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ORIGINAL ARTICLE

Protective Effects of Tyrosol Against DSS-Induced Ulcerative Colitis in Rats

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Abstract— In this study, the effects of tyrosol were investigated in DSS-induced experimental ulcerative colitis model. For this purpose, rats were divided into five groups of seven rats in each: control group, colitis group (DSS-4%), tyrosol group (tyrosol 20 mg/kg), sulfasalazine (sulfasalazine+DSS 100 mg/kg), and treatment group (tyrosol+DSS 20 mg/kg). In the study, the active substances were administered to all animals for a period of 21 days. At the end of the study, malondialdehyde (MDA) levels increased ($p < 0.001$); GSH level ($p < 0.05$) along with GSH.Px ($p < 0.01$) and CAT ($p < 0.001$) activities decreased in the DSS-induced colitis group. However, with the administration of tyrosol, MDA and GSH levels along with GSH.Px and CAT activities came to the same levels as the control group. In the colitis group, an increase occurred in IL-6, COX-2, and NF-κB parameters, which created a significant difference compared to the control group ($p < 0.001$). Similarly, TNF-α levels also significantly increased with the administration of DSS ($p < 0.05$) which created a significant difference compared to the control group, while there was no difference among the other groups. As for the Nrf-2 data, it decreased with the administration of DSS which created a significant difference compared to the control group ($p < 0.05$), while there was no difference in other groups. In the colitis-induced group, IL-6, COX-2, and NF-κB gene expression levels also similarly increased but returned to the normal levels with the administration of tyrosol. In the histopathological scoring, the negativity that increased with the administration of DSS returned to the normal levels with the administration of tyrosol+DSS.

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In conclusion, according to the data obtained, tyrosol fixed the destruction picture in the DSS-induced colitis model, giving rise to thought that it has a protective effect.

KEY WORDS: tyrosol; DSS; colitis; inflammation.

BASIC INFORMATION

Inflammatory bowel disease (IBD) essentially involves Crohn's disease (CD) and ulcerative colitis (UC). Although the etiology of these diseases, characterized by chronic or recurrent immune activation and inflammation in the gastrointestinal tract, cannot be fully explained, it is thought that irregular immune responses and genetic and environmental factors play a role in their ethology [1]. In the studies conducted on this subject, it has been reported that reactive oxygen species significantly affect the occurrence of the disease [1–3]. Unlike CD, inflammation seen in UC is limited to the colon and rectum, mainly affecting the mucosal layer of intestinal wall. Numerous factors such as impaired immune response, mucosal barrier dysfunction, impaired microbiological flora, and genetic susceptibility are considered to contribute to the etiology of UC [3]. UC, characterized by severe damage to colonic mucosa and presence of chronic and recurrent inflammation, may cause abdominal cramps, elevated fever, fatigue, diarrhea, pain, vomiting, weight loss, bloody stool, and some other symptoms [4, 5]. Administration of dextran sulfate sodium (DSS) to rodents through drinking water results in colonic inflammation and resembles the clinical and histopathological characteristics of human UC [6]. Although the mechanism causing intestinal inflammation is not exactly known, it is stated that DSS leads to alteration of the intestinal permeability [7]. It has been shown that the destruction of intestinal barrier defense increases the invasion of antigens and continuously stimulates the immune system by the introduction and activation of inflammatory cells, and the regulation of inflammatory mediators leads to increased ROS (reactive oxygen species) and reduced colonic antioxidant defense [6, 8]. Increased production of reactive oxygen and nitrogen species is closely associated with the destructive effects on DNA, proteins, and lipids that contribute to the initiation and progression of intestinal inflammation by the production of cytokines such as tumor necrosis factor (TNF-α) and interleukins (as IL-6 and IL-1β) [1, 9].

The standard treatment methods for UC, including anti-inflammatory and immunosuppressive drugs, mainly aim at downregulation of inflammation as well as suppression of immune response. Unfortunately, these drugs are associated with undesirable side effects and complications. Therefore, new molecules with high efficacy and safety, preferably of natural origin, are needed in the treatment [5]. Recent studies have focused on alternative natural products and nutritional supplements obtained from plants with anti-inflammatory effect, low toxicity, and minimal side effects [10]. Tyrosol (2-(4-hydroxyphenyl)ethanol) is a well-known phenolic compound with antioxidant properties found in wine, olive oil, and other plant-derived products [11, 12]. The most important source of tyrosol, a natural antioxidant, is olive oil [13]. It is stated that in lipopolysaccharide-induced experimental lung tissue injury in mice, tyrosol reduces the increased lung tissue myeloperoxidase (MPO) activity and iNOS, COX-2, TNF-α, IL-1β, and IL-6 levels and increases the reduced SOD activity and shows an anti-inflammatory effect on inflammation caused by lipopolysaccharide [14].

The aim of this study was to investigate the protective effects of tyrosol, an antioxidant agent, on DSS-induced ulcerative colitis model.

MATERIAL AND METHOD

Experimental Protocol

In the study, Wistar albino male rats weighing 180–250 g were used. The experimental protocol was approved by the Mustafa Kemal University Animal Experimentations Local Ethics Committee, permission number of 2017/9-5 (Hatay, Turkey). The experimental practices were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (12 h of light-12 h of dark and at 21 ± 1 °C). Throughout the experimental practices, the rats were provided with standard commercial feed (pellet feed) and tap water *ad libitum*. The study was continued for 21 days, and the rats were divided into 5 groups of 7 animals in each group. The rats in the control group received no treatment. In the second group (colitis), 4% DSS was given to the animals in drinking water for 7 days between the 15th and 21st days of the study. Tyrosol dissolved in 1 ml of physiological saline solution was administered at a dose of 20 mg/kg once a day by gavage route for 21 days to the third (tyrosol) group. In the fourth

group (sulfasalazine), sulfasalazine was administered to the rats at a dose of 100 mg/kg by gavage between the days 0 and 21. Moreover, DSS was added to the drinking water of the rats at a concentration of 4% between day 15 and day 21 to induce ulcerative colitis. In the fifth group (tyrosol+colitis), tyrosol dissolved in 1 ml of physiological saline solution was administered at a dose of 20 mg/kg once a day by gavage route for 21 days. DSS was added to the drinking water of the rats at a concentration of 4% between day 15 and day 21 to induce ulcerative colitis. Twenty-four hours after the last administration, the rats were decapitated under xylazine (10 mg/kg)-ketamine (60 mg/kg) anesthesia, and 10-cm tissue was taken from the distal colon by washing with physiological saline solution. Half of the tissue samples were kept in 10% neutral formalin solution for histopathological analyses, and the other half was stored at − 80 °C for analyses.

Biochemical Analyses

After the tissues taken for lipid peroxidation and antioxidant activity were homogenized, the analyses were carried out using the spectrophotometric method. The level of lipid peroxidation was measured according to the concentration of thiobarbituric acid reactive substances, and the amount of malondialdehyde (MDA) produced was used as an index of lipid peroxidation. The level of MDA at 532 nm was expressed as nmol/g protein [15]. The level of reduced glutathione (GSH) was measured using the method defined by Sedlak and Lindsay [16]. The level of GSH at 412 nm was expressed as nmol/g protein. The glutathione-peroxidase (GSH.Px) activity was determined according to the method defined by Lawrence and Burk [17]. The GSH.Px activity at 340 nm was expressed as international units per gram protein. The catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm and expressed as kg/protein [18]. The parameters of IL-6, TNF-α, COX-2, NF-κB, and Nrf-2 were determined using commercial kits (ELABSCIENCE), and the results were expressed as protein. The Lowry [18] method was used for protein analyses.

