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Quercetin Attenuates Endoplasmic Reticulum Stress and Apoptosis in TNBS-Induced Colitis by Inhibiting the Glucose Regulatory Protein 78 Activation

Yeter Topçu-Tarladaçalışır[1], Melike Sapmaz-Metin[1], Zeynep Mercan[2], Deniz Erçetin[1]

[1]Department of Histology and Embryology Trakya University Faculty of Medicine, Edirne, Türkiye
[2]Department of Histology and Embryology Gaziantep University Faculty of Medicine, Gaziantep, Türkiye

Background: The inflammatory bowel diseases (IBD) are significantly influenced by apoptosis and endoplasmic reticulum (ER) stress.

Aims: To investigate the effects of quercetin on ER stress-mediated apoptosis in a trinitrobenzene sulfonic acid (TNBS) induced experimental IBD model.

Study Design: In vivo animal experimental study.

Methods: To demonstrate the effect of quercetin in an experimental colitis model, Control, TNBS, and TNBS+quercetin groups were created with 24 Wistar Albino rats. Colitis was induced by intrarectal administration of 25 mg TNBS. In the TNBS+quercetin group, intragastrically 100 mg/kg quercetin was given for 7 days, immediately after colitis induction. In the TNBS-induced experimental IBD model, we evaluated the effects of quercetin on colonic epithelial cell apoptosis, oxidative stress, ER stress, the mitogen-activated protein kinase c-Jun N-terminal kinase, and the nuclear factor kappa B immunoreactivities, the levels of myeloperoxidase and tumor necrosis factor-α, the disease activity index with colonic histopathologic changes.

Results: TNBS administration induced an elevated level of disease activity and oxidative stress indices, inflammation markers, and an increase in the immunoreactivities of nuclear factor kappa B and the mitogen-activated protein kinase c-Jun N-terminal kinase in the colon of the colitis group. Glucose regulatory protein 78, caspase-12 immunoreactivities, and epithelial cell apoptosis also were shown in the colon. However, quercetin improved TNBS-induced histopathological alterations, apoptosis, inflammation, oxidative stress, and ER stress.

Conclusion: This study suggests that quercetin has a regulatory effect on ER stress-mediated apoptosis, and thus may be beneficial in treating IBD.

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic, multifactorial inflammatory diseases of the gastrointestinal system with unknown etiology.[1] Oxidative stress disrupts the mucosal barrier by damaging the digestive tract mucosa, and consequently, facilitates the diffusion of bacterial toxins to the lamina propria. It triggers the disease by stimulating the immune response, leading to the infiltration of immune cells.[2] The increased production of reactive oxygen and nitrogen species by stimulated and infiltrated neutrophils contributes to the progression of the disease.[3] Activation of the nuclear factor kappa B (NF-kB) modulates the production of certain proinflammatory cytokines, thereby contributing to the inflammatory response in the pathogenesis of IBD.[4,5] NF-kB inhibitors reduce the effects of IBD by suppressing proinflammatory cytokine production from intestinal cells and macrophages in experimental models.[6,7] Cell death genes, such as caspases, are involved in apoptosis caused by oxidative stress and inflammation.[8] The endoplasmic reticulum (ER) is highly vulnerable to stresses that disrupt cellular energy and redox state, or Ca^{2+} concentration. They cause ER stress by reducing the protein folding capacity of the ER, leading to the accumulation of unfolded proteins in the organelle lumen. The unfolded protein response (UPR) is an essential survival process as it reduces the accumulation of unfolded proteins. It is mediated by the ER transmembrane receptors: pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). Glucose regulatory protein 78 (GRP78), a chaperone heat shock protein, has a central role in modulating the UPR. In cellular conditions, these ER stress receptors are rendered inactive by binding to GRP78. The accumulation of unfolded proteins results in the dissociation

Corresponding author: Yeter Topçu-Tarladaçalışır, Department of Histology and Embryology Trakya University Faculty of Medicine, Edirne, Türkiye
e-mail: yeter_topcu@yahoo.com

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ORCID iDs of the authors: Y.T.T. 0000-0002-1851-7839; M.S-M. 0000-0001-9623-4116; Z.M. 0000-0002-5293-1865; D.E. 0000-0001-9713-044X.

