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Research Article

Systemic Effects of a Multi-Strain Probiotic Formulation in an Acetic Acid Induced Colonic Ulcer Model in Male Albino Rats

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Abstract | The study aimed at determining the systemic influence of probiotic administration in an experimental pattern of acetic acid-stimulated gastric lesions of the colon in male rats. The animals were selected randomly into four different groups, including healthy control (C), ulcer-induced group (T1), ulcer-induced rats treated with probiotics (T2), and probiotic control group (T3) (n = 6/group). Acetic acid was used to induce colonic ulceration with the probiotics administration remaining daily over 14 days. Hematological variables, liver and kidney biochemical variables, oxidative stress parameters and cytokines of inflammation were measured. The hypothesis was that multi-strain probiotics supplementation would mitigate the systemic inflammatory and oxidative effects that would result after acetic acid-induced colonic injury, such as liver and renal biochemical markers increase, lipid peroxidation amplification, loss of antioxidant defenses, and upregulation of pro-inflammatory cytokines. Probiotic supplementation caused a substantial reduction in these biochemical indices, oxidative, and inflammatory parameters, an indicator of alleviation of systemic perturbations occurring in the wake of intestinal damage. There was a substantial decrease in ALT, AST, creatinine, MDA, TNF-α, IL- 6, and CRP values relative to those of the untreated ulcer group. These results suggest that the systemic effects of experimental colonic ulceration can be regulated by the administration of probiotics.

Novelty Statement | The paper presents a systemic analysis of the multi-strain probiotics in an acetic acid-induced colonic ulcer model, which goes beyond local intestinal effects. It emphasizes the use of probiotics in the regulation of hematological, hepatorenal, oxidative, and inflammatory parameters all at the same time, providing a greater mechanistic understanding of their systemic therapeutic action.

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INTRODUCTION

The current rise in the prevalence of Ulcerative colitis (UC) in the world has escalated the need to seek adjunctive therapeutic options that would help in the regulation of the inflammatory and immune pathways. In this light, probiotics have been subject to a significant amount of attention as potentially beneficial adjunctive treatment due to their capacity to control the microbiota constitution of the gut as well as immune response (Jadhav *et al.*, 2023). According to recent epidemiological evidence, chronic inflammatory bowel disorder has become a major health issue in the world, especially among the adolescents. Ulcerative colitis is regarded as a multi-causal disease with the predisposition which is hereditary, external factors and dysregulation of the immune responses (Derikx *et al.*, 2016). Another key contributor to the progression and development of ulcerative colitis is the intestinal microbiome as a key determinant of the human environment. Emerging

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chronic inflammatory diseases such as ulcerative colitis are associated with dysbiosis, or the disproportionality in the constitution of the gut microbes (Jadhav *et al*., 2023). Such imbalance of microorganisms triggers a series of inflammatory events which destroy the intestinal mucosa and result in chronic inflammation of the intestine. Nevertheless, the existing treatment options are still not optimal, at one year, only about 40 percent of the cases can be put in remission (Rubin *et al*., 2019). Accordingly, growing emphasis has been placed on complementary therapeutic approaches, and probiotics have attracted interest as a complementary clinical intervention. Probiotics represent viable microorganisms that, upon provided in adequate amounts, exert supportive effects on the host's physiological functions. Growing scientific interest has centered on probiotics, particularly regarding their potential as supportive therapeutic agents in inflammatory disorders of the gastrointestinal tract, comprising inflammatory bowel disease. Probiotics influence the course of ulcerative colitis via diverse biological pathways. Their actions involve reestablishing microbial equilibrium within the intestinal ecosystem, enhancing mucosal barrier integrity, and modulating local immune activity in the gastrointestinal tract. Animal studies have shown that probiotics can help with manifestations of UC, including a decline in body mass, diarrhea, hematochezia, and colon shortening. They can also restore the balance between microorganisms in the intestine, strengthen the intestinal barriers and influence immune signaling within the intestinal mucosa (Wan *et al*., 2022). Experimental models provide a controlled platform to evaluate pathophysiological mechanisms and therapeutic interventions, such as physical aspects such as oxidative stress indicators and histopathological changes. These models facilitate mechanistic understanding of probiotic-mediated therapeutic effects. Recent research has shifted from focusing solely on clinical symptom improvement toward more comprehensive mechanistic and systemic evaluations. *Saccharomyces boulardii* is recognized to protect the recipient from inflammatory impairment, which via reducing the heightened sensitivity of the gut and changing the profiles to inflammatory cytokines (Bindels *et al*., 2015). Nevertheless, although the evidence on the intestinal effects of probiotics has been on the rise, there are scarce information on their overall biochemical and inflammatory impact on the body in acute experimental colonic ulcer models. Thus, the present research was undertaken to help fill this knowledge deficit evaluating the systemic effect of multi-strain probiotic supplementation in albino rats in which colonic ulceration was induced by acetic acid. The aim of the investigation was to give a mechanistic understanding of the possible systemic regulatory effects of probiotics in an experimental pattern of intestinal injury.