Histopathological Analyses

The colon tissues taken after systemic necropsy performed for histopathological analyses were washed with physiological saline solution and examined macroscopically. For Morris et al. [19] classification, the severity of macroscopic colonic injury was determined using a 5-point scale: 0, no damage; 1, localized hyperemia, but no ulcer or erosions; 2, ulcer or erosions with insignificant inflammation; 3, ulcer or erosions with inflammation at one site; 4, two or more major sites of ulceration and/or inflammation; 5, two or more major sites of ulceration and inflammation extending more than 1 cm along the length of the colon.

The portions with the most severe macroscopic injury were determined in the tissue samples which were taken from the colons for histopathological evaluation and fixated in 10% formaldehyde solution for 72 h. Within the scope of routine follow-up, the tissue samples were blocked in paraffin after they were dehydrated by passing through a series of alcohol and became transparent by passing through a series of xylol. After 5-μm-thick serial sections were taken from the paraffin blocks in a microtome (Leica RM 2135) and stained according to the hematoxylin–eosin (HE) staining technique, the sections were examined in a light microscope (Nikon 80i-DS-RI2) and photographed. Using the criteria scores of 1 (0–3) = lose of mucosal architecture, 2 (0–3) = cellular infiltration, 3 (0–1) = crypt abscess formation, and 4 (0–1) = goblet cell depletion determined by Appleyard and Wallace [20], microscopic grading of colonic injury was performed.

RNA Isolation and cDNA Synthesis

RNA isolation was performed according to the TRIzol method. For this purpose, approximately 1 ml of TRIzol was used for 50 mg of tissue (ThermoFisher Scientific, USA, cat no. 15596026). By the kit protocol, the samples that were kept at room temperature for about 10 min after chloroform-isopropyl alcohol-ethyl alcohol stages were diluted with 30–100 μl nuclease-free water according to their pellet sizes. After the purity and concentration values of the total RNAs obtained were checked in a nucleic acid spectrophotometer, the isolation of the samples of inappropriate purity and concentration was repeated. Following the spectrophotometric measurements, the samples were evaluated in 1% agarose gel in terms of the integrity of 28S and 18S bands electrophoresis to check the quality of RNA (100 V and 25 min).

After the isolation of RNA, the samples were treated with DNase I for possible DNA contamination (DNase I, RNase-free, Thermo Scientific, USA, cat no. EN0525). After the DNA digestion phase, 1000 μg of total RNA was used and converted into cDNA according to the High-Capacity cDNA Reverse Transcription kit protocol. In the thermal cycler (Biorad T100, USA), the reaction was carried out for 10 min at 25 °C, 120 min at 37 °C, and 5 min at 85 °C. The cDNAs obtained were completed to 150 μl with nuclease-free water and stored at − 80 °C until gene expression studies were performed.

Quantitative Real-Time PCR Analysis

The amplification of the target genes was performed using 10 μl of each cDNA sample with a kit containing SYBR Green I dye (Power SYBR® Green PCR Master, ThermoFisher Scientific, USA, cat no. 4367659). Each sample was studied in duplicate. The reaction performed in qPCR (Rotor-Gene, Qiagen, USA) was programmed as 10 min at 95 °C, followed by 15 s at 95 °C, 60 s at 60 °C and 40 cycles. While amplifying the TNFα, IL-6, NF-κB, COX-2, and Nrf-2 genes involved in the inflammation pathway as target genes, the GAPDH and RPLP0 reference genes were used as internal control. At the end of the qPCR phase, the region multiplied by primers was checked by melting curve analysis. Sequences of the primers used are shown in Table 1.

The Ct values of the RPLP0 and GAPDH genes used as reference genes were used by calculating their geometric means. In the calculation of Ct values obtained, the $2^{-\Delta\Delta Ct}$ method was used [21]. Fold changes were compared with the control group, a p value of < 0.05 was considered significant.

Statistical Analysis

The data obtained were analyzed using the SPSS 23.0 software; the one-way analysis of variance (ANOVA) was used to compare the means of the groups, and the Tukey test was used to determine the differences between the groups.

Table 1. Forward and Reverse Sequences of Primers Used in This Study

Gene	Forward and reverse sequences	P.L.
COX-2	F: 5′-TGTATGCTACCATCTGGCTTCGG-3′ R: 5′-GTTTGGAACAGTCGCTCGTCATC-3′	94
Nrf-2	F: 5′-TTGTAGATGACCATGAGTCGC-3′ R: 5′-TGTCCTGCTGTATGCTGCTT-3′	141
TNFα	F: 5′-GGCATGGATCTCAAAGACAACC-3′ R: 5′-CAAATCGGCTGACGGTGTG-3′	130
RPLP0	F: 5′-CACTGGCTGAAAAGGTCAAGG-3′ R: 5′-GTGTGAGGGGCTTAGTCGAA-3′	187
IL-6	F: 5′-TGATGGATGCTTCCAAACTG-3′ R: 5′-GAGCATTGGAAGTTGGGGTA-3′	230
NF-κB	F: 5′-CTGCGATACCTTAATGACAGCG-3′ R: 5′-AATTTTGGCTTCCTTTCTTGGCT-3′	195
GAPDH	F: 5′-AGTGCCAGCCTCGTCTCATA-3′ R: 5′-TCCCGTTGATGACCAGCTTC-3′	234

P.L.: product length
***The primers were designed by authors in -PrimerBLAST (NCBI)

RESULTS

Biochemical Parameters

The oxidative stress and antioxidant activity markers obtained at the end of the study are given in Table 2. With the administration of DSS, MDA levels increased significantly compared to the control group ($p < 0.001$). However, increased levels of MDA in the fourth group (sulfasalazine) and fifth group (tyrosol+DSS) returned to the levels of the control group. The level of GSH significantly decreased with the administration of DSS ($p < 0.05$), while it was found to come to the same level with the control group in the fourth and fifth groups. GSH.Px ($p < 0.01$) and CAT ($p < 0.001$) activities reduced significantly in the group given DSS compared to the control group, while they came to the same levels as the control group with the administration of sulfasalazine+DSS in the fourth group and with the administration of tyrosol+DSS in the fifth group.

Gene Expression and Protein Levels

It was determined that the 260:280 ratios of RNAs obtained after total RNA isolation was > 1.7 (1.78 ± 0.02), and the concentration values were sufficient for cDNA conversion (161.40 ± 9.54 ng/μL). The analyses were evaluated according to the control as given in Fig. 1. Accordingly, TNF-α, COX-2, IL-6, and NF-κB expressions increased in the rats given DSS. In the groups given DSS+tyrosol, TNF-α, COX-2, and NF-κB gene expressions were the same as control group.

The protein levels are given in Table 3. With the administration of DSS, an increase occurred in IL-6, COX-2, and NF-κB parameters ($p < 0.001$) and TNF-α levels ($p < 0.05$) which created a significant difference compared to the control group. No difference between the other groups was found. Nrf-2protein levels decreased with the administration of DSS which created a significant difference compared to the control group ($p < 0.05$), while there was no difference in other groups (Table 3).

