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Is the Probiotic Mixture Effective in the Treatment of TNBS-induced Experimental Colitis?

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) is an idiopathic disease associated with changes in the immune system and in the intestinal microbiota. The most accepted hypothesis of IBD pathogenesis is thought to be the abnormal immunological response and chronic intestinal inflammation, which is caused by the complex interactions between genetic, environmental factors and the host immune system. Microbial flora is important in the maturation of the immune system. Dysbiosis is defined as changes in intestinal microbiota composition and function. Clinical and experimental studies support that dysbiosis plays a significant role in the etiopathogenesis of IBD. Probiotics are useful live microorganisms that provide the intestinal balance in the host.

In this study, we aimed to evaluate the anti-inflammatory and anti-oxidant activities of *Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum* and *Bifidobacterium longum* bacteria in the experimental colitis model.

Methods: Twenty-four female Wistar-Albino rats and 30 mg 0.5 mL trinitrobenzenesulfonic acid (TNBS) dissolved in 50% ethanol which induced colitis by intrarectal installation. Rats were divided into four groups; healthy control (sham: group A), TNBS colitis (group B), (TNBS + methylprednisolone: group C) and probiotic (TNBS + P: group D). The rats were sacrificed on the 8th day. Macroscopic and microscopic scores, tissue myeloperoxidase (MPO), malondialdehyde (MDA) and superoxide dismutase (SOD) levels were measured.

Results: Macroscopic and microscopic scores levels in group A were significantly lower than in group B, C and D. Macroscopic and microscopic scores levels in group C were significantly lower than in group B. Macroscopic scores were statistically similar between group C and D. There was a statistically significant difference between the groups in terms of median MDA levels and median SOD levels ($p<0.001$). There was no statistically significant difference between the groups in terms of median MPO levels ($p=0.114$). Median MPO levels were 0.27 (0.15-0.30) in group A, 0.44 (0.22-0.61) in group B, 0.28 (0.25-0.50) in group C, and 0.30 (0.25-0.37) in group D ($p=0.114$). Median MDA levels were 1.1 (1.0-2.8) in group A, 4.3 (3.1-5.5) in group B, 3.8 (3.2-4.2) in group C, and 3.9 (3.1-4.2) in group D ($p<0.001$). Median SOD levels were 160.7 (150.1-161.7) in group A, 141.6 (137.9-147.3) in group B, 157.6 (155.2-167.7) in group C, and 164.7 (160.3-168.3) in group D ($p<0.001$). MDA levels were statistically significantly different between each group. These levels were significantly higher in group B, C and D than in group A; statistically similar in group C and D; and statistically higher in group B than in group C and D ($p<0.001$ & $p=0.047$). SOD levels were statistically significantly different between each group. They were significantly lower in group B, C and D than in group B; statistically significantly different in group A, C and D; and statistically higher in group D than in group A and C.

Conclusion: Our study showed that probiotics regulate the balance between anti-oxidant and oxidant systems. Therefore, probiotics can be used as a supportive treatment in inflammatory bowel diseases if promoted by clinical trials.

Keywords: IBD, MDA, MPO, SOD, probiotics

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INTRODUCTION

Probiotics are useful living microorganisms that provide intestinal balance in the host where they are located. The gastrointestinal tract is the colonization site of millions of beneficial or harmful bacteria. Because of its acidic fluid and protective secretions, the stomach contains a small number of bacteria, but certain types of bacteria migrate through the gastrointestinal tract and settle in the intestines, especially the colon (1). 20% of faeces are bacteria mostly from the colon. Bacteria make up most of the flora and more than 60% of the dry weight of the faeces in the columns of normal people. The main bacterial species in the colon are *Lactobacillus, Bifidobacterium, Eubacterium, Streptococcus, Coliforms, Clostridium* and Saccharomyces. A healthy individual has about 100 trillion bacteria in their intestines. Although they are widely consumed with yogurt and fermented foods in the world, it is found and used in different forms such as various dietary supplements and functional foods (2). The microbial flora contributes to the maturation of the immune system and to the formation of the ability to recognize and destroy pathogenic microorganisms taken from food and from outside. It has an important role in the formation of colonic morphology and control of continuous inflammatory response. By creating a barrier to the colonic mucosa, it prevents the entry of microorganisms and allergens into the body (3,4).

