Protective and Therapeutic Effects of Beta Glucan Administration on Experimental Ulcerative Colitis Model Induced by TNBS

TNBS ile Oluşturulan DeneySEL Ulseratif Kolit Modelinde Beta Glukan Uygulamasının Koruyucu ve Terapötik Etkileri

ABSTRACT

Aim: In this study, it was aimed to investigate the effects of beta glucan (BG) on the experimental colitis model created using trinitrobenzene sulfonic acid (TNBS).

Material and Methods: Thirty-two Wistar Albino rats were divided equally into four groups as sham control, TNBS, TNBS-BG3, and TNBS-BG10 groups. While saline was administrated to sham group, TNBS was administered intrarectally to the TNBS groups under anesthesia. BG was administered at a dose of 100 mg/kg by oral gavage, intragastrically, for 3 days (TNBS+3) to the TNBS-BG3 group and for 10 days (7+TNBS+3) to the TNBS-BG10 group. At the end of the study, macroscopic, histological and biochemical tests were applied to the colon tissues taken.

Results: It was determined by histopathological scoring and biochemical results that BG administration caused positive effects on colon damage due to colitis. Malondialdehyde level and myeloperoxidase activity were found to be significantly higher in the TNBS group compared to the other groups (p=0.003 and p<0.001, respectively). Antioxidant levels increased in BG treated groups compared to TNBS group. While this increase was statistically significant among glutathione levels (p<0.001), it was not statistically significant in catalase enzyme activity (p=0.218). BG administration reduced the increase in lipid peroxidation and leukocyte infiltration level in the colon tissue. Positive changes due to the prophylactic effect of BG were determined in histological and biochemical results.

Conclusion: BG administration has been found to show anti-inflammatory and antioxidant properties, and BG has a treatment potential in reducing colon tissue damage due to TNBS-induced colitis.

Keywords: Anti-inflammatory; antioxidant; beta glucan; ulcerative colitis.

ÖZ

Amaç: Bu çalışmada trinitrobenzen sulfonik asit (TNBS) kullamlararak oluşturulan deneySEL kolit modeli üzerinde beta glukan (BG)'n in etkilerinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Otuz iki Wistar Albino sıçan eşi olarak şam kontrol, TNBS, TNBS-BG3 ve TNBS-BG10 gruplarına ayrılmıştır. Şam grubuna serum fizyolojik uygulanırken, TNBS gruplarına anestezi altında intrarektal yol ile TNBS uygulanmıştır. BG 100 mg/kg dozunda, TNBS-BG3 grubuna 3 gün (TNBS+3) süreyle, TNBS-BG10 grubuna ise 10 gün (7+TNBS+3) süreyle, intragastrik şekilde oral gavaj yolu ve erilmişdir. Çalışmanın sonunda alınan kolon dokularına makroskopik, histolojik ve biyokimyasal testler uygulanmıştır.

Bulgular: BG uygulamasının kolite bağlı kolon hasarında olumlu etkileri neden olduğu, histopatolojik skorlama ve biyokimyasal sonucu ile tespit edilmiştir. Malondialdehid seviyesi ve mieloperoksidaz aktivitesinin diğer gruplar ile karşılaştırıldığında TNBS grubunda, anlamlı derecede yüksek olduğu tespit edilmiştir (sirrasıyla p=0.003 ve p<0.001). BG tedavisi uygulanan gruplarda, antioksidan düzey TNBS grubuna göre artış göstermiştir. Bu artış glutatyon düzeyleri arasında istatistiksel olarak anlamlı iken (p<0.001), katalaz enzim aktivitesinde istatistiksel olarak anlamlı olmamış ortaya çıkmıştır (p=0.218). BG uygulaması, kolon dokusundaki lipid peroksidsiyon ve lösöst infiltrasyon düzeyindeki artış azaltmıştır. Histolojik ve biyokimyasal sonuçlarla BG'nin profilaktik etkisine bağlı olarak olumlu değişiklikler tespit edilmiştir.

Sonuç: BG uygulamasının anti-inflamatuar ve antioksidan özellikler gösterdiği tespit edilmiştir ve TNBS ile indukülen kolit sonucu olan kolon dokusunun azaltılması BG tedavisi edici potansiyel taşımaktadır.

