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Effects of Korean Radish on DSS-Induced Ulcerative Colitis in Mice

Hyun-Kyoung Kim

Department of Food Science and Engineering, Seowon University, Cheongju, Korea
Kimhk4@seowon.ac.kr

Abstract

The present study aimed to investigate the comparative evaluation of pharmacological efficacy between sulfasalazine alone and combination with herbal medicine on dextran sodium sulfate (DSS)-induced UC in mice. Balb/c mice received 5% DSS in drinking water for 7 days to induce colitis. Animals were divided into five groups (n = 9): group I-normal group, group II-DSS control group, group III-DSS + sulfasalazine (30 mg/kg), group IV-DSS + sulfasalazine (60 mg/kg), group V-DSS + sulfasalazine (30 mg/kg) + Radish Extract mixture (30 mg /kg) (SRE). DSS-treated mice developed symptoms similar to those of human UC, such as severe bloody diarrhea and weight loss. SRE supplementation, as well as sulfasalazine, suppressed colonic length and mucosal inflammatory infiltration. In addition, SRE treatment significantly reduced the expression of pro-inflammatory signaling molecules through suppression both mitogen-activated protein kinases **(**MAPK) and nuclear factor-kappa B (NF-κB) signaling pathways, and prevented the apoptosis of colon. Moreover, SRE administration significantly led to the up-regulation of anti-oxidant enzyme including SOD and Catalase. This is the first report that Radish extract mixture combined with sulfasalazine protects against experimental UC via the inhibition of both inflammation and apoptosis, very similar to the standard-of-care sulfasalazine.

***Keywords**: Dextran sulfate sodium, ulcerative colitis, Korean radish, MAPK, NF-B, apoptosis t*

1. Introduction

Ulcerative colitis (UC) with high incidences worldwide, commonly referred as chronic inflammatory bowel diseases (IBD) and characterized by a uncontrolled inflammatory condition of the intestinal mucosa [1-4]. The main symptoms of UC are abdominal pain, mucous and bloody diarrhea, weight loss, and anemia. Generally, recommended therapies for UC patients include anti-inflammatory drugs (corticosteroid; prednisolone and aminosalicylic acid; sulfasalazine), immunosuppressants (thiopurines; azathioprine, 6-mercaptopurine and 6-thioguanine), antibiotics (metronidazole, ciprofloxacin) [3-5]. Herein, sulfasalazine, a composed of 5-aminosalicylic acid and sulfapyridine, has been used as a standard-of-care in UC for decades, however, it generates excessive oxidative stress after high dosage and long term intake, resulting in severe adverse symptoms, such as blood disorders, hepatotoxicity, hypospermia, and male infertility [6, 7]. Accordingly, a sole treatment of sulfasalazine is not entirely satisfactory. Therefore, therapeutic strategy for UC has a need to focus on replace current therapy, in addition, the new therapy has to act locally at the site of inflammation to maximize efficacy, to increase convenience, and to minimize side effects [8].

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Corresponding Author: kimhk4@seowon.ac.kr
Tel:+82-43-299-8474, Fax: +82-43-299-8470
Department of Food Science and Engineering, Seowon University, Cheongju, Korea

Korean Radish has been widely used as herbal medicine for treating cold related disorder such as common cold and influenza [9]. Besides, a variety of pharmacological effects have been suggested: anti-inflammatory action, anti-bacterial activity, anti-platelet aggregation, sedative effect, improvement of blood circulation, and inhibition of cancer metastasis [10, 11]. we predicted that Koraen Radish with anti-inflammatory effect and Korean radish with hepatoprotective and renoprotective effects may exert the potential therapeutic benefits in ulcerative colitis. So, the present study was conducted to evaluate the pharmacological effect of sulfasalazine alone and in combination with Korean radish extract Radix mixture in experimentally induced ulcerative colitis in mice.

2. Experiment Materials and Methods

2.1. Materials

DSS (molecular weight: 36,000–50,000 Daltons) was purchased from MP Biologicals (Santa Ana, California, USA). Sulfasalazine (purity ≥ 98 %) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma-Aldrich (St, Louis, MO, USA). The protease inhibitor mixture solution and ethylenediaminetetraacetic acid (EDTA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2′,7′-Dichlorofluorescein diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR, USA). The pierce bicinchoninic acid (BCA) protein assay kit was obtained from Thermo Fisher Scientific (Waltham, MA, USA). ECL Western Blotting Detection Reagents and pure nitrocellulose membranes were supplied by GE Healthcare (Chicago, IL, USA). Rabbit polyclonal antibodies against nuclear factor-kappa B p65 (NF-Bp65; 1:1,000, SC-372), $p47^{phox}$ (1:1,000, SC-14015), Rac 1 (1:1,000, SC-217), superoxide dismutase (SOD; 1:1,000, SC-11407), glutathione peroxidase-1/2 (GPx-1/2; 1:1,000, SC-30147), Bax (1:1,000, SC-7480), Bcl-2 (1:1,000, SC-7382), monocyte chemoattractant peptide-1 (MCP-1; 1:1,000, SC-28879), and intercellular adhesion molecule-1 (ICAM-1; 1:1,000, SC-1511-R); Goat polyclonal antibodies against tumor necrosis factor- (TNF-1:1,000, SC-217), and interleukin-1 (IL-1 ; 1:1,000, SC-1252); mouse monoclonal antibodies against phosphor-extracellular signal-regulated kinase 1/2 (p-ERK1/2; 1:1,000, SC-7383), phosphor-p38 (p-p38; 1:1,000, SC-7973), cyclooxygenase-2 (COX-2; 1:1,000, SC-19999), inducible nitric oxide synthase (iNOS; 1:1,000, SC-7271), histone (1:1,000, SC-8030), and -actin (1:1,000, SC-4778) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Polyclonal antibody against c-Fos (1:1,000, #4384) was obtained from Cell Signaling Technology, Inc. (Cell Signaling, MA, USA). Mouse monoclonal anti-caspase-3 (1:1,000; 3004-100) was purchased from BioVision Inc. (Mountain View, CA, United States). Rabbit polyclonal anti-reduced nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) was purchased from LifeSpan BioSciences (Seattle, WA, United States). Rabbit anti-goat (1:3,000, SC-2774), goat anti-rabbit (1:3,000, SC-2004), and goat anti-mouse (1:3,000, SC-2005) immunoglobulin G (IgG) horseradish peroxidase (HRP)-conjugated secondary antibodies were acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other chemicals and reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

2.2. Test materials

In this study, dried Korean Radish (100 g) was extracted with 10 times of water and boiled in 100°C for 6 h with distilled water. After filtration, the water extracts were evaporated using a rotary evaporator at 45°C.