Inflammatory bowel diseases (IBD) are chronic inflammatory diseases. It has two major types, ulcerative colitis and Crohn's disease. About 3.5 million people are affected in Europe and the United States. Although the incidence of both diseases has increased all over the world, their etiologies remain uncertain and no treatment method providing cure has yet been found (5). The accepted hypothesis of IBD pathogenesis is that complex interactions between genetics, environmental factors and the host immune system lead to abnormal immune responses and chronic intestinal inflammation. Dysbiosis has been described as changes in intestinal microbiota composition and function. Clinical and experimental studies support that dysbiosis plays an important role in the etiopathogenesis of IBD (6). Recently, the replacement of the intestinal microbiota composition with probiotics or fecal transplantation has been the focus of attention among the supportive and therapeutic methods.

In this study, it was aimed to evaluate the anti-inflammatory and antioxidant activities of the bacteria of *Enterococcus Faecium, Lactobacillus Acidophilus, Lactobacillus Rhamnosus, Bifidobacterium Bifidum, Bifidobacterium Longum* in the experimentally created colitis model and the treatment success of probiotics compared to steroid.

METHODS

This study was carried out in Gazi University Faculty of Medicine Experimental Animals Laboratory. Pathological examinations were carried out in Gazi University Faculty of Medicine, Department of Pathology. In this study, a total of 24 Wistar-Albino female rats with the weight of 200-250 grams were used. All animals were fed with standard feed and water before the experiment.

Four different groups were created with 6 rats in each group. The animals were kept in standard humidity, light (12 hours daylight/12 hours dark) and heat conditions (22-24 °C) during the experiment and fed with standard rat food. Wire pads were placed inside the cage to prevent coprophagy. Rats were placed in single cages to determine the amount of food and liquid consumed by each rat. Weights of the rats were recorded every day throughout the study to determine weight changes.

Anesthesia to the rats was provided by injecting intramuscular Ketaminehydrochloride (Ketalar, Parke Davis and Eczacıbaşı, İstanbul) at a dose of 50 mg/kg + Xylazinehydrochloride (Rompun, Bayer HealthCare) at a dose of 5 mg/kg. To create experimental colitis, a mixture of 30 mg (80 mg/kg) trinitrobenzenesulfonic acid (TNBS) (92823, picrylsulfonicacidsolution) + 50% ethanol was used. TNBS was purchased from Sigma (Sigma, La Verpaille-re, France).

Group A (n=6): Sham group; The group receiving intrarectal 2 mL of saline for 7 days,

Group B (n=6): The group developing colitis with TNBS and receiving no treatment,

Group C (n=6): The group given oral Prednisol 2mg/day 24 hours after the development of colitis with TNBS,

Group D (n=6): Twenty-four hours after the development of colitis with TNBS, 1 sachet of oral probiotic per day (*E. Faecium, L. Acidophilus, L. Rhamnosus, B. Bifidum, B. Longum* were given as 3 doses a day with gavage for 7 days.

Monitoring the symptoms: After the animal model was established, rat feces were collected daily. The overall condition of the rats including feces, blood, activity, fur, food intake, and weight was observed.

Surgical method: After anesthesia, which was achieved by applying 50 mg/kg ketamine + 5 mg/kg xylazine intramuscularly, laparotomy was performed with xifopubic median incision in the colitis models and control group, and exploration was performed. Subsequently, transection was performed from the distal of the rectum from the middle of the transverse colon at the lowest possible level and approximately 10 cm colon segment was removed. The removed colon tissue was divided into two equal parts longutidinally. A part of the column sample was detected in 10% formaldehyde. The other half was stored in -80 °C freezer (Sanyo MDF-U70V) until the day of the procedure. Under deep anesthesia (ketamine 45 mg/kg + xylasin 5mg/kg IM intracardiac), 5 cc blood was collected and the rats were sacrificed.

Histopathological Examination Methods

Macroscopic evaluation: Following the longitudinal opening of the removed intestinal sections, they were rapidly washed with normal saline and their macroscopic scorings were done by a pathologist blinded to the groups and treatment for each subject

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individually, as described by Millar et al. (7) and group averages were calculated.

0 = normal mucosa,

1 = mucosal erythema only,

2 = mild mucosal edema, minor bleeding or minor erosions,

3 = moderate edema, bleeding ulcer or erosion,

4 = severe ulcer, erosion, edema and presence of tissue necrosis.

