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Randomized Controlled Trial

The effects of spirulina supplementation on serum iron and ferritin, anemia parameters, and fecal occult blood in adults with ulcerative colitis: A randomized, double-blinded, placebo-controlled trial

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SUMMARY

Background & aims: The present clinical trial aimed to evaluate the efficacy of spirulina administration on serum iron, ferritin, anemia parameters, and fecal occult blood test (FOBT) in adults with ulcerative colitis (UC).

Methods: Eighty participants with UC were randomly assigned to take, either 1 g/day (two 500 mg capsules) spirulina (n = 40) or placebo (n = 40) in a double-blinded clinical trial for eight weeks. Dietary intake, physical activity status, serum iron and ferritin levels, anemia parameters, and FOBT were assessed in each participant at baseline and following the intervention. Seventy-three participants completed the trial.

Results: Our results indicated significantly increased (p = 0.04) serum iron after eight weeks of spirulina supplementation compared to the placebo group. The spirulina group also demonstrated significantly increased mean corpuscular volume (p = 0.004) whereas red blood cell count (p = 0.01) and hematocrit (p = 0.03) were significantly lowered in the placebo group. No significant changes in FOBT outcomes were seen between groups at baseline (p = 0.12) and the end of the trial (p = 0.34). Eight weeks of 1 g/day spirulina supplementation improved anemia parameters in adults with UC compared to placebo.

Conclusions: These outcomes suggest that spirulina administration may be beneficial in the management of anemia in UC. Further clinical trials of longer duration are necessary to corroborate and expand our findings. Registered at: http://www.IRCT.ir (code: IRCT20170802035460N3).

1. Introduction

Ulcerative colitis (UC) is a prevalent type of Inflammatory Bowel Disease (IBD) characterized by long-lasting inflammation, ulcers in the distal part of the intestine, and clinically recurrent phases of aggravation and remission [1]. UC may result in various complications and symptoms such as intermittent diarrhea and constipation, cramping, abdominal, rectal, or joint pain, bleeding, and anemia [2–4]. Among these complications, anemia is the most common (approximately two-thirds of all patients) and is associated with several symptoms including generalized weakness, shortness of breath, dizziness, sleep problems, fatigue, attention deficit, female infertility, recurrent hospitalization, and higher health-care costs [4,5]. Typically, iron supplementation is recommended to treat or manage anemia in UC, however, it may not be well tolerated by all patients leading to several side effects such as diarrhea, dyspepsia, and abdominal pain [6,7]. Therefore, the need

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List of abbreviations

IBD	Inflammatory Bowel Disease
UC	Ulcerative Colitis
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
PCV	Packed Cell Volume
RBC	Red Blood Cell Count
FOBT	Fecal Occult Blood Test
SCCAI	Clinical Colitis Activity Index
WBC	White Blood Cell Count
BMI	Body Mass Index
ELISA	Enzyme-linked Immunosorbent Assay
SD	Standard Deviation
IPAQ	International Physical Activity Questionnaire

for an alternative treatment with notably fewer side effects and lower potential toxicity is warranted in order to manage anemia in this population.

Bioactive plant compounds have been investigated for a range of outcomes in those with UC [8–13]. For example, *Nigella sativa* [8], ginger [10], curcumin [11], and resveratrol [12] may provide useful complementary therapies to improve UC comorbidities and/or their accompanying symptoms. Spirulina (Arthrospira platensis) is a biomass of cyanobacteria (blue-green algae) [14] that has been widely used as a dietary supplement and/or whole food and is considered a beneficial source of essential nutrients, especially phytochemicals (carotenoids and phycocyanins), minerals (calcium and iron), amino acids, essential fatty acids, vitamins (vitamin B12 and provitamin A), and fiber [15–17]. Spirulina supplementation has been suggested as an adjuvant treatment for the management of numerous disorders, owing to its anti-inflammatory, antioxidant, liver-protecting, anti-viral, and microbiome-modulating properties [18–21]. In particular, the nutritional qualities of spirulina have led to its consideration as a dietary supplement in the prevention and treatment of anemia [22–25]. Selmi et al. [23], for example, reported that 12 weeks Spirulina supplementation significantly improved mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) in a cohort of senior citizens. In addition, others have noted that Spirulina supplementation may ameliorate anemia and decrease the maternal mortality rate in pregnant women [26,27]. Emami et al. [24] further indicated that Spirulina adminstration could increase levels of packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), and platelet count in Streptozotocin-induced diabetic rats. However, the effect of Spirulina supplementation on anemia-related parameters in cohorts with UC remains largely understudied.

Hence and due in part to promising emerging in vivo animal model data, the aim of the current randomized, double-blinded, placebo-controlled trial was to evaluate the effect of spirulinapowder supplementation on serum iron and ferritin, anemia parameters, and fecal occult blood test (FOBT) in an adult cohort with UC.

2. Materials and methods

2.1. Participant characteristics

Eighty participants with diagnosed UC (age: 38.64 ± 11.30 years, height: 166 ± 8.57 cm, and BMI: 25.81 ± 4.96 kg/m^2) were referred between May 2020 and January 2021 from the Imam Reza Hospital (Kermanshah, Iran) to enroll in this study.