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of GRP78 from the receptors. The activation of GRP78 initiates the UPR, which may direct the cell to undergo apoptosis. During prolonged ER stress, activated PERK, ATF6, and IRE1 initiate the activation of molecules, such as c-Jun N-terminal kinase (JNK), C/EBP homologous protein (CHOP), and B-cell lymphoma (Bcl-2) proteins, which push the cell to the death pathway. JNK and CHOP activated by ER stress abrogate the antiapoptotic effect of Bcl-2.[9,10] The role of ER stress-mediated apoptosis in IBD has been already reported.[5,11-13] Therapeutic studies on ER stress and apoptosis have become important.[5,12] Nutritional antioxidants are effective and affordable therapeutic alternatives, readily available for use. Quercetin, found in many plants, is a flavonoid known for its antioxidant, antiapoptotic, and anti-inflammatory effects.[14,15] Quercetin also provides a protective effect by reducing ER stress in different pathologies.[16-22] Earlier studies have highlighted quercetin's protective role in the development of IBD by inhibiting oxidative damage and inflammation.[14,23,24] However, there is currently no study assessing the relationship between quercetin and ER stress-mediated apoptosis, a crucial factor in IBD. Therefore, in this study, we investigated the effects of quercetin on ER stress-mediated apoptosis in IBD. We evaluated the impacts of quercetin on histopathologic changes, apoptosis, inflammation, oxidative stress, and ER stress in the 2,4,6 trinitrobenzene sulfonic acid (TNBS)-induced experimental IBD model in rat colon.

MATERIALS AND METHODS

Ethical Approval and Animals

The experiment was approved by the local ethical committee [Local Ethics Committee of Animal Experiments (TUHADYEK) 2018/03]. Twenty-four Wistar albino rats, aged three to four months, were obtained from the Experimental Animal Center of Trakya University for use in this study. The animals were bred and maintained under the standard conditions.

Experimental Protocol

Three groups were formed, including control, TNBS, and TNBS + quercetin groups, each consisting of eight male Wistar albino rats. After a 24-hour fasting period, experimental colitis was induced in the animals using TNBS (Sigma, MA, USA). Each rat in TNBS and TNBS + quercetin groups was anesthetized with ketamine, and a single dose of 25 mg TNBS (dissolved in 0.25 ml 30% ethanol) was administered through a catheter inserted into the colon via the anus to a depth of 8 cm. To prevent early leakage, the rats were maintained in a supine position for 30 seconds.[25] Control rats received physiological saline instead of TNBS. The animals of the TNBS + quercetin group received daily 100 mg/kg quercetin (Alfa Aesar, Ward Hill, Massachusetts, USA) in a volume of 0.5 ml of 1% dimethylsulfoxide (DMSO), intragastrically for seven days, immediately, after TNBS administration.[25] The control group and TNBS group animals were also given 0.5 ml/kg DMSO intragastrically. In terms of the development of colitis, the animal's body weight and stool were followed daily. To evaluate the histopathology of colitis, the disease activity index (DAI) was determined by taking into account weight loss, stool consistency, and bleeding.[26] Seven days after colitis induction, the animals were sacrificed and collected cardiac blood and colon samples.

Microscopic Assessment

Surgeons performed a midline incision of the abdomen and excised a 6 cm portion of the colon located 2 cm above the anal orifice from all animals. The excised colon segments were then cut into small pieces measuring 0.5 cm each. The tissue samples were fixed in a solution of 10% formalin, and then paraffin-embedding procedures were performed.[27] Sections (5 μm thick) of the colonic samples were stained with hematoxylin-eosin, Masson's trichrome, and periodic acid schiff. Colonic pathological changes were graded on a scale from 0 to 4, with modifications made to the criteria based on epithelial morphology and infiltration. Subsequently, the total histopathologic score (E + I) was calculated.

Immunohistoch,emistry

Sections at 5 μm incubated at 56 °C overnight were dewaxed and rehydrated. Sections after antigen retrieval and blocking were incubated in rabbit-polyclonal primary antibodies caspase-12 (1/2000, NBP1-77290, Novus Biologicals, Missouri, USA), GRP78/BİP (1/3000, LS-C312961, Lifespan Biosciences, Washington, USA), NF-κB/p65 (1/100, PA5-16545, Thermo Fisher Scientific, Massachusetts, USA) and p-JNK (1/50, 9251, Cell Signaling Technology, Massachusetts, USA) as described before.[27] AEC chromogen (AEC, ab64252, Abcam, Cambridge, UK), was applied. Sections were counterstained with hematoxylin.

The immunoreactivities of GRP78, NF-κB, caspase 12, and p-JNK in the intestinal tissue were evaluated in five fields/sections under a light microscope by blinded observation at 200x magnification. For each section, an HSCORE value was calculated by multiplying the percentage of immune-positive cells and the intensity of staining and then summing the values.[29]

Terminal Deoxynucleotidyl-transferase-mediated DUTP Nick End Labeling (TUNEL) Assay

TUNEL method that allows in situ recognition of DNA breaks was performed to detect apoptotic cells in the colonic epithelium using ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (S7101-KIT, Merck Millipore, Darmstadt, Germany). Intense brown nuclear staining was identified to detect the number of TUNEL-positive cells. The luminal and glandular apoptotic cells were quantified in randomly selected 20 fields per section under the light microscope through blinded examination at 200x magnification.

