Clinical Trial **Open-Access**

FFHD
Functional Foods in Health and Disease

The effects of probiotics in ulcerative colitis patients: a randomized controlled double blind clinical trial

Babak Tamizifar[1], Awat Feizi[2], Marzieh Rahim Khorasani[3*], Nazila Kassaian[4*], Ali Zamanimoghadam[3], Shayan Arbabnia[5], Peyman Adibi Sede[1]

[1]Isfahan Gastroenterology and Hepatology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; [2]Department of biostatics, Isfahan University of Medical Sciences, Isfahan, Iran; [3]Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; [4]Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; [5]Nutritional Sciences and Food Industry Engineering, Islamic Azad University, Najafabad branch, Isfahan, Iran.

***Corresponding Authors:** Nazila Kassaian, Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran; Marzieh Rahim Khorasani, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Clinical Trial Registry Number: IRCT20210113050024N2, 2021.04.19

Submission Date: April 4th, 2023**;** Resubmission: August 23, 2023; **Acceptance Date:** November 9th, 2023**; Publication Date:** November 15th, 2023

Please cite this article as: Tamizifar B., Feizi A., Khorasani M. R., Kassaian N., Zamanimoghadam A., Arbabnia S., Sede P. A. The Effects of Probiotics in Ulcerative Colitis Patients: A randomized controlled double blind clinical trial. *Functional Foods in Health and Disease* 2023; 13(11): 605-615. DOI: https://www.doi.org/10.31989/ffhd.v13i11.1098

ABSTRACT

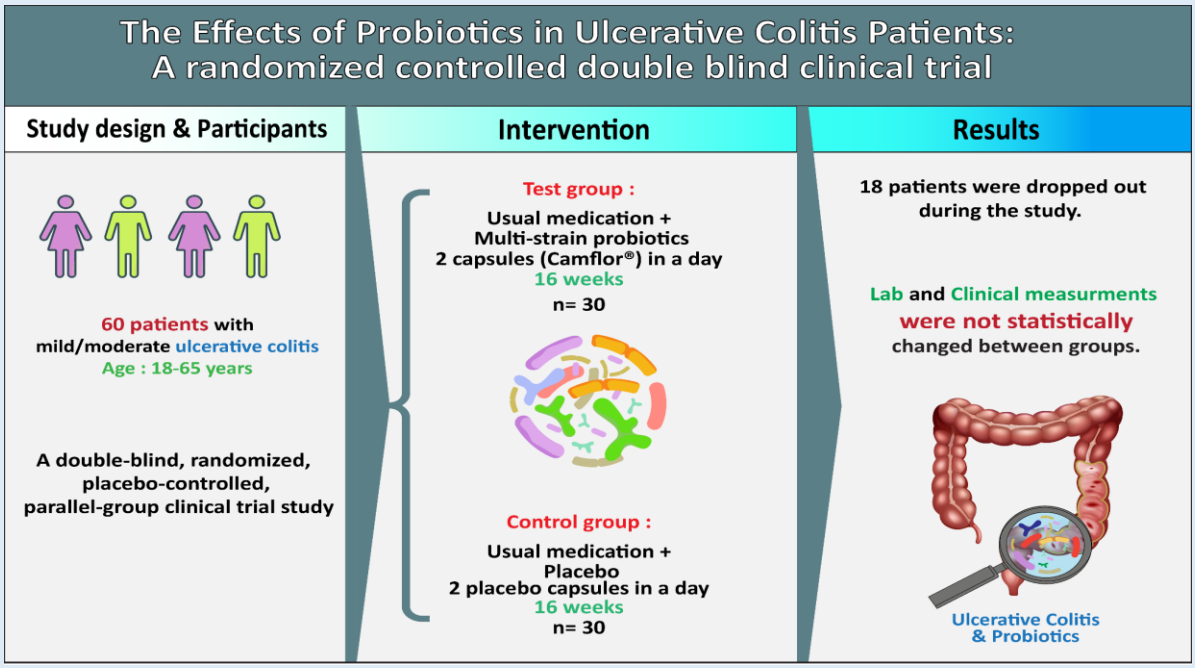
Background: In the recent decade, ulcerative colitis (UC) as a chronic inflammatory bowel disease has a growing incidence and prevalence in the world. Probiotics might be a promising approach to improve ulcerative colitis by favorably modifying the gut microbiota.

