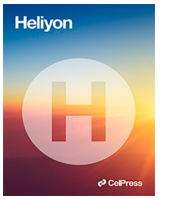
Heliyon 10 (2024) e27383

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Gochujang, a traditional Korean fermented food, protects through suppressed inflammatory pathways and histological structure disruption in dextran sodium sulfate (DSS)-induced colitis mice

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ARTICLE INFO

Keywords:
Gochujang
Colitis
Cytokines
Anti-inflammatory agents
MAP kinases
NF-Kappa B

ABSTRACT

The mechanisms underlying chronic inflammatory diseases remain unclear. Therefore, researchers have explored the mechanisms underlying colitis using diverse materials. Recently, there has been an increasing interest in fermented products and bioconversion materials, their potential efficacy is being actively studied. Gochujang, a traditional Korean fermented product, is crafted by blending fermented Meju powder, gochu (Korean chili) powder, glutinous rice, and salt. In our study, we explored the effectiveness of Gochujang (500 mg/kg; Cheongju and Hongcheon, Korea) in dextran sulfate sodium (DSS)-induced colitis mice model. Gochujang was orally administered for 2 weeks, followed by the induction of colitis using 3% DSS in the previous week. During our investigation, Gochujang variants (TCG22-25, Cheongju and TCG22-48, Hongcheon) did not exhibit significant inhibition of weight reduction ($p = 0.061$) but notably ($p = 0.001$) suppressed the reduction in large intestine length in DSS-induced colitis mice. In the serum from colitis mice, TCG22-48 demonstrated reduced levels of the inflammatory cytokines interleukin (IL)-6 ($p = 0.001$) and tumor necrosis factor (TNF)-α ($p = 0.001$). Additionally, it inhibited the phosphorylation of Erk ($p = 0.028$), p38, and NF-κB ($p = 0.001$) the inflammatory mechanism. In our study, TCG22-25 demonstrated a reduction in the IL-6 level ($p = 0.001$) in serum and inhibited the phosphorylation of p38 and NF-κB ($p = 0.001$). Histological analysis revealed a significant ($p = 0.001$) reduction in the pathological score of the large intestine from TCG22-25 and TCG22-48. In conclusion, the intake of Gochujang demonstrates potent anti-inflammatory effects, mitigating colitis by preventing the large intestine length reduction of animals with colitis, lowering serum levels of TNF-α and IL-6 cytokines, and inhibiting histological disruption and inflammatory mechanism phosphorylation.

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https://doi.org/10.1016/j.heliyon.2024.e27383
Received 23 May 2023; Received in revised form 26 February 2024; Accepted 28 February 2024
Available online 8 March 2024

1. Introduction

Inflammatory bowel disease (IBD), categorized into Crohn's disease (CD) and ulcerative colitis (UC), results from a complex interplay of genetic and environmental factors, along with alterations in the composition and function of intestinal microorganisms [1–3]. The heightened inflammation in the intestine due to IBD can lead to the development of colorectal cancer and have fatal consequences [4]. Variations in intestinal microorganisms have been linked to dietary shifts involving retortable pouches, frozen meals, and shelf-stable ready-to-eat/heated meals, play a role in the IBD [3,5]. Specifically, a decrease in beneficial bacteria capable of fermenting fibers and producing short-chain fatty acids has been reported in the feces of individuals with enteritis compared to healthy individuals [6]. Recently, dextran sulfate sodium (DSS) was been employed to manipulate intestinal microorganisms and IBD [7]. The DSS-induced colitis model exhibits a consistent lesion pattern, closely resembling the histological, physiological, and biochemical characteristics observed in human IBD [8].

Gochujang, a traditional fermented food in Korea, is obtained by mixing gochu (Korean chili pepper) powder, *meju* powder (made from fermented soybean), glutinous rice, starch syrup, and a large amount of salt, followed by fermentation [9]. Fermented sauce and food (such as vinegar, *Doenjang*, and Kimchi) have been reported to be effective in aging, cardiovascular diseases, diabetes, obesity, and gut health [10–16]. It contains capsaicin, isoflavones, and beneficial bacteria (probiotics) that have been reported to have anti-inflammatory, antioxidant, anti-diabetic, anti-obesity, and anti-cancer effects. Joseph et al. (2004) suggested that capsaicin induces the activity of the Liver X receptor involved in the transcription of inflammatory genes (such as iNOS, COX-2, IL-1β, TNF-α, and MMP-9) in macrophages [17]. Isoflavones from soybeans reduce the incidence of DSS-induced murine colitis and lipopolysaccharide-activated RAW267.4 macrophage cells [18]. However, *meju*, an ingredient in *Gochujang*, has been reported to contain aflatoxins and may cause hepatotoxicity and cancer in both humans and animals. Recently, it was shown to cause intestinal epithelial damage and Crohn's disease [19–21]. In a previous study, fermentation products (including those produced by beneficial bacteria) were found to suppress aflatoxin activity [22]. *Gochujang* contains aflatoxins and probiotics, along with other ingredients (such as capsaicin, isoflavones, and probiotics) and has been reported to have anti-inflammatory effects in other diseases [9,23–25].