2.3. Experimental animals and induction of colitis

Animal experiments were carried out according to the “Guidelines for Animal Experimentation” approved by the Ethics Committee of the Daegu Haany University with certificate number DHU2017-044. Eight-week-old male Balb/c mice weighing 22–24 g were purchased from were purchased from Orient (Gyeonggi-do, Korea). Mice were maintained under a 12-h light/dark cycle, housed at a controlled temperature (24±2°C) and humidity (about 60%). After adaptation (1 week), acute colitis was induced by oral administration of 5.0 % (w/v) DSS dissolved in drinking water, for 7 days [15]. For each experiment, the mice

were divided into 5 experimental groups and 36 colitic mice were arbitrarily allocated into 4 groups (n = 9/group). Normal mice received drinking water without DSS throughout the entire experimental period. Sufasalazine was used as a positive reference agent and it was given at 30 or 60 mg/kg/day. The entire colon was removed immediately and examined for gross mucosal injury. The colon tissue was immediately frozen in liquid nitrogen and blood samples were collected by cardiac puncture from anesthetized mice. Subsequently, the esophagus and serum were kept at -80°C until analysis.

2.4. Measurement of ROS level in the serum

Serum ROS level was measured by employing the method of Ali et al. [16]. 25 mM DCFH-DA was added to the serum. After incubation for 30 min, the changes in fluorescence values were determined at an excitation wavelength of 486 nm and emission wavelength of 530 nm.

2.5. Preparation of cytosol and nuclear fractions

Protein extraction was performed according to the method of Komatsu with minor modifications [17]. Colon tissues for cytosol fraction were homogenized with ice-cold lysis buffer A (250 mL) containing 10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, and 1,250 μL protease inhibitor mixture solution. The homogenate incubated at 4°C for 20 min. And then 10% NP-40 was added and mixed well. After centrifugation (13,400×g for 2 min at 4°C) using Eppendorf 5415R (Hamburg, Germany), the supernatant liquid (cytosol fraction) was seperated new e-tube. The left pellets were washed twice by buffer A and discard the supernatant. Next, the pellets were suspended with lysis buffer C (20 mL) containing 50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 1% (v/v) glycerol, and 100 μL protease inhibitor mixture solution suspended and incubated at 4°C for 30 min. After centrifugation (13,400 ×g for 10 min at 4°C), the nuclear fraction was prepared to collect the supernatant. Both cytosol and nuclear fractions were kept at -80°C before the analysis.

2.6. Immunoblotting analyses

For the estimation of c-Fos, NF-Bp65, and histone, 12g of protein from each nuclear fraction was electrophoresed through 10% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE). Separated proteins were transferred to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and then incubated with primary antibodies (c-Fos, NF-Bp65, and histone) and overnight at 4°C. After the blots were washed, they were incubated with anti-rabbit or anti-mouse IgG HRP-conjugated secondary antibody for 1 h at room temperature. In addition, 7.5g protein of each cytosol fraction of NOX4, p47phox, Rac1, Bax, Bcl-2, Caspase 3, SOD, Catalase, GPx-1/2, HO-1, COX-2, iNOS, TNF-IL-1, and β-actin was electrophoresed through 8-12% SDS-PAGE. Each antigen-antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with Sensi-Q 2000 Chemidoc (Lugen Sci Co., Ltd., Gyeonggi-do, Korea). Band densities were measured using ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to histone or β-actin. The protein levels of the groups are expressed relative to those of the normal mouse (represented as 1).

2.7. Hematoxylin and eosin (H/E) stain of colon tissue

For microscopic evaluation, intestine tissue was fixed in 10% neutral-buffered formalin and, after embedding in paraffin, cut into 2m sections and stained using hematoxylin and eosin (H/E) for microscopic evaluation. The stained slices were subsequently observed under an optical microscope and analyzed using the i Solution Lite software program (Innerview Co.).