Total Antioxidant Capacity (TAC) and Total Oxidant Status (TOS)

Following the manufacturer's instructions, the serum levels of TAC and TOS in animals were evaluated by using TAC (RL0017) and TOS kit (RL0024, Rel Assay Diagnostics, Diyarbakır, Türkiye). The TAC test is based on the reduction of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; ABTS) radical, after calibration with Trolox, a vitamin E analog, results are expressed in millimoles of Trolox equivalent per liter (mmol Trolox

equivalent/L).[30] The TOS method is based on the principle that oxidants in the sample oxidize the iron ion chelator complex to ferric ions. Subsequently, these ferric ions form a color with the chromogen in an acidic environment. The assay is calibrated with H_2O_2 and the results are expressed in terms of μM H_2O_2 equivalent per liter (μmol H_2O_2 Equiv/L).[31]

The percentage ratio of TOS to TAC was accepted as the oxidative stress index (OSI).[32] For the calculation, the unit values (mmol Trolox Equiv/L) of TAC were converted to μmol Trolox Equiv/L, and OSI was calculated according to this formula; OSI = (TOS)/(TAC) x 100.

Measurement of Myeloperoxidase (MPO) and Tumor Necrosis Factor-α (TNF-α) Levels

MPO activity as a marker of inflammation and proinflammatory cytokine TNF-α concentration was evaluated in serum samples. The measurements were performed using Enzyme-linked Immunosorbent Assay kits for Rat MPO (E0574Ra) and Rat TNF-α kits (E0764Ra, BT-Lab, Shanghai, China) following the manufacturer's instructions. An absorbance reader (Rel Reader) was employed for the analysis.

Statistical Analysis

The SPSS 20.0 program (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) was used for performing all statistical analyses. The results were expressed as mean ± standard deviation for normally distributed variables and as median (minimum-maximum) for non-normally distributed variables. Normality assumption was assessed using Shapiro-Wilk's test. For comparing two independent groups, the Student's t-test was used. One-way analysis of variance and Kruskal-Wallis tests were used for more than two independent groups, depending on the normality assumption. Tukey, Tamhane, and Multiple comparisons were conducted using Dunn's posthoc tests. A p-value < 0.05 was considered statistically significant.

Disease Findings

Colitis development was monitored through average DAI values, calculated by assessing the following parameters: weight loss, stool consistency, rectal bleeding, and changes in initial and final body weight. Colonic infusion of TNBS resulted in diarrhea and body weight loss in rats. Throughout the experiment, normal defecation was observed in the control group rats, whereas TNBS-administered rats exhibited bloody diarrhea in the initial days. This condition progressed to diarrhea and soft stool in the subsequent days. The intensity of diarrhea was reduced in rats receiving quercetin. The colitis group showed the highest DAI value. However, a lower DAI value was observed in the quercetin group when compared to the colitis group (p = 0.002, Table 1).

OSI Findings

The colonic infusion of TNBS resulted in an elevation in TOS serum levels (p = 0.138) and a reduction in TAC serum levels (p = 0.052). The OSI values, calculated by the ratio of TOS to TAC in the TNBS group, were significantly higher than those in the control group (p = 0.001). Quercetin led to a reduction in colitis-related increase in OSI (p = 0.002, Table 2).

The Inflammatory Response

In the TNBS group, levels of MPO (p = 0.015) and the proinflammatory cytokine TNF-α (p = 0.001) in serum were higher than those in the control group. Quercetin reduced the TNF-α and MPO levels associated with colitis (p = 0.448, p = 0.220 respectively, Table 2).

TABLE 1. Body Weight, DAI, and Histologic Score of Experimental Groups.

	Control	TNBS	TNBS+Quercetin	P
Initial body weight	226.5 ± 13.1	219.1 ± 19.6	212.5 ± 10.2	0.294
Final body weight	231.1 ± 14.5	186.5 ± 18.1[a]	193.8 ± 10.2[a,b]	<0.001
Body weight difference	4.6 ± 2.5	-32.6 ± 6.1[a]	-18.7 ± 5.5[a,b]	<0.001
DAI	-	2.5 ± 0.5	1.3 ± 0.3[b]	0.002
Histologic score	0 (0-1)[c]	8 (8-8)	4.5 (4-5)[d]	<0.001

Data show mean ± SD [a]p < 0.001 versus control group; [b]p < 0.01 versus TNBS group; [c]p < 0.001 versus TNBS group; [d]p < 0.05 versus TNBS group; TNBS, trinitrobenzene sulfonic acid, DAI, disease activity index; SD, standard deviation

TABLE 2. TAC, TOS, OSI, MPO, and TNF-α Levels of Experimental Groups.

