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Original Article

Effect of β-glucan on acetic acid-induced colitis in rats

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Abstract: *Objective:* This study aimed to determine the effects of β-Glucan on colitis induced by acetic acid (AA). *Methods:* A total of 28 Wistar-Albino male rats were divided into four groups. Intrarectal saline were administered in the group 1. AA colitis model was created in the group 2. Intrarectal saline and β-Glucan were administered in the group 3. β-Glucan was given daily after induction of colitis in the group 4. β-Glucan was given by daily gavage for 4 days with 50 mg/kg in the treatment groups. Macroscopically and histologically colon damage was assessed. Biochemically, tumor necrosis factor (TNF) α, interleukin (IL)-1β, IL-6, myeloperoxidase (MPO), malonyldialdehyde (MDA), glutathione (GSH) and superoxid dismutase (SDO) values were measured. *Results:* There was improvement in the macroscopic and histological damage in the colon, but was not statistically significant ($p>0.05$). Biochemically, there was an insignificant difference in MPO, IL-6, TNF α and MDA levels (respectively $p=0.611$, $p=0.540$, $p=0.065$, and $p=0.129$, respectively) while a significant decrease in IL-1β, SOD and GSH levels ($p=0.012$, $p=0.013$, and $p<0.001$, respectively). *Conclusions:* β-Glucan has protective effects in the treatment of ulcerative colitis.

Keywords: Ulcerative colitis, β-Glucan, treatment, rats

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease of the large intestine. However, the etiology of ulcerative colitis has not been fully understood [1]. Increased inflammation and oxidative stress play a major role in the pathogenesis of ulcerative colitis [2]. It is known that inflammatory mediators in this disease are secreted from granulocytes migrated to the inflamed colon mucosa. Inhibition of lipid peroxidation or uptake of free oxygen radicals will greatly contribute to therapeutic treatment and prevention of UC [3]. For this purpose, numerous studies have been conducted to develop an effective pharmacological treatment for UC treatment and prevention [4-6].

β-Glucan, a natural immune booster derived from bread yeast, is a polyglucose molecule with multiple bond structure. In case of infection B-glucan activates white blood cells such as macrophages, granulocytes and monocytes, and therefore initiating the inflammatory cascade , β-Glucan also Helps in the process of healing of damaged tissues in the body. In addition it protects the body from harmful effects of free radicals [7]. It protects the body against infections and cancer development [8]. Depending on the route of administration, it increases macrophage infiltration and collagen deposition, stimulating granulation tissue formation and re-epithelization, thus contributing to wound healing [9].

β-Glucan has antibacterial, antifungal, antiviral, and antitumoral effects [10-13]. However, in addition to all these biological effects, studies on anti-inflammatory activity in colitis are not available. In this study, we aimed to investigate the effect of β-Glucan administration on acetic acid-induced colitis.

Material and methods

The study protocol was approved by Gaziosmanpasa University Medical Faculty Ethical Committee of Experimental Animals (2014-HADYEK-25) and all experimental, surgical and laboratory procedures were carried out at Gazios-

Table 1. Macroscopic evaluation scale

Score	Criterion
0	No damage
1	Focal hyperemia without ulceration
2	Hyperemia or linear ulceration without thickening of colonic wall
3	Linear ulceration with inflammation in one area
4	Ulceration or inflammation in two or more areas
5	Two or more areas of major ulceration and inflammation, one or more sites of damage to the colon segment longer than 1 cm
6-10	The ulcer and inflammation area longer than 2 cm in the colon (the score is increased by 1 unit for every 1 cm damage)

manpasa University Medical Faculty Experimental Research Center and Gaziosmanpasa University Medical Faculty Biochemistry and Histology Laboratories.

Experimental material and laboratory environment

In this experimental study, 28 male Wistar-Albino rats with an average weight of 250-350 g were used. Rats were fed in separate wire cages and ad libitum in standard laboratory conditions (20-24°C, 40-50 humidity, 12 hour light-dark cycles). Rats who were fasted for 24 hours before all surgical procedures were not restricted in their water intake. Rats were randomly assigned to four study groups (7 rats per group) using simple random sampling method.

Surgical procedures and experiment protocol

There was no antibiotic administration to the rats in all groups before or after the surgical procedure. Rats were anesthetized intramuscularly before the experimental surgery with 75 mg/kg ketamine hydrochloride (Ketalar, 500 mg vial, Pfizer Istanbul) and 10 mg/kg xylazine hydrochloride (Rompun 2% flacon, Bayer, Istanbul). Before experimental colitis was formed, the rats were weighed and their weights recorded. To create experimental colitis, a soft 6-mm pediatric nutrition catheter was inserted through the anal canal and pushed 8 cm forward. Transrectally, 1 ml of 4% acetic acid (AA) (pH: 2.3) was slowly applied through the catheter. Before the catheter was withdrawn, 2 mL of air was injected to spread to the entire colon. The catheter was then gently pulled to prevent physical trauma. Rats were held upside down for 30 seconds in order to prevent the material from escaping backwards [14].

Experimental groups were organized as follows: Group 1 (Control Saline): Rats were given saline intrarectally. Group 2 (Control AA): An experimental colitis model was established with AA. Group 3 (β-Glucan-Saline): Rats were given saline intrarectally. 50 mg/kg β-glucan was administered daily for 4 days with gavage. Group 4 (β-Glucan-AA): An experimental colitis model was established with AA. 50 mg/kg β-Glucan was administered daily for 4 days with gavage.

Drug administration

β-Glucan (Imuneks 50 mg, Mustafa Nevzat, Istanbul) in microparticulate form obtained from Saccharomyces cerevisiae yeast was prepared by dissolving in saline. The solution was administered at a dose of 50 mg/kg/day starting from the day of operation to the time of sacrifice with a single dose of gavage every day for 4 days [15].

