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Effects of *Spirulina (Arthrospira) platensis* supplementation on intestinal permeability, antioxidant and inflammatory markers, quality of life and disease severity in patients with constipated-predominant irritable bowel syndrome: a randomized double-blind, placebo-controlled trial

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Abstract

Background Irritable bowel syndrome (IBS) is a chronic, functional gastrointestinal disorder (FGID) which is characterized by chronic pain related to defecation and alteration in GI motility. Recent findings indicated that intestinal barrier dysfunction, hyperpermeability, oxidative stress, and inflammation play a role in IBS pathogenesis. Considering the antioxidant properties of *Spirulina (Arthrospira) platensis* (SP), this study aimed to investigate the effect of SP supplementation on Quality of life (QoL), disease severity, antioxidant capacity, oxidative stress index and intestinal permeability in constipation-dominant IBS (IBS-C) patients.

Methods This study was a parallel randomized, double-blind, placebo-controlled clinical trial involving 60 IBS-C patients aged 18–50 years. The patients were given either 1 g SP (two capsules/day; each capsule contained 500 mg of SP) or placebo for 12 weeks. IBS-QoL, IBS-Severity system score (IBS-SSS), plasma total antioxidant capacity (TAC), malondialdehyde (MDA), and zonulin levels were measured at baseline and the end of the intervention. Univariate comparison and intention-to-treat (ITT) were used for analysis.

Results SP supplementation compared to placebo resulted in a significant increase in QoL score (7.05 ± 2.02 vs. -1.57 ± 2.49; $p = 0.008$), TAC (145.27 ± 30.77 vs. -54.90 ± 45.72; $p < 0.001$) and decrease in IBS-SSS (-32.17 ± 8.96 vs. 1.07 ± 8.49; $p = 0.002$), MDA level (-11.61 ± 2.57 vs. -2.00 ± 2.24; $p < 0.001$) and zonulin level (-0.22 ± 0.05 vs. 0.12 ± 0.07; $p = 0.001$). These results remained significant after adjusting for baseline values.

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Conclusions SP supplementation demonstrated a promising effect in the management of IBS. However, larger trials with a dose-dependent approach in IBS-C and other subtypes of IBS are warranted.

Trial registration The study protocol was approved by the ethical committee at the Isfahan University of Medical Sciences (Registration No. IR.MUI.RESEARCH.REC.1401.370) and registered online at http://www.IRCT.ir (code: IRCT20140208016529N8, approved date 25.04.2023).

Keywords *Spirulina platensis*, *Arthrospira platensis*, Cyanobacteria, Irritable bowel syndrome, Antioxidant capacity, Intestinal permeability

Introduction

Irritable bowel syndrome (IBS) is a complex, chronic, functional gastrointestinal disorder (FGID), which is characterized by chronic pain related to defecation and alteration in gastrointestinal (GI) motility [1]. Moreover, mental health symptoms and comorbidities such as depression and anxiety are common among these patients leading to a reduced Quality of life (QoL) [2]. Previous evidence indicates a high prevalence of depression and anxiety in IBS patients (38 and 40% respectively) [3]. According to the predominant bowel habits, the ROME IV criteria classified IBS as constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), mixed stool pattern IBS (IBS-M) and unclassified IBS (IBS-U). Further classifications have identified two additional subtypes: post-infectious IBS (IBS-PI) and pain-predominant IBS [4, 5]. The incidence of IBS varies between 5 and 10% and is more prevalent in women and younger ages (third and fourth decades of life) [6].

The pathophysiology of IBS is yet to be clear. However, any biochemical or structural pathologies should be ruled out for an individual to be diagnosed with IBS. Recent evidence found that IBS might not be a single disease or solely a somatosensory condition [7]. Findings from Colonoscopy biopsies indicated that intestinal barrier dysfunction, hyperpermeability, and distorted intestinal immune function may have a role in IBS pathogenesis [8, 9]. Some studies have indicated that serum zonulin levels - a marker of hyperpermeability - are elevated in IBS patients [10, 11]. The intestinal epithelium has evolved a compound system to protect the internal environment of the gut, two of which include epithelial cells tight junctions, and immune cells in the mucosa [1]. Barrier dysfunction results in increased gut permeability, the onset of immune cell infiltration, and activation in the mucosa and leads to low-grade inflammation. Inflammation links to visceral hypersensitivity, which plays an important part in pain and other symptoms of IBS [2]. Moreover, inflammation and oxidative stress are linked together. Infiltrated mast cell overreaction in colonic mucosa mediates signaling inflammation to the enteric nervous system (ENS) and subsequently activates the release of several cytokines [12]. This process results in the generation of reactive oxygen species (ROS) in the brain and intestinal cells, which makes IBS a two-way gut-brain barrier disorder [13]. ROS overproduction leads to antioxidant system over-expression [14]. Besides, free radicals production leads to polyunsaturated fatty acids peroxidation in the cells and over-production of lipid peroxidation products namely malondialdehyde (MDA) [15]. One study indicated that chronic constipation could lead to oxidative stress due to a decrease in both enzymatic and non-enzymatic antioxidant complexes [12]. Li et al., also suggested that constipation increases lipid peroxidation as well as the reduction in antioxidant activity in rats [16]. Thus, hemostasis in the antioxidant defense system could be critical in the management of IBS and in preventing symptom recurrence. For many years, researchers have studied several markers of oxidative stress and antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) [13], but in recent decades total antioxidant capacity (TAC) test is used which measures the antioxidant capacity of all antioxidant in biological samples rather than the antioxidant capacity of an individual compound [17]. Studies have shown that in IBS patients serum level of TAC reduces and MDA increases [2].

