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Evaluation of the Efficacy of an Avocado Oil-Based *Lactobacillus rhamnosus* Suspension in DNBS-Induced Colitis in *Sprague-Dawley* Rats

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KEYWORDS

Probiotics

IBD

Lactobacillus rhamnosus

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Oxidative stress

Antioxidant activity

ABSTRACT:

Introduction: Inflammatory bowel disease (IBD) is a persistent inflammatory condition affecting the gastrointestinal tract (GIT), characterized by a spectrum of symptoms ranging from mild abdominal discomfort and diarrhoea to severe manifestations such as rectal bleeding, anemia, and, in extreme cases, fatal outcomes. Disruption of the gut microbiota, or dysbiosis, plays a key role in the onset and progression of IBD. Although corticosteroids remain a mainstay in treatment, their long-term use is associated with adverse effects, prompting interest in safer alternatives such as probiotics.

Objectives: This study aimed to assess the therapeutic efficacy of a *Lactobacillus rhamnosus* suspension formulated in Avocado oil in a rat model of colitis induced by dinitrobenzene sulfonic acid (DNBS).

Methods: Colitis was induced in *Sprague-Dawley* rats via intra-rectal administration of DNBS (120 mg/kg), followed by a 3-day induction period. Post-induction, animals were randomized into treatment groups receiving either oral dexamethasone (2 mg/kg/day) or *L. rhamnosus* suspension (3×10^6 CFU/g/day) for 28 consecutive days. Evaluated parameters included changes in body weight, food and water intake, colon weight, histopathological alterations, colonic mucosal damage index (CMDI), and disease activity index (DAI). Biochemical markers analyzed comprised oxidative stress indicators (nitric oxide [NO], malondialdehyde [MDA]), antioxidant enzymes (glutathione [GSH], superoxide dismutase [SOD]), and the intestinal inflammation marker fecal calprotectin. Statistical analysis was performed using GraphPad Prism, with significance set at $p < 0.05$.

Results: Treatment with both dexamethasone and the *L. rhamnosus* oil suspension significantly prevented DNBS-induced weight loss and improved food and water intake. Both interventions resulted in notable reductions in CMDI and DAI scores, suppressed oxidative stress (lower NO and MDA levels), and enhanced antioxidant defenses (elevated GSH and SOD levels). Fecal calprotectin levels also decreased significantly in both treatment groups. However, dexamethasone consistently produced a more pronounced therapeutic effect across all assessed parameters compared to the probiotic suspension.

Conclusions: The study demonstrates that *L. rhamnosus* suspended in Avocado oil offers protective benefits in DNBS-induced colitis, likely mediated through its antioxidant and anti-inflammatory properties. Although dexamethasone showed superior efficacy, the probiotic formulation presents a promising complementary or alternative therapy for IBD with a potentially better safety profile.

1. Introduction

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract (GIT) [(1,2)]. Based on the location and extent of inflammation, IBD is broadly categorized into two main types: Ulcerative Colitis (UC) and Crohn's Disease (CD) [(2,3)]. UC predominantly affects the inner mucosal lining of the colon and rectum, resulting in inflammation, ulceration, and mucosal injury. In contrast, CD can impact any part of the GIT from the oral cavity to the anus and often involves deeper layers of the intestinal wall, extending beyond the mucosa [(1–3)]. In India, IBD ranks among the most prevalent chronic inflammatory disorders of the digestive system, with a reported prevalence of up to 19.3% [(4)], placing the country among those with the

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highest IBD patient populations globally [5]. Over the past few decades, IBD incidence has risen markedly, a trend attributed to modern sedentary lifestyles, increased exposure to environmental toxins, high-fat and high-carbohydrate diets, chronic alcohol consumption, smoking, and a growing burden of infections [6].

Clinical manifestations of IBD differ depending on disease type and severity but commonly include gastrointestinal symptoms such as diarrhea, abdominal pain, bloating, and anorexia, along with systemic features like fatigue, fever, and general malaise. In more severe cases, complications may involve rectal or gastrointestinal bleeding, anemia, and even fainting episodes [7,8].