Microscopic evaluation: Colon segments fixed in 10% formalin were embedded in paraffin blocks. Sections taken from these blocks with a thickness of 5 µ were stained with hematoxylin-eosin. The prepared preparates were examined for the groups by a blind pathologist under the light microscope and microscopic scoring was performed. The scoring system used by Ackerman et al. (8) was modified and calculated separately for each animal and the average score of each group was obtained.

A: Necrosis depth: none = 0; mucosal = 1; mucosal and submucosal = 2; mucosal, submucosal and muscularispropria = 3; in the entire colon wall = 4,

B: Width of necrosis: none = 0; a small area = 1; a moderate area = 2; a large area = 3; common = 4,

C: The degree of inflammation: none = 0; minimal = 1; mild = 2; moderate = 3; serious = 4,

D: Width of inflammation: none = 0; mucosal = 1; mucosal and submucosal = 2; in mucosal, submucosal and muscularispropria = 3; in the entire colon wall = 4.

Preparation of Tissues for Tissue Myeloperoxidase Activity and Myeloperoxidase Measurment

It is based on the oxidation of H_2O_2 by homogenate and the reduction of O-dianicide and measuring the reduced O-dianicide at 410 nm. 130 mg intestinal mucosa was homogenized with 1.3 mL cold 20 mM EDTA. 1 mL of the homogenate is taken and placed in eppendorf tubes and centrifuged at +4 degrees for 15 minutes at 20.000 g. Supernatant was discarded and it was sonicated for 60 seconds with PELLET 1.3 mL of 50 mM buffer (pH 6), then centrifuged again at +4 degrees at 20.000 g for 15 minutes. The supernatant was transferred to new eppendorfs. The myeloperoxidase (MPO) level was studied from this supernatant. Optical density at 410 nm was read against the blind. That occurring at 1 Unit = min at 37 °C was accepted as optical density change. Specific activity was evaluated as = U/g tissue.

Malondialdehit Level, Homogenizing Tissue Samples for Superoxide Dismutase Activity

Tissue samples were homogenized in ice with 1/10 phosphate buffer saline (pH: 7.4) in cold. Homogenates were centrifuged at 15.000 rpm for 15 minutes in a cooled centrifuge, and tissue malondialdehyde level and tissue superoxide dismutase (SOD) activity were determined from supernatants.

Intestinal Tissue Malondialdehyde Analysis

Tissue malondialdehyde (MDA) levels were determined according to the method described by Ohkawa et al. The principle of this method; After the binding of the homogenate proteins with sodium dodecyl sulfate, the MDA in the sample was performed according to the spectrophotometric measurement of the red color connected to the complex formed by thiobarbituric acid under ambient pH of 3.5.

Superoxide Dismutase Analysis in Colon Mucosa Tissue

The SOD activity was measured spectrophotometrically by this color intensity, as defined by Yi-Sun, by the formation of $O_2^{\cdot}$- with xanthinxanodinoxidase and forming a colored compound with NBT. The greater the SOD activity in the environment, the less the intensity of the resulting color will be, since it will eliminate $O_2^{\cdot}$-'.

Statistical Analysis

Statistical analysis of the data was done using SPSS v.17.0 (SPSS, Inc., Chicago, IL, USA) software. The Kruskal-Wallis test and Mann-Whitney Utest were used as post-hoc tests for the analysis of macroscopic scores, microscopic scores and biochemical parameters between the groups. Data were expressed as mean ± standard deviation. The means of the variables in the groups were drawn by selecting Boxplot. The value of $p<0.05$ between the results was considered statistically significant.

RESULTS

In group A, there was no weight loss, color change in feathers and stool changes in rats, and food intake was normal during the experiment. After the development of the animal model, decreased appetite, increased stool frequency, bloody stool, and coarse hairy appearance were observed in group B, C, and D.

There was no statistically significant difference between the groups in terms of median MPO levels ($p=0.114$).

There was a statistically significant difference between the groups in terms of median MDA levels ($p=0.002$). When the factors causing this difference were examined, it was found that the median MDA levels of group B, C and D were statistically higher compared to group A ($p<0.001$). At the same time, the median MDA levels of group C and D were statistically lower than group B ($p<0.001$ and $p=0.047$). The median MDA level of group D was statistically higher than group C ($p=0.047$) (Figure 1, 2).