Recent clinical studies have shown that nutrients and herbal compounds with antioxidant properties might be beneficial in symptom management, antioxidant status, and inflammatory markers in IBS patients [8, 18, 19]. One such compound is Spirulina platensis (Arthrospira platensis-SP). It is a filamentous blue-green microalga from the Cyanobacteria class. Today, some researchers consider it as a 'superfood' [20]. SP has a high content of protein and multiple micronutrients such as vitamins, minerals, essential fatty acids, phytochemicals and fiber [21]. Besides, SP has shown several health benefits including anti-inflammatory, antioxidant, immunomodulatory, antiviral, and anti-cancer activities [22]. Over the past few years, there has been significant attention to SP as a source of possible treatment for several chronic diseases such as ulcerative colitis, diabetes, obesity, and fatty liver disease [23–26]. These studies have shown significant, advantageous effects of SP on TAC, MDA, and some other antioxidant and oxidant parameters. Furthermore, SP supplementation has provided promising

evidence in improving gut microbiota, antioxidant status, intestinal permeability, and gastric ulcers [27–29]. Previous clinical and rat studies have also shown neuroprotective effects of SP such as reducing stress, fatigue, depression, and anxiety [30–32]. However, studies evaluating the effect of SP in IBS patients are scarce.

Given the lack of a specific treatment for IBS and considering the alteration of antioxidant capacity and intestinal permeability, reduced QoL, and higher prevalence of some psychiatric disorders commonly observed in most IBS patients, this study was conducted to investigate the effect of SP supplementation on QoL, disease severity and plasma TAC, MDA and zonulin levels in IBS-C patients.

Materials and methods

Study design

This was a parallel randomized double-blind placebo-controlled clinical trial (RCT), that investigated the beneficial effects of SP supplementation in individuals with IBS-C for 12 weeks. The study was conducted at the Isfahan Gastroenterology and Hepatology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Ethics

The study protocol was approved by the Medical Ethics Committee at the Isfahan University of Medical Sciences (Registration No. IR.MUI.RESEARCH.REC.1401.370). The.

trial was also registered at the Iranian Registry of Clinical Trials (Registration No. IRCT20140208016529N8). The study was developed and conducted in accordance with the Declaration of Helsinki and was reported based on the Consolidated Standards of Reporting Trials (CONSORT) guidelines. A written informed consent was provided by participants to ensure their awareness of the study details and confirm their voluntary participation before the study.

Subjects and eligibility criteria

Patients who were diagnosed with IBS-C in the ISFUN cohort study were informed of this RCT through phone calls and recruited after comprehensive elaboration on the objectives of the study one moth prior to intervention. Inclusion criteria were as follows: Patients with the age of 18–50 years, were diagnosed with IBS-C according to ROME IV criteria and Bristol Stool Form Scale (BSFS) and then confirmed by a physician in the prior cohort study. physician made sure that all the questions were understood and answered [4]. Moreover, patients who had been diagnosed with or initiated treatment for depression and/or anxiety within the last month before enrollment, suffering from diseases linked to oxidative stress, such as chronic kidney and cardiovascular issues, history of GI surgery, or diseases such as inflammatory bowel disease (IBD), coeliac disease, fecal impaction, hemicolectomy, ileostomy, colostomy, and GI infections were not enrolled in the study.

Exclusion criteria were as follows: disinclination to continue the study, onset of pregnancy during the trial, displaying adverse reaction or allergy to supplementation and starting a new dietary regimen.

Sample size calculation

We utilized the G*Power software to calculate the sample size for our trial. Considering type 1 error of 0.05, 80% statistical power, and an equal number of participants in groups, to detect a clinically significant standardized effect size of at least 0.8 and regarding the primary outcome IBS severity, by taking into account 20% dropout factor, total number of 30 patients were estimated to be enrolled for each group, based on previous studies which they used interventions with potential mechanism of action similar to SP [33].

Random allocation and blinding

Patients who met eligibility criteria were randomly assigned in a 1:1 ratio to receive either SP ($n = 30$) or placebo ($n = 30$) capsules. The randomization process was carried out by a Biostatistician with a permuted block randomization method (block size of 4) using a random number generator from randomizer.org. The allocation of patients to either group was carried out by a research coordinator. To maintain the allocation sequence concealment by the person who assigns patients to groups, sealed opaque envelopes were used and opened successively upon patient enrollment. Moreover, supplement and placebo containers were labeled as A and B by an independent person who was not involved in the study. The patients, researchers, physician associates, and all others who were in communication with patients were blinded to the allocated treatment.

Intervention and follow-up

Both SP and placebo capsules were manufactured by Spiru knowledge-based company (Boushehr, Hormozgan) and were identical in color, weight, taste, odor, shape, size, and packaging. The chemical composition of SP per 1,000 mg (2 capsules) is reported in Table 1. Patients assigned to the intervention group ($n = 30$) were asked to receive SP capsules containing 500 mg of SP, twice a day, after breakfast and dinner. The control group ($n = 30$) was instructed to receive identical placebo capsules, containing corn starch during the same mealtimes. Treatment spanned 12 weeks and patients were requested to return the free containers to improve their compliance with assigned treatments. Furthermore, patients were asked not to change their usual diet, physical activity routine,

Table 1 Chemical composition of Spirulina and per 2 capsules (1 gr)

