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Research Article

Efficacy Evaluation of Probiotic *Lactobacillus rhamnosus* Suspension Formulated with Medium Chain Triglycerides Oil in DNBS-Induced Colitis in Sprague-Dawley Rats

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ABSTRACT

Inflammatory bowel disease (IBD) is a highly prevalent chronic inflammatory disease condition leading to widespread gastrointestinal tract (GIT) damage. Based on the severity, the symptoms of IBD vary ranging from mild and frequent abdominal pain, bloating, and diarrhea to severe bloody stools, anemia, unconsciousness, and sometimes fatal as well. Gut dysbiosis is found to play one of the major roles in the pathogenesis of IBD. The current study aimed to evaluate the effectiveness of a probiotic (*Lactobacillus rhamnosus*) suspension in medium-chain triglycerides (MCT) oil against dinitrobenzene sulfonic acid (DNBS) induced colitis in rats. Colitis was induced in Sprague Dawley (SD) rats by intra-rectal administration of DNBS (120 mg/kg/rat) followed by 3 days of colitis induction. Following colitis induction, rats were evenly randomized to receive either dexamethasone (2 mg/kg/day orally) or *L. rhamnosus* oil suspension (3×10^6 CFU/g/day orally) for 28 consecutive days. The body weight, average food, and water intake, histological analysis, colon weight, biomarkers of oxidative stress (nitric oxide (NO) and malondialdehyde (MDA)), intestinal inflammation (fecal calprotectin), and antioxidant potential (glutathione (GSH) and superoxide dismutase (SOD)). The level of colonic damage was evaluated using the colonic mucosal damage index (CMDI) and disease activity index (DAI) scores. GraphPad Prism software was used for statistical analysis with $p < 0.05$ as the significance threshold. Dexamethasone and *L. rhamnosus* oil suspension significantly prevented body weight and food and water intake reduction. Similarly, dexamethasone and *L. rhamnosus* oil suspension therapy significantly attenuated the DNBS-induced colonic damage level. This effect was accompanied by significant improvement in the antioxidant biomarker levels (GSH and SOD) and reduction in the oxidative stress and intestinal inflammation level. In all the evaluated parameters, dexamethasone therapy was significantly better than the *L. rhamnosus* oil suspension therapy. The results of this study highlight the protective effect of dexamethasone and *L. rhamnosus* oil suspension therapy in a DNBS-induced experimental colitis model, an effect that might be attributed to the therapies' antioxidant and anti-inflammatory potential.

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease condition of the gastrointestinal tract (GIT).[1,2] Based on the location and severity of inflammation and damage, IBD is categorized into two types, namely ulcerative colitis (UC) and Crohn's disease (CD).[2,3] UC primarily involves the inflammation of the inner mucosal lining of the large intestine (colon) and the rectum, leading to inner mucosal lining ulcerations and damage, while on the contrary, the CD can affect any part

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of the entire GIT, from mouth to anus, and the damage can be either on the inner mucosal layer or can spread to deep layers as well.[1–3] IBD is one of the most prevalent chronic inflammatory disease conditions of the GIT in India, with prevalence reaching up to 19.3%.[4] Due to such a high disease burden, India is considered the home to the largest population of IBD around the globe.[5]

The prevalence of IBD has increased drastically in the last two to three decades due to various factors, including a sedentary lifestyle, environmental pollutants, consumption of foods rich in fats and carbohydrates, chronic alcohol and smoking, and an increase in the number of infections as well.[6] The symptoms of IBD can vary depending on the type and severity of the disease, typically including gastrointestinal manifestations (like diarrhea, abdominal pain, bloating, and reduced appetite) and systemic symptoms (like fatigue, fever, and malaise), while severe symptoms include GI bleeding, rectal bleeding, anemia, and feeling of unconsciousness.[7,8] Simultaneously, IBD significantly increases the healthcare burden of individuals which includes chronic medications and hospitalization costs as well.[9,10] Currently, the management of IBD is based on a combination of approaches including non-pharmacological approaches (like exercise, dietary and lifestyle changes, and cessation of alcohol and smoking) and pharmacological medications (including aminosalicylates, corticosteroids, immunosuppressants, and biologics).[11,12] Despite all these treatment options, IBD remains an area of exploration for newer therapeutic targets.

