Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 972-983 | ISSN:2251-6727

Protective Effects of a Bifidobacterium longum Suspension Formulated with Avocado Oil in DNBS-Induced Colitis in Sprague-Dawley Rats

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(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 12 July 2025)

KEYWORDS

Probiotics

Bifidobacteria

IBD

DNBS

GI dysbiosis

Avocado oil

ABSTRACT:

Introduction: Inflammatory bowel disease (IBD) is a chronic condition of the gastrointestinal tract marked by symptoms ranging from diarrhea and abdominal pain to severe bleeding and weight loss. A key factor in IBD pathogenesis is gut dysbiosis. Due to limitations and side effects of current treatments, probiotics are being explored as alternative therapies.

Objectives: This study evaluated the protective effects of a *Bifidobacterium longum* suspension formulated in avocado oil on dinitrobenzene sulfonic acid (DNBS)-induced colitis in *Sprague-Dawley* rats.

Methods: Colitis was induced using a single intrarectal dose of DNBS (120 mg/kg). After three days, rats were treated orally with either *B. longum* oil suspension (2×10^9 CFU/g/day) or dexamethasone (2 mg/kg/day) for 28 days. Key parameters included body weight, food and water intake, colon weight, disease activity index (DAI), and colonic mucosal damage index (CMDI). Biochemical markers assessed included fecal calprotectin, glutathione (GSH), superoxide dismutase (SOD), nitric oxide (NO), and malondialdehyde (MDA). Histological analysis was performed on colon tissues.

Results: DNBS significantly reduced body weight and increased DAI and CMDI scores. Both treatments improved clinical and biochemical markers, reduced inflammation (calprotectin), oxidative stress (NO and MDA), and improved antioxidant levels (GSH and SOD). While dexamethasone showed slightly better results, *B. longum* also conferred significant protection.

Conclusions: *B. longum* formulated in avocado oil effectively attenuates DNBS-induced colitis, likely via anti-inflammatory and antioxidant mechanisms. It may serve as a promising adjunct or alternative therapy for IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder affecting the gastrointestinal tract (GIT) [1,2]. It is broadly categorized into two major types: ulcerative colitis (UC) and Crohn's disease (CD), which differ based on the location and extent of gastrointestinal involvement [2,3]. Crohn's disease may affect any segment of the GIT, from the oral cavity to the anus, and is characterized by patchy, transmural inflammation that can involve deeper layers of the intestinal wall. In contrast, UC is confined to the colon and rectum, causing continuous inflammation and ulceration restricted to the mucosal layer [1-3]. In India, the prevalence of IBD has been reported to be as high as 19.3%, [4] positioning the country as having one of the largest populations affected by IBD worldwide [5]. Over the past two to three decades, the prevalence of inflammatory bowel disease (IBD) has increased significantly, attributed to a range of contributing factors such as sedentary lifestyle, environmental pollution, diets high in saturated fats and refined carbohydrates, chronic alcohol consumption, tobacco use, and rising rates of infectious diseases [6].

The clinical manifestations of IBD are heterogeneous and depend on the type and severity of the disease. Common gastrointestinal symptoms include diarrhea, abdominal pain, bloating, and anorexia, while systemic symptoms may comprise fever, fatigue, and general malaise. In more advanced stages, patients may present

Journal of Chemical Health Risks
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JCHR (2025) 15(4), 972-983 | ISSN:2251-6727

with gastrointestinal or rectal bleeding and anemia [7,8]. IBD also imposes a substantial healthcare burden due to the need for prolonged pharmacotherapy, frequent hospital admissions, and increased utilization of healthcare resources, collectively contributing to elevated direct and indirect healthcare costs [9,10]. Current management approaches involve both pharmacological and non-pharmacological strategies. Pharmacological therapies include aminosalicylates, corticosteroids, immunosuppressive agents, and biologics, whereas non-pharmacological measures comprise dietary modifications, physical activity, and the avoidance of alcohol and tobacco [11,12]. Despite the availability of these treatment modalities, IBD remains a subject of ongoing investigation, highlighting the unmet need for more effective and targeted therapeutic options.