Nutrient content	Spirulina
Energy (kcal)	2
Total fat (g)	0.03
Total carbohydrate (g)	0.2
Protein (g)	0.62
Fiber (mg)	20
B-carotene (IU)	1100
Vitamin E (IU)	0.2
Vitamin K (mcg)	11.2
Vitamin B1 (mg)	0.05
Vitamin B2 (mg)	0.04
Vitamin B3 (mg)	0.16
Vitamin B6 (mg)	0.008
Vitamin B12 (mcg)	1.9
Folic acid (mcg)	96
Calcium (IU)	0.2
Potassium (mcg)	11.2
Magnesium (mg)	0.05
Manganese (mg)	0.04
Sodium (mg)	0.16
Phosphorous (mg)	0.008
Copper (mcg)	1.9
Iron (mcg)	96
Zinc (mg)	1.4
Inositol (mg)	21.74
Chlorophyll (mg)	4
Phycocyanin (mg)	0.07
Zeaxanthin (mg)	15.1
Lutein (mg)	3.6
Moisture (%)	0.81
PH	5.4

and medications during the 12-week timeframe and to report any change. Moreover, they were in direct contact with the main researcher (S.JN) and were required to report any side effects. Also, their adherence was followed by weekly phone calls. Compliance with assigned treatments was assessed by calculating the compliance rate using the following formula: (Tablets taken /Tablets prescribed) × 100. As a result, less than 80% was considered as poor compliance.

Outcome assessment

Information on gender, age, medication, education, disease history, marital status, and socio-economic status was collected through face-to-face interviews.

Anthropometric measurements

Anthropometric measurements were assessed at the beginning and end of the study. Height was evaluated barefoot via a stadiometer, measured to the nearest 0.5 cm. Participants' body weight was measured using a calibrated scale to the nearest 100 g in minimal clothing. Body mass index (BMI) was calculated using the formula: Weight (kg)/ the square of the body height (m2). waist circumference (WC) was assessed using a non-elastic tape, horizontally fixed around the body, measuring a point midway between the lower rib margin and the iliac crest. Hip circumference was measured via a measuring tape, at the highest point above the hips.

Dietary intakes

To assess the dietary intake of patients, they were asked to complete a three-day dietary record at the baseline and end of the intervention (week 12). They were instructed to write down the amounts of consumed foods after the mealtime to diminish their dependence on memory. Energy and nutrient intake were assessed from these 3-day records using Nutritionist IV software (First Databank, Hearst Corp, San Bruno, CA, United States).

Physical activity

Physical activity level was evaluated through a short form of the International Physical Activity Questionnaire (IPAQ-SF) [34]. It is a self-administered questionnaire that assesses the frequency and duration of specific physical activities in 4 domains: moderate activities, vigorous activities, walking, and sedentary behavior. Data from IPAQ-SF was converted into metabolic equivalent minutes per week according to the previous guidelines. The validated Persian-translated version of IPAQ-SF has shown strong internal consistency and reliability among the Iranian population and was used in the present study [35].

Depression, anxiety, and stress scale– 21 (DASS-21)

Mental health issues including anxiety and stress exacerbate IBS symptoms. To ensure that there was not a significant difference between two groups in term of these conditions in the beginning of the study a validated version of DASS-21 was utilized to assess the mental health status of patients [36]. It is a self-report questionnaire, designed to measure three negative emotional states (scales) including depression, anxiety, and stress. Each scale possesses 7 items, which are broken into related subscales. These subscales score from 0 to 3 and were summed up to calculate the overall score for each item. Afterward, these scores are multiplied by 2 to calculate the final score. The total score can range from 0 to 42 and higher scores are representative of severe depression, anxiety, and stress [37].

Study endpoints

The primary outcomes of the study were the change in the IBS quality of life, severity of IBS, and serum zonulin (permeability marker) and the secondary outcomes were the change in the serum total antioxidant capacity

and serum malondialdehyde. All the outcome evaluations were done at baseline and after 12 weeks of intervention.

Irritable bowel syndrome severity scoring system (IBS-SSS) questionnaire

Symptoms severity was assessed through the Irritable Bowel Syndrome Severity Scoring System (IBS-SSS) questionnaire [38]. This is a composite five-item questionnaire with equal weights that considers abdominal pain intensity, number of days with abdominal pain, bloating/distension, satisfaction with bowel habits, and overall IBS effect on global well-being during the past 10 days. Each question scores between 0 and 100, yielding an overall score of 500. Lower scores indicate lower symptom severity [39].

Irritable bowel syndrome quality of life (IBS-QoL) questionnaire

To evaluate patients' quality of life, the IBS-QoL instrument was used [40]. This is a self-report measure, specifically designed for IBS. The IBS-QoL consists of 34 questions and is scored based on a five-point response scale (never to extremely/a great deal). IBS-QoL includes 8 sub-scales: Dysphoria, Health Worry, Interference with Activity, Food Avoidance, Body Image, Relationships, Social Reaction and Sexual worries. To estimate the total score all responses are summed, averaged, and then transformed into a scale between 0 and 100 with higher scores implying better QoL.

Biochemical assessment (Zonulin, TAC, MDA)

A venous blood sample was taken after an overnight fast of 12 h at baseline and end of 12 weeks of intervention. Then the samples were centrifuged at 3500 rpm, and serum was isolated and stored at − 80 °C. Serum levels of Zonulin were measured via the ELISA method, provided by ZelBio kit (Germany). TAC and MDA were assessed by Kiazist life science kits using the CUPric Reducing Antioxidant Capacity and Thiobarbituric acid reactive substance respectively, according to the manufacturer's instruction (Kiazist Life Sciences, Iran).

Data management

Each patient received a unique ID and was informed that all their data would be confidential. All data including questionnaires, records, and laboratory data were coded by a data management team and entered into SPSS, version 20.0 (SPSS Inc., Chicago, IL, United States) and were handed to a study statistician to be analyzed.

Safety evaluations

To keep track of the safety and tolerability of supplements, the main researcher (S.JN.) had phone calls with patients every week and asked about potential adverse effects. Moreover, participants were instructed to promptly inform the researchers of any acute adverse events to the researchers.