Macroscopic Findings

The results of the scoring according to the macroscopic classification criteria are given in Fig. 2. In addition, macroscopic appearances before laparotomy, during laparotomy, and after necropsy are given in Fig. 3. While no macroscopic finding was observed in the colons of the control group and tyrosol and sulfasalazine group rats, the macroscopic examination of the colons of all colitis

Table 2. Mean ± SEM Values of Malondialdehyde (MDA), Reduced Glutathione (GSH) Levels and Glutathione Peroxidase (GSH.Px), and Catalase (CAT) Activities in Colon

Group/variables	MDA (nmol/g prot)	GSH (nmol/g prot)	GSH.Px (IU/g prot)	CAT (ku/g prot)
Control	3.262 ± 0.43^{a}	5.027 ± 0.32^{a}	102.356 ± 3.62^{a}	152.240 ± 4.50^{a}
DSS	5.057 ± 0.40^{b}	1.843 ± 0.34^{b}	73.893 ± 3.13^{b}	87.280 ± 1.80^{b}
Tyrosol	2.235 ± 0.45^{a}	4.255 ± 0.46^{a}	95.390 ± 6.92^{a}	151.993 ± 8.64^{a}
DSS+Sulfasalazine	2.180 ± 0.32^{a}	4.606 ± 0.53^{a}	105.921 ± 3.31^{a}	144.698 ± 2.94^{a}
DSS+Tyrosol	2.327 ± 0.32^{a}	3.915 ± 0.34^{a}	97.068 ± 97.06^{a}	123.573 ± 2.360^{c}
Significance	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.05$

Different letters (a,b,c) within the same column show statistically significant differences between the groups

group rats showed hyperemia, inflammation, and/or ulcerative areas varying in size and severity. No ulcerative area was seen in the tyrosol+DSS and sulfasalazine+DSS groups, while there were mild hyperemic and/or inflammation areas.

Microscopic Findings

The results of the scoring according to the microscopic classification criteria are given in Fig. 4, and the microphotographs of the sections are given in Fig. 5. The microscopic examination of the colons of the control and tyrosol group rats showed crypts with a normal histological structure containing intense goblet cells and unilayered prismatic epithelial cells. The crypts in the colons of the colitis group rats showed very severe erosion, mucosal and submucosal intense inflammatory cell infiltration, hemorrhage, vasculitis, and diffuse submucosal edema. There was no significant difference between tyrosol+DSS and sulfasalazine+DSS groups' purpose in terms of histopathological findings. In both of these groups, the crypts preserved their normal histological structure, while there was markedly mild erosion, mild focal mucosal, and submucosal inflammatory cell infiltration, and mild focal submucosal edema in the crypts, which were less severe findings compared to the colitis group.

DISCUSSION AND CONCLUSION

In this study, oxidative stress and antioxidant activity parameters, IL-6, TNF-α, COX-2, NF-κB and Nrf-2 analyses with mRNA and protein levels were analyzed to demonstrate the protective effects of tyrosol in DSS-induced ulcerative colitis model, and the histopathological data were also evaluated. According to the literature review, this is the first study demonstrating the protective effect of tyrosol on ulcerative colitis.

The pathogenesis of UC is composed of various mechanisms, and DSS-induced colitis models have become more preferable for different research areas. Today, DSS is the most commonly used chemical in colitis model studies. DSS can also damage IECs (intestinal epithelial cells) with sulfated polysaccharides, a sort of toxic

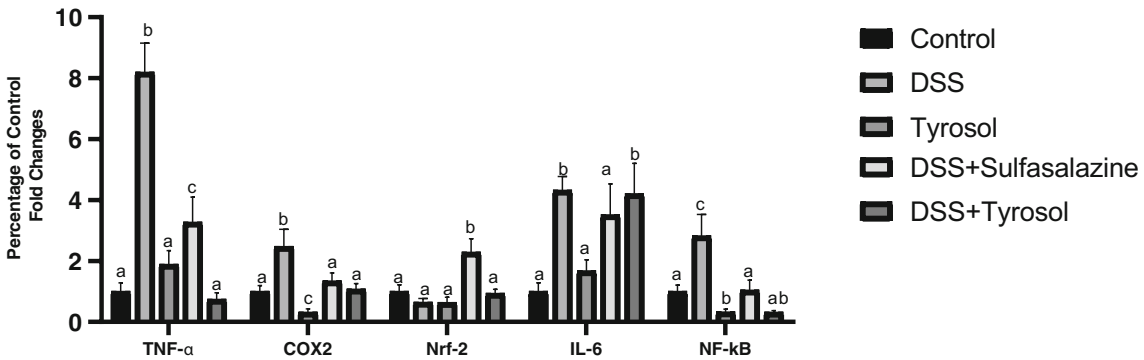

Fig. 1. Fold changes of genes in TNF-α, COX-2, Nrf-2, IL-6, and NF-κB in colon. Different superscript letters (a,b,c) within the same column show statistically significant differences between the groups: $p < 0.05$ (groups compared to the control group).

Table 3. Mean ± SEM Values IL-6, COX-2, TNF-α, NF-κB, and Nrf-2 Levels in Colon

Group/variables	IL-6 (pg/ml)	COX-2 (pg/ml)	TNF-α (pg/ml)	NF-κB (pg/ml)	Nrf-2 (pg/ml)
Control	1.262 ± 0.02^{a}	2.035 ± 0.05^{a}	1.472 ± 0.04^{a}	24.252 ± 1.55^{a}	3.756 ± 0.34^{a}
DSS	1.841 ± 0.12^{b}	2.557 ± 0.04^{b}	2.768 ± 0.36^{b}	65.781 ± 6.22^{b}	2.192 ± 0.15^{b}
Tyrosol	1.341 ± 0.05^{a}	1.964 ± 0.04^{a}	1.461 ± 0.03^{a}	30.594 ± 4.50^{a}	4.515 ± 0.40^{a}
DSS+Sulfasalazine	1.302 ± 0.07^{a}	2.148 ± 0.08^{a}	2.435 ± 0.51^{a}	34.028 ± 2.73^{a}	3.698 ± 0.20^{a}
DSS+Tyrosol	1.142 ± 0.03^{a}	2.012 ± 0.035^{a}	1.322 ± 0.02^{a}	28.575 ± 1.34^{a}	3.608 ± 0.18^{a}
Significance	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.001$	$p < 0.05$

Different letters (a,b) within the same column show statistically significant differences between the groups

substance with anticoagulant properties. The consequences of the resulting damage are similar to UC in humans in terms of symptoms and morphology [22].

In this study, it was found that the expression of inflammatory cytokine increased and a strong inflammatory response occurred in the rats with DSS-induced colitis. The increased oxidative damage in the intestinal mucosa may form an interactive network with IEC, and overactivated interactions may cause the physical barrier to be destroyed or lesions to further worsen. The intestinal barrier is a complex and significant defense system consisting of physical, chemical, biological, and immunological barriers. By this means, it can prevent bacteria or toxins from passing through the intestines to tissues and organs and invading them [23].

Although the underlying pathogenesis of UC is still uncertain, the role of oxidative stress and immunological dysfunction has been proven to be very important in its development.

The deterioration of the antioxidant system of colonic mucosa is associated with oxidative damage to mucosal lipids and proteins and causes mucosal ulceration.