The human GIT is the home to trillions of microbes that are collectively termed as human gut microbiome.[13] These resident microbes play various important functions including digestive functions, preventing pathogens growth, and positively modulating immune system activity in response to dynamic GIT environment.[13,14] Gut dysbiosis, a condition associated with the reduction in the number of beneficial microbes and the subsequent increase in the number of pathogenic microbes, leads to the initiation and/or progression of various disease conditions.[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. Probiotics, as defined by the World Health Organization, are live micro-organisms that, when administered in adequate quantities, can provide various health benefits to the recipient.[13,20] Various experimental and clinical studies have underscored the positive role of probiotics in treating various disease conditions associated with gut dysbiosis.[21-23] While various studies have explored the positive effect of probiotic formulation in experimental studies of induced IBD in various animal models,[24–27] no studies to date have evaluated the effectiveness of probiotic *Lactobacillus rhamnosus* oil suspension formulation in IBD conditions. Hence, the current study evaluated the effectiveness of *L. rhamnosus* 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

The preparation of probiotic oil suspension involved the use of *L. rhamnosus*, 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 ten 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

Healthy adult male and female SD rats (225–250 g weight) were obtained from the Arihant School of Pharmacy & Bio-Research, Gandhinagar (Gujarat, India), and housed in propylene cages with free access to standard chow diet and water. The rats were acclimatized for one week at normal room conditions (temperature: 25 to 30°C; 12:12 h dark/light cycle). The complete experimental procedure was approved by the Institutional Animal Ethics Committee of the Arihant School of Pharmacy & Bio-Research (Proposal No.: ASPBRI/IAEC/2022-23/11).

Induction of Experimental Colitis

Rats were fasted for 12 hours overnight before the induction of colitis with free access to water. Fasted rats were slightly anesthetized using ether. During anesthesia, the rats were regularly observed for their respiratory rate and normal reflexes. Following anesthesia, 1-mL syringe attached to a catheter was inserted gently into the anus to reach the colon area (approximately 8 cm from the anus to reach the splenic flexure). The DNBS was freshly prepared (120 mg/kg/rat dissolved in 50% ethanol) and inserted into the colon area via the syringe, following

which the rats were kept in the trendelenburg position for a few minutes (approximately 5 minutes) to avoid reflux. The signs of colitis were observed within 3 days of the induction procedure.

Experimental Groups

The animals were randomly divided into four groups with 6 rats per group as follows, normal control (NC) group were 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. Following induction, the DC group rats were supplemented with vehicle preparation, the SC group rats were treated with dexamethasone solution (2 mg/kg/day orally), and the T group rats were treated with *L. rhamnosus* oil suspension (3×10^6 colony forming units (CFU)/g/day orally). The therapy was supplemented for 28 days. On the day of the study end (day 28), all the rats were euthanized under anesthesia, the abdomen was dissected and the colon was dissected and removed for further analysis as described below.

Evaluation Parameters

Body weight and food and water intake

During the entire supplementation duration, the amount of food and water intake was noted and the data was used to estimate the average intake of food and water in each group during the entire supplementation period. Similarly, the body weight (in gm) of all the rats was measured at the start of supplementation initiation and at the end of the experiment.

Colonic weight, colonic mucosal damage index and disease activity index

Following euthanasia, the colon was dissected and removed. The colon was freed from any adjacent tissues and opened along the antimesenteric border. The colon was then rinsed properly using saline buffer solution to remove all the colonic matter and then weighed. After weighing, the colon was fixed on a wax block to evaluate the extent of colitis-induced colon damage using the colonic mucosal damage index (CMDI) and disease activity index (DAI) evaluation. The scoring for CMDI ranged from zero (normal mucosa) to four (severe colitis determined by severe hyperemia, necrosis, and ulcers on the mucosal surface with the major ulcerative area extending >40% of the colon), while the scoring of DAI ranged from zero (intact colonic crypt and surface epithelium) to four (loss of entire colonic crypt and surface epithelium).

Tissue homogenate preparation

The tissue homogenate was prepared as per the previously described method. Briefly, the dissected colon was homogenized in an ice-cold phosphate buffer solution (pH 7.4) at a concentration of 50 gm/L. The resultant mixture was centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatant was separated and stored immediately at 20°C for analysis of oxidative stress and inflammatory biomarker levels as described below.