Collection of blood and tissue samples

Before sacrifice, the rats were weighed and their weights were recorded. The sacrificewas performed in accordance with standard laboratory conditions. Before the procedure, the animals were anesthetized with 30 mg/kg hydrochloride and 5 mg/kg xylazine. By opening the abdomen with a midline incision, the heart was reached from the diaphragm. Blood samples were obtained by cardiac puncture, and then the rats were sacrificed by cervical decapitation. Serum was separated and stored at -80°C until analysis day. In all cases, the work was repeated twice. The midline incision was extended to pubis and the rectum was cut from the most distal area possible. From this section, the last 8 cm of the rectum was removed.

Macroscopic colitis evaluation

After the colon was resected, it was opened longitudinally and cleaned with saline. Mucosal lesions were then macroscopically scored. Mo-

Table 2. Comparison of pre-post experiment weight and weight change means according to rats group (Wilcoxon signed rank test)

		Pre-post weight changes				Pre-post weight differences		
		N	Mean ± SD	Median (min-max)	*p* value	Mean ± SD	Median (min-max)	*p* value
Control Saline	Pre	7	283.43±10.11	284 (268-300)	0.271	11.28±27.90	6 (-19, 70)	0.077
	Post	7	294.71±34.83	290 (265-370)				
Control AA	Pre	7	287.14±37.57	270 (250-340)	0.018*	-29.14±24.59	-20 (-68, -9)	
	Post	7	258.00±29.03	250 (235-320)				
β-Glucan Saline	Pre	7	340.57±13.93	342 (312-354)	0.176	-24.00±30.57	-32 (-58, 10)	
	Post	7	316.57±23.68	314 (294-350)				
β-Glucan AA	Pre	7	316.29±43.56	316 (234-376)	0.352	-9.42±56.98	-12 (-62, 106)	
	Post	7	306.86±21.31	306 (272-340)				

*Statistically significant ($p<0.05$); SD: Standard Deviation, Min: Minimum, Max: Maximum.

rris' scoring system was used for colon macroscopic scoring [16] (**Table 1**).

Histopathological evaluation

Histopathological examination was carried out at Gaziosmanpasa University Faculty of Medicine Histology Department. For microscopic evaluation, sections from the most damaged areas of colon were sampled. The tissue samples were fixed in 10% buffered neutral formaldehyde solution for an average of 36 hours. After fixation, the tissues were embedded in paraffin blocks according to routine histological protocol. Tissue specimens embedded in paraffin blocks were cut in 5 μmthickness with a rotary microtome (Leica RM2135, Germany) and placed on standard glass slides. The sections were incubated for approximately 12 hours and the paraffin-melted sections were stained according to Hematoxylin Eosin (H&E) staining protocol. There were 7 animals in each group. Histological analyseswere performed on 10 cross sections of each animal and an average of random 20 microscopic image area of each cross section. Histological analysis was performed by analyzing the microscopic image, which was transferred to the camera (Nikon Ds-Fi1, Japan) integrated with a computer-assisted light microscope (Nikon Eclepse 200 Serial No: T1al 944909, Japan). Microscopic analyses were performed by a non-informed investigator in the form of a blind study based on the coding system. The mucosa, submucosa and adventitia tissues of all colon tissue samples were evaluated microscopically for the intensity of inflammation. Semi quantitative tissue inflammation severity scoring was performed. A 4-level modified scoring scale, shown in the table below, was used for the evaluation of semiquantitative tissue inflammation [17]. According to this scoring system; 0: none, 1: giant cells, occasional lymphocytes, 2: giant cells, plasma cells, eosinophils, neutrophils, 3: many inflammatory cells and microabscess.

Biochemical studies

Blood samples obtained from the rats were placed in vacutainers and allowed to clot for about thirty minutes. The tubes were centrifuged (+4 C0, 4000 rpm) for 10 min to separate the sera. The sera separated into eppendorf tubes were then stored at -80°C until analyzed. GSH (Cayman Chemical, Item No: 7003002, USA) and MDA (Cayman Chemical, Item No: 10009055, USA) levels are measured with calorimetric method. MPO (Cloud-Clone Corp., SEA601Ra, USA), SOD (YH-biosearch, Cat No: YHB2870Hu, China), TNF α (YH-biosearch, Cat No: YHB1098Ra, China), IL-6 (YH-biosearch, Cat No: YHB0630Ra, China) and IL-1β (YH-biosearch, Cat No: YHB0616Ra, China) levels are measured with ELISA method.

Statistical analysis

Statistical analyses were performed by using SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA). The normality distribution was assessed by the Shapiro-Wilk test. Descriptive statistics were presented as mean ± standard deviation, median (min-max). The Kruskal-Wallis test was used because parametric test (ANOVA) assumptions were not provided in the anal-

