

Assessing the beneficial effect of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 on colitis in a rat model

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ABSTRACT

Background and Objectives: Probiotics are effective in improving inflammatory bowel disease (IBD). This study assessed the effect of mesalazine and two candidate probiotics on the improvement of acetic acid (AA)-induced colitis model.

Materials and Methods: *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 were used for IBD model in rat. Twenty-five male Wistar rats weighing 250 ± 50 grams were used in 5 classified groups: Control (CO); Colitis (CL); Colitis, Probiotic (CLP); Colitis, Mesalazine (CLM); Colitis, Probiotic, Mesalazine (CLPM) and the treatment period was 3 weeks. The rats were treated with mesalazine 30 mg/kg and probiotic 10^9 CFU/ml after induction of colitis. Histopathological and immunological analyses were performed to evaluate the effects of probiotic bacteria on IBD.

Results: The results showed that the probiotic bacteria reduced inflammation ($P < 0.05$), extent ($P < 0.01$), crypt abscesses ($P < 0.01$), edema ($P < 0.05$), inflammatory cell infiltration ($P < 0.5$), and increased mucosa ($P < 0.001$) in rats. Mesalazine administration in animals with colitis did not have a significant effect. Administration of probiotics in both CLP and CLPM groups reduced extent, crypt abscesses, edema, and inflammatory cell infiltration and showed an important role in the down-regulation of consolidation of pro-inflammatory factors (TNF α , IL-6, and IL-17), as well as up-regulation of anti-inflammatory factors such as IL-10.

Conclusion: *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 have shown significant potential in alleviating AA-induced colitis symptoms. Their administration leads to a marked reduction in pro-inflammatory cytokines such as TNF- α , IL-17, and IL-6, while enhancing IL-10 levels, indicating their promise as therapeutic candidates for inflammatory bowel disease (IBD).

Keywords: Inflammatory bowel disease; Probiotics; Inflammatory cytokines; Lactic acid bacteria

INTRODUCTION

The term inflammatory bowel disease (IBD) refers to a diverse group of persistent gastrointestinal inflammatory conditions, most notably Crohn's disease

(CD) and ulcerative colitis (UC), both of which are commonly classified as distinct forms of the disorder (1, 2). Investigators posit that four factors may engender IBD: a genetic component, an environmental factor, an improper immune system response, and

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an imbalance of intestinal bacteria. Immune cells generally defend the body from infections, but in IBD patients, the immune system confuses harmless materials in the intestine with foreign materials and launches an attack against them, leading to inflammation. All diseases of this group include mutations of the immune tolerance system of the gastrointestinal tract mucosa (3-5). The symptoms of IBD vary among patients, and may alter over time. The most common symptoms for CD and UC include diarrhea, urgent bowel movements, abdominal pain, cramping, bloody stool, and hematochezia. IBD patients may also report symptoms as lack of appetite, weight loss and fatigue (6-8).

Probiotics help reinforce the intestinal barrier by stimulating mucin production, enhancing tight junction proteins, and supporting the function of Paneth and Goblet cells. In addition, they contribute to gut health by regulating the microbial community, preserving balance, and limiting the proliferation of potentially harmful bacteria (6). Probiotics can stimulate crypt cell proliferation, inhibit apoptosis, and regulate the expression of inflammatory cytokines, thereby modulating intestinal immune responses (9-11). Probiotics could represent an authentic armamentarium to modulate gut microbiota and, possibly to cure IBD (12). Thus, the use of probiotics as a supportive treatment for IBD has gained notable attention in recent years. Scientific studies have identified clear variations in the gut microbiota between IBD-affected individuals or animal models and healthy subjects. These microbial shifts often involve genera such as *Lactobacillus*, *Bifidobacterium*, *Fusobacterium*, *Enterococcus*, *Bacteroides*, and *Streptococcus* (12, 13).

