Original Article

Evaluation Recovery of Ulcerative Colitis with a *Lactobacillus* Cocktail Derived from Traditional Dairy Products: *In vivo* Study

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Abstract

Background: This investigation investigates the anti-inflammatory and fibrinolytic effects of a cocktail of probiotics derived from traditional dairy products in a murine model of ulcerative colitis (UC).

Materials and Methods: A mix of newly isolated probiotics containing *L. plantarum, L. brevis, L. delbrueckii*, and *L. helveticus* was characterized and orally administered to inbred eight-week-old C57BL/6 male mice (n = 6). Clinical symptoms, pathohistological changes, and inflammatory and fibrosis markers were analyzed in the existence and absence of probiotics in colitis mice.

Results: Dairy *lactobacillus* probiotics potently attenuated colitis symptoms by decreasing dextran sulfate sodium (DSS)-induced body weight loss, colon shortening, rectal bleeding, and rectal prolapse. Consistently, a cocktail of probiotics could significantly improve histopathological grading by suppressing crypt loss, mucosal damage, and inflammation scores in colitis tissues. Moreover, the mix of probiotics suppressed pro-inflammatory genes including interleukin (IL)-1β, IL-6, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), and increased anti-oxidant markers and activity such as superoxide dismutase and catalase in colon tissue. Furthermore, compared to the no-treated group, the administration of probiotics reduced fibrosis by decreasing collagen deposition in tissue sections and down-regulating levels of pro-fibrotic genes including alpha-actin-2 (Acta2), collagen (Col) 1a1, and Col 1a2 in colitis tissue homogenates.

Conclusions: The results show the newly isolated cocktail of probiotics elicits a potent protective effect on UC symptoms in mice model. Further study on these probiotics is required to fully explore their effectiveness, strength, and safety considerations.

Keywords: Inflammatory bowel disease, *Lactobacillus*, probiotics, ulcerative colitis

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Introduction

Ulcerative colitis (UC) is the predominant form of inflammatory bowel disease (IBD) marked by inflammation and ulcers in the colon.[1] The main clinical characteristics of UC are colicky abdominal pain, dysentery, rectal bleeding, pyrexia,

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and weight loss.[2] Therapeutic treatments for UC include aminosalicylates, corticosteroids, and immunomodulators that reduce inflammatory responses by decreasing expression of pro-inflammatory cytokines. However, current therapeutics alone are inadequate and of low efficacy, resulting in adverse effects during long-term and high-dose treatments.[3,4] There is therefore a need to identify novel therapeutic treatments with higher efficacy to reduce disease progression and alleviate complications in patients with UC.

The pathogenesis of UC is multifactorial and usually involves genetic, inflammatory, and environmental factors such as stress, smoking, and diet, affecting mucosal immune responses and gut bacterial composition.[5,6] Recent studies indicate that the gut microbiome in colitis patients is notably different from their healthy counterparts, suggesting the presence of a relationship between the intestinal microbiome and colitis pathogenesis.[7–10] In this regard, the strategy of manipulating the intestinal microbiota and using probiotics that are effective on the intestinal microbial composition has shown promising results in the treatment of UC.[11,12] Probiotics are live microorganisms that are not easily digested and, when consumed in appropriate quantities, can provide advantages to the host. Probiotics cause a positive change in the intestinal flora by changing the type and number of bacteria in the digestive tract. In addition, probiotics improve intestinal barrier function by reducing permeability and increasing tight junctions.[13,14] Probiotics also have anti-inflammatory action and regulate the host's immune responses. Regarding their proven health benefits, various species of probiotics have been shown to improve the function of the gut microbiota and affect inflammatory diseases of the gastrointestinal tract.[15–17]

In this investigation, we examined the therapeutic potency of a cocktail of four probiotic strains containing *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus helveticus*, and *Lactobacillus delbrueckii*, isolated from traditional dairy products of northeastern Iran alone or in combination with the standard 5-aminosalicylic acid (mesalazine) in a dextran sulfate sodium (DSS)-induced colitis mouse model.

Materials and Methods

Reagents

DSS was bought from Cayman Chemical in Ann Arbor, MI, USA. The real-time polymerase chain reaction (RT-PCR) materials, such as the RNA extraction kit, cDNA synthesis kit, and SYBR green, were obtained from Yekta Tajhiz Azma in Tehran, Iran. Enzyme-linked immunosorbent assay (ELISA) kits were sourced from ZellBio Company in Lonsee, Germany. All the remaining chemicals were purchased from Sigma Aldrich in Missouri, USA.

Assessment of probiotic properties

Identification at the molecular level

Molecular identification of probiotics was previously performed via 16s rRNA gene sequencing as defined.[18]

Biochemical tests

Following inoculating bacteria into de Man-Rogosa-Sharpe (MRS) broth (Merck, Germany), they were cultured on MRS agar (Ibresco, Italy) plates. Next, Gram staining and oxidase and catalase (CAT) tests were performed for purified colonies.[19,20]

Antimicrobial efficacy of probiotics

Isolated strains were evaluated for antibacterial activities against main pathogenic microorganisms including *Staphylococcus aureus* (ATCC 25923), *Salmonella enterica subsp. enterica serotype Typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (PTCC 1338), and *Enterococcus faecalis* (ATCC 29212), using a previously described disk diffusion method (Tagg and McGiven).[21]

Screening of antibiotic resistance

The antibiotic resistance profiles of the strains were evaluated following the protocols outlined by the Clinical and Laboratory Standards Institute (CLSI). Antibiotic discs were positioned on MRS agar plates, and the diameter of the inhibition zones was measured with a caliper.[22–27]