The human gut microbiome comprises a diverse and dynamic community of trillions of microorganisms residing within the gastrointestinal tract (GIT)[13]. These commensal microbes have evolved to adapt to the fluctuating environment of the GIT and perform several essential physiological functions, including nutrient metabolism, protection against pathogenic colonization, and regulation of host immune responses [13,14]. Disruption of this microbial equilibrium, a condition referred to as gut dysbiosis, is characterized by a decline in beneficial microbial populations and a concurrent proliferation of potentially harmful species [13,15]. This imbalance has been associated with the pathogenesis of various gastrointestinal and systemic disorders. Notably, gut dysbiosis has been strongly implicated in the development and progression of inflammatory bowel disease (IBD), thereby representing a potential therapeutic target in disease prevention and management [16-19].

Probiotics, as defined by the World Health Organization, are live microorganisms that confer health benefits to the host when administered in adequate amounts [13,20]. A growing body of experimental and clinical evidence supports the therapeutic potential of probiotics in managing conditions associated with gut dysbiosis [21-23]. Numerous studies have investigated the beneficial effects of probiotics in animal models of experimentally induced inflammatory bowel disease (IBD) [24-27]; however, to date, no study has specifically evaluated the therapeutic efficacy of a *Bifidobacterium longum* formulation administered as an oil-based suspension. Accordingly, the present study was designed to assess the protective effects of *B. longum* formulated in a stable avocado oil suspension in a dinitrobenzene sulfonic acid (DNBS)-induced colitis model in *Sprague-Dawley* (SD) rats.

2. Objectives

The current investigation was carried out to investigate the protective effects of a *Bifidobacterium longum* suspension formulated in Avocado oil on dinitrobenzene sulfonic acid (DNBS)-induced colitis in *Sprague-Dawley* rats.

3. Methods

3.1 Preparation and Characterization of *Bifidobacterium longum* Suspension in Avocado Oil:

The probiotic oil-based suspension was prepared using *Bifidobacterium longum*, silicon dioxide, avocado oil, and selected pharmaceutical excipients. Initially, the probiotic powder and excipients were accurately weighed and passed through an appropriate mesh sieve to ensure uniform particle size distribution. A predetermined volume of avocado oil was employed as the suspending medium, into which silicon dioxide was added. The mixture was stirred using a magnetic stirrer at 750 rpm for 10 minutes to achieve uniform dispersion. Following this, the probiotic powder and remaining excipients were gradually incorporated into the silicon dioxide–avocado oil mixture and thoroughly blended to obtain a homogeneous suspension. The final formulation appeared as a pale yellow suspension with a characteristic odor of avocado oil, indicating the absence of degradation or chemical interaction among the constituents. Visual examination confirmed that the formulation was free from foreign particulate matter and exhibited no signs of phase separation.

3.2 Assessment of Viable Colony-Forming Units (CFUs) in the Probiotic Oil Suspension:

The viable count of probiotic cells in the prepared oil suspension was determined using a standard microbial assay. A 100 µL aliquot of the formulation was aseptically transferred onto sterile Petri dishes and uniformly spread across the surface. Sterile de Man, Rogosa, and Sharpe (MRS) agar medium previously sterilized by boiling and cooled to an appropriate temperature was then poured into the plates and allowed

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to solidify at room temperature for 15–30 minutes. The inoculated plates were subsequently incubated at room temperature for 24 hours under anaerobic conditions. Following incubation, viable colony-forming units (CFUs) were enumerated using a calibrated digital colony counter to quantify the number of viable *Bifidobacterium longum* cells present in the formulation.

3.3 Animals

Healthy adult male and female Sprague-Dawley (SD) rats, weighing between 225–250 g, were procured from Arihant School of Pharmacy & Bio-Research, Gandhinagar, Gujarat, India. The animals were housed in standard polypropylene cages under controlled laboratory conditions, with ad libitum access to a standard pellet diet and drinking water. Prior to the commencement of the study, all animals were acclimatized for a period of one week under standardized environmental conditions, including a temperature range of 25–30 °C and a 12-hour light/dark cycle. All experimental procedures were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Ethics Committee of Arihant School of Pharmacy & Bio-Research (Approval No.: ASPBRI/IAEC/2022-23/11).