There was a statistically significant difference between the groups in terms of median SOD levels ($p<0.001$). When the conditions causing the difference in question were examined, it was found that the median SOD level of group B was statistically lower compared to group A and the median SOD level of group D was statistically significantly higher ($p<0.001$). At the same time, the median SOD levels of group C and D were statistically higher than group B ($p<0.001$). The median SOD level of group D was statistically higher than group C ($p<0.001$). The median SOD

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levels were statistically similar between group A and group C (p=0.106) (Table 1).

In group A, there was no statistically significant decrease in body weight on the 8th day compared to the 1st day. In group B, there was a statistically significant decrease in body weight on the 8th day compared to the 1st day (p<0.001). There was a statistically significant decrease in body weight on the 8th day compared to the 1st day in group C (p<0.001). In group D, there was a statistically significant decrease in body weight on the 8th day compared to the 1st day (p<0.001).

There was a statistically significant difference among the groups in terms of weight loss (p<0.001). When the factors causing the difference in question were examined, it was found that, the average weight loss in groups B, C and D was statistically higher compared to group A (p<0.001). The mean weight loss between group B and C, group B and D, and group C and D were found to be statistically similar (p=0.550; p=0.077 and p=0.608) (Table 2).

There was a statistically significant difference among the groups in terms of median macroscopy scores (p<0.001). In the investigation of this difference, it was found that the median macroscopy scores of group B, C and D were statistically higher compared to group A (p<0.001). At the same time, the median macroscopy score of group B was statistically higher than that of group C and D (p=0.002 and p<0.001). The median macroscopy scores were statistically similar between group C and D (p=0.093) (Table 3).

DISCUSSION

Probiotics have regulatory effects on the intestinal mucosa. These effects include receptor antagonism, receptor expression, binding and expression of adapter proteins, expression of negative regulator signal molecules, induction of microRNAs, endotoxin tolerance, and stimulation of the secretion of

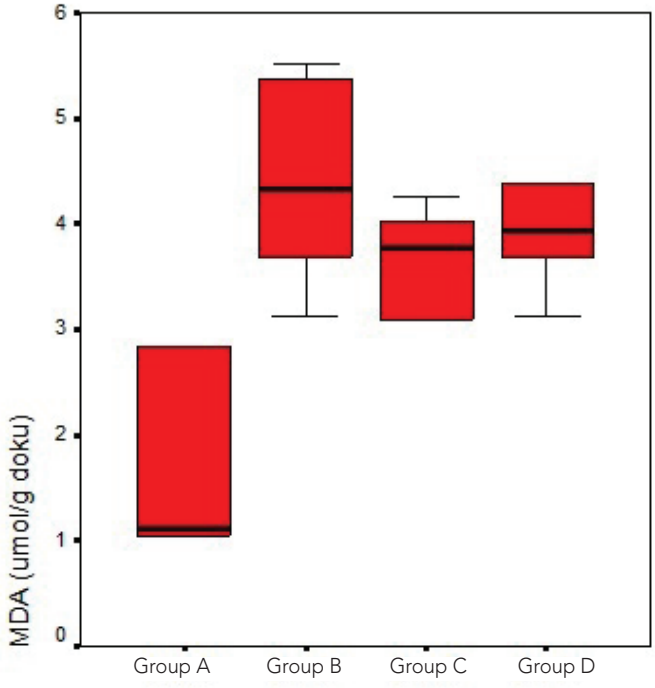

Figure 1. Malondialdehyde levels according to the groups
MDA: Malondialdehyde

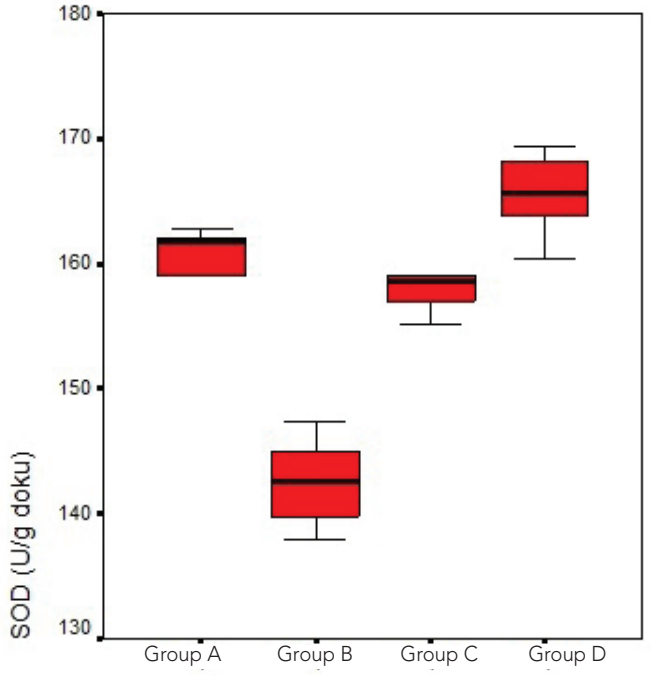

Figure 2. Superoxide dismutase levels according to the groups
SOD: Superoxide dismutase