In further, gut microbial dysbiosis is linked to abnormal immune responses, which are often accompanied by aberrant production of inflammatory cytokines. These cytokines can affect how different inflammatory markers are produced and released, including both pro-inflammatory agents like Interferon- γ (IFN- γ), IL-1 β , various interleukins (IL-1, IL-2, IL-6, IL-12, IL-17), and tumor necrosis factor- α (TNF- α), as well as anti-inflammatory ones such as IL-4 and IL-10. Other related compounds include platelet-activating factor, eicosanoids, nitrogen-based metabolites, and reactive oxygen species (14, 15). This study aims to evaluate the positive impact of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 both isolated from traditional

dairy products on colitis in a rat model.

MATERIALS AND METHODS

Bacteria. *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 isolated from Varze-ghan-Azarbayjan traditional dairy products (16) and deposited in microbial collection of Tabriz University were used in this study.

Probiotic preparations. The bacterial strains were activated and cultured in MRS (de Man, Rogosa and Sharpe) broth. For probiotic evaluation in rats, the bacterial strains were incubated in MRS broth at 37°C for 48 hours. After incubation, the cells were harvested by centrifugation at 4000 rpm for 10 minutes.

Animals. A total of 25 adult male Wistar rats (weighing 250-300g) were procured from the Pasteur Institute in Tehran, Iran. The animals were housed in groups of five per cage under controlled room temperature conditions (22-25°C), with a 12-hour light/dark cycle and unrestricted access to standard food and water.

Induction of experimental colitis. All animal procedures in this study were carried out following the ethical standards set by the Biomedical Research Ethics Committee of Tabriz University, with formal approval granted under code IR.TABRIZU.REC.1399.039. Twenty-five male Wistar rats weighing almost 280 g were randomly divided into five groups according to the time of treatment (3 weeks): Control (CO); Colitis (CL); Colitis, Probiotic (CLP); Colitis, Mesalazine (CLM); Colitis, Probiotic, Mesalazine (CLPM). The animals were kept in the vivarium of Natural Sciences Faculty of Tabriz University, in plastic boxes of 47 cm $\times$ 34 cm $\times$ 18 cm lined. The model chosen for the induction of colitis was adapted from those described by Manna et al. and Azza Abdel-fattah et al. (17, 18). Distal colitis was experimentally induced by administering a 4% solution of acetic acid (AA) in saline directly into the colon. The solution was injected with a PE-50 cannula into the colon 8cm near the anus. To prevent immediate expulsion of the AA solution, the cannula was retained in the colon for 15 seconds prior to removal. Three weeks after induction of colitis, all rats were anesthetized by intraperitoneal injection of sodium pentobarbital

(50mg/kg) and sacrificed by cardiac puncture. Then their blood was drawn into a vacutainer containing heparin, centrifuged at 3000 g for 10 minutes, and the plasma was subsequently stored at -80°C. Mesalazine was purchased from a pharmacy in Tabriz. Each tablet contains 500 mg of Mesalazine.

Oral administration of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 and mesalazine through gavage. To investigate the therapeutic effects of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 combination and mesalazine against colitis induced by AA, the animals were randomly divided into five groups (1-5), with five rats per group. The empirical plan was as follows:

1- Control group (CO): The animals were intact, 2- Colitis group (CL): In this group, colitis was induced with AA, and no medication was received, 3- Probiotics group (CLP): In this group, after induction of colitis, the combination of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 was administered daily, delivering approximately 10^9 CFU/kg of body weight (BW) (15) via gavage for 3 weeks. 4- Mesalazine group (CLM): Mesalazine, an effective drug for the treatment of colitis, was considered as a positive control. In this group, after induction of colitis, mesalazine was administered daily at a dose of 30 mg/kg BW via gavage for 3 weeks. 5- Probiotic and Mesalazine group (CLPM): In this group, after induction of colitis, animals received both drugs concurrently; The administered dosages were 30 mg/kg BW for mesalazine and 10^9 CFU/kg BW for the probiotic strains (*L. plantarum* MS1 and *L. delbrueckii* YN1), both delivered daily via gavage. Meanwhile, the typical group was maintained under standard feeding conditions, including a normal diet and free access to water from weeks 1 to 3. The colitis group or the AA-treated group was fed a normal diet and drank water during the analysis. All groups, except the control, received daily gavage for three weeks: either probiotics (10^9 CFU/kg BW), mesalazine (30 mg/kg BW), or a combination of both, depending on the treatment protocol. The ten-ml inocula were centrifuged at 3000rpm for 10 min, washed twice with 15 mL of sterilizing 0.9% saline peptone water, and resuspended in 2 mL 0.9% peptone saline. Then the number of cells was measured by the plate colony-counting method. Moreover, its absorbance was evaluated at 600 nm wavelength by using an Eppendorf BioPhotometer. During this experiment, the re-