Acid and bile salt resistance assays

The bacteria surviving in acidic conditions and their resistance to the bile salts were assessed using methods developed by Afshari *et al*. (2022). Following the growth of each strain on the MRS agar plate, it was moved to a sterile saline solution (0.85%) to form a 1.0 McFarland suspension. A 10 µl aliquot of the suspension was then applied to agar plates containing varying concentrations of Ox-bile (0.3%, and 1.0%, w/v, Sigma-Aldrich). The plates were anaerobically incubated at 37°C and assessed after 24 hours. Plates without any bacterial growth were classified as negative, while those with colonies were considered positive. Plates lacking Ox-bile served as the control.[28]

Tolerance to simulated gastric and intestinal conditions

A 30 ml overnight culture of each strain in MRS broth was centrifuged at 8,000 ×g for five minutes at 4°C. The supernatant was decanted, and the collected cells were washed twice with 10 ml of 50 mM phosphate-buffered saline (PBS) (pH = 6.5) before being resuspended in 3 ml of PBS buffer. Subsequently, 1 ml of each strain containing 9 log CFU/ml of bacteria was mixed with 9 ml of simulated gastric fluid (composed of pepsin (3 g/L) from Sigma-Aldrich at pH = 2.5) and then incubated at 37°C for three hours. Following this, the suspension was centrifuged at 3,800 rpm for 10 minutes, the supernatant was discarded, and the pellets were washed with PBS. The pellet was resuspended in simulated intestinal fluid containing pancreatin 0.1% w/v (from Sigma-Aldrich) and bile salt 0.15% w/v, at pH = 8.0, and incubated for three hours at 37°C. Post-incubation, the surviving bacterial counts were enumerated and expressed as log CFU/ml.[29]

Hemolysis assay

The *Lactobacillus* isolates were grown in MRS medium for 18–24 hours at 37°C. The streak plate technique was utilized on 5% sheep blood agar plates to assess hemolytic activity.[30]

Cocktail of Lactobacillus probiotics

Lactobacillus strains isolated from yogurt\milk were cultured in MRS broth and collected by centrifugation (6000 g, 4°C, 7 min). The cell pellet was washed and re-suspended in PBS. Next, the concentration of the cocktail was adjusted to 1×10^3 CFU/mL in PBS and administered by oral gavage to mice daily for 10 days.[31]

Animals

Male C57BL/6 mice, eight weeks old and inbred, were procured from the Bo Ali Research Institute in Mashhad, Iran, and housed under Institutional Animal Care Guidelines. The mice were kept under standard conditions, including a 22–25°C temperature range, humidity maintained at 55–60%, a 12-hour light/dark cycle, and unrestricted access to food and water. All animal procedures followed the Care and Use of Laboratory Animals guidelines authorized by the ethics committee of the Mashhad University of Medical Sciences. The study was approved with the ID IR.MUMS.MEDICAL.REC.4010834.

Colitis model and experimental protocol

Thirty C57BL/6 mice were randomly divided into five groups and housed in separate cages. One group, serving as the control, received only drinking water for 10 days (n = 6). The other groups were administered a dextran sodium sulfate (DSS) solution (5%) in their drinking water for seven days to induce symptoms resembling UC in the experimental animals. Specifically, the colitis group received DSS for the initial seven days and normal drinking water from days 7 to 10 (n = 6). The mesalazine group was given DSS solution for the first seven days and then mesalazine (100 µL/mouse/day, administered via oral gavage) from day 4 to day 10 (n = 6). The probiotics mixed group received DSS solution for the initial seven days and probiotics (200 µL/mouse/day, administered via oral gavage) from days 4 to 10 (n = 6). Lastly, the combination group was treated with DSS solution for the first 7 days and received both mesalazine (100 µL/mouse/day, oral gavage) and probiotics mixed (200 µL/mouse/day, oral gavage) from days 4 to 10 (n = 6).[32] A diagram illustrating the experimental protocol is shown in Figure 1a. Subsequently, the mice were anesthetized via an intraperitoneal injection of a combination of ketamine and xylazine administered into the right abdominal area before being euthanized. Following euthanasia, the colon and spleen were harvested, and their weights and lengths were documented. The collected tissues were either fixed in 10% formalin for histological analysis or preserved in liquid nitrogen for subsequent investigations.

Evaluation of disease activity index and spleen index

To investigate the therapeutic effects of probiotics, body weight, stool characteristics, rectal bleeding, and rectal prolapse were evaluated daily as described in the disease activity index (DAI) in Table 1. The weight of the spleen was measured, and the spleen index was calculated as the ratio of the spleen weight to the body weight.[18,33-35]

Table 1: Disease activity index (DAI) was scored at the time of procedure

	DAI			
Score	Rectal bleeding	Stool consistency	Rectal prolapse	Lose weight
0	None	Normal	None	<5%
1	Red	Soft	Sign of prolapse	5–10%
2	Dark red	Very soft	Clear prolapse	10–15%
3	Gross bleeding	Diarrhea	Extensive prolapse	>15%

Histopathological evaluation of colons

Samples of tissue from the distal colon were collected, fixed in formalin, embedded in paraffin, sectioned, and stained using hematoxylin-eosin (H and E) and Masson's trichrome methods. The tissue samples were then analyzed under a light microscope and evaluated based on established histopathological guidelines, with scoring done according to the criteria outlined in Table 2.[36]

Assessment of oxidative stress

To measure the levels of markers for oxidative stress, the levels of superoxide dismutase (SOD) and CAT enzymes were quantified in the colon tissue following the methodology outlined in previous studies.[1,37]

Real-time PCR

Quantitative RT-PCR was conducted following established protocols.[38] The expression levels of genes related to inflammation and fibrosis were analyzed using specific primers sourced from Macrogen (Seoul, Korea) [see Table 3].