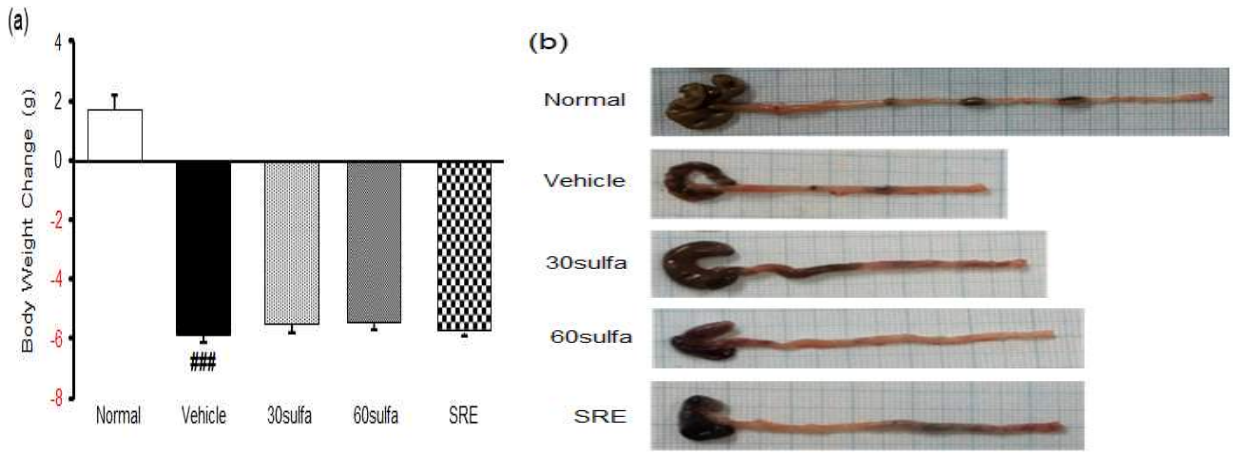
2.8. Statistical analysis

The data are expressed as the mean ± S.E.M. Significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA). Values of $p < 0.05$ were considered significant.

3. Result and Discussion

3.1. Effects of Ulcerative colitis (UC) and anti-inflammatory

Ulcerative colitis (UC), chronic and complex autoimmune inflammatory disorders, is associated with a diverse dysfunction led to overproduction of inflammatory cells and cytokine. Especially, the continuous progression of UC increases a risk for development of colorectal cancer (CRC). Accordingly, new complement remedy of sulfasalazine, which mainly used in the treatment of UC, however, had various side effects can be alleviated a deterioration of UC symptoms and use for a long-term in safety. The present study revealed, for the first time, comparative evaluation of a pharmacological efficacy between sulfasalazine combined with Radish Extract mixture (SRE) and sulfasalazine alone in a mouse model of UC. As shown in Figure 1, DSS control group significantly increased body weight loss and decreased colon length ($P < 0.001$) in comparision with normal group. Results in previous studies had showed that length of colon was negatively correlated with severity of experimental colitis. Sulfasalazine or SRE administration led to anti-inflammatory effects, including reduced body weight loss and less shortening of the colon length, whereas there were few significance in body weight change and colon length among the DSS-treated groups (Figure 1a, c). Colonic inflammation involves the disruption of the apparatus of colonic mucosa and ulceration, resulting in the infiltration of inflammatory cells such as inflammatory monocytes and macrophages and thickening of the lamina propria. To investigate mucosal inflammation, we performed H/E staining. Colons in normal group exhibited with normal crypt morphology, abundant goblet cells, no signs of mucosal thickening, and complete absence of ulceration. On the contrary, microscopic damage was lower in with SRE or sulfasalazine group than those in DSS control group (Figure 1d).



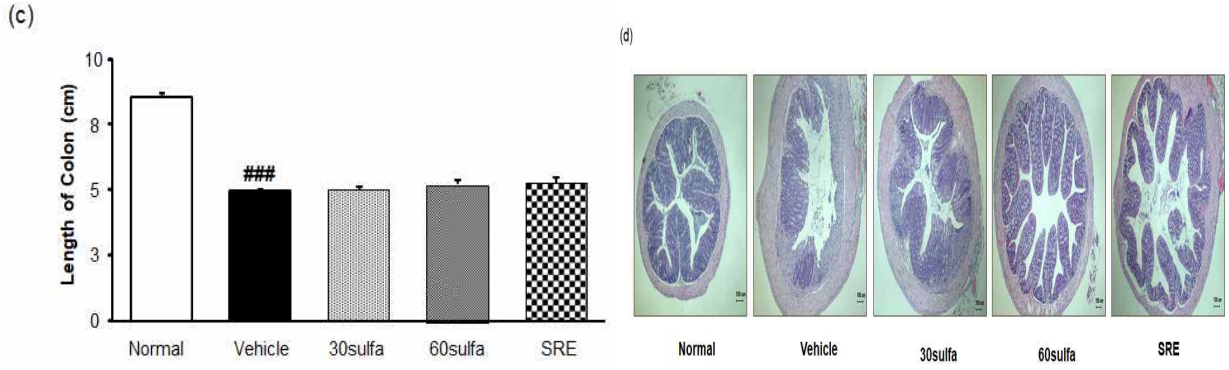


Figure 1. SRE alleviated dextran sodium sulfate-induced experimental colitis. (a) Body weight changes after induction of colitis by dextran sodium sulfate (DSS); (b) Macroscopic appearance; and (c) Length of colon (d) H/E staining of colon magnification×40. Data are mean ± S.E.M. (n=7). Significance: ###P <0.001 versus normal rats.

3.2. Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are generated as part of normal oxidative metabolism, yet cell damage is induced by their excess formation. Moreover, redox-active sulfur species, which are the wildly known pathway of free radical generation by oxygen species, have been characterized as part of a sulfate assimilation pathway. This reactions also involve the metabolism of sulfinic and sulfonic acids that are oxidized sulfur molecules. Since DSS is so highly sulfated, we estimate that it may lead a sulfate load on cells and that this is associated with an elevation of the noticeable ROS, leading to acceleration of an inflammatory cascade. The reported clinical data show that ROS increases in colitis patients, causing oxidative cellular damage and promoting carcinogenesis. Previous studies indicated the importance of ROS-induced oxidative stress in the development of UC. Besides, the key producers of ROS are NADPH oxidase enzymes including NOX4, $p47^{phox}$, and Rac 1. Overproduction of ROS via NADPH oxidase has been implicated in tissue damage observed in chronic inflammatory disorders and play vital roles in various biological activities, including host defense, cell growth and differentiation, stimulation of pro-inflammatory genes, and cell death. In present study, the DSS injury was markedly higher than those of normal group ($P < 0.001$), whereas, the elevated level of serum ROS were significantly decreased lower to the levels of normal group both SRE and sulfasalazine ($P < 0.001$) (Figure 2a). The protein expressions of both NOX4 and $p47^{phox}$, the markers of NADPH oxidase activity, in the colon were augmented in the DSS control group (vs. normal group, $P < 0.05$, $P < 0.01$, resp.). However, SRE-treated group had significantly down-regulated NADPH oxidase, whereas sulfasalazine-treated group decreased without significance. Otherwise, Rac 1 expression showed a tendency to a decrease (Figure 2b). In general, ROS are known to be neutralized by the endogenous antioxidant enzymes. SOD converts O^{2-} to H_2O_2, which is subsequently neutralized to water by Catalase and GPx-1/2. The activity of enzymic antioxidants such as SOD, Catalase, and GPx-1/2 was decreased in DSS-induced group. Herein, SRE administration significantly increased the activity of SOD and Catalase except for GPx-1/2 (without significance) (Figure 3). These findings indicated that SRE treatment of colitis may be reducing the extent of colonic injury by its antioxidant effect. Especially, SRE supplementation was superior to those when sulfasalazine alone (Figure 3).