Colonic mucosal oxidative stress and inflammatory biomarker analysis

For malondialdehyde (MDA) level estimation, briefly, 1.0-mL of tissue homogenate was mixed with 0.2 mL of 4% w/v sodium dodecyl sulphate, 1.5 mL of 20% acetic acid in 0.27M hydrochloric acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid in a clean test tube. The entire mixture was mixed and heated in a water bath at 85°C for 1-hour. The developed solution was then analyzed at 532 nm using an ultraviolet (UV) visible spectrophotometer. The entire process was performed using 1.0 mL of distilled water for the preparation of a blank sample. MDA was calculated using the molar extraction coefficient of $1.56 \times 10^5\ M^{-1}.cm^{-1}$ or from the standard curve and reported as µg/mL.

For nitric oxide (NO) level estimation, briefly, 1.0 mL of tissue homogenate was mixed with 1.0 mL sulphanilamide solution (1% sulphanilamide in 5% phosphoric acid previously equilibrated at room temperature) and incubated for 5 to 10 minutes at room temperature protected from light. Following incubation, 1.0 mL of NED solution (0.1% N-1-naphthyl ethylenediamine dihydrochloride in water, previously equilibrated at room temperature) was added to the incubated mixture mixed and again incubated for 5 to 10 minutes at room temperature protected from light. The final solution is a purple/magenta colored solution which was used for absorbance reading at 540 nm within 30 minutes of the mixture preparation. The 1.0 mL of distilled water was used for the preparation of a blank sample using the above-discussed process. The quantity of NO was evaluated using the standard curve method and the values were expressed in µmol/mL.

For superoxide dismutase (SOD) level estimation briefly, 1.0 mL of the tissue homogenate was mixed with 0.1 mL ethylenediaminetetraacetic acid (EDTA) solution (1×10^{-4} M), 0.5 mL carbonate buffer (pH 9.7), and 1.0 mL epinephrine solution (3×10^{-3} M) in a clean test tube. The optical density of the final mixture was read at 480 nm for 3 minutes at intervals of 30 seconds. Blank sample was prepared using 1.0 mL of distilled water instead of the tissue homogenate and following the same above-discussed procedure. The observed results were expressed as units per tissue gram (U/gm tissue).

For glutathione (GSH) level estimation, briefly, 1.0 mL of tissue homogenate was mixed with 1-mL TCA solution (10% trichloroacetic acid in water). The mixture was cooled for 10 minutes, centrifuged at 2000 rpm for 10 minutes, and the supernatant collected. To 0.5 mL of the collected supernatant, 4.0 mL DTNB solution (0.6% 5,5'-Dithiobis-2-nitrobenzoic acid in 1% sodium citrate solution) and 1.5 mL phosphate buffer solution were added. The mixture was

mixed well and kept at room temperature for 5 minutes. The intensity of color developed was analyzed at 412 nm using distilled water mixture (prepared using the same method) as a blank mixture. The observed readings were reported as µg/mL.

For fecal calprotectin level estimation, the feces of the animals before the euthanasia were collected from the cages and mixed with saline solution (1-mL/gm feces). The mixture was mixed and stored in a freezer until analysis. Fecal calprotectin level was measured using standard analysis kits and procedures as per the manufacturer's protocol. The fecal calprotectin level was recorded as ng/mL.

Histopathological analysis of colonic mucosal layer

Using 10% neutral buffered formalin solution, the collected colonic tissues were fixed and embedded in paraffin solution, cut into 3 µm sections and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) solution and periodic acid Schiff regent for histopathological assessment of mucosal layer cellular integrity, goblet cells structure, intestinal tight junctions, and change in mucosal layer due to inflammatory cells infiltration and oxidative stress.

Statistical Analysis

The complete results were expressed in mean ± SD. The GraphPad Prism software (Desktop version 9.0.0; GraphPad Software Inc., San Diego, CA, USA) was used for conducting the statistical analysis using two-way ANOVA with the post-hoc Tukey test. $p < 0.05$ was considered as the threshold for statistical significance for all the evaluated parameters.