Methods: A double-blind, randomized, placebo-controlled, parallel-group clinical trial was conducted on sixty patients with mild/moderate ulcerative colitis. Participants were administered either placebo (n = 30) or a multi-strain probiotic (n= 30) for 16 weeks. Clinical disease status, via Lichtiger and Mayo questionnaires, was assessed at baseline and after 8 and 16 weeks of intervention. Fecal calprotectin was measured before and after the study period. Within and between groups, comparisons were made using per-protocol (PP) and intention-to-treat (ITT) approaches, and a P-value≤0.05 was considered a statistically significant level.

Results: Of the sixty patients who agreed to participate in the study, 18 dropped out during the study due to low compliance and gastrointestinal complications. The two groups were comparable in baseline variables (P>0.05). During the study, the within and between groups' differences of calprotectin and Mayo scores were not statistically significant. Although the mean score of Lichtiger was significantly decreased in the probiotic group during the study period (*P* = 0.001), no statistically significant differences compared with the placebo group were seen.

Conclusion: Our study elucidated that probiotic supplementation does not significantly improve UC patients, which may be due to the strain and dose administered. Future research should focus on the best effective strains and doses for ulcerative colitis.

Keywords: probiotic, ulcerative colitis, IBD, clinical trial



INTRODUCTION

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease is a complex chronic inflammatory disorder of the gastrointestinal tract. The worldwide incidence and prevalence of IBD are continuously growing in recent decades (1). This phenomenon may be associated with a conversion of lifestyle, which alters the gut microbiome. The crosstalk between the gut immune system and the gut microbiome is vital for maintaining homeostasis of the GIT, and an alteration of composition and diversity of the gut microbiota could have a crucial role in the IBD pathogenesis (2).

Unlike CD, which can affect different parts of the gastrointestinal tract, UC has a characteristic effect on

the colon (3, 4). Ulcerative colitis is associated with genetic factors, dysfunction of intestinal epithelial cells, dysfunction of the host immune system, and an imbalance of gut microbiota (5).

The current hypothesis is that the dysregulated immune response of the mucosa predisposes it to inflammation of the intestines (6). It is not yet clear whether these abnormalities are the cause or result of UC's severe systemic inflammatory response. However, it is well documented that the bacterial microbial flora changes in patients with active disease. Current studies have reported significant differences in gut microbiota, with a particular focus on the effects of pro-inflammatory intertype on mucosal layers and disease activity (7).

Regulation of the gut flora can be done by either antibiotics or functional foods. According to the European Consensus definition, food can be considered functional if it beneficially affects target functions beyond basic nutritional effects, either by reducing the risk of disease or improving health (8). The global interest in functional foods has been on the rise recently (9). It is widely acknowledged that probiotics, considered a type of functional food, have the potential to assist in treating Ulcerative Colitis (UC) as a conventional method. Several pieces of evidence have indicated the impact of multi-strain probiotics on the intestinal microbial flora, particularly in cases of dysbiosis where the presence of pathogenic bacteria disrupts the normal concentration of beneficial flora (10, 11). The standard treatment for UC patients relies on initial treatment with anti-inflammatory agents such as corticosteroids, biologic agents, and 5-aminosalicylic acids (5-ASA), as well as symptomatic treatment and hydration with antidiarrheal medicine. These treatments are not always credible in controlling the clinical course of the disease (12, 13) and have some side effects in patients who cannot tolerate existing treatments (14).

As a result, new treatment options are constantly being sought. Probiotics are live microbial supplements which may affect the host by enhancing intestinal barrier function, improving intestinal microbial balance, and improving the local immune response (15).

Probiotics, as a rational option for a positive effect on the clinical course of the disease, maintain the normal intestinal flora and reduce the existing inflammatory processes and increase the function of the epithelial defense barrier (16).

Studies around the world have examined the effect of probiotics on modifying intestinal microbial flora and their beneficial effects on the host. However, due to the diversity of bio drugs in terms of species and dosage in existing research, as well as conflicting results, more extensive investigations are needed to confirm the influential role of probiotics in the treatment and alleviation of the complications associated with ulcerative colitis (17, 18).The present study was carried out to assess the effects of a multi-strain probiotic supplement on the disease status and specific inflammation index in patients with UC through RCT.