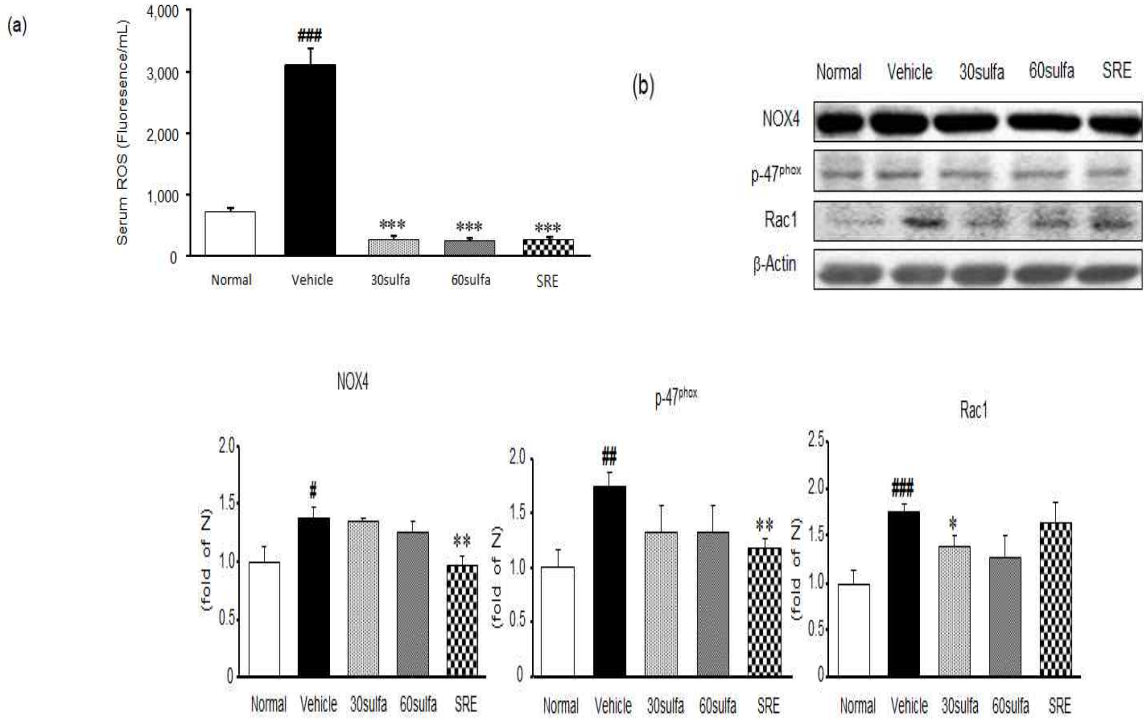


Figure 2. SRE decreased serum ROS and NADPH oxidase activity in colon. (a) serum ROS (b) NOX4, p47^{phox}, and Rac 1 protein expressions. Normal, normal mice; Vehicle, DSS control mice; 30sulfa; sulfasalazine 30 mg/kg-treated mice; 60sulfa, sulfasalazine 60 mg/kg-treated mice. Data are mean ± S.E.M. (n=7) Significance: $^{\#}$P <0.05, $^{\#\#}$P <0.01, $^{\#\#\#}$P <0.001 versus normal mice and **P <0.01, ***P <0.001 versus DSS control mice.

