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Effects of flaxseed and flaxseed oil supplement on serum levels of inflammatory markers, metabolic parameters and severity of disease in patients with ulcerative colitis

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ABSTRACT

Objective: The present study aimed to evaluate the possible effect of grounded flaxseed and flaxseed oil on serum levels of inflammatory markers, metabolic parameters, and the severity of disease in patients with UC.

Methods: In this open-labeled randomized controlled trial, 90 UC patients were randomly assigned to one of the 3 groups for 12 weeks: grounded flaxseed (GF; 30 g/day), flaxseed oil (FO; 10 g/day) and control group. The weight, waist circumference, systolic and diastolic blood pressure, serum inflammatory markers (interleukin-6 (IL-6), interferon gamma (INF-γ), transforming growth factor beta (TGF-β), and Erythrocyte Sedimentation Rate (ESR)), and fecal calprotectin were measured at the baseline and end of the study.

Results: Totally, 75 patients (43 men and 32 women) with a mean age of 31.54 ± 9.84 years participated in the present study. Comparing the change of the variables indicated a significant decrease in fecal calprotectin ($P < 0.001$), Mayo score ($P < 0.001$), ESR ($P < 0.001$), INF-γ ($P < 0.001$), IL-6 ($P < 0.001$), waist circumference ($P = 0.02$), Diastolic Blood Pressure (DBP) ($P < 0.001$), and Systolic Blood Pressure (SBP) ($P < 0.001$) and a significant increase in TGF-β ($P < 0.001$) and Inflammatory Bowel Disease Questionnaire-Short form (IBDQ-9) score ($P < 0.001$) in the GF and FO groups compared to the control. No difference was obvious between the FO and GF groups except for TGF-β.

Conclusion: The present study showed that both flaxseed and flaxseed oil, attenuate inflammatory markers, disease severity, blood pressure, and WC. However, the effect of flaxseed on weight and BMI was not evident.

1. Introduction

Inflammatory bowel disease (IBD) refers to diseases that cause inflammation of the intestinal wall. Crohn disease (CD) and ulcerative colitis (UC) are two of the most important subtype of these diseases. IBD characterized by chronic uncontrolled inflammation of the intestinal mucosa.[1–3] Osteopenia, skin and joint disease, growth impairment and puberty latency are among the complications of the IBD.[4] This disease affects more than 10 million people around the world.[5] Over the past two decades, the incidence of IBD has increased dramatically in Asia; however, there was a plateau or declining trend in Europe.[6] In general, the prevalence of IBD is increasing rapidly in developing countries, and unfortunately, in the near future, there will be a major health challenge in this regard.[4]

Inflammation is a key factor in the pathology of IBD. The most common cause of gastrointestinal (GI) inflammation is the inadequate response of the immune system to the intestinal mucosa.[7] The existence of a balance between inflammatory and anti-inflammatory processes can improve the integrity of the intestinal mucosa.[2] Several cytokines are involved in the pathogenesis of IBD and the regulation of these cytokines function could be a therapeutic target for IBD patients.[3] The most important cytokines in this process are inflammatory mediators,

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including interleukin-1 (IL-1), INF-γ, tumor necrosis factor alpha (TNF-α), IL-6, and C-reactive protein (CRP) and anti-inflammatory mediators, including interleukin-10 (IL-10) and transforming growth factor beta (TGF-β).[3,8]

Previous studies suggested both genetic and environmental triggers as risk factors in the pathogenesis of IBD.[9] Among environmental factors, infectious diseases, childhood nutrition, tonsil and appendix removal, diet, microbial changes in diet and intestine, socioeconomic status, medication use, smoking, lack of physical activity, and intestinal pathogens were known to be associated with IBD.[10,11] Evidence shows that the expression of IBD could be affected by dietary constituents such as fat, refined sugar, fruits, vegetables and fiber.[12] Also, there is an inverse association between omega-3 fatty acids intake and the expression of the IBD.[13]

Flaxseed is a rich source of omega-3 fatty acids (α-linolenic acid; ALA), phytoestrogens and soluble fiber.[14] Studies in the animal models of colitis and IBD showed the potential effects of flaxseed or ALA on INF-γ, TNF-α, IL-6, NF-κB and intestinal permeability.[1,14,15] Studies showed that Flaxseed extract improve disease severity through down-regulating inflammatory cytokines such IFN-γ and TNF-α and reduce neutrophil infiltration.[14] Contrary to the common belief, the association of other dietary components such as Probiotic, SFA, PUFAs and n-3 fatty acids with disease activity in IBD patients has been controversy.[16,17]

Also, fiber supplementation was associated with lower IL-6, IL-8, TNF-α, and NF-κB through more production of short chain fatty acids (SCFAs) by anaerobic bacterial fermentation.[18] It has been suggested that flaxseed may alter susceptibility to gut-associated diseases by modulating the colonic microenvironment in mice.[19] In a recent animal study, protein hydrolysates and phenolic fractions isolated from flaxseed had a protective effect on UC.[20]

The effects of this complex food on IBD have not yet been evaluated. Since the pathogenesis of UC is related to inflammatory mediators, we assumed that supplementation with flaxseed may act as a novel adjunctive therapeutic strategy for patients with IBD. However, it is not known definitively that observed effects are related to ALA or other active compounds that present in the whole flaxseed. To the best of our knowledge, there is no study on the effects of flaxseed or flaxseed oil on IBD severity and its outcomes in human subjects. The present study aimed to evaluate the possible effect of grounded flaxseed and flaxseed oil on serum levels of inflammatory markers, metabolic parameters and the severity of disease in patients with UC.