	Control	TNBS	TNBS+Quercetin	P
TAC (mmol trolox equiv./l)	1.6 ± 0.4	0.7 ± 0.2	1.3 ± 0.6	0.054
TOS (μmol H_2O_2 equiv./l)	11.3 ± 3.4	16.6 ± 2.3	12.6 ± 4.5	0.140
OSI	0.7 ± 0[a]	2.7 ± 0.7	1 ± 0.4[a]	0.001
MPO (ng/ml)	48.3 ± 13.9[b]	116.1 ± 33.2	81.7 ± 29.4	0.019
TNF-α (pg/ml)	857.7 ± 202.2[a]	2560 ± 557.5	2173.3 ± 458.4[c]	0.001

Data show mean ± SD; [a]p < 0.01 versus the TNBS group; [b]p < 0.05 versus the TNBS group; [c]p < 0.01 versus the control group; TNBS, trinitrobenzene sulfonic acid; TAC, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index; MPO, myeloperoxidase; TNF-α, tumor necrosis factor-α; SD, standard deviation.

Histopathological Findings

Light microscopic examinations revealed that the colon tissue of the animals in the control group exhibited normal histology (Figure 1 a, d, g). However, TNBS caused ulcerations in the rat colon. Intact and eroded areas co-existed side by side. Ulceration areas were characterized by inflammation. Histological examination revealed edema extending to the submucosa, mucosal ulceration, erosion, infiltration, hemorrhage, distortion of the crypts, and deterioration in the surface epithelium with observed goblet cell depletion. Transmural inflammation characterized by neutrophilic and lymphocytic infiltration along with thickening of the colon wall, was also detected (Figure 1 b, e, h). The TNBS group had a higher microscopic score compared with the control ($p < 0.001$, Table 1). The quercetin reduced these abnormal histologic damages (Figure 1 c, f, ı) and as a result, the microscopic score ($p = 0.045$, Table 1).

TUNEL Findings

Table 3 represents the numbers of TUNEL-positive apoptotic epithelial cells for the three groups. A negligible number of apoptotic cells were observed in the control group sections (Figure 2 a, d). Colitis induction with TNBS led to an increase in cell apoptosis in the colon ($p < 0.001$, Figure 2 b, d). Quercetin reduced the epithelial apoptosis in the colon tissue with colitis, preventing programmed cell death ($p < 0.001$, Figure 2 c, d).