Beyond its clinical burden, IBD also poses significant economic challenges due to prolonged treatment regimens and recurrent hospital admissions [9,10]. Current therapeutic strategies incorporate both pharmacological and non-pharmacological interventions. These include lifestyle adjustments, dietary changes, and cessation of smoking and alcohol, alongside medications such as aminosalicylates, corticosteroids, immunosuppressants, and biologic agents [11,12]. However, limitations in efficacy and safety continue to drive the search for novel and more effective treatment options.

The human gastrointestinal tract hosts trillions of microorganisms collectively referred to as the gut microbiome [13]. These microbes play crucial roles in digestion, immune regulation, and protection against pathogenic bacteria [13,14]. Disruption of this microbial balance referred to as gut dysbiosis is characterized by a reduction in beneficial microbes and overgrowth of harmful species, and is increasingly recognized as a key factor in the pathogenesis of IBD and other inflammatory disorders [13,15]. A growing body of evidence supports gut dysbiosis as a critical therapeutic target in IBD management [16–19].

Probiotics, defined by the World Health Organization as live microorganisms that provide health benefits when administered in adequate amounts, have garnered attention for their potential to restore gut microbial balance [13,20]. Both experimental and clinical studies have highlighted the therapeutic potential of probiotics in conditions associated with dysbiosis, including IBD [21–23]. While various probiotic formulations have shown promise in preclinical models of IBD [24–27], no study has yet evaluated the therapeutic potential of an avocado oil based *Lactobacillus rhamnosus* suspension in this setting.

Accordingly, the present study aimed to investigate the protective efficacy of a *Lactobacillus rhamnosus* suspension formulated in avocado oil in a dinitrobenzene sulfonic acid (DNBS)-induced colitis model using *Sprague-Dawley* rats.

2. Objectives

This study was conducted to evaluate the protective effects of a *Lactobacillus rhamnosus* suspension formulated in avocado oil against dinitrobenzene sulfonic acid (DNBS)-induced colitis in *Sprague-Dawley* rats.

3. Methods

3.1 Preparation and Characterization of *Lactobacillus rhamnosus* Suspension in Avocado Oil:

The oil-based probiotic suspension was prepared using *Lactobacillus rhamnosus*, silicon dioxide, avocado oil, and selected pharmaceutical excipients. To begin, the probiotic powder and excipients were accurately weighed and sieved through an appropriate mesh to ensure uniform particle size distribution. Avocado oil served as the suspending medium. Silicon dioxide was added to the oil and stirred at 750 rpm for 10 minutes using a magnetic stirrer to ensure even dispersion. Following this, the probiotic powder and remaining excipients were gradually added to the silicon dioxide–avocado oil mixture and thoroughly blended to obtain a uniform suspension. The final product was a pale yellow suspension with the characteristic aroma of avocado oil, indicating stability and the absence of any degradation or chemical interaction between the ingredients. Visual examination confirmed that the formulation was free of foreign matter and showed no evidence of phase separation.

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

The viable cell count of *Lactobacillus rhamnosus* in the formulated oil suspension was determined using a standard microbiological method. A 100 µL aliquot of the suspension was aseptically transferred onto sterile Petri dishes and evenly spread across the surface. Sterile de Man, Rogosa, and Sharpe (MRS) agar previously

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sterilized by boiling and cooled to a suitable temperature was then poured into the plates and allowed to solidify at room temperature for 15–30 minutes. The plates were incubated under anaerobic conditions at room temperature for 24 hours. Following incubation, the number of viable colony-forming units (CFUs) was quantified using a calibrated digital colony counter to assess the viable *Lactobacillus rhamnosus* population in the suspension.