2. Materials and methods

2.1. Participants

Subjects were recruited from UC patients referring to gastroenterology and liver diseases clinic of Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. UC should have been confirmed by the gastroenterologist through histopathology for at least 3 months before study beginning. Fig. 1 shows the flowchart of the participant's enrollment. We recruited 90 subjects (48 male and 42 female) aged 18 and 55 years old, with BMI > 20 kg/m^2. Exclusion criteria were having other intestinal disorders, autoimmune diseases, cancer, inflammatory and infectious diseases, pregnancy or lactating, change in dose or type of medication in last 3 months, adherence to a specific diet, using omega-3 supplement in last 3 months, using anti-inflammatory drugs (corticosteroids, immune-modulators (such as Azathioprine, 6-mercaptopurine, Methotrexate and Cyclosporine A), and anti-TNF-α medications(such as Adalimumab, Certolizumab pegol and Infliximab)) in the baseline or during the study, and unwilling to participate.

2.2. Study design

An open-labeled randomized controlled trial was carried out to investigate the effect of flaxseed and flaxseed oil for 12 weeks in IBD patients. The calculated sample size was 25 subjects according to the study of Samsami-kor et al [21] Considering 10% of drop-out, 30 patients were included in each group. The study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences and carried out in accordance with the Helsinki Declaration (IRCT registration no. IRCT20180311039043N1). Informed consent taken from the participants before the start of the study.

2.3. Study protocol

After describing the methods and objectives of the study to the patients and signing the informed consent by them, baseline information including age, sex, education level, diseases history, dietary supplements, and drug use, smoking status, anthropometric measures, blood tests, dietary intake and severity of the disease was collected through interview. Then, participants were randomly assigned to 3 groups if they met the inclusion criteria: 1) receiving 30 g per day of grounded flaxseed (GF), 2) receiving 10 g per day of flaxseed oil (FO), and 3) control group that only received medical advice and routine medications.

The flaxseed was provided from a farm in Khoy, West Azerbaijan province of Iran. The energy, fat, ALA, protein, carbohydrate, and fiber content of the flaxseed were 450 kcal, 41 g, 21.5 g, 20 g, 29 g, and 28 g per 100 g of flaxseed, respectively. The whole flaxseed was cleaned, milled and packed (250 g each pack) with a 15 g measure. Subjects in the GF group were asked to use one serving (15 g) of grounded flaxseed mixed in a glass of cold water after breakfast and one serving at the evening with an hour interval of taking medications. Packages were given to the participants at the start, 4^{th} and 8^{th} weeks of the study. Patients in the FO group received 10 g/day of oil (contains 57.5% ALA, 17.2% oleic acid, 15.2% linoleic acid, 5.1% palmitic acid, 4% stearic acid, and 1% other fatty acids) that was provided by Barij Essence company (Tehran, Iran). They consumed 10 g flaxseed oil at lunch or dinner After 12 weeks of the study, the patients referred to the clinic and were again subjected to anthropometric measurements, blood tests, stool sample, and completed food recalls and severity of the disease questionnaire.

2.4. UC diagnosis and severity of the disease

In this study, the definitive diagnosis of UC was based on pathology, colonoscopy, and biopsy assessment. The UC was distinguished from CD by its specific characteristics including the presence of intestinal fistula in other parts (e.g. skin or intestine or colon), sever fistula around the anus, chronic inflammation in the small intestine or upper GI tract (confirmed by a professional radiologist by MR enterography, CT scan or biopsy), and the presence of a specific pathology such as granuloma or post-surgical biopsy confirming the inflammation of the entire intestinal wall. In addition, the remission and flare-up of the disease were determined by the gastroenterologist based on laboratory findings and colonoscopy. Patients in the active phase of the disease (mild to moderate) were included in the study.

The severity of the disease was evaluated using the Mayo score instrument.[22] The total score of Mayo ranges between 0–12 and the higher rates represent more severity of the disease. The inflammatory bowel disease questionnaire-short form (IBDQ-9) was used to measure the quality of life of the participants.[23] This questionnaire includes 9 questions regarding four aspects of gastrointestinal, systemic, emotional and social disturbances. Each question scores between 1–7 and the total score ranges between 9-63. The higher score indicates better life quality in the subjects.