sults of colony counts and absorbance were used to adjust the concentration of *L. plantarum* MS1 and *L. delbrueckii* YN1 to gavage. The fresh inoculum was prepared and adapted daily prior to administration. Additionally, throughout the experimental period, all groups were maintained on a standard diet and had unrestricted access to drinking water.

Sample collection. After 3 weeks, all animals were killed by ether overdose at the end of the experiment. Colon fragments were opened in an ice bath after being washed with normal saline, subsequently, they were evaluated and scored in terms of histopathology. Serum isolated from blood was assayed to evaluate the level of inflammatory cytokines. The serum was separated (3000 rpm, 10 min, 4°C) and stored at -80°C.

Histologic examination. For histological assessment, intestinal samples were fixed in 10% (v/v) buffered formalin, embedded in paraffin, and sectioned at a thickness of 3 μ m using a rotary microtome. The sections were stained with hematoxylin and eosin (HE) for routine microscopic evaluation. Slides were examined under a NIKON Labophot binocular microscope at 200 $\times$ magnification. Histological parameters were assessed according to the criteria outlined in Table 1.

Measurement of cytokine in the serum. The serum concentrations of IL-6, IL-17, TNF- α , and IL-10 were measured using enzyme-linked immunosorbent assay (ELISA) kits (Beijing Bai Ao Lai Bo Co., Ltd., Beijing, China) according to the protocol provided by the manufacturer.

Statistical analysis. Statistical analyses were performed using SPSS version 16. Data normality was assessed prior to analysis, and group comparisons were conducted via one-way ANOVA followed by Tukey's post hoc test. Results were reported as mean $\pm$ standard error, and differences were considered statistically significant at $p < 0.05$.

RESULTS

Effects of AA treatment in rat model. Administration of acetic acid induced moderate colitis in rats. Colitis was associated with diarrhea, one of the

Table 1. Histological grading of colitis (12)

Feature graded	Grade	Description
Inflammation	0 1 2 3	None Slight Moderate Severe
Extent	0 1 2 3	Mucosa and Submucosa Transmural No tissue repair Surface epithelium not intact
Regeneration	3 2 1 0	Regeneration with crypt depletion Almost complete regeneration Complete regeneration or normal tissue
Crypt damage	0 1 2 3 4	None Basal 1/3 damaged Basal 2/3 damaged Only surface epithelium intact Entire crypt and epithelium lost
Percent involvement	1 2 3 4	1-25% 26-50% 51-75% 76-100%
Mucus cell depletion	0 1 2 3 4	Preserved mucus cell Mild depletion in a few cells Moderate depletion (less than 50% of cells) Severe depletion or complete disappearance of mucosa
Crypt abscesses	0 1 2 3 4	No abscess 1-3 abscesses/slide 4-9 abscesses/slide 10 or more abscesses/slide
Inflammatory cysts	0 1 2 3 4	No cyst 1-3 cysts/slide 4-9 cysts/slide 10 or more cysts/slide
Mucosal Atrophy	0 1 2 3 4	Normal thickness Mild atrophy (less than 10%) Moderate atrophy (10-50%) Severe atrophy (>50%)
Edema (submucosa)	0 1 2 3 4	Normal thickness Mild edema (submucosal expansion less than 10%) Moderate edema (submucosal expansion, 10-100%) Severe edema (submucosal expansion > 100%)
Inflammatory cell infiltration	0 1 2 3 4	No Inflammatory cell infiltration Mild Inflammatory cell infiltration; few scattered cells Moderate: distributed but not dense Inflammatory cell infiltration
Vascular dilatation	0 1 2 3	Inflammatory cell infiltration Normal blood vessels Mild dilatation of single blood vessels Moderate dilatation of several blood vessels Severe dilatation of caecal blood vessels