ELISA assay

The tissue concentration of tumor necrosis factor-alpha (TNF-α) was assessed in colon tissue samples from mice. Specimens were homogenized in PBS and protein concentration was measured by the bicinchoninic acid assay (BCA) method. Tissue TNF-α levels were counted using an ELISA kit as expressed (Elabscience Biotechnology Co., Ltd., Wuhan, China).

Data analysis

The data are shown as mean values with a standard error of the mean and were statistically analyzed using Tukey's multiple comparison tests, Student's *t*-test, or analysis of variance (ANOVA). Statistical analyses were conducted employing Statistical Package for the Social Sciences (SPSS) version 20 software (IBM, Chicago).

Results

Biochemical tests

The biochemical test results indicated that all four isolated strains identified as *Lactobacillus* are gram-positive, CAT-negative, and oxidase-negative bacteria.

Antibacterial activity of probiotics strains

Antibacterial tests against various pathogenic organisms showed that all four strains have antibacterial activity against

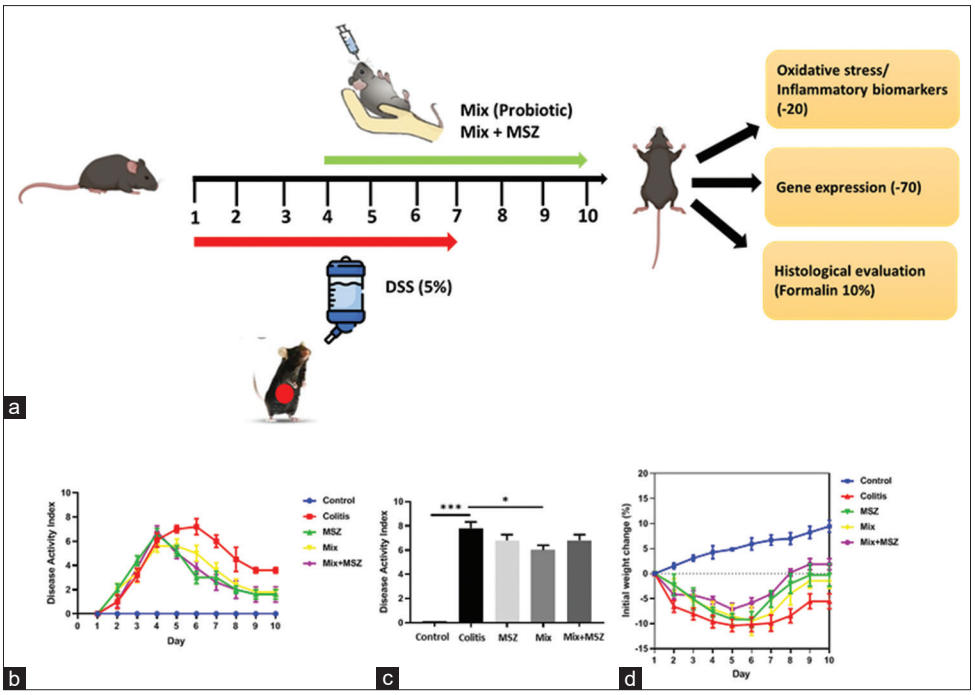

Figure 1: Schematic representation of the experimental design of the study and protective effects of probiotics on colitis clinical symptoms. (a) Schematic representation of the murine colitis model and experimental protocol. (b) The inhibitory effect of probiotic mix on DAI at different time points is presented. (c) The highest DAI during the experiment period (10 days) is shown in each group. (d) The effects of probiotic mix (200 μL/kg/day) alone or in combination with MSZ on body weight loss in DSS-treated mice. MSZ: mesalazine

Table 2: Colorectal tissue damage was scored according to histopathological criteria

Score	0	1	2	3	4
Inflammation	None	Mild	Moderate	Severe	
Mucosal damage	None	Mucus layer	Submucosa	Muscular and serosa	100% with epithelium lose
Crypt loss	None	1/3	2/3	100% + intact epithelium	76–100%
Pathological change range	None	1–25%	26–50%	51–75%	

Table 3: qPCR primers sequence

Gene	Source	Primer	Sequence
GAPDH	Mouse	Forward	CAACGACCCCTTCATTGACC
Col 1a1	Mouse	Reverse	CTTCCCATTCTCGCCTTGA
Col 1a2	Mouse	Forward	AATGGTGAGACGTGGAAACC
IL-1β	Mouse	Reverse	GACAGTCCAGTTCTTCATTGCA
TNF-α	Mouse	Forward	GTTCTCAGGGTAGCCAAGGT
α-SMA	Mouse	Reverse	CCTTCAAAACCAAAGTCATAGCC
IFN-γ	Mouse	Forward	GACTTCACCATGGAATCCGT
IL-6	Mouse	Reverse	TGCTCATTCACGAAAAGGGA
		Forward	AGGCTGTCGCTACATCACTG
		Reverse	CTCTCAATGACCCGTAGGGC
		Forward	CCCAGACATCAGGGAGTAATGG
		Reverse	TCTATCGGATACTTCAGCGTCA
		Forward	TGGCTGTTTCTGGCTGTTAC
		Reverse	CTCTTTTCTTCCACATCTATGCC
		Forward	TCTATACCACTTCACAAGTCGGA
		Reverse	GAATTGCCATTGCACAACTCTTT

Staphylococcus aureus (ATCC 25923) and *Salmonella enterica subsp. enterica serotype Typhimurium* (ATCC 14028). Also, *L. brevis, L. helveticus*, and *L. delbrueckii* strains have antibacterial activity against *Pseudomonas aeruginosa* (ATCC 9027), while *L. plantarum* showed no inhibitory effect on *Pseudomonas aeruginosa* (ATCC 9027). All four strains exhibited no antimicrobial activity against *Enterococcus faecalis* (ATCC 29212). Moreover, among the four strains, only *L. delbrueckii* exerted antimicrobial activity against *Escherichia coli* (PTCC 1338) and showed the highest inhibitory effect on standard pathogens [Table 4].