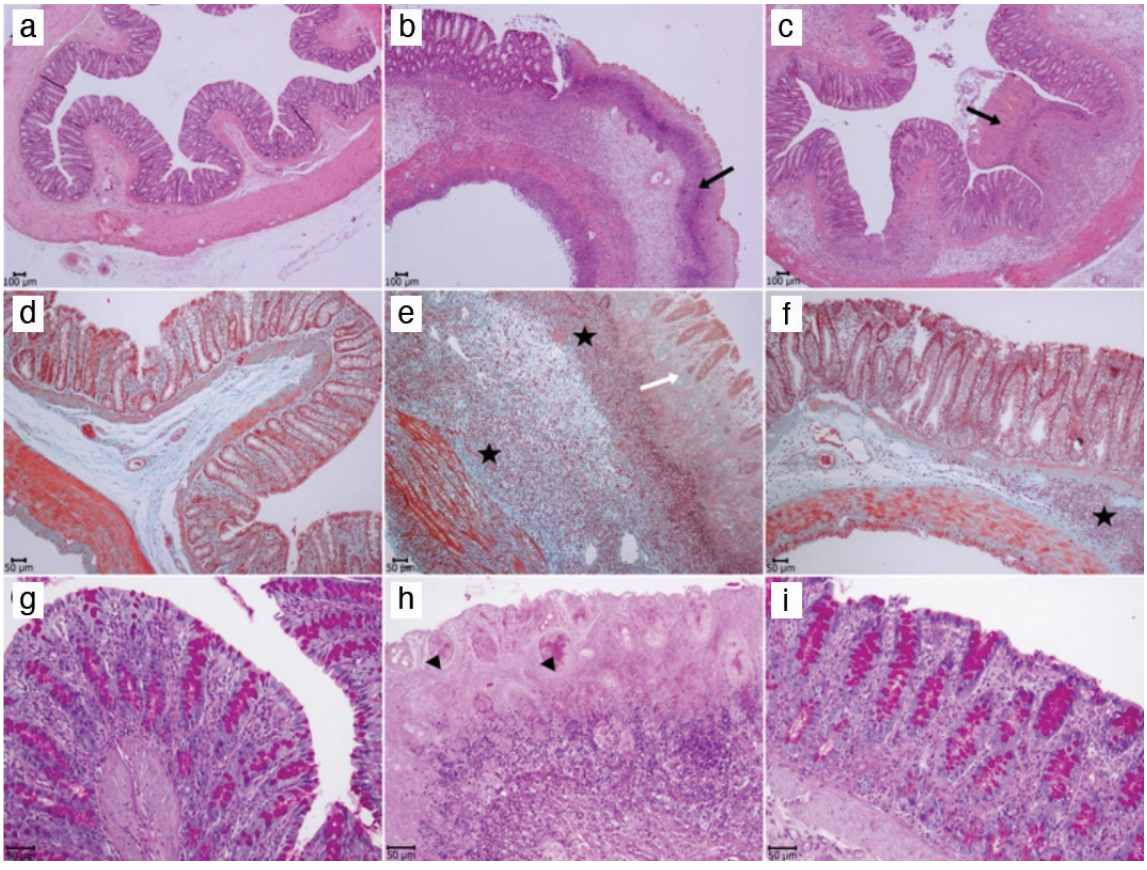

FIG. 1. Representative micrographs of rat colon sections stained with H&E (a, b, c), Masson's trichrome (d, e, f), and PAS (g, h, ı). (a, d, g) The control group showed normal colonic structure. (b, e, h) The TNBS group showed ulcerations (arrows), epithelial cell and goblet loss (arrowheads), crypt disruption (white arrow) in the mucosa, and widespread inflammation (asterisks) with significant hemorrhage and edema in the submucosa. (c, f, ı) The TNBS + quercetin group showed slight ulcerations and inflammation.

TABLE 3. The Number of TUNEL-Positive Apoptotic Cells, HSCORE Results of Caspase-12, p-JNK, and GRP78 of All Experimental Groups.

	Control	TNBS	TNBS+Quercetin	P
Apoptotic cell number	6.7 ± 0.6^{a}	49 ± 2	$17.5 \pm 1.4^{a,b}$	<0.001
Caspase-12	5.2 ± 2.6^{a}	160 ± 16.7	$81.7 \pm 9.8^{a,b}$	<0.001
p-JNK	2.5 ± 4.2^{a}	140 ± 19	$53.3 \pm 7.5^{a,b}$	<0.001
GRP78	4 ± 3.2^{a}	106.7 ± 16.3	$70 \pm 8.9^{b,c}$	<0.001

Data show mean ± SD; $^{a}p < 0.001$ versus the TNBS group; $^{b}p < 0.001$ versus the control group; $^{c}p < 0.01$ versus the TNBS group; TNBS, trinitrobenzene sulfonic acid; SD, standard deviation.

NF-κB Immunoreactivity Findings

The NF-κB immunoreactivity with cytoplasmic localization was detected in superficial and glandular epithelial cells in the colon (Figure 3 a-c). An increase in NF-κB immune expression was observed in all TNBS-applied groups compared to the control group ($p < 0.001$) and the highest HSCORE value was detected in the colitis group. This value was lower in the quercetin group when compared to the colitis group ($p < 0.001$, Figure 3 d).