3.4 Induction of Colitis Using DNBS:

Prior to the induction of colitis, rats were fasted for 12 hours with free access to water. Mild anesthesia was induced using diethyl ether, and animals were continuously monitored for respiratory rate and reflex responses to ensure adequate anesthetic depth. Following anesthetization, a 1.0 mL syringe fitted with a flexible catheter was carefully inserted into the rectum to a depth of approximately 8 cm, reaching the region of the splenic flexure. A freshly prepared solution of dinitrobenzene sulfonic acid (DNBS; 120 mg/kg body weight in 50% ethanol) was administered intrarectally. To minimize reflux and ensure uniform distribution, the animals were maintained in the Trendelenburg position for approximately five minutes post-administration. Clinical manifestations of colitis, including weight loss, diarrhea, and rectal bleeding, typically developed within three days following DNBS administration.

3.5 Experimental Groups:

The animals were randomly assigned into four groups (n = 6 per group): normal control (NC), disease control (DC), standard control (SC), and test (T) groups. The NC group received an intrarectal administration of normal saline solution (1 mL/kg) along with the vehicle. Colitis was induced in the DC, SC, and T groups by intrarectal administration of DNBS solution. Following induction, the SC group was orally treated with dexamethasone at a dose of 2 mg/kg/day, while the T group received an oral dose of *Bifidobacterium longum* oil suspension containing 2×10^9 colony-forming units (CFU)/g/day. The DC group was administered the vehicle orally as a supplement. Treatments were administered once daily for 28 consecutive days. On day 28, all animals were euthanized, and their abdomens were opened to carefully dissect and collect colon tissues for further analyses.

3.6 Evaluation parameters:

3.6.1 Body Weight and Food and Water Intake:

Food and water consumption were monitored daily for each group throughout the supplementation period. The recorded data were used to calculate the average intake per group. Additionally, the body weight of each rat (grams) was measured at the beginning and at the end of the experimental period.

3.6.2 Colonic Weight, Colonic Mucosal Damage Index, and Disease Activity Index:

After euthanasia, the colon was carefully dissected and excised. The tissue was then opened longitudinally along the antimesenteric border following removal of any adherent tissues. The colon was thoroughly rinsed with saline buffer to remove residual contents before being weighed. Subsequently, the colon was placed on a wax block for macroscopic evaluation of tissue damage using the Disease Activity Index (DAI) and the Colonic Mucosal Damage Index (CMDI). The DAI is scored from 0 to 4, where 0 indicates intact colonic crypts and surface epithelium, and 4 represents severe colitis characterized by pronounced hyperemia, necrosis, and mucosal ulceration affecting more than 40% of the colon. Similarly, the CMDI ranges from 0, denoting normal mucosa, to 4, indicating complete loss of colonic crypts and surface epithelium.

3.6.3 Tissue Homogenate Preparation:

The excised colon tissue was homogenized at a concentration of 50 g/L in ice-cold phosphate-buffered saline (pH 7.4). The homogenate was then centrifuged at

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JCHR (2025) 15(4), 972-983 | ISSN:2251-6727

3000 rpm for 10 minutes at 4 °C. The supernatant was carefully collected and immediately stored at –20 °C for subsequent analysis of oxidative stress markers and inflammatory biomarkers.

3.6.4 Colonic Mucosal Oxidative Stress and Inflammatory Biomarkers Analysis:

3.6.4.1 Malondialdehyde (MDA) Estimation:

Malondialdehyde (MDA) levels were quantified using the thiobarbituric acid reactive substances (TBARS) assay. Briefly, 1.0 mL of tissue homogenate was mixed with 0.2 mL of 4% (w/v) sodium dodecyl sulfate, 1.5 mL of 20% acetic acid prepared in 0.27 M hydrochloric acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid. The mixture was thoroughly vortexed and incubated in a water bath at 85 °C for 1 hour. After incubation, the absorbance of the resulting solution was measured at 532 nm using a UV-visible spectrophotometer. A blank sample containing 1.0 mL of distilled water in place of homogenate was used for baseline correction. MDA concentration was expressed as µg/mL and calculated either from a standard curve or by applying the molar extinction coefficient of $1.56 \times 105\ M^{-1} \cdot cm^{-1}$.