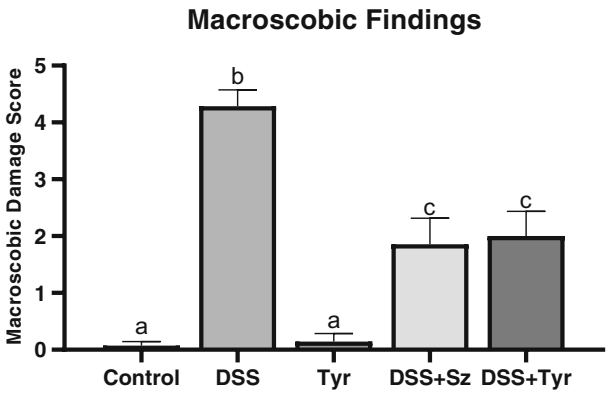

Fig. 2. Score graph according to macroscopic classification criteria of colon tissue according to groups. Different superscript letters (a,b,c) within the same column show statistically significant differences between the groups: $p < 0.001$.

Meanwhile, the damaged mucosal barrier causes the mucosal immune system to be exposed to lumen antigens, then the change and regulation of immune response, and the progression of the disease [24]. With the increase in free radicals, the integrity of the intestinal mucosal barrier is impaired, and inflammatory mediators are stimulated due to lipid peroxidation and oxidative stress [25–27]. In the experimental UC model studies, it was found that GSH [27–29] levels and GSH.Px [29], SOD [30], and CAT [31] activities, which are antioxidant activity parameters, decreased. Similarly, in our study, it was found that MDA levels increased and GSH level and GSH.Px and CAT activities decreased in the group given DSS (colitis group).

Since oxidative stress and inflammatory responses play an important role in the occurrence of UC [32], natural products with antioxidant effect have drawn more interest in recent years. Antioxidant effects of tyrosol have been shown in various previous studies [33–35]. In this study, MDA, an oxidative stress marker, decreased in the tyrosol+DSS and sulfasalazine+DSS groups, while GSH levels and GSH.Px and CAT activities increased and have the same levels as the control group. This indicates that the active ingredient of tyrosol has a protective effect close similar to sulfasalazine in preventing oxidative damage.

Proinflammatory cytokines such as IL-1β, IL-6, and TNF-α are the most important inflammatory mediators [36] that regulate acute phase protein synthesis in inflammation. In addition, COX-2 is also known to be one of the inflammatory proteins expressed in high amounts in inflammation areas. Activation of these produces an inflammatory response that may affect the integrity of colon mucosa and contributes to the progression of intestinal damage, and their expression increases in IBD and colitis. Nuclear translocation of NF-κB is associated with the transactivation of target genes related to inflammatory cytokines and promotes the inflammatory process in UC patients [37]. In IBD, it increases the expression of many pro-inflammatory cytokines including NF-κB, IL-1β, IL-6, and TNF-α as well as the expression of proinflammatory

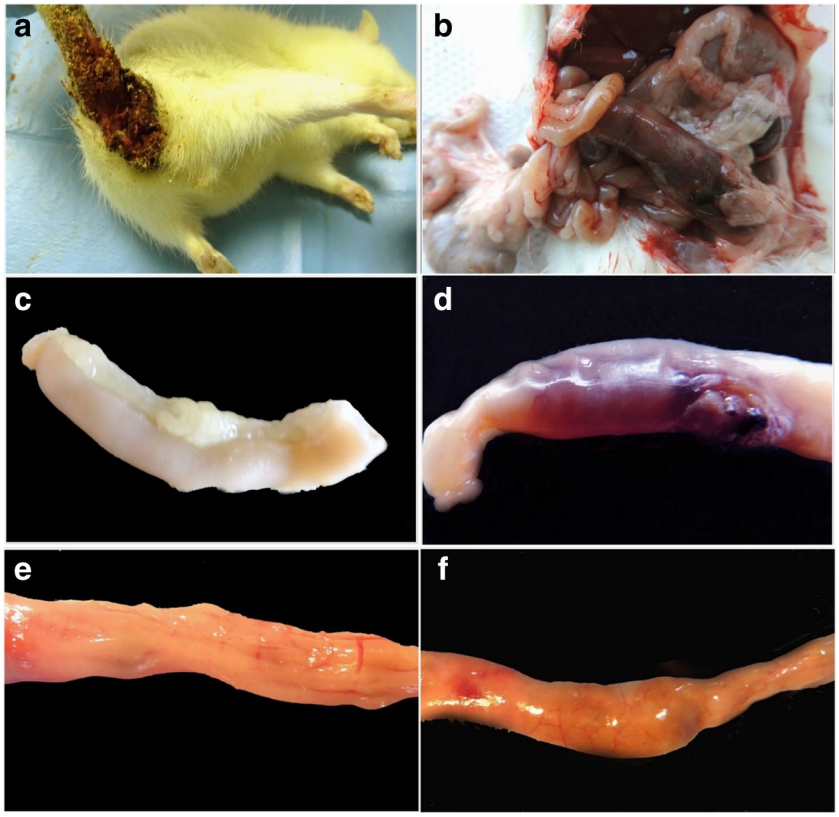

Fig. 3. Colitis group rat: **a** hemorrhagic appearance of colon before laparotomy; **b** appearance of colon during laparotomy. After necropsy macroscopic appearance of the colon: **c** control group; **d** colitis group; **e** colitis+tyrosol group; **f** colitis+sulfasalazine group.

mediators such as COX-2 and nitric oxide synthase (iNOS) [38]. In various studies, it has been reported that the administration of DSS in mice and rats causes an increase in the TNF-α [39], IL-6 [40, 41], IL-1β [40, 42], NF-κB [43, 44], and COX-2 levels of the colonic tissue. In our study, TNF-α and IL-6 levels were found to be increased in the DSS-induced colitis group (Table 3). Similar increases also occurred in TNF-α mRNA expressions. The increase in

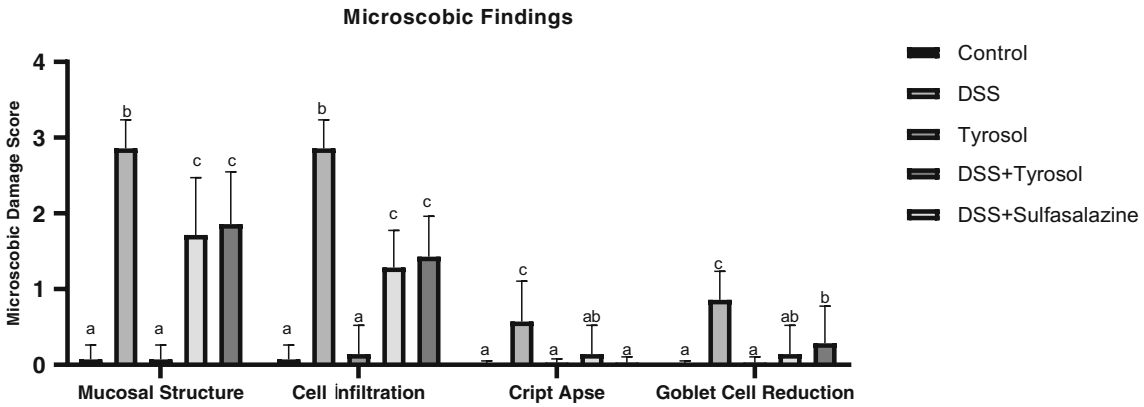

Fig. 4. Score graphs according to microscopic classification criteria of colon tissue according to groups. Different superscript letters (a,b,c) within the same column show statistically significant differences between the groups: $p < 0.001$.