3.3 Animals

Healthy adult male and female *Sprague-Dawley* (SD) rats, weighing between 225 and 250 grams, were procured from the Arihant School of Pharmacy & Bio-Research, Gandhinagar, Gujarat, India. The animals were housed in standard polypropylene cages under controlled laboratory conditions, with unrestricted access to a standard pellet diet and drinking water. Prior to the initiation of the study, all rats underwent a one-week acclimatization period in an environment maintained at 25–30 °C with a 12-hour light/dark cycle. All experimental procedures were conducted in accordance with institutional ethical 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 colitis induction, the rats were fasted for 12 hours while being allowed unrestricted access to water. Mild anesthesia was induced using diethyl ether, and animals were continuously monitored for respiratory rate and reflex activity to ensure adequate anesthetic depth. Once anesthetized, a 1.0 mL syringe fitted with a flexible catheter was gently inserted into the rectum to a depth of approximately 8 cm, targeting the splenic flexure. A freshly prepared solution of dinitrobenzene sulfonic acid (DNBS; 120 mg/kg body weight in 50% ethanol) was then administered intrarectally. To minimize reflux and ensure uniform distribution of the solution, the animals were maintained in the Trendelenburg position for approximately five minutes post-administration. Clinical symptoms of colitis—including weight loss, diarrhea, and rectal bleeding typically manifested within three days following DNBS exposure.

3.5 Experimental Groups:

The animals were randomly assigned to four groups (n = 6 per group): normal control (NC), disease control (DC), standard control (SC), and test (T). The NC group received an intrarectal administration of normal saline (1 mL/kg) along with the vehicle. Colitis was induced in the DC, SC, and T groups via intrarectal administration of DNBS solution. Following induction, the SC group was orally administered dexamethasone at a dose of 2 mg/kg/day, while the T group received an oral dose of *Lactobacillus rhamnosus* oil suspension containing 3×10^6 colony-forming units (CFU)/g/day. The DC group was given the vehicle orally to serve as a control. All treatments were administered once daily for 28 consecutive days. On day 28, all animals were euthanized, and the abdominal cavity was opened for careful dissection and collection of colon tissues for subsequent analysis.

3.6 Evaluation parameters:

3.6.1 Body Weight and Food and Water Intake:

Food and water intake were recorded daily for each group throughout the supplementation period, and the data were used to calculate the average consumption per group. Additionally, the body weight of each rat (in grams) was measured at both the beginning and 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 removed. The tissue was opened longitudinally along the antimesenteric border, and any adherent tissue was gently cleared. The colon was then thoroughly rinsed with saline buffer to eliminate residual contents and subsequently weighed. For macroscopic evaluation of tissue damage, the colon was placed on a wax block and assessed using the Disease Activity Index (DAI) and the Colonic Mucosal Damage Index (CMDI). The DAI is rated on a scale from 0 to 4, with 0 indicating intact colonic crypts and surface epithelium, and 4 indicating severe colitis marked by extensive hyperemia, necrosis, and mucosal ulceration involving more than 40% of the colon. Likewise, the CMDI score ranges from 0 (normal mucosa) to 4 (complete loss of colonic crypts and surface epithelium).

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3.6.3 Tissue Homogenate Preparation:

The excised colon tissue was homogenized in ice-cold phosphate-buffered saline (pH 7.4) at a concentration of 50 g/L. The homogenate was then centrifuged at 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 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 determined 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 vortexed thoroughly and incubated in a water bath at 85 °C for 1 hour. After incubation, absorbance 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 calculated either from a standard curve or by using the molar extinction coefficient of 1.56×10^5 $M^{-1} \cdot cm^{-1}$ and expressed in µg/mL.