3.6.4.2 Nitric Oxide (NO) Estimation:

Nitric oxide levels were determined using the Griess reaction. Briefly, 1.0 mL of tissue homogenate was mixed with 1.0 mL of sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid, equilibrated at room temperature) and incubated for 5–10 minutes at room temperature in the dark. Subsequently, 1.0 mL of NED solution (0.1% N-1-naphthylethylenediamine dihydrochloride in water, equilibrated at room temperature) was added, and the mixture was further incubated for 5–10 minutes protected from light. After 30 minutes, the resulting purple/magenta-colored solution was used to measure absorbance at 540 nm. A blank sample was prepared by substituting the homogenate with 1.0 mL of distilled water and processed similarly. Nitric oxide concentration was quantified using a standard curve and expressed as µmol/mL.

3.6.4.3 Superoxide Dismutase (SOD) Assay:

Superoxide dismutase (SOD) activity was determined by mixing 1.0 mL of tissue homogenate 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) in a clean test tube. The optical density of the reaction mixture was measured at 480 nm at 30-second intervals over 3 minutes. A blank sample was prepared by substituting the tissue homogenate with 1.0 mL of distilled water and processed similarly. SOD activity was expressed as units per gram of tissue (U/g tissue).

3.6.4.4 Glutathione (GSH) Estimation:

Glutathione levels were estimated by mixing 1.0 mL of tissue homogenate with 1.0 mL of 10% trichloroacetic acid (TCA). After cooling the mixture for 10 minutes, it was centrifuged at 2000 rpm for 10 minutes, and the supernatant was collected. Subsequently, 0.5 mL of the supernatant was combined with 1.5 mL of phosphate buffer and 4.0 mL of DTNB solution (0.6% 5,5'-dithiobis-2-nitrobenzoic acid in 1% sodium citrate). The mixture was thoroughly mixed and incubated at room temperature for 5 minutes. Absorbance was measured at 412 nm against a blank prepared with distilled water processed identically. Glutathione concentration was expressed as µg/mL.

3.6.4.5 Fecal Calprotectin Content Estimation:

Fecal samples were collected from the animals prior to euthanasia and homogenized with saline solution at a ratio of 1 mL per gram of feces to estimate fecal calprotectin levels. The homogenate was thoroughly mixed and stored at –20 °C until analysis. Fecal calprotectin concentrations were determined using a commercially available assay kit following the manufacturer's instructions. Results were expressed as ng/mL.

3.6.4.6 Histopathological Analysis of the Colonic Mucosal Layer:

Excised colonic tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned into 3 µm thick slices, which were subsequently mounted on glass slides. For histopathological evaluation, sections were stained with Hematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS) reagents. These stains facilitated assessment of intestinal tight junction integrity, goblet cell morphology, mucosal cellular architecture, and mucosal alterations associated with oxidative stress and inflammatory cell infiltration.

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3.7 Statistical Analysis:

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA with multiple comparisons by the Tukey method, employing GraphPad Prism software (version 9.0.0; GraphPad Software Inc., San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

4. Results

4.1 Body Weight and Dietary Intake Assessment:

Weight loss is a common clinical manifestation of inflammatory bowel disease (IBD), often attributed to reduced food and water intake, increased fecal output, and blood loss. In the present study, rats with DNBS-induced colitis exhibited a significant reduction in body weight compared to the normal control group ($p < 0.05$; Fig. 1). Treatment with dexamethasone or *Bifidobacterium longum* oil suspension significantly attenuated this weight loss, with dexamethasone demonstrating a more pronounced protective effect than the *B. longum* suspension ($p < 0.05$).

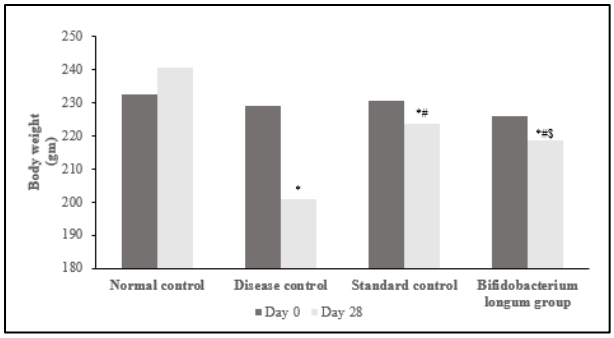

Fig.1: Change in body weight during study duration. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively.