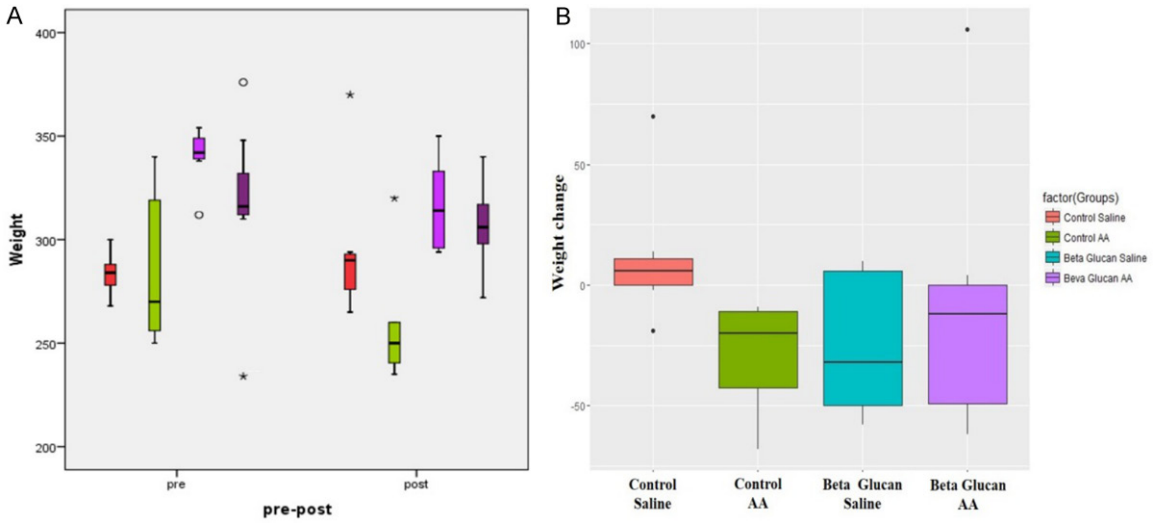

Figure 1. Boxplots of weight measurements for pre-post experiment (A) and weight change means (B) according to rat groups.

Table 3. Comparison of Macroscopic damage score meansaccording to rats group (Kruskal-Wallis test)

	N	Mean ± SD	Median (min-max)	*p* value
Control Saline	7	0.14±0.37	0 (0, 1)	<0.001*
Control AA	7	6.14±1.21	7 (4, 7)	
β-Glucan-Saline	7	0.14±0.37	0 (0, 1)	
β-Glucan-AA	7	2.71±0.48	3 (2, 3)	

*Statistically significant (p<0,001); SD: Standard Deviation, Min: Minimum, Max: Maximum.

ysis of Macroscopic damage score, Inflammatory score and Biochemical Parameters in more than one independent group sample mean comparisons. Post-hoc tests were used to determine the significance levels originated after the variance analysis. For the mean comparison between the two dependent groups, normalized t-test was used, Wilcoxon signed rank test was used for comparison of pre-post experiment weight and weight change means according to rats group when no normality assumption was provided. The statistical significance level was accepted as p<0.05.

Results

There were no complication or death during or after the surgical procedures.

Macroscopic findings

The weight measurements done before and after the experiment were compared for weight loss evaluation. There were no statistically significant diff erences in weight loss between Control Saline, β-Glucan Saline and β-Glucan AA groups; The only statistically significant difference in weight loss was observed with the Control AA group (p=0.271, p=0.176, p=0.352>0.05 and p=0.018<0.05, respectively). The average comparison results are presented in **Table 2**. The boxplot before and after the experiment in rat groups are shown in **Figure 1A**. Two in the Control Saline group, 4 in the β-Glucan Saline group, 5 in the β-Glucan group and all rats in the Control AA group had weight loss.

By the difference between before and after weight, a new variance was determined as the weight change and the weight change measurements were compared in the rat groups. The average comparison results are presented in **Table 2**. When **Table 2** was examined, it was seen that there was no statistically significant difference between the weight change averages according to the groups (p>0.05). The average change boxplot for the weight change are presented in **Figure 1B**.

When the groups were examined in terms of macroscopic damage score, statistically signifi-

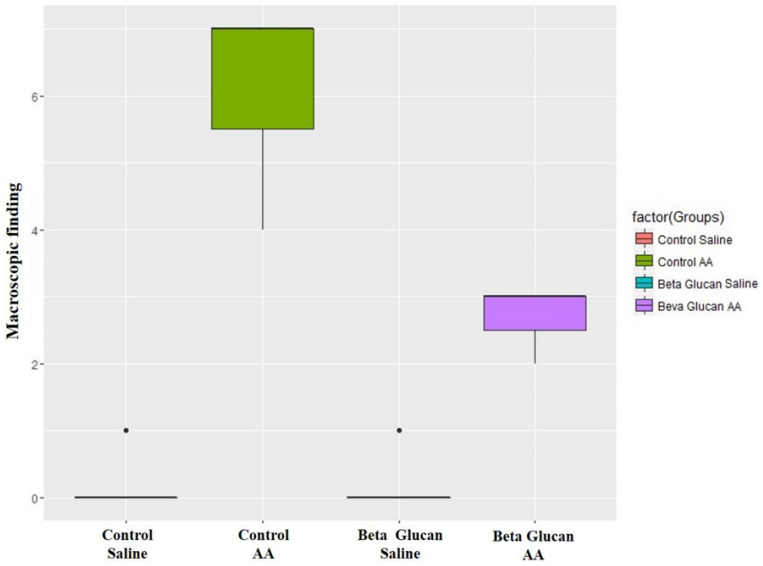

Figure 2. Boxplot for macroscopic finding according to rat groups.

cant difference was observed (p<0.001). The average macroscopic damage scores of the groups are shown in **Table 3**. Differences in the macroscopic findings were found between Control Saline-Control AA and β-Glucan Salin-Control AA groups (p<0.001, p<0.001, respectively) by using post-hoc tests. The macroscopic damage score was the highest in the Control AA group (6.14±1.21), while the β-Glucan AA group was lower other groups but not statistically significant (2.71±0.48). For the macroscopic findings, the mean change boxplot are presented in **Figure 2**.