The inclusion criteria were: diagnosis of UC based on colonoscopy, clinical records, and pathology; age 18–65 years; having symptoms of active mild-to-moderate UC disease (score on the Clinical Colitis Activity Index between 5 and 12) [28]. Exclusion criteria for initial and/or continued participation included: pregnancy or breastfeeding; current antidepressant and/or anxiety drug administration; anemia; anxiety; consuming antioxidant and omega-3 supplements within three months; smoking and alcohol consumption; diagnosed heart disease; liver, kidney, or other cancer diseases; thyroid and parathyroid disease; gastrointestinal diseases; poor study compliance (falling below 90% compliance with supplementation).

2.2. Experimental setting

Eighty adults with active mild to moderate UC were randomly allocated to either the spirulina supplementation intervention group (n = 40) or the placebo control group (n = 40) for 8 weeks. All study parameters were evaluated twice: immediately prior to the intervention (baseline) and at trial cessation (post-intervention). The evaluated parameters included: anthropometry, dietary intake, physical activity, iron, ferritin, anemia-related markers, and FOBT. All participants were requested to continue their usual physical activity, dietary intake, and medication regimen throughout the study period. Spirulina and placebo supplementation compliance were evaluated through weekly phone calls and by monitoring the number of participants who saved packages.

This study was conducted in accordance with the Helsinki Declaration and written informed consent was signed by all participants prior to the initiation of the clinical trial. The research protocol for this clinical trial was confirmed by the ethical committee at the Kermanshah University of Medical Sciences (code: KUMS.REC.1398.1141, approval date April. 04 2020) and registered at: http://www.IRCT.ir (code: IRCT20170802035460N3).

2.3. Randomization and blinding

A simple randomization method using a random number table was used where participants, laboratory staff, and researchers, were blinded to the supplement allocation for the duration of the trial intervention. At no time during the intervention were the investigators and/or participants aware of which treatment was being provided to study participants. Patients, researchers, and laboratory staff were blinded to the treatment assignment.

2.4. Spirulina supplementation

The intervention received a 500 mg capsule of spirulina twice per day; one each before lunch and dinner for eight weeks. The control group similarly received two capsules daily of placebo each containing 500 mg corn starch devoid of chlorophyll in a similar color, size, and shape compared to spirulina capsules over the same study duration. The selected dose and time of ingestion were based on previous research [29–33].

The spirulina powder was produced by Javane Sabz company, Shiraz, Iran. The chemical composition of spirulina per 100 g is reported in Table 1. All chemical analytical procedures were completed in the Beh-azma laboratory (Iran) in compliance with assessment methods recommended by the Association of Analytical Communities. In order to control consistency, the capsules containing spirulina powder and placebo were prepared specifically for the present study under sterile conditions and with accurate determination of supplement weight and quality.

Table 1
Chemical composition of Spirulina per 100-g of product weight.

Nutrients content	
Energy (kcal)	378
Carbohydrate (g)	15.5
Protein (g)	64
Fat (g)	8.5
Fibre (g)	7.2
Ca (mg)	186
Fe (mg)	128
Zn (mg)	265
Mg (mg)	1.2
B6 (μg)	85
B9 (μg)	91
B12 (μg)	310
Phycocyanin (mg)	1500
Chlorophyll (mg)	800
Beta-carotene (mg)	288
Moisture (%)	5.8
Total ash (%)	4.2
Heavy metals and toxins	
Lead (ppm)	0.133
Arsenic (ppm)	0.127
Mercury (ppm)	0.016
Cadmium (ppm)	0.008
Aflatoxin (ppb)	0.043

2.5. Sample size calculation

The sample size was calculated considering a statistical power of 5% and 80%, respectively. The minimum detectable effect size (i.e. Δ of clinical response) was considered to be 0.3 according to similar clinical trials in UC patients [34,35]. The effective sample size was determined to be 33 participants in each intervention/placebo group and after assuming an approximate 20% dropout rate, 40 participants were established for each group.

2.6. Outcome measurements

Participants' height was evaluated via a nonelastic wall-mounted stadiometer and measured to the nearest 0.5 cm. Body mass was assessed with participants dressed in minimal clothing using a digital scale with an accuracy of ±0.1 kg. Body mass index (BMI) was subsequently calculated as weight (kg)/square of height (m).

To assess the dietary intake of each participant, 3-day food diaries (including one weekend day) were recorded. Nutrient intakes were computed using Nutritionist IV software (First Databank, San Bruno, CA) upon modification for Iranian foods. Physical activity levels were evaluated via the short form of the International Physical Activity Questionnaire (IPAQ) [36].

Participant blood samples were obtained between 08:00 and 10:00 am after overnight fasting (12 h) at baseline and following the intervention. Blood samples were centrifuged (3500 rpm) and aliquoted for storage at −80 °C temperature until further analysis.

Participants' feces were collected in a clean, dry specimen collection container and analyzed for FOBT at pre- and post-intervention utilizing the FOBT Cassette (Acro Biotech, USA). The FOBT Cassette as primary outcome is a visual immune-chromatographic test for the qualitative detection of blood hemoglobin in fecal samples. The test uses a double-antibody sandwich assay to selectively detect fecal occult blood at 100 ng/ml or higher, or 10 μg/g feces. The accuracy of the test is not affected by the diet of the patients. All procedures were conducted based on manufacturer instructions and standards for the determination of FOBT.

Secondary outcome containing cell blood count and other anemia parameters including hemoglobin, hematocrit, MCV, MCH, and MCHC were measured by a hematology analyzer (Mindray BC-3000 Plus, China). Serum iron was measured using a fully automatic biochemistry analyzer (Sinnowa D280, China).The normal ranges for iron were established at 50–150 μg/dL for women and 50–180 μg/dL for men. Serum ferritin concentrations were evaluated using the enzyme-linked immunosorbent assay (ELISA) method with the intra- and inter-assay CV less than 5% and human ferritin RIA test kit (PADYABTEB, Iran) following manufacturer guidelines.

