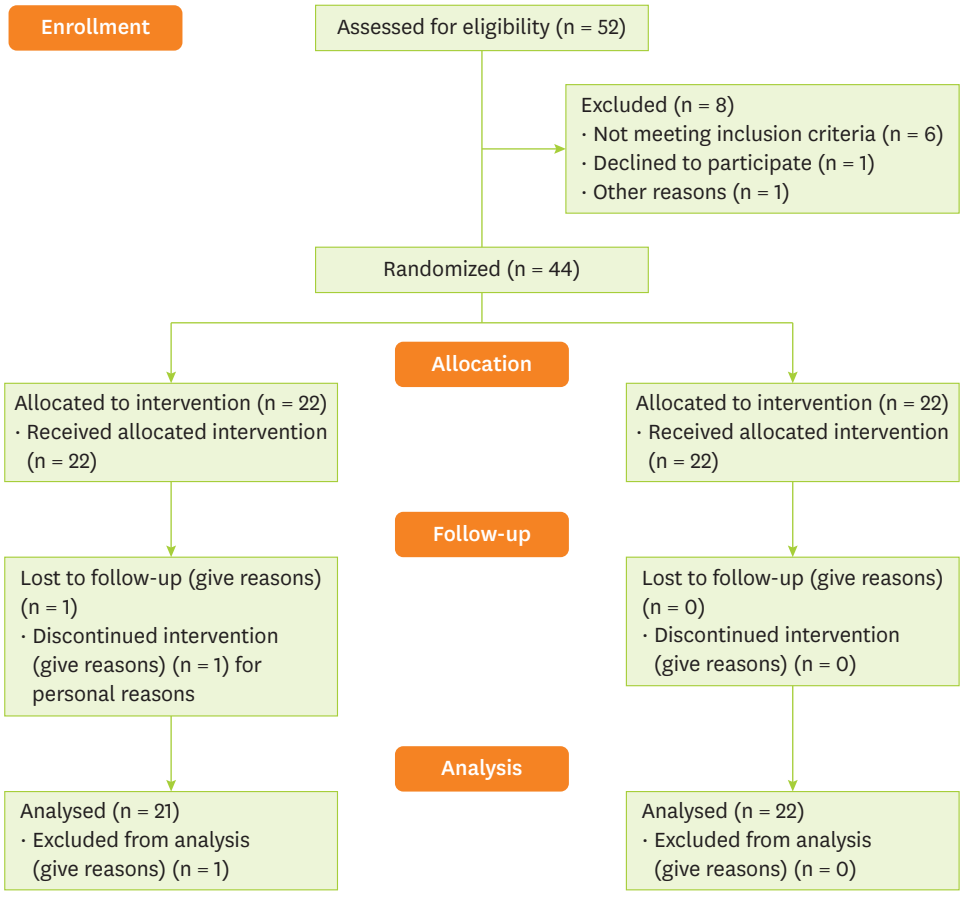

Figure 1. Trial profile and design.

the EA and placebo groups in terms of weight (63.67 ± 7.91 vs. 63.48 ± 9.14), BMI (23.81 ± 1.34 vs. 23.75 ± 1.42 kg/m^2), and physical activity (37.53 ± 2.76 vs. 36.21 ± 2.91) at the beginning of the study ($p > 0.05$; **Table 1**). However, there was no significant difference in BMI, weight, and physical activity between the patients (**Table 1**). In this study, we did not receive any adverse effects reports from patients about EA consumption.