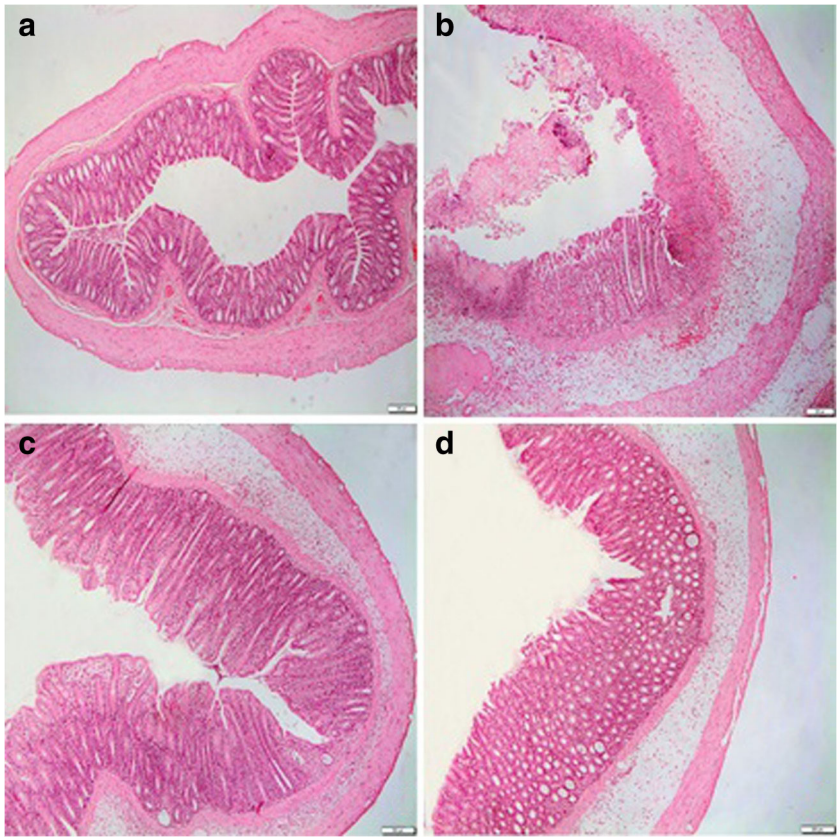

Fig. 5. **a** Normal microscopic view of the control group colon. Crypts with normal histological structure including intensive goblet cells and single-layer prismatic epithelial cells. H&E × 100. **b** Colitis group microscopic appearance of the damage in the colon. Very violent erosion in the colon crypts, desquame epithelial cells in the lumen, mucosal and submucosal intense inflammatory cell infiltration, and diffuse hemorrhage, submucosal edema, and vasculitis. H&E × 100. **c** Microscopic appearance of colitis+tyrosol group colon damage. Erosion, mild mucosal, and submucosal inflammatory cell infiltration, focal submucosal edema in the colon crypts. H&E × 100. **d** Microscopic appearance of colitis+sulfasalazine group colon damage. Erosion, mildly mucosal and submucosal inflammatory cell infiltration, and focal submucosal edema. H&E × 100.

TNF-α may be caused by loss of mucin-producing goblet cells, reduction of colonic mucus layer thickness, exposure of colonic mucosa to lumen antigens, and colonic mucosal inflammation [45]. The increased monocyte/macrophage infiltration into the colonic mucosa in DSS-induced colitis rats may cause enhanced IL-6 [46]. However, the levels of IL-6 gene expression increased about 4 times in the DSS-induced colitis group, whereas no decrease was found in the tyrosol-treated group, and it was found that the protein levels were approximately the same as in the DSS group ($p > 0.05$). It is known that the protein levels are brought under control by some posttranslational mechanisms. In the study by Wei et al. [47], it was reported that the levels of IL-6 gene expression were brought under control by some miRNAs in the let-7 (Lethal 7) family under various physiological conditions. Accordingly, it was thought that the protein levels of this interleukin with increased activity at mRNA level might have been brought under control by some posttranslational mechanisms.

NF-κB, which is a transcription factor and is mostly found in passive form in the cytoplasm, is involved in the initiation of inflammatory and immune response by transferring to the nucleus as a result of inflammation. NF-κB plays an important role in maintaining the integrity of homeostasis and mucosal barrier functions in the intestine [48]. Increased NF-κB in the intestinal mucosal cells in relation to this condition is closely associated with IBD [49]. In our study, it was found that the levels of NF-κB protein similarly increased in the DSS-induced colitis group. With the induction of colitis, NF-κB is activated and increases the expression of proinflammatory gene that is effective on the mucosal inflammation process [50]. Studies have shown that agents

targeting NF-κB reduce the severity of intestinal inflammation [51, 52]. Similarly, there was no increase in the tyrosol+DSS group and similar results were obtained with the control group. When the activity of NF-κB at mRNA level was analyzed, it was found that there was a significant decrease in the tyrosol+DSS group compared to the control group (0.31 ± 0.06-fold; $p < 0.05$), and that the control and tyrosol+DSS groups were similar in terms of protein amounts. The synthesis of mRNA can be inhibited by various molecular mechanisms. However, depending on the half-life of existing mRNAs in the cell environment, the process of protein synthesis continues in varying periods [53]. While the levels of mRNA were low in the groups treated with tyrosol+DSS and tyrosol alone, it was thought that the reason for the high protein level might have been caused by this.

Several studies have shown that Nrf-2 activators have a protective effect against oxidative stress and inflammatory responses by suppressing NF-κB-mediated signaling activation [54–56]. Increased Nrf-2 levels are known to induce a strong anti-inflammatory response, as well as induction of antioxidant enzymes. Nrf-2 is a significant transcription factor involved in the expression of enzymes, including GPx, GSH, and HO-1, so these enzymes reduce inflammatory pathway activation and oxidative stress [57]. This indicates that Nrf-2 plays an important role in the prevention and treatment of IBD, including UC. Similarly, it was found in our study that Nrf-2 levels decreased in the DSS-induced colitis group. However, this was found to be not similar in mRNA expression level. It was determined that Nrf-2 gene expression levels were expressed more than 2 times (2.28 ± 0.45) in the positive control group treated with sulfozalasine alone ($p < 0.05$). As expected, there was a decrease in the mRNA levels of the colitis group, while this decrease was not statistically significant, and Nrf-2 gene expression levels were similar to the control group. It was found that Nrf-2 mRNA activity increased only in the sulfosalazine group. When the gene expression levels in the group 5 were analyzed, it was seen that the expression levels in the tyrosol+DSS group were the same as the control group. This gave rise to the thought that the suppression of Nrf-2 induced by inflammation could be limited by the use of tyrosol.