Anahtar kelimeler: Anti-inflamatuar; antioksidan; beta glukan; ulseratif kolit.
INTRODUCTION

Inflammatory bowel disease (IBD) is a group of diseases of unknown etiology, caused by genetic, environmental and immunological causes. IBD is a disease that affects the gastrointestinal system with chronic, widespread, recurrent gastrointestinal inflammation and has a clinical course with intestinal and extra-intestinal involvement (1). IBD gathers ulcerative colitis and Crohn's disease, although these two diseases have similar clinical course and treatment methods; complications differ from each other in that they are localized to different parts of the intestine (2). In the pathogenesis of IBD, the effect of reactive oxygen species (ROS) on the increase of tissue damage in many inflammatory disease groups, including colitis, has been found (3,4). Mucosal damage in IBD causes leukocyte migration to the intestinal mucosa. As a result of neutrophil and macrophage infiltration, oxidative damage in the mucosa increases and the increase in leukocyte migration together with the release of proinflammatory cytokines turns into a vicious circle (5). In ulcerative colitis, inflammation generally starts from the rectal mucosa and progresses uninterruptedly proximally and is observed as a disease that keeps the mucosa without bypassing (6). Environmental factors, genetic predisposition, changes in intestinal microflora and immunological response are thought to play a role in the etiopathogenesis of IBD (7). ROS contribute to the destruction of the mucosal barrier by disrupting the protective layer in the colonic epithelial tissue. It causes leukocyte infiltration and passage of bacterial toxins into the lamina propria (8). Although oxidative activities continue in the body, antioxidant capacity, which is one of the body's defense mechanisms, protects tissues from ROS under physiological conditions. However, when the production rates of oxygen radicals exceed the capacity of the endogenous antioxidant defense mechanisms, they can destroy the tissue. Endogenous mechanisms for defense have many radical scavengers and enzymes, including catalase (CAT), glutathione (GSH), superoxide dismutase and peroxidases (8-11). Free oxygen radicals cause lipid membrane peroxidation, damage to protein and DNA, and damage to cells and tissues. Neutrophil migration in inflamed tissue facilitates the emergence of strong cytotoxic oxidants with the effect of myeloperoxidase (MPO) enzyme (12). Beta glucan (BG) is a polysaccharide found in baker's yeast, cereals and fungi, and is involved in the structure of the cell wall (13). When BG is taken orally, it is absorbed from the gastrointestinal tract and plays a role in stimulating the immune system by binding to receptors on macrophages, neutrophils, and natural killer cells (14,15). It is known that BG has antioxidant properties and increases the level of antioxidant enzymes and reduces oxidant substances (16). It has been understood that BGs stimulate collagen tissue synthesis by increasing NF-1 activity in fibroblasts and play a role in wound healing by increasing the amount of procollagen mRNA and type 1 and type 3 collagen levels (17).

In this study, the antioxidant and anti-inflammatory effects of BG given by gastric gavage were investigated in an experimental colitis model created with trinitrobenzene sulphonic acid (TNBS), by measuring histopathological evaluations, and malondialdehyde (MDA), MPO, CAT, and GSH levels in the colon tissue by ELISA method.

MATERIAL AND METHODS

The studies were carried out using 32 Wistar Albino female rats aged between 8 and 10 weeks, after the approval which was obtained from the Experimental Research Ethics Committee of Aydın Adnan Menderes University, dated 14.08.2015 and numbered 64583101/2015/094. The rats were randomly divided into 4 equal experimental groups as sham group, TNBS group, TNBS-BG3 group, and TNBS-BG10 group. Except for the rats in the sham group, all subjects were exposed to experimental colitis. 2,4,6-trinitrobenzene sulphonic acid, purchased from Sigma company (Sigma, La Verpilliere, France) and prepared by dissolving 25 mg in 37% ethanol, BG is Imuneks brand (Imuneks brand (Sigma, La Verpilliere, France) isolated from Saccharomyces cerevisiae). Mustafa Nevzat İlaç San. A.Ş., Istanbul, Turkey was prepared by dissolving the preparation in drinking water.