Results

Body Weight and Dietary Change Measurement

Reduction in body weight is one of the common features of IBD, which is attributed to an increase in feces output, reduced food and water intake, and loss of blood as well. Similarly, animals with DNBS-induced colitis showed a significant reduction in body weight compared to normal control animals ($p < 0.05$; Fig. 1). As shown in Fig. 1, treatment with dexamethasone and *L. rhamnosus* oil suspension significantly prevented the reduction in body weight compared to DC group. Comparing the effect, dexamethasone was found to be significantly better than

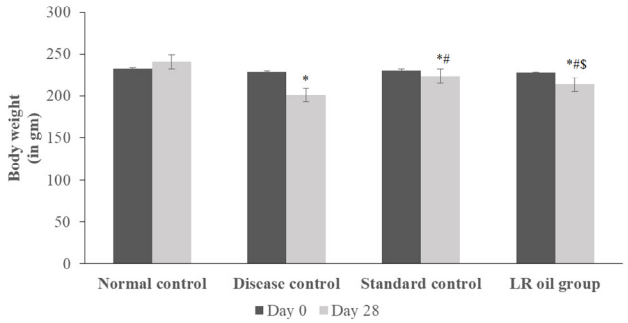

Fig. 1: Change in body weight during study duration. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively

L. rhamnosus oil suspension therapy ($p < 0.05$). Similarly, significant reduction in overall food and water intake was observed in DC group rats compared to control group rats ($p < 0.05$; Fig. 2). The effect of dexamethasone and *L. rhamnosus* oil suspension on food and water intake levels showed similar trend compared to body weight, with significant improvement in overall food and water intake in both the groups compared to DC group, while significantly more pronounced effect observed in the dexamethasone-treated group compared to *L. rhamnosus* oil suspension group (Fig. 2).

Colonic damage scores and histological evaluation

The intra-rectal DNBS therapy resulted in significant damage to the colonic mucosal area, as observed by the significant increase in the CMDI and DAI scores (Table 1). Compared to a control group, rats with DNBS-induced colitis showed a significant increase in the CMDI score

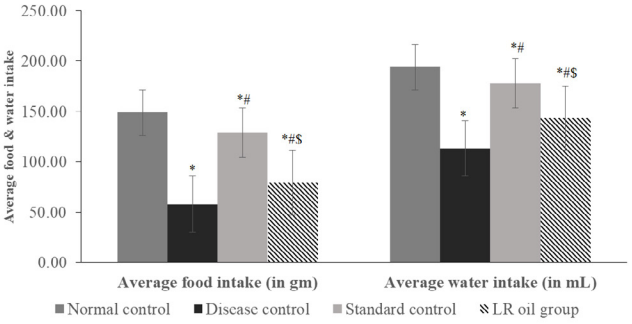

Fig. 2: Change in average food and water intake. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively

Table 1: Macroscopic colonic damage scores

	Normal group	*Disease control*	*Standard control*	*L. rhamnosus oil suspension group*
CMDI score	0.112 ± 0.042	2.228 ± 0.064*	0.555 ± 0.037$^{*\#}$	1.167 ± 0.033$^{*\#\$}$
DAI score	0.152 ± 0.011	3.338 ± 0.219*	1.337 ± 0.096$^{*\#}$	2.213 ± 0.245$^{*\#\$}$

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.

(0.112 v/s 2.228; $p < 0.05$) and the DAI score (0.152 v/s 3.338; $p < 0.05$) indicating significant mucosal damage. Supplementation with dexamethasone and *L. rhamnosus* oil suspension significantly prevented the DNBS-induced mucosal damage. As presented in Table 1, dexamethasone therapy significantly improved the overall CMDI score (0.555 v/s 2.228; $p < 0.05$) and the DAI score (1.337 v/s 3.338; $p < 0.05$) compared to the DC group. Similarly, the *L. rhamnosus* oil suspension therapy showed significant improvement in the overall CMDI score (1.167 v/s 2.228; $p < 0.05$) and the DAI score (2.213 v/s 3.338; $p < 0.05$) compared to DC group, respectively. Comparing the effectiveness, dexamethasone therapy showed a statistically significant trend in better improvement in colonic mucosal damage prevention compared to *L. rhamnosus* oil suspension group for both the CMDI and DAI scores (Table 1). As presented in Fig. 3A, the microscopic evaluation of normal control group showed intact colonic mucosal barrier with preserved barrier integrity with no alteration in the morphology of goblet cells, while DNBS-induced colitis resulted in visible mucosal damage with ruptured goblet cells and infiltration of inflammatory cells into the mucosal layer (Fig. 3B). Treatment with dexamethasone and *L. rhamnosus* oil suspension prevented the DNBS-induced colon damage. As presented in Fig. 3C, dexamethasone-treated rats showed preserved cellular integrity and goblet cell morphology, along with mild damage and inflammatory cell infiltration, while as shown in Fig. 3D, *L. rhamnosus* oil suspension-treated rats showed slightly altered cellular integrity with mild inflammatory cells infiltration.