Statistical analysis

Continuous and categorical baseline variables are presented as mean ± standard deviation (SD) and frequency (percentage) and were compared between two groups using independent samples t-test and chi-square test respectively. The normality of continuous variables was assessed using the Shapiro-Wilk test and Q-Q plot. Differences within and between groups were compared using paired sample test and independent samples t-test respectively. The analysis of covariance (ANCOVA) was used to adjust baseline value of the measured outcomes. Regarding categorical binary and ordinal outcomes, two groups were compared using the Pearson chi-square and linear by linear association chi-square test. All the statistical analyses were conducted using SPSS software version 20.0 and p-values < 0.05 were considered statistically significant. we controlled the false discovery rate (FDR) for the primary outcomes using the Benjamini-Hochberg procedure to ensure the precision and validity of our statistical test results.

Results

A total of 70 patients were assessed for the study. After excluding 10 participants due to not meeting inclusion criteria ($n = 6$) and declining to participate ($n = 4$), 60 patients were randomized to SP and placebo groups. Fifty-four patients completed the 12-week study (27 patients in the SP group and 27 patients in the placebo group). Three participants were lost to follow-up from SP group: personal reasons ($n = 3$) and 3 from the placebo group: stomach cramp ($n = 2$) and personal reasons ($n = 1$) (Fig. 1). Patients' compliance with the intervention was > 80% in both groups. All 60 patients were included in the final analysis using Intention to Treat (ITT) analysis.

General characteristics of the participants at baseline and end of the study are shown in Table 2. No significant differences were detected between the characteristics of participants between the SP and placebo groups at baseline and after 12 weeks of intervention (P-values > 0.05).

The mean energy, macronutrient, and selected micronutrient intake at the baseline and at end of the intervention are presented in Table 3. No statistically significant differences were observed between groups at baseline and after 12 weeks of intervention (P-values > 0.05).

The effect of SP supplementation on biochemical variables and IBS-SSS and IBS-QoL are presented in Table 4.

Primary outcomes

At the end of the 12 weeks trial, the between-group comparison showed that participants in the SP group had a

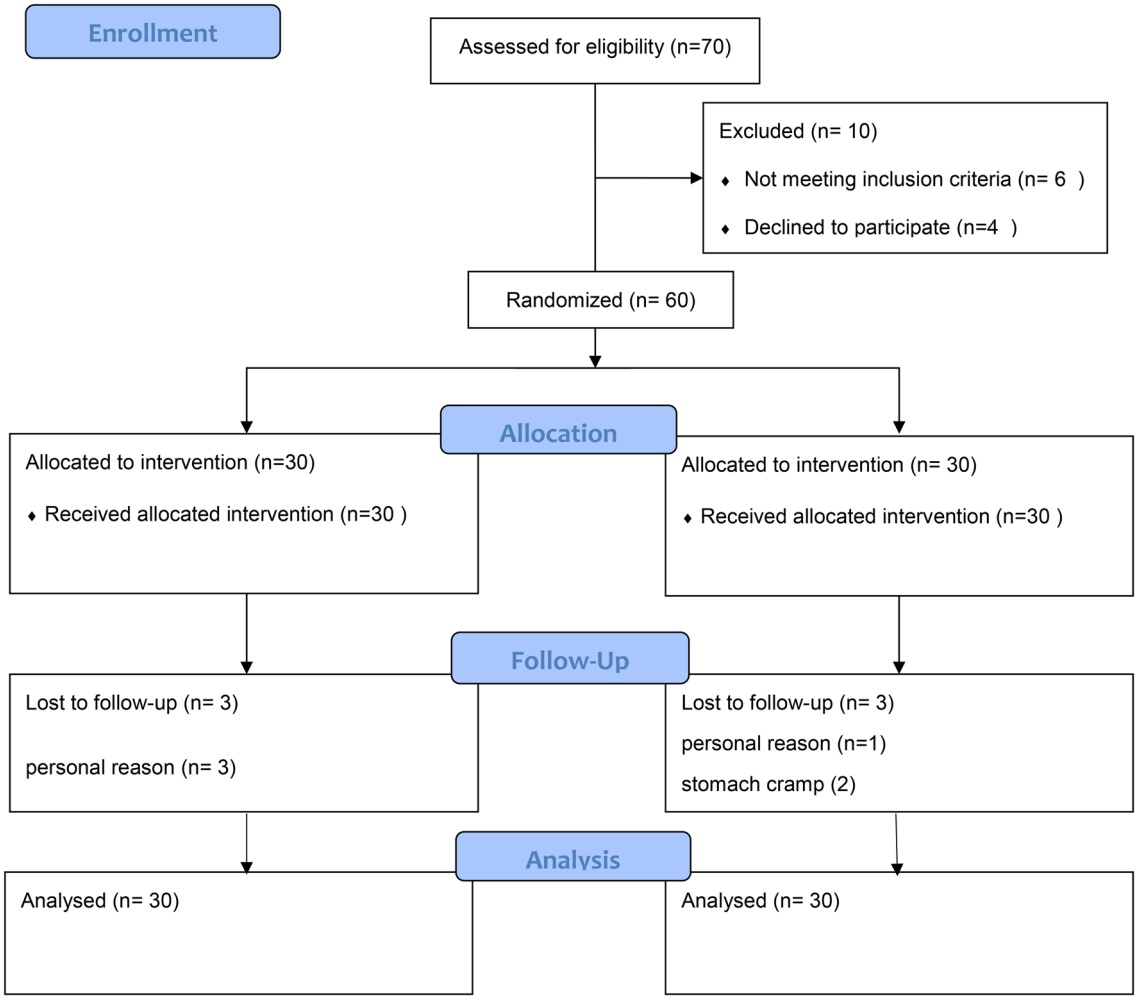

Fig. 1 Study flow diagram of study recruitment

significantly lower Zonulin (−0.22 ± 0.05 vs. 0.12 ± 0.07; $p = 0.001$), overall IBS-SSS score (−32.17 ± 8.96 vs. 1.07 ± 8.49; $p = 0.01$) and higher IBS-QoL score (7.05 ± 2.02 vs. −1.57 ± 2.49; $p = 0.008$) compared to placebo group.