It has been reported that the colonic tissue of the DSS-administered mice showed a protective effect against DSS-induced ulcerative colitis by means of extra-virgin olive oil inhibiting the PPARγ (peroxisome proliferator-activated receptor gamma) and NF-κB pathways which was added to the diet where there were significant increases in TNF-α, COX-2, and iNOS protein levels [58]. In various studies, it has been reported that the administration of tyrosol, one of the important antioxidant components of olive oil, has anti-inflammatory effects [34, 35, 59]. In our study, the increased IL-6, NF-κB, TNF-α, and Cox-2 and decreased Nrf-2 protein levels with the administration of DSS similarly returned to normal levels with the administration of tyrosol.

DSS-induced colitis studies have reported that the use of various antioxidant agents have shown a decrease in histological findings, decreased inflammation in the mucosa and regeneration of the epithelium [29, 60]. Superoxide dismutase, catalase [61], vitamin E, and selenium [62, 63] have been shown to be useful in reducing the occurrence of colonic inflammation in studies. In this study, DSS-induced colitis rats showed very severe erosion, mucosal and submucosal inflammatory cell infiltration, severe widespread hemorrhage, goblet cell damage, widespread submucosal edema, and vasculitis. On the other hand, colon tissues of the tyrosol-treated group with DSS were found to have a normal histological structure, crypts with mild signs of mucosal and submucosal inflammatory cell infiltration and focal submucosal edema were observed in crypts that were less severe than the colitis group. These histopathological results showed that the antioxidant agents used in the prevention of colitis were compatible with the results of the abovementioned studies. Both macroscopic and microscopic findings in the histopathological evaluations confirm the occurrence of damage in the DSS-induced colitis model but show that the administration of tyrosol reverses this destruction picture.

In conclusion, it was seen that oxidative damage increased, histopathological disorders increased, and inflammation parameters increased in DSS-induced colitis model, and these findings were also similar in gene expression. However, the fact that the treatment with tyrosol exhibited therapeutic effects in the specified parameters gives rise to the belief that this antioxidant agent is effective in the treatment of colitis. However, the use of tyrosol active ingredient in different studies and at different doses and times will provide a better understanding of the effects of this substance.

REFERENCES

1. Moura, Fabiana Andréa, Kívia Queiroz de Andrade, Juliana Célia Farias dos Santos, Orlando Roberto Pimentel Araújo, and Marília Oliveira Fonseca Goulart. 2015. Antioxidant therapy for treatment of inflammatory bowel disease: Does it work? *Redox Biology* 6: 617–639.
2. Ahmet, U.Y.A.R., Hüseyin EMLİK, and Y.A.M.A.N. Turan. Deneysel Ülseratif Kolit Üzerine Yüksek Karbonhidratlı, Yüksek

Yağlı ve Aralıklı Beslemenin Etkisi. *Harran Üniversitesi Veteriner Fakültesi Dergisi* 7 (1): 7–14.
3. Yadav, Vipul, Felipe Varum, Roberto Bravo, Esther Furrer, Daniela Bojic, and Abdul W. Basit. 2016. Inflammatory bowel disease: Exploring gut pathophysiology for novel therapeutic targets. *Translational Research* 176: 38–68.
4. Nowarski, Roni, Ruaidhrí Jackson, Nicola Gagliani, Marcel R. de Zoete, Noah W. Palm, Will Bailis, Jun Siong Low, Christian C.D. Harman, Morven Graham, and Eran Elinav. 2015. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell* 163 (6): 1444–1456.
5. Ko, K. 2014. Joshua, and Kathy K Auyeung. *Inflammatory bowel disease: etiology, pathogenesis and current therapy. Current pharmaceutical design* 20 (7): 1082–1096.
6. Vicario, María, Concepció Amat, Montserrat Rivero, Miquel Moretó, and Carme Pelegrí. 2007. Dietary glutamine affects mucosal functions in rats with mild DSS-induced colitis. *The Journal of Nutrition* 137 (8): 1931–1937.
7. Solomon, Livingstone, Sorsiah Mansor, Peter Mallon, Eilish Donnelly, Margaret Hoper, Morris Loughrey, Stephen Kirk, and Keith Gardiner. 2010. The dextran sulphate sodium (DSS) model of colitis: An overview. *Comparative Clinical Pathology* 19 (3): 235–239.
8. Damiani, Carlos R., César A.F. Benetton, Cristhopher Stoffel, Katrine C. Bardini, Vilson H. Cardoso, Gabriela Di Giunta, Ricardo A. Pinho, Felipe Dal-Pizzol, and Emilio L. Streck. 2007. Oxidative stress and metabolism in animal model of colitis induced by dextran sulfate sodium. *Journal of Gastroenterology and Hepatology* 22 (11): 1846–1851.
9. Geronikaki, Athina A., and Antonios M. Gavalas. 2006. Antioxidants and inflammatory disease: Synthetic and natural antioxidants with anti-inflammatory activity. *Combinatorial Chemistry & High Throughput Screening* 9 (6): 425–442.
10. Sakthivel, K.M., and C. Guruvayoorappan. 2013. Amentoflavone inhibits iNOS, COX-2 expression and modulates cytokine profile, NF-κB signal transduction pathways in rats with ulcerative colitis. *International Immunopharmacology* 17 (3): 907–916.
11. Bu, Youngmin, Seongjoo Rho, Jinmo Kim, Mi Yeon Kim, Dae Hee Lee, Sun Yeou Kim, Hoyoung Choi, and Hocheol Kim. 2007. Neuroprotective effect of tyrosol on transient focal cerebral ischemia in rats. *Neuroscience Letters* 414 (3): 218–221.
12. St-Laurent-Thibault, C., M. Arseneault, F. Longpre, and Charles Ramassamy. 2011. Tyrosol and hydroxytyrosol two main components of olive oil, protect N2a cells against amyloid-β-induced toxicity. Involvement of the NF-κB signaling. *Current Alzheimer Research* 8 (5): 543–551.
13. Chandramohan, Ramasamy, Settu Saravanan, and Leelavinothan Pari. 2017. Beneficial effects of tyrosol on altered glycoprotein components in streptozotocin-induced diabetic rats. *Pharmaceutical Biology* 55 (1): 1631–1637.
14. Kim, Yeon-Yong, Soyoung Lee, Min-Jong Kim, Byeong-Cheol Kang, Hima Dhakal, Young-Ae Choi, Pil-Hoon Park, Hyukjae Choi, Tae-Yong Shin, and Hyun Gyu Choi. 2017. Tyrosol attenuates lipopolysaccharide-induced acute lung injury by inhibiting the inflammatory response and maintaining the alveolar capillary barrier. *Food and Chemical Toxicology* 109: 526–533.
15. Placer, Zdenek A., Linda L. Cushman, and B. Connor Johnson. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Analytical Biochemistry* 16 (2): 359–364.
16. Sedlak, Jozef, and Raymond H. Lindsay. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* 25: 192–205.
17. Lawrence, Richard A., and Raymond F. Burk. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and Biophysical Research Communications* 71 (4): 952–958.
18. Lowry, Oliver H., Nira J. Rosebrough, A. Lewis Farr, and Rose J. Randall. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193: 265–275.
19. Morris, Gerald P., Paul L. Beck, Margaret S. Herridge, William T. Depew, Myron R. Szewczuk, and John L. Wallace. 1989. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 96 (2): 795–803.
20. Appleyard, Caroline B., and John L. Wallace. 1995. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 269 (1): G119–G125.
21. Livak, Kenneth J., and Thomas D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *methods* 25 (4): 402–408.
22. Gaudio, Eugenio, Gennaro Taddei, Antonella Vetuschi, Roberta Sferra, Giuseppe Frieri, Giuseppe Ricciardi, and Renzo Caprilli. 1999. Dextran sulfate sodium (DSS) colitis in rats (clinical, structural, and ultrastructural aspects). *Digestive Diseases and Sciences* 44 (7): 1458–1475.
23. Balfe, Aine, Grainne Lennon, Aonghus Lavelle, Neil G. Docherty, J. Calvin Coffey, Kieran Sheahan, Desmond C. Winter, and P. Ronan O'Connell. 2018. Isolation and gene expression profiling of intestinal epithelial cells: Crypt isolation by calcium chelation from in vivo samples. *Clinical and Experimental Gastroenterology* 11: 29–37.
24. Piechota-Polanczyk, Aleksandra, and Jakub Fichna. 2014. The role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. *Naunyn-Schmiedeberg's Archives of Pharmacology* 387 (7): 605–620.
25. Vargas Robles, Hilda, Alí Francisco Citalán Madrid, Alexander García Ponce, Angelica Silva Olivares, Mineko Shibayama, Abigail Betanzos, Leonardo Del Valle Mondragón, Porfirio Nava, and Michael Schnoor. 2016. Experimental colitis is attenuated by cardioprotective diet supplementation that reduces oxidative stress, inflammation, and mucosal damage. *Oxidative Medicine and Cellular Longevity* 2016: 1–9.
26. Fu, Yan, Huan-qing Wu, Huai-liang Cui, Yue-yun Li, and Chang-zheng Li. 2018. Gastroprotective and anti-ulcer effects of oxymatrine against several gastric ulcer models in rats: Possible roles of antioxidant, antiinflammatory, and prosurvival mechanisms. *Phytotherapy Research* 32 (10): 2047–2058.
27. Arafa, Hossam M.M., Ramadan A. Hemeida, Ali I.M. El-Bahrawy, and Farid M.A. Hamada. 2009. Prophylactic role of curcumin in dextran sulfate sodium (DSS)-induced ulcerative colitis murine model. *Food and Chemical Toxicology* 47 (6): 1311–1317.
28. Kabeya, Luciana M., Carolina N. Fuzissaki, Micássio F. Andrade, Ana Elisa C.S. Azzolini, Silvia H. Taleb-Contini, Roberta B. Vermelho, Joao Luis C. Lopes, and Yara Maria Lucisano-Valim. 2013. 4-methylcoumarin derivatives inhibit human neutrophil oxidative metabolism and elastase activity. *Journal of Medicinal Food* 16 (8): 692–700.
29. Rajendiran, Vijayabharathi, Vidhya Natarajan, and Sivasithamparam Niranjali Devaraj. 2018. Anti-inflammatory activity of Alpinia officinarum hance on rat colon inflammation and tissue damage in DSS induced acute and chronic colitis models. *Food Science and Human Wellness* 7 (4): 273–281.
30. Samman, Fatma S., Samah M. Elaidy, Soha S. Essawy, and Mohammad S. Hassan. 2018. New insights on the modulatory roles of metformin or alpha-lipoic acid versus their combination in dextran sulfate sodium-induced chronic colitis in rats. *Pharmacological Reports* 70 (3): 488–496.