hallmarks of the disease. Intra-rectal administration of acetic acid caused a slight decrease in intestinal mucosa, and increased indexes such as; extent (($P<0.01$), crypt damage ($P<0.01$), percent involvement ($P<0.05$), mucosa cell depletion ($P<0.01$), crypt abscess ($P<0.02$), inflammatory cysts ($P<0.001$), mucosal atrophy ($P<0.5$), edema ($P<0.001$), vascular dilatation ($P<0.03$) and severe inflammation ($P<0.001$) in the colitis group in comparison to the control group (Fig. 1).

Histologic examination. Intra-rectal 4% acetic acid solution administration for 1 days induced an acute colitis in Wistar rats (Fig. 2B). In Histologic exam-

inations the control group was completely normal (Fig. 2A). Intra-rectal administration of acetic acid caused severe decrease in intestinal mucosa ($P<0.05$), and increased indexes such as; extent ($P<0.05$), crypt damage ($P<0.01$), percent involvement ($P<0.02$), mucosa cell depletion ($P<0.01$), crypt abscess ($P<0.02$), inflammatory cysts ($P<0.001$), mucosal atrophy ($P<0.5$), edema ($P<0.001$), vascular dilatation ($P<0.001$) and severe inflammation ($P<0.05$) in the colitis group compared to the control group. The group treated with the combination of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 (CLP) reduced indexes such as; inflammation ($P<0.05$), extent ($P<0.05$), crypt abscesses ($P<0.05$),

