Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : http://ijpsdronline.com/index.php/journal

Research Article

Protective Effect of *Bifidobacterium longum* Suspension Formulated with Medium Chain Triglycerides Oil in DNBS-Induced Colitis in Sprague-Dawley Rats

Dharmeshkumar B. Kheni[1*], Varun P. Sureja[1], Shrikalp S. Deshpande[2], Vishal P. Dubey[3], Jignesh J. Kansagra[3], Aditi H. Bariya[4]

[1]*Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India.*
[2]*K.B. Institute of Pharmaceutical Education and Research, Affiliated to Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India.*
[3]*Sundyota Numandis Probioceuticals Pvt. Ltd., Ahmedabad, Gujarat, India.*
[4]*Arihant School of Pharmacy & Bio-Research, Ahmedabad, Gujarat, India.*

ARTICLE INFO

Article history:
Received: 12 February, 2025
Revised: 09 March, 2025
Accepted: 19 March, 2025
Published: 30 March, 2025

Keywords:
Probiotic, *Bifidobacterium longum*, Dexamethasone, Inflammatory bowel disease, Oxidative stress, Inflammation, Gut dysbiosis.

DOI:
10.25004/IJPSDR.2025.170210

ABSTRACT

One of the most common chronic inflammatory diseases that causes extensive damage to the gastrointestinal tract (GIT) is inflammatory bowel disease (IBD). IBD symptoms can range in severity from mild and frequent diarrhea, bloating, and abdominal pain to severe bloody stools, anemia, unconsciousness, and occasionally even death. One of the main contributing factors to the pathophysiology of IBD is gut dysbiosis. The purpose of the current study was to determine how well a probiotic (*Bifidobacterium longum*) solution in medium-chain triglycerides (MCT) oil protected rats from colitis caused by dinitrobenzene sulfonic acid (DNBS). Sprague Dawley (SD) rats were given DNBS (120 mg/kg/rat) intrarectally to induce colitis, which was then allowed to persist for three days. Rats were randomly assigned to receive either *B. longum* oil suspension (3 x 10^6 CFU/g/day orally) or dexamethasone (2 mg/kg/day orally) for 28 days in a row after colitis induction. Body weight, average intake of food and water, histological analysis, colon weight, intestinal inflammatory biomarkers (fecal calprotectin), antioxidant potential (glutathione (GSH) and superoxide dismutase (SOD)), and biomarkers of oxidative stress (nitric oxide (NO) and malondialdehyde (MDA)). The disease activity index (DAI) and colonic mucosal damage index (CMDI) scores were used to assess the extent of colonic damage. The statistical analysis was conducted using GraphPad Prism software, with a significance threshold of $p < 0.05$. Body weight and food and water intake were significantly reduced in the DNBS-induced colitis group, while it was prevented by dexamethasone and *B. longum* oil suspension. Similarly, the degree of colonic damage brought on by DNBS was considerably reduced by dexamethasone and *B. longum* oil suspension therapy. This impact was followed by considerable improvement in the antioxidant biomarker levels (GSH and SOD) and reduction in the oxidative stress and intestinal inflammation level. Dexamethasone treatment outperformed *B. longum* oil suspension therapy in every analyzed metric. The study's findings demonstrate the protective effects of *B. longum* oil suspension therapy and dexamethasone in a DNBS-induced experimental colitis model. This effect may be explained by the anti-inflammatory and antioxidant properties of the treatments.

INTRODUCTION

Inflammatory bowel disease (IBD) is defined as a chronic inflammatory disease condition of the gastrointestinal tract (GIT).[1,2] IBD is categorized into two types, ulcerative colitis (UC) and Crohn's disease (CD), depending the location and severity of the disease.[2,3] While CD can affect any part of the entire GIT, from mouth to anus, and damage can occur on the inner mucosal layer or spread

***Corresponding Author:** Mr. Dharmeshkumar B. Kheni
Address: Kadi Sarva Vishwavidyalaya, Gandhinagar, Gujarat, India.
Email ✉**:** dharmeshbkheni341@gmail.com
Tel.: +91-9904081889

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

to deep layers, UC primarily involves inflammation of the inner mucosal lining of the large intestine (colon) and the rectum, resulting in inner mucosal lining ulcerations and damage.[1–3] With a frequency of up to 19.3%, IBD is one of the most common chronic inflammatory diseases of the gastrointestinal tract in India.[4] India is thought to have the highest number of IBD patients worldwide due to its high disease burden.[5]

Due to a number of variables, such as a sedentary lifestyle, environmental pollution, intake of foods high in fats and carbs, chronic alcohol and smoking, and a rise in infections, the prevalence of IBD has significantly grown over the past two to three decades.[6] Depending on the type and severity of the disease, IBD symptoms can vary. Generally, gastrointestinal symptoms (such as diarrhea, abdominal pain, bloating, and decreased appetite) and systemic symptoms (such as fever, fatigue, and malaise) are present, while severe symptoms include GI bleeding, rectal bleeding, anemia, and fatigue.[7,8] Simultaneously, IBD significantly increases the healthcare burden of individuals which includes chronic medications and hospitalization costs as well.[9,10] Currently, pharmacological medications (such as aminosalicylates, corticosteroids, immunosuppressants, and biologics) and non-pharmacological strategies (such as exercise, dietary and lifestyle modifications, and quitting alcohol and smoking) are used to treat IBD.[11,12] Despite all these treatment options, IBD remains an area of exploration for newer therapeutic targets.