2.5. Blood and stool sampling and biochemical assessment

Blood and stool sampling was performed at the beginning and the

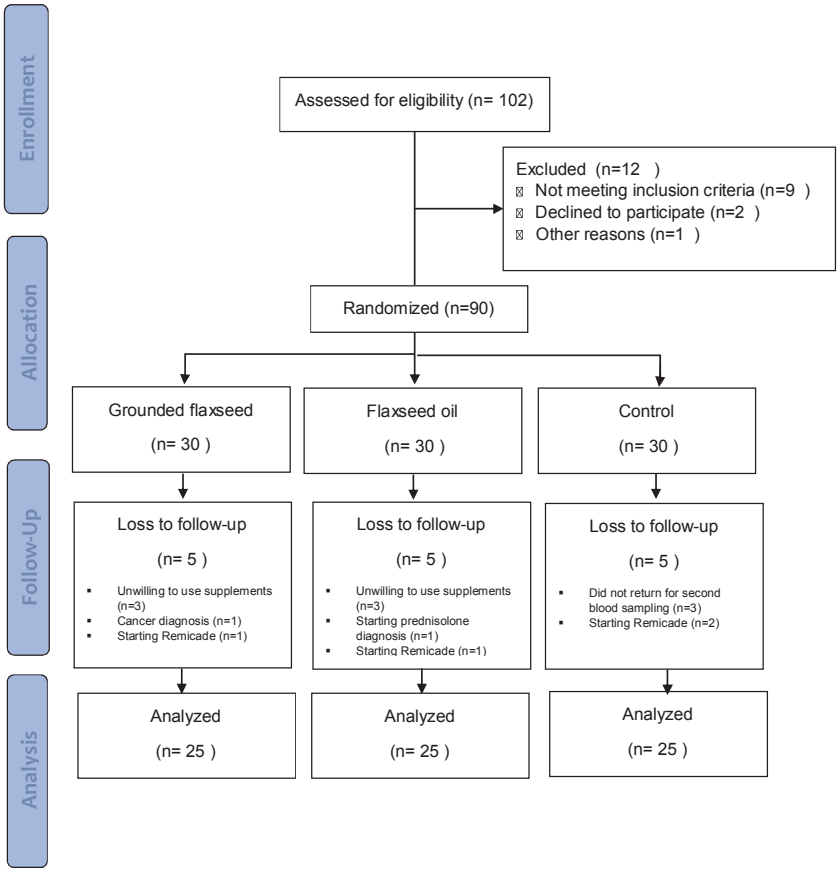

Fig. 1. Flow-diagram of the study participants.

end of the study. Stool samples were stored in -20 °C until biochemical analysis. In each stage, after 10–12 hours of fasting, 15 cc of blood samples were taken. To separate the serum, 10 cc of the blood samples were centrifuged at room temperature with 3000 rpm for 10 min and the isolated serum was stored at -80 °C until the biochemical tests were carried out. Also, 5 cc of blood samples were discharged into the EDTA tube to separate the plasma sample. An ELISA kit was used to assess the fecal concentration of calprotectin (Buhlmann Co., Switzerland). Serum concentrations of inflammatory factors (IFN-γ, IL-6, TGF-β) were assessed using ELISA kits (Diaclone Research, Besançon, France). The quantitative photometric method was used to measure the erythrocyte sedimentation rate (ESR; TEST-1 system; Alifax, Polverara, Italy)

2.6. Anthropometric measurements

A Seca scale was used to measure weight with a precision of 100 g, with the minimum dress and without shoes. The height was measured using a Seca stadiometer in a standing position next to the wall and without shoes, with a precision of 0.1 cm. Then BMI was calculated by dividing the weight (kg) by the square of height (m^2). Waist circumference was measured using a flexible tape at the midpoint of lowest rib and the iliac crest hip bone, with a precision of 0.1 cm.

2.7. Dietary intake assessment

To evaluate the dietary intake of participants in terms of energy, micronutrients, and macronutrients, three (two consecutive days and a day-off) 24 -h food recalls were completed, at the beginning and the end of the study. Then, each food item entered to Nutritionist IV software (1997, First DataBank Inc., San Bruno, CA) and mean intake of energy, micronutrients, and macronutrients were calculated at the baseline and after 3 months of the study.

2.8. Compliance

Patients were asked to return the former packages at each visit in order to assess their compliance. Patients who have not consumed more than 10% of the flaxseed or flaxseed oil were excluded from the study.