Induction of Colitis

Animals that will cause colitis with TNBS were fasted 24 hours before the application and their colons were emptied by triggering the defecation reflex. Intrarectal administrations were given under ketamine (75 mg/kg) and xylazine (8 mg/kg) anesthesia, by entering 0.8 ml of TNBS through a polyethylene cannula 8 cm from the anal orifice. Animals in the sham group were given physiological saline instead of TNBS.

Experimental Groups

The groups were organized as given below.

Sham Control Group: Saline was administered rectally under ketamine and xylazine anesthesia through the cannula. The rats were sacrificed 3 days after this treatment.

TNBS Group: Experimental colitis was formed by rectally giving 25 mg of TNBS dissolved in 0.8 ml 37% ethanol through a cannula under ketamine and xylazine anesthesia. The rats were sacrificed 3 days after this treatment.

TNBS-BG3 Group: At a dose of 100 mg/kg was given intrastragically via an oral gavage needle for 3 days from TNBS administration to sacrifice. After 3 days of treatment, the rats were sacrificed.

TNBS-BG10 Group: At a dose of 100 mg/kg was given intrastragically via an oral gavage needle for 7 days before TNBS administration and for 3 days until sacrifice.

At the end of the experiment, the rats were sacrificed by the cervical dislocation method while under ketamine xylazine anesthesia. Columns approximately 10 cm long were cut open longitudinally, the tissue was washed with physiological saline and made ready for histopathological and biochemical examination.

Histopathological Evaluation

Histopathological evaluation was performed using standard tissue fixation and sectioning procedures. Colon tissue taken from rats was kept in 10% neutral formaldehyde. The fixation time was 24 hours, and the temperature was 4 °C. Colon tissues were washed in ethanol for dehydration and cleared in xylene. Paraaffin blocks were used for embedding the tissues and 5 µm thick random sections were taken using a microtome (Leica model RM 2135, Leica, Germany). Hematoxylin-eosin procedure was used for staining these sections and the stained sections were examined under a light microscope.
Screenshots were captured using an Olympus DP20 digital camera connected to an Olympus BX51 microscope. Changes in histopathological parameters were scored appropriately. The degree of change in the columns was evaluated on a scale of 0 to 3 (18): These scores mean: 0, no change; 1, slight damage; 2, moderate damage; 3, severe damage. The evaluated parameters with respect to the change were inflammatory cell infiltration, submucosal edema, mucosal hemorrhage, and damage/necrosis. Scoring of tissue samples was done by a blinded observer.

Biochemical Evaluation
A 2-cm segment of the colon was separated and used for biochemical evaluations. These tissues were homogenized in pH 7.0 50 mM phosphate buffer at 0-4 °C (w/v=1/10). Analyzes were performed by ELISA method using kits from BioVision Incorporated, California, USA.

MDA Activity: Homogenized tissue samples and Biovision Lipid Peroxidation Colorimetric/Fluorometric Detection Kit were used for MDA level.

MPO Activity: Homogenized tissue samples and Biovision Myeloperoxidase Activity Colorimetric Detection Kit were used for MPO activity.

GSH Level: Homogenized tissue samples and Biovision Glutathione Fluorometric Detection Kit were used for GSH level.

CAT Activity: Homogenized tissue samples and Biovision Catalase Activity Colorimetric/Fluorometric Detection Kit were used for CAT activity.

Statistical Analysis
All statistical analyses were performed via IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The distributions of biochemical measurements (GSH, MDA, CAT and MPO) were evaluated by the Shapiro-Wilk’s test and skewness/kurtosis statistics.

Homogeneity of the variances among groups for these measurements were examined by Levene’s test. All these measurements were provided by mean±standard deviation (mean±SD). The four groups were compared with respect to the biochemical measurements by ANOVA. Tukey’s HSD test was performed, if necessary. A p-value <0.05 was accepted as statistically significant.

RESULTS
Histopathological Results
The histopathological results are given in Table 1. No damage was detected in the sham control group (Figure 1). In the colitis group, intense mucosal damage and necrosis, moderate inflammation, edema, and hemorrhage were detected (Figure 2). Moderate mucosal damage, inflammation, and hemorrhage were seen in the TNBS-BG3 group (Figure 3). Although there was no necrosis in the TNBS-BG10 group, mild edema and inflammation were detected (Figure 4).