3.6.4.2 Nitric Oxide (NO) Estimation:

Nitric oxide levels were measured using the Griess reaction. In brief, 1.0 mL of tissue homogenate was combined with 1.0 mL of sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid, equilibrated to room temperature) and incubated in the dark at room temperature for 5–10 minutes. Next, 1.0 mL of NED solution (0.1% N-1-naphthylethylenediamine dihydrochloride in water, also equilibrated to room temperature) was added, and the mixture was incubated for an additional 5–10 minutes, protected from light. After 30 minutes, the purple/magenta-colored reaction product's absorbance was measured at 540 nm. A blank sample was prepared by substituting the homogenate with 1.0 mL of distilled water and processed identically. Nitric oxide concentration was determined from a standard curve and expressed in 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 absorbance of the mixture was measured at 480 nm every 30 seconds over a 3-minute period. A blank, prepared by substituting the tissue homogenate with 1.0 mL of distilled water, was processed in the same way. SOD activity was expressed as units per gram of tissue (U/g tissue).

3.6.4.4 Glutathione (GSH) Estimation:

Glutathione levels were measured by combining 1.0 mL of tissue homogenate with 1.0 mL of 10% trichloroacetic acid (TCA). The mixture was cooled for 10 minutes, then centrifuged at 2000 rpm for 10 minutes, and the supernatant was collected. Next, 0.5 mL of the supernatant was mixed 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). After thorough mixing, the solution was incubated at room temperature for 5 minutes. Absorbance was recorded at 412 nm against a blank prepared with distilled water processed in the same manner. Glutathione concentration was expressed in µg/mL.

3.6.4.5 Fecal Calprotectin Content Estimation:

Fecal samples were collected from the animals prior to euthanasia and homogenized in saline at a ratio of 1 mL per gram of feces to assess fecal calprotectin levels. The homogenate was thoroughly mixed and stored at –20 °C until analysis. Fecal calprotectin concentrations were determined using a commercial assay kit following the manufacturer's instructions, with results reported in ng/mL.

3.6.4.6 Histopathological Analysis of the Colonic Mucosal Layer:

The excised colon tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned into 3 µm-thick slices, which were then placed on glass slides. For histopathological examination, the sections were stained with Hematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS). These staining techniques facilitated the assessment of intestinal tight junction

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integrity, goblet cell morphology, mucosal cellular architecture, and mucosal alterations associated with oxidative stress and inflammatory cell infiltration.

3.7 Statistical Analysis:

Results are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test, conducted with GraphPad Prism software (version 9.0.0; GraphPad Software Inc., San Diego, CA, USA). A p-value less than 0.05 was considered statistically significant.

4. Results

4.1 Body Weight and Dietary Intake Assessment:

Weight loss is a common clinical feature of inflammatory bowel disease (IBD), often caused by reduced food and water intake, increased fecal output, and blood loss. In this study, rats with DNBS-induced colitis exhibited a significant decline in body weight compared to the normal control group ($p < 0.05$; Fig. 1). Treatment with both dexamethasone *and Lactobacillus rhamnosus* oil suspension significantly mitigated this weight loss, with dexamethasone showing a notably greater protective effect than the *L. rhamnosus* suspension ($p < 0.05$).

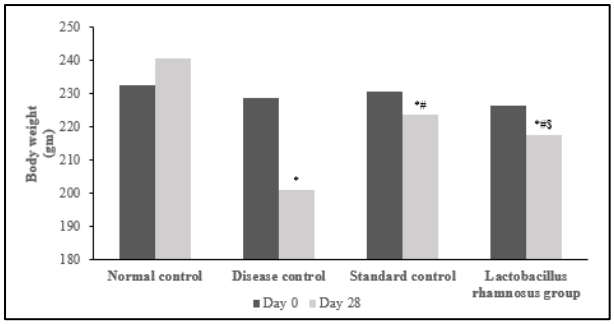

Fig. 1: Change in body weight during study duration. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively.

Similarly, the disease control (DC) group showed a significant decrease in food and water intake compared to the normal controls ($p < 0.05$; Fig. 2). Both treatment groups demonstrated significant improvements in consumption compared to the DC group, with dexamethasone-treated rats exhibiting a more pronounced increase than those receiving the *L. rhamnosus* oil suspension. These alterations in dietary intake closely paralleled the changes observed in body weight.