In our study, we hypothesized that the anti-inflammatory efficacy of traditional Korean *Gochujang* could reduce the incidence of colitis and evaluated it in DSS-induced colitis mice. These results demonstrated that *Gochujang* inhibited pro-inflammatory cytokines in the serum, length of the large intestine (LI), and disruption of the intestinal structure.

2. Materials and methods

2.1. Preparation of gochujang

Gochujang used in this study was obtained from traditional *Gochujang* (TCG22-25, Cheongju, Chungcheongbuk-do, Korea; TCG22-48, Hongcheon, Gangwon-do, Korea) from the Microbial Institute for Fermentation Industry (MIFI). TCG22-25 is composed of 40% gochu powder, 20% glutinous rice powder, 15% fermented soybean (*Meju*), 15% water, and 10% solar salt, whereas TCG22-48 is composed of 50% gochu powder, 20% starch syrup, 10% sticky rice cake, 5% soy sauce (*Ganjang*), 5% fermented soybean, and 10% solar salt. All samples were ground in distilled water (150 g/350 mL) using a blender. After the supernatant was collected, it was dried (57 °C for 24 h), powdered, and orally administered at a concentration of 500 mg/kg.

2.2. Chemical analysis of gochujang

The water content, crude protein, total lipid content, carbohydrate and calorie of the TCG22-25 and TCG22-48 were analyzed by methods on the Korean Ministry of Food and Drug Safety disclosed Notification No. 2021-54 in Food Code. The water content was determined by drying at 95 °C until reaching a constant weight. Using Kjeldahl method measured the crude protein content of Gochujang. And total fat content of gochujang was determined using the Soxhlet method. Sodium content was quantified using inductively coupled plasma mass spectrometry. Calories were calculated in kilocalories (kcal) by Atwater's coefficient.

2.3. Biogenic amines and aflatoxin analysis of gochujang

Biogenic amines (histamine and tyramine) and aflatoxins of TCG22-25 and TCG22-48 were analyzed using High Performance Liquid Chromatography (HPLC, Agilent 1200 series) equipped with Capcellpak C18 column for biogenic amines, and Shiseido UC 120 column for aflatoxin. To identify components of TCG22-25 and TCG22–48, H_2O solution (0.1%) was used as solvent A, and HPLC-grade acetonitrile was used as solvent B (elution conditions: 0–10 min, 55 % B; 10–15 min, 65% B; 15–20 min, 80% B; 20–30 min, 90% B; 45 min, 100% B; flow rate, 1 mL/min; injection volume, 20 μL (biogenic amine) and 10 μL (aflatoxin); wavelength, 245 nm (biogenic amine) and 450 nm (aflatoxin) visible; and column temperature 27 °C.

2.4. Microbial analysis of gochujang

Microbial analysis of *Gochujang* was measured as previously described using Next-Generation Sequencing (NGS) [26]. DNA from *Gochujang* (TCG22-25 and TCG22-48) was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany). Amplification of the V3–V4 regions of the targeting primers. Sequencing was performed on the Illumina MiSeq platform at MIFI (Sunchang, South Korea).

2.5. DSS-induced colitis model and tissue preparation

All the experiments were conducted following the National Institutes of Health Guidelines for the Care and Use of Animals. This study was approved by the Institutional Animal Care and Use Committee of INVIVO Co., Ltd. (IV-RA-13-2204-08). C57BL/6 mice (6 weeks old, male) was purchased from Orient BIO Co. (Sungnam, Kyoung Gi-DO, Korea). After stabilization period, the mice were divided into five groups (Normal, Control, TCG22-25, TCG22-48 and SSZ). The normal group received drinking water, and the control group, *Gochujang* (500 mg/kg/day)-treated group and SZZ (60 mg/kg/day)-treated group received drinking water containing 3% [w/v] DSS for 7 days (Fig. 1A). Mice were monitored body weight, rectal bleeding, food intake, and water intake throughout the DSS treatment period. The mice were kept in a temperature-controlled room with a 12 h light and 12 h dark cycle.