The supplementation's side effects during the intervention were assessed via telephone. The third person, who was not aware of the content and process of the study, called and asked about any side effects such as any allergic, itching, flatulence, causing abdominal cramps, nausea, anaphylaxis, or serious adverse events.

2.7. Statistical methods

Data analyses were conducted using SPSS Version 22 (Inc., Chicago IL., USA). Q–Q plot and normality tests were used to evaluate the normality of distribution. All variables were reported as mean ± standard deviation (SD). Participants' demographic characteristics and micro-nutrient and macro-nutrient intakes were compared between the spirulina and placebo groups using either an independent t-test or Mann–Whitney U test for quantitative and Chi-square for qualitative variables. Analysis of within-group differences was carried out using paired t-tests [37]. Multiple linear regression (adjusted for a mean value of iron, magnesium, and zinc intake) was applied to determine post-intervention differences between the spirulina and placebo groups. We performed subgroup analysis based on gender. Bonferroni correction was used for multiple-comparison adjustments [37]. A p-value $\leq$0.05 for all analyses was considered statistically significant in the present study.

3. Results

3.1. Participants' dropout and supplementation compliance rates

Among the eighty participants who were initially registered in the present clinical trial, there was an 8.8% dropout rate, as 4 participants were withdrawn for non-adherence to the supplementation regimen and 3 for personal reasons (Fig. 1). Thus, data analyses were performed on 73 participants (n = 36 in the spirulina group; n = 37 in the placebo group) who had a supplementation compliance rate of >90%.

3.2. Baseline characteristics

No significant differences in baseline characteristics of participants between the intervention and control groups were observed for age (P = 0.52), sex (P = 0.72), height (P = 0.50), weight (P = 0.43), BMI (P = 0.73), RBC (P = 0.39), WBC (P = 0.79), ferritin (P = 0.73), serum iron (P = 0.76), hemoglobin (P = 0.25), hematocrit (P = 0.80), MCV (P = 0.95), MCH (P = 0.56), MCHC (P = 0.20), disease duration (P = 0.12), dose of mesalazine (P = 0.41), family UC history (P = 0.72), FOBT (P = 0.12) and current medication (P > 0.05), (Table 2).

Moreover, the dietary intake items (P > 0.05) and physical activity (P = 0.36) were not differed significantly between two group (Table 3).

The efficacy of spirulina supplementation on anemia-related parameters is illustrated in Table 4. Within-group comparisons following 8 weeks of spirulina supplementation revealed no

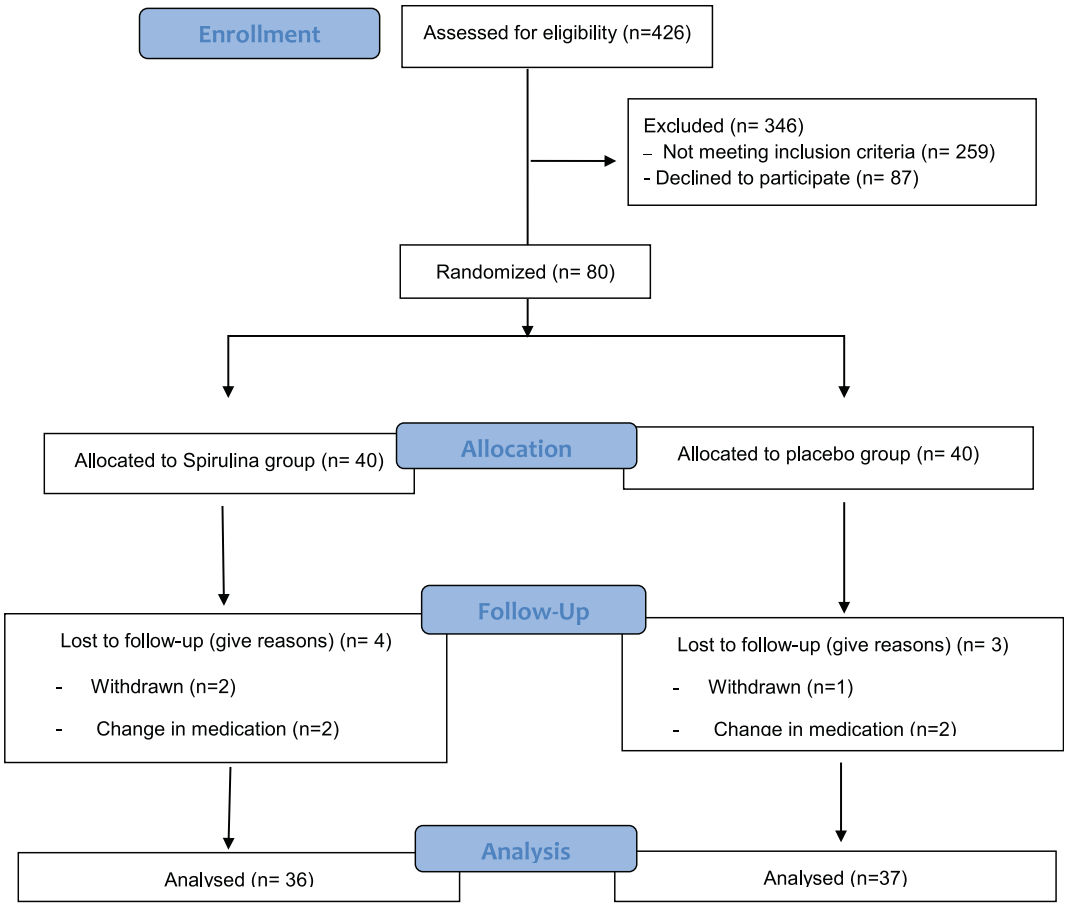

Fig. 1. Participants' flow diagram.