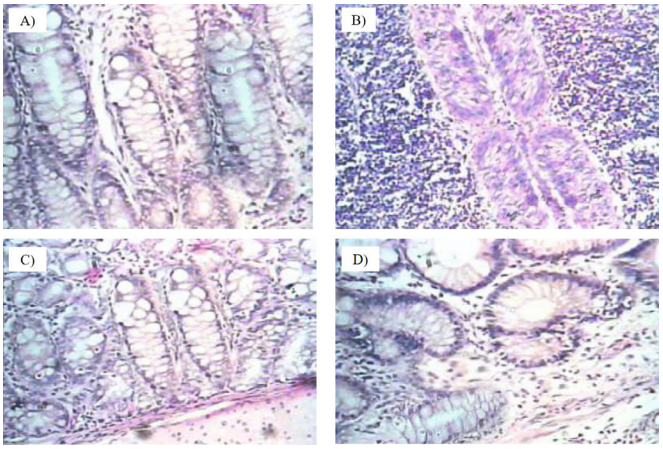

Fig. 3: Histological evaluation of colonic tissue of (A) Normal control group, (B) Disease control group, (C) Standard (dexamethasone) group, and (D) *L. rhamnosus* oil suspension group

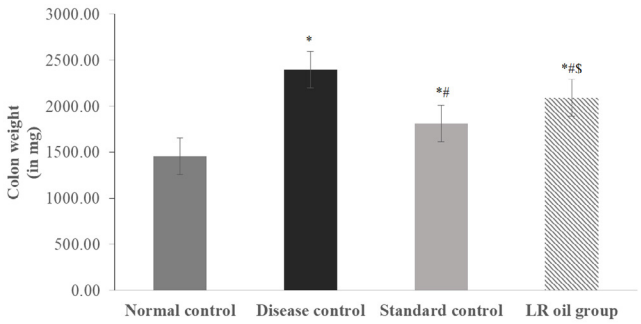

Fig. 4: Change in colon weight. *,#,$ indicates $p < 0.05$ v/s normal control group, disease control group, and standard (dexamethasone) group, respectively

Colon weight and colon inflammation level

The damage associated with IBD in the colon is associated with an increase in the infiltration of inflammatory cells and oxidative stress mediators, resulting in damage to the overall mucosal barrier, swelling of the colonic tissues, and an increase in the overall colon weight. Similar observations were observed in the DNBS-treated rats which showed significant increase in the overall colon weight compared to control group ($p < 0.05$; Fig. 4). As shown in Fig. 4, supplementation with dexamethasone and *L. rhamnosus* oil suspension significantly prevented the increase in the colon weight compared to the DC group, with significantly better effect observed in the dexamethasone-treated rats compared to the *L. rhamnosus* oil suspension-treated rats. To evaluate whether the observed effect of dexamethasone and *L. rhamnosus* oil suspension is due to their anti-inflammatory activity, we evaluated the level of calprotectin in the feces as a biomarker of intestinal inflammation. As per our hypothesis, rats in the DC group showed significantly increased fecal calprotectin levels compared to the normal control group (Table 2). Dexamethasone and *L. rhamnosus* oil suspension therapy, due to their anti-inflammatory potential, showed a significant reduction in the fecal calprotectin level compared to the DC group ($p < 0.05$ for both groups v/s DC group), with significantly better effect of dexamethasone compared to the *L. rhamnosus* oil suspension group (Table 2).

Measurement of colonic oxidative stress level

The effect of DNBS-induced colitis on the level of oxidative stress biomarkers and the efficacy of dexamethasone and *L. rhamnosus* oil suspension in preventing oxidative stress is presented in Table 2. Compared to a control group, rats with DNBS-induced colitis exhibited significant increase in the level of NO (1088.25 v/s 189.45 µmole/mL; $p < 0.05$) and MDA (1.46 v/s 0.21 µg/mL; $p < 0.05$), while significant reduction in the GSH level (82.38 v/s 354.12 µg/mL; $p < 0.05$) and SOD level (3.27 v/s 15.05 U/gm tissue; $p < 0.05$) at end of study duration. Supplementation with dexamethasone significantly improved 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 reduced the oxidative stress level (NO: 235.14 v/s 1088.25 µmoles/mL, $p < 0.05$; MDA: 0.11 v/s 1.46 µg/mL, $p < 0.05$) compared to DC group. Similarly, supplementation with *L. rhamnosus* oil suspension significantly prevented the oxidative stress (NO: 650.22 v/s 1088.25 µmoles/mL, $p < 0.05$; MDA: 0.55