The human gut microbiome is the collective term to indicate the trillions of microbes that reside in the human GIT.[13] In response to the dynamic GIT environment, these resident microorganisms perform a number of critical roles, such as aiding in digestion, inhibiting the growth of pathogens, and favorably regulating immune system activity.[13,14] Various disease disorders are initiated and/or progressed by gut dysbiosis, a state where the number of beneficial microorganisms decreases and the number of harmful microbes increases.[13,15] Numerous studies have highlighted one of the major roles of gut dysbiosis in the pathogenesis of IBD condition,[16–19] making gut dysbiosis an attractive therapeutic target for the management of IBD. According to the World Health Organization, probiotics are live microorganisms that, when given in sufficient amounts, can offer a number of health advantages to the recipient.[13,20] The beneficial effects of probiotics in treating a range of illnesses linked to gut dysbiosis have been highlighted by numerous experimental and clinical investigations.[21–23] While several research has investigated the beneficial effects of probiotic formulation in animal models used in experimental studies of caused IBD,[24–27] no studies to date have evaluated the effectiveness of probiotic *Bifidobacterium longum* oil suspension formulation in IBD conditions. Hence, the current study evaluated the effectiveness of *B. longum* probiotic formulated as stable oil suspension using medium-chain triglycerides (MCT) oil as a medium in dinitrobenzene sulfonic acid (DNBS)-induced colitis model in Sprague Dawley (SD) rats.

Materials And Methods

B. longum Oil Suspension Preparation and Characterization Method

The preparation of probiotic oil suspension involved the use of *B. longum*, silicon dioxide, MCT oil, and other excipients. Briefly, the excipients and probiotics were dispensed and sieved for particle uniformity. In a defined volume of MCT oil, silicon dioxide was added and mixed at 750 rpm for 10 minutes using a magnetic stirrer. In the prepared suspension, probiotics along with other excipients were then added and mixed uniformly to form the final suspension. The developed final suspension was a pale yellow color suspension with a characteristic MCT oil odor, indicating no deterioration or cross-reaction of any of the ingredients added to the suspension. The final suspension was visually inspected and found to be free from any foreign particulate matter.

The prepared probiotic oil suspension was used for the assay procedure. Briefly, 100 µL of probiotic oil suspension was taken in a sterile petri-plate. The oil was evenly distributed on the plate surface. Previously boiled and cooled sterile agar/MRS broth medium was then added to the probiotic plate and allowed to solidify at normal room temperature for 15 to 30 minutes. The plates were then incubated at room temperature for 24 hours following which the total colonies formed in the plate were counted using a calibrated colony counter machine.

Animals

The Arihant School of Pharmacy & Bio-Research, Gandhinagar (Gujarat, India) provided healthy adult male and female SD rats (225–250 gm weight), which were kept in propylene cages with unrestricted access to water and a normal chow diet. For a week, the rats were acclimated to standard room settings, which included a temperature range of 25 to 30°C and a 12:12 hours cycle of light and dark. The Institutional Animal Ethics Committee of Arihant School of Pharmacy & Bio-Research (Proposal No.: ASPBRI/IAEC/2022-23/11) authorized the entire experimental protocol.

Induction of Experimental Colitis

Rats were given free access to water and fasted for 12 hours the night before colitis was induced. Ether was used to mildly anesthetize the fasted rats. The rats' normal reflexes and respiration rate were monitored often while they were under anesthesia. After anesthesia, a 1.0 mL syringe with a catheter was carefully placed into the anus to reach the colon (the splenic flexure is about 8 cm from the anus). The rats were placed in the Trendelenburg

posture for a short while (about five minutes) to prevent reflux after the DNBS, which was freshly prepared (120 mg/kg/rat dissolved in 50% ethanol), was administered into the colon area using a syringe. Within three days of the induction treatment, the symptoms of colitis were noticed.

Experimental Groups

The animals were randomly divided into four groups with 6 rats per group as follows, the normal control (NC) group was treated with intra-rectal normal saline solution (1-mL/kg) and supplemented with vehicle, disease control (DC), standard control (SC), and test (T) groups were treated with intra-rectal DNBS solution for colitis induction. After induction, the SC group rats received treatment with dexamethasone solution (2 mg/kg/day orally), the T group rats received treatment with *B. longum* oil suspension (3×10^6 colony forming units (CFU)/g/day orally), and the DC group rats were given vehicle preparation as a supplement. For 28 days, the therapy was supplemented. All of the rats were put to sleep on the last day of the study (day 28), and their abdomens and colons were dissected and taken out for additional examination as detailed below.

Evaluation Parameters

Body weight and food and water intake

The amount of food and water consumed by each group for the whole supplementation period was recorded, and the data was used to calculate the average consumption of these substances for each group. At the beginning of supplementation and at the conclusion of the experiment, the body weight (in grams) of every rat was also measured.

Colonic weight, colonic mucosal damage index and disease activity index

The colon was dissected and removed after euthanasia. The colon was opened at the antimesenteric border after being separated from any surrounding tissues. After thoroughly rinsing the colon with saline buffer solution to get rid of any remaining colonic matter, the colon was weighed. Following weighing, the colon was placed on a wax block so that the disease activity index (DAI) and colonic mucosal damage index (CMDI) could be used to assess the degree of colon damage brought on by colitis. While the DAI score ranged from zero (intact colonic crypt and surface epithelium) to four (severe colitis defined by severe hyperemia, necrosis, and ulcers on the mucosal surface with the major ulcerative area extending >40% of the colon), the CMDI score ranged from zero (normal mucosa) to four (loss of entire colonic crypt and surface epithelium).

Tissue homogenate preparation

The dissected colon was homogenized at a concentration of 50 gm/L in an ice-cold phosphate buffer solution (pH 7.4). The resulting mixture was centrifuged for 10 minutes at 4°C and 3000 rpm. As explained below, the supernatant was removed and promptly kept at 20°C for the study of oxidative stress and inflammatory biomarker levels.

Colonic mucosal oxidative stress and inflammatory biomarker analysis

In a clean test tube, 1.0 mL of tissue homogenate, 0.2 mL of 4% w/v sodium dodecyl sulfate, 1.5 mL of 20% acetic acid in 0.27M hydrochloric acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid were combined to estimate the quantity of malondialdehyde (MDA). After mixing the entire mixture, it was cooked for one hour at 85°C in a water bath. A UV visible spectrophotometer was then used to analyze the produced solution at 532 nm. 1.0 mL of distilled water was used throughout the procedure to prepare the blank sample. MDA was expressed as µg/mL and was computed using the standard curve or the molar extraction coefficient of 1.56×10^5 $M^{-1}.cm^{-1}$.