Table 1. The comparison of baseline characteristics of the participants

Characteristics	Placebo (n = 21)	Ellagic acid (n = 22)	P1
Age (yr)	35.29 ± 5.84	34.55 ± 4.93	0.611
Weight (kg)			
Baseline	63.48 ± 9.14	63.67 ± 7.91	0.941
2 mon change	63.03 ± 8.45	63.66 ± 7.81	0.799
P2	0.843	0.903	
BMI (kg/m^2)			
Baseline	23.75 ± 1.42	23.81 ± 1.34	0.827
2 mon change	23.61 ± 1.36	23.81 ± 1.39	0.690
P2	0.813	0.890	
Physical activity (met-h/week)			
Baseline	36.21 ± 2.91	37.53 ± 2.76	0.136
2 mon change	36.72 ± 3.03	38.20 ± 2.80	0.104
P2	0.603	0.189	

Data are expressed as means ± standard deviation. P1: comparison of the mean of baseline characteristics between the 2 groups of ellagic acid and placebo (independent samples t-test). P2: comparison of mean of baseline characteristics in each group at baseline and end of study (paired samples t-test).

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The mean energy and macronutrient intake at the study's baseline were shown in **Table 2.** There were no statistically significant differences between the groups in terms of average daily intake the energy, protein, fat, saturated fatty acids, unsaturated fatty acids, and some micronutrients ($p > 0.05$).

The effect of EA supplementation on oxidative stress and inflammatory biomarkers in IBS patients has been summarized in **Table 3.** EA consumption reduced CRP, and IL-6 levels in

Table 2. The comparison of the in dietary intake at the baseline and the end of the study in patients with irritable bowel syndrome

Variables	Placebo (n = 21)	Ellagic acid (n = 22)	P1
Energy (kcal)			
Baseline	1,908.27 ± 380.67	1,961.31 ± 367.27	0.307
End	1,890.49 ± 401.19	1,973.20 ± 360.09	0.299
P2	0.503	0.499	
Protein (g)			
Baseline	74.34 ± 17.14	76.41 ± 19.02	0.314
End	73.63 ± 16.97	76.88 ± 21.10	0.300
P2	0.407	0.480	
Carbohydrate (g)			
Baseline	248.83 ± 41.93	254.67 ± 42.97	0.540
End	245.52 ± 41.53	256.21 ± 43.10	0.492
P2	0.607	0.643	
Fat (g)			
Baseline	71.47 ± 16.22	72.14 ± 20.48	0.716
End	68.55 ± 37.01	71.31 ± 24.11	0.695
P2	0.291	0.382	
Saturated fatty acids (g)			
Baseline	19.27 ± 5.00	19.45 ± 5.72	0.705
End	18.17 ± 6.40	20.08 ± 3.23	0.612
P2	0.415	0.541	
Monounsaturated fatty acid (g)			
Baseline	27.19 ± 7.09	28.02 ± 6.08	0.749
End	26.11 ± 5.03	28.91 ± 6.11	0.546
P2	0.617	0.719	
Polyunsaturated fatty acid (g)			
Baseline	22.09 ± 8.13	22.85 ± 7.29	0.780
End	21.19 ± 6.12	21.01 ± 4.18	0.803
P2	0.307	0.243	
Fiber (g)			
Baseline	6.39 ± 0.91	6.48 ± 1.04	0.407
End	6.03 ± 2.24	6.71 ± 2.17	0.354
P2	0.207	0.210	
Vitamin C (mg)			
Baseline	68.09 ± 17.09	69.47 ± 13.27	0.704
End	66.28 ± 47.11	68.27 ± 9.67	0.501
P2	0.570	0.609	
Vitamin E (IU)			
Baseline	6.39 ± 0.11	6.79 ± 0.27	0.405
End	5.83 ± 0.91	6.80 ± 0.40	0.102
P2	0.311	0.821	
Selenium			
Baseline	119.47 ± 29.12	120.08 ± 21.14	0.457
End	118.6 ± 27.13	119.13 ± 23.04	0.263
P2	0.302	0.451	

Data are expressed as means ± standard deviation.
P1: comparison of the mean of dietary intake between the 2 groups of ellagic acid and placebo (independent samples t-test). P2: comparison of mean of baseline characteristics in each group at baseline and end of study (paired samples t-test).

