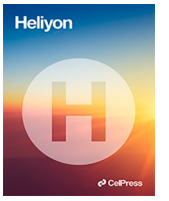
Heliyon 9 (2023) e12778

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Anti-inflammatory effect and signaling mechanism of 8-shogaol and 10-shogaol in a dextran sodium sulfate-induced colitis mouse model

Ha-Rim Kim, Eun-Mi Noh *, Seon-Young Kim

Jeonju AgroBio-Materials Institute, Jeonju, 54810, Republic of Korea

ARTICLE INFO

Keywords:
Inflammation
Dextran sulfate sodium
Colitis
Gingerol
8-Shogaol
10-Shogaol

ABSTRACT

Ethnopharmacological relevance: Ginger (*Zingiber officinale* Roscoe) has been used for food and applied in Ayurvedic medicine in India for thousands of years. With a reputation for strong anti-inflammatory properties, it has been used for to treat colds, migraines, nausea, arthritis, and high blood pressure in China and Southeast Asia. The physiological activity of ginger is attributed to its functional components, including gingerol and shogaol, and their derivatives.

Aim of the study: We aimed to investigate the effects of 8- and 10-shogaol and their bioactive signaling mechanisms in a dextran sodium sulfate (DSS)-induced colitis mouse model. The anti-colitis efficacy of 6-, 8-, and 10-derivatives of gingerol and shogaol was comparatively analyzed.

Materials and methods: Colitis was induced by providing mice with drinking water containing 5% DSS (w/v) for 8 days. The 6-, 8-, and 10-derivatives of gingerol and shogaol were orally administered for two weeks at a dose of 30 mg/kg. Changes in body weight and disease activity index were measured. The levels of pro-inflammatory cytokines, iNOS and COX-2, as well as the phosphorylation of NF-κB were analyzed using ELISA, PCR, or western blotting. Mucin expression and mRNA levels were measured using alcian blue staining and PCR, respectively. The tight-junction-associated proteins occludin and ZO-1 were assessed using immunohistological staining.

Results: The 6-, 8-, and 10-derivatives of gingerol and shogaol exhibited anti-inflammatory effects by regulating NF-κB signaling. Among the compounds administered, 10-shogaol was the most effective against DSS-induced inflammation. Comparative analysis of the chemical structure showed that shogaol, a dehydrated analog of gingerol, was more effective. 6- and 10-shogaol showed similar effects on DSS-induced morphological changes in the colonic mucus layer, mucin expression, and tight junction proteins.

Conclusions: 6-, 8-, and 10-Gingerol and 6-, 8-, and 10-shogaol significantly improved the clinical symptoms and intestinal epithelial barrier damage in DSS-induced colitis in mice. The derivatives effectively inhibited DSS-induced inflammation through the regulation of NF-κB signaling. Moreover, 10-shogaol showed the most potent anti-inflammatory effect among the six compounds used in this study. The results indicate that 8- and 10-shogaol, both main ingredients in ginger, may serve as therapeutic candidates for the treatment of colitis.

* Corresponding author. Jeonju AgroBio-Materials Institute, 111-27 Wonjangdong-gil, Deokjin-gu, Jeonju, 54810, Republic of Korea.
E-mail addresses: loiter@jami.re.kr (E.-M. Noh), seon02@jami.re.kr (S.-Y. Kim).

https://doi.org/10.1016/j.heliyon.2022.e12778
Received 10 November 2022; Received in revised form 23 December 2022; Accepted 30 December 2022
Available online 5 January 2023

1. Introduction

Inflammatory bowel disease (IBD) is characterized by chronic recurrent intestinal inflammation, and includes ulcerative colitis (UC) and Crohn's disease [1]. UC causes persistent inflammation and ulcers throughout the colon and rectal mucosa [2]. The main symptoms of UC are weight loss, diarrhea, and rectal bleeding [3]. The upregulation of specific proteins, such as cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), influences immune dysregulation in UC [4,5]. In addition, nuclear factor-kappa B (NF-κB) participates in the pathogenesis of UC by controlling the activation of various pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) [6,7].

Despite research on the pathogenesis of UC and efforts to discover new therapeutics, a curative treatment has not yet been developed. Pharmacological treatment of IBD is frequently accompanied by complications or side effects. Long-term use of drugs such as aminosalicylate and corticosteroids for the treatment of UC is limited because of their considerable side effects [8]. Therefore, the development of safer and more effective drugs is necessary. Many studies on natural products and their functional compounds have been conducted during efforts to identify a potential treatment for UC [9-11].

Ginger (*Zingiber officinale* Roscoe) has long been used as a traditional food in worldwide [12]. It has been used as a diuretic, an antiemetic, and an herbal medicine for various diseases, such as abdominal pain, diarrhea, nausea, asthma, and arthritis [13,14]. In a dextran sodium sulfate (DSS)-induced mouse colitis model, ginger delayed colitis progression through weight loss, decreased the disease activity index (DAI), and regulated iNOS expression [15]. Allah et al. reported that ginger administration significantly prevented acetic acid-induced colitis by reducing NF-κB expression and colonic levels of TNF-α, IL-10, and total peroxide [16]. Ginger is rich in active phenolic and terpene compounds such as gingerol, shogaol, and paradol. These compounds exhibit antioxidant, anti-inflammatory, antibacterial, and anticancer properties [17]. Gingerol is abundant in fresh ginger but is converted to shogaol through dehydration at high temperatures. The double bond of shogaol has been reported to increase its biological activity by facilitating free-radical scavenging [8]. Because gingerol and shogaol are structurally similar, various studies have compared their pharmacological effects [18,19].

Several studies have reported that gingerols and shogaols ameliorated IBD symptoms via their antioxidant and anti-inflammatory activities [20–22]. Zhang et al. showed that 6-, 8-, and 10-gingerol improved DSS-induced UC in mice through antioxidant and anti-inflammatory effects [23]. The results showed that 6-, 8-, and 10-gingerol significantly reduced elevated DSS-induced TNF-α and IL-1β levels in the serum of UC mice. Moreover, Hui et al. reported that 6-shogaol repairs mucosal tissue damage in UC mice by modulating Notch signaling [24]. To the best of our knowledge, no study has reported the use of 8- and 10-shogaol for the treatment of colitis. Based on previous reports, 8- and 10-shogaol are expected to exert similar pharmacological effects in the treatment of UC. Therefore, we investigated the anti-colitis effect and molecular mechanisms of 8- and 10-shogaol using a DSS-induced colitis mouse model and compared the efficacy of 6-, 8-, and 10-gingerol and 6-, 8-, and 10-shogaol.

2. Methods and materials

2.1. Reagents

6-Gingerol (Cat No. CFN99931), 8-gingerol (Cat No. CFN99131), and 10-Gingerol (Cat No. CFN99132) and 6-shogaol (Cat No. CFN99531), 8-shogaol (Cat No. CFN92299), and 10-shogaol (Cat No. CFN92300) (purity: ≥98%) were purchased from ChemFaces Biochemical Co., Ltd. (Wuhan, China). The DSS was obtained from MP Biomedicals (Cat No. 9011-18-1; Irvine, CA, USA). Commercial enzyme-linked immunosorbent assay (ELISA) kits were provided by R&D Systems (Minneapolis, MN, USA). In this study, we used anti-iNOS (Cat No. #13120), anti-phospho–NF–κB (Cat No. #3031), anti–NF–κB (Cat No. #3033), and anti-β-actin (Cat No. #3700) antibodies from Cell Signaling Technology (Danvers, MA, USA) and anti-COX-2 (Cat No. ab15191), anti-occludin (Cat No. ab216327) and anti-ZO-1 (Cat No. ab216880) antibodies from Abcam (Cambridge, UK). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise indicated.