Table 2
Participants' properties at baseline.

Variables	Spirulina group (n = 36)	Placebo group (n = 37)	p^a
Age (years)	11.67 ± 37.77	39.48 ± 11.03	0.52
Sex (female/male)	18/18	20/17	0.72
Height (cm)	166.73 ± 8.38	165.37 ± 8.82	0.50
Weight (kg)	75.33 ± 13.59	69.72 ± 14.54	0.43
BMI (kg/m2)	26.01 ± 4.41	25.61 ± 5.05	0.73
RBC ($*10^6/\mu L$)	4.48 ± 0.55	5.07 ± 1.18	0.39
WBC ($*10^3/\mu L$)	7.01 ± 1.90	7.14 ± 2.22	0.79
Ferritin (μg/L)	45.03 ± 42.76	42.04 ± 32.63	0.73
Serum Iron (μg/dL)	88.69 ± 47.67	92.61 ± 62.97	0.76
Hemoglobin (g/dL)	13.9 ± 4.82	12.92 ± 1.79	0.25
Hematocrit (%)	40.01 ± 4.49	39.72 ± 4.61	0.80
MCV (fL)	82.47 ± 8.97	82.58 ± 7.65	0.95
MCH (pg)	27.24 ± 2.21	26.74 ± 3.10	0.56
MCHC (%)	32.77 ± 2.25	31.98 ± 2.94	0.20
Disease duration (year)	7.16 ± 5.59	5.21 ± 5.02	0.12
Dose of Mesalazine (mg/day)	2277.77 ± 1614.41	1959.45 ± 1180.73	0.41
Family history n (%)	8 (22.2)	7 (18.9)	0.72
FOBT Positive n (%)	26 (72.2)	21 (56.7)	0.12
Current medication n (%)			
Mesalazine (oral)	30 (83.3)	32 (86.4)	0.23
Mesalazine (rectal)	11 (30.5	12 (32.4)	0.32
Sulfasalazine	6 (16.6)	3 (8.1)	0.69
Prednisolone	3 (8.3)	3 (8.1)	0.35
Azathioprine	6 (16.6)	5 (13.5)	0.62

Note: Variables are expressed as mean ± SD. Abbreviations: BMI, Body mass index; RBC, Red Blood Cell Count; WBC, White blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; FOBT, Fecal occult blood test. [a] p values resulted from independent t tests for quantitative and Chi-square for qualitative variables between the two groups.

Table 3
Baseline dietary intake and physical activity levels of participants.

Variables	Spirulina group (n = 36)	Placebo group (n = 37)	*p*
Energy (kcal/day)	2138.92 ± 540.58	2104.41 ± 445.74	0.97[b]
Carbohydrate (g/day)	311.60 ± 105.13	325.77 ± 113.78	0.51[b]
Protein (g/day)	91.39 ± 31.84	92.15 ± 25.15	0.74 [b]
Fat (g/day)	60.97 ± 19.57	59.69 ± 20.26	0.78[a]
Saturated fats (g/day)	19.47 ± 7.37	20.55 ± 5.90	0.53[b]
Cholesterol (mg/day)	406.12 ± 295.03	365.78 ± 188.80	0.85[b]
Linolenic fat (g/day)	0.35 ± 0.31	0.34 ± 0.28	0.87[b]
Linoleic fat (g/day)	7.67 ± 4.47	7.78 ± 4.25	0.93[b]
EPA-Omega 3 (g/day)	0.04 ± 0.10	0.03 ± 0.10	0.89[b]
DHA-Omega 3 (g/day)	0.07 ± 0.16	0.07 ± 0.12	0.32[b]
Polyunsaturated fats (g/day)	15.16 ± 10.97	12.30 ± 8.14	0.33[b]
Monounsaturated fats (g/day)	19.56 ± 8.04	17.49 ± 6.80	0.23[a]
Dietary fibre (g/day)	15.64 ± 7.06	17.98 ± 17.55	0.45[b]
Arginine (mg/day)	433.87 ± 492.11	401.12 ± 391.46	0.90[b]
Alanine (mg/day)	401.30 ± 484.18	400.02 ± 390.28	0.50[b]
Glutamic Acid (mg/day)	2264.44 ± 2119.63	1890.66 ± 1541.69	0.66[b]
Leucine (mg/day)	5526.92 ± 1970.38	5388 ± 1407.34	0.71[a]
Methionine (mg/day)	1764.70 ± 704.83	1705.22 ± 472.82	0.64[b]
Calcium (mg/day)	814.46 ± 269.71	891.71 ± 415.17	0.44[b]
Phosphorus (mg/day)	1160.54 ± 416.85	1202.33 ± 367.22	0.65[a]
Iron (mg/day)	16.48 ± 5.58	18.83 ± 5.34	0.07[a]
Copper (mg/day)	1.11 ± 0.58	1.13 ± 0.44	0.32[a]
Magnesium (mg/day)	208.60 ± 83.16	238.96 ± 64.90	0.08[a]
Zinc (mg/day)	8.77 ± 2.74	9.82 ± 2.53	0.09[a]
Selenium (mg/day)	0.11 ± 0.05	0.09 ± 0.04	0.23[b]
B6 (mg/day)	1.37 ± 0.59	1.63 ± 0.92	0.16[b]
B9 (Ug/day)	301.03 ± 150.05	311.56 ± 128.90	0.42[b]
B12 (Ug/day)	4.57 ± 2.33	4.82 ± 2.37	0.57[b]
Vitamin C (mg/day)	152.71 ± 72.19	143.49 ± 109.27	0.40[b]
Vitamin E (mg/day)	2.73 ± 1.80	2.50 ± 0.87	0.94[b]
Lutein (mg/day)	890.52 ± 524.64	804.85 ± 447.95	0.46[b]
Lycopene (mg/day)	1505.78 ± 1124.94	1543.48 ± 1993.32	0.22[b]
α −Carotene (mg/day)	1815.62 ± 1436.28	1878.21 ± 980.41	0.36[b]
β-Carotene (mg/day)	207.19 ± 124.75	217.71 ± 62.97	0.11[b]
β-Cryptoxanthin (mg/day)	90.48 ± 78.23	93.52 ± 75.81	0.80[b]
α-Tocopherol (mg/day)	4.49 ± 1.37	4.19 ± 1.38	0.35[a]
Physical activity level (MET/h/day)	24.65 ± 1.69	23.97 ± 1.93	0.36[b]