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Table 3. Changes in baseline to endpoint measures for oxidative stress and inflammatory biomarkers in 2 groups

Characteristics	Placebo (n = 21)	Ellagic acid (n = 22)	P1
IL-6			
Baseline	9.46 ± 0.99	9.65 ± 0.64	0.440
2 mon change	9.25 ± 1.00	6.66 ± 0.54	< 0.001
P2	0.711	< 0.001	
Mean change	−0.21 ± 0.01	−2.99 ± 0.10	0.002
CRP (μM)			
Baseline	12.12 ± 0.72	11.77 ± 0.82	0.140
2 mon change	12.00 ± 0.79	7.95 ± 0.33	< 0.001
P2	0.270	< 0.001	
Mean change	−0.12 ± 0.07	−3.82 ± 0.49	0.001
TAC (μmol/L)			
Baseline	1.28 ± 0.48	1.08 ± 0.20	0.080
2 mon change	1.25 ± 0.35	3.02 ± 0.62	< 0.001
P2	0.555	< 0.001	
Mean change	−0.30 ± 0.13	1.94 ± 0.42	< 0.001
MDA (μmol/L)			
Baseline	2.17 ± 0.24	2.55 ± 0.59	0.008
2 mon change	2.06 ± 0.18	0.89 ± 0.17	< 0.001
P2	0.509	< 0.001	
Mean change	−0.11 ± 0.06	−1.66 ± 0.42	< 0.001

P1: comparison of the mean of oxidative stress biomarkers and inflammatory factors between 2 groups (independent samples t-test). P2: comparison of the mean of oxidative stress biomarkers and inflammatory factors in each group at the baseline and end of the study (paired samples t-test).
CRP, C-reactive protein; IL-6, interleukin-6; MDA, malondialdehyde; TAC, total antioxidant capacity.

the intervention group, and this change was significant compared to placebo group changes ($p < 0.05$). Also, as is clear from our results, the intervention group increased TAC (3.02 ± 0.62 vs. 1.25 ± 0.35) and decreased MDA amounts in comparison to the placebo group (0.89 ± 0.17 vs. 2.06 ± 0.18), and these differences were statistically significant ($p < 0.05$).

The amount and changes in quality of life scores at the beginning and the end of the study are summarized in **Table 4**. In the intervention group, the overall score of IBS-QOL significantly decreased, and quality of life was increased ($p < 0.05$), but there were no significant changes in the placebo group. According to the study data in **Table 4**, statistically, significant changes are seen in various components of quality of life, including dysphoria, social reaction, health worries, food avoidance, social reaction, relationships, and sexual subscales in the EA group as compared to the placebo group ($p < 0.05$).

Safety and adverse events

No side effects were reported in the study. Also, there were no cointerventions in this study.

DISCUSSION

Our findings provide support for the anti-inflammatory and anti-oxidative properties of EA in patients with IBS. IBS as a gastrointestinal disease is a complex of symptoms described by the abdominal pain and changes in bowel habits, frequency, and form of stool, without any indications for other diseases to cause such symptoms [21]. This condition can considerably affect health-related quality of life [22]. Other scientific evidence showed that reactive oxygen metabolites have a possible role in the cause of almost diseases. Numerous productions of reactive oxygen metabolites have been shown to be responsible for the secretion of electrolytes and water that can result in diarrhea. Moreover, disturbance in redox balance can also change

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Table 4. Changes in baseline to endpoint measures for IBS-QOL in 2 groups