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$^{*\#}$	650.22 ± 3.45$^{*\#\$}$
MDA level (µg/mL)	0.21 ± 0.05	1.46 ± 0.08*	0.11 ± 0.02$^{*\#}$	0.55 ± 0.05$^{*\#\$}$
GSH level (µg/mL)	354.12 ± 6.19	82.38 ± 2.93*	212.48 ± 4.50$^{*\#}$	145.10 ± 5.12$^{*\#\$}$
SOD level (U/gm tissue)	15.05 ± 2.46	3.27 ± 0.46*	12.56 ± 2.45$^{\#}$	6.69 ± 0.39$^{*\#\$}$
Fecal calprotectin level (ng.mL)	2910.00 ± 115.29	24866.17 ± 649.37*	9534.17 ± 246.02$^{*\#}$	16530.00 ± 163.42$^{*\#\$}$

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.

v/s 1.46 µg/mL, $p < 0.05$) and improved the antioxidant levels (GSH: 145.10 v/s 82.38 µg/mL, $p < 0.05$; SOD: 6.69 v/s 3.27 U/gm tissue, $p < 0.05$) compared to the DC group. Dexamethasone therapy was found to be significantly better than *L. rhamnosus* oil suspension in reducing oxidative stress and improving antioxidant potential ($p < 0.05$ for all evaluated parameters).

Discussion

Many previous studies have supported the role of DNBS in inducing colitis via altering the gut microbiome leading to gut dysbiosis.[28-30] The current study aimed to evaluate the effectiveness of *L. rhamnosus* suspension in MCT oil in a DNBS-induced colitis model using SD rats and dexamethasone as standard control therapy. The results of the current study provide preliminary insights into the positive effect of *L. rhamnosus* oil suspension in the treatment of colitis. Based on the currently available results, it can be postulated that *L. rhamnosus* oil suspension therapy can significantly prevent the damage of intestinal mucosa by reducing oxidative stress and inflammation and increasing the endogenous antioxidant potential.

Many studies have highlighted and confirmed one of the major roles of gut dysbiosis in the pathogenesis of IBD.[16–19] Gut dysbiosis refers to the increase in the number of pathogenic microbes and reduction in the number of beneficial microbes in the GIT. Gut dysbiosis plays a complex role in the pathogenesis and progression of the IBD condition, including the alteration in the gut microbiome-intestinal epithelial interaction, abnormal activation of the immune system, and epithelial damage due to pathogenic activity playing important roles.[16-19] The gut microbiome plays an important role in maintaining overall intestinal health like the *Firmicutes* species are involved in the production of short-chain fatty acids (SCFAs) like butyrate and in reducing the activity of pro-inflammatory cytokines, and the *Proteobacteria* species help in preventing the colonization and growth of pathogens by producing various types of bacteriocins, the *Actinobacteria* species helps in improving the overall intestinal barrier function, and the *Bacteroidetes* supports the maturation of intestinal epithelial cells and helps in nutrients absorption as well.[31, 32] Gut dysbiosis leads to the overall reduction in all the beneficial microbes further reducing the activity of these microbes in supporting the overall intestinal health, leading to a chain of reactions, ultimately damaging the intestinal mucosal barrier.[31,32] Probiotics have been shown to improve the overall gut microbiome by reducing the number of pathogenic microbes and thus restoring the normal gut microbiome.[33] The positive effect of *L. rhamnosus* oil suspension in the current study might involve its positive effect in improving the overall gut microbiome, which further supports overall intestinal health.