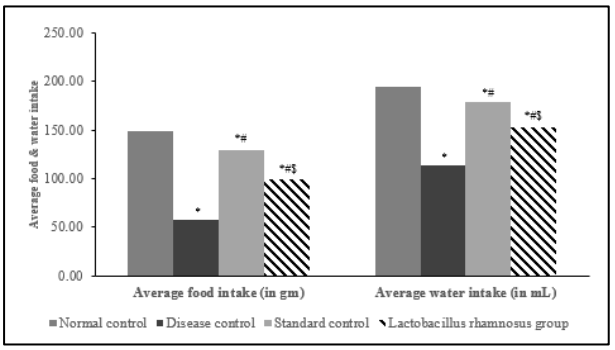

Fig. 2: Change in average food and water intake. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively.

4.2 Colonic Damage Scores and Histological Evaluation:

Intrarectal administration of DNBS caused a significant increase in both the Colonic Mucosal Damage Index (CMDI) and Disease Activity Index (DAI), indicating substantial damage to the colonic mucosa (Table 1). The CMDI score rose sharply 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 increased significantly from 0.152 to 3.338 ($p < 0.05$), confirming severe mucosal injury following colitis induction.

Treatment with dexamethasone or *Lactobacillus rhamnosus* oil suspension significantly alleviated DNBS-induced mucosal damage. Dexamethasone notably reduced the CMDI score (0.555 vs. 2.228; $p < 0.05$) and the DAI score (1.337 vs. 3.338; $p < 0.05$) compared to the disease control group. Likewise, administration of the *L. rhamnosus* oil suspension significantly decreased CMDI (0.781 vs. 2.228; $p < 0.05$) and DAI (1.752 vs. 3.338; $p < 0.05$) scores relative to the disease control. However, dexamethasone demonstrated superior protective effects over the *L. rhamnosus* suspension, as reflected by significantly lower CMDI and DAI scores (Table 1).

Histological examination corroborated these findings. The normal control group displayed intact colonic mucosa with well-preserved epithelial cells and goblet

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cell morphology, with no evidence of inflammatory infiltration (Fig. 3A).

Table 1: Macroscopic colonic damage scores

	Normal group	**Disease control**	**Standard control**	***L. rhamnosus* oil suspension group**
CMDI score	0.112 ± 0.042	2.228 ± 0.064*	0.555 ± 0.037$^{*\#}$	0.781 ± 0.039$^{*\#\$}$
DAI score	0.152 ± 0.011	3.338 ± 0.219*	1.337 ± 0.096$^{*\#}$	1.752 ± 0.163$^{*\#\$}$

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.

In contrast, the DNBS-induced colitis group exhibited extensive mucosal damage, disruption of goblet cells, and pronounced inflammatory cell infiltration (Fig. 3B). Dexamethasone treatment preserved mucosal architecture, maintained normal goblet cell morphology, and showed only mild inflammatory infiltration (Fig. 3C). The *L. rhamnosus* oil suspension group showed partial preservation of epithelial structure, minor goblet cell alterations, and mild inflammatory infiltration (Fig. 3D), indicating moderate protective effects.

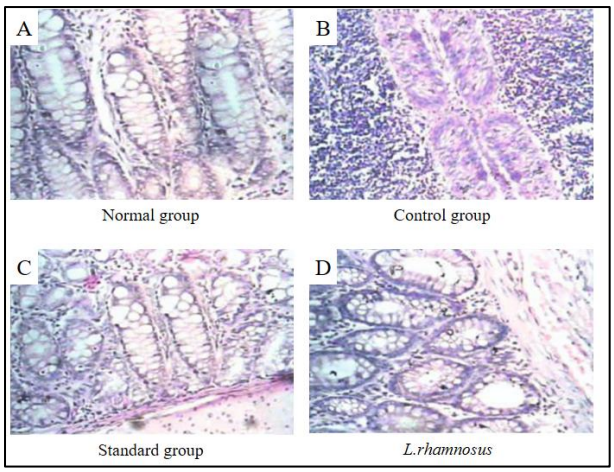

Fig. 3: Histological evaluation of colonic tissue of (A) Normal control group, (B) Disease control group, (C) Standard (dexamethasone) group, and (D) L. rhamnosus oil suspension group.