Note: Note: Variables are expressed as mean ± SD.
[a] p values resulted from independent t tests.
[b] p values resulted from Mann–Whitney U test.

Table 4
The effects of Spirulina administration on anemia parameters.

Variables	Normal range	Spirulina group (n = 36)				Placebo group (n = 37)				
		Baseline	End of trial	Change	*p*[a]	Baseline	End of trial	Change	*p*[a]	*p*[b]
RBC ($*10^6$/μL)	W: 4.2 to 5.4 $*10^6$/μL M: 4.7 to 6.1 $*10^6$/μL	4.88 ± 0.55	4.99 ± 0.63	0.10 ± 0.47	0.18	5.07 ± 1.18	4.84 ± 0.84	−0.23 ± 0.53	0.01	0.01
WBC ($*10^3$/μL)	W/M: 4.5 to 11 $*10^3$/μL	7.01 ± 1.90	6.54 ± 2.45	−0.47 ± 2.15	0.19	7.14 ± 2.22	6.64 ± 2.13	−0.50 ± 2.42	0.21	0.65
Ferritin (μg/L)	W: 24 to 307 μg/L M: 24 to 336 μg/L	45.03 ± 42.76	49.31 ± 36.91	4.28 ± 39.89	0.52	42.03 ± 32.63	36.82 ± 36.55	−5.21 ± 21.46	0.14	0.19
Serum Iron (μg/dL)	W:50 to 150 μg/dL M:50 to 180 μg/dL	88.69 ± 47.67	100.21 ± 41.15	11.52 ± 33.97	0.05	92.61 ± 62.97	85.86 ± 52.32	- 6.74 ± 35.11	0.25	0.04
Hemoglobin (g/dL)	W: 12.1 to 15.1 g/dL M: 13.8 to 17.2 g/dL	13.90 ± 4.82	13.91 ± 4.55	0.01 ± 1.17	0.94	12.92 ± 3.93	12.86 ± 3.86	−0.05 ± 3.76	0.93	0.68
Hematocrit (%)	W: 36% to 48% M: 41% to 53%	40.01 ± 3.99	40.42 ± 3.98	0.41 ± 3.20	0.44	39.72 ± 4.61	38.69 ± 3.90	−1.02 ± 3.09	0.05	0.03
MCV (fL)	W/M: 80 to 100 fL	82.47 ± 8.97	81.69 ± 8.57	- 0.77 ± 0.04	0.12	82.58 ± 7.65	83.63 ± 7.03	1.05 ± 2.34	0.01	0.004
MCH (pg)	W/M: 25.4 to 34.6 pg	27.24 ± 4.21	27.30 ± 4.66	0.05 ± 2.40	0.89	26.74 ± 3.10	26.25 ± 3.28	−0.48 ± 1.86	0.11	0.32
MCHC (%)	W/M: 31%–36%	32.77 ± 2.25	32.72 ± 2.14	- 0.05 ± 1.16	0.79	31.98 ± 2.94	31.70 ± 2.96	−0.28 ± 1.62	0.29	0.68

Note: Variables are expressed as mean ± SD. Abbreviations: RBC, Red Blood Cell Count; WBC, White blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; W, Women; M, Men.
[a] Obtained from paired t test.
[b] Obtained from multiple linear regression test, adjusted for mean value of iron, magnesium and zinc intake.

significant differences in RBC (4.88 ± 0.55 × 10^6/μL vs 4.99 ± 0.63 × 10^6/μL; P = 0.18), WBC (7.01 ± 1.90 × 10^3/μL vs 6.54 ± 2.45 × 10^3/μL; P = 0.19), ferritin (45.03 ± 42.76 μg/L vs 49.31 ± 36.91 μg/L; P = 0.52), serum iron (88.69 ± 47.67 μg/dL vs 100.21 ± 41.15 μg/dL; P = 0.05), hemoglobin (13.90 ± 4.82 g/dL vs 13.91 ± 4.55 g/dL; P = 0.94), hematocrit (40.01 ± 3.99% vs 40.42 ± 3.98%; P = 0.44), MCV (82.47 ± 8.97 fL vs81.69 ± 8.57 fL; P = 0.12), MCH (27.24 ± 4.21 pg vs 27.30 ± 4.66 pg; P = 0.89), and