MATERIALS AND METHODS

Study design: In this 16-week parallel-group, placebo-controlled, randomized, double-blind clinical trial, ulcerative colitis patients in the mild to moderate stage were selected between June 2021 and March 2022 based on inclusion and exclusion criteria. The participants were recruited from the Inflammatory Bowel Diseases Clinic of Gastroenterology and Hepatology Research Center. The study was designed based on the CONSORT 2010 guideline (19).

Questionnaires of disease symptoms (Lichtiger and Mayo score) of patients were obtained at the beginning, the middle, and the end of the study. Moreover, stool samples were taken from patients at baseline and at the end of the study to measure fecal calprotectin.

INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria: Mild to moderate ulcerative colitis patients of both sexes, aged 18 to 65 years.

Exclusion criteria: Use of antibiotics, probiotics, or prebiotics during the last three months and study period, being pregnant or lactation.

Sample size: Regarding the type one error rate $\alpha = 0.05$ ($Z = 1.96$), and $1 - \beta = 0.80$ ($Z = 0.84$) for detecting an effect size of at least $\Delta=0.75$(20) about the impacts of probiotic supplementation on improving inflammatory variables in UC IBD individuals, 25 subjects were determined. For compensating possible attrition, 20% additional samples were recruited, in which a final 30 subjects in each study group or 60 subjects were considered for study participation. The Average concentration of the "good" flora.

Randomization and blinding: After reviewing eligibility criteria and obtaining written informed consent, patients were randomly assigned into two equal groups using a random allocation sequence via computer-generated random numbers. The random sequence was generated by someone not involved in the study using SPSS (SPSS Inc., Chicago, IL, USA). The participants were allocated to treatment or placebo supplements for four months using a simple sampling method. Participants and all researchers, including the interviewer, laboratory staff, outcome assessors, and data analyst, were blinded to the allocation of the supplements until all data analyses were completed. The supplements which were assigned A or B labels were identical. The producing company pharmacist was responsible for delivering the blinded supplements. The blinding codes were broken after the statistical analyses.

Clinical assessment of disease status: Clinical evaluation was done with the Lichtiger Colitis Activity Index (LCAI) and the Mayo score. In the beginning, after two months, and at the end of the study, patients were completed with disease symptoms using these questionnaires.

The Lichtiger questionnaire, which is a tool used in clinical research to measure and quantify the impact of symptoms of UC, included eight questions that ask about the number of bowel movements, nocturnal diarrhea, blood discharge, fecal incontinence, heart pain, and colic, general condition, pain when putting your hand on the abdomen, and the anti-diarrheal drugs that the patient uses. These eight questions were scored separately; their total was considered Lichtiger's score. A score >10 points defined severe acute colitis (21, 22).

The Mayo clinical questionnaire consisted of three parts. The first question was about the number of bowel movements, the second was about the amount of bleeding from the anus, and the third was about the physician's opinion regarding the severity of the disease. All three questions are scored from zero to three, and finally, the sum of these three numbers was considered as the Mayo score for each patient (22, 23).

Laboratory assessment: Fecal samples were taken from patients at the beginning and the end of the study (At week 16), and a specific inflammatory factor for IBD (Calprotectin) was measured by auto analysis method (kit: Calperest NG, Lat No: 011895, Eurospital Diagnostic Company, Trieste, Italy) for each patient.

Supplement administration: Probiotics and placebos in capsules were produced in the same shape in the Fara Daru Fanavar Mehr, Tehran, Iran. They were delivered to the patients in compliance with the principles of storage. The probiotic product was a Lyophilized Multi-strain Probiotic Mixture called Camflor. Each capsule contained eight trains, including *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Bifidobacterium longum*, *Bifidobacterium*

breve, Bifidobacterium infantis and *Streptococcus thermophiles* (4.5 x1011 CFU for each capsule). The placebo included corn starch and maltodextrin in the exact shape of probiotic capsules. The capsules were stored in a dried place under 20° centigrade. Subjects were instructed to ingest two capsules a day in addition to their usual medication after dinner and lunch to minimize the killing of the probiotics by gastric acid.

The supplements were delivered to the participants for 120 days. In addition, the participants were advised not to modify their medication and lifestyle during the study. One of the researchers was in weekly contact with the participants to address any concerns or side effects during the study. Returned tablet counts were used for compliance assessment. Non-compliance was defined as having more than a 10% missed supplement dose. Everyone who was noncompliant or had severe adverse effects was excluded from the study.