Histopathological findings

When the groups were examined in terms of inflammation score, there was a significant difference between the groups (p<0.001). The lowest value was in the Control Saline group (0.32±0.12). In microscopic analysis of colon tissues of the control group rats, it was observed that the intensity of inflammatory cells and the integrity of tissues were in normal colon tissue appearance. The highest value was in the Control AA group (2.02±0.61) in terms of the mean of the inflammation score. With AA administration, it was determined that inflammatory cell densities were markedly increased in all layers of the organ wall. There were a number of diffuse inflammatory cells, especially eosinophills and neutrophills, and areas of intense microabscesses.

In addition, in the groups treated with AA relative to other groups as an indicator of impaired tissue integrity, the amount of tissue falling into a microscopic field of view was even greater. There were also intraluminal erosions where the mucosal crypts are usually lost by foliage along with the underlying muscularis mucous membranes. As a result of the microscopic analyzes performed in the treated groups, it was found that these tissue damages were reduced to some extent, and also the inflammation score intensities were decreased. In the β-Glucan AA group, the inflammation score decreased (1.32±0.35), although it was not statistically significant. In general, it was seen that tissue integrations were preserved together with all layers (**Figure 3**).

Descriptive statistics for the inflammation score according to the groups are shown in **Table 4**. There was a significant difference between the groups according to the results of the mean inflammation comparison (p<0.001). Differences in the inflammation averages were found between the groups β-Glucan Saline-β-Glucan AA, β-Glucan Saline-Control AA and Control Saline-Control AA (p=0.007, p<0.001, p=0.011, respectively). The mean change box plot for the inflammation average are presented in **Figure 4**.

Biochemical findings

Descriptive statistics and mean comparisons for the research parameters according to the groups are shown in **Table 5**. There was no statistically significant difference for MPO, IL-6, TNF α and MDA parameters according to control and glucan groups (p=0.611, p=0.540, p=0.065, p=0.129>0.05, respectively).

Other variables; the mean values of IL-1β, SOD and GSH were statistically significant (*p* values: p=0.012, p=0.013 and p<0.001, respectively) in comparison with control and glucan groups. According to the results of multiple comparison

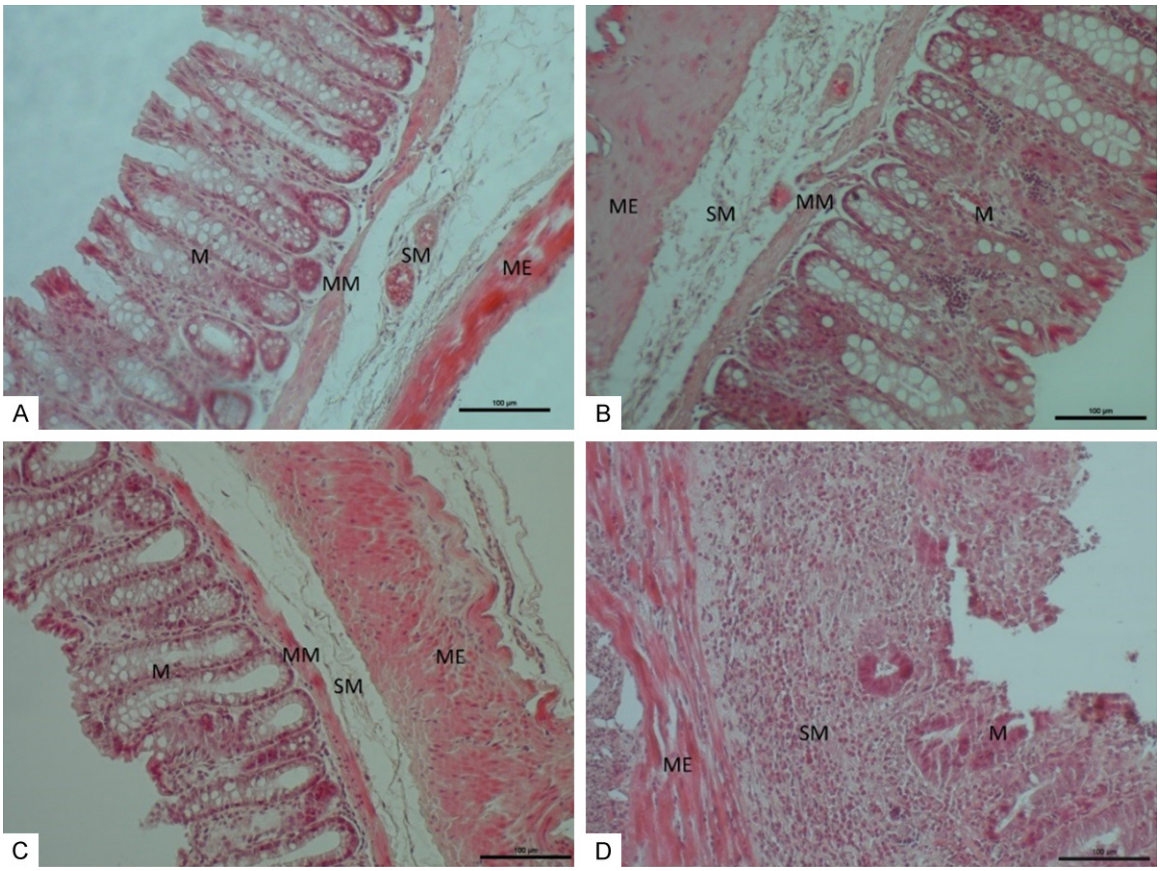

Figure 3. Histological sections of the groups (A): Control saline, (B): Beta-glucan AA, (C): Beta-glucansaline, (D): Control AA. M: mucosa, MM: muscularis mucosa, SM: submucosa, ME: muscularisexterna (H&E, Bar: 100 µm).