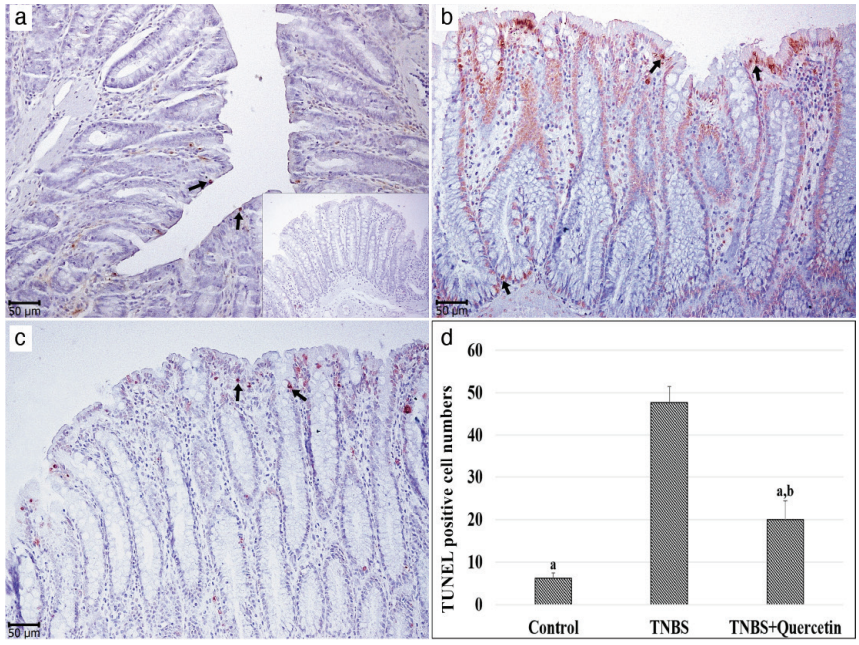

FIG. 2. TUNEL micrographs of colon tissue in all groups (a, b, c). (a) Control group (Inset: Negative control was performed using distilled water in place of terminal deoksinükleotidil transferase). (b) TNBS group. (c) TNBS + quercetin group. TUNEL-positive (arrows) apoptotic nuclei (brown), blue-purple staining nonapoptotic nuclei hematoxylin counter-staining. (d) Graphs of apoptotic cell number for groups (mean ± SD). ([a]p < 0.001 compared to TNBS group), ([b]p < 0.001 compared to the control group).
SD, Standard deviation

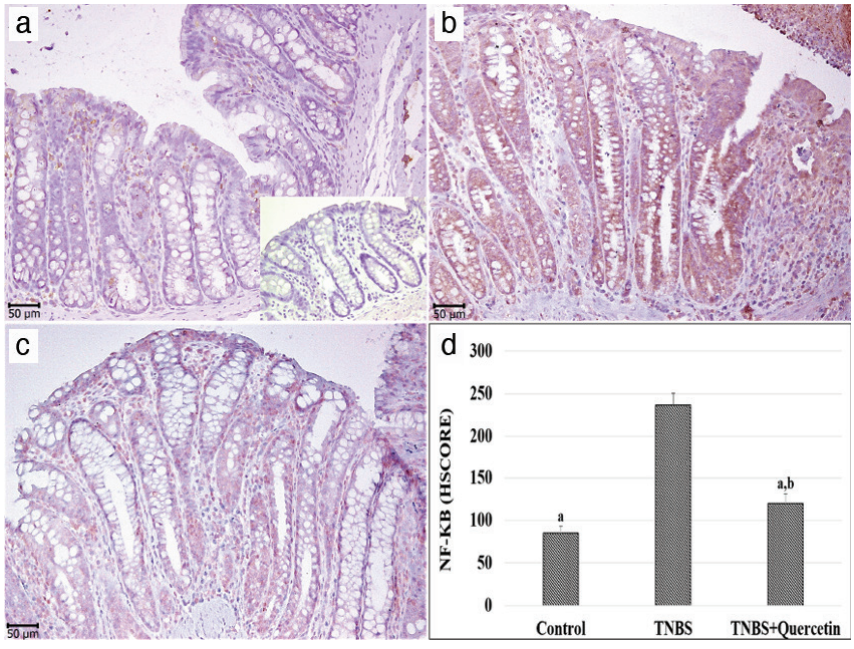

FIG. 3. Micrographs of NF-kB immunoreactivity in colon tissue all in groups. (a) Control group (Inset: Negative control was performed using phosphate buffer saline in place of primary antibody). (b) TNBS group. (c) TNBS + quercetin group. Hematoxylin counterstain. (d) Graphs of HSCORE for NF-κB immunoreactivity (mean ± SD). (ap < 0.001 compared to TNBS group), (bp < 0.001 compared to the control group).
SD, Standard deviation