MCHC (32.77 ± 2.25% vs 32.72 ± 2.14%; P = 0.79) after eight weeks of spirulina supplementation. Furthermore, within-group comparisons in the placebo group indicated a significant increase in MCV (82.58 ± 7.65 fL vs 83.63 ± 7.03 fL; P = 0.01) and a significant decrease in RBC (5.07 ± 1.18 × 10^6/μL vs 4.84 ± 0.84 × 10^6/μL; P = 0.01) pre-to post-intervention. After correcting for multiple comparisons, a significant increase in serum iron (P = 0.04) was seen after 8-weeks of spirulina supplementation compared to the control group. Moreover, a significant increase in MCV (P = 0.004) and a significant reduction in RBC (P = 0.01) and hematocrit (P = 0.03) were observed in the control group compared to the spirulina group (Table 4). Sub-group analyses indicated that a significant increase in serum iron (P = 0.04) was seen after 8-weeks of spirulina supplementation compared to the control group in women. Furthermore, a significant increase in MCV (P < 0.05) was found in the control group compared to the spirulina group in both sexes (Tables 5 and 6).

Finally, data analysis revealed no significant changes in FOBT outcomes after 8-weeks spirulina supplementation in comparison to the placebo group (Table 7).

3.3. Side effects

No participant in the present study reported any allergic or serious adverse events during the intervention period. A small number of participants (N = 6) reported mild bloating during the early stages of spirulina supplementation; however, all such side effects were resolved in advance of post-intervention data collection. Due to the lack of side effects of the supplementation and the good compliance of people, we found no need for interim analysis and stopping guidelines.

4. Discussion

Anemia is considered to be one of the primary contributors to low quality of life [38], high hospital admission rates [39], and early mortality [40] in adults with UC. Due to the multifactorial etiology of anemia in UC, management of this condition is often complicated or even neglected over symptom relief. It appears that iron, folate, and cobalamin deficiencies, inflammation, hemolysis, and myelosuppression are involved in the pathophysiology of UC-related anemia [41]. Spirulina has been suggested to have hematopoietic effects due to its high iron, folate, and cobalamin content [42], anti-inflammatory [18] and anti-hemolytic [43] properties, and its ability to reduce myelosuppression [44,45]. Consequently, we investigated the effect of spirulina supplementation on iron status, complete blood count, and FOBT in participants with UC. Results from this study indicated that 8-weeks supplementation with 1 g/d spirulina significantly improved serum iron levels and prevented worsening of RBC, HCT, and MCV compared to the placebo group. Besides, sub-group analyses indicated that a significant increase in serum iron was seen after 8-weeks of spirulina supplementation compared to the control group in women. Furthermore, a significant increase in MCV was found in the control group compared to the spirulina group in both sexes. However, no significant between-group differences were observed in serum ferritin, WBC, HGB, MCH, MCHC, and FOBT during the trial.

Table 5
The effects of Spirulina administration on anemia parameters in women.

Variables	Normal range	Spirulina group (n = 18)				Placebo group (n = 37)				
		Baseline	End of trial	Change	p^a	Baseline	End of trial	Change	p^a	p^b
RBC (*10^6/μL)	4.2 to 5.4 *10^6/μL	4.71 ± 0.48	4.81 ± 0.66	0.10 ± 0.44	0.34	5.11 ± 1.58	4.83 ± 1.09	−0.27 ± 0.64	0.07	0.09
WBC (*10^3/μL)	4.5 to 11 *10^3/μL	6.26 ± 0.35	5.98 ± 0.54	−0.27 ± 1.70	0.51	7.53 ± 2.16	6.70 ± 1.93	−0.83 ± 2.84	0.21	0.46
Ferritin (μg/L)	24 to 307 μg/L	37.54 ± 26.06	54.97 ± 59.17	17.43 ± 62.60	0.25	47.94 ± 36.82	40.23 ± 38.46	−7.70 ± 22.26	0.14	0.19
Serum Iron (μg/dL)	50 to 150 μg/dL	89.80 ± 52.43	105.29 ± 37.80	15.48 ± 39.78	0.12	88.47 ± 63.93	80.29 ± 53.74	8.17 ± 40.82	0.38	0.04
Hemoglobin (g/dL)	12.1 to 15.1 g/dL	14.40 ± 6.35	14.35 ± 6.21	−0.04 ± 0.88	0.83	12.79 ± 1.63	13.09 ± 4.43	0.30 ± 5.04	0.79	0.92
Hematocrit (%)	36% to 48%	39.45 ± 3.00	39.16 ± 2.70	−0.28 ± 2.04	0.55	39.06 ± 4.32	37.66 ± 3.70	1.40 ± 3.22	0.07	0.19
MCV (fL)	80 to 100 fL	84.45 ± 7.60	83.66 ± 7.90	−0.78 ± 2.85	0.26	82.88 ± 7.10	83.82 ± 7.01	0.94 ± 1.84	0.03	0.05
MCH (pg)	25.4 to 34.6 pg	27.81 ± 3.46	27.85 ± 3.62	0.03 ± 2.21	0.95	26.97 ± 3.05	26.28 ± 3.51	0.69 ± 1.71	0.08	0.38
MCHC (%)	31%–36%	32.81 ± 1.88	32.94 ± 1.71	0.12 ± 1.26	0.67	32.55 ± 1.28	31.86 ± 1.89	−0.69 ± 1.68	0.08	0.16

Note: Variables are expressed as mean ± SD. Abbreviations: RBC, Red Blood Cell Count; WBC, White blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration.
[a] Obtained from paired t test.
[b] Obtained from multiple linear regression test, adjusted for mean value of iron, magnesium and zinc intake.