Materials and Methods

Experimental animals

The use of adult male albino rats in the current investigation was acquired in a certified animal breeding institute. The animals used were 8-10 weeks old and were in the range of body weights of between 180-220g when the experiment began. Control environmental conditions were kept in which the rats were housed in polypropylene cages, a 12-h light/dark cycle, relative humidity of 55 ± 5 and surrounding temperature of 22±2 °C. *Ad libitum* feeding was given to the participants using regular pellet diet in the laboratory and tap water. All animals were made to acclimate to laboratory conditions before experimental procedures commenced and this took one week.

Probiotic preparation

The probiotic product consisted of a multi-strain combination including *Lactobacillus acidophilus* NCFM (2 × 10^9 CFU/g), *Bifidobacterium lactis* Bi-07 (1× 10^9 CFU/g), and *Lactobacillus plantarum* LP-115 (1×10^9 CFU/g). The bacterial strains were supplied by the microbiology laboratory culture repository, Department of Biology, University of Al-Qadisiyah, and had been previously characterized for key probiotic attributes, including tolerance to acidic conditions, resistance to bile salts, epithelial adhesion capacity, and antimicrobial activity.

For experimental use, isolates were cultivated in De Man, Rogosa and Sharpe (MRS) broth at 37°C for 24 h under anaerobic conditions. Following incubation, bacterial cells were collected by centrifugation at 4,000 × g for 15 min at 4°C, rinsed with phosphate-buffered saline (PBS; pH 7.4), and reconstituted in sterile saline to achieve a final density of nearly 1 × 10^{10} CFU/mL. Bacterial viability prior to administration was verified using standard plate count methodology on MRS agar.

Experimental protocol: Using a random allocation protocol, the animals were distributed into four separate study cohorts (six rats per cohort) as detailed below:

Group C (Control): Animals received standard diet and vehicle (sterile saline, 1 mL/kg body weight) by oral gavage for 14 sequential days.

Group T1 (Ulcer-Induced): Animals received standard diet and vehicle for 14 days. On day 8, colon ulcers were induced using acetic acid (4% v/v, 2 mL instillation).

Group T2 (Probiotic-Treated): Animals received probiotic formulation (1 × 10^{10} CFU/mL, 1 mL/kg body weight) by oral gavage for 14 sequential days. On day 8, colon ulcers were induced using acetic acid (4% v/v, 2 mL instillation).

Group T3 (Probiotic Control): Animals received probiotic formulation (1 × 10^{10} CFU/mL, 1 mL/kg body weight) by oral gavage for 14 sequential days without ulcer induction.

The research population was made up of twenty-four rats which were stratified to form four different experimental arms and each arm comprised of six rats. Assignments to the respective arms were done by a simple randomization procedure to reduce the chances of selection bias. The sample size (six rats per arm) was informed by the well-known protocols of inducing ulcers in the literature of earlier studies and was considered sufficient to identify any significant biological difference in the experimental conditions.

Colonic ulceration induction

After an overnight fast in which the two groups (T1 and T2) were allowed free access to water, ulcerative lesions were induced experimentally in the T1 and T2 groups by intracolonic injection of 4 percent (v/v) acetic acid (2 mL). This procedure was conducted under light anesthesia with ketamine (50 mg/kg) and xylazine (5 mg/kg). Lubricated flexible catheter was carefully placed 6 cm deep into the rectocolonic area and the acetic acid solution was administered to C and T1 groups and the probiotic preparation was offered to T2 and T3 groups 6 h after inducing an ulcer. The treatments were carried on throughout the experimental period of 14 days.