2.9. Statistical methods

Quantitative and qualitative variables are presented as mean or geometric mean (standard deviation) and frequency (%), respectively. Qualitative variables were compared using the chi-square test. Kolmogorov–Smirnov test was used to test the normality of quantitative variables. One-way analysis of variance and LSD post-hoc test were used to compare groups in terms of quantitative variables. Also, analysis of covariance (ANCOVA) was used to adjust the effect of confounding variables (dietary intake of energy, protein, fat, polyunsaturated fatty acids, omega 3 polyunsaturated fatty acids, and omega 6 polyunsaturated fatty acids). To assess the change of variables over the study period in each group the paired sample *t*-test was used. The P-value < 0.05 was considered as statistically significant. All statistical analyses were performed using SPSS software version 24 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY).

3. Results

3.1. Study baseline characteristics

The baseline characteristics of the participants are outlined in Table 1. Totally, 75 patients (43 men and 32 women) with a mean age of 31.54 ± 9.84 years participated in the present study. There was no significant difference in age (P = 0.60), height (P = 0.72), diagnosis duration (P = 0.73), gender (P = 0.95), education level (P = 0.43),

Table 1
Baseline characteristics of the participants [1].

Variable[2]		Total (n = 75)	GF (n = 25)	FO (n = 25)	Control (n = 25)	p-value[3]
Age (years)		31.54 (9.84)	29.92 (9.14)	32.20 (9.88)	32.52 (10.65)	0.60
Height (cm)		165.41 (5.69)	164.98 (6.30)	165.22 (5.72)	166.03 (5.17)	0.72
Diagnosis duration (years)		5.17 (2.62)	4.94 (2.24)	5.77 (2.59)	4.82 (2.98)	0.73
Gender	Male	43 (57.3)	15 (60.0)	14 (56.0)	14 (56.0)	0.95
	Female	32 (42.7)	10 (40.0)	11 (44.0)	11 (44.0)	
Education	Diploma or lower	3 (4.0)	2 (8.0)	0 (0.0)	1 (4.0)	0.43
	Academic	44 (58.7)	12 (48.0)	15 (60.0)	17 (68.0)	
	Post-graduate	28 (37.3)	11 (44.0)	10 (40.0)	7 (28.0)	
Smoking	Yes	10 (13.3)	4 (16.0)	4 (16.0)	2 (8.0)	0.63
	No	65 (86.7)	21 (84.0)	21 (84.0)	23 (92.0)	
Ethnicity	Fars	43 (57.3)	15 (60.0)	14 (56.0)	14 (56.0)	0.94
	Other	32 (42.7)	10 (40.0)	11 (44.0)	11 (44.0)	
Marital statues	Married	33 (44.0)	12 (48.0)	9 (36.0)	12 (48.0)	0.61
	Single or divorced	42 (56.0)	13 (52.0)	16 (64.0)	13 (52.0)	
Alcohol consumption	Yes	7 (9.3)	3 (12.0)	2 (8.0)	2 (8.0)	0.85
	No	68 (90.7)	22 (88.0)	23 (92.0)	23 (92.0)	

[1] GF, grounded flaxseed; FO, flaxseed oil.
[2] Data are presented as mean (SD) for quantitative and frequency (%) for qualitative variables.
[3] Calculated using one-way ANOVA or chi-square.

smoking (P = 0.63), ethnicity (P = 0.94), marital status (P = 0.61), and alcohol consumption (P = 0.85) between three groups of GF, FO and control.

3.2. Compare dietary intakes of the participants

Dietary intakes of groups are compared in Table 2. According to this table, subjects in the control group had a lower intake of protein compared to GF and FO groups at the baseline (P = 0.002) and end of the study (P < 0.001). Moreover, the paired *t*-test showed a significant increase in the intake of PUFA (P = 0.001), n-3 PUFA (P < 0.001), and n-6 PUFA (P < 0.001) in the GF group over the study duration. Also, the FO group had a significantly higher intake of fat at the end of the study compared to the baseline (P = 0.003). No significant difference or change was observed in carbohydrate and energy intake (P > 0.05).

3.3. Effect of flaxseed on the metabolic parameters

Table 3 compares the metabolic parameters between GF, FO, and control groups at the baseline and following 12 weeks of the study. No

Table 2
- Comparison of dietary intakes between groups at the baseline and following intervention[1].