Secondary outcomes

The between-group comparison at the end of the 12 weeks trial, showed that participants in the SP group had a significantly lower MDA (−11.61 ± 2.57 vs. −2.00 ± 2.24; $P < 0.001$), and higher TAC (145.27 ± 30.77 vs. -54.90 ± 45.72; $p < 0.001$) level compared to placebo group.

All differences remained significant after adjusting for the baseline amounts.

Adverse effects

There were no adverse effects following SP supplementation.

Discussion

This randomized, double-blind, placebo-controlled clinical 12-week trial involving 60 IBS-C patients indicated that 1000 mg/d SP supplementation reduced serum Zonulin, MDA, and IBS-SSS and improved serum TAC levels and IBS-QoL compared to the control group. Moreover, there were within-group significant differences in changes in all factors in the SP group.

Our study showed that SP supplementation can improve antioxidant capacity and alleviate oxidant status in patients with IBS-C. To the best of our knowledge, no study has been conducted concerning the effect of SP effect in IBS before the present study. However, there are some prior studies investigating the effect of SP on antioxidant capacity and oxidant status in other diseases. For instance, Moradi et al. indicated that 8 weeks of supplementation with SP in ulcerative colitis patients showed an increase in serum TAC [23]. Mazloomi et al. reported a significant increase in TAC and a decrease in MDA serum levels after 8 weeks of supplementation

Table 2 General characteristics of study subjects

Variables		Spirulina group ($n=30$)	Placebo group ($n=30$)	P^*
Age (year)		39 ± 4.38	38.77 ± 4.22	0.83
Height (m)		1.69 ± 0.09	1.68 ± 0.09	0.68
Weight (kg)				
Baseline		76.86 ± 15.98	75.15 ± 13.17	0.65
End		76.55 ± 16.11	75.60 ± 13.33	0.80
BMI (kg/m2)				
Baseline		26.72 ± 4.09	26.57 ± 4.02	0.88
End		30.99 ± 3.45	31.72 ± 4.52	0.49
Waist circumference (cm)				
Baseline		90.13 ± 11.35	89.85 ± 11.50	0.92
End		88.50 ± 11.55	89.26 ± 11.82	0.80
Hip circumference (cm)				
Baseline		104.20 ± 8.66	103.90 ± 8.30	0.89
END		103.02 ± 8.62	103.21 ± 1.39	0.93
Physical activity (MET/min/week)				
Baseline		1784.28 ± 1809.25 922.50 (2384.25) **	2348.16 ± 1968.47 2303 (2988) **	0.32 #
End		1638.70 ± 1789.64 786.00 (1176.38) **	2585.58 ± 2872.76 2300 (2625) **	0.09 #
gender	Female	17 (56.7)	16 (53.3)	0.79
	Male	13 (43.3)	14 (46.7)	
occupation	Employee	9 (30)	6 (20)	0.46
	Freelance	11 (36.7)	16 (53.3)	
	College student	1 (3.3)	0 (0)	
	Housewife	9 (30)	8 (26.7)	
Education status	Under diploma	4 (13.3)	4 (13.3)	0.52
	Diploma	8 (26.7)	12 (40.0)	
	University graduated	18 (60)	14 (47.7)	
Marital status	Married	25 (83.3)	26 (86.7)	0.72
	Single	5 (16.7)	4 (13.3)	
Familial history of IBS	No	21 (70)	19 (63.3)	0.58
	Yes	9 (30)	11 (36.7)	
Familial history of chronic disease	No	22 (73.3)	20 (66.7)	0.57
	Yes	8 (26.7)	10 (33.3)	
Multivitamin Use	No	25 (83.3)	27 (90)	0.45
	Yes	5 (16.7)	3 (10)	
Anxiety	normal	5 (16.7)	7 (23.3)	0.32
	Mild	1 (3.3)	2 (6.7)	
	Moderate	4 (13.3)	8 (26.7)	
	Severe	20 (66.7)	13 (43.3)	
Stress	Normal	2 (6.7)	3 (10)	0.90
	Mild	3 (10)	4 (13)	
	Moderate	8 (26.7)	7(23)	
	High	17 (56.7)	16 (53)	

Quantitative variables are expressed as mean ± SD/ **median (IQR) and qualitative variables are expressed as n (%)

*P-value resulted from independent samples t-test/ #Mann–Whitney U for continuous and chi-squared tests for categorical variable

with 20 g/d SP sauce in nonalcoholic fatty liver disease patients [24]. Moreover, Aleid et al. revealed that supplementation of gastric ulcerated rats with SP resulted in a significant decrease in serum MDA and an increase in reduced glutathione (GSH) and superoxide dismutase (SOD) levels in serum and stomach tissues [41]. Results of a meta-analysis of 8 RCT studies conducted in various health conditions by Naeini et al. showed a marginally significant effect on TAC after supplementation with SP [42]. On the other hand, results from a meta-analysis of 9 studies by Mohiti et al. revealed no significant effect on MDA concentration after supplementing by SP [43].