2.2. Animals

Specific pathogen-free (SPF)-grade BALB/c mice (male, 5-week-old) were purchased from Damul Science (Daejeon, Korea) and acclimated for 1 week. The mice were housed in a room maintained under a 12 h light/dark cycle at 22 ± 2 °C and a relative humidity of 55 ± 5%. All experimental procedures were approved by the Animal Care Committee of Jeonju AgroBio-Materials Institute (JAMI IACUC 2021001, Jeonju, Korea).

2.3. Experimental groups

The animals were divided into seven groups (five mice/group) according to the treatment: N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS) groups. The samples were dissolved in olive oil and orally administered at 30 mg/kg once daily for 2 weeks before DSS treatment. The N and NC groups received olive oil as a vehicle.

2.4. Induction of colonic inflammation

Acute colitis was induced using DSS. Mice were allowed to consume drinking water containing 5% (w/v) DSS (36–50 kDa) for 8 consecutive days and were sacrificed one day after this period. After treatment with DSS had begun, the body weight and disease activity index (DAI) were measured each day before administration of the samples. The assigned DAI score was dependent on the stool characteristics and ranged from 0 (normal) to 4 (maximal disease activity) [25] (Table 1).

2.5. Evaluation of biomarkers in serum and colon tissue

The serum levels of tumor necrosis factor (TNF)-α (Cat No. #MTA00B), interleukin (IL)-1 β (Cat No. #MLB00C), IL-6 (Cat No, #M6000B), and interferon (IFN)-gamma (Cat No. #MIF00) were determined using commercial ELISA kits, in accordance with the manufacturer's instructions. The mRNA expression levels of the genes *iNOS*, *COX-2*, *MUC2*, and *MUC3* were determined using quantitative real-time reverse-transcription PCR (qRT-PCR). Colon tissue was intercepted and homogenized in ice-cold TRIzol reagent (Cat No. TR118; MRC, Cincinnati, OH, USA). Subsequently, cDNA was synthesized by reverse transcription of 1 μg of RNA samples using the BioFact™ 2 × RT Pre-Mix (Cat No. BR441-096; BioFact, Daejeon, Korea). RT-qPCR was performed using a TB Green® Premix Ex Taq™ II kit (Cat No. RR820A; TaKaRa, Japan). The primer sequences used are listed in Table 2. Relative mRNA levels were calculated using the comparative Ct method with β-actin as the reference gene. The colon tissues were homogenized in ice-cold RIPA buffer (Cat No. 89901; Invitrogen, CA, USA) containing protease and phosphatase inhibitor cocktail (Cat No. 78440; Thermo Fisher Scientific, Waltham, MA, USA). Protein samples (20 μg per lane) were separated using SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Cat No. #1620174; Bio-Rad, Munich, Germany). The blots were analyzed using the specified antibodies and developed using an enhanced chemiluminescence (ECL) system (Cat No. RPN2235; Amersham, Buckinghamshire, UK). Quantitative analysis was performed using densitometric scanning (Amersham Imager 600, GE Healthcare, Buckinghamshire, UK).

2.6. Histology

Mouse colon tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections were prepared into 4 μm thick slices stained with hematoxylin and eosin (H&E) or alcian blue (AB). For immunohistochemical (IHC) analysis of ZO-1 and occludin, the sections were deparaffinized, rehydrated, and incubated overnight at 4 °C with antibodies, followed by treatment using the anti-rabbit Envision Plus Polymer Kit (Cat No. K4065; Dako, Glostrup, Denmark). The sections were stained with hematoxylin. The morphological features of the stained sections and the IHC expression were observed using an optical microscope (Olympus, Tokyo, Japan) and photographed. Histological scores were measured and calculated as the averages of the grades for each parameter, as previously reported [26].

2.7. Statistical analysis

Data are expressed as the means ± standard deviation (SD), and all statistical analyses were performed using the Sigmaplot v16.0 software (Systat Software Inc., San Jose, CA, USA). Statistical analysis was performed to identify the differences, followed by one-way analysis of variance (ANOVA) and Duncan's multiple comparison test. A value of $P < 0.05$ was considered to indicate significant difference. Exact *P*-values for each group were provided in Supplementary File.

3. Results

3.1. Preventive effect of 8- and 10-shogaols on DSS-induced colitis

The chemical structures of the compounds used in this study are shown in Fig. 1A. According to previous reports, DSS-induced mice show disease progression to weight loss, colon shortening, and bloody stool [27]. After oral administration of 6-, 8-, and 10-gingerol and 6-, 8-, and 10-shogaol at a dose of 30 mg/kg for two weeks, drinking water containing 5% DSS was supplied for 8 days to induce UC. During the two-week treatment period, none of the compounds induced body weight changes (Fig. 1B), eating disorders, or abnormal behaviors (data not shown). In the DSS-treated (NC, negative control) group, body weight (Fig. 2A and B) and colon length (Fig. 2E and F) were significantly decreased, and DAI was increased compared to the normal (N) group (Fig. 2C and D). In contrast, administration of 6-,8-, and 10-gingerol (6G, 8G, and 10G experimental groups) and 6-,8-, and 10-shogaol (6S, 8S, and 10S

Table 1
Disease Activity Index (DAI) score.

Weight Loss (%)	Shape of stool	Occult Blood/Bloody Stool	Score
0	Normal	Negative	0
1–5	Soft stool	Negative	1
6–10	Soft stool	Occult blood	2
11–15	Diarrhea	Occult blood	3
>15	Diarrhea	Bloody stool	4

Table 2
Primer sequences.

Gene	Sequences
iNOS	F: 5′-CGAAACGCTTCACTTCCAA-3′
	R: 5′-TGAGCCTATATTGCTGTGGCT-3′
COX-2	F: 5′-TTTGGTCTGGTGCCTGGTC-3′
	R: 5′-CTGCTGGTTTGGAATAGTTGCTC-3′
MUC2	F: 5′-GCAGTCCTCAGTGGCACCTC-3′
	R: 5′-CACCGTGGGGCTACTGGAGAG-3′
MUC3	F: 5′-CGTGGTCAACTGCGAGAATGG-3′
	R: 5′-CGGCTCTATCTCTACGCTCTC-3′
β-actin	F: 5′-CGGTTCCGATGCCCTGAGGCTCTT-3′
	R: 5′-CGTCACACTTCATGATGGAATTGA-3′