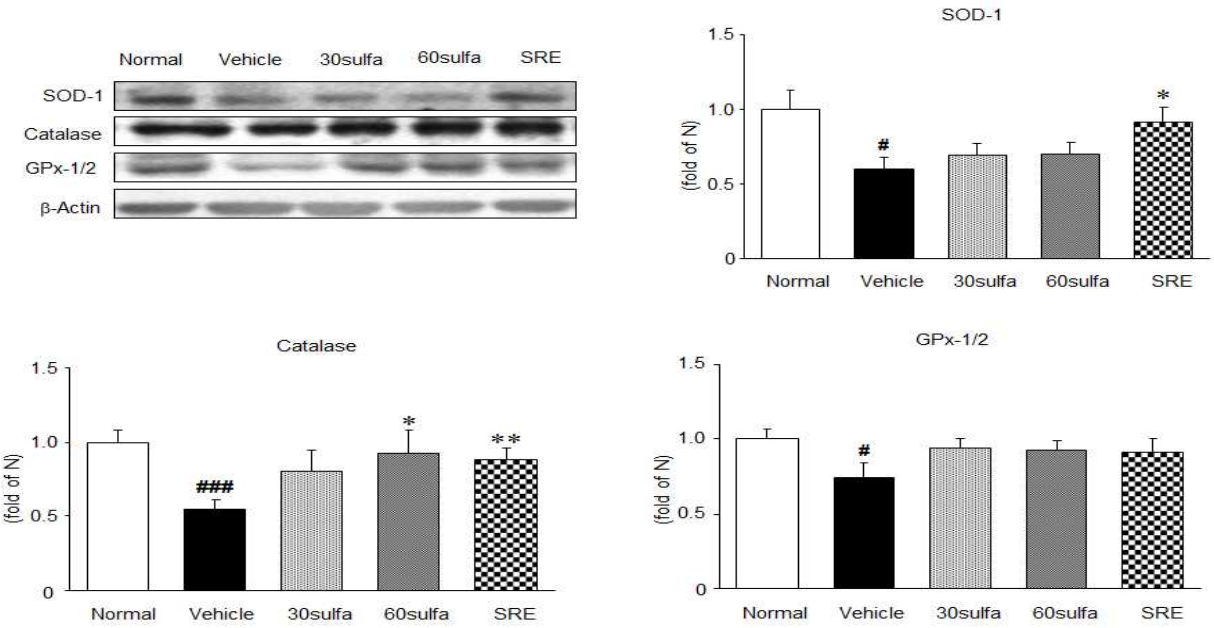


Figure 3. Effect of SRE on anti-oxidant proteins in DSS-induced colitis mice. SOD, Catalase, GPx-1/2 protein expressions. Normal, normal mice; Vehicle, DSS control mice; 30sulfa; sulfasalazine 30 mg/kg-treated mice; 60sulfa, sulfasalazine 60mg/kg-treated mice. Data are mean ± S.E.M. (n=7) Significance: $^{\#\#}$P <0.01, $^{\#\#\#}$P <0.001 versus normal mice and *P <0.05, **P <0.01 versus DSS control mice.

3.3. ROS overexpression activates MAPK including p38 and ERK1/2 and NF-κB

ROS overexpression activates MAPK including p38 and ERK1/2. The MAPK cascades on p38 and ERK1/2 are proving to play major roles in the regulation of intracellular metabolism and gene or protein expression in many parts, including disease, apoptosis, and cellular responses to external stresses. Furthermore, the phosphorylation of p38 and ERK1/2 MAPK are also implicated by leading to the activation of nuclear transcriptions factors. In this study, increased expressions of ERK1/2 and p38 were observed in colon of DSS control group ($P < 0.05$). As expected, SRE and sulfasalazine treatment were decreased via inhibition of their upstream c-Fos protein expression. Herein, SRE supplementation significantly attenuated activation of not ERK1/2 but p38 ($P < 0.05$) (Figure 4). As an important nuclear transcription factor, NF-κB controls several important physiological processes, as well as immune and inflammatory responses. Prior to activation, NF-κB is complexed with IκBα, an inhibitory protein keeping NF-κB inactive state in the cytoplasm. Induced by various stimuli, NF-κB is released and translocates from cytoplasm into the nucleus due to IκBα phosphorylation, ubiquitinylation, and degradation. Attempts to control mucosal inflammation through the use of agents that suppress the NF-κB pathway have achieved some success in mouse models. Similarly, SRE treatment has been shown to suppress the activation of NF-κB by inhibition of IκBα phosphorylation. Above all, SRE supplementation was much lower than sulfasalazine alone. ($P < 0.01$) (Figure 5).