Antibiotic resistance screening

Assessing the susceptibility of the strains to various commercial antibiotics showed that all strains were sensitive to erythromycin, ampicillin, chloramphenicol, and tetracycline but highly resistant to ciprofloxacin, followed by gentamicin. The results of the antibiotic sensitivity test were classified based on the size of the inhibition zone (mm) around the paper disc containing the microbial cell-free supernatant: sensitive (>12 mm), intermediate (10–11 mm), and resistant (>9 mm). The findings of the resistance test are presented in Tables 5 and 6.

Table 4: Antibacterial activity of *Lactobacillus* strains against pathogenic strains

Strain	*Salmonella enterica serotype Typhimurium*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Enterococcus faecalis*	*Staphylococcus aureus*
L. plantarum	Sensitive	Resistant	Resistant	Resistant	Sensitive
L. brevis	Sensitive	Resistant	Sensitive	Resistant	Sensitive
L. helveticus	Sensitive	Resistant	Sensitive	Resistant	Sensitive
L. delbrueckii	Sensitive	Sensitive	Sensitive	Resistant	Sensitive

The inhibition zone (mm) around the paper disc containing the microbial cell-free supernatant was classified as sensitive, >12 mm; intermediate, 10–11 mm; resistant, <9 mm

Table 5: The bacterial antibiotic resistance patterns (mm) based on the CSLI guidelines

Antibiotics	Resistant (R)	Intermediate (I)	Sensitive (S)
Ciprofloxacin (5 µg)	≤15	16–20	≥21
Erythromycin (15 µg)	≤15	16–20	≥21
Gentamicin (10 µg)	≤12	13–14	≥15
Vancomycin (30 µg)	≤9	10–11	≥12
Tetracycline (30 µg)	≤18	19–22	≥23
Clindamycin (2 µg)	≤15	16–18	≥19
Ampicillin (10 µg)	≤12	13–15	≥16
Chloramphenicol (30 µg)	≤12	13–17	≥18
Sulfamethoxazole/ trimethoprim (25 µg)	≤10	11–15	≥16

Table 6: The bacterial antibiotic resistance patterns based on the diameter (mm) of the inhibition zones

Antibiotic	*L. helveticus*	*L. delbrueckii*	*L. brevis*	*L. plantarum*
Ciprofloxacin	0(R)	15(R)	0(R)	0(R)
Erythromycin	17(I)	31(S)	24(S)	23(S)
Gentamicin	13(I)	0(R)	4(R)	10(R)
Vancomycin	0(R)	25(S)	24(S)	0(R)
Tetracycline	21(I)	30(S)	28(S)	20(I)
Clindamycin	10(R)	8(R)	18(I)	19(S)
Ampicillin	27(S)	27(S)	26(S)	32(S)
Chloramphenicol	30(S)	26(S)	26(S)	27(S)
sulfamethoxazole/ trimethoprim	30(S)	0(R)	0(R)	16(S)

Sensitive (S), intermediate (I), and resistant (R)

Table 7: Acid and bile tolerance of isolates after four hours

Species	0.3% bile	1% bile	pH 3.0
L. plantarum	+	−	+
L. brevis	+	+	−
L. helveticus	+	+	+
L. delbrueckii	+	−	+

Bacterial growth: +, no growth: −

Acid and bile salt resistance assays

The ability to survive in the stomach and intestine is a vital characteristic necessary for probiotics. The growth of the four selected isolates was influenced differently by acidic conditions and exposure to bile salts. As shown in Table 7, two isolates were tolerant of bile salt and three were tolerant of low pH.

Tolerance to the GIT (Gastrointestinal Tract) condition

The upper gastrointestinal tract consists of the stomach, which has a low pH, and the small intestine, which contains bile salts and digestive enzymes. For a probiotics strain to be effective, it needs to pass through this tract while remaining alive and without experiencing a significant decrease in numbers [Table 8]. In this particular study, the probiotics strains were initially inoculated at a concentration of 9 log CFU/ml. Following exposure to simulated gastric and intestinal conditions, variations in the number of probiotics strains were observed, depending on the kind of strain. Among the strains tested, *L. helveticus* exhibited the highest resistance to the simulated gastric and intestinal conditions when compared to the standard strain.

Hemolysis assay

To assess the safety of the bacteria, the hemolytic activity of the lactobacilli was examined. Our findings indicated that there was no hemolytic activity observed for both α- and β-hemolysis, demonstrating that the strains did not cause damage to red blood cells (RBCs) and were deemed safe.

Probiotics reduced the severity of clinical symptoms associated with UC in a mouse model

Mice treated with DSS showed clinical symptoms of UC including DAI and body weight loss. Our findings demonstrate that the administration of probiotics with or without mesalazine can significantly reduce the DAI parameters including stool consistency and rectal bleeding [Figure 1b and c]. To delve deeper and explore the protective function of probiotics in UC, body weight loss was compared between different groups. Administration of probiotics and/or mesalazine successfully mitigated body weight loss when compared to the control group [Figure 1d]. Furthermore, treatment with probiotics reduced colonic shortening [Figure 2a] and colon weight loss [Figure 2b] in colitis mice. Additionally, probiotics enhanced the colon weight to colon length ratio, which is an indicator of inflammation and tissue swelling [Figure 2b–d]. Similarly, probiotic treatment enhanced spleen weight [Figure 3a–b] and spleen-to-body weight ratio [Figure 3c]. Enhanced spleen weight is considered a marker of splenic macrophage infiltration. These findings indicate the therapeutic properties of probiotics in reducing clinical symptoms of UC, either alone or in combination with mesalazine.