Processing and collection of samples

Day 15, the animals were put on a 24-h fast and anesthetized intraperitoneally using ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood samples were retrieved through cardiac puncture and were subdivided into two. A portion was put into EDTA-containing tubes to be assessed hematologically, the other portion was left to clot at ambient temperature in 30 min. The clotted samples were centrifuged at 3,000xg at 4o C in 15 min to separate serum, which was saved to undergo biochemical analyses. Cervical dislocation was used to complete euthanasia. A longitudinal incision was made in the colon and ice-cold phosphate-buffered saline (PBS) was used to empty the lumen and allow examination of any obvious lesions. The samples were snap-frozen in liquid nitrogen and kept at -80 C awaiting further analyses.

Hematological assessment

The parameters of the peripheral blood were measured by assessing complete blood count (CBC) and using automated hematology system (Sysmex XN-1000, Sysmex Corporation, Japan). Important indices were measured and recorded such as hemoglobin level, erythrocyte count and leukocyte count, which were to be examined later.

Assessment of hepatic and renal biomarkers

In accordance with the instructions of the supplier, serum biochemical indicators of liver and kidney functions were determined through measuring the value of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine by using standardized colorimetric assay kits (Biosystems S.A., Barcelona, Spain). A semi-automated clinical chemistry analyzer was used to perform the analytical procedures (Erba Chem 7, Erba Diagnostics, Germany).

Determination of oxidative stress

Colonic tissue homogenates were prepared with the following tool: A mortar and pestle.

To facilitate measure the indices of oxidative stress, the colonic samples were subjected to get the tissue homogenates. In a nutshell, homogenization of specimens was done at a ratio of 10% (w/v) in frosty phosphate-buffered saline (PBS; pH 7.4) with 1 mM of EDTA, in Teflon glass homogenizer. The homogenates were then centrifuged at 10,000 x g over 15 min at a temperature of 4 °C and the resulting supernatant was carefully harvested in order to determine the oxidative stress biomarkers.

Estimation of lipid peroxidation

Lipid peroxidation was determined by evaluating the concentration of malondialdehyde (MDA) as recommended by thiobarbituric acid reactive substances (TBARS) procedure by Ohkawa *et al.* (1979). In order to perform the assay, aliquots of tissue supernatant were gently mixed with sodium dodecyl sulfate (8.1%), acetic acid (20% pH 3.5), and thiobarbituric acid (0.8%), bringing the total volume of the mixture to 0.2 mL.The mixture was heated to 95 o C in 60 min to promote the formation of chromogen, at which point it was allowed to cool to ambient temperature. The products of the reaction were filtered with n-butanol (15:1, v/v) and centrifuged at 4,000 × g during 10 min to separate the organic layer. Absorbance of the top layer was spectrophotometrically at 532 nm. The outcomes were presented in the form of nanomoles MDA per milliliter (nmol/mL).

Activity of superoxide dismutase (SOD)

The superoxide dismutase activity was measured in the colonic tissue tissues based on the technique described by Kakkar *et al.* (1984). This was determined by the ability of SOD to inhibit the degradation of nitroblue tetrazolium (NBT) catalyzed by the superoxide anions produced in the reaction system in the presence of 0.1 mL of tissue supernatant, 1.2 mL of sodium pyrophosphate buffer (0.052 M; pH 8.3), 0.1 mL of phenazine methosulfate (186 μM) and 0.3 mL of nitroblue tetrazolium (The mixture was reacted with the addition of 0.2 mL of NADH (780 μM) and left to react at 30C, 90 seconds. The reaction was stopped by adding 1 mL of glacial acetic acid.The chromogenic product was then extracted with 4 mL of n-butanol. After centrifugation, the top organic layer was separated and its absorbance determined spectrophotometrically at 560 nm. The enzymatic activity was determined and given in units per milliliters (U/mL).

Determination of reduced glutathione (GSH)

Intracellular reduced glutathione values were quantified using the colorimetric method described by Ellman (1959). Briefly, 0.5 mL of tissue homogenate was precipitated with an equal volume of 5% trichloroacetic acid, followed by centrifugation at 3,000 × g for 10 min to obtain a clear supernatant. An aliquot (0.1 mL) of the supernatant was then mixed with 0.5 mL of Ellman's reagentprepared from 5,5′-dithiobis-(2-nitrobenzoic acid) dissolved in 0.1% sodium citrate and 3 mL of phosphate buffer (0.2 M; pH 8.0). The formation of the yellow-colored chromophore was measured spectrophotometrically at 412 nm. Glutathione concentrations were calculated and reported as nmol/mL.

Measurement of inflammatory biomarkers

Circulating levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) were quantified in serum samples using commercially available ELISA kits, strictly adhering to the protocols provided by the manufacturers. All biochemical and hematological assessments were conducted by investigators who were unaware of the experimental allocations to ensure objectivity and minimize bias.