Table 4. Descriptive statistics for the Inflammatory score; 0, 1, 2, 3 and Comparison of Inflammatory score means according to rats group (N=7) (Kruskal-Wallis test)

		Inflammation score					*p* value
		0	1	2	3	Average	
Control Saline	Mean ± SD	33.71±11.78	16.14±9.08	0.43±1.13	0	0.32±0.12	<0.001*
	Median (min-max)	32 (22-58)	10 (7-29)	0 (0-3)	0	0,31 (0.21-0.57)	
Control AA	Mean ± SD	2.86±5.24	11.43±12.10	17.57±9.34	15±13.01	2.02±0.61	
	Median (min-max)	0 (0-14)	10 (0-36)	13 (5-30)	12 (1-34)	1.98 (1.17-2.86)	
β-Glucan Saline	Mean ± SD	45.71±12.6	11.14±2.79	0	0	0.20±0.03	
	Median (min-max)	50 (22-63)	12 (6-14)	0	0	0.21 (0.14-0.25)	
β-Glucan AA	Mean ± SD	8.29±7.15	28.71±14.91	19.86±10.49	4.29±5.37	1.32±0.35	
	Median (min-max)	9 (1-18)	20 (17-58)	21 (6-39)	2 (0-12)	1.38 (0.88-1.8)	

*Statistically significant ($p<0.001$); SD: Standard Deviation, Min: Minimum, Max: Maximum.

post-hoc tests; there was a significant difference for IL-1β found to be between β-Glucan AA-Control AA, β-Glucan AA-Control Saline, β-Glucan Salin-Control AA and β-Glucan Saline-Control Saline groups (p=0.019, p=0.018, p=0.021 ve p=0.019, <0.001 respectively). Mean change boxplots for MPO, IL-1β, IL-6 and TNF α are presented in **Figure 5A-D**, respectively. The difference for the SOD variant was found to be between β-Glucan AA-Control Saline, β-Glucan AA-Control AA, β-Glucan Saline-Control Saline and β-Glucan Saline-Control AA groups (p=0.017, p=0.013, p=0.029 and p=0.024<0.05, respectively). Differences for the GSH variant were found between Beta-glucan Saline-Control Saline, β-Glucan Saline-

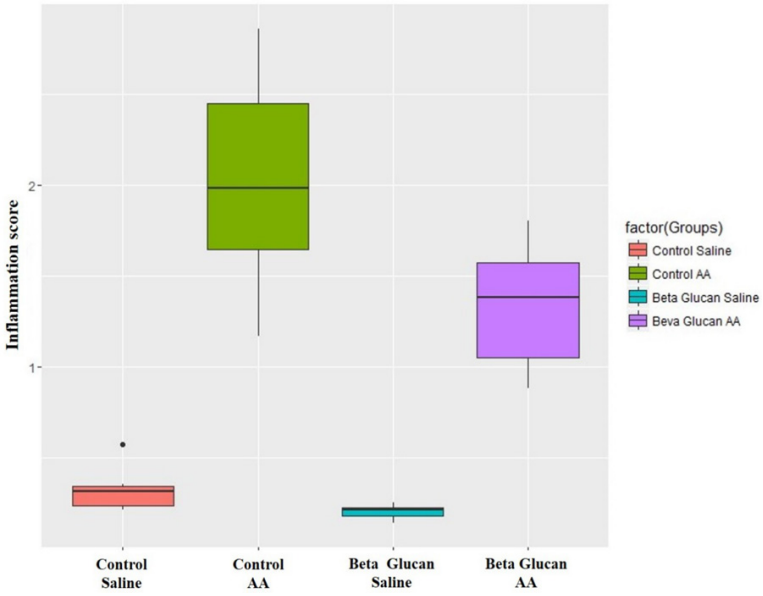

Figure 4. Boxplot for the inflammation score average according to rat groups.

Control AA and β-Glucan AA-Control AA groups ($p=0.024$, $p=0.001$ and $p=0.005<0.05$, respectively). The mean change box plots for SOD, GSH and MDA are presented in **Figure 6A-C**, respectively.

Discussion

In experimental studies, many different substances were investigated in terms of efficacy in different colitis models. We used a transrectal acetic acid model for colitis induction in our study [18]. 4% AA leads to inflammatory histopathological findings similar to those of the human when contacted with the rats colon mucosa for about 15 seconds [19].

The antioxidant properties of many substances have been experimentally investigated, and the treatment response was assessed with macroscopic and microscopic scoring systems [5, 20, 21]. β-Glucan helps heal damaged tissues in the body. Besides, protect the body from harmful effects of free radicals. Glucan has been shown to decrease malnutrition after major thoracic and gastrointestinal operations, to strengthen the immunity system, to have a positive effect on macrophage and leukocyte functions, and to reduce wound infections and death relatively [22, 23]. In our study, macroscopically localized hyperemia of the colon mucosa, the presence of linear ulcers and inflammation, histopathologically significant loss of mucosal structure, cellular infiltration, goblet cell depletion and crypt abscesses demonstrate the presence of inflammatory bowel disease. The group with the highest macroscopic colitis grading score was Control AA group (6,14±1,21). In the β-Glucan AA group, the colitis score (2,71±0,48) was not statistically significant but decreased. In addition, weight loss was observed in the rats due to inflammation and diarrhea in the colitis group formed by AA. This loss was not significant but decreased with β-Glucan therapy. All rats in the control AA group experienced weight loss, while in the β-Glucan AA group 5 rats experienced weight loss. However, the average weight loss was less than in the colitis group. In our study, it was determined that β-Glucan usage positively affected the macroscopic score and microscopic evaluation scores in the parameters evaluating the colon histology in the AA-induced colitis model, but the difference was not statistically significant.