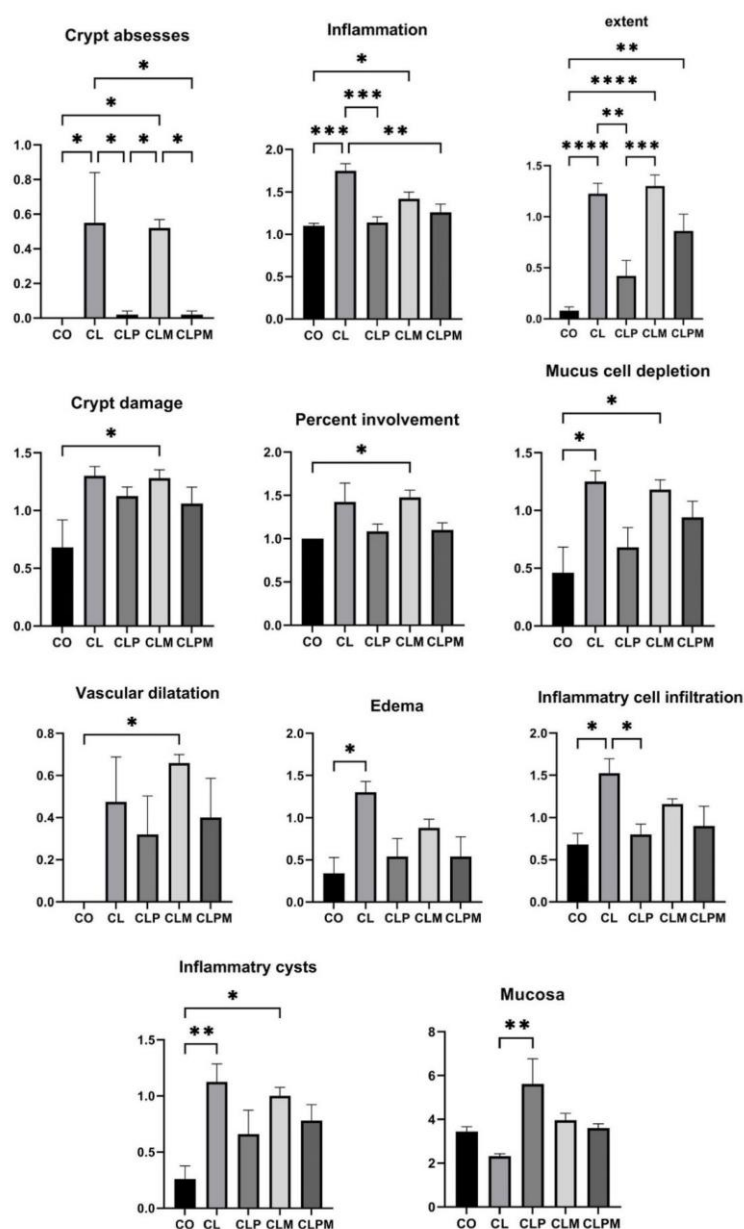


Fig. 1. Histopathological examination of different groups CO, CL, CLP, CLM, CLPM. *Significantly different compared with CO group, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

edema ($P < 0.001$), inflammatory cell infiltration ($P < 0.05$) and increase mucosa ($P < 0.001$) compared to the colitis group. Mesalazine (CLM) administration in animals with colitis did not have a significant effect. Co-administration of mesalazine with the combination of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 (CLPM) reduced indexes such as extent ($P < 0.05$), crypt abscesses ($P < 0.05$), edema ($P < 0.01$), inflammatory cell infiltration ($P < 0.001$) (Fig. 1).

Changes in the serum cytokines in rats after treatment. The effects of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 on the alteration of pro-inflammatory cytokine levels, IL-17, IL-6, TNF- α and anti-inflammatory cytokine levels IL-10 in the serum of the AA-induced rats are shown in Fig. 3.

Pro-inflammatory cytokines levels TNF- α , IL-17 and IL-6 levels in serum were significantly increased in the colitis group (CL) $P < 0.001$ compared to the CO