Characteristics	Placebo (n = 21)	Ellagic acid (n = 22)	P1
IBS-QOL overall score			
Baseline	119.9 ± 9.02	123.18 ± 4.89	0.144
2 mon change	114.57 ± 8.25	78.31 ± 6.15	< 0.001
P2	0.152	< 0.001	
Mean change	−5.33 ± 0.77	−44.87 ± 1.26	< 0.001
Dysphoria			
Baseline	33.29 ± 5.56	33.05 ± 3.76	0.870
2 mon change	31.43 ± 4.85	16.68 ± 3.65	< 0.001
P2	0.617	< 0.001	
Mean change	0.14 ± 0.71	−16.37 ± 0.11	< 0.001
Social reaction			
Baseline	13.57 ± 3.88	14.36 ± 1.59	0.382
2 mon change	13.00 ± 3.20	6.77 ± 1.44	< 0.001
P2	0.804	< 0.001	
Mean change	−0.57 ± 0.68	−7.59 ± 0.15	< 0.001
Health worries			
Baseline	10.81 ± 2.50	11.14 ± 1.72	0.790
2 mon change	10.05 ± 2.03	6.82 ± 1.50	< 0.001
P2	0.719	< 0.001	
Mean change	−0.76 ± 0.47	−4.32 ± 0.22	0.001
Body image			
Baseline	12.19 ± 2.69	12.73 ± 1.85	0.454
2 mon change	11.34 ± 2.27	10.80 ± 1.25	0.500
P2	0.240	0.073	
Mean change	−0.85 ± 0.42	−1.93 ± 0.60	0.109
Relationships			
Baseline	10.19 ± 1.50	10.64 ± 1.84	0.408
2 mon change	10.38 ± 2.01	8.64 ± 1.43	0.002
P2	0.734	0.001	
Mean change	0.19 ± 0.11	−2.00 ± 0.41	0.001
Sexual			
Baseline	6.57 ± 1.07	6.91 ± 1.19	0.336
2 mon change	6.76 ± 1.26	4.41 ± 0.59	< 0.001
P2	0.802	< 0.001	
Mean change	0.19 ± 0.19	−2.50 ± 0.60	< 0.001
Food avoidance			
Baseline	6.76 ± 1.44	6.73 ± 1.12	0.930
2 mon change	6.52 ± 1.63	4.91 ± 0.68	< 0.001
P2	0.850	< 0.001	
Mean change	−0.24 ± 0.19	−1.82 ± 0.44	0.002
Interference with activity			
Baseline	26.52 ± 5.04	25.64 ± 3.21	0.499
2 mon change	25.24 ± 5.15	19.27 ± 4.15	0.021
P2	0.063	0.020	
Mean change	−1.28 ± 0.11	−6.37 ± 0.94	0.039

P1: comparison of the mean of IBS-QoL between 2 groups (independent samples t-test). P2: comparison of the mean of IBS-QoL in each group at the baseline and end of the study (paired samples t-test).
IBS-QOL, quality of life was assessed using a self-report questionnaire for irritable bowel syndrome patients.

the expression of immune and inflammatory markers [23]. The results of scientific studies confirmed the role of dietary polyphenols in improving TAC and decreasing the molecular degradation caused by oxidative stress. These mechanisms result in high immune responses, especially in those patients with inflammatory diseases [24]. The anti-inflammatory properties of EA as a potent polyphenol are shown in scientific research. This property can improve oxidative stress status and reduce the tissue damage to the colon [25]. For the first time in this randomized clinical trial, oral supplementation of EA was investigated on oxidative stress index, inflammatory factors, and quality of life in IBS patients.

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Results of the present study indicated that EA supplement therapy for 8 weeks decreased serum levels of the MDA, a marker of oxidative stress, and increased TAC. Various rodent models showed the anti-oxidative effect of EA by detoxification function and modifying MDA formation in the mucosa [26]. In the study of Shukla et al. [27], a model of rheumatoid arthritis was used to assess the effect of EA supplement. Based on their results, 13.6 mg/kg of EA decreased IL-6 levels in arthritic joints and reduced MDA in rats' plasma and colon mucosa [27]. Also, findings in the study of Toklu et al. [28] are consistent with our findings. Administration of 50 mg/kg of pomegranate peel extract containing EA for 28 days in an animal model with liver fibrosis decreased MDA levels [28]. An important issue emerging from Yüce et al. [29] was the protective role of EA against cisplatin-induced oxidative stress in rats' liver and heart tissue. EA (10 mg/kg) in the animals that received cisplatin decreased MDA levels, resulting in lower toxicity.