2.6. Complete blood cell (CBC) count analysis

After respiratory anesthesia, whole blood from mice was collected from the abdominal vena cava and divided into ethylenediaminetetraacetic acid-coated tubes (DB Caribe, Ltd., USA). The total numbers of white blood cells (WBCs), lymphocytes, granulocytes, and mid-sized cells were analyzed using a Mindray BC-2800 hematological analyzer. (Mindray, Bath, UK).

2.7. Pro-inflammatory cytokine levels in serum from DSS-induced colitis mice

Whole blood was coagulated at room temperature for 30 min and then separated in a centrifuge at 3000 rpm for 15 min at 4 °C to collect serum. The levels of TNF-α and IL-6 in serum were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (TNF-α, # BMS607HS; IL-6, # BMS603HS, Invitrogen).

2.8. Histological analysis

LI tissues were harvested for hematoxylin and eosin (H&E) staining. Its was fixed in 10% formalin and embedded in paraffin. Paraffin sections were cut to a thickness of 4 μm and stained with H&E. Stained LIs were visualized using microscope (Nikon, TE-2000, Tokyo, Japan). The microscopic LI tissue damage (histological score) was measured as previously described [[27]]. Scores were assigned as follows: 0 = normal, 1 = hyperproliferation, irregular crypts, and goblet cell loss; 2 = crypt loss (10%–50%); 3 = crypt loss (50%–90%); 4 = complete crypt loss; 5 = small-to medium-sized ulcer (<10 crypt); and 6 = large ulcer (≥10 crypt). The infiltration of inflammatory cells was assigned scores separately for the mucosa (0 = normal, 1 = mild, 2 = moderate, 3 = severe), submucosa (0 = normal, 1 = mild, 2 = severe), and muscle/serosa (0 = normal, 1 = severe).

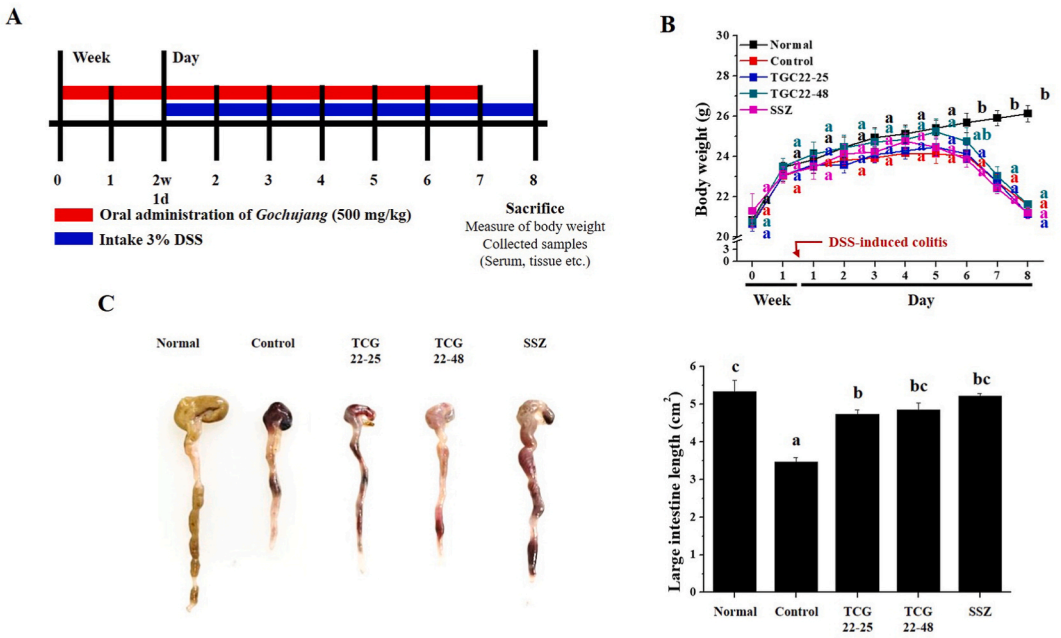

Fig. 1. Effects of *Gochujang* on dextran sulfate sodium (DSS)-induced colitis symptoms in mice. (A) Diagram of the animal experimental design, (B) body weight, (C) representative image (left), large intestine length (cm^2, right) Results are expressed as the mean ± SE ($n = 7$ per group). Bars labeled with different superscripts are significantly different ($p < 0.05$, *vs.* control).

2.9. Western blot

Mouse LI tissues were lysed by PRO-PREP™ Protein Extraction Solution (iNtRON, Cat No. 17081). The total protein concentration was determined using the Bradford assay kit (Sigma-Aldrich, USA). Equal amount of protein (10 μg/well) was blotted on polyvinylidene fluoride membranes (PVDF, Bio-Rad, CA, USA). The protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF. Specific antibodies (phospho-Erk, Erk, phospho-p38, p38, phospho–NF–κB, and nuclear factor kappa B (NF-κB); Cell Signaling Technology, MA, USA, β-actin; Santa Cruz Biotechnology Co., Ltd., TX, USA) were used to bind the target proteins. The latter was detected using a chemiluminescence reagent (SURMODICS IVD, Inc., USA) and visualized and analyzed using a C-Digit 3600 imaging system (LI-COR, NE, USA) equipped with an Image Lab.