4.2 Colon Weight and Inflammation Assessment:

Colon damage in inflammatory bowel disease (IBD) is commonly associated with increased oxidative stress and infiltration of inflammatory cells, which compromise the mucosal barrier, cause tissue edema, and result in increased colon weight. In this study, rats treated with DNBS showed a significant increase in colon weight compared to the normal control group ($p < 0.05$; Fig. 4). Treatment with either dexamethasone or *Lactobacillus rhamnosus* oil suspension significantly reduced this increase in colon weight compared to the disease control (DC) group, with dexamethasone exhibiting a more pronounced effect than the *L. rhamnosus* suspension (Fig. 4).

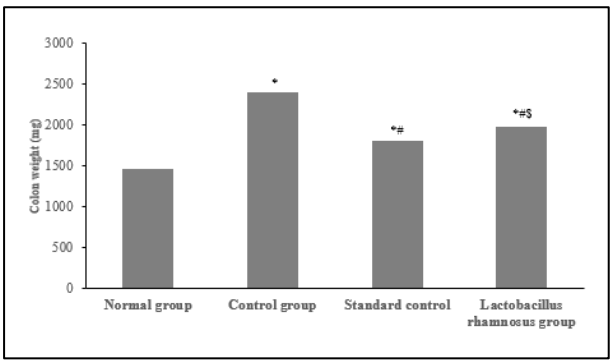

Fig. 4: Change in colon weight. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively.

To further assess the anti-inflammatory effects of the treatments, fecal calprotectin—a well-established marker of intestinal inflammation—was measured. Fecal calprotectin levels were significantly elevated in the DC group compared to the normal control group ($p < 0.05$; Table 2), confirming active intestinal inflammation. Both dexamethasone and *Lactobacillus rhamnosus* oil suspension treatments significantly lowered fecal calprotectin levels relative to the DC group ($p < 0.05$), indicating their anti-inflammatory efficacy. Notably, dexamethasone induced a significantly greater reduction in calprotectin levels compared to the *L. rhamnosus* oil suspension (Table 2).

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

	Normal group	Disease control	Standard control	*L. rhamnosus* oil suspension group
NO level (µmol/mL)	189.45 ± 3.63	1088.25 ± 9.29*	235.14 ± 8.95*#	370.60 ± 5.92*#$
MDA level (µg/mL)	0.21 ± 0.05	1.46 ± 0.08*	0.11 ± 0.02*#	0.27 ± 0.09*#$
GSH level (µg/mL)	354.12 ± 6.19	82.38 ± 2.93*	212.48 ± 4.50*#	165.75 ± 3.95*#$
SOD level (U/gm tissue)	15.05 ± 2.46	3.27 ± 0.46*	12.56 ± 2.45#	8.93 ± 0.42*#$
Fecal calprotectin level (ng.mL)	2910.00 ± 115.29	24866.17 ± 649.37*	9534.17 ± 246.02*#	12830.00 ± 794.29*#$

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.

4.3 Measurement of Colonic Oxidative Stress Levels:

Table 2 illustrates the impact of DNBS-induced colitis on oxidative stress markers and the protective effects of dexamethasone and *Lactobacillus rhamnosus* 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 such as nitric oxide (NO: 1088.25 vs. 370.60 µmol/mL; $p < 0.05$) and malondialdehyde (MDA: 1.46 vs. 0.27 µg/mL; $p < 0.05$) were significantly elevated in the DNBS-treated group.