Findings from preclinical studies illustrated that two active compounds in SP i.e. phycocyanin and β-carotene

Table 3 Baseline dietary intake of the participants

Variable	Spirulina (*N* = 30)	Placebo (*N* = 30)	*P*[a]
Energy (kcal/day)			
Baseline	2087.54 ± 885.07	2340.74 ± 999.25	0.26
End	2068.78 ± 815.07	2329.08 ± 998.91	0.27
Carbohydrate (g/day)			
Baseline	262.16 ± 103.43	299.04 ± 128.98	0.23
End	260.12 ± 98.67	299.10 ± 128.12	0.19
Protein (g/day)			
Baseline	77.81 ± 40.38	83.43 ± 37.10	0.58
End	75.80 ± 35.40	79.43 ± 37.51	0.70
Fat (g/day)			
Baseline	84.60 ± 43.10	95.36 ± 43.22	0.34
End	83.09 ± 40.39	93.67 ± 43.39	0.33
Chol (g/d)			
Baseline	245.02 ± 136.89	270.75 ± 155.39	0.50
End	243.84 ± 115.71	278.50 ± 158.38	0.33
SFA (g/d)			
Baseline	19.69 ± 10.69	21.89 ± 10.48	0.42
End	17.34 ± 9.40	22.64 ± 10.23	0.36
PUFA (g/day)			
Baseline	30.62 ± 15.89	33.48 ± 15.60	0.48
End	32.56 ± 14.39	34.24 ± 16.37	0.50
MUFA (g/day)			
Baseline	22.48 ± 12.02	25.05 ± 12.57	0.42
End	21.47 ± 10.36	23.16 ± 12.83	0.33
Fiber (g/d)			
Baseline	13.72 ± 6.16	15.29 ± 7.29	0.37
End	16.42 ± 7.18	15.26 ± 8.43	0.46
Vitamin B1 (mg/d)			
Baseline	1.59 ± 0.65	1.78 ± 0.81	0.21
End	1.64 ± 0.45	1.61 ± 0.73	0.36
Vitamin B2 (mg/d)			
Baseline	1.29 ± 0.66	1.47 ± 0.6	0.28
End	1.26 ± 0.78	1.30 ± 0.8	0.33
Vitamin B3 (mg/d)			
Baseline	19.18 ± 9.65	20.23 ± 10.19	0.68
End	20.43 ± 8.36	20.18 ± 9.47	0.63
Pantothenic acid (mg/d)			
Baseline	4.03 ± 1.97	4.48 ± 1.80	0.36
End	5.50 ± 2.45	4.11 ± 1.20	0.29
Vitamin B6 (mg/d)			
Baseline	1.19 ± 0.63	1.53 ± 0.93	0.10
End	1.30 ± 0.57	1.68 ± 0.85	0.16
Biotin (µg/d)			
Baseline	19.31 ± 7.09	22.27 ± 10.76	0.21
End	19.91 ± 6.73	20.38 ± 8.43	0.27
Folate (µg/d)			
Baseline	237.91 ± 116.42	274.53 ± 120.47	0.24
End	240. 84 ± 111.32	276.00 ± 114.34	0.19
Vitamin B12 (µg/d)			
Baseline	4.00 ± 2.71	4.38 ± 2.98	0.62
End	5.34 ± 3.12	4.23 ± 3.67	0.52
Vitamin C (mg/day)			
Baseline	95.32 ± 60.03	116.36 ± 61.67	0.19
End	93.45 ± 57.24	119.56 ± 53.17	0.20
Vitamin A (µg/d)			

Table 3 (continued)

Variable	Spirulina (*N* = 30)	Placebo (*N* = 30)	*P*[a]
Baseline	552.17 ± 276.58	655.80 ± 314.03	0.21
End	546.19 ± 325.45	648.72 ± 300.42	0.24
Vitamin D (µg/d)			
Baseline	1.02 ± 1.05	1.25 ± 0.10	0.39
End	9.68 ± 1.06	1.21 ± 0.16	0.35
Vitamin E (mg/day)			
Baseline	2.83 ± 0.96	3.28 ± 1.39	0.15
End	2.95 ± 0.84	3.05 ± 1.20	0.11
αTocopherol (mg/d)			
Baseline	9.31 ± 4.45	10.08 ± 4.59	0.51
End	11.24 ± 5.14	9.34 ± 5.67	0.36
Zinc (mg/day)			
Baseline	8.20 ± 4.31	9.08 ± 3.82	0.41
End	8.50 ± 3.24	8.86 ± 2.43	0.38
Calcium (mg/d)			
Baseline	700.00 ± 371.65	775.57 ± 291.21	0.23
End	686.21 ± 325. 43	732.46 ± 300.34	0.21
Magnesium (mg/day)			
Baseline	191.60 ± 83.37	220.48 ± 90.32	0.20
End	201.47 ± 74.68	221.94 ± 86.74	0.24
Iron (mg/d)			
Baseline	19.64 ± 9.90	23.62 ± 17.87	0.29
End	20.54 ± 8.95	26.86 ± 19.45	0.32
Sodium (mg/d)			
Baseline	3281.60 ± 1453.47	3490.55 ± 1628.56	0.60
End	3156.93 ± 1411.71	3528.58 ± 1548.42	0.59
Potassium (mg/d)			
Baseline	2484.65 ± 1211.99	2891.55 ± 1171.93	0.19
End	2310.43 ± 1367.82	2784. 19 ± 1134.67	0.27
Phosphorus (mg/d)			
Baseline	937.46 ± 449.42	1066.17 ± 422.64	0.26
End	1079 ± 378.69	1258.93 ± 460.52	0.36
Tryptophan (mg/d)			
Baseline	649.45 ± 352.08	676.86 ± 288.44	0.74
End	670.35 ± 345.10	680.45 ± 290.45	0.76
Isoleucine (mg/d)			
Baseline	2705.31 ± 1492.61	2838.19 ± 1204.34	0.71
End	2684.35 ± 1476.98	2865 ± 1267.31	0.83
Leucin (mg/d)			
Baseline	4507.99 ± 2448.53	4748.26 ± 2015.24	0.68
End	4426.75 ± 2497.46	4735.19 ± 1952.89	0.59
Lysin (mg/d)			
Baseline	4154.68 ± 2456.25	4306.64 ± 1916.98	0.79
End	4186.76 ± 2383.67	4385.54 ± 1878.67	0.57
Methionine (mg/d)			
Baseline	1344.98 ± 760.48	1404.45 ± 609.37	0.74
End	1326.12 ± 740.35	1378.35 ± 679.65	0.61
Cysteine (mg/d)			
Baseline	733.64 ± 368.93	778.40 ± 329.69	0.62
End	750.63 ± 372.49	790.40 ± 311.23	0.60
Tyrosine (mg/d)			
Baseline	1975.98 ± 1072.97	2089.00 ± 873.41	0.65
End	1934.96 ± 986.23	2102.32 ± 649.16	0.58