Statistical analysis: Recorded data, including outcome data and adverse events, were double entered on SPSS software Version 18 (SPSS Inc., Chicago, IL, USA). The normality of continuous data was evaluated using the Kolmogorov-Smirnov test, and the Q-Q plot and data were managed for the presence of outliers, violations of normality, and missing data. Quantitative normally/abnormally distributed data were presented as the mean ± standard deviation (SD) or median (minimum-maximum) and categorical data as frequency (percentage). Participants' essential characteristics, including age, gender, disease duration, location of primary diagnosis, and medications in the two groups, were compared using independent samples t-student or Chi-squared tests. The analyses of primary study outcomes were done by both per protocol (PP) for participants who completed the intervention with >90% product compliance and without severe complication and Intention to treat (ITT) for all participants by imputing missing value by mean for normally distributed data and median for non-normal ones. To test our hypothesis that probiotic supplementation may improve primary outcomes in IBD patients, intra and inter-group changes were compared by paired t-test, Wilcoxon Signed Ranks Test, independent t-test (Mann-Whitney U for non-normal data), and Repeated Measured ANOVA. Sphericity assumption in the repeated measure of the Muchly test checked ANOVA, and when it was violated, a multivariate approach was adopted. In all tests, a $P<0.05$ was considered statistically significant.

Ethical aspects: The research protocol was approved by the Ethical Research Board of the Isfahan University of Medical Sciences, Iran (approval number: IR.MUI.REC.1399.988). The study was registered in clinicaltrials.gov as follows: IRCT20210113050024N2.

Obtaining informed consent was done by observing all the rules of ethics in research for clinical studies, including providing complete information, the right to withdraw from cooperation, voluntary entry, and ease of access to facilitators for the subjects.

Code availability: After the statistical analysis, the blinding codes were provided to the researchers. The datasets used and analyzed during the current investigation and support data are available upon reasonable request.

RESULTS

Seventy patients with ulcerative colitis were invited to come to this investigation. However, ten subjects were excluded because they needed to fulfill the inclusion criteria or decline to participate. Of 60 individuals who agreed to participate, 18 patients dropped out during the study. The most reasons for the attrition were low compliance (one in the probiotic group and 8 in the placebo group), gastrointestinal complications including diarrhea, constipation, and flatulence (2 in the probiotic group and 2 in the placebo group), and Anus burning (3 in probiotic group and 2 in placebo group). See the CONSORT flow diagram in Fig.1.