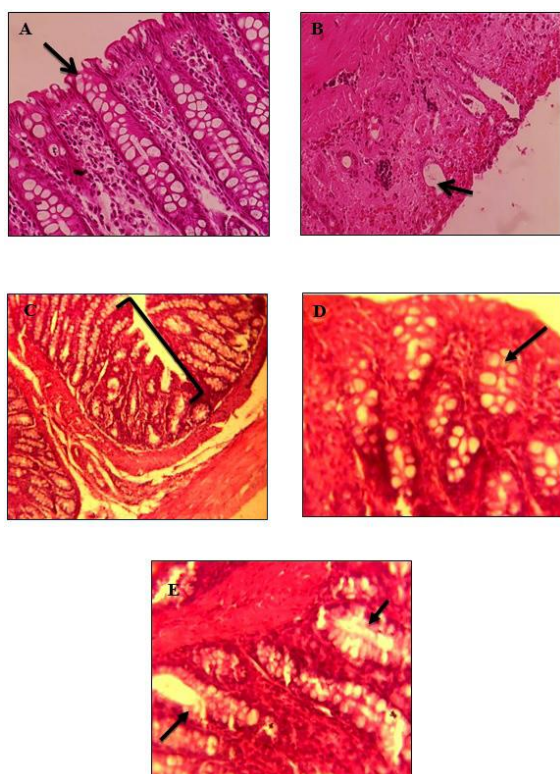


Fig. 2. Each section represents the changes of colon tissue in a group of 5 rats on the 4th after surgery and acetic acid injection (except for control normal rats). Eosin-hematoxylin-stained sections are shown A: Control rat; Normal mucosa with the absence of ulceration and inflammation, significant mucin production in the glands, mild edema and vasodilation are visible under the mucosa (H&E stain, $\times 400$). B: Colitis rat; Partial reduction of intestinal mucosa, increased indexes such as; extent, crypt damage, percent involvement, mucosa cell depletion, crypt abscess, inflammatory cysts, mucosal atrophy, edema, vascular dilatation and severe inflammation in the mucosa are visible (H&E stain, $\times 40$). C: Rat treated with 10^9 CFU/kg body weight *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 one day after acetic acid injection; mucosa with superficial ulceration, cysts with solitary abscess, mild inflammatory cell infiltration, mild mucosal atrophy, mild edema, and submucosa vascular dilatation are visible (H&E stain, $\times 40$). D: Rat treated with 30 mg/kg mesalazine one day after acetic acid instillation; did not have a significant effect (H&E stain, $\times 400$). E: Rat treated with 30 mg/kg body weight mesalazine and 10^9 CFU/kg BW *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 one day after acetic acid instillation; reduced indexes such as extent, crypt abscesses, edema, inflammatory cell infiltration are visible (H&E stain, $\times 400$).

group. The combination of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 administration reduced TNF- α ($P < 0.001$), IL-17 ($P < 0.001$) and IL-6 levels ($P < 0.001$).

Anti-inflammatory IL-10 levels in serum significantly decreased in the colitis group (CL) compared to the control group (CO) ($P < 0.05$). The combination of *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 increased IL-10 levels. The level of IL-10 in the CLP group had a significant increase compared to the two groups of CLM and CLMP.

DISCUSSION

The histopathological properties and inflammatory intermediaries of colitis caused by AA are very similar to the human IBD. In addition, AA-induced colitis is widely used as an experimental model for IBD due to its simplicity (19). The rectal administration of acetic acid may result in epithelial injury, leading to ulcer formation and inflammation in the colonic mucosa. This process facilitates the migration of neutrophils into the affected tissue, triggering the release of inflammatory cytokines and initiating acute pathological alterations in the colon (19). Microscopic analysis demonstrated that AA can cause cryptic damage, inflammation, inflammatory cell penetration, and submucosal edema and bleeding. Increased indexes included; extent, crypt damage, percent involvement, mucosa cell depletion, crypt abscess, inflammatory cysts, mucosal atrophy, vascular dilatation and severe inflammation in colon tissue (Fig. 2). Among therapeutic groups (CLP, CLM, CLPM), the CLP group showed a significant recovery of colon mucosal damage from colitis caused by AA by reducing submucosal edema as also by reduced indicators such as; inflammation, extent, crypt abscess, edema, and inflammatory cell penetration compared to the colitis group.

The onset of colitis damages the epithelial barrier function, increasing intestinal permeability and allowing antigens to enter. This leads to an immune response involving the movement of lymphocytes and macrophages into the tissue and the release of inflammatory cytokines such as TNF- α , IL-6, and IL-17. These cytokines are small proteins that influence immune activity. IL-6, IL-17, and TNF- α promote inflammation, while IL-10 has anti-inflammatory effects (20, 21).