Statistical analysis

Quantitative findings were presented as mean values accompanied by standard deviation (SD). The assumption of normal distribution was verified using the Shapiro–Wilk test. Intergroup comparisons were performed using one-way analysis of variance (ANOVA), and significant differences were further examined through the least significant difference (LSD) post hoc procedure. Effect magnitude was determined using eta squared (η^2). Statistical significance was established at a threshold of $p < 0.05$. Data processing and analyses were carried out using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA).

Results

Hematological parameters

The impact of probiotic treatment on hematological parameters in male rats with induced colon ulcer is represented in Table 1. One-way ANOVA showed a significant treatment-related effect on hemoglobin values ($F(3,20) = 28.9$, $p < 0.0025$, $\eta^2 = 0.81$). Control group (C): values of all parameters were within the reference range whereas the induction of ulcer (T1) proved to induce significant changes in hematological profile. Hemoglobin was significantly lowered ($p<0.0025$) in the ulcer-induced group (T1, 11.7 ± 0.8 g/dL) than in C (14.2 ± 0.5 g/dL). Probiotic treatment improved hemoglobin levels in ulcerated rats. Hemoglobin values in the probiotic control group (T3: 14.0 ± 0.4 g/dL) did not differ significantly from the control cohort. Red blood cell (RBC) counts exhibited a pattern similar to hemoglobin levels, although in this case ulcer induction led to a notable reduction (T1, 5.9 ± 0.5 $\times 10^{12}$/L) relative to the control cohort (C, 7.8 ± 0.4 $\times 10^{12}$/L). Intergroup comparison through one-way ANOVA indicated a significant effect on erythrocyte count ($F(3,20) = 34.7$, $p < 0.0019$, $\eta^2 = 0.84$). The gradual increase in RBC count observed in the probiotic-treated groups T2 (6.7 ± 0.3 $\times 10^{12}$/L) and T3 (7.5 ± 0.4 $\times 10^{12}$/L). RBC values in T3 were consistent with control values. The WBC count was markedly greater ($p<0.0018$) in ulcer-induced group relative with control level (T1: 12.5 ± 1.2 $\times 10^9$/L and C: 7.2 ± 0.9 $\times 10^9$/L), which implied inflammatory reaction to the ulceration injury. A statistically meaningful intergroup difference was detected for WBC count ($F(3,20) = 41.2$, $p < 0.0018$, $\eta^2 = 0.86$). Leukocytosis was significantly reduced in the probiotic-treated groups, and WBC levels in the probiotic control group (T3: 7.3 ± 0.8 $\times 10^9$/L) were consistent with measurements recorded in controls.

Table 1: The effect of probiotic on Hematological Parameters in male Rats with Colon Ulcer-Induced

Parameter	C	T1	T2	T3	p-value	η^2	$LSD_{0.05}$
Hemoglobin (g/dL)	14.2 ± 0.5^a	11.7 ± 0.8^c	13.1 ± 0.6^b	14.0 ± 0.4^a	0.0025	0.81	0.74
RBC Count ($\times 10^{12}$/L)	7.8 ± 0.4^a	5.9 ± 0.5^c	6.7 ± 0.3^b	7.5 ± 0.4^a	0.0019	0.84	0.52
WBC Count ($\times 10^9$/L)	7.2 ± 0.9^b	12.5 ± 1.2^a	8.6 ± 0.7^b	7.3 ± 0.8^b	0.0018	0.86	1.05

Data are presented as mean ± SD (n = 6 per group), Different superscript letters indicate significantly different ($p < 0.05$). C: control group; T1: ulcer-induced group; T2: probiotic-treated ulcer group; T3: probiotic control group.

Table 2: Effect of probiotic on Liver and Renal Function tests in male rats with colon ulcer-induced.

Parameter	C	T1	T2	T3	p-value	η^2	$LSD_{0.05}$
Liver function							
ALT (U/L)	32.4 ± 4.5^c	65.2 ± 7.3^a	42.1 ± 5.6^b	33.2 ± 4.7^c	0.0011	0.86	6.84
AST (U/L)	28.7 ± 3.8^c	57.6 ± 6.2^a	38.5 ± 4.9^b	29.3 ± 3.9^c	0.0031	0.85	5.72
Kidney function							
Creatinine (mg/dL)	0.7 ± 0.1^c	1.4 ± 0.2^a	0.9 ± 0.1^b	0.7 ± 0.1^c	0.0022	0.88	0.16
Urea (mg/dL)	32.5 ± 4.2^c	58.7 ± 6.5^a	41.3 ± 5.1^b	33.2 ± 4.5^c	0.0019	0.87	6.27

Data are presented as mean ± SD (n = 6 per group), Different superscript letters indicate significantly different ($p < 0.05$). C: control group; T1: ulcer-induced group; T2: probiotic-treated ulcer group; T3: probiotic control group.