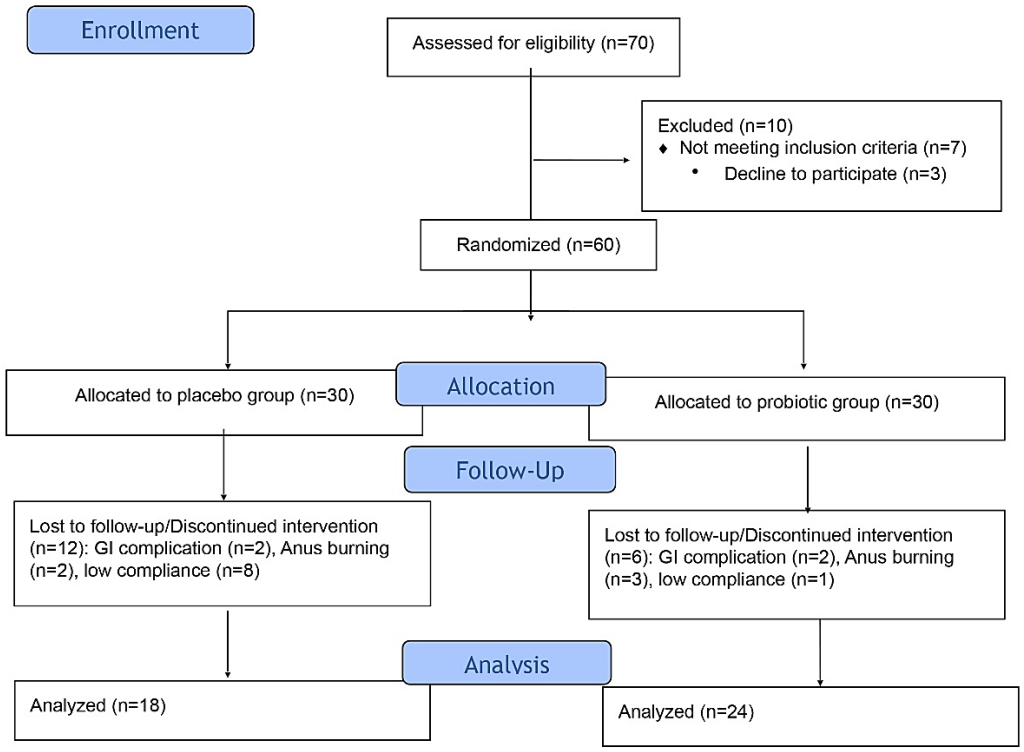


Figure 1. CONSORT 2010 flow diagram

At baseline, there were no statistically significant differences in age, gender, duration of IBD disease, and location of primary diagnosis between the two groups. Among medications used by study participants, the frequency of 5-aminosalicylic acid (5-ASA) users in the probiotic group was significantly more than the placebo group (P<0.05) (Table 1).

Table 1. Baseline characteristics of the two groups (mean values ± standard deviations and number with percentages)

Variable		Groups		P-value
		Probiotic(n=30)	Placebo(n=30)	
Age (Mean years ±SD)		44.7±11.8	44.1±13.6	0.84
Gender N (%)	Female(n=34)	20 (66.7)	14 (46.7)	0.19
	Male(n=26)	10 (33.3)	16 (53.3)	
disease extension (Mean years ±SD)		7.5±5	8±4.8	0.66
Location of primary diagnosis N (%)	extensive colitis	16(53.3)	12(40)	0.43
	Proclitic	6 (20)	5 (16.7)	
	Recto sigmoiditis	8 (26.7)	13 (43.3)	
Medication N (%)	Immunomodulators	10 (23.25)	5 (15.63)	0.11
	Biologics	4 (9.3)	6 (18.75)	0.32
	5-aminosalicylic acid	29 (67.45)	21(65.62)	0.03*

Derived from Independent sample t-student and Chi-square tests.

The Wilcoxon Signed Ranks Test showed that although fecal calprotectin was decreased during the study in both groups, the within groups' reductions were not statistically significant, and the differences between the two groups were not also significant via both ITT and PP analysis (Table2).

Table2. The impacts of Probiotic/Placebo supplementation on fecal calprotectin in patients with ulcerative colitis

Group	Fecal Calprotectin Levels(mg/kg)		$P_{within\ groups}$*(PP**)	$P_{between\ groups}$*(PP**)
	Before study	After study		
Probiotic				0.47(0.23)
Mean ±SD	221.9±535.7	186.5±314	0.29(0.094)	
Median (Min-Max)	34.6(0-2468)	24(2-1026)		
Placebo			0.93(0.9)	
Mean ±SD	188.6±323.5	132.4±235.7		
Median (Min-Max)	30(0-1313)	25.8(0-1131)		

Derived from Wilcoxon Signed Ranks Test

*P-value extracted from Intention to treat analysis; **P-value extracted from per-protocol analysis.

The mean score of Lichtiger was significantly decreased in the probiotic group (P = 0.001) by ITT analysis during the study period. However, the observed changes from baseline were not statistically different in the two groups (Table 3).

Moreover, the rectal bleeding, assessed by the clinical Mayo scale, revealed no significant differences within and between the two groups (Table 4).

Table 3. The effects of probiotic/placebo supplementation on the disease status (from Lichtiger questionnaire) in patients with ulcerative colitis

Group	Time			*P_{time}	*$P_{time*group}$	*P_{group}
	At first (n=60)	At 8 weeks (n=43)	At 16 weeks (n=42)			
Probiotic (Mean±SD)	4.3±2.4	4.4±1.9	3.1±1.4	0.001	0.62	0.66
Placebo (Mean±SD)	4.7±4.2	3.6±2.3	2.7±1.6	0.14		
**P-value	0.65	0.14	0.37			

The analysis has done based on Intention-to-treat (ITT)

*Derived from Repeated Measured ANOVA test; **Derived from Mann-Whitney U test.

Table 4. The effects of probiotic/placebo supplementation on rectal bleeding assessed by Mayo scale in patients with ulcerative colitis.