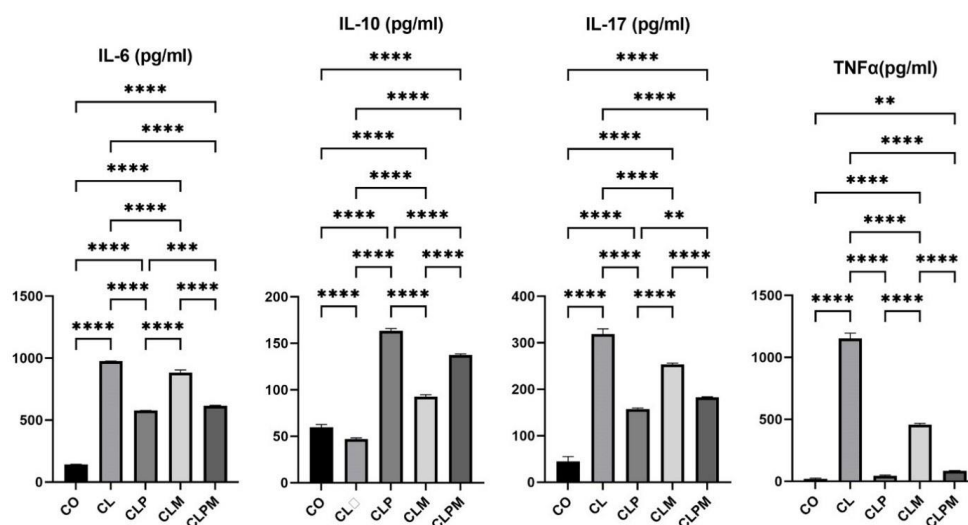


Fig. 3. Serum concentration of IL-6, IL-10, IL-17 and TNF- α in various groups (CO, CL, CLP, CLM and CLPM) of animal models with experimental colitis treated with *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 and mesalazine. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

CLP, CLM, and CLPM groups remarkably decreased the secretion of TNF- α , IL-6, and IL-17 compared with the colitis group (Fig. 3). Also, the TNF- α , IL-17 and IL-6 levels in probiotic (CLP) and the simultaneous administration of mesalazine and probiotic (CLPM) were significantly different from the mesalazine group alone. Therefore, probiotics (*Lactobacillus Plantarum* MS1 and *Lactobacillus delbrueckii* YN1) can return the secretion of inflammatory cytokines to retreat colitis caused by AA. Dicksved et al. Studies suggest that probiotics (including *Lactobacilli* and *Bifidobacteria*) play a crucial role in preserving mucosal barrier function and modulating immune responses. They encourage anti-inflammatory activity and prevent the growth of pathogenic microbes in the intestinal flora (22, 23).

There is evidence that intestinal bacteria and probiotics may change the expression and distribution of sturdy connecting proteins or reduce oxidative stress thus strengthening the barrier of the intestine; for example, the nonpathogenic *Escherichia coli* strain Nissle 1917, which is known to be safe and effective in maintenance of clinical remission of ulcerative colitis in humans (23, 24). Many probiotics and prebiotics were shown to modulate the intestinal mucosal and systemic immune system (25).

A clinical trial investigated the impact of a one-month probiotic yogurt regimen on inflammation in 20 patients with IBD compared to 20 healthy participants as the control group. The yogurt contained

Lactobacillus rhamnosus GR-1 and *Lactobacillus reuteri* RC-14. Findings revealed a reduction in the proportion of TNF- α -producing immune cells, such as monocytes, in both groups. Unlike our results, the TNF- α level showed no significant changes in the CL group, but the TNF- α serum levels in the control group declined (26). Zhu et al. conducted an analysis of IL-17 gene expression using datasets from the Gene Expression Omnibus (GEO) repository and observed a marked increase in expression levels among patients with UC and CD compared to healthy controls. These results reinforce the link between IL-17 activity and intestinal inflammation (27).

In a study to evaluate probiotics in UC patients they found the metabolites produced by *Lactobacillus fermentum* F-B9-1 decreased the level of pro-inflammatory cytokines IL-6 and IL-1 β and improved the normal gut microbiome. Their results also indicated that exopolysaccharide and proteins present in the metabolites are the main components of this probiotic for inhibiting inflammation in colitis. (28). A randomized controlled trial involving 30 patients with UC demonstrated post-intervention changes in mRNA expression levels of several cytokines, notably TNF- α , IL-1, and IL-17, three key mediators of inflammation (28, 29). The Oral consumption of *Lactobacillus suntoryeus* Hy7801 in TNBs-caused colitis mice significantly reduced the expression of IL-1 β , TNF- α and IL-6 in the intestine (30). Biopsies obtained from colon-based UC patients with *Bifido-*

bacterium and lower TNF- α concentrations were cultivated in probiotic-cultivated epithelium cells (31). In another clinical study involving oral probiotic intervention in patients with UC, post-intervention analysis revealed a significant downregulation in the mRNA expression of NF- κ B and TNF- α (32).