Table 3: Effect of Probiotic on Lipid Peroxidation and antioxidant status in male rats with Colon Ulcer Induced.

Parameter	C	T1	T2	T3	p-value	η^2	$LSD_{0.05}$
MDA (nmol/mL)	2.3 ± 0.4^c	6.7 ± 0.9^a	3.8 ± 0.6^b	2.4 ± 0.5^c	0.0023	0.89	0.78
SOD (U/mL)	125.6 ± 15.2^a	78.3 ± 10.5^c	112.4 ± 14.7^b	126.1 ± 15.5^a	0.0017	0.83	17.26
GSH (nmol/mL)	42.5 ± 5.6^a	18.7 ± 3.2^c	35.6 ± 4.7^b	43.2 ± 5.8^a	0.0032	0.88	5.94

Data are presented as mean ± SD (n = 6 per group), Different superscript letters indicate significantly different ($p < 0.05$). C: control group; T1: ulcer-induced group; T2: probiotic-treated ulcer group; T3: probiotic control group.

Table 4: Effects of probiotic on serum inflammatory cytokines on male rat with colon ulcer-induced.

Parameter	C	T1	T2	T3	p-value	η^2	$LSD_{0.05}$
TNF-α (pg/mL)	15.2 ± 2.1^c	45.6 ± 3.7^a	22.3 ± 2.5^b	16.1 ± 1.8^c	0.0027	0.90	3.18
IL-6 (pg/mL)	18.7 ± 2.3^c	52.4 ± 4.1^a	27.6 ± 3.2^b	19.2 ± 2.1^c	0.0013	0.89	3.62
CRP (mg/L)	3.2 ± 0.5^c	12.7 ± 1.6^a	5.4 ± 0.8^b	3.5 ± 0.6^c	0.0014	0.89	1.12

Data are presented as mean ± SD (n = 6 per group), Different superscript letters indicate significantly different ($p < 0.05$). C: control group; T1: ulcer-induced group; T2: probiotic-treated ulcer group; T3: probiotic control group.

Liver function parameters

Serum ALT levels were markedly increased in the ulcer-related group (T1: 65.2 ± 7.3 U/L) compared with the control group (C: 32.4 ± 4.5 U/L; $p < 0.0011$). One-way ANOVA demonstrated a meaningful therapeutic effect on ALT values ($F(3,20) = 39.5, p < 0.0011, \eta^2 = 0.86$). Probiotic administration significantly reduced ALT levels in ulcerated rats (T2: 42.1 ± 5.6 U/L) compared with the untreated ulcer group (T1). ALT values in the probiotic control group (T3: 33.2 ± 4.7 U/L) were consistent with baseline levels of control cohort. The trends for AST levels were also similar to those of ALT in all groups. Serum AST levels were significantly elevated in the ulcer-induced group (T1: 57.6 ± 6.2 U/L) compared with the control group (C: 28.7 ± 3.8 U/L; $p < 0.0031$). One-way ANOVA demonstrated a markedly group influence on AST values ($F(3,20)= 36.8, p < 0.0031, \eta^2= 0.85$). The concurrent elevation of transaminases associated with systemic inflammatory responses and oxidative processes secondary to colonic ulceration. Probiotic treatment significantly reduced AST levels in ulcerated rats (T2: 38.5 ± 4.9 U/L) compared with the untreated ulcer group. AST values in the probiotic control group (T3: 29.3 ± 3.9 U/L) were comparable relative to control values, indicating that probiotic supplementation did not adversely affect liver enzyme levels in healthy animals.