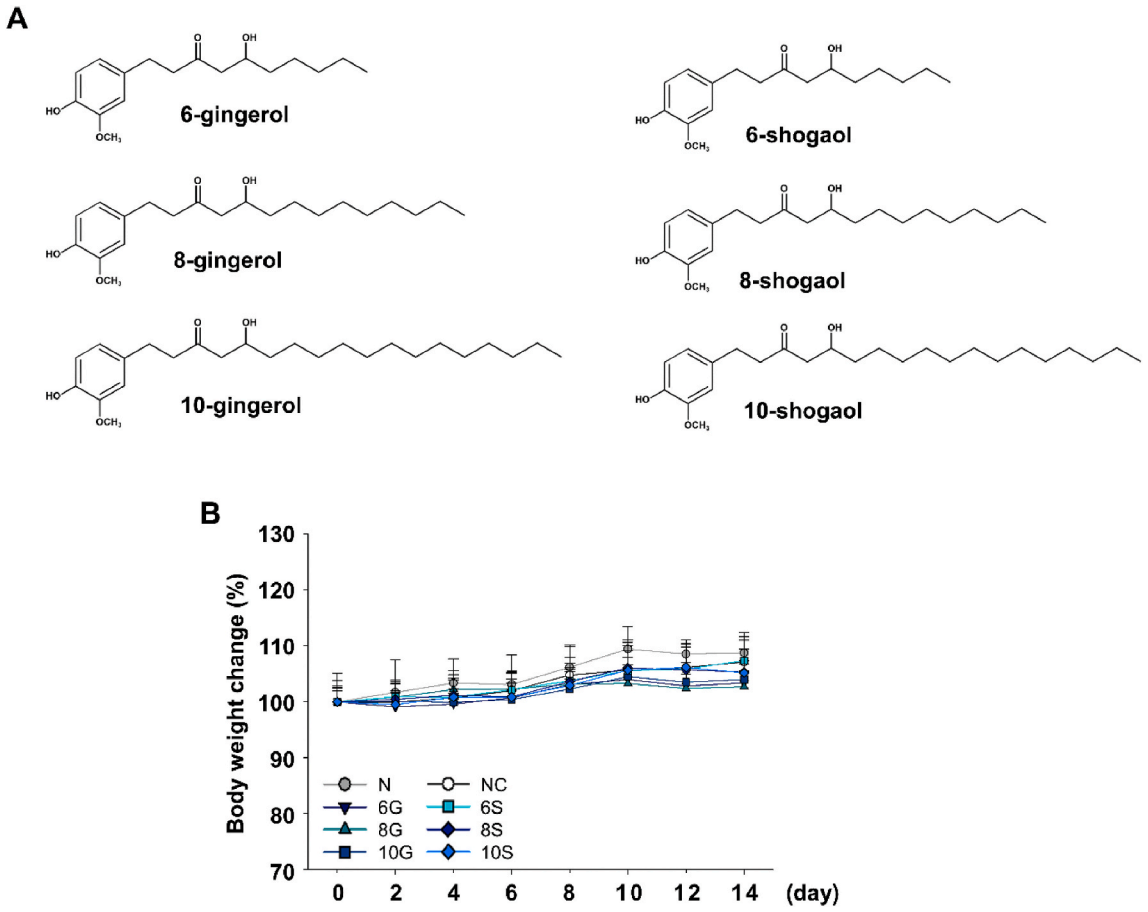


Fig. 1. Structure of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols. (A) The chemical structures of ginger compounds used in this study. (B) Changes in body weight of mice. 6-, 8-, and 10-Gingerols and 6-, 8-, and 10-shogaols were orally administered (30 mg/kg) for 2 weeks before DSS treatment. The body weight was assessed. The groups were: N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS). All values represent the mean ± SD (n = 5 per group). The data were analyzed by Duncan's multiple comparison test, but no significant differences were observed.

experimental groups) remarkably prevented DSS-induced weight loss (Fig. 2B), colon length reduction (Fig. 2E and F), and increase in DAI (Fig. 2C and D) compared to the NC treatment. The 8S and 10S groups showed greater effect than those treated with the other compounds, and 10S significantly inhibited DSS-induced weight loss compared with 10G (Fig. 2B). The DSS-induced decrease in colon length was significantly inhibited in all groups treated with gingerol and shogaol derivatives compared to that in the NC group. In addition, analogs with the same alkyl side chain were more effective in the shogaol-treated group than in the gingerol-treated group (Fig. 2D and C). Specifically, the 8S group showed significant inhibition of DSS-induced colon length reduction compared with the 8G group (Fig. 2E and F). Therefore, 8S and 10S produced a therapeutic effect on DSS-induced UC, similar to other analogs.

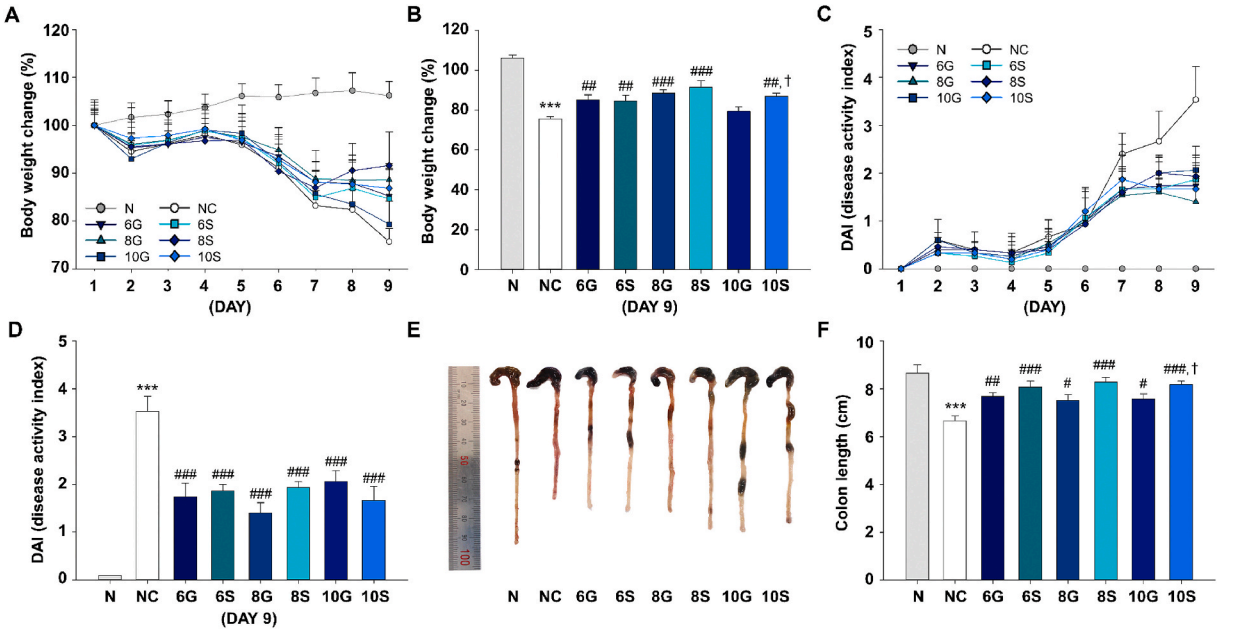


Fig. 2. Effects of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols on body weight, DAI and colon length in DSS-induced colitis. (A) Change in body weight. (B) Change in body weight at 9 days in induced colitis. (C) Disease activity index. (D) Disease activity index at 9 days in induced colitis. (E) Colon paragraph. (F) Colon length. N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS) groups are shown. All values represent the mean ± SD (n = 5 per group). The data were analyzed by Duncan's multiple comparison test. $^{***}P < 0.001$ versus N group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus NC. $^{\dagger}P < 0.05$ versus gingerol of equal alkyl chain length.

3.2. Effect of 8- and 10-shogaols on DSS-induced pro-inflammatory cytokine production

Pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IFN-γ, have been reported to play key roles in the pathogenesis of UC [28]. DSS significantly increased the levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IFN-γ in mouse serum (Fig. 3). After administration of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols for approximately 2 weeks, the production levels of IL-1β, IL-6, and IFN-γ decreased in all groups. Furthermore, DSS-induced TNF-α production was significantly decreased in the 8S and 10S groups compared to that in the NC group, and the 10S group showed the most effective results (Fig. 3A). Among the target cytokines, the IL-1β, IL-6, and IFN-γ levels were the lowest in the 10G, 10G, and 10S groups, respectively (Fig. 3B–D).