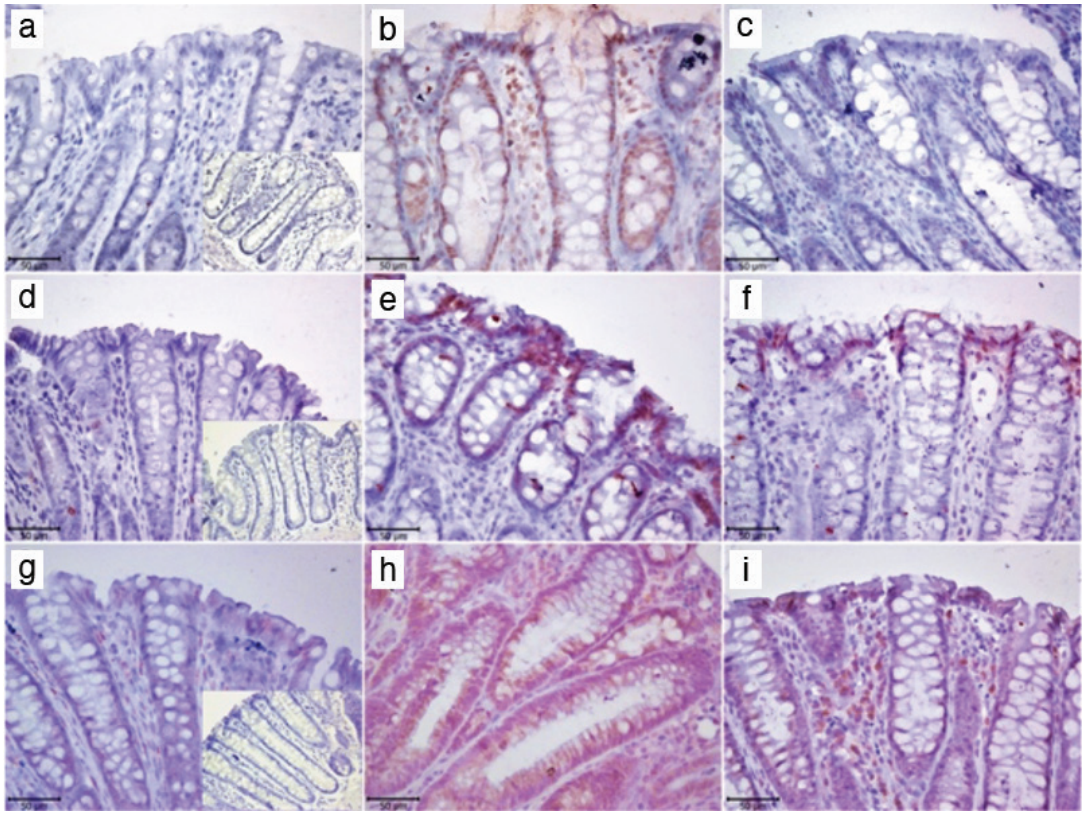

FIG. 4. Micrographs of immunohistochemical staining for caspase-12 (a, b, c), p-JNK (d, e, f), and GRP78 (g, h, ı) in colon tissue. (a, d, g) Control group. (b, e, h) TNBS group (c, f, ı) TNBS + quercetin group. Insets: Negative controls were performed using phosphate buffer saline in place of primary antibodies. Hematoxylin counterstain.

Caspase-12, GRP78, and P-JNK Immunoreactivity Findings

The caspase-12 (Figure 4 a-c), p-JNK (Figure 4 d-f), and GRP78 (Figure 4 g-ı) immunoreactivities were observed in superficial and glandular epithelial cells of rat colon. An increase was detected in the immunoexpression of caspase-12, GRP78, and p-JNK in all TNBS-administered groups compared with the control group ($p < 0.001$). The HSCORE values of p-JNK ($p < 0.001$), caspase-12 ($p < 0.001$), and GRP78 ($p = 0.004$) were lowered in the quercetin group when compared to the colitis group (Table 3).

DISCUSSION

Quercetin showed protective effects against ER stress-mediated colonic epithelial cell apoptosis in the experimental IBD model, suggesting its potential benefits for the recovery of IBD. In this study, we used intracolonic, single dose TNBS/ethanol administration to induce colitis. TNBS interacts with colon tissue proteins after ethanol disrupts the intestinal barrier. This interaction triggers a mucosal immune response, leading to IBD by rendering these proteins immunogenic within the host immune system. Alterations in intestinal epithelial function accompanying IBD are linked with the extraordinary production of reactive oxygen species (ROS).[2] Colitis development was monitored daily by observing the body weight and fecal condition of the animals, with the subsequent calculation of average DAI values. Consistent with previous studies,[25,33] a significant weight loss with bloody diarrhea was observed in subjects following the induction of colitis. This scenario is believed to arise from pain-related limitation of movement as well as a decrease in fluid absorption, stemming from the direct disruption of the intestinal epithelial barrier.[2] In addition, intracolonic TNBS administration induced extensive inflammation, bleeding, crypt distortion, goblet cell depletion, surface epithelial disruption, patch-like ulcerations, along with edema extending to the submucosa, and transmural inflammation. In line with these macroscopic and histopathologic findings, the DAI score and colonic microscopic damage score of the subjects with colitis were significantly higher than the controls. Quercetin reduced these pathological findings and histological damages, consistent with the data from previous studies.[23,25] In our study, oxidative stress resulting from the rectal infusion of TNBS was demonstrated by OSI (TOS/TAC ratio). A high OSI value was obtained, serving as an indicator of the imbalance between prooxidants and antioxidant capacity. We also detected increased serum MPO and TNF-a levels due to inflammation. An increased level of oxidative stress together with an increase in the levels of inflammation markers, MPO and TNF-α, have been reported previously.[14,23,25] Activation of NF-kB plays a major role in IBD pathogenesis.[4] The overexpression and activation of NF-kB in intestinal epithelial cells have been reported in experimental IBD models.[6,7] In parallel with these findings, we demonstrated an increase in NF-κB immunoreactivity in the TNBS-induced colonic mucosa, which has a regulatory