Inflammation has a special role in the pathogenesis of IBS that can worsen the symptoms by activating the visceral sensory system and perturbing different reflexes of the gastrointestinal [30], and the expression of pro-inflammatory mediators such as cytokines can increase pain by activating intracellular signaling and sensitizing the nervous system [31]. So it is possible to hypothesize that EA may ameliorate inflammatory symptoms due to its properties. Our study showed that oral EA supplement reduced serum levels of the inflammatory markers (IL-6, CRP) after 8 weeks. Marta et al. [15] showed that EA decreased inflammation, histological scores and disease severity through improvements in the inflammatory profile of mediators such as TNF-α, IL-6, and interferon gamma in mice with UC. Moreover, down regulation of some mediators such as cyclooxygenase-2 and inducible NO synthase were reported [32]. Also, the report of Umesalma et al. [33] on EA confirmed previous findings and contributed to our results that EA efficiently reduced the expressions of IL-6 in 1,2-dimethylhydrazine-induced rats. The results obtained by Esmaeilinezhad et al. [34] showed that receiving daily pomegranate juice for 8 weeks improved metabolic, oxidative, inflammatory, and BP outcomes in females with polycystic ovary syndrome.

Contrary to these findings, a meta-analysis of 5 prospective trials did not support the possible effect of pomegranate juice on plasma CRP levels [35]. Rosillo et al. [14] studied the effect of enriched PE on colon inflammation and demonstrated the therapeutic and protective function of EA through suppressing leukocyte infiltration and proinflammatory cytokines activation. Based on Larrosa et al. [36], dietary supplementation of EA has protective function in colitis through alleviation of inflammatory response and production of proinflammatory cytokines which results in lower colonic damage. A proposed mechanism for EA, which is demonstrated among animal models of colitis, is through suppression of some signaling pathways such as nucleus factors, IKB (an enzyme complex that is involved in propagating the cellular response to inflammation), and NF-κB (a protein complex that controls cytokine production), MAPK-ERK (a chain of proteins that communicates a signal from a receptor to the DNA), and JNK (responsive to stress stimuli, such as cytokines) [37]. In this study, quality of life was increased significantly in the EA group. Since EA has some therapeutic effects against IBS disease, there might be possible correlations between the consumption of EA and the quality of life in those patients suffer from it. Nevertheless, a limited number of studies have assessed these correlations. The quality of life is strongly related to condition and severity of IBS so it requires accurate treatment and monitoring. Based on different evidences, psychological complications such as depression and anxiety, which are common signs in IBS patients, lead to impairment of quality of life [38]. The hypothalamic pituitary adrenal axis, the core endocrine stress system, can be

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activated by psychological stressors [39], which provides the gut immune system and the brain connection [26]. Since previous studies used several pomegranate products such as husk, seeds, or pulp, it wasn't possible to see the exact effect of EA among other bioactive compounds. So one of the strengths of this study is that for the first time the effect of pure supplement of EA was investigated in patients with IBS. Also, the design of this study as a double-blind randomized clinical trial that had parallel groups, making the results of this study remarkable. However, the present study, like other clinical trials, had limitations such as single dose, budget deficit, and lower factor measurements. To obtain a perfect picture of EA treatment, it would be necessary to execute a randomized clinical trial with a greater number of participants and different doses.

CONCLUSIONS

Summing up the results, it can be concluded that dietary polyphenols like EA can contribute to the maintenance of gut health by the modulation of inflammation and oxidative stress. The results of our study provide evidence to support the view that EA can play an important role in enhancing the quality of life among IBS patients. Nevertheless, further studies are needed to provide additional evidence.

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