For nitric oxide (NO) estimation, 1.0 mL of tissue homogenate and 1.0 mL of sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid previously equilibrated at room temperature) were combined. The mixture was then incubated for 5 to 10 minutes at room temperature, shielded from light. After the mixture was incubated, 1.0 mL of NED solution (0.1% N-1-napthylethylenediamine dihydrochloride in water, previously equilibrated at room temperature) was added, and it was once more incubated for 5 to 10 minutes at room temperature under light protection. After 30 minutes of combination production, the final solution, which has a purple/magenta color, was utilized to measure absorbance at 540 nm. The blank sample was prepared using the previously described procedure with 1.0 mL of distilled water. The standard curve method was used to evaluate the amount of NO, and the results were represented in µmol/mL.

In order to estimate the quantity of superoxide dismutase (SOD), 1.0 mL of tissue homogenate was combined in a clean test tube with 0.1 mL of ethylenediaminetetraacetic acid (EDTA) solution (1×10^{-4} M), 0.5 mL of carbonate buffer (pH 9.7), and 1.0 mL of epinephrine solution (3×10^{-3} M). For three minutes, at 30-second intervals, the optical density of the finished combination was measured at 480 nm. The identical process described above was used to prepare the blank sample, substituting 1.0 mL of distilled water for the tissue homogenate. Units per tissue gram (U/gm tissue) were used to express the obtained results.

For glutathione (GSH) estimation, 1.0 mL of tissue homogenate and 1.0 mL of TCA solution (10% trichloroacetic acid in water) were combined. After 10 minutes of cooling, the mixture was centrifuged for 10 minutes at 2000 rpm, and the supernatant was gathered. 1.5 mL of phosphate buffer solution and 4.0 mL of DTNB solution (0.6% 5,5'-Dithiobis-2-nitrobenzoic acid in 1% sodium

citrate solution) were added to 0.5 mL of the recovered supernatant. After thoroughly mixing the concoction, it was allowed to sit at room temperature for 5 minutes. Using a distilled water mixture made the same way as the blank mixture, the color intensity created was measured at 412 nm. The measured values were expressed as µg/mL. The feces of the animals before to euthanasia were taken out of their cages and combined with saline solution (1-mL/gm feces) in order to estimate the fecal calprotectin content. Before being analyzed, the mixture was combined and kept in a freezer. Using common analysis tools and following the manufacturer's instructions, the amount of fecal calprotectin was determined. The amount of calprotectin in the feces was measured in ng/mL.

Histopathological analysis of colonic mucosal layer

The recovered colonic tissues were preserved and immersed in paraffin solution, cut into 3 µm slices, and then placed on glass slides using a 10% neutral buffered formalin solution. For histopathological evaluation of intestinal tight junctions, goblet cell structure, mucosal layer cellular integrity, and changes in mucosal layer due to oxidative stress and inflammatory cell infiltration, the sections were stained with hematoxylin and eosin (H&E) solution and periodic acid Schiff regent.

Statistical Analysis

The mean ± SD was used to express the entire set of results. The statistical analysis was performed using the two-way ANOVA with a post-hoc Tukey test in GraphPad Prism (Desktop version 9.0.0; GraphPad Software Inc., San Diego, CA, USA). For each of the assessed parameters, $p < 0.05$ was regarded as the threshold for statistical significance.

Results

Body Weight and Dietary Change Measurement

One of the typical symptoms of IBD is weight loss, which is caused by decreased food and water intake, increased fecal output, and blood loss. The body weight of animals with DNBS-induced colitis was also significantly lower than that of normal control animals ($p < 0.05$; Fig. 1). When compared to the DC group, treatment with dexamethasone plus *B. longum* oil suspension greatly inhibited the decrease in body weight (Fig. 1). The results showed that dexamethasone was substantially more effective than *B. longum* oil suspension therapy ($p < 0.05$). Similarly, rats in the DC group consumed significantly less food and water overall than rats in the control group ($p < 0.05$; Fig. 2). When compared to body weight, the effects of dexamethasone and *B. longum* oil suspension on food and water intake levels showed a similar trend. Both groups' overall food and water intake significantly improved when compared to the DC group, but the dexamethasone-treated group's effect was noticeably more pronounced than that of the *B. longum* oil suspension group (Fig. 2).

Colonic Damage Scores and Histological Evaluation

The CMDI and DAI values significantly increased after intra-rectal DNBS therapy, indicating severe damage to the colonic mucosal region (Table 1). The CMDI score (0.112 v/s 2.228; $p < 0.05$) and DAI score (0.152 v/s 3.338; $p < 0.05$) of rats with DNBS-induced colitis were significantly higher than those of the control group, suggesting severe mucosal injury. Dexamethasone and *B. longum* oil suspension supplementation considerably reduced the mucosal damage brought on by DNBS. Table 1 shows that the total CMDI score (0.555 v/s 2.228; $p < 0.05$) and the DAI score (1.337 v/s 3.338; $p < 0.05$) were considerably improved by dexamethasone medication as compared to the DC group. Comparing the *B. longum* oil suspension therapy to the DC group, the DAI score (2.043 v/s 3.338; $p < 0.05$) and total CMDI score (1.057 v/s 2.228; $p < 0.05$) both significantly improved. Compared to the *B. longum* oil suspension group, dexamethasone medication demonstrated a statistically significant trend in improving colonic mucosal injury prevention for both the CMDI and DAI scores (Table 1). In contrast to DNBS-induced colitis, which caused visible mucosal damage with ruptured goblet cells and infiltration of inflammatory cells into the mucosal layer (Fig. 3B), the microscopic evaluation of the normal control group revealed an intact colonic mucosal barrier with preserved barrier integrity and no change in the morphology of goblet