Colitis can generate disruption to the intestinal epithelium and boost its permeability. In addition, antigens can enter the intestinal lumen and accumulate lymphocytes and macrophages, resulting in inflammatory cytokines such as TNF- α , IL-6 and IL-17. In our study, CLP, CLM and CLPM groups decreased the secretion of TNF- α , IL-6 and IL-17 compared to AA alone. Also, the level of TNF- α , IL-17 and IL-6 in probiotic (CLP) and double administration of mesalazine and probiotic (CLPM) was different compared to the mesalazine group alone. Therefore, probiotics such as *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 can reduce the secretion of inflammatory cytokines to reduce the development of colitis caused by AA (33). It has been reported that probiotics (for example *Lactobacillus* and *Bifidobacterium*) can penetrate the mucosal barrier, protect the intestines and the function of the immune system, strengthen anti-inflammatory responses, and inhibit the growth of harmful bacteria. There is evidence that gut bacteria and probiotics may express and distribute alter the tight junction proteins or reduce oxidative stress and thus strengthen the intestinal barrier.

In animal models with experimental colitis, there was a significant difference ($P<0.05$) in the serum concentrations of IL-10, IL-17, IL-6, and TNF- α cytokines between the control group (CO) and the treatment groups (CL, CLP, CLM, and CLPM) after testing with probiotic bacteria. The level of pro-inflammatory cytokines TNF- α , IL-17 and IL-6 in serum increased significantly in the colitis group compared to the control group ($P<0.001$).

Considering the TNF α serum concentration in different groups (CO, CL, CLP, CLM and CLPM), there was a significant difference between the control group and the CLP group ($P<0.001$). Also, the CO group had a significant difference with other groups in this index ($P<0.05$).

Evaluation of IL-10 serum concentration in different groups (CO, CL, CLP, CLM and CLPM) showed a significant difference between the control group and the CLP group ($P<0.001$) and also, the CO group with other groups in this index ($P<0.05$). In IBD, IL-

10 was observed to be upregulated due to its anti-inflammatory effect. IL-10 serum concentration reduces inflammation in the mucosa, but it is not strong enough to suppress it. In the present study, IL-10 serum level was significantly higher in the CL group than in the CO group. Probiotics have been shown to affect the body's immune system by increasing IL-10 levels, however, this effect isn't consistent across all probiotic species and dosages. The effective range of IL-10 for this benefit remains uncertain.

Similar results were obtained for IL-6 and IL-17 serum concentrations in different groups. In examining the control group with the CLP group, there was a significant difference in the amount of IL-6 and IL-17 ($P<0.001$). Also, the CO group had a significant difference with other groups in this index ($P<0.05$).

In the current study, the serum concentration of TNF- α , IL-6 and IL-17 reduced after intervention in CLP and CLPM groups. In IBD, it was observed that IL-10 was adjusted because of its anti-inflammatory effect. Serum concentration IL-10 reduces inflammation in the mucosa, but it is not strong enough to conceal it.

Despite the encouraging results in the present study, the administration of probiotics as a medical intervention requires further testing to obtain confirmatory results and to identify the active components and their mechanisms of action. To confirm the colonization of probiotics in the gut, tracking target bacteria in feces or directly examining intestinal tissue samples using specific probes for probiotics can be employed. Additionally, purification and identification of the active components of these probiotics can help in understanding their pathways and mechanisms of action, which can be considered in future studies.

CONCLUSION

Lactobacillus plantarum MS1 and *Lactobacillus delbrueckii* YN1 have been used as probiotics to treat colitis in the rat model. Our results showed that *Lactobacillus Plantarum* MS1 and *Lactobacillus delbrueckii* YN1 can greatly reduce the symptoms of AA -induced colitis. They also reduced the concentration of inflammatory pre-inflammatory factors (TNF- α , IL-17 and IL-6) and increased IL-10 levels in colitis, showing that *Lactobacillus plantarum* MS1 and *Lactobacillus delbrueckii* YN1 may have

potential use in the treatment of IBD.

Despite the favorable results observed, prescribing probiotics as a clinical treatment requires validation through more rigorous trials. Importantly, intestinal homeostasis seems to be governed by the equilibrium between stimulatory and suppressive immune responses in the gut, which may be positively influenced by probiotic administration.

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