2.10. Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM), and differences between groups were analyzed using one-way ANOVA (Duncan's multiple-range test). All analyses were performed using SPSS (version 23.0; SPSS, Inc. USA). Each value represents the mean of at least three independent experiments for each group. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Components and chemical of gochujang

First, we analyzed the ingredients of *Gochujang* (TCG22-25, Cheongju and TCG22-48, Hongcheon) from two different regions. Biogenic amines investigated that TCG22-25 contained 135.4 ± 2.9 mg/kg and 0.8 ± 0.3 mg/kg of histamine and tyramine, respectively, and TCG22-48 contained 0.5 ± 0.1 mg/kg and 9.7 ± 3.0 mg/kg of histamine and tyramine, respectively. TCG22-48 contained 25.82 ± 0.66 μg/kg of aflatoxin, but also showed many beneficial bacteria (71.72 ± 1.39%) in the microbial distribution (Table 1). The chemical analysis of *Gochujang* was investigated as TCG22-25 and TCG22-48 of water content (41.60 ± 0.9% and 41.56 ± 0.6%), sodium content (2.637 ± 0.24 g and 2.901 ± 0.16 g), carbohydrate (52.96 ± 1.42 g and 49.44 ± 0.95 g), crude protein (6.69 ± 0.30 g and 5.34 ± 0.09 g), crude fat (1.46 ± 0.23 g and 2.60 ± 0.03 g) and calorie (251.74 ± 7.41 kcal and 242.52 ± 3.97 kcal), respectively (Table 2).

3.2. Effect of gochujang on DSS-induced colitis mice

To determine the effectiveness of *Gochujang* (TCG22-25 and TCG22-48) in DSS-induced colitis, we fed only *Gochujang* (500 mg/kg) for 2 weeks, followed by 3% DSS with *Gochujang* for 7 days (Fig. 1A). The weight was measured before oral administration. As shown in Fig. 1B, body weight decreased significantly in the control group (only 3% DSS) compared to the normal group, and it was observed that the weight also decreased in *Gochujang*-treated groups. These results demonstrated that weight recovery was not affected. Rectal bleeding and diarrhea were also observed (Supplementary Table 1). The stool condition did not change, during the 2 weeks of oral administration of only *Gochujang*. After supplying 3% DSS, diarrhea and bloody stools were observed for 5 days. Rectal bleeding and diarrhea were observed due to the intake of 3% DSS, and *Gochujang* did not affect rectal bleeding and diarrhea. Both water and dietary intake decreased significantly compared with those in the normal group (Supplementary Fig. 1). In Fig. 1C confirmed that the large intestine length was reduced by 3% DSS (normal group vs only DSS group, 6.05 ± 0.13 cm vs 3.68 ± 0.06 cm). Intake of TCG22-25 (4.80 ± 0.66 cm) and TCG22-48 (4.97 ± 0.16 cm) significantly inhibited the reduction of the large intestine length.

3.3. CBC count in DSS-induced colitis mice

CBC is widely used to monitor health, diagnose diseases, and acts as an inflammatory biomarker. Blood from mice with DSS-induced colitis was collected after respiratory anesthesia and CBC performed (Fig. 2). Compared to the normal group, the control group (only 3% DSS) showed a significant increase in WBCs, granulocytes, lymphocytes, and mid-sized cells. TCG 22–25 significantly reduced the number of granulocytes, and the number of mid-sized cells decreased, similar to the positive control group (SSZ). TCG 22–48 was also found to decrease SSZ levels.

Table 1
Information and component of *Gochujang*.

Gochujang	Region (in Korea)	Biogenic amine (mg/kg)		Total Aflatoxin (μg/kg)	Microbial distribution (%)	
		Histamine	Tyramine		Beneficial bacteria	Harmful bacteria
TCG22–25	**Cheongju, Chungcheongbuk-do**	**135.4 ± 2.9**	**0.8 ± 0.3**	**N.D**	**1.17 ± 0.41**	**0.09 ± 0.01**
TCG22–48	**Hongcheon, Gangwon-do**	**0.5 ± 0.1**	**9.7 ± 3.0**	**25.82 ± 0.66**	**71.72 ± 1.39**	**0.21 ± 0.00**

Table 2
Chemical analysis of *Gochujang*.