Table 3 (continued)

Variable	Spirulina (*N* = 30)	Placebo (*N* = 30)	*P*[a]
Valin (mg/d)			
Baseline	3133.45 ± 1669.54	3327.17 ± 1392.35	0.63
End	3076.62 ± 1634.32	3256.78 ± 1367.51	0.46

CHO: carbohydrate; Chol: cholesterol; SFA: saturated fatty acid; PUFA: polyunsaturated fatty acid; MUFA: monounsaturated fatty acid

Variables are expressed as mean ± SD

[a] Obtained from independent t-test

are accountable for the anti-inflammatory and antioxidant properties of SP. Phycocyanin exerts its antioxidant effects by scavenging free radicals such as hydroxyl, alkoxyl, and peroxyl radicals inhibiting the expression of inducible nitric oxide synthase (iNOS) and reducing nitrite production [44]. Furthermore, its anti-inflammatory effects may be due, in part to selective inhibition of cyclooxygeanase-2 (COX-2) expression and reducing the activities of inflammatory markers such as IL-1β, TNF-α, and IL-6 as was seen in damaged colonic cells in a study by Abdel-Daim et al. in colitis induced rats [43, 45, 46]. β-carotene, the other main antioxidant compound of SP seems to directly block the accumulation of ROS in cells and inhibits the expression of proinflammatory cytokines (COX-2, TNF-α, and IL-1β) and iNOS genes. It also reduces oxygen-mediated lipid peroxidation [47, 48]. All of the mentioned mechanisms can result in increase in TAC and decrease in MDA levels. Accordingly, SP might have beneficial effects in alleviating the IBS symptoms associated with low-grade inflammation and oxidation.

Considering the notion of increased intestinal permeability in IBS patients, the findings of our study showed that supplementation with SP resulted in significantly reduced permeability marker i.e., zonulin. It is a protein in intestinal cells which modulates integrity of intestine and induces breakdown of these tight junctions between epithelial cells which can lead to the initiation of inflammatory responses and is used as a marker for intestinal permeability [49]. There is currently no prior clinical study examining the effect of spirulina on zonulin; however, Yu et al. showed a significant decrease in intestinal permeability and tight junction proteins such as zona occludens 1 (ZO-1), Claudin-1, and Occludin after supplementation with SP in high-fat diet-fed rats [27]. Moreover, Wang et al. reported that SPS-3-1 purified from SP improved tight junction integrity, in Caco-2 intestinal cells [50]. Also, Chandrarathna et al. reported that Spirulina-derived Modified Pectin and Modified Pectin Nanoparticles enhanced gut morphometry, gut microbiome, and immune responses [51]. It has been proposed that SP can invert the detrimental effect of lipopolysaccharides (LPS) on the intestinal epithelial barrier by decreasing tight junction proteins and consequently reducing gut permeability [52, 53]. This also results in the suppression of inflammatory response by blocking the TLR4/MyD88/nuclear factor kappa B (NF-κB) signaling pathway which plays a central role in oxidative stress [50]. Furthermore, SP supplementation increases short-chain fatty acid production, and gut microbiota diversity and enhances the composition of gut microbiota, and therefore, decreases gut permeability [53–55]. Overall, SP can reduce intestinal permeability by effecting tight junction proteins such as zonulin, inflammatory responses and gut microbiota. However, it is worth noting that Lacerda et al. questioned the use of serum zonulin for the evaluation of tissue permeability along the intestinal tract and they hypothesized that zonulin acts in specific parts such as the small bowel [56]. Nevertheless, it needs to be clarified in further studies.

Given that IBS is characterized by a set of symptoms, improvement in intestinal permeability and antioxidant status of the body by SP supplementation, may lead to a reduction in symptoms, decrease in the severity of symptoms and enhancement of the patient's QoL as was seen in the present study. Moradi et al. in an RCT, indicated that SP for 8 weeks increased QoL of ulcerative colitis [31]. A systematic review conducted by Sorrenti et al., of the impact of SP on brain health, reported that SP as a superfood is high in nutrients and chemicals such as vitamin B12, magnesium, phosphorous, manganese, branched chain amino acids, and Gamma-linolenic acid (GLA), have shown neuroprotective effects via synthesis of neurotransmitters and energy production [57]. Based on a body of evidence, psychological conditions such as depression, anxiety, and fatigue are common in IBS patients which results in increased IBS symptoms and reduced QoL in these patients [58]. Activation of the hypothalamic pituitary adrenal axis by psychological stressors leads to an increase in intestinal permeability [59]. SP has demonstrated antidepressant effects similar to fluoxetine through its antioxidant properties by reducing MDA and increasing catalase and SOD levels [60]. Moreover, high content of tryptophan (90 mg per 10 g) in SP may act as a precursor in 5-hydroxytryptamine or serotonin biosynthesis. Furthermore, phycocyanin in SP can show anxiolytic effects through interplay with the GABA receptor [61]. Therefore, SP may reduce symptom severity and increase QoL of IBS patients by alleviating stress, anxiety and depression. Hense, IBS as a gut-brain axis disorder can benefit from the mental health effects