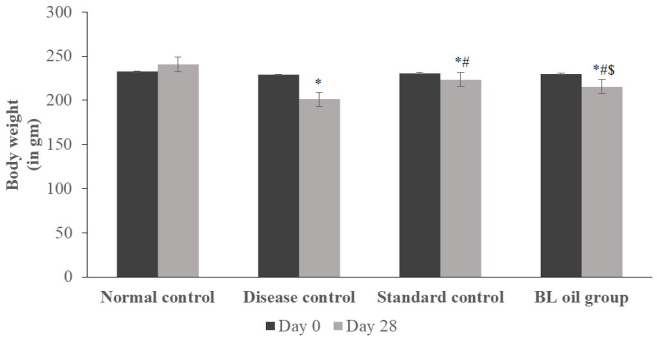

Fig. 1: Change in body weight during study duration. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively

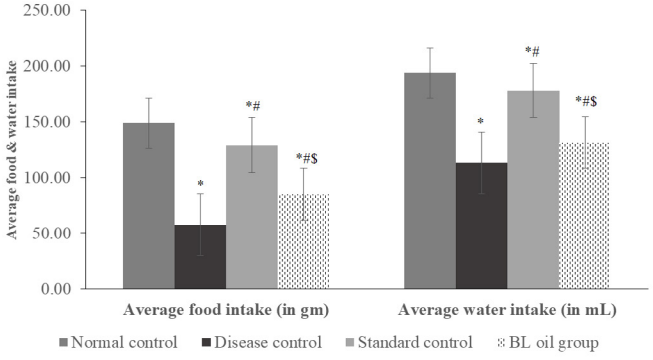

Fig. 2: Change in average food and water intake. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively

Table 1: Macroscopic colonic damage scores

	Normal group	*Disease control*	*Standard control*	*B. longum oil suspension group*
CMDI score	0.112 ± 0.042	2.228 ± 0.064*	0.555 ± 0.037$^{*\#}$	1.057 ± 0.063$^{*\#\$}$
DAI score	0.152 ± 0.011	3.338 ± 0.219*	1.337 ± 0.096$^{*\#}$	2.043 ± 0.177$^{*\#\$}$

Data presented as mean ± SD. CMDI: Colonic mucosal damage index, DAI: Disease activity index. *, #, $ indicates $p < 0.05$ v/s normal control group, disease control group, and standard control group, respectively.

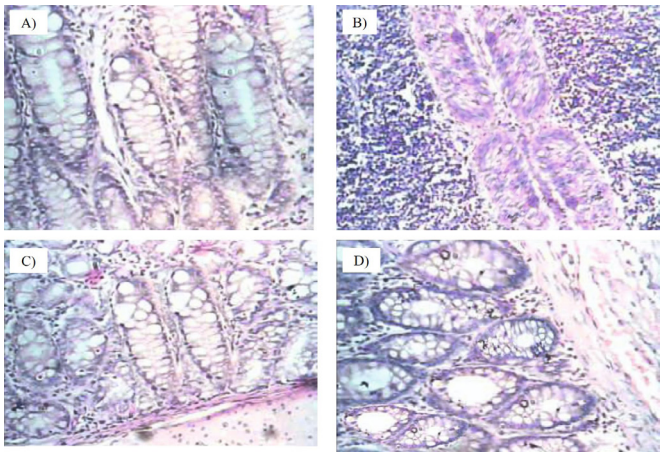

Fig. 3: Histological evaluation of colonic tissue of (A) Normal control group, (B) Disease control group, (C) Standard (dexamethasone) group, and (D) *L. rhamnosus* oil suspension group

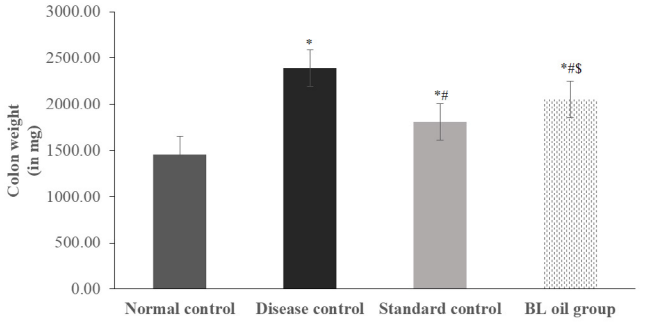

Fig. 4: Change in colon weight. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively

cells, as shown in Fig. 3A. Dexamethasone and *B. longum* oil suspension treatment stopped the colon damage brought on by DNBS. Rats treated with dexamethasone displayed preserved cellular integrity and goblet cell morphology, along with mild damage and infiltration of inflammatory cells, as shown in Fig. 3C. In contrast, rats treated with *B. longum* oil suspension displayed slightly altered cellular integrity and mild infiltration of inflammatory cells, as shown in Fig. 3D.

Colon Weight and Colon Inflammation Level

Increased oxidative stress mediators and inflammatory cell infiltration are linked to IBD-related colon damage, which damages the entire mucosal barrier, causes the colonic tissues to expand, and raises the weight of the colon overall. Rats treated with DNBS exhibited similar findings, with their overall colon weight significantly increasing in comparison to the control group ($p < 0.05$; Fig. 4). Supplementing with dexamethasone and *B. longum* oil suspension considerably inhibited the rise in colon weight when compared to the DC group, as illustrated in Fig. 4. The dexamethasone-treated rats showed a much stronger effect than the *B. longum* oil suspension-treated rats. We assessed the amount of calprotectin in the feces as a biomarker of intestinal inflammation in order to determine if the impact of dexamethasone and *B. longum* oil suspension is attributable to their anti-inflammatory properties. In accordance with our prediction, rats in the DC group had fecal calprotectin levels that were noticeably higher than those in the normal control group (Table 2). Because of their anti-inflammatory properties, dexamethasone and *B. longum* oil suspension therapy significantly decreased the fecal calprotectin level when compared to the DC group ($p < 0.05$ for both groups v/s DC group). Dexamethasone's effect was also significantly better than that of the *B. longum* oil suspension group (Table 2).