3.3. Effects of 8- and 10-shogaols on iNOS and COX2 expression and NF-κB signaling pathway in DSS-induced colitis

iNOS induces an immune response mediated by NO production, while COX-2 is an inducible enzyme induced by pro-inflammatory cytokines [4,5]. These factors are strongly induced in DSS-induced UC [28]. The DSS-treated group showed significant upregulation of iNOS and COX-2 mRNA and protein expression compared to the NC group (Fig. 4A). Mice treated with 6-,8-, and 10-gingerols and 6-, 8-, and 10-shogaols showed remarkably downregulated iNOS and COX-2 mRNA and protein expression compared with the DSS-induced control mice (Fig. 4A and B). Among the treated compounds, 10S was the most effective in downregulating DSS-induced COX-2 expression (Fig. 4C and D). Previous studies have shown that NF-κB is an important transcription factor that acts as a pivotal mediator of the inflammatory response through the activation of iNOS, COX-2, and pro-inflammatory cytokines [7]. We investigated whether the anti-inflammatory effects of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols involved NF-κB signaling pathways in the colitis mouse model. Treatment with DSS stimulated phosphorylation of NF-κB in colonic tissue; however, this effect was significantly reduced by the administration of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols (Fig. 4C and D). In particular, 10S showed the highest effectiveness in blocking DSS-induced NF-κB phosphorylation (Fig. 4C and D), similar to its inhibitory effect on COX-2 expression. These results indicate that the anti-inflammatory effects of 8S, 10S, and their analogs are related to the NF-κB signaling pathway in DSS-induced colitis.

3.4. Effects of 8- and 10-shogaols on colonic histological damage and mucin in DSS-induced colitis

The histopathology of the colon was assessed using H&E and Alcian blue (AB) staining of tissue sections. The DSS-treated group showed deterioration of the crypts, loss of goblet cells, submucosal edema, and infiltration of inflammatory cells compared to the N group (Fig. 5A). All six compounds prevented DSS-induced intestinal damage, significantly protected against colonic inflammation,

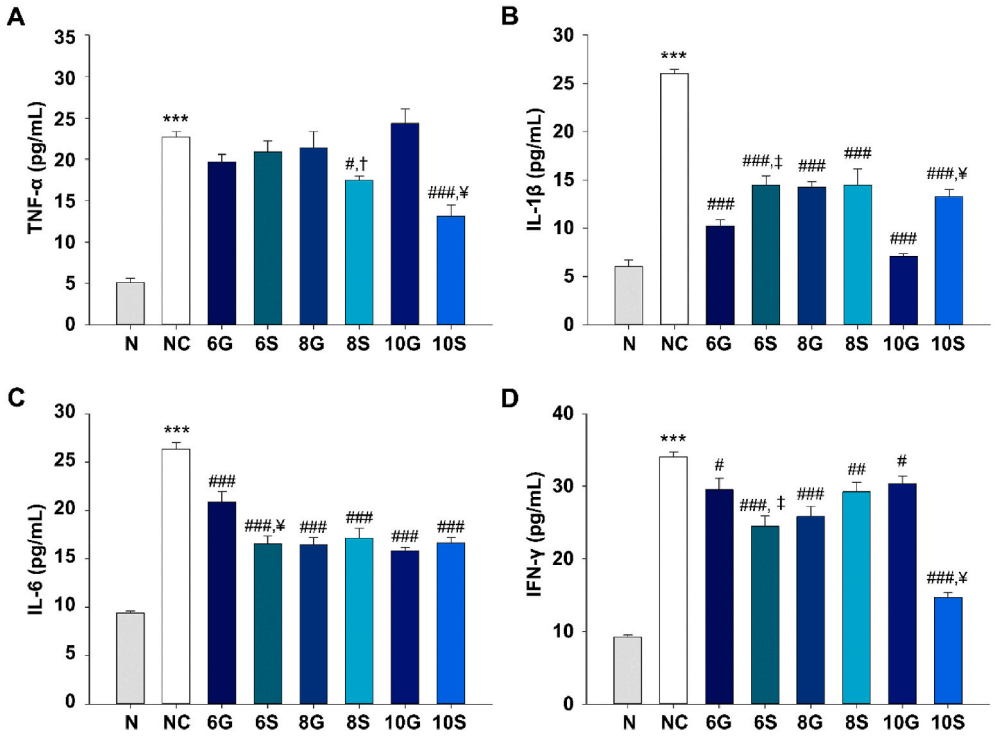


Fig. 3. Effects of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols on pro-inflammatory cytokines in DSS-induced colitis. Levels of (A) tumor necrosis factor (TNF)-α, (B) interleukin (IL)-1β, (C) IL-6, and (D) interferon (IFN)-gamma in DSS-induced colitis mouse serum. N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS) groups are shown. All values represent the mean ± SD (n = 5 per group). Data were analyzed using Duncan's multiple comparison test. $^{***}P < 0.001$ versus N group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus NC. $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$ and $^{¥}P < 0.001$ versus gingerol of equal alkyl chain length.

and significantly reduced the histology score (Fig. 5A and C). Based on the histological scores, the compounds can be arranged in the following order according to their protective effect: 6S > 10S > 8S > 6G > 10G > 8G (Fig. 5C). AB staining was performed to determine mucus levels in intestinal goblet cells. Consistent with the anti-inflammatory effect of the test compounds, the groups administered gingerol and shogaol derivatives showed significantly increased AB-stained mucus in colitis compared to the DSS-treated group (Fig. 5B). Intestinal epithelial cells are affected by the main constituents of the mucus layer (MUC2) and membrane-bound mucin (MUC3) in the colon. The reduced levels of MUC2 and MUC3 mucin by DSS were attenuated upon treatment with 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols; 6S and 10S were the most effective in regulating DSS-induced MUC2 and MUC3, respectively (Fig. 5D and E).

3.5. Effects of 8- and 10-shogaols on intestinal barrier function in DSS-induced colitis

Tight junction (TJ) proteins are essential for the maintenance of the intestinal epithelial barrier. Therefore, the expression of the TJ proteins ZO-1 and occludin was assessed using immunohistochemistry. In the normal group, ZO-1 and occludin were uniformly expressed around the superficial membrane and the crypts of the epithelium. However, DSS treatment compromised the colon barrier integrity and crypt structure, resulting in decreased TJ protein expression. In contrast, the administration of gingerol and shogaol derivatives caused the preservation and maintenance intestinal barrier integrity associated with high expression levels of ZO-1 and occluding along epithelial membranes and intact colonic crypts (Fig. 6A and B). Protection against DSS-induced loss of TJ proteins was more significant in the 10S group than in the other groups (Fig. 6C and D). These results indicate that the administration of 10S and 8S protects against DSS-induced disruption of intestinal barrier function by preventing the loss of TJ proteins.

4. Discussion

In this study, we demonstrated, for the first time that 8-shogaol and 10-shogaol produced promising therapeutic effects in mice with DSS-induced colitis. The animal model of DSS-induced colitis is the most widely used chemically inducible model of intestinal inflammation because of its similarities to UC [29,30]. Administration of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols for 2 weeks before DSS treatment significantly suppressed clinical symptoms of colitis and protected against mucosal damage. Consistent with our results, 6-, 8-, and 10-gingerol, and 6-shogaol have been previously reported to improve DSS-induced colitis in mice [23,31].