Variable[2]		GF (n = 25)	FO (n = 25)	Control (n = 25)	P[3]
Energy (kcal)	Baseline	2251.78 (265.29)	2338.26 (267.25)	2367.91 (261.55)	0.27
	12th week	2223.85 (205.97)	2307.32 (241.24)	2366.20 (229.00)	0.08
	Changes	−27.93 (89.33)	−30.93 (77.56)	−1.70 (81.03)	0.39
	P[4]	0.13	0.06	0.91	
Protein (gr)	Baseline	68.69 (17.76) [a]	68.04 (14.20) [b]	55.13 (10.34) [a,b]	0.002
	12th week	69.09 (16.61) [a]	67.50 (13.96) [b]	54.67 (8.10) [a,b]	< 0.001
	Changes	0.40 (3.71)	−0.53 (3.21)	−0.45 (2.81)	0.53
	P[4]	0.59	0.41	0.42	
Carbohydrate (gr)	Baseline	334.45 (46.66)	344.36 (45.83)	335.61 (49.38)	0.72
	12th week	335.11 (48.29)	345.97 (43.02)	340.08 (45.98)	0.70
	Changes	0.65 (11.09)	1.61 (12.17)	4.47 (23.02)	0.69
	P[4]	0.77	0.51	0.34	
Fat (gr)	Baseline	65.10 (13.12)	61.49 (11.88)	62.84 (14.19)	0.61
	12th week	65.26 (12.20)	62.86 (11.45)	62.77 (13.76)	0.72
	Changes	0.16 (4.46)	1.37 (2.10)	−0.07 (1.13)	0.18
	P[4]	0.85	0.003	0.75	
PUFA (gr)	Baseline	12.37 (2.19)	13.80 (1.99)	13.21 (2.04)	0.06
	12th week	12.92 (2.28)	13.83 (2.01)	13.34 (1.96)	0.31
	Changes	0.54 (0.72) [a, b]	0.02 (0.47) [a]	0.13 (0.45) [b]	0.004
	P[4]	0.001	0.80	0.12	
n-3 PUFA (gr)	Baseline	0.63 (0.17)	0.67 (0.19)	0.67 (0.18)	0.72
	12th week	0.65 (0.17)	0.67 (0.20)	0.68 (0.18)	0.84
	Changes	0.02 (0.02)	−0.0006 (0.02)	0.01 (0.02)	0.07
	P[4]	< 0.001	0.91	0.13	
n-6 PUFA (gr)	Baseline	1.30 (0.45)	1.63 (0.59)	1.51 (0.51)	0.08
	12th week	1.41 (0.47)	1.70 (0.57)	1.55 (0.48)	0.15
	Changes	0.11 (0.13)	0.06 (0.21)	0.04 (0.14)	0.36
	P[4]	< 0.001	0.15	0.15	

[a, b, c] similar letters show significant differences.
[1] GF, grounded flaxseed; FO, flaxseed oil; PUFA, polyunsaturated fatty acid; n-3 PUFA, omega-3 polyunsaturated fatty acid; n-6 PUFA, omega-6 polyunsaturated fatty acid.
[2] data are presented as mean (SD).
[3] Calculated using one-way ANOVA.

Table 3
- Comparison of the metabolic parameters between groups at the baseline and following intervention[1].

Variable[2]		GF (n = 25)	FO (n = 25)	Control (n = 25)	P[3]	P- adjusted[4]
Weight (kg)	Baseline	64.07 (10.24)	65.51 (8.90)	65.21 (8.14)	0.84	0.053
	12th week	63.37 (8.81)	65.08 (8.39)	65.67 (8.10)	0.60	0.09
	Changes	−0.70 (1.97)	−0.42 (1.17)	0.45 (4.2)	0.31	0.40
	P[5]	0.09	0.08	0.59		
BMI (kg)	Baseline	23.45 (2.82)	23.94 (2.44)	23.60 (2.12)	0.77	0.93
	12th week	23.22 (2.41)	23.79 (2.27)	23.80 (2.54)	0.63	0.77
	Changes	−0.22 (0.73)	−0.14 (0.42)	0.19 (1.66)	0.33	0.43
	P[5]	0.13	0.09	0.55		
WC (cm)	Baseline	90.92 (13.19)	97.89 (14.69)	96.16 (9.15)	0.13	0.46
	12th week	89.91 (12.17)	97.07 (14.08)	96.45 (9.27)	0.07	0.44
	Changes	−1.00 (1.84) [a]	−0.82 (0.97)	0.29 (2.13) [a]	0.02	0.04
	P[5]	0.01	< 0.001	0.49		
DBP (mmHg)	Baseline	8.59 (0.47)	8.52 (0.55)	8.49 (0.55)	0.77	0.64
	12th week	7.93 (0.31) [a]	7.96 (0.46) [b]	8.32 (0.43) [a, b]	0.002	0.03
	Changes	−0.66 (0.42) [a]	−0.55 (0.32) [b]	−0.16 (0.29) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.009		
SBP (mmHg)	Baseline	13.14 (1.13)	13.28 (1.13)	13.20 (1.18)	0.91	0.93
	12th week	12.19 (7.76) [a]	12.10 (9.85) [b]	12.90 (9.55) [a, b]	0.004	0.08
	Changes	−0.95 (0.57) [a]	−1.18 (0.74) [b]	−0.29 (0.52) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.009		

[a, b, c] similar letters show significant differences.
[1] GF, grounded flaxseed; FO, flaxseed oil; BMI, body mass index; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure.
[2] Data are presented as mean (SD) or geometric mean (SD).
[3] Calculated using one-way ANOVA.
[4] Calculated using ANCOVA, adjusted for the effect of energy, carbohydrate, protein, fat, total PUFA, n-3 PUFA, and n-6 PUFA.
[5] Calculated using paired sample *t*-test.