Kidney function parameters

A notable rise in circulating creatinine levels was detected in the ulcer-induced cohort (T1: 1.4 ± 0.2 mg/dL) compared with the control group (C: 0.7 ± 0.1 mg/dL; $p < 0.0022$). One-way ANOVA indicated a meaningful therapeutic influence on serum creatinine values ($F(3,20) =48.3, p < 0.0022, \eta^2= 0.88$). Probiotic treatment significantly reduced serum creatinine levels in ulcerated rats (T2: 0.9 ± 0.1 mg/dL) compared with the untreated ulcer group. Creatinine values in the probiotic control group (T3: 0.7 ± 0.1 mg/dL) were comparable to those of the control cohort. Blood urea values were significantly increased in the ulcer-induced treatment (T1: 58.7 ± 6.5 mg/dL) compared with the control group (C: 32.5 ± 4.2 mg/dL; $p < 0.0019$). A significant group difference was observed for serum urea levels ($F(3,20)= 44.7, p < 0.0019, \eta^2= 0.87$). Probiotic treatment significantly reduced serum urea levels in ulcerated rats (T2: 41.3 ± 5.1 mg/dL) compared with the untreated ulcer group. Urea levels in the probiotic control group (T3: 33.2 ± 4.5 mg/dL) were equivalent to those of the control cohort, reflecting no significant influence of probiotic supplementation on renal biochemical parameters in healthy animals.

Effect of probiotic on antioxidants indices in colon-related ulcer male rats

Malondialdehyde (MDA)

Serum MDA values were significantly increased in the ulcer-induced treatment (T1: 6.7±0.9 nmol/mL) relative to the control cohort (C: 2.3±0.4 nmol/mL; $p < 0.0023$). One-way ANOVA indicated a meaningful therapeutic influence on MDA values ($F(3,20)= 52.6, p < 0.0023, \eta^2= 0.89$). Probiotic treatment significantly reduced MDA levels in ulcerated rats (T2: 3.8±0.6 nmol/mL) compared with the untreated ulcer group (T1), suggesting attenuation of oxidative stress. Although MDA levels remained slightly elevated compared with control values, the reduction was statistically significant relative to the untreated ulcer group. MDA levels in the probiotic control group (T3: 2.4±0.5 nmol/mL) exhibited no significant deviation from those of the control cohort, revealing that probiotic supplementation exhibited no increase oxidative stress in healthy animals.

Superoxide dismutase (SOD)

Serum SOD activity was significantly reduced in the ulcer-induced group (T1: 78.3 ± 10.5 U/mL) relative with the control cohort (C: 125.6 ± 15.2 U/mL; $p < 0.0017$). One-way ANOVA demonstrated a markedly group influence on SOD activity ($F(3,20)= 32.4, p < 0.0017, \eta^2= 0.83$). Probiotic treatment significantly increased SOD activity in

ulcerated rats (T2: 112.4 ± 14.7 U/mL) compared with the untreated ulcer group (T1), suggesting partial restoration of antioxidant capacity. However, the T2 exhibited a slightly lower SOD activity than the control, even though they were much higher than that of T1, suggesting that there was a partial but significant recovery of antioxidant ability. SOD activity in the probiotic control group (T3: 126.1 ± 15.5 U/mL) was comparable to that of the control cohort, reflecting that probiotic supplementation did not adversely affect antioxidant status in healthy animals.

Reduced glutathione (GSH)

The tendency in GSH concentrations was similar as SOD activities among all the experimental groups. Serum GSH levels were significantly reduced in the ulcer-induced group (T1: 18.7 ± 3.2 nmol/mL) compared with the control group (C: 42.5 ± 5.6 nmol/mL; $p < 0.0032$). Probiotic treatment significantly increased GSH levels in ulcerated rats (T2: 35.6 ± 4.7 nmol/mL) compared with the untreated ulcer group, suggesting improvement of antioxidant status. One-way ANOVA indicated a meaningful therapeutic influence on GSH values ($F(3,20)= 47.9, p < 0.0032, \eta^2= 0.88$). This remarkable recovery of non-enzymatic antioxidant capacity amounted to a restoration level of 83.8% towards normal control values. GSH levels in the probiotic control group (T3: 43.2 ± 5.8 nmol/mL) were consistent with those of the control cohort, reflecting no significant influence of probiotic supplementation on antioxidant status in healthy animals.

Pro-inflammatory cytokines

Tumor necrosis factor-alpha (TNF-α)

Serum TNF-α levels were markedly elevated in the ulcer-induced cohort (T1: 45.6 ± 3.7 pg/mL) relative to the control cohort (C: 15.2 ± 2.1 pg/mL; $p < 0.0027$). One-way ANOVA demonstrated a marketable group influence on TNF-α values ($F(3,20)= 61.2, p < 0.0027, \eta^2 = 0.90$). Probiotic treatment significantly reduced TNF-α levels in ulcerated rats (T2: 22.3 ± 2.5 pg/mL) relative to the untreated ulcer torment, suggesting attenuation of the inflammatory activity. TNF-α values in the probiotic control group (T3: 16.1 ± 1.8 pg/mL) were consistent with those of the control cohort, reflecting no pro-inflammatory influence of probiotic supplementation in healthy animals.