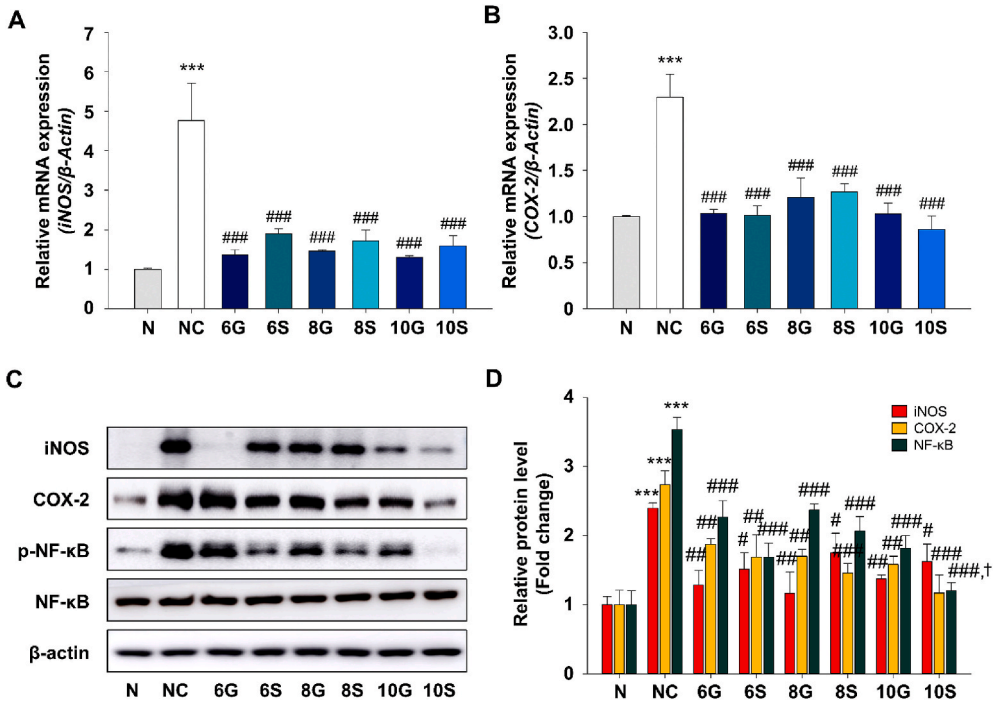


Fig. 4. Effect of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols on NF-κB signaling pathway in DSS-induced colitis. The mRNA levels of (A) iNOS and (B) COX-2 were analyzed using quantitative real-time PCR. The protein levels of (C) iNOS and COX-2, as well as phosphorylation of NF-κB in the colon were analyzed by immunoblotting. (D) Densitometry of each band quantified using image analysis. The band density of iNOS and COX-2 was normalized using β-actin, and phosphorylated NF-κB normalized to total NF-κB. N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS) groups are shown. All values represent the mean ± SD (n = 5 per group). Data were analyzed using Duncan's multiple comparison test. $^{***}P < 0.001$ versus N group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus NC. $^{\dagger}P < 0.05$ versus gingerol of equal alkyl chain length. The uncropped images of (C) were referred in Supplemental file.

However, the protective effects of 8- and 10-shogarol against colitis have not yet been established. The effects of 8- and 10-shogarol were evaluated using a mouse model of DSS-induced colitis and compared to that of their analogs. We found that 8- and 10-shogarol ameliorated colitis, as indicated by effects on weight loss, colon length, histological characteristics, and DAI scores. Specifically, 8- and 10-shogaol more effectively improved colonic length shortening and DAI scores than did the other analogs. The shogaol derivatives were more effective than the corresponding gingerol derivative in treating the symptoms of UC caused by DSS; our results were similar to those in previous reports [18,32].

Pro-inflammatory cytokines, including IL-1β, TNF-α, IFN-γ, and IL-6, are important mediators of inflammation and are elevated in DSS-induced colitis [28]. IL-1β plays a critical role in the pathogenesis and progression of acute and chronic inflammation through neutrophil activation [33]. TNF-α disrupts the epithelial barrier to induce apoptosis of epithelial cells and secretion of chemokines from intestinal epithelial cells; it is a major target for the initial treatment of colitis and colon lesions [34]. IL-1β and IL-6 are the key mediators of UC. IL-1β receptor antagonists and anti-murine IL-1β antibodies have been reported to inhibit pathological symptoms, including colon inflammatory cell infiltration and IL-6 mRNA expression, in an animal model of colitis [35,36]. 6-Gingerol, 8-gingerol, and 10-gingerol reduce IL-1β and TNF-α levels in the serum of rats [23]. 6-Shogaol inhibited the production of TNF-α and IL-6 in human mast cells and endometriotic lesions [22]. In this study, the increase in inflammatory cytokines induced by DSS intake was inhibited by the administration of 6-, 8-, and 10-gingerol and 6-, 8-, and 10-shogaol. When comparing the inhibitory effects of DSS-induced pro-inflammatory cytokine production between analogs, 10-shogaol was the most effective for TNF-α and IFN-γ, while10-gingerol was the most effective for IL-6 and IL-1β. These results suggest that increasing the alkyl chain length of gingerol or shogaol may produce a potent effect on the improvement of DSS-induced inflammation.

iNOS and COX-2 are signaling factors that induce oxidative stress and are closely related to the initiation and development of inflammation [4]. Krieglstein et al. reported that DSS-induced colitis was significantly attenuated in iNOS-knockout mice [37]. In addition, COX-2 is expressed at low levels in the healthy intestine but is significantly elevated in Crohn's disease and UC [5]. In this study, 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols inhibited DSS-induced iNOS and COX-2 expression in colon tissue, and 10-shogaol completely suppressed the DSS-induced increase in the protein and mRNA expression of COX-2. Consistent with our results, 10-shogaol, 8-shogaol, and 10-gingerol have been reported to inhibit COX-2 with the compounds arranged in descending order of inhibitory activity as follows: 10-shogaol > 8-shogaol >10-gingerol [38]. Collectively, our results suggest that the potential for 8- and 10-shogaol to be used as therapeutic agents for improving inflammation by modulating COX-2 activity.