significant change was observed in weight and BMI after 12 weeks of intervention in any of the 3 groups. In contrast, SBP and DBP were reduced significantly after 12 weeks in all three groups. Also, it has been revealed a significant reduction in WC in the GF and FO groups (P = 0.01 and P < 0.001, respectively), but not in the control group (P = 0.49). There were no significant differences in the weight (P = 0.84), BMI (P = 0.77), WC (P = 0.13), DBP (P = 0.77), and SBP (P = 0.91) before study between groups. After 12 weeks of intervention, no significant difference was observed between groups in weight (P = 0.60), BMI (P = 0.63), and WC (P = 0.07). However, subjects in the control group had a significant higher DBP (P = 0.002) and SBP (P = 0.004) at 12th week. Also comparing the change of these variables from baseline showed a significant difference between groups in WC (P = 0.02), DBP (P < 0.001), and SBP (P < 0.001). In the ANCOVA model adjusted for the effect of energy, protein, carbohydrate, total fat, PUFAs, n-3 PUFAs, and n-6 PUFAs dietary intake, most of the P-values significance remained unchanged, except for SBP at 12th week (P = 0.004 to P = 0.08).

3.4. Effect of flaxseed on the serum levels of inflammatory markers and disease severity

Table 4 shows the serum levels of inflammatory markers (ESR, IL-6, INF-γ, and TGF-β) and disease severity criteria (Mayo score, IBDQ-9, and fecal calprotectin) at the baseline and following 12 weeks of intervention.

There was a significant reduction in the serum levels of ESR (P < 0.001), INF-γ (P < 0.001), and a significant increase in the TGF-β (P < 0.001) in the GF and FO groups, but not in the control group. Also, a significant reduction was observed in serum IL-6 after 12 weeks, in all three groups. At the beginning of the study, there was no significant difference in the ESR (P = 0.66), INF-γ (P = 0.27), and TGF-β (P = 0.26) between groups. However, subjects in the GF group had higher levels of IL-6 compared to the control (11.94 ± 1.55 vs. 7.61 ± 1.63 pg/dL; P = 0.01). After the 12th week, subjects in control group had higher serum levels of ESR (P < 0.001), INF-γ (P < 0.001), and lower serum levels of TGF-β (P < 0.001) compared to the GF and FO groups. Moreover, the FO group, but not the GF, had significantly lower levels of the IL-6 compared to the control (P = 0.001). Comparing the change of these variables indicated a significant decrease in ESR (P < 0.001), INF-γ (P < 0.001), and IL-6 (P < 0.001) and a significant increase in TGF-β (P < 0.001) in the GF and FO groups compared to the control. Also, supplementing by grounded flaxseed resulted in a greater increase in TGF-β compared to the flaxseed oil (173.29 ± 101.60 vs 103.42 ± 77.97; P = 0.007). As shown in Table 4 ANCOVA did not change the significance of the differences after adjusting for the effect of dietary intakes.

The results showed a significant decrease in fecal calprotectin (P < 0.001) and Mayo score (P < 0.001) and a significant increase in IBDQ-9 score (P < 0.001) after 12 weeks of intervention compared to the baseline in the GF and FO groups. No significant change was observed in the IBDQ-9 score (P = 1.00), Mayo score (P = 0.78), and fecal calprotectin (P = 0.32) after 12 weeks compared to the beginning of the study in the control group. Comparing groups showed that subjects in the GF and FO groups had a higher IBDQ-9 score (P < 0.001) and lower fecal calprotectin (P = 0.008) and Mayo score (P < 0.001) at the 12th week of the study compared to the control. In addition, greater reduction in the Mayo score and fecal calprotectin and more increase in the IBDQ-9 score were observed in the GF and GF groups compared to the control group over the study period. No difference was obvious between the FO and GF groups in this regard. Adjusting for dietary intake did not change the significance of the differences.

4. Discussion

The primary aim of the present study was to investigate the effect of flaxseed or flaxseed oil on serum levels of inflammatory markers, metabolic parameters and the severity of disease in patients with UC. According to the results, both flaxseed and flaxseed oil, attenuate inflammatory markers, disease severity, blood pressure, and WC. However, effect of the flaxseed, neither whole flaxseed nor oil, on weight and BMI was not evident.

UC is a chronic inflammatory condition with intermittent relapse and remitting courses.[24] During a relapse, neutrophil granulocyte infiltrates into the mucosal tissue and they will be observed in the mucosa and intestinal lumen.[25] Fecal calprotectin, a protein that represents

Table 4
Comparison of inflammatory markers and severity of disease between groups at the baseline and following intervention[1].