When the microscopic parameters were compared, it was found that the scores on the β-Glucan group were lower than the control group Şener and coworkers [24] reported that β-Glucan significantly reduced interstitial inflammatory infiltration, tubular epithelial degeneration, Bowman's capsule degeneration, and glomerular necrosis in kidney tissue and preserved normal renal tissue histopathologically. Bedirli and coworkers [7] investigated the effect of β-Glucan on lung injury in an experimental sepsis model; and reported significant healing on pulmonary infiltration, congestion and intraalveolar hemorrhage. Mladenovic and coworkers [25] showed that proinflammatory cytokines such as IL-6, and TNF α increased in the intestinal mucosa in the colitis model they formed with acetic acid. Other studies have shown that β-Glucan does not stimulate IL-1β, IL-6, and TNF α proinflammatory cytokine production, but increases the microbicidal effects of neutrophils and macrophage [26, 27] have shown that β-Glucan stimulates IL-1β, IL-6,

Table 5. Mean Comparisons for Biochemical Parameters (Kruskal-Wallis test)

	G	N	Mean ± SD	Median (min-max)	*p* value
MPO (pg/mL)	1	7	4543.33±1640.15	4227.49 (2225.42-6931.25)	0,611
	2	7	3724.12±756.94	3703,93 (2613.16-5004.22)	
	3	7	3694.19±856.27	3701.77 (2171.71-4787.85)	
	4	7	3488.48±658.14	3640.40 (2626.13-4239.17)	
IL-1β (pg/mL)	1	7	16.16±2.50	16.83 (11.02-18.26)	0,012*
	2	7	16.20±0.91	16.28 (15.17-17.61)	
	3	7	13.98±0.91	14.01 (12.24-15.19)	
	4	7	13.90±1.61	13.87 (11.98-16.52)	
IL-6 (ng/L)	1	7	224.80±9.34	225 (209.67-234.33)	0,540
	2	7	227.38±9.67	225 (212.67-239.33)	
	3	7	221.09±9.10	220.33 (209.67-233.00)	
	4	7	219.38±16.41	218.33 (198.00-244.67)	
TNF α (ng/L)	1	7	209.79±7.54	208.66 (198.33-219.33)	0,065
	2	7	203.85±13.02	196.33 (191.33-220.67)	
	3	7	186.90±14.54	186 (167.67-209)	
	4	7	197±33.23	176 (171.33-260.67)	
SOD (U/L)	1	7	624.29±18.38	620 (603-647)	0,013**
	2	7	630.29±32.21	628 (592-682)	
	3	7	591.29±18.64	594 (555-611)	
	4	7	572±52.44	584 (481-629)	
GSH (uM)	1	7	1.29±0.32	1.23 (0.85-1.86)	<0,001***
	2	7	1.6±0.25	1.66 (1.21-2.01)	
	3	7	0.62±0.25	0.65 (0.18-0.95)	
	4	7	0.76±0.05	0.74 (0.71-0.87)	
MDA (uM)	1	7	3.67±0.13	3.65 (3.51-3.86)	0,129
	2	7	3.48±0.38	3.51 (2.94-3.96)	
	3	7	3.87±1.09	3.9 (2.59-5.1)	
	4	7	3.15±0.34	3.25 (2.57-3.63)	

G: Groups, SD: Standard Deviation, Min: Minimum, Max: Maximum; *Statistically significant $p<0.05$; **Statistically significant $p<0.01$; ***Statistically significant $p<0.001$.

IL-10 levels and suppresses TNF α levels. In our study, decrease in IL-1β, IL-6 and TNF α levels was observed with β-Glucan and the decrease in IL-1β level was also statistically significant. The absence of significant decrease in biochemical findings of TNF α and IL-6 levels, which are thought to play a key role in the pathogenesis of inflammatory bowel disease suggests that different mediators may also play a role in acute colonic inflammation.

Oxidative stress plays a key role in the pathogenesis of UC [2]. In colonic mucosa biopsies of inflammatory bowel patients, oxidative stress has been increased and antioxidant defenses have been shown to be reduced [6]. When the colon is inflamed, the free oxygen radicals break down the antioxidant system and cause oxidative damage [28]. In addition, lipid peroxidation triggers the formation of lipid peroxide radicals and thus plays an important role in the inflammatory process [29]. β-Glucan is a very potent antioxidant, which removes free oxygen radicals [30]. Antioxidant enzymes such as SOD are part of the natural cell defense system against damage caused by the superoxide radical. A cellular antioxidant in the structure of metalloenzymes with critical presupposition, which converts superoxide radicals formed in situations like oxidative stress to molecular oxygen and hydrogen peroxides [31]. Hagar and coworkers [32] demonstrated that the activity of the antioxidants in the mucosa is accompanied by a decrease in the experimental colitis