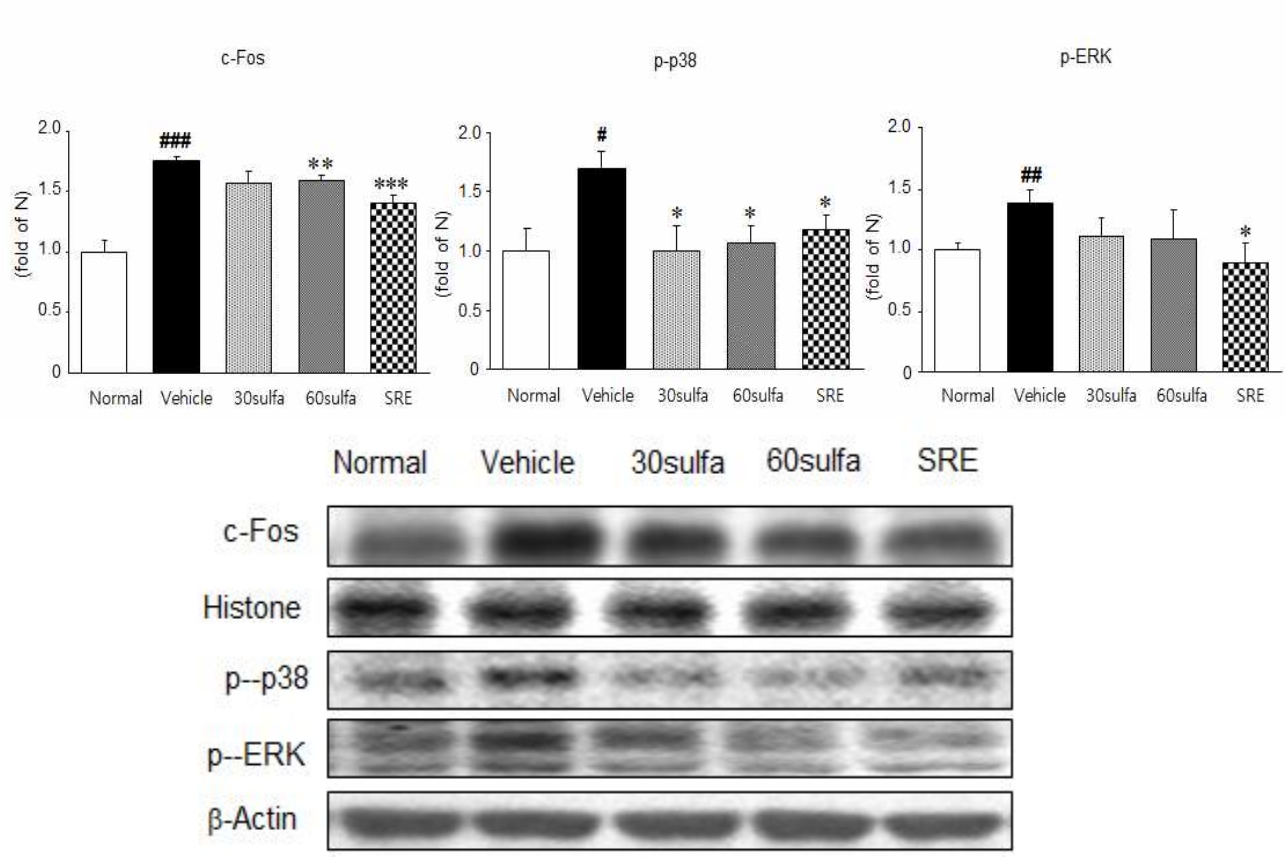


Figure 4. c-Fos, p-p38, and p-ERK protein expressions in DSS-induced colitis. Normal, normal mice; Vehicle, DSS control mice; 30sulfa; sulfasalazine 30 mg/kg-treated mice; 60sulfa, sulfasalazine 60 mg/kg-treated mice. Data are mean ± S.E.M. (n=7) Significance: $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ versus normal mice and $^{*}P < 0.05$, $^{***}P < 0.001$ versus DSS control mice.

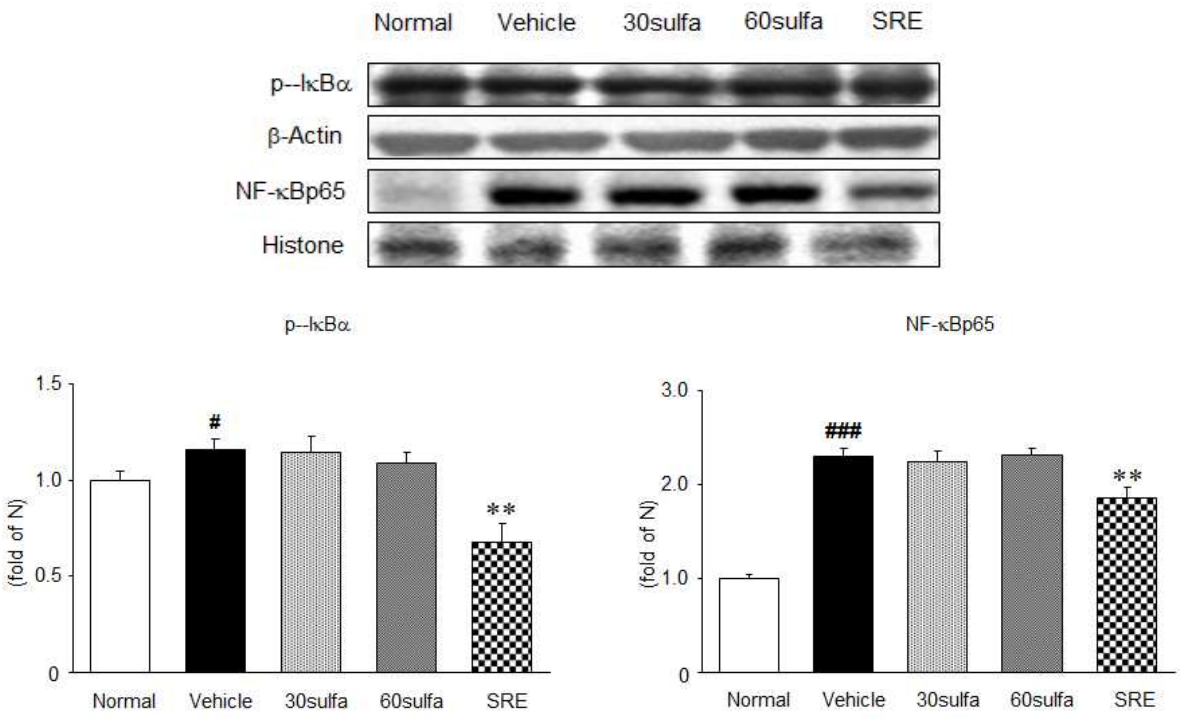


Figure 5. p- IκBα and NF-κBp65 protein expressions in DSS-induced colitis. Normal, normal mice; Vehicle, DSS control mice; 30sulfa; sulfasalazine 30 mg/kg-treated mice; 60sulfa, sulfasalazine 60 mg/kg-treated mice. Data are mean ± S.E.M. (n=7) Significance: #P <0.05, ###P <0.001 versus normal mice and **P <0.01 versus DSS control mice.

3.4. Pro-inflammatory

NF-κB participates in controlling the activation of various pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) and cytokines such as IL-1, and tumor necrosis factor-α (TNF- α), supporting a critical role in the pathogenesis of UC. As the result, the activation of NF-κB results in disruption of the oxidant/antioxidant balance. TNF-α is crucial in recruiting immune cells at the sites of damaged tissues and in the pathogenesis of UC. TNF- α and IL-1β as well as COX-2 and iNOS were noticeably amplified in DSS control group. Our results also indicate that SRE significantly inhibits the induction of COX-2 and iNOS expressions and the production of pro-inflammatory cytokines such as TNF-α and IL-1β. These protein levels were down-regulated to nearly normal levels (Figure 6). MCP-1 promotes monocyte infiltration into inflamed tissues and elevated levels of MCP-1 can be found in the intestinal mucosa of IBD patients. Accordingly, reduced MCP-1 by SRE treatment might reduce the attraction of inflammatory cells into the intestine and thereby decrease inflammatory responses (Figure 6). Several studies have reported that TNF- α causes an increase in endothelial permeability and then leads to neutrophils recruitment to the gut in part through stimulating the synthesis of intracellular adhesion molecule (ICAM). ICAM-1 is up-regulated at sites of inflammation. Similar to this study, DSS control group significantly increased compared with normal group, whereas SRE treatment showed a tendency to a decrease.