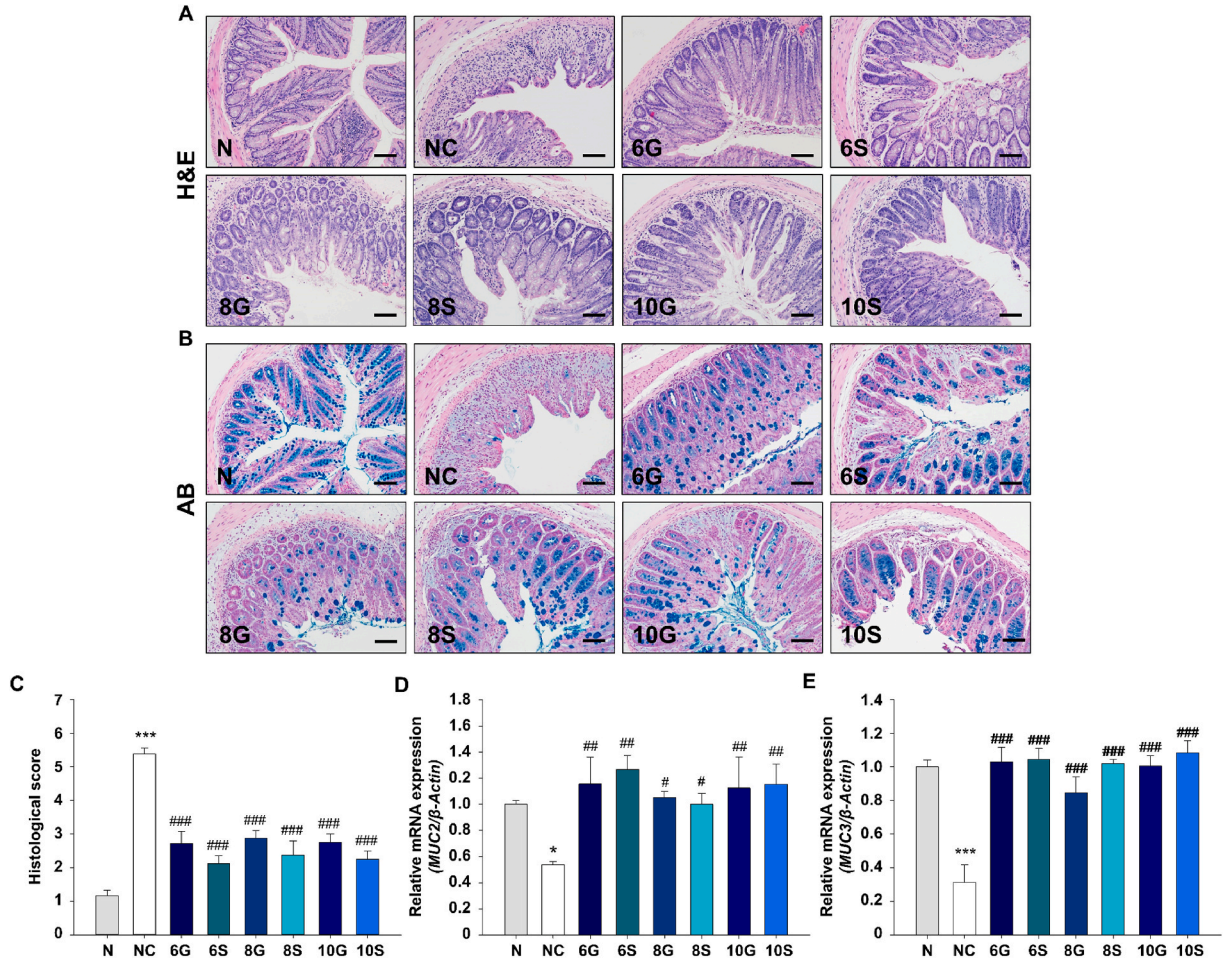


Fig. 5. Effect of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols on histological evaluation in DSS-induced colitis. Representative (A) H&E staining and (B) Alcian blue staining images of colon tissue. Magnification: 200× and, scale bar: 50 μm. The (C) histological score was assessed using H&E staining (n = 8 per group). The mRNA levels of (D) MUC2 and (E) MUC3 were analyzed in colon tissue using quantitative real-time PCR (n = 3 per group). N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS) groups are shown. All values represent the mean ± SD (n = 5 per group). Data were analyzed using Duncan's multiple comparison test. $^{***}P < 0.001$ versus N group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus NC. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In addition, NF-κB activation is an important transcription factor in IBD pathogenesis. It has been shown in regulate the expression of pro-inflammatory cytokines and inflammatory proteins such as COX-2 and iNOS [6]. Ginger extract and 6-gingerol, a functional component of ginger, have been reported to have protective effects in inflammatory arthritis and DSS-induced UC by regulating the NF-κB pathway [21,39]. We found that administration of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols inhibited DSS-induced phosphorylation of NF-κB in colonic tissue, with 10-shogaol being the most effective.

Changes in pathological structures, such as crypt destruction, ulceration, inflammatory cell infiltration, and depletion of colonic mucus, are important factors in assessing and determining the effectiveness of colitis treatment [40]. Ginger and 6-gingerol alleviate lipopolysaccharide-induced intestinal barrier damage [41], and shogaol suppresses the expression of intestinal stem cell markers in colitis [31]. In addition, 6-, 8-, and 10-gingerol have been shown to prevent DSS-induced colonic tissue damage, such as crypt destruction, goblet cell depletion, and severe mucosal ulceration [23]. In our study, DSS treatment resulted in inflammatory cell infiltration and goblet cell loss, whereas 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols effectively protected against DSS-induced intestinal damage. The mucus layer is important in protection, secretion, and absorption. Epithelial mucins are a large group of high molecular weight glycoproteins (comprising more than 20 identified genes) that impart viscosity to the mucus [42]. Mucin expression was found to exhibit a negative correlation with the severity of UC, and deficiency of MUC2 and MUC3 expression was observed in the goblet cells of UC patients [43]. The DSS treatment group showed a significant reduction in MUC2 and MUC3, and pretreatment with 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols significantly prevented it. 6-Shogaol and 10-shogaol exhibited the strongest effects on MUC2 and MUC3, respectively. These results suggest that shogaol is more effective in controlling MUC than gingerol; however, the effect was not dependent on the alkyl side chain of each derivative. The TJ complex is a protein cluster that forms a physiologically