Interleukin-6 (IL-6)

Serum IL-6 values were markedly elevated in the ulcer-induced group (T1: 52.4 ± 4.1 pg/mL) compared with the control group (C: 18.7 ± 2.3 pg/mL; $p < 0.0013$). One-way ANOVA demonstrated a markable group influence on IL-6 values ($F(3,20)= 58.7, p < 0.0013, \eta^2 = 0.89$). Probiotic treatment significantly reduced IL-6 levels in ulcerated rats (T2: 27.6 ± 3.2 pg/mL) relative to the untreated ulcer treatment. IL-6 values in the probiotic control cohort (T3: 19.2 ± 2.1 pg/mL) were comparable to those of the control group.

C-reactive protein (CRP)

Serum CRP values were significantly elevated in the ulcer-induced group (T1: 12.7 ± 1.6 mg/L) relative to the control cohort (C: 3.2 ± 0.5 mg/L; $p < 0.0014$). One-way ANOVA demonstrated a meaningful intergroup disparity in CRP values ($F(3,20)= 54.1, p < 0.0014, \eta^2= 0.89$). Probiotic treatment significantly reduced CRP levels in ulcerated rats (T2: 5.4 ± 0.8 mg/L) compared with the untreated ulcer group, suggesting attenuation of systemic inflammation. CRP values in the probiotic control group (T3: 3.5 ± 0.6 mg/L) were constituent to those of the control cohort, reflecting no inflammatory influence of probiotic supplementation in healthy animals.

Discussion

The current paper has shown that the administration of a multi-strain probiotic formulation was linked with the regulation of systemic biochemical and inflammatory changes caused by acetic acid-induced colonic ulceration in male rats. To systemically indicate the effects of intestinal inflammation, hematological alterations noted after inducing ulcers, e.g. a decrease in hemoglobin levels and the number of erythrocytes, are caused by inflammatory-mediated inhibition of erythropoiesis, mucosal hemolysis, and the influence of cytokines on iron metabolism (Muthas *et al.*, 2017). Intestinal barrier damage can also augment systemic inflammatory reaction and hematological disproportion (Shaw *et al.*, 2019). The above enhancement in the hemoglobin concentration, RBC count, and leukocyte profile after administration of probiotics indicates that regulation of gut microbiota and inflammatory signaling pathways could be involved in the restoration of hematological homeostasis. These results can be compared to the emerging evidence that shows that probiotic interventions may modulate the host inflammatory and oxidative pathways that are not located in the gastrointestinal tract (Estevinho *et al.*, 2024).

The increase of hepatic transaminases after induction of the ulcer indicates that the inflammation of the intestines can be spread outside the colon and affect other metabolic organs of the body. Higher ALT and AST levels are usually related to inflammatory and oxidative stress and could indicate secondary changes in hepatic biochemical parameters and not to direct structural damage. The breakdown of the integrity of the intestinal barriers can lead to the translocation of the bacterial components and inflammatory mediators into the circulation and the activation of the gut-liver axis (Fumery *et al.*, 2018).

The effect of the decrease in transaminase levels, which were observed under the influence of probiotics, could be associated with the change in intestinal microbiota structure and the inhibition of systemic inflammatory cues. The newest findings suggest that probiotic treatments

have the potential to modulate hepatic and renal metabolic and inflammatory pathways (Zhang *et al*., 2022). Equally, the reduction of serum creatinine and urea in animals treated with probiotics indicates an improvement in biochemical parameters of the kidney, which may be achieved by controlling the gut-kidney axis and decreasing the concentration of inflammatory mediators in the blood (Liu *et al*., 2024). Nonetheless, these results are to be taken as functional biochemical relationships, but not as the evidence of structural organ protection.

The augmentation of lipid peroxidation alongside the diminution of endogenous antioxidant defenses which occurred following the induction of ulcers is indicative that there exists an oxidative imbalance that is correlated with intestinal inflammation. The large production of reactive oxygen species (ROS) in response to inflammatory stimuli, which leads to oxidative stress, can also contribute to additional injury to tissues. The increase in the levels of MDA and the decrease in the SOD and GSH activity are in line with the increase in oxidative burden due to colonic ulceration.