Dexamethasone treatment markedly restored antioxidant levels, with glutathione (GSH) increasing to 212.48 µg/mL from 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 compared to 3.27 U/g tissue in the DC group ($p < 0.05$). Simultaneously, oxidative stress markers were significantly reduced, with nitric oxide (NO) dropping from 1088.25 µmol/mL to 235.14 µmol/mL ($p < 0.05$) and malondialdehyde (MDA) decreasing from 1.46 µg/mL to 0.11 µg/mL ($p < 0.05$). Similarly, treatment with *Lactobacillus rhamnosus* oil suspension significantly enhanced antioxidant status (GSH: 165.75 vs. 82.38 µg/mL; SOD: 8.93 vs. 3.27 U/g tissue; $p < 0.05$) and reduced oxidative stress markers (NO: 370.60 vs. 1088.25 µmol/mL; MDA: 0.27 vs. 1.46 µg/mL; $p < 0.05$) relative to the DC group.

However, dexamethasone demonstrated significantly greater efficacy than *Lactobacillus rhamnosus* oil suspension in elevating antioxidant defenses and lowering oxidative stress markers ($p < 0.05$ for all parameters measured).

5. Discussion

Several prior studies have demonstrated that dinitrobenzene sulfonic acid (DNBS) induces colitis by disrupting the gut microbiota and causing dysbiosis [28–30]. In the present study, *Sprague-Dawley* (SD) rats were used to evaluate the therapeutic potential of a *Lactobacillus rhamnosus* suspension formulated in avocado oil in a DNBS-induced colitis model, with dexamethasone serving as the standard control treatment. The results provide preliminary evidence that the *L. rhamnosus* oil suspension may help mitigate colitis. Based on these findings, it is suggested that the *L. rhamnosus* oil suspension exerts protective effects on the intestinal mucosa by reducing oxidative stress and inflammation while enhancing the body's endogenous antioxidant defenses.

Numerous studies have emphasized the crucial role of gut dysbiosis in the onset and progression of

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inflammatory bowel disease (IBD) [16–19]. Gut dysbiosis is characterized by an imbalance in the gastrointestinal microbial community, featuring an overgrowth of harmful pathogens and a reduction in beneficial microbes. This disruption of the microbial ecosystem contributes to IBD development by impairing the balanced interaction between the gut microbiota and the intestinal epithelium, provoking abnormal immune reactions, and promoting epithelial damage caused by pathogenic microorganisms [16–19].

The gut microbiome plays a vital role in maintaining intestinal health. For example, Firmicutes species produce short-chain fatty acids (SCFAs) such as butyrate, which help inhibit pro-inflammatory cytokine activity. Proteobacteria produce various bacteriocins that prevent the colonization and proliferation of harmful pathogens. Actinobacteria contribute to maintaining the integrity of the intestinal barrier, while Bacteroidetes support nutrient absorption and promote 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 series of events that compromise the intestinal mucosal barrier [31,32]. Probiotics have been shown to help restore microbial balance by reducing harmful bacteria and promoting the growth of beneficial microbes, thus enhancing overall gut health [33]. In this study, the beneficial effects observed with *Lactobacillus rhamnosus* oil suspension may be attributed to its capacity to modulate the gut microbiota, thereby supporting improved intestinal health.

Oxidative stress is a key factor in the development and progression of inflammatory bowel disease (IBD) [34]. It occurs when there is an excessive production of reactive oxygen species (ROS), which damage cellular components such as proteins, lipids, polysaccharides, and nucleic acids [35]. This oxidative imbalance activates the pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, leading to elevated levels of various pro-inflammatory cytokines [34,35]. These cytokines infiltrate the intestinal epithelium, exacerbating inflammation and accelerating tissue injury. Moreover, NF-κB activation and the increase in cytokine production disrupt mitochondrial function, resulting in further ROS generation and heightened oxidative stress, creating a vicious cycle [36–38]. This interaction between inflammation and oxidative damage ultimately causes significant impairment of the intestinal epithelial barrier, which is a hallmark of IBD pathogenesis [34–39].

Probiotics are widely recognized for their antioxidant and anti-inflammatory properties that help alleviate oxidative stress and inflammation [40,41]. Additionally, multiple studies have emphasized that gut dysbiosis contributes to exacerbating oxidative stress-induced damage to intestinal tissue [42,43].