Measurement of Colonic Oxidative Stress Level

Table 2 shows how DNBS-induced colitis affects oxidative stress biomarker levels and how well dexamethasone and *B. longum* oil suspension work to mitigate oxidative stress. At the end of the study period, rats with DNBS-induced colitis showed a significant decrease in GSH (82.38 v/s 354.12 µg/mL; $p < 0.05$) and SOD level (3.27 v/s 15.05 U/gm tissue; $p < 0.05$), but a significant increase in NO (1088.25 v/s 189.45 µmole/mL; $p < 0.05$) and MDA (1.46 v/s 0.21 µg/mL; $p < 0.05$). When compared to the DC group, the antioxidant potential (GSH: 212.48 v/s 82.38 µg/mL, $p < 0.05$; SOD: 12.56 v/s 3.27 U/gm tissue, $p < 0.05$) and oxidative stress level (NO: 235.14 v/s 1088.25 µmoles/mL, $p < 0.05$; MDA: 0.11 v/s 1.46 µg/mL, $p < 0.05$) were significantly improved by supplementation with dexamethasone. Similarly, supplementing with *B. longum* oil suspension improved antioxidant levels (GSH: 154.90 v/s 82.38 µg/mL, $p < 0.05$; SOD: 5.99 v/s 3.27 U/gm tissue, $p < 0.05$) and significantly reduced oxidative stress (NO: 632.22 v/s 1088.25 µmoles/mL, $p < 0.05$; MDA: 0.42 v/s 1.46 µg/mL, $p < 0.05$). In terms of lowering

Table 2: Antioxidant, oxidative stress, and intestinal inflammation biomarker levels

	Normal group	*Disease control*	*Standard control*	*B. longum oil suspension group*
NO level (µmol/mL)	189.45 ± 3.63	1088.25 ± 9.29*	235.14 ± 8.95*#	632.22 ± 7.93*#$
MDA level (µg/mL)	0.21 ± 0.05	1.46 ± 0.08*	0.11 ± 0.02*#	0.42 ± 0.05*#$
GSH level (µg/mL)	354.12 ± 6.19	82.38 ± 2.93*	212.48 ± 4.50*#	154.90 ± 4.19*#$
SOD level (U/gm tissue)	15.05 ± 2.46	3.27 ± 0.46*	12.56 ± 2.45#	5.99 ± 0.36*#$
Fecal calprotectin level (ng.mL)	2910.00 ± 115.29	24866.17 ± 649.37*	9534.17 ± 246.02*#	15305.50 ± 887.91*#$

Data presented as mean ± SD. NO: Nitric oxide, MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase. *, #, $ indicates $p < 0.05$ v/s normal control group, disease control group, and standard control group, respectively.

oxidative stress and enhancing antioxidant capability, dexamethasone therapy was found to be substantially superior to *B. longum* oil suspension ($p < 0.05$ for all assessed parameters).

Discussion

Numerous earlier investigations have shown evidence that DNBS can cause colitis by changing the gut microbiota and resulting in dysbiosis.[28–30] Using SD rats and dexamethasone as a conventional control treatment, the current study sought to assess the efficacy of *B. longum* suspension in MCT oil in DNBS-induced colitis models. The current study's findings offer initial evidence of the beneficial effects of *B. longum* oil suspension in the management of colitis. It is hypothesized that *B. longum* oil suspension therapy can considerably minimize intestinal mucosal damage by lowering oxidative stress and inflammation and raising endogenous antioxidant potential based on the results currently available.

One of the key roles of gut dysbiosis in the pathophysiology of IBD has been emphasized and validated by numerous research.[16–19] The term "gut dysbiosis" describes the GIT's increased pathogenic microbial population and decreased helpful microbial population. The pathophysiology and course of IBD are complicated by gut dysbiosis, which includes changes in the gut microbiome-intestinal epithelial interaction, aberrant immune system activation, and epithelial damage brought on by pathogenic activity.[16–19]

The gut microbiome is crucial for preserving intestinal health overall. For example, the species of *Firmicutes* produce short-chain fatty acids (SCFAs) like butyrate and lower the activity of pro-inflammatory cytokines; the species of *Proteobacteria* prevent pathogen colonization and growth by producing different kinds of bacteriocins; the species of *Actinobacteria* enhance the function of the intestinal barrier; and the species of *Bacteroidetes* support the maturation of intestinal epithelial cells and aid in the absorption of nutrients.[31,32] Gut dysbiosis causes a general decrease in all the good bacteria, which in turn lowers their activity in promoting intestinal health overall. This sets off a series of events that ultimately harm the intestinal mucosal barrier.[31,32] By lowering the quantity of harmful bacteria and so reestablishing the normal gut microbiome, probiotics have been demonstrated to enhance the gut microbiome as a whole.[33] The beneficial impact of *B. longum* oil suspension in the present investigation may be related to its enhancement of the gut microbiota, which in turn improved intestinal health in general.

One of the key elements in the onset and advancement of IBD is oxidative stress.[34] In general, oxidative stress is defined as a situation when there is a marked increase in reactive oxygen species (ROS), which causes cellular damage, including damage to the cell's proteins, lipids, polysaccharides, and genetic material.[35] The pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway is triggered by oxidative stress, which increases the synthesis of different pro-inflammatory cytokines.[34, 35] Pro-inflammatory cytokine infiltration in the intestinal epithelial region causes inflammation, which intensifies the process of cellular destruction. Furthermore, the mitochondrial chain reaction is further damaged by the NF-κB pathway and inflammatory cytokines, which increases the production of ROS and oxidative stress.[36–38] IBD is the result of extensive intestinal epithelial barrier damage brought on by this cycle of oxidative stress and inflammation.[34, 39] While probiotic use is known to have antioxidant and anti-inflammatory effects that decrease the level of inflammation and oxidative stress [40,41], many studies have indicated the function of gut dysbiosis in the state of oxidative stress-induced intestinal damage.[42,43] An elevated NO level in intestinal tissue is a biomarker of inflammation and oxidative stress linked to IBD.[44] Increased generation of NO results from the activation of inducible nitric oxide synthase (iNOS) and damage to mucosal endothelial cells caused by elevated ROS and pro-inflammatory cytokines.[44] These NO molecules function similarly to ROS when endogenous antioxidants are depleted, intensifying the oxidative stress and inflammatory pathways. By generating SCFAs (mostly butyrate), which lessen iNOS over-expression and activation in the intestinal epithelium, the gut microbiota has been shown to limit NO generation in the intestinal

tissues.[45] The generation of SCFAs along with concurrently elevated oxidative stress and inflammation in gut dysbiosis causes the intestines to produce more NO, which in turn causes extensive intestinal damage and IBD. *B. longum* oil suspension administration dramatically lowered the tissue NO level in the current investigation, confirming the function of probiotics in lowering oxidative stress and the inflammatory cascade. Comparing the *B. longum* oil suspension therapy to the disease control group, the former considerably decreased the tissue MDA level. One often-used indicator of oxidative stress is MDA.[34,46] According to a number of studies, patients with IBD have significantly higher tissue and serum levels of MDA, and the severity of the disease is positively connected with these levels.[34] The level of MDA was found to be dramatically lowered with supplementation with *B. longum* oil suspension, suggesting a significant reduction in oxidative stress.