Oxidative stress is one of the critical factors involved in the development and progression of IBD.[34] Oxidative stress generally refers to the condition in which the number of reactive oxygen species (ROS) increases significantly leading to cellular damage, including damage to the proteins, lipids, polysaccharides, and genetic material of the cell.[35] Oxidative stress leads to the activation of pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, which enhances the production of various pro-inflammatory cytokines.[34,35] Infiltration of pro-inflammatory cytokines in the intestinal epithelial area leads to inflammation which further enhances the cellular damage process. Additionally, the NF-κB pathway and the inflammatory cytokines further damage the mitochondrial chain reaction leading to enhanced ROS production, further enhancing the oxidative stress.[36–38] This cycle of oxidative stress and inflammation ultimately leads to widespread damage of the intestinal epithelial barrier leading to IBD.[34,39] Numerous studies have supported the role of gut dysbiosis in the oxidative stress-induced intestinal damage condition,[40,41] while the use of probiotics is known to have an antioxidant and anti-inflammatory effect that suppresses the oxidative stress and inflammation level.[42,43]

Increased levels of NO in intestinal tissue a biomarkers of oxidative stress and inflammation associated with IBD condition.[44] The increased level of ROS and pro-inflammatory cytokines leads to the damage of mucosal

endothelial cells and activation of inducible nitric oxide synthase (iNOS), which leads to the increased production of NO.[44] Under the influence of reduced endogenous antioxidants, these NO molecules work similarly to ROS thereby potentiating the oxidative stress and inflammatory pathways. The gut microbiome is found to suppress the production of NO by producing SCFAs (mainly butyrate) that reduce the over-expression and activation of iNOS in the intestinal epithelium, thereby preventing the overproduction of NO in the intestinal tissues.[45] In gut dysbiosis, the production of SCFAs and the simultaneous increased oxidative stress and inflammation leads to increased NO production in the intestine, leading to widespread intestinal damage and IBD. In the current study, supplementation with *L. rhamnosus* oil suspension significantly reduced the tissue NO level, thereby supporting the role of probiotics in reducing oxidative stress and inflammatory cascade. Similarly, the *L. rhamnosus* oil suspension therapy reduced the tissue MDA level significantly compared to the disease control group. MDA is a widely accepted biomarker of oxidative stress.[34,46] Various studies have found that the tissue and serum level of MDA is significantly increased in patients with IBD, and their level is positively correlated with the severity of the disease.[34] It was observed that supplementation with *L. rhamnosus* oil suspension significantly reduced the level of MDA, indicating a significant reduction in oxidative stress. Antioxidants (including GSH and SOD) play a very crucial role in maintaining the level of oxidative stress in the body.[47-49] By scavenging the free radicals and inhibiting the over-activation of enzymes responsible for ROS production, antioxidants are responsible for maintaining a balance in cellular oxidative stress to support normal cellular health, growth, development, and functioning.[50] In the current study, animals in the disease control group exhibited significantly reduced GSH and SOD levels, an observation in line with previous experimental and clinical studies results.[51] Alternatively, supplementation with *L. rhamnosus* oil suspension significantly improved the GSH and SOD levels. This observation is in line with the results of previous studies that have underscored the potential of probiotics therapy in improving the antioxidant potential.[52] Fecal calprotectin on the other hand is a well-known sensitive biomarker of intestinal inflammation which is found to be significantly increased in IBD condition.[53] Supplementation of *L. rhamnosus* oil suspension significantly reduced the fecal calprotectin level, thereby supporting the anti-inflammatory potential of the probiotic as well, an observation similar to the results of previous studies.[54]

The current study has several strengths, including the first study to evaluate the efficacy of *L. rhamnosus* probiotic in MCT oil suspension in reducing the severity of DNBS-induced colitis in SD rats. The results of the current study were in line with the observations of the previous studies, thereby strengthening the results of the current study. Secondly, we evaluated the level of oxidative stress and inflammation which enabled us to identify the mechanism of action involved in the beneficial effect of *L. rhamnosus* oil suspension in preventing colitis-induced intestinal damage. Simultaneously, the current study has a few limitations too, including the lack of standard therapies used as a control group. While we used dexamethasone as one of the control groups in our study, various other standard therapies (like sulfasalazine) are currently used in IBD. Future studies with different standard control therapies might help us to better identify and compare the effectiveness of *L. rhamnosus* oil suspension in experimental colitis. Secondly, our study evaluated the biomarkers of oxidative stress and inflammation, while other mechanisms of action (like immune system modulation and gut microbiome modulation) also might be responsible for the observed efficacy of *L. rhamnosus* oil suspension.

Conclusion

To conclude, the administration of *L. rhamnosus* oil suspension or dexamethasone appears to have a beneficial effect in DNBS-induced experimental colitis. The preventive effect might be attributed to the antioxidant and anti-inflammatory potential of *L. rhamnosus* oil suspension and dexamethasone therapies. Based on the results of the current study, future experimental and clinical studies are warranted to support the results of the current study.

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