Gochujang	Water content (%)	Sodium content (mg/100g)	Carbohydrate (g/100 g)	Crude Protein (g/100 g)	Crude Fat (g/100 g)	Calorie (kcal/100 g)
TCG22-25	**41.60 ± 0.9**	**2.64 ± 0.24**	**52.96 ± 1.42**	**6.69 ± 0.30**	**1.46 ± 0.23**	**251.74 ± 7.41**
TCG22-48	**41.56 ± 0.6**	**2.90 ± 0.16**	**49.44 ± 0.95**	**5.34 ± 0.09**	**2.60 ± 0.03**	**242.52 ± 3.97**

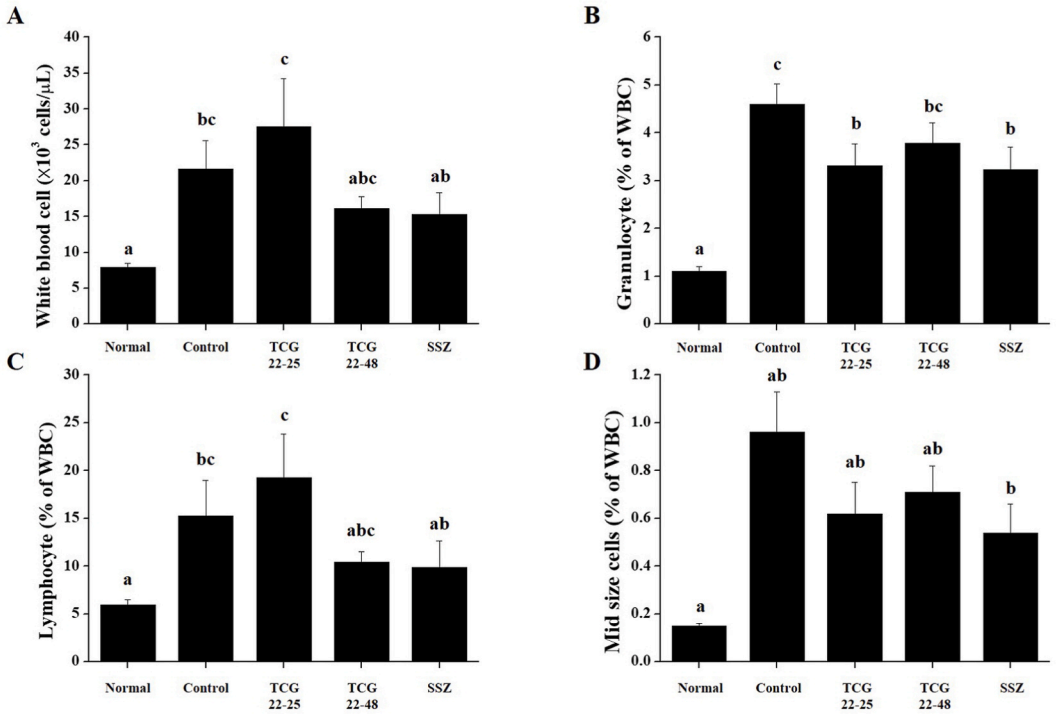

Fig. 2. Effects of *Gochujang* on whole blood of DSS-induced colitis mice. The quantities of (A) total WBCs, (B) granulocytes, (C) lymphocytes, and (D) mid-sized cells (Mid) in the whole blood of DSS-induced colitis mice were assessed utilizing the BC-2800Vet system. Values are presented as mean ± SE ($n = 7$ per group). Bars labeled with different superscript numerals indicate $p < 0.05$.

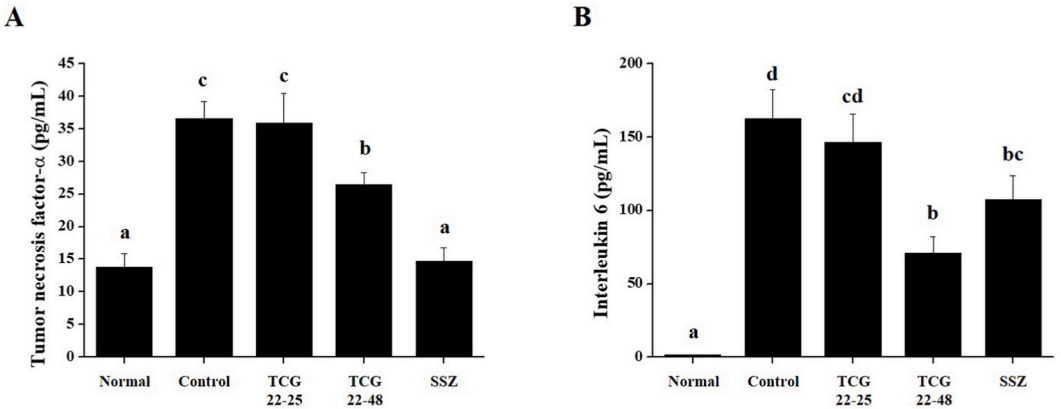

Fig. 3. Effects of *Gochujang* on TNF-α and IL-6 levels in the serum of DSS-induced colitis mice. (A) TNF-α and (B) IL-6. Serum TNF-α and IL-6 levels were assayed using an ELISA kit. Values are presented as mean ± SE ($n = 7$ per group). Bars labeled with different superscript numerals indicate $p < 0.05$.