Variable[2]		GF (n = 25)	FO (n = 25)	Control (n = 25)	P[3]	P- adjusted[4]
ESR (mm/hr)	Baseline	27.40 (3.42)	27.36 (3.49)	26.17 (3.59)	0.37	0.66
	12th week	20.41 (2.11) [a]	19.76 (2.23) [b]	25.75 (4.14) [a, b]	< 0.001	< 0.001
	Changes	−6.99 (1.69) [a]	−7.59 (2.17) [b]	−0.41 (2.49) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.41		
IL-6 (pg/dL)	Baseline	11.94 (1.55) [a]	9.02 (1.58)	7.61 (1.63) [a]	0.005	0.01
	12th week	4.76 (1.93)	3.32 (1.75) [a]	6.35 (1.75) [a]	0.001	0.003
	Changes	−7.32 (2.59) [a]	−6.18 (3.09) [b]	−1.14 (1.96) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	< 0.001		
INF-γ(pg/dL)	Baseline	33.17 (8.40)	35.17 (8.99)	30.76 (7.46)	0.17	0.27
	12th week	19.98 (2.57) [a]	21.08 (2.46) [b]	28.83 (7.14) [a, b]	< 0.001	< 0.001
	Changes	−13.18 (7.33) [a]	−14.08 (7.13) [b]	−1.93 (5.99) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.12		
TGF-β (pg/dL)	Baseline	41.67 (3.03)	27.38 (2.27)	42.94 (2.94)	0.22	0.26
	12th week	228.14 (1.75) [a, b]	111.05 (2.18) [a, c]	49.40 (2.73) [b, c]	< 0.001	< 0.001
	Changes	173.29 (101.60) [a, b]	103.42 (77.97) [a, c]	−1.27 (48.29) [b, c]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.38		
Calprotectin	Baseline	616.40 (140.82)	658.60 (174.65)	625.45 (179.46)	0.64	0.94
	12th week	424.20 (98.46) [a]	484.20 (157.23) [b]	602.32 (185.43) [a, b]	< 0.001	0.008
	Changes	−192.20 (81.02) [a]	−174.4 (187.26) [b]	−23.13 (115.17) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.32		
IBDQ-9 score	Baseline	39.88 (4.43)	40.00 (3.29)	42.08 (3.52)	0.07	0.16
	12th week	48.96 (2.76) [a]	48.08 (1.41) [b]	42.08 (3.22) [a, b]	< 0.001	< 0.001
	Changes	9.08 (2.51) [a]	8.08 (2.64) [b]	0.001 (2.73) [a, b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	1.00		
Mayo score	Baseline	5.15 (1.32)	5.10 (1.41)	4.95 (1.43)	0.89	0.89
	12th week	3.66 (1.25) [a]	3.78 (1.41) [b]	4.90 (1.47) [a, b]	0.006	0.007
	Changes	−1.60 (0.86) [a]	−1.36 (0.70) [b]	0.05 (0.76) [a,b]	< 0.001	< 0.001
	P[5]	< 0.001	< 0.001	0.78		

[a, b, c] similar letters show significant differences.

[1] GF, grounded flaxseed; FO, flaxseed oil; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; INF-γ, interferon-gamma; TGF-β, transforming growth factor beta; IBDQ-9, inflammatory bowel disease questionnaire-short form.

[2] Data are presented as mean (SD) or geometric mean (SD).

[3] Calculated using one-way ANOVA.

[4] Calculated using ANCOVA, adjusted for the effect of energy, carbohydrate, protein, fat, total PUFA, n-3 PUFA, and n-6 PUFA.

[5] Calculated using paired sample *t*-test.

60% of the neutrophil granulocyte, indicates migration of the neutrophils to GI tract.[26] In addition, it seems that a network of regulatory cytokines is involved in the GI cells integrity. The imbalance between pro-inflammatory (including IL-6 and INF-γ) and anti-inflammatory (including TGF-β) cytokines disturbs cells function and exacerbates the symptoms of IBD.[3] Thus, these cytokines, as well as calprotectin and other inflammatory markers (such as hs-CRP and ESR) show disease activity in UC patients.[3,27] Current medical treatments focus on diminishing GI inflammation to improve mucosal health and intestinal function.[28]