Similarly, the disease control (DC) group showed a significant decrease in food and water consumption relative to the normal controls ($p < 0.05$; Fig. 2). Both treatment groups exhibited a significant improvement in dietary intake compared to the DC group, with the dexamethasone-treated group showing greater enhancement than the *B. longum* oil suspension group. The patterns observed in dietary intake closely corresponded with the changes in body weight.

4.2 Colonic Damage Scores and Histological Evaluation:

Intra-rectal administration of DNBS resulted in a significant increase in both the Colonic Mucosal Damage Index (CMDI) and Disease Activity Index (DAI), reflecting marked injury to the colonic mucosa **(Table 1)**. The CMDI score increased significantly from 0.112 in the normal control group to 2.228 in the DNBS-treated disease control group ($p < 0.05$). Similarly, the DAI score rose significantly from 0.152 to 3.338 ($p < 0.05$), confirming extensive mucosal damage following colitis induction.

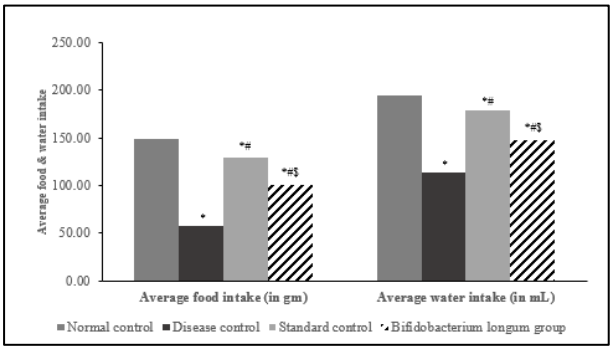

Fig. 2: Change in average food and water intake. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively.

Supplementation with dexamethasone or *Bifidobacterium longum* oil suspension significantly attenuated DNBS-induced mucosal injury. Dexamethasone treatment markedly reduced the Colonic Mucosal Damage Index (CMDI) score (0.555 vs. 2.228; $p < 0.05$) and Disease Activity Index (DAI) score (1.337 vs. 3.338; $p < 0.05$) compared to the disease control group. Similarly, administration of *B. longum* oil suspension significantly decreased CMDI (0.741 vs. 2.228; $p < 0.05$) and DAI (1.552 vs. 3.338; $p < 0.05$) scores relative to the disease control group. However, dexamethasone exhibited superior protective effects compared to *B. longum* oil suspension, as indicated by significantly lower CMDI and DAI scores **(Table 1).**

Histological analysis corroborated these findings. In the normal control group, the colonic mucosa exhibited intact architecture, with well-preserved epithelial cells and goblet cell morphology, and absence of inflammatory infiltration (Fig. 3A). In contrast, the DNBS-induced colitis group demonstrated extensive

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mucosal disruption, goblet cell rupture, and marked infiltration of inflammatory cells (Fig. 3B). Treatment with dexamethasone preserved mucosal architecture, maintained normal goblet cell structure, and showed only mild inflammatory cell infiltration (Fig. 3C). The *B. longum* oil suspension group exhibited partial preservation of epithelial integrity, minor goblet cell alterations, and mild inflammatory infiltration (Fig. 3D), indicating moderate protective effects.

4.2 Colon Weight and Inflammation Assessment:

Colon damage in inflammatory bowel disease (IBD) is commonly associated with elevated oxidative stress and infiltration of inflammatory cells, which compromise the mucosal barrier, induce tissue edema, and consequently increase colon weight. In the present study, DNBS-treated rats showed a significant increase in colon weight compared to the normal control group ($p < 0.05$; Fig. 4). Administration of dexamethasone or *Bifidobacterium longum* oil suspension significantly attenuated this increase in colon weight relative to the disease control (DC) group, with dexamethasone exerting a more pronounced effect than *B. longum* (Fig. 4).