Biochemical Results
The results of the biochemical measurements are given in Table 2. Accordingly, GSH level (p<0.001), MDA level (p=0.003) and MPO activity (p<0.001) showed statistically significant difference between groups, while CAT activity was found to be similar between groups (p=0.218). The post-hoc test revealed that the mean GSH level was significantly higher in sham group compared to all other groups (all p values <0.001) and in TNBS-BG10 group compared to TNBS group (p=0.018). The mean MDA level and mean MPO activity in sham group (p=0.008 and p<0.001, respectively), and also in TNBS-BG10 group (p=0.005 and p=0.014, respectively) were significantly lower than those in TNBS group. There was also a significant difference between sham group and TNBS-BG3 group, indicating lower MPO activity in sham group (p=0.037).

Table 1. The histological (microscopic) scores for each parameter in groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham Control</th>
<th>TNBS</th>
<th>TNBS-BG3</th>
<th>TNBS-BG10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal Damage / Necrosis</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Edema</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

TNBS: trinitrobenzene sulfonic acid, BG: beta glucan
DISCUSSION

In this study, we found that the mean GSH and CAT levels were significantly higher in sham group compared to others. Also, MPO and MDA levels were significantly lower in sham group compared to others. This indicates that oxidative damage was observed in the biochemical results of the TNBS-treated groups. Furthermore, histological examination results support the biochemistry results. Oxidative damage and histological tissue damage decreased with BG administration.

Jurjus et al. (19) reported that the inflammatory process and wound development in TNBS colitis may be due to disruption of the intestinal mucosal barrier depending on the dose of the chemical applied. Likewise, Millar et al. (20) reported that widespread mucosal edema, necrosis and ulcerations were observed in the TNBS colitis model. In addition, according to current studies, Xue et al. (21) used the chemical OxaZolone to induce experimental ulcerative colitis. Shanmugam et al. (22) used indomethacin, and Matowea et al. (23) and Guazelli et al. (24) used acetic acid for induction of colitis. Their results support the results of TNBS-induced ulcerative colitis and our results. Significant histopathological changes were detected in all experimental groups in which colitis was induced by TNBS colitis model used in our study. Among the TNBS-treated groups, necrosis and hemorrhage were not observed in the mucosa in the TNBS-BG10 group, and inflammation was observed to be more limited than in the other TNBS-treated groups.

Nosálová et al. (25) reported that N-acetyl cysteine treatment showed antioxidative properties in acetic acid-induced colitis model. Ademoğlu et al. (26), on the other hand, investigated the antioxidative effects of vitamin E and selenium on ulcerative colitis in their experimental study and concluded that MDA decreased significantly in the experimental group given vitamin E and selenium together. Ek et al. (27) determined that caffeic acid phenethyl ester (CAPE) administered to rats with colitis with TNBS caused a decrease in MPO levels in the treatment group. Ige et al. (28) in their study in which they associated the effects of ulcerative colitis induced by acetic acid with maize consumed in the diet, showed that while the MPO values of the colitis group increased significantly compared to the control group, the MPO levels of the rats fed a diet supplemented with maize at an appropriate rate approached the values of the control group. Motawea et al. (23), in their study investigating the effects of oleuropein against acetic acid-induced ulcerative colitis, found that MDA and MPO levels, which increased significantly in the colitis group, decreased in the oleuropein-administered groups and approached the results of the control group. With our study findings, it was observed that BG reduced lipid peroxidation with its antioxidant property, and the lowest MDA level was found in the TNBS-BG10 group. In addition, a statistically significant increase was found in the colon tissue MPO activity values in the colitis group, which was applied TNBS, and it was observed that the values approached the control group with the treatment.