In the present study, UC disease severity (assessed by the Mayo score) and quality of life (assessed by IBDQ-9), were improved following the flaxseed supplementation, both in grounded flaxseed and flaxseed oil. In addition, reduced levels of the inflammatory markers were observed in patients that receive flaxseed oil or whole grounded flaxseed. Also, TGF-β, a protein that regulates macrophage functions and promotes the resolution of the UC, increased by flaxseed supplementation. However, consumption of the grounded flaxseed was more effective in the increasing of the TGF-β compared to the flaxseed oil. It appears that inflammatory markers and severity of the UC are related according to the present and previous studies. Although the literature search did not find any study investigating the effect of flaxseed on UC human subjects, the present study is in line with previous studies in the animal models. Palla et al [15] suggest a protective effect of flaxseed extract (150–500 mg/kg for 7 days) on mucosal function and disease activity by modulating INF-γ, TNF-α, IL-17, and other anti-inflammatory mechanisms. Another study demonstrated that replacing 10% of calories with grounded flaxseed in healthy male mice for 3 weeks modulated colonic microenvironment and improved gut health and susceptibility to disease.[19] Moreover, recently a study on the animal model showed that phenolic fractions and protein hydrolysates isolated from flaxseed attenuated TNF-α, INF-γ, IL-17, colon inflammation, and T-cells proliferation.[20] In addition, human studies in other disease showed a similar effect of flaxseed on inflammatory markers.[29–31] There are some other studies that did not find any association between flaxseed consumption and IL-6, [32–34] INF-γ, [35] and TGF-β. [36] However, it should be noted that each disease may have different pathophysiology and comparisons and interpretations should be made with caution. A part of the protective effects observed by flaxseed could be attributed to the modulation of gut microbiota.[20] Microbial metabolism on flaxseed produces a metabolite called Entrolignan.[37] These compounds prevent I-κB degradation and NF-κB activation and eventually reduce TNF-α level.[38] Also, the phenolic compound of the flaxseed may decrease oxidative stress in the lumen and attenuate intestinal inflammation.[15,20] At the other extreme, flaxseed contains omega-3 fatty acids, phytoestrogens and soluble fiber that several studies suggested they have anti-inflammatory effects.[1,14,15,18] ALA elongates to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and decrease arachidonic acid utilization in cell membrane. Previous studies showed that n-3 PUFAs reduce inflammatory eicosanoids, leukotriene B4 (LTB4) and prostaglandins (PGE2 and PGD2), production.[1] Also, evidence show a reduction in pro-inflammatory cytokines (IL-6, IL-8, and TNF-α) following n-3 PUFA supplementation.[39,40] It has been shown that n3-PUFAs activate PPAR-γ, a nuclear receptor that regulates NF-κB-mediated gene expression.[41] Inhibition of NF-κB activation by ALA can downregulate inflammatory response and oxidative stress.[1] Thus, flaxseed may have protective effects against IBD through several mechanisms.

In our study, 12 weeks of flaxseed consumption did not change body weight or BMI. Similarly, in the study of Khandouzi et al [30],

consumption of flaxseed (30 g/day) for 12 weeks in coronary artery disease patients did not change weight and BMI. Also, Mirfatahi et al [31] did not find any effect of flaxseed oil (6 g/day for 8 weeks) on body weight and BMI in hemodialysis patients. Moreover, Wu et al [42] observed no differences between intervention by flaxseed (30 g/day), walnut (30 g/day), or lifestyle counseling for 12 weeks in weight, BMI and WC. In contrast, the present study found that the mean WC of the subjects in the GF and FO groups reduced at the end of the study compared to the control. The study of Akrami et al [43] found a significant reduction in weight and WC in subjects with metabolic syndrome after receiving 25 ml/day of the flaxseed oil for 7 weeks. Also, in the study of Yari et al[44] consumption of 30 g/day brown milled flaxseed resulted in lower body weight, waist circumference, and BMI. Flaxseed may help in weight management due to its fiber content.[45] Also, omega-3 fatty acids (ALA) present in flaxseed oil may ameliorate central obesity.[46] On the other hand, the high content of energy in flaxseed from fat can neutralize these beneficial effects.[46] However, in the present study, the relationship between flaxseed consumption and weight or BMI did not change after adjusting for the effect of the energy intake or other nutrients.

A significant reduction was observed in the SBP and DBP following grounded flaxseed or flaxseed oil consumption. This was in line with the study of West et al [47], Skoczyńska et al, [48] Akrami et al, [43] Di et al, [49] and Javidi et al. [50] Evidence suggests the beneficial effect of the flaxseed on flow-mediated dilation and endothelial function.[30,47] ALA that presents in the flaxseed oil may improve blood pressure by improving endothelial function. [47] Also, it has been reported that the lignans of the flaxseed improves aortic stiffness and may affect blood pressure by this mechanism.[51] At the other hand, there is an inverse correlation between endothelial function and the pro-inflammatory biomarkers such as IL-6 and TNF-α.[52] As described earlier, flaxseed oil [53] and powder, [34] both can reduce circulating levels of inflammatory markers.

The main limitation of the present study is the open-labeled design due to the lack of an appropriate placebo for the grounded flaxseed or oil. To the best of our knowledge, this is the first study investigating the effect of flaxseed on UC patients. Controlled clinical trial design, using both forms of whole grounded and oil of the flaxseed and high compliance of the participants are among the strength of this study.

5. Conclusion

In conclusion, the present study showed that both grounded flaxseed and flaxseed oil can reduce pro-inflammatory markers and improve the severity of disease in UC patients. Also, the results suggested a beneficial effect of flaxseed on central obesity and blood pressure in participants. However, no change was observed in weight and BMI following intervention by grounded flaxseed or flaxseed oil.

Declaration of Competing Interest

The authors disclose no conflict of interest.

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