role for many proinflammatory cytokines. Quercetin inhibited oxidative stress and colonic activation of NF-kB. Consistent with our findings, studies on various experimental colitis models have shown the protective effect of quercetin in IBD, attributed to its antioxidant properties and NF-kB inhibition.[14,24,34] ROS formation and activation of NF-κB lead to gene expressions related to proinflammatory mediators and apoptosis in damaged cells.[8] B-cell lymphoma (Bcl-2) family members,[35] kinases (JNK),[11,] and caspases (caspases 3, 9, 12)[36] are involved in ER stress-induced apoptosis. ER stress resulting from the accumulation of misfolded or unfolded proteins triggers the UPR pathway, reduces the accumulation of unfolded proteins, and regulates ER functions. If the activation of UPR cannot cope with ER stress, it creates death signals and leads the cell to apoptosis.[9,10] ER stress not only triggers GRP78 gene expression but also facilitates NF-κB activation through increased $Ca2^{+}$ flux and production of ROS.[37] GRP78 is up-regulated during ER stress and plays a role in ER stress-mediated apoptosis through PERK, ATF6, and IRE1 pathways.[38-40] ER stress, inflammation, and oxidative stress are important signals for JNK activation.[38] Previous studies have demonstrated JNK activation and GRP78 up-regulation in colon tissue of colitic animals as well as in patients with IBD.[12,41,42] The activation of JNK serves as a death signal by regulating members of the *BCL2* gene family and induces cell death through caspases.[9,43] The caspase-12 is an ER membrane-associated protein that is normally found in the form of an inactive procaspase. During ER stress, it dissociates from the ER membrane, then activates and triggers apoptosis.[36,44] Consistent with previous studies,[12,41,42] we observed an increase in mucosal immunoreactivity of GRP78, p-JNK, and caspase 12. As an indicator of ER stress-induced apoptosis, we illustrated an elevated number of apoptotic cells in the epithelium of the TNBS-mediated colitic rat colon using the TUNEL method. We also demonstrated that this induction is markedly abolished by quercetin.

The tight junctions between the apical membranes of enterocytes and the mucus layer produced by goblet cells prevent the passage of microorganisms in the intestinal lumen to the lower layers.[45] Secretory cells of the intestines, which produce large amounts of complex proteins associated with mucosal defense, are susceptible to ER stress.[46] Goblet cells produce a glycoprotein mucin 2 (MUC2) which is the main constituent of the intestinal mucus barrier.[41] Tawiah et al.[47] observed that increased levels of misfolded MUC2, mediated by increased ROS, triggered goblet cell apoptosis. Heazlewood et al.[41] showed that intestinal mucin tends to misfold or undergo abnormal assembly, which causes ER stress, morphological changes in goblet cells, early goblet cell apoptosis, and the development of chronic intestinal inflammation. Electron microscopic studies in IBD patients and animal models revealed the presence of dilated ER-containing goblet cells which is the ultrastructural appearance of misfolded proteins.[41,42] Previous studies have investigated the role of apoptosis-mediated epithelial cell death and colonic lesions in TNBS colitis.[48-50] The inhibiting effect of quercetin on ER stress has been demonstrated in various pathological conditions.[16-22] Liu et al.[17] showed that quercetin inhibits ER stress during lead induction by regulating the antioxidant activity of the liver and IRE1/JNK signaling pathway in rats. Similarly, Woo et al.[22] reported that in the amyloid-beta-injected mouse brain, quercetin reduced oxidative stress parameters, ER stress markers, and protein expression levels of proapoptotic molecules, such as JNK. Wang et al.[16] reported the beneficial effect of quercetin on testosterone secretion disorders caused by diabetes, attributing this effect to the inhibition of ER stress.

Our results support the notion that quercetin may offer benefits in IBD, showcasing a protective effect. This is attributed not only to its anti-inflammatory and antioxidant effects but also to its regulatory influence on ER stress-mediated apoptosis. Thus, the results of this study will enhance the knowledge of quercetin and ER stress-mediated apoptosis in IBD. However, in the study, the effectiveness of quercetin was not compared with ER stress inhibitors or reference agents used in treatment. Planning future studies in this direction will enhance the potential clinical utility of quercetin.

Ethics Committee Approval: Trakya University, Local Ethics Committee of animal experiments (TUHADYEK 2018/03)-28.02.2018.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authorship Contributions: Concept- Y.T-T., M.S-M., Z.M.; Design- Y.T-T., M.S-M., Z.M.; Data Collection or Processing- Y.T-T., M.S-M., Z.M., D.E.; Analysis or Interpretation- Y.T-T., M.S-M., Z.M., D.E.; Literature Search- Y.T-T., M.S-M.; Writing- Y.T-T., M.S-M.

Conflict of Interest: No conflict of interest was declared by the authors.

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