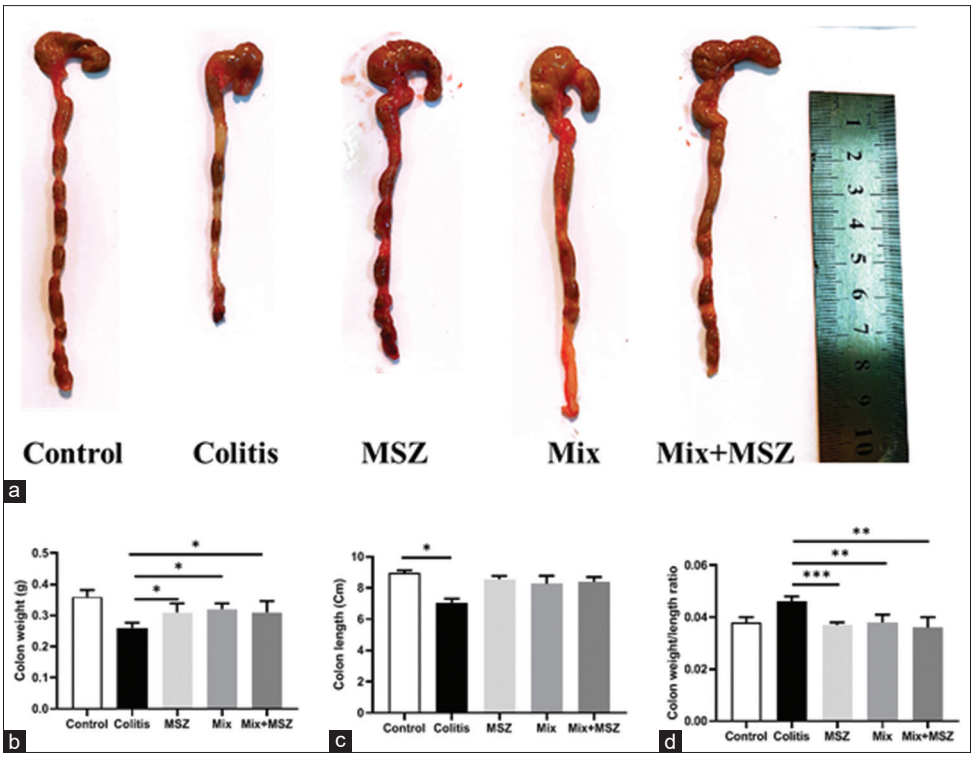

Figure 2: Probiotics attenuated colon shortening in colitis mice. (a) Probiotic mix efficacy against DSS-induced colon shortening was evaluated. (b) Colon weights, (c) colon length, and (d) colon weight to colon length ratio as a marker of inflammation were compared between the control group, probiotic–treated, and untreated colitis mice. $^{**}P < 0.01$, $^{***}P < 0.001$. Data were presented as mean ± SEM (Structural equation modeling); DAI, colon weight, and length were analyzed by one-way ANOVA followed by a post-hoc LSD (Least Significant Difference) test. Data are representative of three independent experiments with six mice in each group (n = 6)

Table 8: The resistance of Lactobacillus spp. to simulated gastric and intestinal fluid (log/ml)

Strain	Gastric		Intestine	
	0 (hrs)	2 (hrs)	4 (hrs)	6 (hrs)
L. helveticus	7.477	6.560	4.356	3.736
L. brevis	7.280	6.571	3.435	0
L. delbrueckii	7.338	4.657	3.259	0
L. plantarum	8.134	3.435	3.259	0

Probiotics reduced colon tissue damage and inflammation

Next, we compared the colon histopathological differences between probiotic-treated and untreated groups. As shown in Figure 4, treatment with probiotics clearly reduces the histopathological score [Figure 4f] by decreasing inflammation [Figure 4b], mucosal damage [Figure 4c], crypt loss [Figure 4d], and pathological change [Figure 4e] in DSS-induced colitis. To evaluate the anti-inflammatory mechanism of probiotics, the expression level of pro-inflammatory cytokines was compared between different groups. Our results indicate that treatment with probiotics significantly reduced mRNA expression of interleukin-1β (IL-1β), interleukin-6 (IL-6), TNF-α, and interferon-gamma (IFN-γ) [Figure 5a]. Consistent with this, probiotics administration significantly reduced the protein levels of TNF-α compared to the positive control group, as shown in the ELISA assay results [Figure 5b].

To further investigate the anti-inflammatory functions of the probiotics cocktail, we evaluated the modulatory role of probiotics on oxidant/anti-oxidant status in colitis tissue homogenates. Treatment with probiotics significantly elevated SOD and CAT activities, indicating the antioxidant properties of probiotics in colitis tissues [Figure 5c and d].

Probiotics reduced fibrosis in colon tissue

The histopathological staining findings demonstrated that probiotics administration decreased collagen buildup and fibrosis in the colon tissue when compared to the colitis control group [Figure 6a–b]. Consistently, treatment with probiotics significantly down-regulated the expression of pro-fibrotic genes including alpha-actin-2 (Acta 2), collagen type 1 alpha 1 (Col1a1), and 2 (Col1a2) [Figure 6c] in tissue samples. These data clearly support the presence of anti-fibrotic mechanisms in probiotics against DSS-induced colitis.