3.4. Pro-inflammatory cytokine levels in the serum of DSS-induced colitis mice

In the serum of mice with DSS-induced colitis, decreased levels of cytokines (such as IL-1β, IL-6, and TNF-α) under pathological conditions indicated anti-inflammatory effects [28]. DSS-induced colitis mice exhibited a notable rise in TNF-α and IL-6 levels compared to normal mice (Fig. 3). While TCG 22-25-intake did not alter TNF-α and IL-6 levels in the serum of DSS-induced colitis mice, TCG 22-48-intake significantly reduced IL-6 and TNF-α levels compared to the control group. These results suggest that TCG 22–48 prevents inflammation through the downregulation of IL-6 and TNF-α levels in the serum of DSS-induced colitis mice (Fig. 3A and B).

3.5. Histological studies on LI in DSS-induced colitis mice

Representative images of LI in DSS-induced colitis mice are shown in Fig. 4. In the normal group, epithelial erosion, inflammatory cell infiltration and lesions, epithelial erosion, and ulcerations were not observed (Fig. 4A). In the control group (only 3% DSS), the epithelial barrier collapsed due to the loss of goblet cells and crypts in the mucous membrane. Inflammatory cells invaded submucosal tissues, leading to severe submucosal edema (Fig. 4B). In the *Gochujang* group treated with DSS, inflammation in the LI tissue decreased compared to the control group, with no histological evidence of inflammation observed in the LI group (Fig. 4C and D). Histological scores for LI tissues in all groups were measured. Of the 12 points in total, the highest score (11.43 ± 0.20) was observed in control group, and the scores of TCG 22–25 (9.86 ± 0.51), TCG 22–48 (9.29 ± 0.42), and SSZ (7.71 ± 0.61) were significantly lower than those of control group (Fig. 4F). As shown in Fig. 4, TCG 22–25 and TCG 22–48 protected the LI tissue collapsed by 3% DSS.

3.6. Protein expression in the LI of DSS-induced colitis mice

The mitogen-activated protein kinases (MAPKs) and NF-κB pathways, major inflammatory pathways that are significantly activated in DSS-induced UC mice [23]. Representative images of protein expression are shown in Fig. 5. MAPK pathways (Erk and p38) were significantly activated in the DSS group. As anticipated, *Gochujang* suppressed the phosphorylation of p38 and Erk in the LI tissue of mice with DSS-induced colitis (Fig. 5B and C). JNK phosphorylation remains unaffected (data not shown). Additionally, phosphorylation of NF-κB p-65 significantly increased in the control group compared to the normal group (Fig. 5D) and was suppressed by *Gochujang* in LI tissue of DSS-induced colitis mice. These results suggest that *Gochujang* induces the inhibition of MAPKs and the NF-κB pathway, demonstrating effectiveness against colitis.