3.5. Apoptosis

Apoptosis is considered to prevent excessive accumulation of non-functional cells in the tissue. Excessive exposure of intestinal mucosa to ROS under inflammatory conditions increases epithelial cell apoptosis, which is likely to change epithelial barrier integrity and contributes to intestinal damage. Bcl-2 is

regarded as a pro-survival molecule, whereas Bax is a pro-apoptotic molecule as it binds to and antagonizes the effects of Bcl-2. Thus, Caspase-3 activation is an important event in cell death. SRE showed a substantial down-regulation of pro-apoptotic genes, such as Bax and Caspase 3 ($P < 0.001$, $P < 0.05$, resp). Meanwhile, the Bcl-2 protein expression during UC didn't show a marked difference as only a mild increase (Figure 7).

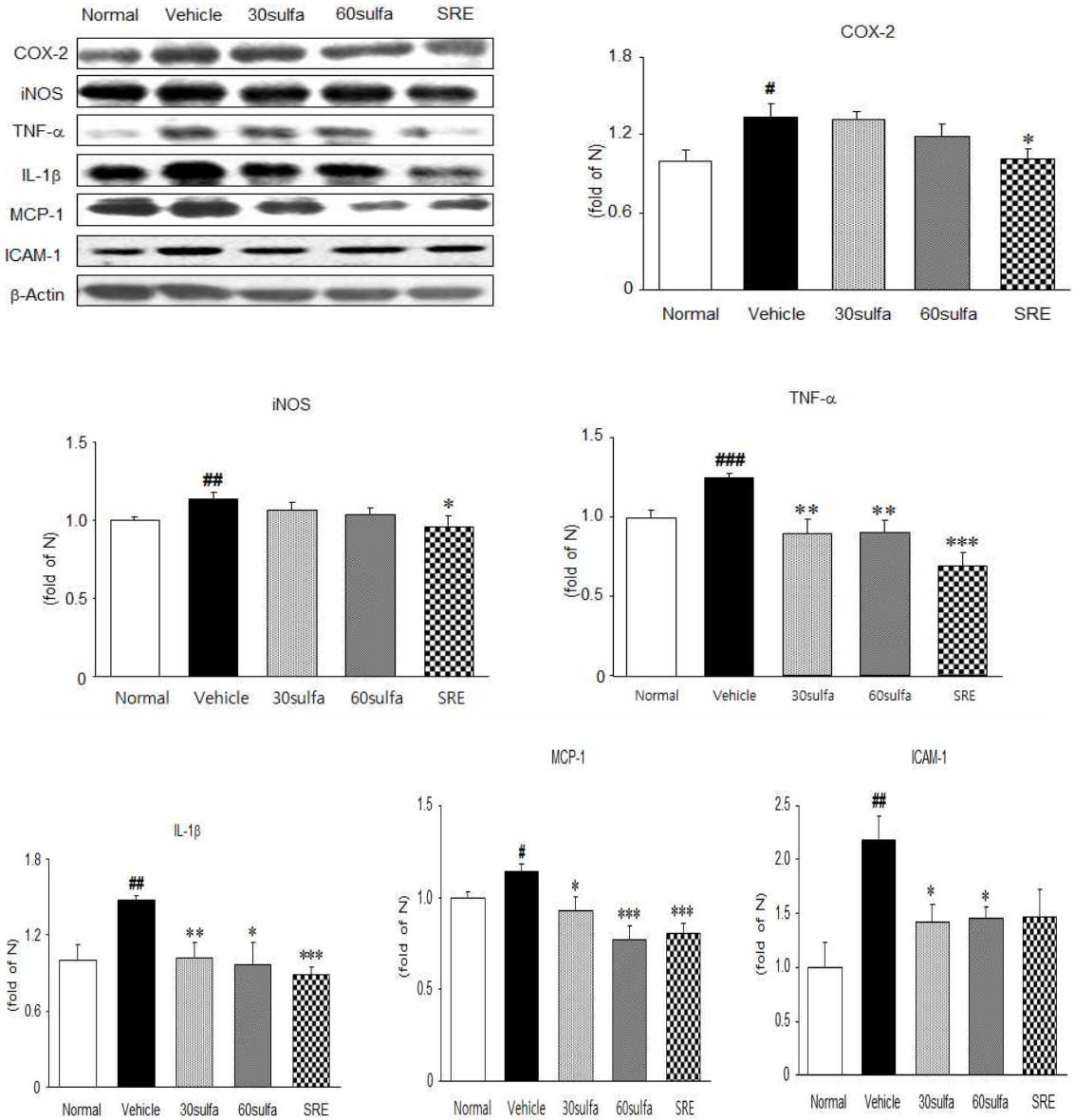


Figure 6. COX-2, iNOS, MCP-1, ICAM-1, TNF- , and IL-1 protein expressions in DSS-induced colitis. Normal, normal mice; Vehicle, DSS control mice; 30sulfa; sulfasalazine 30 mg/kg-treated mice; 60sulfa, sulfasalazine 60mg/kg-treated mice. Data are mean ± S.E.M. (n=7) Significance: ##P <0.01, ###P <0.001 versus normal mice and *P <0.05, ***P <0.001 versus DSS control mice.