Discussion

We demonstrated the therapeutic potency of a probiotic cocktail of four *Lactobacillus* strains isolated from traditional dairy products in northeastern Iran. Our macroscopic and microscopic findings showed that treatment with these probiotics significantly improved the DAI and colon histopathological scores in a DSS-induced colitis mouse by

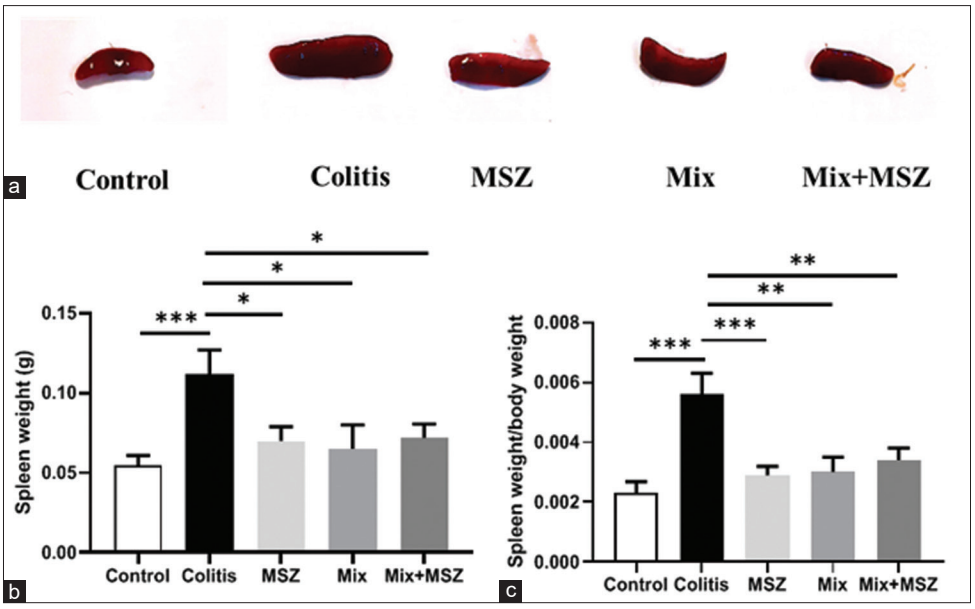

Figure 3: Probiotics reduced DSS-induced spleen tissue inflammation. (a) Protective effects of probiotic mix against DSS-induced spleen inflammation. (b) Spleen weight and (c) spleen weight to body weight ratio as a marker of inflammation were compared between the control group, probiotic-treated, and untreated colitis mice. $^{**}P < 0.01$, $^{***}P < 0.001$

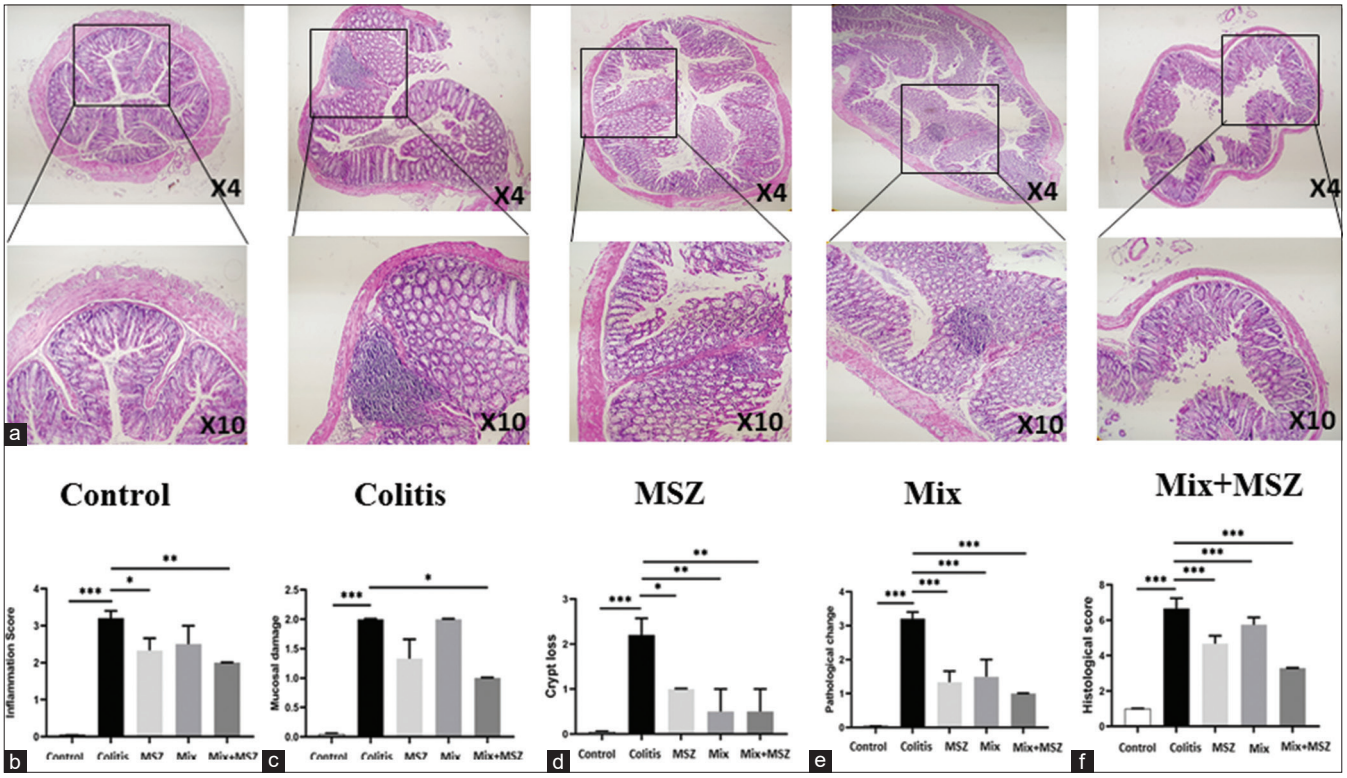

Figure 4: Probiotic reduced DSS-induced colon tissue inflammation. (a) Hematoxylin and eosin (H and E)-stained sections of colons from indicated groups of mice showing representative histopathological damage and crypt loss induced by DSS. (b) Quantifying the effect of probiotic mix on the inflammation score, (c) mucosal damage, (d) crypt loss, (e) pathological changes, and (f) histological score in DSS-induced colitis mice

inducing antioxidant, anti-inflammatory, and anti-fibrosis responses. Interestingly, we have shown that the therapeutic effects of mesalazine can be improved in combination with traditional probiotics by attenuating disease progression, fibrosis, and inflammatory responses.