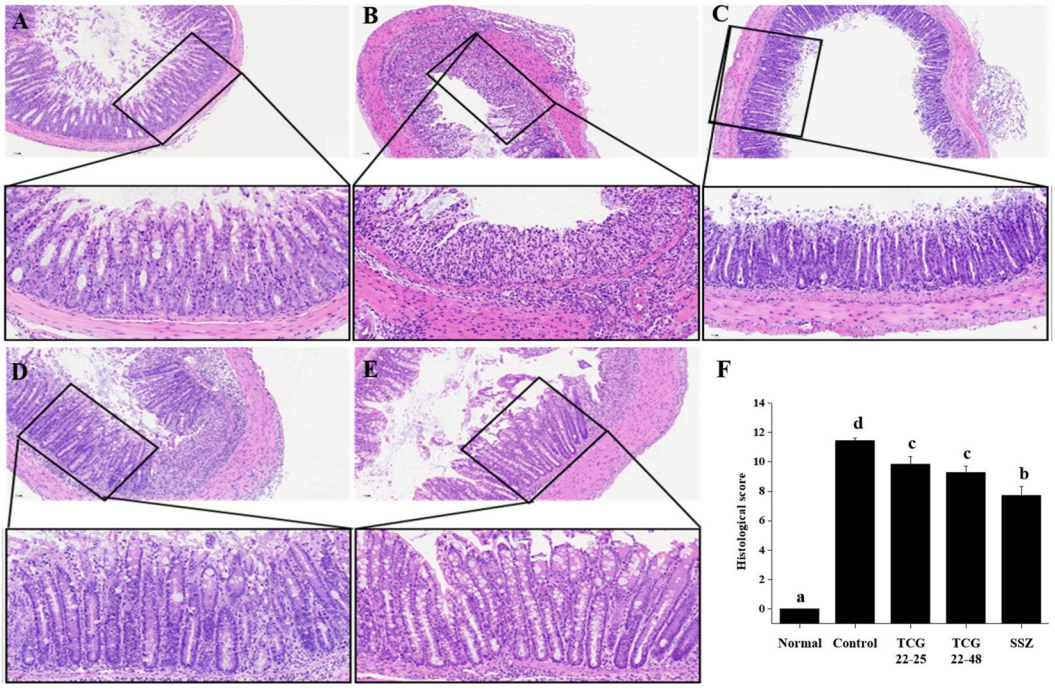

Fig. 4. Histology of large intestine (LI) form DSS-induced colitis mice sections after hematoxylin and eosin (H&E) staining. A set of 35 LI tissues were subjected to H&E staining to examine various treatment groups: (A) normal, (B) control, (C) TCG 22–25, (D) TCG 22–48, and (E) sulfasalazine (SSZ). (magnification, 10 × and 20 × ; scale bar = 50 μm). (F) histological score of total LI tissues ($n = 7$ per group). Bars labeled with different superscript numerals indicate $p < 0.05$.

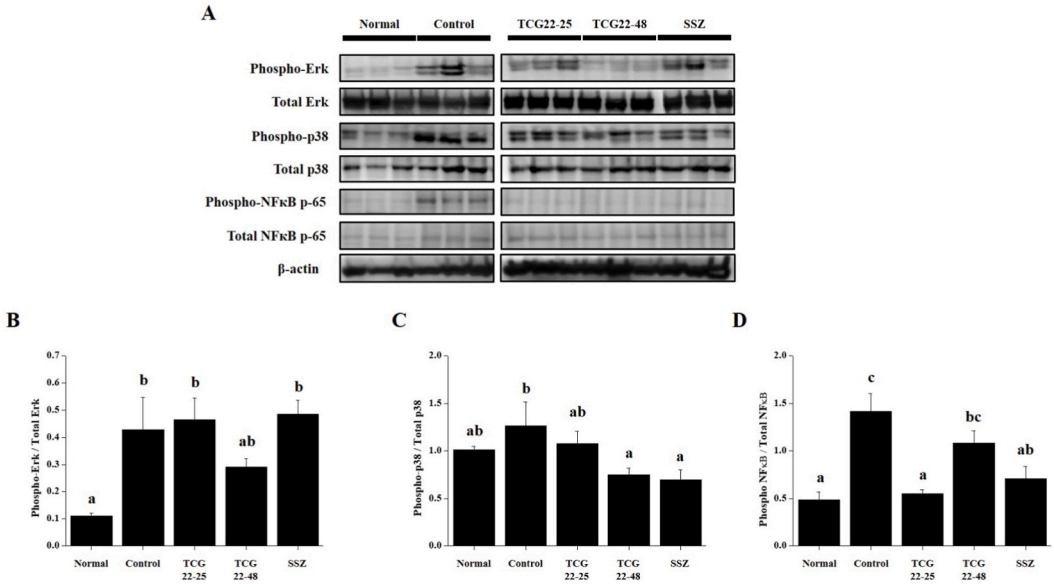

Fig. 5. *Gochujang* suppressed the MAPKs and NF-κB signaling pathway. (A) The phosphorylation of p65 and MAPKs (Erk and p38) was detected by western blotting. (B–D) The relative density of each signaling band was calculated. β-actin was used as the protein loading control. Bars labeled with different superscript numerals indicate $p < 0.05$.

4. Discussion

IBD is notably prevalent in young people, is on the rise globally [29]. UC, characterized by inflammation of the colon mucosa, particularly in the proximal part of the LI in the rectum, and is influenced by genetic factors, intestinal microbial changes, and immunological abnormalities [3–5]. The DSS-induced colitis model, is well-suited for studying colitis due to its uniform lesion form in the colon, rapid interference with the intestinal barrier function, and emulation of the clinical histological characteristics of IBD through local inflammation simulation [8]. Consequently, numerous researchers have explored the treatment and pathogenesis of DSS-induced colitis utilizing animal models. Recently, there has been considerable interest in improvements through fermentation and bioconversion [30]. Fermented or bioconversion materials have been reported for their heightened impact on ingredients and beneficial bacteria (prebiotics and probiotics) showcasing effectiveness in antioxidant, anti-inflammatory, skin moisturizing, elasticity, atopic dermatitis, and influencing cardiovascular health [30–32].