To further assess the anti-inflammatory effects of the treatments, fecal calprotectin; a validated biomarker of intestinal inflammation was quantified.

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$^{*\#}$	0.741 ± 0.054$^{*\#\$}$
DAI score	0.152 ± 0.011	3.338 ± 0.219*	1.337 ± 0.096$^{*\#}$	1.552 ± 0.132$^{*\#\$}$

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.

Fecal calprotectin levels were significantly elevated in the disease control (DC) group compared to the normal control group ($p < 0.05$; Table 2), confirming active intestinal inflammation. Treatment with dexamethasone or *Bifidobacterium longum* oil suspension significantly decreased fecal calprotectin concentrations relative to the DC group ($p < 0.05$), indicating their anti-inflammatory efficacy. Notably, dexamethasone exhibited a significantly greater reduction in calprotectin levels than the *B. longum* oil suspension (Table 2).

4.3 Measurement of Colonic Oxidative Stress Levels:

Table 2 summarizes the impact of DNBS-induced colitis on oxidative stress biomarkers and the protective effects of dexamethasone and *Bifidobacterium longum* oil suspension against oxidative damage. At the conclusion of the study, rats with DNBS-induced colitis exhibited a significant reduction in antioxidant biomarkers, including glutathione (GSH: 82.38 vs. 354.12 µg/mL; $p < 0.05$) and superoxide dismutase (SOD: 3.27 vs. 15.05 U/g tissue; $p < 0.05$), compared to the normal control group. Conversely, oxidative stress markers, namely nitric oxide (NO: 1088.25 vs. 189.45 µmol/mL; $p < 0.05$) and malondialdehyde (MDA: 1.46 vs. 0.21 µg/mL; $p < 0.05$), were significantly elevated in the DNBS group.

Dexamethasone supplementation significantly restored antioxidant levels, with glutathione (GSH) increasing to 212.48 µg/mL compared to 82.38 µg/mL in the disease control (DC) group ($p < 0.05$), and superoxide dismutase (SOD) activity rising to 12.56 U/g tissue versus 3.27 U/g tissue in the DC group ($p < 0.05$). Concurrently, oxidative stress markers were markedly reduced, as nitric oxide (NO) decreased to 235.14 µmol/mL from 1088.25 µmol/mL ($p < 0.05$) and malondialdehyde (MDA) declined to 0.11 µg/mL from 1.46 µg/mL ($p < 0.05$). Similarly, treatment with *Bifidobacterium longum* oil suspension significantly enhanced antioxidant status (GSH: 195.25 vs. 82.3 µg/mL; SOD: 10.52 vs. 3.27 U/g tissue; $p < 0.05$) and reduced oxidative stress markers (NO: 381.42 vs. 1088.25 µmol/mL; MDA: 0.3 vs. 1.46 µg/mL; $p < 0.05$) compared to the DC group.

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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.

However, dexamethasone demonstrated significantly greater efficacy than *Bifidobacterium longum* oil suspension in enhancing antioxidant defenses and reducing oxidative stress markers ($p < 0.05$ for all evaluated parameters).

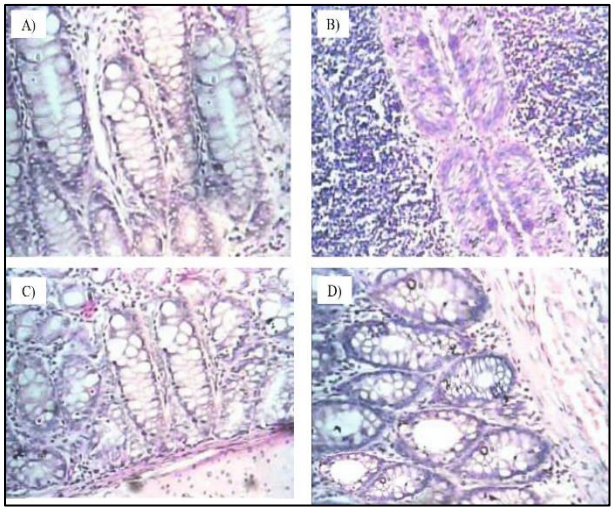

Fig. 3: Histological evaluation of colonic tissue of (A) Normal control group, (B) Disease control group, (C) Standard (dexamethasone) group, and (D) *B. longum* oil suspension group.