31. Petronilho, Fabricia, Monique Michels, Lucinéia G. Danielski, Mariana Pereira Goldim, Drielly Florentino, Andriele Vieira, Mariana G. Mendonça, Moema Tournier, Bárbara Piacentini, and Amanda Della Giustina. 2016. Diphenyl diselenide attenuates oxidative stress and inflammatory parameters in ulcerative colitis: A comparison with ebselen. *Pathology-Research and Practice* 212 (9): 755–760.
32. Almeer, Rafa S., Sahar M. Mahmoud, Hatem K. Amin, and Ahmed E. Abdel Moneim. 2018. Ziziphus spina-christi fruit extract suppresses oxidative stress and p38 MAPK expression in ulcerative colitis in rats via induction of Nrf2 and HO-1 expression. *Food and Chemical Toxicology* 115: 49–62.
33. Loru, Debora, Alessandra Incani, Monica Deiana, G. Corona, Angela Atzeri, M.P. Melis, Antonella Rosa, and M.A. Dessì. 2009. Protective effect of hydroxytyrosol and tyrosol against oxidative stress in kidney cells. *Toxicology and Industrial Health* 25 (4–5): 301–310.
34. Je, In-Gyu, Duk-Sil Kim, Sung-Wan Kim, Soyoung Lee, Hyun-Shik Lee, Eui Kyun Park, Dongwoo Khang, and Sang-Hyun Kim. 2015. Tyrosol suppresses allergic inflammation by inhibiting the activation of phosphoinositide 3-kinase in mast cells. *PLoS One* 10 (6): e0129829.
35. Cicerale, S., L.J. Lucas, and R.S.J. Keast. 2012. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. *Current Opinion in Biotechnology* 23 (2): 129–135.
36. Pan, Jinmei, Qian Xiang, and Simon Ball. 2000. Use of a novel real-time quantitative reverse transcription-polymerase chain reaction method to study the effects of cytokines on cytochrome P450 mRNA expression in mouse liver. *Drug Metabolism and Disposition* 28 (6): 709–713.
37. Bonizzi, Giuseppina, and Michael Karin. 2004. The two NF-κB activation pathways and their role in innate and adaptive immunity. *Trends in Immunology* 25 (6): 280–288.
38. Tak, Paul P., and Gary S. Firestein. 2001. NF-κB: A key role in inflammatory diseases. *The Journal of Clinical Investigation* 107 (1): 7–11.
39. Witaicenis, Aline, Ellen Cristina Souza de Oliveira, Alexandre Tanimoto, Sofia Fernanda Gonçalves Zorzella-Pezavento, Silvio Luis de Oliveira, Alexandrina Sartori, and Luiz Claudio Di Stasi. 2018. 4-methylesculetin, a coumarin derivative, ameliorates dextran sulfate sodium-induced intestinal inflammation. *Chemico-Biological Interactions* 280: 59–63.
40. Jang, Sun-Hee, Jisang Park, Sae-Hae Kim, Kyung-Min Choi, Eun-Sil Ko, Jeong-Dan Cha, Young-Ran Lee, Hyonseok Jang, and Yong-Suk Jang. 2017. Oral administration of red ginseng powder fermented with probiotic alleviates the severity of dextran-sulfate sodium-induced colitis in a mouse model. *Chinese Journal of Natural Medicines* 15 (3): 192–201.
41. Andrade, Maria Emília Rabelo, Patrícia Aparecida Vieira de Barros, Penélope Lacrísio dos Reis Menta, Grazielle Mara Ferreira Costa, Sued Eustáquio Mendes Miranda, Paola Caroline Lacerda Leocádio, Camila Megale de Almeida-Leite, Simone de Vasconcelos Generoso, Jacqueline Isaura Alvarez Leite, and Valbert Nascimento Cardoso. 2019. Arginine supplementation reduces colonic injury, inflammation and oxidative stress of DSS-induced colitis in mice. *Journal of Functional Foods* 52: 360–369.
42. Mai, Chu-Tian, Mei-Mei Wu, Chun-Li Wang, Zi-Ren Su, Yuan-Yuan Cheng, and Xiao-Jun Zhang. 2019. Palmatine attenuated dextran sulfate sodium (DSS)-induced colitis via promoting mitophagy-mediated NLRP3 inflammasome inactivation. *Molecular Immunology* 105: 76–85.
43. Tsang, Siu Wai, Siu Po Ip, Justin Che-Yuen Wu, Siew-Chien Ng, Ken Kin-Lam Yung, and Zhao-Xiang Bian. 2015. A Chinese medicinal formulation ameliorates dextran sulfate sodium-induced experimental colitis by suppressing the activity of nuclear factor-kappaB signaling. *Journal of Ethnopharmacology* 162: 20–30.
44. Lee, Hyung-Jun, Hee Geum Lee, Ki-Seok Choi, Young-Joon Surh, and Hye-Kyung Na. 2013. Diallyl trisulfide suppresses dextran sodium sulfate-induced mouse colitis: NF-κB and STAT3 as potential targets. *Biochemical and Biophysical Research Communications* 437 (2): 267–273.
45. Sands, Bruce E., and Gilaad G. Kaplan. 2007. The role of TNFα in ulcerative colitis. *The Journal of Clinical Pharmacology* 47 (8): 930–941.
46. Tokuyama, Hirotake, Satoshi Ueha, Makoto Kurachi, Kouji Matsushima, Fuminori Moriyasu, Richard S. Blumberg, and Kazuhiro Kakimi. 2005. The simultaneous blockade of chemokine receptors CCR2, CCR5 and CXCR3 by a non-peptide chemokine receptor antagonist protects mice from dextran sodium sulfate-mediated colitis. *International Immunology* 17 (8): 1023–1034.
47. Wei, Y.B., J.J. Liu, J.C. Villaescusa, E. Åberg, S. Brené, G. Wegener, A.A. Mathé, and C. Lavebratt. 2016. Elevation of Il6 is associated with disturbed let-7 biogenesis in a genetic model of depression. *Translational Psychiatry* 6 (8): e869.
48. Jobin, Christian, and R. Balfour Sartor. 2000. The IκB/NF-κB system: A key determinant of mucosal inflammation and protection. *American Journal of Physiology-Cell Physiology* 278 (3): C451–C462.
49. Schreiber, S., S. Nikolaus, and J. Hampe. 1998. Activation of nuclear factor κB in inflammatory bowel disease. *Gut* 42 (4): 477–484.
50. Atreya, I., R. Atreya, and M.F. Neurath. 2008. NF-κB in inflammatory bowel disease. *Journal of Internal Medicine* 263 (6): 591–596.
51. Du, Xiaowan, Wei Chen, Yufeng Wang, Chen Chen, Lei Guo, Rui Ju, Juan Li, Dechang Zhang, Lei Zhu, and Caiying Ye. 2017. Therapeutic efficacy of carboxyamidotriazole on 2, 4, 6-trinitrobenzene sulfonic acid-induced colitis model is associated with the inhibition of NLRP3 inflammasome and NF-κB activation. *International Immunopharmacology* 45: 16–25.
52. Fu, Yong, Junting Ma, Xiafei Shi, Xiang-Yun Song, Yaping Yang, Shuke Xiao, Jiahuang Li, Gu Wei-Jin, Zhen Huang, and Junfeng Zhang. 2017. A novel pyrazole-containing indolizine derivative suppresses NF-κB activation and protects against TNBS-induced colitis via a PPAR-γ-dependent pathway. *Biochemical Pharmacology* 135: 126–138.
53. Alberts, Bruce, Dennis Bray, Karen Hopkin, Alexander D. Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 2013. Essential Cell Biology. New York: *Garland Science*.
54. Han, Chang Woo, Min Jung Kwun, Kyun Ha Kim, Jun-Yong Choi, Sei-Ryang Oh, Kyung-Seop Ahn, Jang Hoon Lee, and Myungsoo Joo. 2013. Ethanol extract of Alismatis Rhizoma reduces acute lung inflammation by suppressing NF-κB and activating Nrf2. *Journal of Ethnopharmacology* 146 (1): 402–410.
55. Wang, Wen-chen, Yan-min Xia, Bo Yang, Xiang-ni Su, Jia-kuan Chen, Wei Li, and Tao Jiang. 2017. Protective effects of tyrosol against LPS-induced acute lung injury via inhibiting NF-κB and AP-1 activation and activating the HO-1/Nrf2 pathways. *Biological and Pharmaceutical Bulletin*: b16–b00756.
56. Tekeli, İbrahim Ozan, Ahmet Ateşşahin, Fatih Sakin, Abdullah Aslan, Songül Çeribaşı, and Mustafa Yipel. 2019. Protective effects of conventional and colon-targeted lycopene and linalool on ulcerative colitis induced by acetic acid in rats. *Inflammopharmacol* 27 (2): 313-322.
57. Fan, Rong, Tong Pan, An-Li Zhu, and Mei-Hong Zhang. 2017. Anti-inflammatory and anti-arthritic properties of naringenin via attenuation of NF-κB and activation of the heme oxygenase HO)-