The use of probiotics was linked to the partial restoration of antioxidant parameters, which implied the regulation of redox homeostasis. These effects have been suggested to be mediated by a number of mechanisms such as the amplification of endogenous antioxidant enzyme activity, modulation of inflammatory signal transduction, and indirectly by stabilizing the gut microbiota (Wang *et al*., 2022). The recovery of GSH levels can also indicate the enhancement of the capacities of cellular detoxification and preservation of the intracellular redox balance (Jeon *et al*., 2020). These results confirm the possibility of probiotics to mediate oxidative pathways associated with intestinal inflammation.

TNF-α, IL-6 and CRP response after induction of ulcers indicates the activation of the systemic inflammation pathways in response to colonic injury. TNF-α and IL-6 are pro-inflammatory cytokines which are key mediators of mucosal immune response and which are linked to extraintestinal inflammatory responses, whereas CRP is a downstream of acute-phase systemic inflammation. The same findings are aligned with the immunopathological processes in which inflammatory bowel disease is described (Pais *et al*., 2024).

The reduction in circulating cytokines and CRP levels observed after probiotic administration suggests modulation of inflammatory signaling cascades beyond the local intestinal environment. Previous studies have demonstrated that probiotic strains can attenuate TNF-α and IL-6 production and regulate NF-κB–mediated inflammatory pathways (Wang *et al*., 2019). Probiotic-mediated immune modulation may also involve enhancement of regulatory immune responses and increased production of anti-inflammatory metabolites such as short-chain fatty acids. Although inflammatory markers were not completely normalized, the observed attenuation indicates partial control of systemic inflammation rather than complete resolution.

The present findings suggest that probiotic supplementation may contribute to modulation of systemic inflammatory and oxidative responses associated with experimental colonic injury. Improvements observed in hematological, biochemical, oxidative, and inflammatory parameters indicate that probiotic administration was associated with attenuation of systemic alterations secondary to intestinal inflammation; however, these findings should be interpreted within the biochemical scope of the present experimental design. These observations are consistent with previous reports suggesting that probiotics may influence immune regulation and inflammatory signaling pathways in inflammatory bowel disease (Danese *et al*., 2016; Shaw *et al*., 2019).

Despite some of the initial clinical studies conducted to determine the potential utility of probiotics in inflammatory bowel disease (Estevinho *et al*., 2024), the optimal dosage planning, strain-specific effects, and long-term treatment outcomes have not been fully determined. Thus, probiotic supplementation cannot be regarded as a substitute to the already existing therapies but rather a complement to the conventional treatment. Notably, the fact that no adverse changes in the probiotic control group were observed in terms of biochemical parameters can be deemed as a clear indication of the overall tolerability of the formulation under the parameters examined in the present experimental model.

This was done in an acute acetic acid induced experimental model which might not be a complete solution to the chronic and the relapsing characteristic of the human inflammatory bowel disease. Also, a lack of macroscopic ulcer scoring and histopathological analysis of colon, liver, or kidney tissues do not allow direct confirmation of structural tissue recovery. Thus, the result must be understood as biochemical correlations throughout the system and not as a clear indication of tissue-level recovery. There was only a single probiotic dose that was considered which excludes the dose response analysis. Future studies need to include long-term experimental models, multi-dose models, and histological studies in order to better understand strain-specific pathways and maximize treatment regimens. In addition, the standardized probiotic formulations and individualized microbiome profiles will be necessary in order to make a translation to clinical practice.

Conclusion

Summing up, the current research proves that the use of multi-strain probiotic formulation was linked to the amelioration of systemic biochemical, oxidative, and inflammatory changes provoked by acetic acid-induced colonic ulceration in male rats. The supplementation with probiotics was associated with a regulation of hematological parameters, liver and renal biochemical indices, antioxidant status, and levels of pro-inflammatory cytokines. These observations imply that the administration of probiotics can be useful in the mitigation of the systemic effects of intestinal inflammation. Nevertheless, the findings are to be seen in the framework of an acute experimental model, and additional studies are needed to explain the long-term outcomes, strain-specific processes, and clinical relevance of human inflammatory bowel disease.

Declarations

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Ethical approval and IRB approval

The University of Al-Qadisiyah gave the experimental protocol an ethical approval of the Institutional Animal Ethics Committee (Approval No. 322/2024). All animal research activities were performed in compliance with the domestic laws on the treatment and use of laboratory animals and with the ARRIVE reporting guidelines.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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