Group	Time			*P_{time}	*$P_{time*group}$	*P_{group}
	At first (n=60)	At 8 weeks (n=43)	At 16 weeks (n=42)			
Probiotic (Mean±SD)	2.1±1.3	2.0±1.4	1.8±1.1	0.17	0.79	0.99
Placebo (Mean±SD)	2.2±2.2	2.1±2.0	2.0±1.9	0.11		
**P-value	0.93	0.82	0.66			

The analysis has done based on Intention-to-treat (ITT)

*Derived from Repeated Measured ANOVA test; ** Derived from Mann-Whitney U test.

Subgroup analysis was done based on the type of medications used to control its possible confounding effect. The data analysis demonstrated no changes in the results in 5-ASA users and not users. No, severe adverse effect has been reported from the participants.

DISCUSSION

Functional foods are described to natural or processed foods that provide positive effects on the health through optimizing the capacity of the immune system, as well as alterations in the hemostasis of biochemical parameters and neuronal functions. Functional foods including nutraceuticals, prebiotics, probiotics, and synbiotics have been studied to improvement of some chronic diseases in the past 2 decades (24, 25), There is high discrepancies about the adverse effects of probiotics in different studies which may be due to schedules across studies know the safety and effective doses of this functional food.

This study was a prospective, placebo-controlled, double-blind study to assess the efficacy of a multi-strain probiotic agent including *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve* and *Streptococcus thermophiles* (4.5×10^{11} CFU for each capsule) in patients with mild/moderate UC.

The main finding in present study was that despite the slight improvement in Lichtiger score, this agent has no significant effect in treating UC patients compared with a placebo.

During the last 20 years, the treatment of IBD with probiotics has been suggested in some studies (26). Probiotics may modulate the immune system by enhancing mucosal barrier function or T cell responses, increasing the anti-inflammatory factor interleukin, and decreasing pro-inflammatory cytokines. However, there is still little convincing data regarding probiotics in ulcerative colitis (27). On the other hand, there is some evidence which has shown that probiotics may be responsible for excessive immune stimulation in susceptible individuals (28). The reported evidence of conventional probiotics is vary depending on the dose and strain of the probiotics administered and the stage and type of disease (29). In our study, the attrition due to complications of the intervention in the probiotic group was almost the same as in the placebo group and no severe side effect was seen during the intervention with probiotic or placebo. However, the non-compliance in

the placebo group was notable, possibly attributed to the higher satisfaction reported by patients who consumed probiotics.

The novel approach to selecting the next generation of probiotics for each disorder may help us unlock further benefits. For example, it has been understood that *Faecalibacterium prausnitzii* has beneficial effects on inflammatory diseases like ulcerative colitis. Future studies are needed to evaluate which probiotics formulations will be effective on specific humans' health and disease (30).

Moreover, it has been shown that some of the functional foods like curcumin and extra virgin olive oil have the potential of anti-inflammation and may be beneficial in the treatment of ulcerative colitis as a complementary medicine (31).

More interventional studies will provide an integrated dietary strategy with the selected probiotic strains for modulating the gut microbiota and making improvement in clinical practice.

Limitation: The high loss to follow-ups due to non-compliance in the placebo group and the occurrence of gastrointestinal adverse effects in both groups were the predominant limitations in this study.

Strength: In this study, the discrepancies in the composition of the probiotic and placebo groups were minimized because of the excellent definition of the population by the inclusion and exclusion criteria. Also, the sampling method and data collection processes in this study cause the quality of the study and the generalizability of the findings. Moreover, most studies on the effects of probiotics on IBD patients have been focused on questionnaire data. This is one of the limited studies in which both lab data and questionnaire data have been reported.

CONCLUSION

Our study elucidated that although this probiotic agent, has had a scanty improvement in clinical status and patient satisfaction, it does not have a considerable effect on ulcerative colitis improvement.

Future research should focus on the best effective strains and doses for ulcerative colitis management. Metagenomics studies may help to identify specific probiotic strains with biological effects. The possibility of combining the best next generation probiotics with synergistic effects may be an exciting future alternative to the current pharmacological treatment for patients with UC. Also, the functional foods which have anti-inflammation effects can be present as a complementary medicine for UC patients.

Abbreviations: IBD: inflammatory bowel disease, UC: ulcerative colitis

Acknowledgements: We would like to thank all research participants for their cooperation and the Gastroenterology and Hepatology Research Center for their cooperation.

Author contributions: PA, NK and BT designed and authored the study. NK, MRK, SA and AZM were the main investigators. AF has developed statistical design and analysis. All authors saw the final version of the manuscript and approved it.