Table 1. Measurements of inflammation markers according to the groups

Groups	MPO (U/g tissue)	MDA (umol/g tissue)	SOD (U/g tissue)
Group A (control)	0.27 (0.15-0.30)	1.1 (1.0-2.8)[a,b,c]	160.7 (150.1-161.7)[a,c]
Group B (TNBS)	0.44 (0.22-0.61)	4.3 (3.1-5.5)[a,d,e]	141.6 (137.9-147.3)[a,d,e]
Group C (TNBS + MP)	0.28 (0.25-0.50)	3.8 (3.2-4.2)[b,d,f]	157.6 (155.2-167.7)[d,f]
Group D (TNBS + P)	0.30 (0.25-0.37)	3.9 (3.1-4.2)[c,e,f]	164.7 (160.3-168.3)[c,e,f]
p	0.114†	**0.002†**	**<0.001†**

†Kruskal-Wallis test, [a]The difference between group A and B is statistically significant (p<0.001), [b]The difference between group A and C is statistically significant (p<0.001), [c]The difference between group A and D is statistically significant (p<0.001), [d]The difference between group B and group C is statistically significant (p<0.001), [e]The difference between group B and group D is statistically significant (p<0.05), [f]The difference between group C and D is statistically significant (p<0.05).
MPO: Myeloperoxidase, MDA: malondialdehyde, SOD: superoxide dismutase, MP: methylprednisolone, P: probiotic

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immunomodulatory proteins, lipids and metabolites to modulate the immune system. Probiotics not only have activating effects on the immune system, but they also have a suppressive effect. It can affect hemostasis, inflammation and immunopathology pathways with direct or indirect effect on the immune system (1). Probiotics pass the testinal epithelium and reach the M-cells, which are immunomodulators in the payer plates. M-cells transmit probiotic bacteria and their soluble proteins to mucosalenfoid tissue and initiate immunregulation. Antimicrobial activity increases and as a result, mucosal immunoglobulin A also increases. It activates goblet cells located in the mucosa, stimulates mucous secretion, prevents the pathogens to attach the epithelium and to settle in the tissue. It enhances mucosal integrity by strengthening the connections between epithelial cells. It inhibits the binding of lipopolysaccharides to the CD14 receptor, reducing nuclear factor-κβ activation and the production of proinflammatory cytokines. It has regulatory effects on B and T lymphocyte functions located in laminapropria. They reduce the secretion of tumor necrosis factor-alpha and interferon gamma, which play a central role in the formation of testinal inflammation of probiotics. They stimulate regulatory T-cells via interleukin-10 and transforming growth factor-beta. Thus, they display a regulatory effect on inflammation. Lactobacillus and Bifidobacterium species are quite common probiotic species and they are found naturally in the gastrointestinal tract. These bacteria are beneficial bacteria that help the digestive process (9).

IBD are chronic inflammatory diseases that progress with remission and activation. The etiopathogenesis of IBD is still not fully known. It is accepted that it occurs as a result of genetic and environmental factors, and mutual interaction between luminalmicroflora and immune system. Although antibiotics and immunomodulating drugs have an important role in the treatment of these diseases, the ideal treatment method for IBD is still under discussion. In recent years, doubts about that intestinal microbiota changes trigger susceptibility to many gastrointestinal diseases have increased. As a result, attention has focused on the use of probiotics in treatment.

Numerous studies on colitis models formed with TNBS and on patients with IBD have also confirmed anti-inflammatory activities of probiotics (4,9,10). Although the mechanisms regarding how probiotics reduce IBD symptoms are not known, it is known that they have a role in the change of the composition of bacteria in the gastrointestinal system; they compete in holding the epithelium with pathogenic microorganisms and prevent their infection development; they regulate the function of mucosal immune cells; they stimulate the formation of antimicrobial factors such as bacteriocin, hydrogen peroxide, acetic acid, and lactic acid, and inhibit the proliferation of pathogenic microbes; they increase the barrier functions of the mucosa by increasing the inter-epithelial connections and providing mucosal integrity; and they induce T-cell apoptosis (11).