5. Discussion

Several previous studies have demonstrated that dinitrobenzene sulfonic acid (DNBS) induces colitis through disruption of the gut microbiota and induction of dysbiosis (28-30). In this study, *Sprague-Dawley* (SD) rats were used to evaluate the therapeutic efficacy of *Bifidobacterium longum* suspension in avocado oil in a DNBS-induced colitis model, with dexamethasone serving as the standard control treatment. The results provide preliminary evidence supporting the potential of *B. longum* oil suspension to ameliorate colitis. Based on these findings, it is postulated that *B. longum* oil suspension exerts protective effects on the intestinal mucosa by reducing oxidative stress and inflammation, while enhancing endogenous antioxidant defenses.

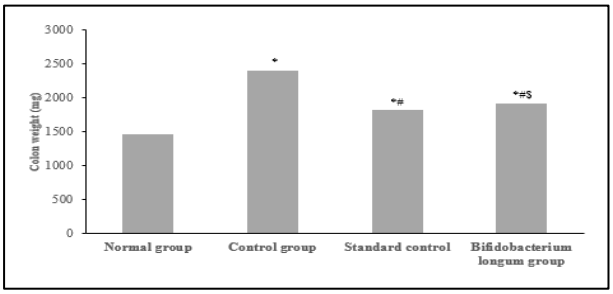

Fig. 4: Change in colon weight. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively.

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Numerous studies have underscored the critical role of gut dysbiosis in the pathophysiology of inflammatory bowel disease (IBD) [16-19]. Gut dysbiosis is defined as an imbalance in the gastrointestinal microbial community, characterized by an increase in pathogenic microorganisms and a concomitant reduction in beneficial microbes. This microbial imbalance contributes to the onset and progression of IBD by disrupting the homeostatic interaction between the gut microbiota and the intestinal epithelium, inducing aberrant immune responses and promoting epithelial damage mediated by harmful microbes [16-19].

The gut microbiome plays a fundamental role in maintaining intestinal health. For example, Firmicutes species produce short-chain fatty acids (SCFAs), such as butyrate, which suppress pro-inflammatory cytokine activity. Proteobacteria synthesize various bacteriocins that inhibit the colonization and proliferation of pathogenic microorganisms. Actinobacteria contribute to maintaining the integrity of the intestinal barrier, while Bacteroidetes facilitate nutrient absorption and support the maturation of intestinal epithelial cells [31,32].

Gut dysbiosis, characterized by a reduction in beneficial bacterial populations, impairs these protective and regulatory functions, leading to a cascade of events that compromise the intestinal mucosal barrier [31,32]. Probiotics have been shown to restore microbial balance by reducing harmful bacterial populations and promoting the growth of beneficial microbes, thereby improving overall gut health[33]. In the present study, the beneficial effects observed with *Bifidobacterium longum* oil suspension may be attributed to its capacity to modulate the gut microbiota, ultimately enhancing intestinal health.

Oxidative stress is a key factor in the initiation and progression of inflammatory bowel disease (IBD) [34]. It is defined as a state characterized by excessive generation of reactive oxygen species (ROS), resulting in cellular damage to proteins, lipids, polysaccharides, and nucleic acids [35]. Oxidative stress activates the pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which promotes the production of various pro-inflammatory cytokines [34,35]. These cytokines infiltrate the intestinal epithelium, exacerbating inflammation and accelerating cellular injury. Moreover, activation of the NF-κB pathway and elevated cytokine levels impair mitochondrial function, thereby amplifying ROS production and oxidative stress in a self-perpetuating cycle [36-38]. This reciprocal interaction between inflammation and oxidative damage culminates in severe disruption of the intestinal epithelial barrier, a hallmark of IBD pathogenesis [34-39].

Although probiotics are widely recognized for their antioxidant and anti-inflammatory properties that contribute to the mitigation of oxidative stress and inflammation [40,41] several studies have also emphasized the role of gut dysbiosis in exacerbating oxidative stress-associated intestinal injury [42,43].