Funding: This project was funded by Isfahan University of Medical Sciences. The funding agency played no role in the decision to submit the manuscript, design, collection and analysis, data interpretation, manuscript writing, or publication.

Conflict of interest: The authors declare no competing interests.

Data statement: The data are available upon request from the corresponding author.

REFERENCES

1. Andoh A, Nishida A. Alteration of the Gut Microbiome in Inflammatory Bowel Disease. Digestion. 2023;104(1):16-23. DOI: https://doi.org/10.1159/000525925
2. Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology. 2017;152(2):327-39. e4. DOI: https://doi.org/10.1053/j.gastro.2016.10.012
3. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases With Time, Based on Systematic Review. Gastroenterology. 2012;142(1):46-54. e42. DOI: https://doi.org/10.1053/j.gastro.2011.10.001
4. Kumar V AA, Fausto N, Aster JC. Robbins and Cotran pathologic basis of disease, professional edition e-book: Elsevier health sciences; 2014.
5. Sairenji T, Collins KL, Evans DV. An Update on Inflammatory Bowel Disease. Prim Care. 2017;44(4):673-92. DOI: https://doi.org/10.1016/j.pop.2017.07.010
6. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448(7152):427-34. DOI: https://doi.org/10.1038/nature06005
7. Almeida MG, Kiss DR, Zilberstein B, Quintanilha AG, Teixeira MG, Habr-Gama A. Intestinal mucosa-associated microflora in ulcerative colitis patients before and after restorative proctocolectomy with an ileoanal pouch. Dis Colon Rectum. 2008;51(7):1113-9. DOI: https://doi.org/10.1007/s10350-008-9347-8
8. Tursi A, Brandimarte G, Papa A, Giglio A, Elisei W, Giorgetti GM, et al. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol. 2010;105(10):2218-27. DOI: https://doi.org/10.1038/ajg.2010.218
9. Youovop AJ, Takuissu G, Christelle MB, Nwang F, Françoise N, Mbong Angie M-A, et al. The effects of Dyglomera ® (dichrostachys glomerata extract) on body fat percentage and body weight: a randomized, double- blind, placebo-controlled clinical trial. Functional Foods in Health and Disease. 2023; 13:334-46. DOI: https://doi.org/10.31989/ffhd. v13i6.1088
10. Mack DR. Probiotics in inflammatory bowel diseases and associated conditions. Nutrients. 2011;3(2):245-64. DOI: https://doi.org/10.3390/nu3020245
11. D'Haens GR, Sartor RB, Silverberg MS, Petersson J, Rutgeerts P. Future directions in inflammatory bowel disease management. J Crohns Colitis. 2014;8(8):726-34. DOI: https://doi.org/10.1016/j.crohns.2014.02.025
12. Langan RC, Gotsch PB, Krafczyk MA, Skillinge DD. Ulcerative colitis: diagnosis and treatment. Am Fam Physician. 2007;76(9):1323-30. PMID: 18019875
13. MacDermott RP, Green JA, Ashley CC. What is the optimal therapy for severe ulcerative colitis? Inflammatory Bowel Diseases. 2008;14(suppl_2): S228-S31. DOI: https://doi.org/10.1002/ibd.20514
14. Miele E, Pascarella F, Giannetti E, Quaglietta L, Baldassano RN, Staiano A. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. Am J Gastroenterol. 2009;104(2):437-43. DOI: https://doi.org/10.1038/ajg.2008.118
15. Kaur L, Gordon M, Baines PA, Iheozor-Ejiofor Z, Sinopoulou V, Akobeng AK. Probiotics for induction of remission in ulcerative colitis. Cochrane Database Syst Rev. 2020;3(3): Cd005573. DOI: https://doi.org/10.1002/14651858.CD005573.pub3
16. Halloran K, Underwood MA. Probiotic mechanisms of action. Early Hum Dev. 2019; 135:58-65. DOI: https://doi.org/10.1016/j.earlhumdev.2019.05.010
17. Derwa Y, Gracie DJ, Hamlin PJ, Ford AC. Systematic review with meta-analysis: the efficacy of probiotics in inflammatory bowel disease. Aliment Pharmacol Ther. 2017;46(4):389-400. DOI: https://doi.org/10.1111/apt.14203
18. Jakubczyk D, Leszczyńska K, Górska S. The Effectiveness of Probiotics in the Treatment of Inflammatory Bowel Disease (IBD)-A Critical Review. Nutrients. 2020;12(7). DOI: https://doi.org/10.3390/nu12071973
19. Cuschieri S. The CONSORT statement. Saudi J Anaesth. 2019;13(Suppl 1): S27-s30. DOI: https://doi.org/10.4103/sja.SJA_559_18
20. Iheozor-Ejiofor Z, Kaur L, Gordon M, Baines PA, Sinopoulou V, Akobeng AK. Probiotics for maintenance of remission in ulcerative colitis. Cochrane Database Syst Rev. 2020;3(3): Cd007443. DOI: https://doi.org/10.1002/14651858.CD007443.pub3