Maintaining the body's level of oxidative stress depends heavily on antioxidants, such as GSH and SOD.[47–49] Antioxidants maintain a balance in cellular oxidative stress to support normal cellular health, growth, development, and functioning by scavenging free radicals and preventing the over-activation of enzymes that produce ROS.[50] Animals in the disease control group in the current investigation showed significantly lower levels of GSH and SOD, which is consistent with the findings of earlier experimental and clinical studies.[51] As an alternative, the GSH and SOD levels were markedly raised by supplementing with *B. longum* oil suspension. This finding is consistent with the findings of earlier research that highlighted the potential of probiotic therapy to enhance antioxidant capacity.[52] Conversely, a well-known sensitive biomarker of intestinal inflammation, fecal calprotectin, is reported to be markedly elevated in IBD.[53] The fecal calprotectin level was dramatically decreased by supplementing with *B. longum* oil suspension, which supports the probiotic's anti-inflammatory properties as well. This finding is consistent with findings from other studies.[54]

One of the study's many advantages is that it is the first to assess how well the probiotic *B. longum* in MCT oil solution works to lessen the severity of DNBS-induced colitis in SD rats. The present study's findings were supported by the observations of earlier research since they were consistent with the current study's findings. Second, we assessed the degree of inflammation and oxidative stress, which allowed us to pinpoint the mechanism of action behind the advantageous effect of *B. longum* oil suspension in averting intestinal damage brought on by colitis. However, there are also some drawbacks to the current study, such as the absence of a control group consisting of traditional medicines. Although dexamethasone was one of the control groups in our study, sulfasalazine and other common treatments are being utilized for IBD. The usefulness of *B. longum* oil suspension in experimental colitis may be better identified and compared in future research using various conventional control therapies. Second, our study assessed the indicators of inflammation and oxidative stress; however, the observed effectiveness of *B. longum* oil suspension may also be due to other mechanisms of action, such as immune system and gut microbiota modification.

Conclusion

In conclusion, dexamethasone or *B. longum* oil suspension seems to be helpful in treating experimental colitis brought on by DNBS. The anti-inflammatory and antioxidant properties of dexamethasone treatments and *B. longum* oil suspension may be responsible for the preventative effect. Future experimental and clinical research is necessary to support the current study's findings in light of its findings.

Acknowledgments

We authors would like to thank the K.B. Institute of Pharmaceutical Education and Research (Affiliated with Kadi Sarva Vishwavidyalaya) for providing us the opportunity to work and contribute to this project.

References

1. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clinic Proceedings. 2019;94(1):155–165. Available from: doi.org/10.1016/j.mayocp.2018.09.013.
2. Zhang YZ, Li YY. Inflammatory bowel disease: Pathogenesis. World Journal of Gastroenterology. 2014;20(1):91-99. Available from: doi.org/10.3748/wjg.v20.i1.91.
3. Vermeire S, Van Assche G, Rutgeerts P. Classification of inflammatory bowel disease: the old and the new. Current opinion in gastroenterology. 2012;28(4):321–326. Available from: doi.org/10.1097/MOG.0b013e328354be1e.
4. Ray G. Inflammatory bowel disease in India - Past, present and future. World Journal of Gastroenterology. 2016;22(36):8123–8136. Available from: doi.org/10.3748/wjg.v22.i36.8123.
5. Kedia S, Ahuja V. Epidemiology of Inflammatory Bowel Disease in India: The Great Shift East. Inflammatory Intestinal Diseases. 2017;2(2):102–115. Available from: doi.org/10.1159/000465522.
6. Dam AN, Berg AM, Farraye FA. Environmental Influences on the Onset and Clinical Course of Crohn's Disease—Part 1: An Overview of External Risk Factors. Gastroenterology & Hepatology. 2013;9(11):711-717.
7. Davis J, Kellerman R. Gastrointestinal Conditions: Inflammatory Bowel Disease. FP essentials. 2022;516:23–30.
8. Singh S, Blanchard A, Walker JR, Graff LA, Miller N, Bernstein CN. Common Symptoms and Stressors Among Individuals With Inflammatory Bowel Diseases. Clinical Gastroenterology and Hepatology. 2011;9(9):769–775. Available from: doi.org/10.1016/j.cgh.2011.05.016.
9. Linschoten RCA, Visser E, Niehot CD, Woude CJ, Hazelzet JA, Noord D, et al. Systematic review: societal cost of illness of inflammatory bowel disease is increasing due to biologics and varies between continents. Alimentary Pharmacology & Therapeutics. 2021;54(3):234–248. Available from: doi.org/10.1111/apt.16445.
10. Coward S, Windsor J, Benchimol E, Bernstein C, Avina-Zubieta A, Bitton A, et al. THE COST OF INFLAMMATORY BOWEL DISEASE: A POPULATION-BASED ANALYSIS OF ADMINISTRATIVE DATA. Inflammatory Bowel Diseases. 2024;30(Supplement_1):S40. Available from: doi.org/10.1093/ibd/izae020.084.