Table 6
The effects of Spirulina administration on anemia parameters in men.

Variables	Normal range	Spirulina group (n = 18)				Placebo group (n = 17)				
		Baseline	End of trial	Change	p^a	Baseline	End of trial	Change	p^a	p^b
RBC (*10^6/μL)	4.7 to 6.1 *10^6/μL	5.05 ± 0.13	5.16 ± 0.13	0.11 ± 0.50	0.37	5.05 ± 0.44	4.84 ± 0.42	−0.17 ± 0.38	0.08	0.08
WBC (*10^3/μL)	4.5 to 11 *10^3/μL	7.76 ± 0.47	7.09 ± 0.60	−0.67 ± 2.55	0.28	6.67 ± 2.26	6.56 ± 2.41	−0.11 ± 1.83	0.81	0.76
Ferritin (μg/L)	24 to 336 μg/L	52.51 ± 54.46	53.81 ± 24.55	1.29 ± 56.83	0.92	35.08 ± 26.28	39.34 ± 35.34	4.25 ± 19.12	0.37	0.49
Serum Iron (μg/dL)	50 to 180 μg/dL	87.59 ± 43.90	95.14 ± 44.75	7.55 ± 6.49	0.26	97.48 ± 63.41	92.42 ± 51.23	−5.06 ± 28.10	0.46	0.67
Hemoglobin (g/dL)	13.8 to 17.2 g/dL	13.40 ± 2.66	13.47 ± 1.92	0.07 ± 1.42	0.83	13.08 ± 1.99	12.61 ± 1.86	−0.47 ± 1.11	0.10	0.22
Hematocrit (%)	41% to 53%	40.57 ± 6.45	41.68 ± 4.69	1.11 ± 3.98	0.25	40.51 ± 4.95	39.91 ± 3.88	−0.59 ± 2.98	0.42	0.23
MCV (fL)	80 to 100 fL	80.49 ± 9.99	79.73 ± 8.98	−0.76 ± 3.06	0.31	82.23 ± 8.45	83.41 ± 7.28	1.18 ± 2.88	0.11	0.02
MCH (pg)	25.4 to 34.6 pg	26.67 ± 4.88	26.75 ± 5.56	0.07 ± 2.65	0.91	26.47 ± 3.24	26.22 ± 3.09	−0.25 ± 2.04	0.61	0.93
MCHC (%)	31%–36%	32.73 ± 2.62	32.50 ± 2.53	−0.27 ± 0.25	0.38	31.32 ± 4.08	31.52 ± 3.93	1.19 ± 1.45	0.59	0.59

Note: Variables are expressed as mean ± SD. Abbreviations: RBC, Red Blood Cell Count; WBC, White blood cell; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration.
[a] Obtained from paired t test.
[b] Obtained from multiple linear regression test, adjusted for mean value of iron, magnesium and zinc intake.

Table 7
Fecal occult blood test among trial groups.

Evolutions	Spirulina (n = 36)		Placebo (n = 37)		p-value[a]
	Negative	Positive	Negative	Positive	
Baseline	10 (27.7)	26 (72.3)	16 (43.2)	21 (56.7)	0.12
End of trial	11 (30.5)	25 (69.5)	14 (37.8)	23 (62.1)	0.34

Note: Variables are expressed as n (%).
[a] p values resulted from Chi-square test.

4.1. Iron status

A significant between-group difference in serum iron was noted in the present study in participants receiving spirulina compared to the placebo intervention. This result has important clinical ramifications because iron deficiency is the leading cause of anemia in those with UC [41]. Spirulina is an excellent source of iron, providing 12 times more iron than any other food [46], and has higher iron bioaccessibility and bioavailability than ferrous sulfate [47,48]. In addition, spirulina contains vitamin C and beta-carotene [49]. These two micronutrients increase iron uptake through converting the insoluble form of iron (Fe^{+3}) to the more soluble form (Fe^{+2}), maintaining the iron released from food in a soluble form during digestion before entering intestinal cells, and overcoming the inhibition of iron absorption by potent inhibitors [50,51]. Moreover, spirulina is free of oxalate, a strong iron chelator [48]. It seems reasonable, therefore, that serum iron increased over 8 weeks in participants supplemented with spirulina. However, other indicators of anemia status such as serum ferritin levels, a reliable indicator of body iron stores, were not significantly altered in the present study. Possible explanations for such a result may be due to intermittent bleeding of the colonic mucosa in UC as well as the relatively shorter duration of spirulina supplementation (8-weeks). Similarly, another clinical trial found no significant effect on serum ferritin levels after 3-month supplementation with spirulina because of blood loss due to possible hookworm infection in young anemic Indian women [52].