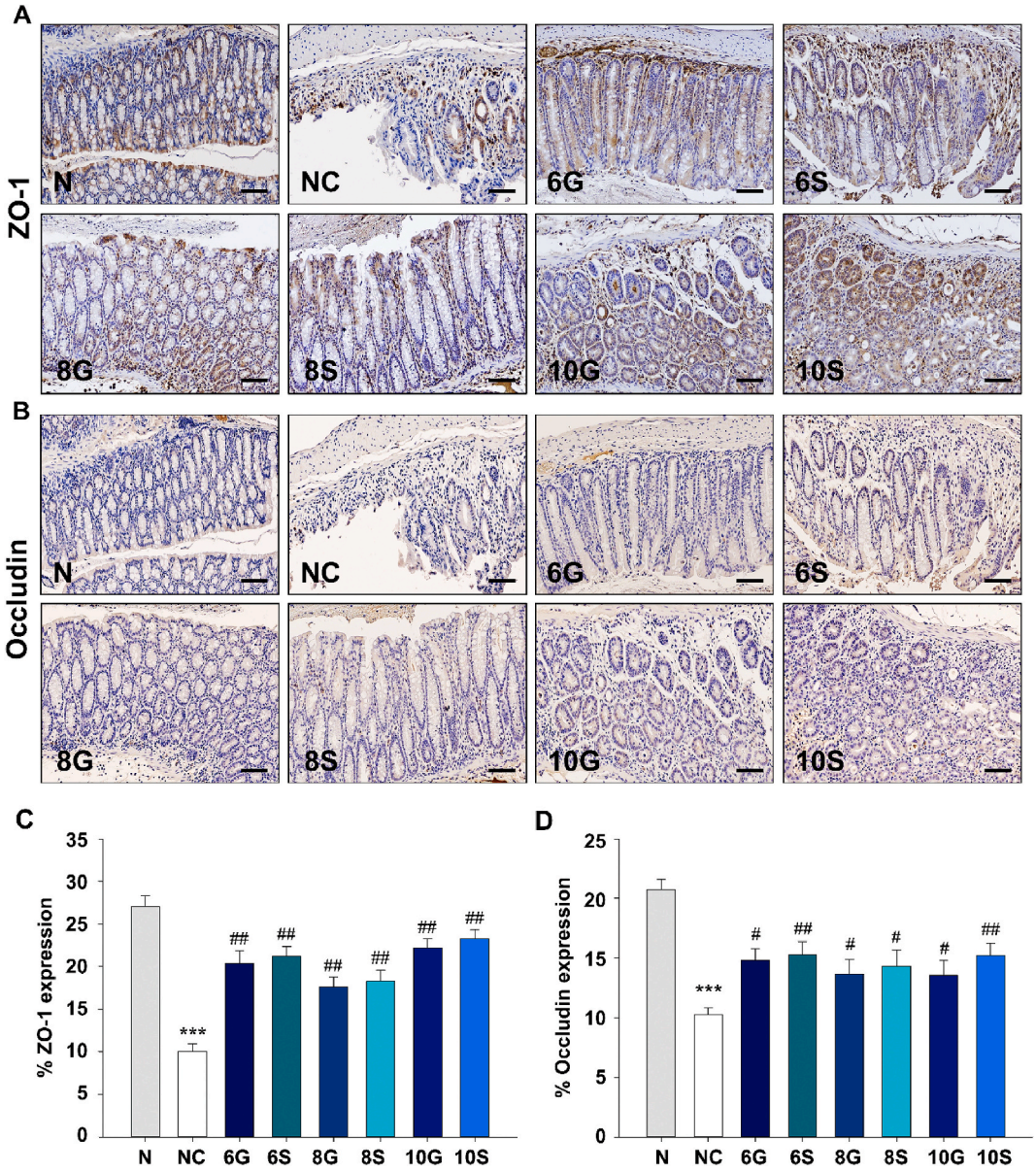


Fig. 6. Effects of 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols on occludin and ZO-1 in DSS-induced colitis. Immunohistochemical analysis of tight junction proteins (A) ZO-1 and (B) occludin in the colon tissue. Magnification: 200× and, scale bar: 50 μm. (C, D) Histological score was assessed (n = 4 per group). N (normal), NC (5% DSS; negative control), 6G (6-gingerol + 5% DSS), 8G (8-gingerol + 5% DSS), 10G (10-gingerol + 5% DSS), 6S (6-shogaol + 5% DSS), 8S (8-shogaol + 5% DSS), and 10S (10-shogaol + 5% DSS) groups are shown. All values represent the mean ± SD (n = 5 per group). Data were analyzed using Duncan's multiple comparison test. $^{***}P < 0.001$ versus N group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus NC.

active barrier in intestinal epithelial cells, and ZO-1 and occludin are key proteins of TJ [44]. Ginger extract and 6-gingerol have been reported to regulate TJ proteins [45,46]. In this study, 6-, 8-, and 10-gingerols and 6-,8-, and 10-shogaols inhibited DSS-induced ZO-1 and occludin expression in colon tissue, with 10-shogaol being the most effective. These results suggest that 10-shogaol is the most effective among gingerol and shogaol compounds in protecting against DSS-induced intestinal barrier injury.

Since the DSS-induced colitis model causes acute colitis, we designed a pretreatment model with gingerol and shogaol for 2 weeks to evaluate the effect. In order to confirm the effect of this experimental design, further studies using other colitis models and comparative analysis with previous studies are needed.

5. Conclusions

In this study, 6-, 8-, and 10-gingerols and 6-, 8-, and 10-shogaols showed significant anti-inflammatory effects through the NF-κB signaling pathway and intestinal epithelial barrier regulation in DSS-induced colitis. Among them, 10-shogaol was found to be the most effective in suppressing colitis symptoms and inflammation and more effective than the corresponding gingerol analog. Therefore, our results suggest that 8-shogaol and 10-shogaol can be used as natural source-derived therapeutic agents for colitis.

Declaration of competing interest

All authors declare that there is no conflict of interest.

Abbreviations

AB	alcian blue
COX-2	cyclooxygenase-2
DAI	disease activity index
DSS	dextran sodium sulfate
H&E	hematoxylin and eosin
IBD	inflammatory bowel disease
IFN	interferon
IL	interleukin
iNOS	inducible nitric oxide synthase
NF-κB	nuclear factor-κB
TNF-α	tumor necrosis factor-α
TJ	tight junction
UC	ulcerative colitis

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2022.e12778.

References

[1] T.S. Furey, P. Sethupathy, S.Z. Sheikh, Redefining the IBDs using genome-scale molecular phenotyping, Nat. Rev. Gastroenterol. Hepatol. 16 (5) (2019) 296–311.
[2] C. Chelakkot, J. Ghim, S.H. Ryu, Mechanisms regulating intestinal barrier integrity and its pathological implications, Exp. Mol. Med. 50 (8) (2018) 1–9.
[3] W. Strober, I. Fuss, P. Mannon, The fundamental basis of inflammatory bowel disease, J. Clin. Invest. 117 (3) (2007) 514–521.
[4] G. Kolios, V. Valatas, S.G. Ward, Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle, Immunology 113 (4) (2004) 427–437.
[5] D. Wang, R.N. Dubois, The role of COX-2 in intestinal inflammation and colorectal cancer, Oncogene 29 (6) (2010) 781–788.
[6] I. Atreya, R. Atreya, M.F. Neurath, NF-kappaB in inflammatory bowel disease, J. Intern. Med. 263 (6) (2008) 591–596.
[7] P.P. Tak, G.S. Firestein, NF-kappaB: a key role in inflammatory diseases, J. Clin. Invest. 107 (1) (2001) 7–11.
[8] Y. Li, Y. Hong, Y. Han, Y. Wang, L. Xia, Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1011 (2016) 223–232.
[9] S.M.A. Bastaki, N. Amir, E. Adeghate, S. Ojha, Lycopodium mitigates oxidative stress and inflammation in the colonic mucosa of acetic acid-induced colitis in rats, Molecules 27 (9) (2022).
[10] M.P. Utrilla, M.J. Peinado, R. Ruiz, A. Rodriguez-Nogales, F. Algieri, M.E. Rodriguez-Cabezas, A. Clemente, J. Galvez, L.A. Rubio, Pea (Pisum sativum L.) seed albumin extracts show anti-inflammatory effect in the DSS model of mouse colitis, Mol. Nutr. Food Res. 59 (4) (2015) 807–819.
[11] S. Yang, F. Li, S. Lu, L. Ren, S. Bian, M. Liu, D. Zhao, S. Wang, J. Wang, Ginseng root extract attenuates inflammation by inhibiting the MAPK/NF-kappaB signaling pathway and activating autophagy and p62-Nrf2-Keap1 signaling in vitro and in vivo, J. Ethnopharmacol. 283 (2022), 114739.
[12] S. Chrubasik, M.H. Pittler, Addendum to a recent systematic review on ginger, Forsch Komplementarmed Klass Naturheilkd 12 (3) (2005) 168, author reply 168-169.
[13] M. Afzal, D. Al-Hadidi, M. Menon, J. Pesek, M.S. Dhami, Ginger: an ethnomedical, chemical and pharmacological review, Drug Metabol. Drug Interact. 18 (3–4) (2001) 159–190.
[14] L. Khodaie, O. Sadeghpoor, Ginger from ancient times to the new outlook, Jundishapur J. Nat. Pharm. Prod. 10 (1) (2015), e18402.
[15] S. Guo, W. Geng, S. Chen, L. Wang, X. Rong, S. Wang, T. Wang, L. Xiong, J. Huang, X. Pang, Y. Lu, Ginger alleviates DSS-induced ulcerative colitis severity by improving the diversity and function of gut microbiota, Front. Pharmacol. 12 (2021), 632569.
[16] E.S.H. Abd Allah, R. Makboul, A.O. Mohamed, Role of serotonin and nuclear factor-kappa B in the ameliorative effect of ginger on acetic acid-induced colitis, Pathophysiology 23 (1) (2016) 35–42.
[17] Q.Q. Mao, X.Y. Xu, S.Y. Cao, R.Y. Gan, H. Corke, T. Beta, H.B. Li, Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe), Foods 8 (6) (2019).
[18] A. Ghasemzadeh, H.Z.E. Jaafar, A. Baghdadi, A. Tayebi-Meigooni, Formation of 6-, 8- and 10-shogaol in ginger through application of different drying methods: altered antioxidant and antimicrobial activity, Molecules 23 (7) (2018).
[19] S.M. Zick, Z. Djuric, M.T. Ruffin, A.J. Litzinger, D.P. Normolle, S. Alrawi, M.R. Feng, D.E. Brenner, Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects, Cancer Epidemiol. Biomarkers Prev. 17 (8) (2008) 1930–1936.
[20] S.M. Hassan, A.H. Hassan, The possibility of using shogaol for treatment of ulcerative colitis, Iran. J. Basic Med. Sci. 21 (9) (2018) 943–949.