11. Duff W, Haskey N, Potter G, Alcorn J, Hunter P, Fowler S. Non-pharmacological Therapies for Inflammatory Bowel disease: Recommendations for self-care and Physician Guidance. World Journal of Gastroenterology.2018;24(28):3055–3070. Available from: doi.org/10.3748/wjg.v24.i28.3055.
12. Leitner GC. Pharmacological- and non-pharmacological therapeutic approaches in inflammatory bowel disease in adults. World Journal of Gastrointestinal Pharmacology and Therapeutics. 2016;7(1):5-20. Available from: doi.org/10.4292/wjgpt.v7.i1.5.
13. Kheni DB, Sureja VP, Deshpande SS, Dubey VP, Kansagra JJ. A Systematic Mapping Review of In-Vitro and In-Vivo Evidences Exploring The Role of Strain-Specific Probiotic Bifidobacterium longum W11: International Journal of Pharmaceutical Sciences and Drug Research.2024;127–134. Available from: doi.org/10.25004/IJPSDR.2024.160117
14. Jandhyala SM. Role of the Normal Gut Microbiota. World Journal of Gastroenterology. 2015;21(29):8787- 8803. Available from: doi.org/10.3748/wjg.v21.i29.8787
15. Bidell MR, Hobbs ALV, Lodise TP. Gut microbiome health and dysbiosis: A clinical primer. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2022;42(11):849–857. Available from: doi.org/10.1002/phar.2731
16. Santana PT, Rosas SLB, Ribeiro BE, Marinho Y, de Souza HSP. Dysbiosis in Inflammatory Bowel Disease: Pathogenic Role and Potential Therapeutic Targets. International Journal of Molecular Sciences. 2022;23(7):3464. Available from: doi.org/10.3390/ijms23073464
17. Ram Hari Dahal, Kim S, Yu Kyung Kim, Eun Soo Kim, Kim J. Insight into gut dysbiosis of patients with inflammatory bowel disease and ischemic colitis. Frontiers in Microbiology. 2023; 14:1174832. Available from: doi.org/10.3389/fmicb.2023.1174832
18. Shan Y, Lee M, Chang EB. The Gut Microbiome and Inflammatory Bowel Diseases. Annual Review of Medicine. 2022;73:455–468. Available from: doi.org/10.1146/annurev-med-042320-021020.
19. Lal S, Kandiyal B, Ahuja V, Takeda K, Das B. Gut microbiome dysbiosis in inflammatory bowel disease. Progress in Molecular Biology and Translational Science.2022;192(1):179-204. Available from: doi.org/10.1016/bs.pmbts.2022.09.003
20. Fijan S. Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. International Journal of Environmental Research and Public Health. 2014;11(5):4745–4767. Available from: doi.org/10.3390/ijerph110504745
21. Ma T, Shen X, Shi X, Sakandar HA, Quan K, Li Y, et al. Targeting gut microbiota and metabolism as the major probiotic mechanism - An evidence-based review. Trends in Food Science & Technology. 2023;138:178–198. Available from: doi.org/10.1016/j.tifs.2023.06.013
22. Chandrasekaran P, Weiskirchen S, Ralf Weiskirchen. Effects of Probiotics on Gut Microbiota: An Overview. International journal of molecular sciences. 2024;25(11):6022. Available from: doi.org/10.3390/ijms25116022
23. Li C, Niu Z, Zou M, Liu S, Wang M, Gu X, et al. Probiotics, prebiotics, and synbiotics regulate the intestinal microbiota differentially and restore the relative abundance of specific gut microorganisms. Journal of Dairy Science. 2020;103(7):5816–5829. Available from: doi.org/10.3168/jds.2019-18003
24. Ahn SI, Cho S, Choi NJ. Effect of dietary probiotics on colon length in an inflammatory bowel disease-induced murine model: A meta-analysis. Journal of dairy science. 2020;103(2):1807–1819. Available from: doi.org/10.3168/jds.2019-17356
25. Zhang T, Zhang J, Duan L. The Role of Genetically Engineered Probiotics for Treatment of Inflammatory Bowel Disease: A Systematic Review. Nutrients. 2023;15(7):1566. Available from: doi.org/10.3390/nu15071566
26. Huang Y, Peng S, Zeng R, Yao H, Feng G, Fang J. From probiotic chassis to modification strategies, control and improvement of genetically engineered probiotics for inflammatory bowel disease. Microbiological Research. 2024;289:127928. Available from: doi.org/10.1016/j.micres.2024.127928
27. Pesce M, Seguella L, Del Re A, Lu J, Palenca I, Corpetti C, et al. Next-Generation Probiotics for Inflammatory Bowel Disease. International Journal of Molecular Sciences.2022;23(10):5466. Available from: doi.org/10.3390/ijms23105466.
28. Khafipour A, Eissa N, Munyaka PM, Rabbi MF, Kapoor K, Kermarrec L, et al. Denosumab Regulates Gut Microbiota Composition and Cytokines in Dinitrobenzene Sulfonic Acid (DNBS)-Experimental Colitis. Frontiers in Microbiology. 2020;11:1405. Available from: doi.org/10.3389/fmicb.2020.01405.
29. Zhou Y, Xu H, Xu J, Guo X, Zhao H, Chen Y, et al. F. prausnitzii and its supernatant increase SCFAs-producing bacteria to restore gut dysbiosis in TNBS-induced colitis. AMB Express. 2021;11(1):33. Available from: doi.org/10.1186/s13568-021-01197-6.
30. Son M, Park IS, Kim S, Ma HW, Kim JH, Kim TI, et al. Novel Potassium-Competitive Acid Blocker, Tegoprazan, Protects Against Colitis by Improving Gut Barrier Function. Frontiers in Immunology. 2022:13:870817. Available from: doi.org/10.3389/fimmu.2022.870817.
31. Maciel-Fiuza MF, Muller GC, Campos DMS, do Socorro Silva Costa P, Peruzzo J, Bonamigo RR, et al. Role of gut microbiota in infectious and inflammatory diseases. Frontiers in Microbiology. 2023;14:1098386. Available from: doi.org/10.3389/fmicb.2023.1098386.
32. Valdes AM, Walter J, Segal E, Spector TD. Role of the Gut Microbiota in Nutrition and Health. British Medical Journal. 2018; 361:k2179. Available from: doi.org/10.1136/bmj.k2179.
33. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therapeutic Advances in Gastroenterology. 2013;6(1):39–51. Available from: doi.org/10.1177/1756283X12459294.
34. Muro P, Zhang L, Li S, Zhao Z, Jin T, Mao F, et al. The emerging role of oxidative stress in inflammatory bowel disease. Frontiers in Endocrinology. 2024;15:1390351. Available from: doi.org/10.3389/fendo.2024.1390351.
35. Liu P, Li Y, Wang R, Ren F, Wang X. Oxidative Stress and Antioxidant Nanotherapeutic Approaches for Inflammatory Bowel Disease. Biomedicines. 2021;10(1):85. Available from: doi.org/10.3390/biomedicines10010085.
36. Morgan MJ, Liu Z. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Research. 2011;21(1):103–115. Available from: doi.org/10.1038/cr.2010.178.
37. Khansari N, Shakiba Y, Mahmoudi M. Chronic Inflammation and Oxidative Stress as a Major Cause of Age- Related Diseases and Cancer. Recent Patents on Inflammation & Allergy Drug Discovery. 2009;3(1):73–80. Available from: doi.org/10.2174/187221309787158371.
38. Ramos-González EJ, Bitzer-Quintero OK, Ortiz G, Hernández-Cruz JJ, Ramírez-Jirano LJ. Relationship between inflammation and oxidative stress and its effect on multiple sclerosis. Neurología. 2024;39(3): :292-301. Available from: doi.org/10.1016/j.nrleng.2021.10.010.
39. Tian T, Wang Z, Zhang J. Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. Oxidative Medicine and Cellular Longevity. 2017:2017:4535194. Available from: doi.org/10.1155/2017/4535194.
40. Hamid Mostafavi Abdolmaleky, Zhou JR. Gut Microbiota Dysbiosis, Oxidative Stress, Inflammation, and Epigenetic Alterations in Metabolic Diseases. Antioxidants.2024;13(8):985. Available from: doi.org/10.3390/antiox13080985.
41. Li L, Peng P, Ding N, Jia W, Huang C, Tang Y. Oxidative Stress, Inflammation, Gut Dysbiosis: What Can Polyphenols Do in Inflammatory Bowel Disease? Antioxidants. 2023;12(4):967. Available from: doi.org/10.3390/antiox12040967.
42. Li Q, Zheng T, Ding H, Chen J, Li B, Zhang Q, et al. Exploring the Benefits of Probiotics in Gut Inflammation and Diarrhea—From an Antioxidant Perspective. Antioxidants. 2023 ;12(7):1342. Available from: doi.org/10.3390/antiox12071342.
43. Ballini A, Santacroce L, Cantore S, Bottalico L, Dipalma G, Topi S, et al. Probiotics Efficacy on Oxidative Stress Values in Inflammatory Bowel Disease: A Randomized Double-Blinded Placebo-Controlled