Elevated levels of nitric oxide (NO) in intestinal tissues are recognized as key biomarkers of inflammation and oxidative stress in inflammatory bowel disease (IBD) [44]. The overproduction of NO is primarily mediated by the upregulation of inducible nitric oxide synthase (iNOS), often triggered by mucosal endothelial damage induced by excessive reactive oxygen species (ROS) and pro-inflammatory cytokines [44]. In conditions of antioxidant depletion, NO exerts pro-oxidant effects analogous to ROS, thereby intensifying oxidative stress and promoting further activation of inflammatory signaling pathways. The intestinal microbiota plays a regulatory role in NO synthesis through the production of short-chain fatty acids (SCFAs), particularly butyrate, which has been shown to suppress iNOS expression and activity in the intestinal epithelium [45]. However, gut dysbiosis marked by altered SCFA profiles and heightened oxidative and inflammatory states results in uncontrolled NO production, contributing significantly to intestinal injury and the pathogenesis of IBD.

In the present study, administration of *Bifidobacterium longum* oil suspension significantly reduced colonic nitric oxide (NO) levels, supporting the probiotic's role in attenuating oxidative stress and inflammatory responses. Furthermore, treatment with *B. longum* oil suspension markedly decreased tissue malondialdehyde (MDA) concentrations compared to the disease control group. MDA is a widely recognized biomarker of oxidative stress [34,36] and elevated tissue and serum levels have been closely associated with disease severity in inflammatory bowel disease (IBD) [34]. The observed decline in MDA levels following *B. longum* oil supplementation indicates a substantial reduction in

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oxidative damage, further reinforcing the antioxidant potential of the formulation in the DNBS-induced colitis model.

Antioxidants, including glutathione (GSH) and superoxide dismutase (SOD), play a pivotal role in modulating oxidative stress within the body [47-49]. These compounds act by scavenging free radicals and inhibiting the overactivation of enzymes responsible for the generation of reactive oxygen species (ROS), thereby preserving cellular redox homeostasis and supporting normal cellular function, growth, and development [50]. In the present study, animals in the disease control group demonstrated significantly decreased levels of GSH and SOD, consistent with findings from prior experimental and clinical investigations [51]. Conversely, administration of *Bifidobacterium longum* oil suspension resulted in a marked increase in GSH and SOD concentrations, supporting previous reports that suggest the efficacy of probiotic interventions in enhancing antioxidant defences [52].

Moreover, fecal calprotectin, a well-established and sensitive biomarker of intestinal inflammation, is typically elevated in individuals with inflammatory bowel disease (IBD) [53]. In the current study, supplementation with *Bifidobacterium longum* oil suspension resulted in a significant reduction in fecal calprotectin levels, further substantiating the probiotic's anti-inflammatory properties in alignment with findings from previous investigations [54].

A notable strength of the present study is that it is the first to assess the therapeutic efficacy of *Bifidobacterium longum* formulated in an avocado oil suspension in mitigating the severity of 2,4-dinitrobenzenesulfonic acid (DNBS)-induced colitis in Sprague-Dawley (SD) rats. The findings are in concordance with previous research, thereby reinforcing the outcomes observed in this investigation. Furthermore, by evaluating key markers of inflammation and oxidative stress, the study elucidated potential mechanisms underlying the protective effects of *B. longum* oil suspension against colitis-associated intestinal injury.

Despite its strengths, the present study has certain limitations. A major limitation is the absence of a comparison group treated with conventional inflammatory bowel disease (IBD) therapies. Although dexamethasone was included as a reference treatment, other commonly used agents such as sulfasalazine were not evaluated. Inclusion of a broader range of standard therapies in future studies would allow for a more comprehensive assessment of the therapeutic potential of *Bifidobacterium longum* oil suspension. Additionally, while the study focused on markers of inflammation and oxidative stress, the observed protective effects may also involve alternative mechanisms, including immunomodulation and alterations in the gut microbiota, which merit further investigation.

In summary, both dexamethasone and *Bifidobacterium longum* oil suspension demonstrated efficacy in ameliorating DNBS-induced experimental colitis. The protective effects of these treatments are likely mediated through their anti-inflammatory and antioxidant activities. Further experimental and clinical studies are required to validate and extend the findings of the present investigation.

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