[21] Y. Sheng, T. Wu, Y. Dai, L. Xu, Y. Zhong, Y. Xue, Y. Tian, 6-gingerol alleviates inflammatory injury in DSS-induced ulcerative colitis mice by regulating NF-kappaB signaling, Ann. Palliat. Med. 9 (4) (2020) 1944–1952.
[22] Y. Sohn, N.Y. Han, M.J. Lee, H.J. Cho, H.S. Jung, [6]-Shogaol inhibits the production of proinflammatory cytokines via regulation of NF-kappaB and phosphorylation of JNK in HMC-1 cells, Immunopharmacol. Immunotoxicol. 35 (4) (2013) 462–470.
[23] F. Zhang, N. Ma, Y.F. Gao, L.L. Sun, J.G. Zhang, Therapeutic effects of 6-gingerol, 8-gingerol, and 10-gingerol on dextran sulfate sodium-induced acute ulcerative colitis in rats, Phytother. Res. 31 (9) (2017) 1427–1432.
[24] Y. Hui, S.-G. Yan, Q. Wang, J.-T. Li, H.-L. Wei, Y.-P. Shan, Effects of 6-Shogaol on Notch signaling pathway in colonic epithelial cells of ulcerative colitis mice, Chin. J. Appl. Physiol. 36 (1) (2020) 90–93.
[25] P. Alex, N.C. Zachos, T. Nguyen, L. Gonzales, T.E. Chen, L.S. Conklin, M. Centola, X. Li, Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis, Inflamm. Bowel Dis. 15 (3) (2009) 341–352.
[26] U. Erben, C. Loddenkemper, K. Doerfel, S. Spieckermann, D. Haller, M.M. Heimesaat, M. Zeitz, B. Siegmund, A.A. Kuhl, A guide to histomorphological evaluation of intestinal inflammation in mouse models, Int. J. Clin. Exp. Pathol. 7 (8) (2014) 4557–4576.
[27] M.L. Clapper, H.S. Cooper, W.C. Chang, Dextran sulfate sodium-induced colitis-associated neoplasia: a promising model for the development of chemopreventive interventions, Acta Pharmacol. Sin. 28 (9) (2007) 1450–1459.
[28] F. Biasi, G. Leonarduzzi, P.I. Oteiza, G. Poli, Inflammatory bowel disease: mechanisms, redox considerations, and therapeutic targets, Antioxidants Redox Signal. 19 (14) (2013) 1711–1747.
[29] B. Bang, L.M. Lichtenberger, Methods of inducing inflammatory bowel disease in mice, Curr. Protoc. Pharmacol. 72 (2016), 5 58 51-55 58 42.
[30] S. Wirtz, V. Popp, M. Kindermann, K. Gerlach, B. Weigmann, S. Fichtner-Feigl, M.F. Neurath, Chemically induced mouse models of acute and chronic intestinal inflammation, Nat. Protoc. 12 (7) (2017) 1295–1309.
[31] S.M.A. Hassan, A.H. Hassan, Effect of shogaol on the expression of intestinal stem cell markers in experimentally induced colitis in BALB/c mice, Anal. Cell Pathol. 2019 (2019), 5134156.
[32] S. Sang, J. Hong, H. Wu, J. Liu, C.S. Yang, M.H. Pan, V. Badmaev, C.T. Ho, Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from Zingiber officinale relative to gingerols, J. Agric. Food Chem. 57 (22) (2009) 10645–10650.
[33] F. Sanchez-Munoz, A. Dominguez-Lopez, J.K. Yamamoto-Furusho, Role of cytokines in inflammatory bowel disease, World J. Gastroenterol. 14 (27) (2008) 4280–4288.
[34] O.H. Nielsen, New strategies for treatment of inflammatory bowel disease, Front. Med. 1 (2014) 3.
[35] S. Dionne, I.D. D'Agata, J. Hiscott, T. Vanounou, E.G. Seidman, Colonic explant production of IL-1and its receptor antagonist is imbalanced in inflammatory bowel disease (IBD), Clin. Exp. Immunol. 112 (3) (1998) 435–442.
[36] K.H. Kwon, A. Murakami, R. Hayashi, H. Ohigashi, Interleukin-1beta targets interleukin-6 in progressing dextran sulfate sodium-induced experimental colitis, Biochem. Biophys. Res. Commun. 337 (2) (2005) 647–654.
[37] C.F. Krieglstein, W.H. Cerwinka, F.S. Laroux, J.W. Salter, J.M. Russell, G. Schuermann, M.B. Grisham, C.R. Ross, D.N. Granger, Regulation of murine intestinal inflammation by reactive metabolites of oxygen and nitrogen: divergent roles of superoxide and nitric oxide, J. Exp. Med. 194 (9) (2001) 1207–1218.
[38] R.B. van Breemen, Y. Tao, W. Li, Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale), Fitoterapia 82 (1) (2011) 38–43.
[39] B. Oz, C. Orhan, M. Tuzcu, N. Sahin, I.H. Ozercan, P. Demirel Oner, S.S. Koca, V. Juturu, K. Sahin, Ginger extract suppresses the activations of NF-kappaB and Wnt pathways and protects inflammatory arthritis, Eur. J. Rheumatol. 8 (4) (2021) 196–201.
[40] S.M.A. Bastaki, M.M. Al Ahmed, A. Al Zaabi, N. Amir, E. Adeghate, Effect of turmeric on colon histology, body weight, ulcer, IL-23, MPO and glutathione in acetic-acid-induced inflammatory bowel disease in rats, BMC Compl. Alternative Med. 16 (2016) 72.
[41] X.X. Guo, Y.D. Zhang, T.C. Wang, X.L. Wang, Y.Y. Xu, Y. Wang, J. Qiu, Ginger and 6-gingerol prevent lipopolysaccharide-induced intestinal barrier damage and liver injury in mice, J. Sci. Food Agric. 102 (3) (2022) 1066–1075.
[42] M.A. McGuckin, R. Eri, L.A. Simms, T.H. Florin, G. Radford-Smith, Intestinal barrier dysfunction in inflammatory bowel diseases, Inflamm. Bowel Dis. 15 (1) (2009) 100–113.
[43] A.E. Dorofeyev, I.V. Vasilenko, O.A. Rassokhina, R.B. Kondratiuk, Mucosal barrier in ulcerative colitis and Crohn's disease, Gastroenterol. Res. Pract. 2013 (2013), 431231.
[44] L.S. Poritz, K.I. Garver, C. Green, L. Fitzpatrick, F. Ruggiero, W.A. Koltun, Loss of the tight junction protein ZO-1 in dextran sulfate sodium induced colitis, J. Surg. Res. 140 (1) (2007) 12–19.
[45] M.S. Kim, J.Y. Kim, Ginger attenuates inflammation in a mouse model of dextran sulfate sodium-induced colitis, Food Sci. Biotechnol. 27 (5) (2018) 1493–1501.
[46] J. Luettig, R. Rosenthal, I.M. Lee, S.M. Krug, J.D. Schulzke, The ginger component 6-shogaol prevents TNF-alpha-induced barrier loss via inhibition of PI3K/Akt and NF-kappaB signaling, Mol. Nutr. Food Res. 60 (12) (2016) 2576–2586.