Table 4 The effects of 12 weeks' spirulina supplementation on oxidative stress and inflammatory markers

Outcome variables	Spirulina (*N* = 30)				Placebo (*N* = 30)			P^b	P^c
	Baseline	12th week	Change	P^a	Baseline	12th week	Change	P^a		
Zonulin (ng/mL)	2.11 ± 0.06	1.9 ± 0.05	−0.22 ± 0.05	< 0.001	1.95 ± 0.06	2.07 ± 0.06	0.12 ± 0.07	0.35	< 0.001	0.001
TAC (nmol/mL)	1976.17 ± 63.29	2121.43 ± 63.39	145.27 ± 30.77	< 0.001	2010.93 ± 107.50	1956.03 ± 89.48	-54.90 ± 45.72	0.65	0.002	< 0.001
MDA (nmol/mL)	78.19 ± 2.49	66.58 ± 2.33	−11.61 ± 2.57	< 0.001	83.22 ± 3.34	81.22 ± 3.11	−2.00 ± 2.24	0.63	0.011	< 0.001
IBS-SSS	172.27 ± 12.13	140.10 ± 10.00	−32.17 ± 8.96	0.001	174.36 ± 15.75	175.43 ± 13.78	1.07 ± 8.49	0.90	0.009	0.002
IBS-QoL	31.37 ± 2.96	38.43 ± 2.99	7.05 ± 2.02	0.002	31.62 ± 3.17	30.05 ± 3.50	−1.57 ± 2.49	0.66	0.009	0.008

TAC total antioxidant capacity, MDA Malondialdehyde, SP Spirulina, PL Placebo, IBS-SSS irritable bowel syndrome severity system score, QoL quality of life

Variables are expressed as mean ± SE

[a] Paired t-test was used to compare outcomes in pre and post-test interventions

[b] independent samples t-test was used to compare the mean change between the two groups

[c] Obtained from ANCOVA in the adjusted models (adjusted for baseline values)

All presented p-values adjusted for controlling false discovery rate by Benjamini-Hochberg method

of SP, at least in some respects. Moreover, Ma et al. expressed that SP relieves Diphenoxylate-induced constipation in mice. They supposed that SP may improve peristalsis by the anti-constipation action of some SP monosaccharides [62]. Given that constipation is one of the main distressing issues in IBS-C patients, it seems that SP can potentially help alleviate this symptom and improve their QoL.

Our study had some strengths. Firstly, this is the first RCT to investigate the efficacy of SP supplementation among IBS patients. Secondly, we exclusively included IBS-C patients, and this homogenous patient population could prevent us from generalizing the results to all subtypes of IBS, as the efficacy of SP supplementation could be different in those populations. Nevertheless, despite the strength and novelty of the present study, several limitations have to be mentioned. We could have evaluated the patients' fecal microbiome to comprehend the beneficial effect of SP in intestinal dysbiosis but, due to the limited budget, it was not feasible. Moreover, due to this, being the first study of SP in IBS, supplementation was done with a cautious dose of 1000 mg. Another limitation of the study is the patients' past knowledge of the disease and learning to self-manage the complications of the condition over time. In due course, future works should consider the additional confounding factors. Lastly, Recent research [63] mentioned the value of assessment of patients response rates to the intervention through methods such as visual analogous scale (VAS) which has predefined standard values for response rate. Future studies might benefit from response rates to provide additional insights into treatment efficacy.

Conclusion

In summary, the present RCT demonstrated that SP supplementation significantly increased serum TAC and quality of life in IBS patients and reduced intestinal permeability, MDA, and disease severity. Our findings suggested that SP could be beneficial in the management of IBS. However, larger RCTs with a dose-dependent approach in IBS-C and other subtypes of IBS are warranted.

Abbreviations

IBS	Irritable bowel syndrome
FGID	functional gastrointestinal disorder
SP	Spirulina (Arthrospira) platensis
QoL	Quality of life
IBS-C	constipation-dominant IBS
IBS-SSS	IBS-Severity system score
TAC	Plasma total antioxidant capacity
MDA	Malondialdehyde
ITT	Intention-to-treat
IBS-D	Diarrhea-predominant IBS
IBS-M	Mixed stool pattern IBS
IBS-U	Unclassified IBS
IBS-PI	Post-infectious IBS
RCT	Randomized double-blind placebo-controlled clinical trial

BSFS	Bristol Stool Form Scale
IBD	Inflammatory bowel disease
BMI	Body mass index
WC	waist circumference
IPAQ-SF	International Physical Activity Questionnaire
ANCOVA	The analysis of covariance
iNOS	Inducible nitric oxide synthase
COX-2	Cyclooxygeanase-2
ZO-1	Zona occludens
LPS	Lipopolysaccharides
NF-κB	Nuclear factor kappa B
GLA	Gamma-linolenic acid

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Author contributions
Study design: MH.E, M.B, P.A and S.JN; Data gathering: S.N, P.H; Statistical analysis: AF, S.JN, M.B; Drafting the manuscript: S.JN, MH.E; Critically revised and edited the final manuscript: M.B, P.H, M.S. All authors have read and approved the final manuscript before submission.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All study protocols were carried out according to the Declaration of Helsinki. All patients provided written informed consent before participation in the trial. The study protocol was approved by the ethical committee at the Isfahan University of Medical Sciences (Registration No. IR.MUI.RESEARCH.REC.1401.370) and registered online at http://www.IRCT.ir (code: IRCT20140208016529N8, approved date 25.04.2023).

Consent for publication
All authors approved the final version of the manuscript, and agreed for all aspects of the work to be published.

Competing interests
The authors declare no competing interests.

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