21. Schoepfer AM, Beglinger C, Straumann A, Safroneeva E, Romero Y, Armstrong D, Schmidt C, Trummler M, Pittet V, Vavricka SR. Fecal calprotectin more accurately reflects endoscopic activity of ulcerative colitis than the Lichtiger Index, C-reactive protein, platelets, hemoglobin, and blood leukocytes. Inflamm Bowel Dis. 2013 Feb;19(2):332-41. DOI: https://doi.org/10.1097/MIB.0b013e3182810066
22. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. Inflamm Bowel Dis. 2008;14(12):1660-6. DOI: https://doi.org/10.1002/ibd.20520
23. Higgins PDR, Harding G, Revicki DA, Globe G, Patrick DL, Fitzgerald K, et al. Development and validation of the Ulcerative Colitis patient-reported outcomes signs and symptoms (UC-pro/SS) diary. J Patient Rep Outcomes. 2017;2(1):26. DOI: https://doi.org/10.1186/s41687-018-0049-2
24. Granato D, Barba FJ, Busać Kovačević D, Lorenzo JM, Cruz AG, Putnik P. Functional Foods: Product Development, Technological Trends, Efficacy Testing, and Safety. Annu Rev Food Sci Technol. 2020; 11:93-118. DOI: https://doi.org/10.1146/annurev-food-032519-051708
25. Risso D, Laurie I, Sarkkinen E, Karnik K. The effect of polydextrose on fecal bulk and bowel function in mildly constipated healthy adults: a double-blind, placebo controlled study. Functional Foods in Health and Disease. 2023; 13:240. DOI: https://doi.org/10.31989/ffhd. v13i5.1099
26. Chapman TM, Plosker GL, Figgitt DP. Spotlight on VSL#3 probiotic mixture in chronic inflammatory bowel diseases. BioDrugs. 2007;21(1):61-3. DOI: https://doi.org/10.2165/00063030-200721010-00007
27. Agraib LM, Yamani MI, Rayyan YM, Abu-Sneineh AT, Tamimi TA, Tayyem RF. The probiotic supplementation role in improving the immune system among people with ulcerative colitis: a narrative review. Drug Metab Pers Ther. 2021. DOI: https://doi.org/10.1515/dmdi-2021-0150
28. Zhang XF, Guan XX, Tang YJ, Sun JF, Wang XK, Wang WD, Fan JM. Clinical effects and gut microbiota changes of using probiotics, prebiotics or synbiotics in inflammatory bowel disease: a systematic review and meta-analysis. Eur J Nutr. 2021;60(5):2855-75. DOI: https://doi.org/10.1007/s00394-021-02503-5
29. Štofilová J, Kvaková M, Kamlárová A, Hijová E, Bertková I, Guľašová Z. Probiotic-Based Intervention in the Treatment of Ulcerative Colitis: Conventional and New Approaches. Biomedicines. 2022;10(9). DOI: https://doi.org/10.3390/biomedicines10092236
30. Santonicola A, Molinari R, Piccinocchi G, Salvetti A, Natale F, Cimmino G. Role of a novel nutraceutical composition for irritable bowel syndrome management: symptoms relief and unexpected triglycerides-lowering effect. Functional Foods in Health and Disease. 2023; 13:82.
31. Dore MP, Rocchi C, Longo NP, Scanu AM, Vidili G, Padedda F, Pes GM. Effect of Probiotic Use on Adverse Events in Adult Patients with Inflammatory Bowel Disease: a Retrospective Cohort Study. Probiotics Antimicrob Proteins. 2020;12(1):152-9. DOI: https://doi.org/10.1007/s12602-019-9517-0