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Anti-Colitic Effects of *Kanjangs* (Fermented Soy Sauce and Sesame Sauce) in Dextran Sulfate Sodium-Induced Colitis in Mice

Jia-Le Song,[1] Jung-Ho Choi,[2] Jae-Hoon Seo,[2] Yaung-Iee Lim,[3] and Kun-Young Park[1]

[1]*Department of Food Science and Nutrition, Kimchi Research Institute, Pusan National University, Busan, South Korea.*
[2]*Daesang R&D, Echeon, South Korea.*
[3]*Department of Food and Nutrition, Sungshin Women's University, Seoul, South Korea.*

ABSTRACT This study was conducted to investigate the preventive effects of different *kanjangs* (Korean soy sauces), including acid-hydrolyzed soy sauce (AHSS), fermented soy sauce (FSS), and fermented sesame sauce (FSeS), on 2% dextran sulfate sodium (DSS)-induced ulcerative colitis in C57BL/6J mice. The fermented sauces, particularly FSeS, significantly suppressed DSS-induced body weight loss, increased colon length, and decreased colon weight/length ratios. Histological observations suggested that the fermented sauces prevented edema, mucosal damage, and the loss of crypts induced by DSS compared to the control mice and animals fed AHSS. FSeS and FSS decreased the serum levels of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-6, and IL-17α. mRNA expression of these cytokines as well as that of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in colon mucosa was also inhibited by the two sauces. Our results suggest that fermented sauces, especially FSeS, exert an anticolitic effect partially by reducing the serum levels of proinflammatory cytokines and inhibiting the mRNA expression of these factors in the colon tissue of mice treated with DSS. However, AHSS did not protect against DSS-induced colitis. In addition, low-dose treatment (4 mL/kg) with the fermented sauces resulted in greater anticolitic effects than consumption of a high quantity (8 mL/kg) of the sauces.

KEY WORDS: • ***colitis*** • ***dextran sulfate sodium*** • ***fermented sesame sauce*** • ***fermented soy sauce*** • **kanjang** • ***pro-inflammatory cytokines***

INTRODUCTION

Soy sauce is a traditional fermented soy-based liquid condiment with a salty taste that is widely consumed in Asia. This sauce is known as *kanjang* in South Korea, *jiangyou* in China, and *shoyu* in Japan. In South Korea, soy sauces can be classified as fermented soy sauce (FSS), acid-hydrolyzed soy sauce (AHSS; also called chemical soy sauce), and mixed soy sauce. FSS is prepared by fermenting protein-rich soybeans with *Aspergillus oryzae* (or *Aspergillus sojae*) in the presence of sodium chloride at 30°C for over 6 months. AHSS is manufactured from defatted soybeans or other protein-rich soybeans that are treated with 18% food-grade hydrochloric acid for 15–20 h with heating (107°C). The solution is then neutralized with sodium hydroxide or sodium carbonate, mixed with active carbon, and finally filtered to remove insoluble materials.[1] Mixed soy sauce is produced by combining FSS and AHSS at an appropriate ratio. Fermented sesame sauce (FSeS), a new type of liquid condiment, is prepared in a manner similar to that of FSS[2] and has also gained popularity in South Korea. FSS is known as a traditional functional food with antimutagenic[3,4] and antioxidant[5–7] properties. FSS was also found to exert anti-tumor effects against benzo[α]pyrene-induced forestomach neoplasia in female ICR mice,[8,9] spontaneous liver tumors in female C3H/HeN mice,[10] and liver tumors in neutron-irradiated male B6C3F1 mice.[10] Despite these favorable properties, the anti-inflammatory activities of FSS and other fermented sauces have not been studied.