Treatment with DSS induces mucosal damage and disrupts epithelial cell membranes by elevating reactive oxygen species (ROS) in the intestinal tissue.[4,36] Disturbance in the cell membrane facilitates the infiltration of immune cells into submucosal layers that induce inflammatory reactions

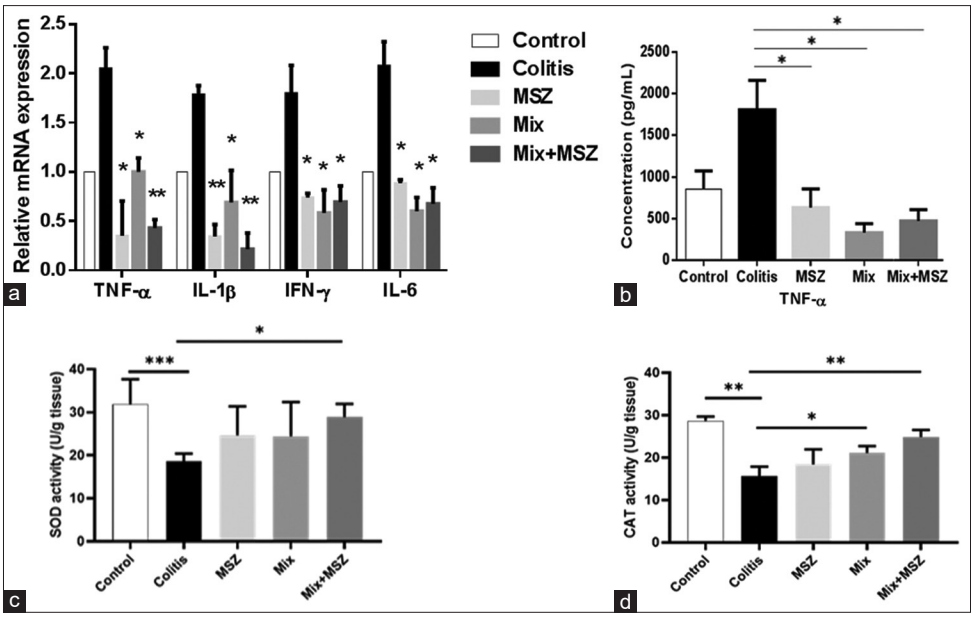

Figure 5: Anti-inflammatory and anti-oxidant effects of probiotic on colitis. (a) Probiotic mix significantly decreased expression levels of pro-inflammatory genes in colitis mice compared to the untreated colitis group. (b) Probiotics significantly reduced the protein expression of TNF-α in colon tissues. (c) SOD activity and (d) CAT activity were compared between different groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data were presented as mean ± SEM. Data are representative of three independent experiments (n = 6)

by releasing various growth factors and proinflammatory cytokines, including TNF-α, IL-1β, and IL-6.[33,34] Recent findings revealed that the upregulation of these proinflammatory cytokines is directly related to the severity of inflammatory reactions and DAI in UC.[35,39] Therefore, the expression levels of proinflammatory cytokines like TNF-α may be considered as potential biomarkers for evaluating colitis-associated inflammatory responses.[40]

Consistently, our results indicated that administration of DSS induces colitis clinical symptoms and upregulates proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ. Emerging evidence demonstrates the therapeutic potency of probiotics in colitis patients by downregulating proinflammatory mediators like TNF-α and IL-6.[41,42] Recent findings show that *Lactobacillus plantarum* has significant protective properties against inflammatory responses by suppressing TNF-α and IL-8 in DSS-induced colitis.[43,44] Consistently, Hegazy and El-Bedewy[45] demonstrated that two probiotics of *Lactobacillus strains* (*L. delbrueckii* and *L. fermentum*) have significant anti-inflammatory effects in colitis mice. Their findings revealed that administration of these probiotics significantly attenuates inflammation by reducing IL-6, TNF-α, and NF-kB (p65) in colon tissues. In agreement with these studies, Pan *et al*.[46] reported that treatment with *Lactobacillus plantarum* ZS62 reduces inflammatory processes and modulates oxidant/anti-oxidant balance in DSS-induced colitis by downregulating IL-1β, IL-6, IL-12, and TNF-α and regulating oxidant-anti-oxidant balance. Consistent with these findings, our results revealed that probiotics with or without mesalazine can significantly reduce inflammatory responses and improve oxidant/anti-oxidant status by suppressing proinflammatory mediators and enhancing the activities of anti-oxidant mediators including CAT and SOD enzymes in colon tissues. To further investigate the anti-inflammatory effects of probiotics in UC, we evaluated various inflammatory indicators like colon weight-to-length ratio and spleen-to-body weight ratio. Treatment with probiotics significantly improved these inflammatory markers either alone or in combination with mesalazine.

Fibrosis, another key pathological feature of colitis, is associated with the proliferation of fibroblast cells and the upregulation of several growth factors and profibrotic genes including Acta2, Col1a1, and Col1a2.[34,47,48] Enhanced expression of these profibrotic genes causes excessive production of extracellular matrix (ECM), collagen deposition, and fibrosis in the injured area.[1,49] There are several studies supporting the protective effects of probiotics in the fibrosis process.[50,51] In an animal study, the anti-fibrotic properties of *Lactobacillus acidophilus* were evaluated on liver and colon fibrosis. Their results indicate that *L. acidophilus* attenuates fibrosis by suppressing fibrotic markers such as TGF-β1, Acta2, and collagen.[52] In another study, the anti-inflammatory and anti-fibrotic effects of *Lactiplantibacillus plantarum* IMC513 were investigated in a colitis model. Results showed that *Lactiplantibacillus plantarum* decreased the expression of Acta2 and collagen I–III. Further results of Masson's trichrome staining revealed that administration of *Lactiplantibacillus plantarum* significantly attenuates fibrosis and collagen deposition in colitis tissues.[53] Consistently, our results demonstrated that probiotics significantly decrease the expression of fibrotic markers including Acta2, Col1a1, and Col1a2 either alone or in combination with Mesalazine.