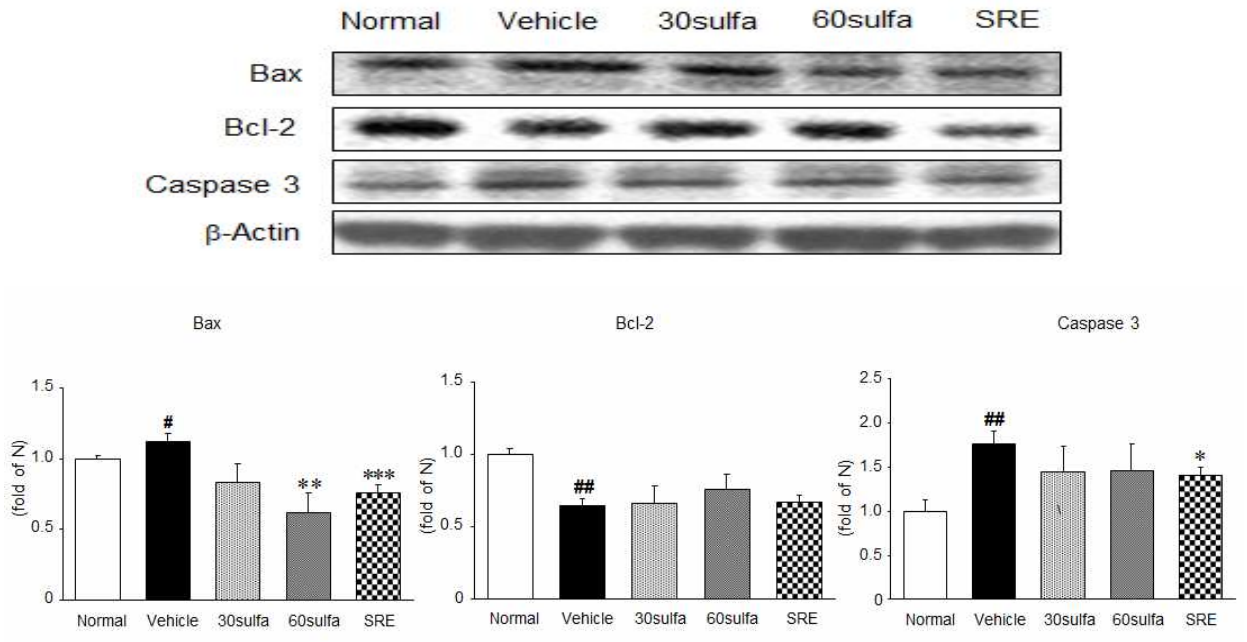


Figure 7. Bax, Bcl-2, and Caspase 3 protein expressions in DSS-induced colitis. Normal, normal mice; Vehicle, DSS control mice; 30sulfa; sulfasalazine 30 mg/kg-treated mice; 60sulfa, sulfasalazine 60mg/kg-treated mice. Data are mean ± S.E.M. (n=7) Significance: #P <0.05, ##P <0.01 versus normal mice and *P <0.05, ***P <0.001 versus DSS control mice.

5. Conclusion

Sulfasalazine or SRE administration led to anti-inflammatory effects, including reduced body weight loss and less shortening of the colon length, whereas there were few significance in body weight change and colon length among the DSS-treated groups. Colonic inflammation involves the disruption of the apparatus of colonic mucosa and ulceration, resulting in the infiltration of inflammatory cells such as inflammatory monocytes and macrophages and thickening of the lamina propria. Colons in normal group exhibited with normal crypt morphology, abundant goblet cells, no signs of mucosal thickening, and complete absence of ulceration. On the contrary, microscopic damage was lower in with SRE or sulfasalazine group than those in DSS control group. The DSS injury was markedly higher than those of normal group , whereas, the elevated level of serum ROS were significantly decreased lower to the levels of normal group both SRE and sulfasalazine . The protein expressions of both NOX4 and $p47^{phox}$, the markers of NADPH oxidase activity, in the colon were augmented in the DSS control group. However, SRE-treated group had significantly down-regulated NADPH oxidase, whereas sulfasalazine-treated group decreased without significance. Otherwise, Rac 1 expression showed a tendency to a decrease. In general, ROS are known to be neutralized by the endogenous antioxidant enzymes. SOD converts O^{2-} to H^2O^2, which is subsequently neutralized to water by Catalase and GPx-1/2 . The activity of enzymic antioxidants such as SOD, Catalase, and GPx-1/2 was decreased in DSS-induced group. Herein, SRE administration significantly increased the activity of SOD and Catalase except for GPx-1/2 (without significance). These findings indicated that SRE treatment of colitis may be reducing the extent of colonic injury by its antioxidant effect. Especially, SRE supplementation was superior to those when sulfasalazine alone.

The activation of NF-κB results in disruption of the oxidant/antioxidant balance. TNF-α is crucial in recruiting immune cells at the sites of damaged tissues and in the pathogenesis of UC. TNF- α and IL-1β as well as COX-2 and iNOS were noticeably amplified in DSS control group. Our results also indicate that SRE significantly inhibits the induction of COX-2 and iNOS expressions and the production of pro-inflammatory

cytokines such as TNF-α and IL-1β. These protein levels were down-regulated to nearly normal levels . MCP-1 promotes monocyte infiltration into inflamed tissues and elevated levels of MCP-1 can be found in the intestinal mucosa of IBD patients . Accordingly, reduced MCP-1 by SRE treatment might reduce the attraction of inflammatory cells into the intestine and thereby decrease inflammatory responses . Several studies have reported that TNF-α causes an increase in endothelial permeability and then leads to neutrophils recruitment to the gut in part through stimulating the synthesis of intracellular adhesion molecule (ICAM). ICAM-1 is up-regulated at sites of inflammation. Similar to this study, DSS control group significantly increased compared with normal group, whereas SRE treatment showed a tendency to a decrease. SRE showed a substantial down-regulation of pro-apoptotic genes, such as Bax and Caspase 3. Meanwhile, the Bcl-2 protein expression during UC didn't show a marked difference as only a mild increase.

In conclusion, the present findings suggest that SRE is an effective inhibitor of DSS-induced colitis in mice. The administration of SRE to mice treated with DSS attenuated acute inflammation and apoptosis in the colon. Above all, SRE may exert the similar protective effect by sulfasalazine alone. Accordingly, SRE may be a promising herbal formula combined with sulfasalazine in the treatment of ulcerative colitis field.

Acknowledgments

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