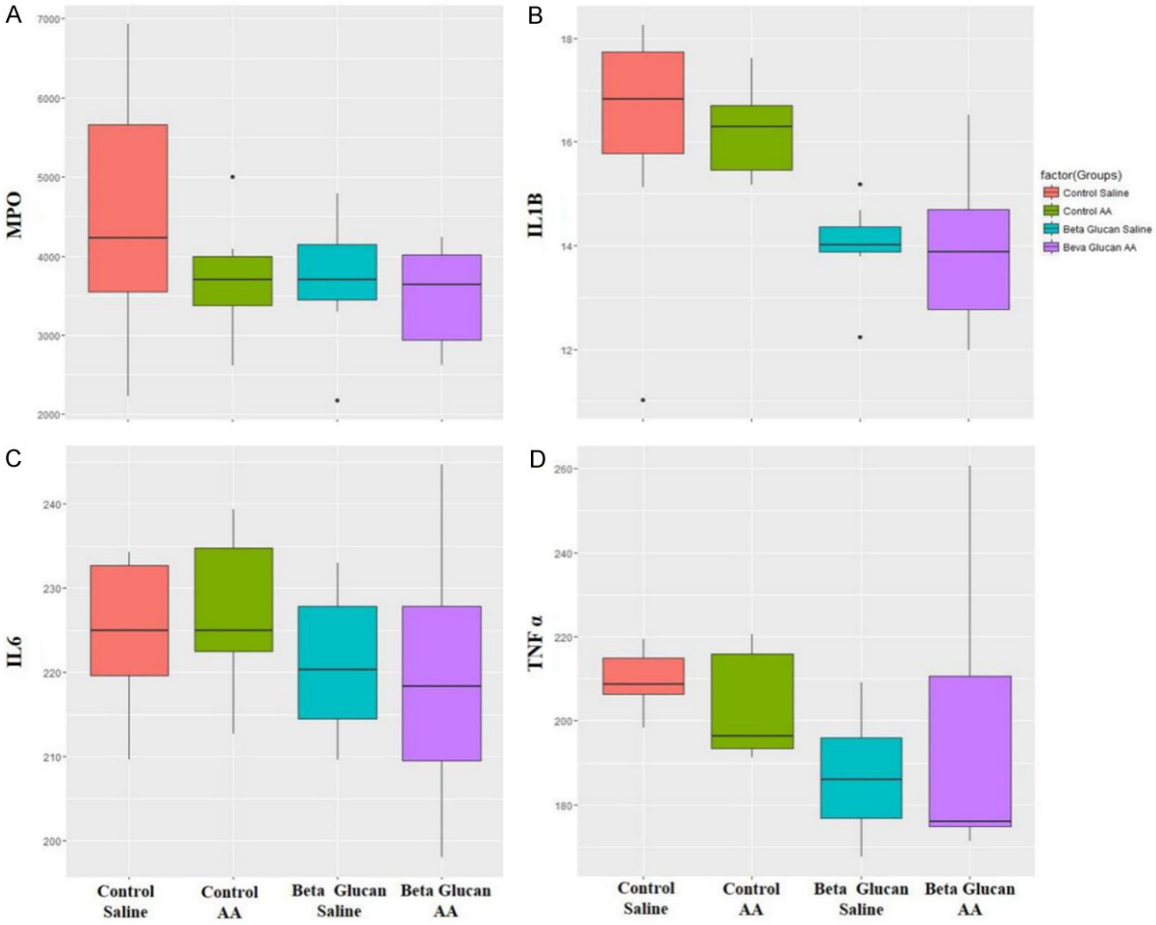

Figure 5. Boxplots for MPO (A), IL1B (B), IL6 (C) and TNF α (D) measurements according to rat groups.

model. Kapan and coworkers [33] reported that the renal tissue SOD levels in the experimental aortic ischemia reperfusion model were significantly elevated in the ischemia reperfusion group compared to the control group. Mun and coworkers [34] reported that tissue SOD and catalase levels were elevated in the experimental kidney ischemia reperfusion model. Bayrak and coworkers [35] reported that β-Glucan significantly reduced SOD levels in experimental kidney ischemia reperfusion injury in rats. These findings support that β-Glucan significantly reduces antioxidant oxidative stress. In our study, SOD and GSH levels were significantly higher in the AA colitis group when compared with the β-Glucan AA group.

MPO is an indirect indicator of inflammation, showing neutrophil infiltration in tissues in experimental studies with different antiinflammatory agents in different colitis models, and is an enzyme that responds rapidly in the acute phase [5, 20, 21]. High MPO activity indicates neutrophil infiltration and tissue damage [7]. Babayigit and coworkers [36] reported that β-Glucan significantly reduced pulmonary tissue MPO levels and corrected lung damage in acute lung injury in experimental sepsis model. Toklu and coworkers [37] reported a significant decrease in renal MPO levels with inhibition of neutrophil infiltration in experimental oxidative organ damage. In the study of Şener and coworkers [15], it was determined that β-Glucan lowered the MPO level in the rats in which the pressure ulcer was formed. In our study, it was determined that the beta glucan decreased the MPO levels even though it was not statistically significant. Our results show that β-Glucan inhibits activation of colonic leukocyte infiltration and has protective effect on mucosa. However, there is a need for new efforts in this regard.