Gochujang is a traditional Korean food is fermented by mixing gochu powder, salt, and glutinous rice. Besides *Gochujang*, traditional Korean sauces, incorporate soybean paste and soy sauce, both crafter through fermentation. Research has indicated that fermented foods contribute to the modulation of intestinal bacteria and overall health [9–11]. We sought to illustrate the effectiveness of *Gochujang* through assessment of weight change, hematological analysis, and colon morphological analysis in a DSS-induced colitis model. The characteristics of DSS-induced colitis were visible only in the LI. Initial lesions occurred in the left LI and led to bloody stools and weight loss. Additionally, plasma cells and lymphocytes infiltrate the colon and progress to chronic inflammation, and colon length reduction is observed during autopsy [18]. In this study, DSS-induced colitis for 7 days with 3% DSS resulted in weight loss, bloody stools, and colon reduction. Oral administration of *Gochujang* did not suppress weight loss but suppressed the reduction in colon length (Fig. 1D). Additionally, histological analysis of LI from DSS-induced colitis mice showed that *Gochujang* inhibited large intestinal structural disruptions caused by DSS (Fig. 4). Although TCG 22–48 contains aflatoxin (Table 1), there was little damage to the intestinal epithelial layer (Fig. 4D). This outcome suggests that the substantial production of beneficial bacteria, facilitated by the fermentation of *Gochujang* prevented damage to the intestinal epithelial layer by DSS and aflatoxins (Fig. 4). Cytokines TNF-α and IL-6 are important mediators of inflammation in patients with UC. TNF-α has been shown to increase intestinal permeability in patients with IBD by increasing the expression of epithelial myosin light-chain kinase [33]. Therefore, TNF-α inhibitors are important for the treatment of IBD. IL-6 has been reported to modulate intrinsic epithelial tight junctions by activating claudin-2. In a clinical study, anti-IL-6 receptor monoclonal antibody therapy showed significant clinical improvement in patients with CD [[34]]. Therefore, we investigated TNF-α and IL-6 levels in the serum of mice with DSS-induced colitis. After the oral administration of *Gochujang*, TNF-α and IL-6 levels decreased in the serum (Fig. 3 A and B). These results suggest that *Gochujang* protects against UC by reducing the levels of pro-inflammatory cytokines in the serum. The inflammatory reactions in the body involve several mechanisms. Representative mechanisms related to inflammation include the phosphorylation of MAPKs (Erk, JNK, and p38) and NF-κB. A previous study indicated that elevated phosphorylation of MAPKs and NF-κB was associated with inflammation, while inhibition of phosphorylation in MAPKs and NF-κB was considered anti-inflammatory [18,23]. We investigated the phosphorylation of MAPKs and NF-κB in the colon tissue of mice with DSS-induced colitis. Our findings revealed that *Gochujang* suppressed the phosphorylation of Erk, p38, and NF-κB. However, it did not affect JNK phosphorylation (data not shown).

5. Conclusion

Our study demonstrated that *Gochujang*, a traditional Korean fermented product, protects against the reduction of LI structure, lowers inflammatory cytokines TNF-α and IL-6 in the serum, and inhibits phosphorylation of inflammatory pathways (MAPKs and NF-κB) in mice with DSS-induced colitis. However, its worth noting that alfatoxin generation through fermentation offsets its efficacy due to the simultaneous production of beneficial bacteria.

Ethics statement

Animal experiments reported here were approved by the University of Florida Animal Care and Use Committee (IV-RA-13-2204-08, approved on 28 Aprile 2022)

Data availability

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Hak Yong Lee: Writing – original draft, Investigation. **Young Mi Park:** Investigation, Data curation. **Dong Yeop Shin:** Investigation, Data curation. **Hai Min Hwang:** Investigation. **Hanna Jeong:** Investigation. **Su-Ji Jeong:** Formal analysis. **Hee-Jong Yang:** Formal analysis. **Myeong Seon Ryu:** Formal analysis. **Ji Won Seo:** Formal analysis. **Do-Youn Jeong:** Formal analysis. **Byeong Soo Kim:** Writing – review & editing. **Jae Gon Kim:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by “functional research of fermented soybean food (safety monitoring)” under the Ministry of Agriculture, Food and Rural Affairs and partly Korea Agro-Fisheries and Food trade corporation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27383.

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