Pilot Study. Endocrine, Metabolic & Immune Disorders - Drug Targets. 2019;19(3):373–81. Available from: doi.org/10.2174/1871530319666181221150352.
44. Nesina Avdagić, Asija Zaciragic, Nermina Babić, Mirsada Hukić, Seremet M, Orhan Lepara, et al. Nitric oxide as a potential biomarker in inflammatory bowel disease. Bosnian Journal of Basic Medical Sciences.2013;13(1):5–9. Available from: doi.org/10.17305/bjbms.2013.2402.
45. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, et al. Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. Science. 2017;357(6351):570–575. Available from: doi.org/10.1126/science.aam9949.
46. Cordiano R, Di Gioacchino M, Mangifesta R, Panzera C, Gangemi S, Minciullo PL. Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. Molecules.2023;28(16):5979. Available from: doi.org/10.3390/molecules28165979.
47. Aquilano K, Baldelli S, Ciriolo MR. Glutathione: new roles in redox signaling for an old antioxidant. Frontiers in Pharmacology. 2014;5:196. Available from: doi.org/10.3389/fphar.2014.00196.
48. Younus H. Therapeutic potentials of superoxide dismutase. International Journal of Health Sciences.2018;12(3):88–93.
49. Korczowska-Łącka I, Słowikowski B, Piekut T, Hurła M, Banaszek N, Szymanowicz O, et al. Disorders of Endogenous and Exogenous Antioxidants in Neurological Diseases. Antioxidants. 2023;12(10):1811. Available from: doi.org/10.3390/antiox12101811.
50. Lee S, Hu L. Nrf2 activation through the inhibition of Keap1–Nrf2 protein–protein interaction. Medicinal Chemistry Research.2020;29(5):846-867. Available from: doi.org/10.1007/s00044-020-02539-y.
51. Moura FA, de Andrade KQ, dos Santos JCF, Araújo ORP, Goulart MOF. Antioxidant therapy for treatment of inflammatory bowel disease: Does it work? Redox Biology. 2015;6:617–639. Available from: doi.org/10.1016/j.redox.2015.10.006.
52. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant Properties of Probiotic Bacteria. Nutrients. 2017;19;9(5):521. Available from: doi.org/10.3390/nu9050521.
53. Pathirana WGW, Chubb SP, Gillett MJ, Vasikaran SD. Faecal Calprotectin. The Clinical Biochemist Reviews. 2018;39(3):77–90.
54. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-Inflammatory and Immunomodulatory Effects of Probiotics in Gut Inflammation: A Door to the Body. Frontiers in Immunology.2021;12:578386. Available from: doi.org/10.3389/fimmu.2021.578386.

HOW TO CITE THIS ARTICLE: Kheni DB, Sureja VP, Deshpande SS, Dubey VP, Kansagra JJ, Bariya AH. Protective Effect of *Bifidobacterium longum* Suspension Formulated with Medium Chain Triglycerides Oil in DNBS-Induced Colitis in Sprague-Dawley Rats. Int. J. Pharm. Sci. Drug Res. 2025;17(2):194-202. **DOI:** 10.25004/IJPSDR.2025.170210