In this study, we preferred the colitis model formed with TNBS due to the development of chronic inflammation, no occurrence of spontaneous remission, and development of inflammation similar to IBD pathogenesis. Subsequently, we examined the colon samples taken from rats microscopically and macroscopically. Activation of neutrophils, macrophages, lymphocytes, and mast cells for any reason and the formation of reactive oxygen metabolites, which occur as a result of oxidative stress in the tissue, cause mucosal impairment and ulceration, and form the pathogenesis of intestinal inflammation.

Therefore, in this study, we determined the SOD, MPO, and MDA tissue levels, which are inflammation, oxidative stress and fibrotic markers, and compared the groups in terms of the tissue levels of these molecules. In this way, we investigated the effects of probiotics on inflammatory and antioxidant markers, which are expected to exist on the mucosa at the very early stage of inflammation.

When the sham group (group A), control group (group B), group receiving steroid treatment (group C), and group taking probiotic

Table 3. Macroscopy scores according to the groups

Groups	Macroscopy score
Group A	0 (0-0)[a,b,c]
Group B	3.5 (2-4)[a,d,e]
Group C	2 (1-3)[b,d]
Group D	2 (2-3)[c,e]
p	**<0,001†**

†Kruskal-Wallis test, [a]The difference between group A and B is statistically significant (p<0.001), [b]The difference between group A and C is statistically significant (p<0.001), [c]The difference between group A and D is statistically significant (p<0.001), [d]The difference between group B and C is statistically significant (p=0.002), [e]The difference between group B and D is statistically significant (p<0,001)

Table 2. Body weights on the 1st and 8th days according to the groups

	1st day	8th day	p†	Change	p‡
Body weight	-	-	-	-	**<0.001**
Group A	227.3±6.8	224.0±6.6	0.118	-3.3±1.2[a,b,c]	-
Group B	233.7±9.2	176.7±14.0	**<0.001**	-57.0±14.0[a]	-
Group C	226.1±9.4	178.3±10.2	**<0.001**	-47.8±18.1[b]	-
Group D	230.0±8.9	190.7±13.0	**<0.001**	-39.3±6.0[c]	-

†Dependent t-test, comparisons of body weights on the 1st and 8th days among the groups, ‡One-way ANOVA, comparisons among the groups in terms of changes in body weight on the 8th day compared to the 1st day, [a]The difference between group A and group B is statistically significant (p<0.001), [b]The difference between group A and group C is statistically significant (p<0.001), [c]The difference between group A and group D is statistically significant (p<0.001)

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(group D) were compared in terms of weight differences on the 1st and 8th days, there was a statistically significant difference among the groups in the weight measurements on the 1st and 8th days. When the groups were compared among themselves, while there was a significant difference in weight loss between the sham group and colitis forming groups, the weight loss was statistically similar among the control group, standard group and test group. This supported the subjects to be selected homogeneously. This may be due to the fact that the rats were not fed 24 hours before the colitis induction and the oral intake of rats receiving anesthesia was reduced because they were administered IR TNBS.

Compared to the sham group, colitis formed groups showed a marked difference in the macroscopic appearance of the colon. In colitis-formed groups, the colon was necrozed, edematous, and rigid, and wall thickness was increased. In groups with colitis, the uterus was adhered to the colon, the spleen was small in size, and the pancreas was hyperemic. In the sham group, the colon was completely normal in macroscopic and microscopic appearance, and the pathological features observed in the groups where colitis was formed in the uterus, spleen and pancreas were not found. While there was microscopically prominent colonic inflammation, erosion, ulceration, necrosis in colitis groups, no pathological findings were found in preparates belonging to the sham group. This difference confirmed us that TNBS was an effective chemical agent in creating a chronic colitis model.