Karabeyoğlu et al. (29) examined the effect of ethyl pyruvate in the experimental colitis model created in rats and studied the glutathione levels in the colon tissue to investigate the antioxidant property of ethyl pyruvate. GSH levels in the treatment group were found to be significantly higher than in the plain colitis group. Shanmugam et al. (22) in their study investigating the effects of Passiflora subpeltata Ortega on experimental colitis, found that the GSH levels, which decreased significantly in the colitis group, increased with the administration of Passiflora subpeltata Ortega depending on the dose they applied. Kuralay et al. (30) reported that CAT activity did not change with colitis in acetic acid-induced colitis model. In the study conducted by Yildiz et al. (31), it was found that there was no significant difference in CAT levels between the colitis group and the treatment group in which resveratrol was applied in the colitis model induced by TNBS. In our study, no statistically significant difference was found between the groups in terms of CAT levels. The inability to detect a change in CAT level is due to the more effective functioning of other cellular antioxidant systems. Şener et al. (32) investigated the antioxidant properties of BG and its protective role against oxidative organ damage in the heart, brain, kidney and liver in rats in which they caused sepsis as a result of cecal ligation and piercing and observed that it reduced TNF-α levels in the organs after sepsis. Kayali et al. (33) stated that β-glucan reduces oxidative damage in experimentally induced spinal cord injury. Şener et al. (34) investigated the protective and therapeutic effects of β-glucan on bladder and kidney tissues in their study of nicotine-induced oxidative damage. They found that the events increase the activities of antioxidant enzymes. Tatlı-Seven et al. (35) investigated the effects of BG against lead acetate-induced hepatic damage and reproductive damage and determined that it showed protective and healing properties against different tissues and organs.

CONCLUSION

In the results obtained in our study, the antioxidant effect of β-glucan was found to be compatible with the literature. BG increased the level of glutathione, which is an indicator

Table 2. Comparison of the biochemical measurements between groups

<table>
<thead>
<tr>
<th></th>
<th>Sham Control</th>
<th>TNBS</th>
<th>TNBS-BG3</th>
<th>TNBS-BG10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (nmol/mg), mean±SD</td>
<td>16.575±2.629</td>
<td>9.304±1.085</td>
<td>11.463±1.885</td>
<td>12.338±1.411</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MDA (nmol/g), mean±SD</td>
<td>239.538±19.715</td>
<td>265.600±12.762</td>
<td>252.263±11.951</td>
<td>238.350±13.748</td>
<td>0.003</td>
</tr>
<tr>
<td>CAT (μ/ml), mean±SD</td>
<td>4.540±0.664</td>
<td>4.116±0.956</td>
<td>3.918±0.740</td>
<td>3.762±0.635</td>
<td>0.218</td>
</tr>
<tr>
<td>MPO (U/g), mean±SD</td>
<td>10.131±1.427</td>
<td>13.683±1.176</td>
<td>12.129±1.605</td>
<td>11.393±1.333 &lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

TNBS: trinitrobenzene sulfonic acid, BG: beta glucan, GSH: glutathione, MDA: malondialdehyde, CAT: catalase, MPO: myeloperoxidase, SD: standard deviation, Tukey HSD post hoc test results; GSH, Sham vs TNBS: p<0.001, Sham vs TNBS-BG3: p=0.001, Sham vs TNBS-BG10: p=0.001, TNBS vs TNBS-BG3: p=0.135, TNBS vs TNBS-BG10: p=0.018, TNBS-BG3 vs TNBS-BG10: p=0.835, MDA, Sham vs TNBS: p=0.008, Sham vs TNBS-BG3: p=0.336, Sham vs TNBS-BG10: p<0.001, TNBS vs TNBS-BG3: p=0.379, TNBS vs TNBS-BG10: p=0.005, TNBS-BG3 vs TNBS-BG10: p=0.263, MPO, Sham vs TNBS: p=0.001, Sham vs TNBS-BG3: p=0.037, Sham vs TNBS-BG10: p=0.290, TNBS vs TNBS-BG3: p=0.140, TNBS vs TNBS-BG10: p=0.014, TNBS-BG3 vs TNBS-BG10: p=0.178
of antioxidant parameter, decreased the levels of MDA and MPO, and caused improvement in histopathological scores. In this way, it has been shown by our study that BG can be a therapeutic agent. Treatment with BG has the potential to reduce colon tissue damage in TNBS-induced colitis in rats. Therefore, with these features, BG can be considered as a new and effective adjunctive therapy in the prevention and treatment of IBD.

**Ethics Committee Approval:** The study was approved by the Ethics Committee of Aydın Adnan Menderes University (14.08.2015, 64583101/2015/094).

**Conflict of Interest:** None declared by the authors.

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