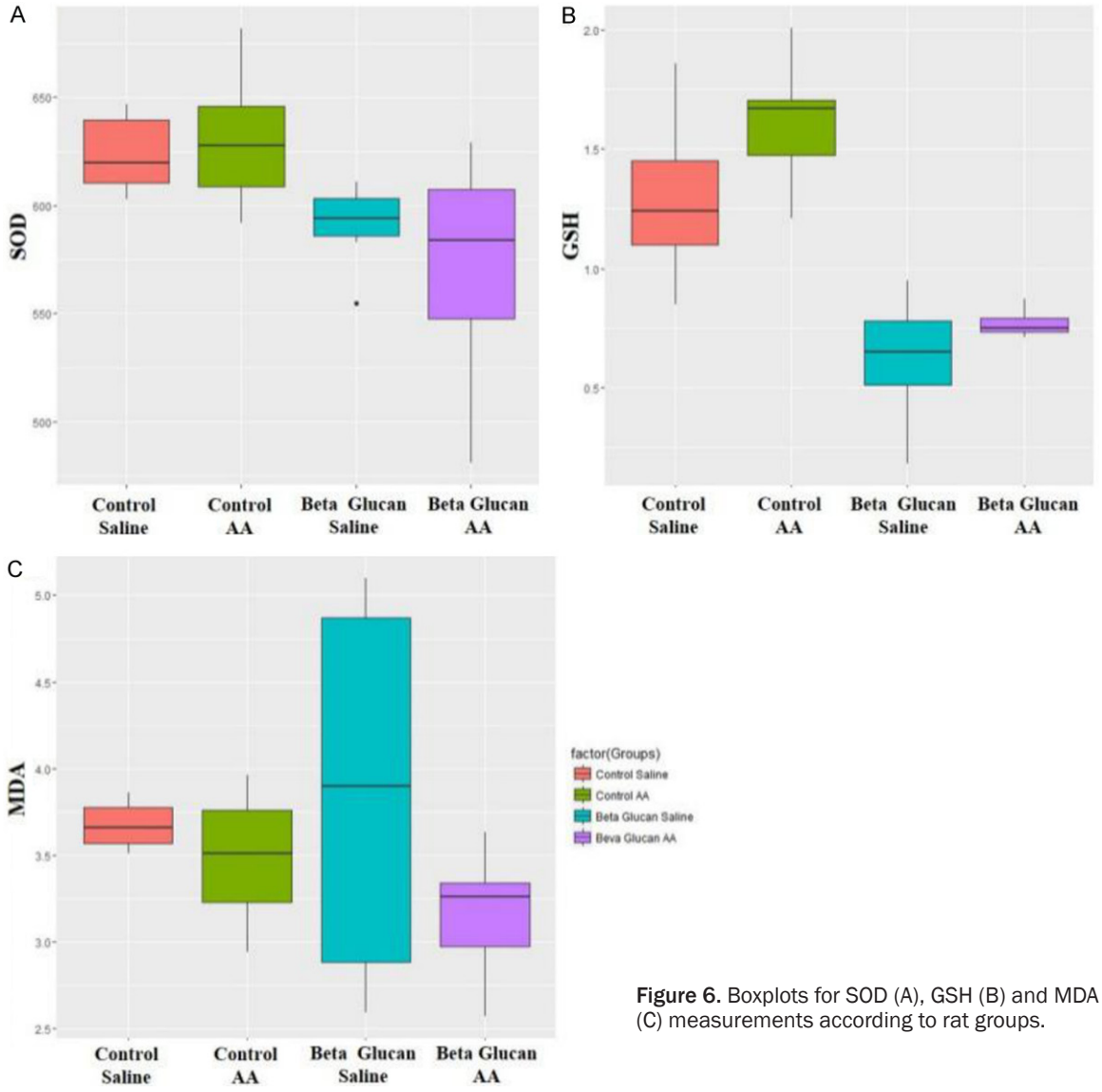

Figure 6. Boxplots for SOD (A), GSH (B) and MDA (C) measurements according to rat groups.

MDA is the latest product of late-onset cellular and intracellular lipid peroxidation [38]. MDA increase reflects the level of lipid peroxidation in the tissues and is considered as an indicator of tissue damage. The activated neutrophils leave the circulation and enter the intestinal mucosa and submucosa in acute inflammation. Then contribute to intestinal damage by over production of Lipid mediators, lactoferrin proteases, reactive oxygen and nitrogen derivatives. Lipid peroxidation of the cell membrane is an important step in the mucosal damage [39]. β-Glucan has been shown to reduce damage and MDA levels in other tissues [35]. Toklu and coworkers [37] reported that β-Glucan significantly reduced renal MDA levels in oxidative damage caused by burn injury in rats. Sener and coworkers [40] reported that β-Glucan significantly reduced renal and bladder MDA levels in nicotine-induced oxidative damage. Bolcal and coworkers [41] also reported that β-Glucan significantly reduced muscle tissue MDA levels in experimental lower limb IR injury. Kayali and coworkers [13] found no significant changes in tissue MDA levels in experimental spinal cord injury and reported this as a relationship with ischemia. In our study, we found a decrease in tissue MDA levels in the β-Glucan AA group, although not significant, compared to the control AA group. These results support that

β-Glucan reduces lipid peroxidation by protecting cell integrity in damaged colon mucosa.

GSH is an important intracellular protective compound against harmful stimuli, including oxidative stress, and protects organs against oxidative damage. In the study of Sener and coworkers [40] tissue GSH levels were found to be decreased in the kidney and bladder by chronic nicotine administration. It was also found that β-Glucan administration significantly increased tissue GSH levels. In Toklu and coworkers [37] study, it was reported that β-Glucan increased GSH level in acetaminophen induced liver toxicity. Another study by Toklu and coworkers [42] showed that the use of β-Glucan in local and systemic treatment leads to an early and late decrease with GSH levels in thermal injury model. In our study, it was also found that GSH level in serum decreased statistically significantly by β-Glucan administration against acetic acid-induced colonic damage. This result can be explained by the antioxidant properties of β-Glucan.

There is no current study in the literature that investigates the effect of β-Glucan in AA-induced colitis in rats. A similar study found that there was a decrease in macroscopic colonic damage score with β-Glucan. However, β-Glucan could not prevent the increase in MPO level in the damaged area. The MPO level in the segment of the colon without inflammatory macroscopic damage was significantly lower. Histologically, this finding has been confirmed. The authors have shown that with these results β-Glucan can be used as an immunomodulator in the treatment of UC [43].

Our study showed that the experimental colitis model formed with AA caused oxidative stress and lipid peroxidation. β-Glucan has been shown to cause improvement in colon damage, macroscopically and histopathologically, in this experimental colitis model. In addition, there was no significant change biochemically in MPO, IL-6, TNF α and MDA levels, but there was a significant decrease in IL-1β, SOD and GSH levels. β-Glucan has been shown to have protective effect in this experimental colitis model but further clinical trials are needed for β-Glucan to be used for the treatment of colitis and to be included in standard treatment protocols.

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Disclosure of conflict of interest

None.

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