Elevated nitric oxide (NO) levels in intestinal tissues serve as key biomarkers of inflammation and oxidative stress in inflammatory bowel disease (IBD) [44]. This excessive NO production primarily results from the upregulation of inducible nitric oxide synthase (iNOS), which is often triggered by mucosal endothelial injury caused by high levels of reactive oxygen species (ROS) and pro-inflammatory cytokines [44]. When antioxidant defenses are compromised, NO behaves as a pro-oxidant like ROS, intensifying oxidative stress and further stimulating inflammatory signaling pathways. The intestinal microbiota plays a regulatory role in NO production through the release 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 leads to uncontrolled NO production, greatly contributing to intestinal damage and IBD progression.

In this study, treatment with *Lactobacillus rhamnosus* oil suspension significantly reduced colonic nitric oxide (NO) levels, demonstrating the probiotic's effectiveness in mitigating oxidative stress and inflammatory responses. Furthermore, *L. rhamnosus* oil suspension markedly lowered tissue malondialdehyde (MDA) levels compared to the disease control group. Since MDA is a well-established marker of oxidative stress [34,36] and its elevated levels in tissue and serum are closely associated with the severity of inflammatory bowel disease (IBD) [34], the observed decrease in MDA after *L. rhamnosus* oil treatment indicates a significant reduction in oxidative damage. This finding further supports the antioxidant potential of this formulation in the DNBS-induced colitis model.

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Antioxidants like glutathione (GSH) and superoxide dismutase (SOD) are vital for managing oxidative stress in the body [47–49]. They work by neutralizing free radicals and inhibiting the overactivation of enzymes that generate reactive oxygen species (ROS), thereby preserving cellular redox balance and promoting normal cell function, growth, and development [50]. In this study, the disease control group exhibited significantly decreased levels of GSH and SOD, consistent with previous experimental and clinical findings [51]. Conversely, treatment with *Lactobacillus rhamnosus* oil suspension resulted in a significant increase in GSH and SOD levels, supporting earlier evidence that probiotic therapies can effectively boost antioxidant defenses [52].

Additionally, fecal calprotectin a well-established and sensitive marker of intestinal inflammation is typically elevated in patients with inflammatory bowel disease (IBD) [53]. In this study, treatment with *Lactobacillus rhamnosus* oil suspension significantly reduced fecal calprotectin levels, further confirming the probiotic's anti-inflammatory properties and aligning with findings from previous studies [54].

A major strength of this study is that it is the first to assess the therapeutic potential of *Lactobacillus rhamnosus* formulated in an avocado oil suspension for alleviating 2,4-dinitrobenzenesulfonic acid (DNBS)-induced colitis in *Sprague-Dawley* (SD) rats. The findings are consistent with previous research, further reinforcing the conclusions of this investigation. Moreover, by evaluating key markers of inflammation and oxidative stress, the study provides insight into the potential mechanisms through which the *L. rhamnosus* oil suspension offers protection against colitis-associated intestinal damage.

Although this study has several strengths, it also has some limitations. One major limitation is the absence of a comparison group receiving conventional inflammatory bowel disease (IBD) treatments. While dexamethasone served as a reference therapy, other commonly used drugs like sulfasalazine were not included. Future research incorporating a broader range of standard treatments would provide a more comprehensive assessment of the therapeutic potential of *Lactobacillus rhamnosus* oil suspension. Additionally, although this study primarily focused on markers of inflammation and oxidative stress, the observed protective effects may also involve other mechanisms such as immunomodulation and alterations in the gut microbiota, which should be explored in further studies.

In summary, both dexamethasone and *Lactobacillus rhamnosus* oil suspension effectively mitigated DNBS-induced experimental colitis, likely through their anti-inflammatory and antioxidant actions. Further experimental and clinical studies are necessary to validate and expand upon these findings.

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