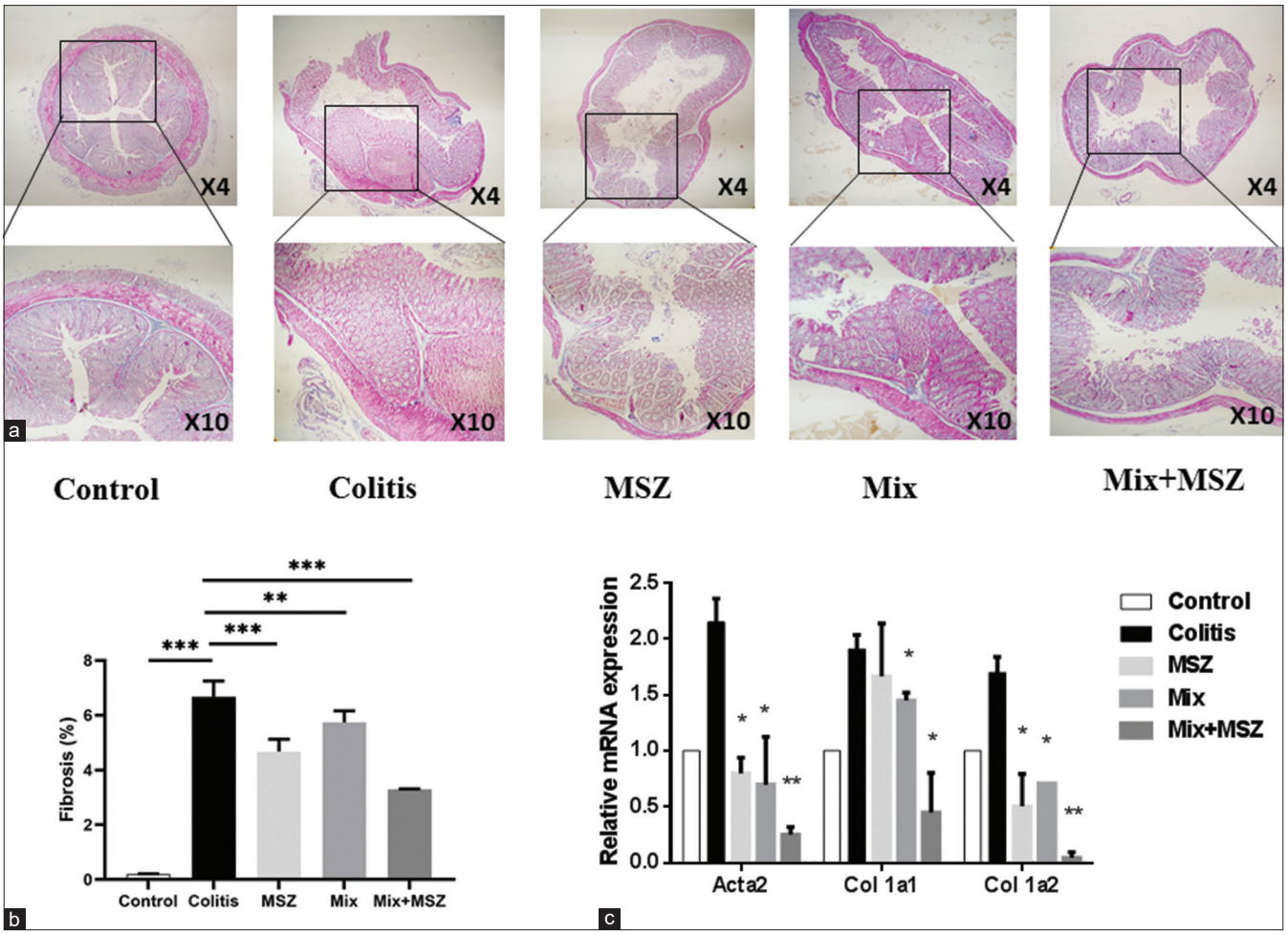

Figure 6: Probiotics attenuate fibrosis and reduce collagen content. (a) Collagen fiber was detected using Masson's trichrome staining. Mix alone or in combination with mesalazine reduces collagen deposition. (b) Digital image analysis of collagen deposition and fibrosis. (c) qRT-PCR results showed the potential effect of Mix on reducing pro-fibrotic genes including Acta2, Col1a1, and Col1a2. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data were presented as mean ± SEM. Data are representative of three independent experiments with six mice in each group (n = 6)

In addition, analysis of Masson's trichrome-staining results indicates that treatment with probiotics potently reduces collagen deposition in DSS-induced colitis tissues.

Conclusion

In this study, we demonstrated that the mixture of four probiotic strains isolated from traditional dairy products including *L. plantarum, L. brevis, L. delbrueckii,* and *L. helveticus* had significant inhibitory effects on the inflammatory and fibrotic responses in DSS-induced colitis mice model. Further preclinical studies are required to elucidate the molecular mechanism of probiotic-mediated attenuation of colitis. The results and analyses presented herein support the therapeutic potential of probiotics formulations as a new and alternative strategy for UC and other diseases in the future.

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Conflicts of interest

The authors have no conflicts of interest.

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