1/related factor 2 pathway. *Pharmacological Reports* 69 (5): 1021–1029.
58. Sánchez-Fidalgo, Susana, Ana Cárdeno, Marina Sánchez-Hidalgo, Marina Aparicio-Soto, and Catalina Alarcón de la Lastra. 2013. Dietary extra virgin olive oil polyphenols supplementation modulates DSS-induced chronic colitis in mice. *The Journal of Nutritional Biochemistry* 24 (7): 1401–1413.
59. Luo, Gang, Yinuo Huang, Dapeng Mo, Ning Ma, Feng Gao, Ligang Song, Xuan Sun, Xiaotong Xu, Lian Liu, and Xiaochuan Huo. 2018. Tyrosol attenuates pro-inflammatory cytokines from cultured astrocytes and NF-κB activation in in vitro oxygen glucose deprivation. *Neurochemistry International* 121: 140–145.
60. Huang, Tzou-Chi, Shinn-Shyong Tsai, Li-Fang Liu, Yu Lin Liu, Hung-Jen Liu, and Kuo Pin Chuang. 2010. Effect of Arctium lappa L. in the dextran sulfate sodium colitis mouse model. *World journal of gastroenterology: WJG* 16 (33): 4193–4199.
61. Yavuz, Yunus, Meral Yüksel, Berrak Ç. Yeĕn, and İnci Alican. 1999. The effect of antioxidant therapy on colonic inflammation in the rat. *Research in Experimental Medicine* 199 (2): 101–110.
62. Ademoglu, E., Y. Erbil, B. Tam, U. Barbaros, E. Ilhan, V. Olgac, and U. Mutlu-Turkoglu. 2004. Do vitamin E and selenium have beneficial effects on trinitrobenzenesulfonic acid-induced experimental colitis. *Digestive Diseases and Sciences* 49 (1): 102–108.
63. Isozaki, Yutaka, Norimasa Yoshida, Masaaki Kuroda, Tomohisa Takagi, Osamu Handa, Satoshi Kokura, Hiroshi Ichikawa, Yuji Naito, Takeshi Okanoue, and Toshikazu Yoshikawa. 2006. Effect of a novel water-soluble vitamin E derivative as a cure for TNBS-induced colitis in rats. *International Journal of Molecular Medicine* 17 (3): 497–502.