Consistent with our findings, several human and animal studies have shown the beneficial effects of spirulina supplementation on iron status [51,53–57]. Consumption of a diet high in spirulina content recovered rats from iron-deficiency anemia [51,53], improved iron storage in rats during pregnancy and lactation [54], and improved serum levels of iron, transferrin, and ferritin in cobalamin-deficient rats [55]. Moreover, supplementation with spirulina was shown to be significantly more effective at increasing serum iron and ferritin levels than vitamins and minerals in moderately malnourished children [56]. Moreover, spirulina supplementation was found to be as efficient as ferrous sulfate supplementation in the treatment of iron-deficiency anemia in adult females [57]. In contrast to our findings, a prior investigation reported that 3-months supplementation with 2 g/d spirulina resulted in a significant decrease in plasma iron levels in people with obesity [15], however, this result may be caused by dysregulation of iron metabolism in obesity [58]. It has been noted that adiposity-related inflammation can increase hepcidin concentrations subsequently leading to decreased dietary iron absorption, increased internalization and degradation of ferroportin, and reduced circulating iron levels [59]. Also hypothesized was that the iron-chelating ability of phycocyanin, a pigment–protein complex produced by spirulina, may exacerbate the dysregulated metabolism of iron in subjects with obesity [15]. More work needs to be done in this area as the present study did not stratify participants into cohorts based on body mass index or other markers of body composition, yet overall, spirulina supplementation seems to preserve serum iron levels in those with UC.

4.2. Complete blood count

As illustrated in Table 4 between-group differences in complete blood count parameters were statistically significant for RBC, HCT, and MCV. These results suggest that spirulina supplementation may stimulate erythropoiesis and consequently stabilize certain hematological parameters in UC despite recurrent gastrointestinal bleeding. In fact, spirulina contains several essential nutrients for erythropoiesis including amino acids, cobalamin, folate, pyridoxine, iron, copper, and zinc [42]. Furthermore, spirulina has antioxidant and anti-inflammatory properties that help to inhibit eryptosis and hemolysis [18,60] These effects may be exerted through inhibiting lipid peroxidation and DNA damage, activating cellular antioxidant enzymes, increasing the activity of superoxide dismutase and catalase, scavenging free radicals, and regulating the signaling pathways of the extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, inhibitor of nuclear factor κB, and nuclear factor erythroid 2-related factor 2 [18,48,61,62]. In addition, the high antioxidant content of spirulina can augment the function of hematopoietic stem cells [63,64]. Moreover, phycocyanin pigment of spirulina may increase hematopoiesis by stimulating the production of erythropoietin hormone in bone marrow [65].

Although previous animal and human studies have consistently reported that supplementation with spirulina can reverse anemia (particularly iron-deficiency anemia [66–68]) to an extent, the effect of spirulina administration on various hematological indices proves inconsistent [23,25,43,55,69–75]. For instance, 2-months supplementation with 1–2 g/d spirulina did not significantly affect hemoglobin and MCV in a participant cohort with diabetes-induced anemia [74]. However, 30-day supplementation with 10 g/d spirulina significantly increased hemoglobin and MCV in malnourished children [75]. It is feasible that the dose and duration of spirulina supplementation are critical and may be responsible for the inconsistency across studies. This notion is illustrated across several animal studies that indicate hematological parameters respond to spirulina supplementation in a dose-dependent manner [43,69,70]. Further, differences in the underlying cause of anemia in participant cohorts may explain varying hematological outcomes and status. For example, MCV is decreased in iron deficiency anemia but increased in cobalamin and folate deficiency anemia [76] and thus spirulina supplementation may not address hematological status in all manners, particularly when dose and/or dosage varies.

4.3. Fecal occult blood test

In this clinical trial, 8-week supplementation with 1 g/d spirulina did not significantly change the number of positive fecal occult blood tests in adults with UC. Previous animal studies have shown that spirulina administration can significantly reduce the UC activity index by inhibiting the overproduction of pro-inflammatory cytokines and intracellular reactive oxygen species and enhancing the expression of antioxidant enzymes and tight junction proteins in the colonic mucosal barrier [77,78]. However, in accordance with our results, there was no significant histopathological alteration in colonic mucosal and submucosal hemorrhage in rats with acetic acid-induced UC following spirulina consumption [79]. Further clinical trial studies with longer follow-up duration are needed to evaluate changes in the fecal occult blood of colitis patients after spirulina supplementation.

5. Strengths and limitations

To the best of our knowledge, this is the first randomized double-blind placebo-controlled trial that examined the effect of

spirulina supplementation on iron status, complete blood count, and fecal occult blood test in adults with UC. Therefore, it is our hope that the present study may serve as a foundation for future investigations in this field. Nevertheless, the current study has the following limitations: first, the duration of this clinical trial was relatively short and did not allow for a complete discernment of the influence of spirulina on anemia status in UC; second, the dose–response effect of spirulina supplementation was not evaluated in the present study; third, the sample size was relatively small (n = 73); and fourth, FOBT has certain advantages such as a lower financial burden to administer, but a commensurate lower specificity, sensitivity, and predictive value than a fecal immunochemical test for detection of colonic bleeding [80].

6. Practical implications statement

- Spirulina may be suggested as a well-tolerated dietary supplement in adults with mild to moderate UC.
- Supplementation with spirulina may be considered a viable approach to maintaining iron status in adults suffering from UC.
- Spirulina supplementation shows promising potential as adjuvant therapy in the prevention and management of anemia in adults with UC.

7. Conclusion

In conclusion, spirulina supplementation may improve iron status and anemia-related markers despite recurrent gastrointestinal bleeding in adults with UC. Further well-designed and conducted randomized and placebo-controlled trials are warranted to corroborate and expand upon these findings.

Authors' contributions

SM and MZ designed this study. SM, FP and MZ contributed in trial operation. SM and FP performed the statistical analysis and interpretation of data. SM, ST, PA and SF wrote the manuscript. MZ, MN, RB, and AW critically revised the manuscript. All authors approved the final version of the manuscript.

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Declaration of competing interest

The authors declare that they have no competing interests.

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