Inflammatory process in IBD and experimental colitis occurs by infiltration of leukocytes such as neutrophils, monocytes and lymphocytes into the colon mucosa. Leukocytes are the main source of free oxygen radicals. Reactive oxygen radicals cause cell and tissue level damage by doing peroxidation of lipid membranes, protein denaturation and DNA damage. MPO is the most frequently released enzyme from azurophilic granules, found in neutrophils (12). Neutrophil infiltration in inflamed tissue facilitates the emergence of potent cytotoxic oxidants through the MPO enzyme (13). Studies have shown that MPO activity correlates with the severity of tissue damage in TNBS colitis and increases more in the colitis groups than in the control groups, and is also a marker of infiltration of neutrophils (13,14). Domek et al. (15) investigated MPO activities in the treatment groups according to colitis groups in the rats that they formed as colitis model and suggested that neutrophil infiltration and activation played an important role and this was determined by the level of MPO in the tissue. Karmeli et al. (16) compared the effects of cyclooxygenase-2 inhibitors in the colitis model in rats and stated that with treatment, MPO activities decreased 61% compared to subjects in the colitis model. In our study, the MPO levels of the group receiving steroid and the group receiving probiotics were very close to the sham group. The MPO level of the group taking probiotics was higher than the group taking steroids. Although MPO level was higher in the experimental colitis model compared to other groups, no statistically significant difference was found. Although there was no significant difference in MPO values among the groups, MPO values were lower in the sham and treatment groups than in the colitis model.

MDA increase is the most important laboratory indicator of lipid peroxidation in tissues in clinical and experimental studies (17). In acute inflammation, activated neutrophils leave the circulation and enter the mucosa and submucosa of the intestine, causing excessive production of lipid mediators, lactoferrin, proteases, reactive oxygen and nitrogen derivatives that contribute to inflammatory damage (18). Aytac et al. (19) showed that ilioprost significantly reduced tissue MDA levels in the colitis model they formed with acetic acid. In our study, the MDA level was significantly higher in the colitis group and the groups that formed colitis and received treatment compared to the sham group, and the MDA level was lower in the probiotic group compared to the steroid group, and this was statistically significant. When MPO and MDA levels were evaluated, low values compared to the control group supported the probability of probiotics to have anti-inflammatory activity, but not as much as steroid.

SOD is the major defense system against superoxide anions. SOD catalyzes the conversion of superoxide to hydrogen peroxide. It is the primary protector against oxidant molecules. Three types of SOD have been identified; those located in mitochondria-SOD, in cytosol [(Cu), Zn-SOD], and in extracellular matrix [(EC)-SOD]. EC-SOD is released from endothelial and stromal cells. It tends to decrease in IBD patients. Cu, Zn-SOD is released from the epithelium dominantly and decreases in inflammation. Plasma and tissue levels are low in IBD patients. SOD activity in normal intestinal mucosa is lower than in the liver and lung. This level decreases even more in inflammatory conditions (20). Kuralay et al. (21) showed that SOD levels decreased in response to oxidative stress in the experimental colitis model. Grisham et al. (22) found low SOD activity in the colitis model they created with TNBS. Patel et al. (23), in an experimental study, they compared the sham group given 3 groups as 500 mg/kg, 1 mg/kg, 5 mg/kg IR in the colitis model stimulated with PAR-2 agonistitrypsin TNBS and the groups given only TNBS and they examined MPO, MDA, SOD levels. While oxidative enzymes, macroscopic score and microscopic score were high in the group applied 5 mg/kg, antioxidant enzyme levels were low.

In our study, a statistically significant difference was detected among the groups in terms of SOD levels. Compared to the sham group, the median SOD level was statistically lower in the colitis-formed group not receiving treatment. The median SOD level of the probioticic receiving group was statistically higher than the other groups. Also, remarkably, the SOD level was higher in the probiotic group than the sham group. This situation supported the antioxidant activity of probiotics.

Our study supports that probiotics regulate the balance between antioxidant and oxidant systems.

CONCLUSION

As a result, probiotics can be used as supportive therapy to classical therapy with their antioxidant effects. However, clinical studies are needed to show that probiotics regulate mucosal defense systems with changes in microbiota and may be effective in treatment.

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Ethics Committee Approval: This study was carried out in Gazi University Faculty of Medicine Experimental Animals Laboratory.

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Peer-review: Internally peer-reviewed.

Author Contributions: Surgical and Medical Practices - Ö.G.U., E.K., Ö.E., M.A.; Concept - Ö.G.U., E.K., M.A.; Design - Ö.G.U., E.K., B.E., M.A.; Data Collection and/or Processing - Ö.G.U., E.K., C.Y., Ö.E., M.A.; Analysis and/or Interpretation - Ö.G.U., E.K., B.E., C.Y., Ö.E., M.A.; Literature Search - Ö.G.U., E.K., B.E., M.A.; Writing Manuscript - Ö.G.U., E.K.

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