Inflammatory bowel diseases (IBDs) are defined as chronic inflammation of the gastrointestinal tract, and include ulcerative colitis (UC) and Crohn's disease (CD). These conditions represent a significant public health problem in Western societies and affect 1 in 1000 individuals.[11] In South Korea, the incidence of UC is lower compared with Western nations but is increasing rapidly. According to an epidemiological study conducted from 1986 to 2005, the mean annual incidence rates of CD and UC in the Songpa-Kangdong district (Seoul, South Korea) significantly increased from 0.05 and 0.34 per 100,000 inhabitants, respectively, in 1986–1990 to 1.34 and 3.08 per 100,000 individuals, respectively, in 2001–2005.[12]

The etiology of UC remains unclear. Generally, UC pathogenesis is believed to involve complex interactions

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Address correspondence to: Kun-Young Park, PhD, Department of Food Science and Nutrition, Kimchi Research Institute, Pusan National University, Jangjeon-dong 30, Geumjung-gu, Busan 609-735, South Korea, E-mail: kunypark@pusan.ac.kr

between the intestinal microbial environment, persistent pathogenic infections, defective mucosal barrier function, and dysregulation of the colonic mucosal immune system along with genetic and environment factors.[13] Currently, it is widely accepted that both UC and CD are caused by inflammation-related cytokine-driven mixed infiltrates in the intestinal mucosa.[14] In cases of animal and human UC, some proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-1β, IL-6, and IL-17α also play crucial roles in disease pathogenesis.[14–16]

In the present study, a murine model of dextran sulfate sodium (DSS)-induced UC was used to evaluate the anti-inflammatory effects of AHSS, FSS, and FSeS. This mouse model exhibited many symptoms similar to those of human UC patients.[17] In addition, the underlying mechanisms of action were explored.

MATERIALS AND METHODS

Sauce samples and chemical reagents

AHSS, FSS, and FSeS were provided by Daesang Food Co., Ltd. (Echeon, South Korea). The FSS was prepared as follows: the cooked soybeans were mixed with roasted wheat and fermented for 72 h at 30°C with *Aspergillus oryzae* to prepare meju, which was mixed with a brine solution (15–20% NaCl) and fermented for 180 days at 30°C.[1] The manufacturing process of FSeS was similar to the FSS. The roasted and crushed defatted sesame seeds were mixed with *A. oryzae* and fermented for 2–5 days at 15°C and 3–5 days at 45°C. The brine solution (15–20% NaCl) was added and fermented for 45 days at 30°C.[2] The fermented soy or sesame sauce was finally processed by pressing, filtration, pasteurization, and packing. Different from the fermented sauces, the manufacturing process of acid dehydrolyzed soy sauce is produced by boiling defatted soybean or gluten in hydrochloric acid (18%, food grade) and then neutralizing the solution with sodium hydroxide. Following filtration and pasteurization, the acid dehydrolyzed soy sauce was prepared.[1] The sauce samples were filtered through 0.45-μm syringe filters (Whatman International, Maidstone, Kent, United Kingdom) and stored at 4°C until further use. The Trizol reagent, oligodT_{18} primer, dNTPs, murine maloney leukemia virus (MMLV) reverse transcriptase, RNase inhibitor, ethidium bromide (EtBr), and agarose were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). DSS (molecular weight: 36,000–50,000) was obtained from MP Biomedical (Solon, OH, USA). Dinitrosalicylic acid (DNS) and Folin–Denis reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade.

Chemical composition analyses

Salinity, pH, reducing sugar levels, amino type nitrogen concentrations, and total polyphenol contents of the three sauce samples were measured in triplicate. pH of the sauce samples was directly measured using a SevenEasy™ S20 pH meter (Mettler-Toledo GmbH, Schwerzenbach, Switzerland), and salinity was evaluated by volumetric titration with silver nitrate ($AgNO_3$) using the Mohr method.[18] Reducing sugar contents were analyzed with the Miller methods[19] using the DNS reagent. Amino nitrogen levels were determined, as previously described, by Beddows *et al.*[20] with some modifications. Diluted sauce samples (20 mL) were mixed with 20 mL of formalin solution (pH 7.4), and the pH was adjusted to 8.4 by titrating with 0.1 M NaOH. The volume of NaOH used was recorded to determine the total amino nitrogen content of the samples. Total polyphenolic contents of the sauces were measured, as previously described, by Taga *et al.*[21] with modifications. A 0.5-mL aliquot of Folin–Denis reagent (previously diluted 50-fold with distilled water) was added to 200 μL of each sauce. Next, 0.75 mL of sodium carbonate (7.5%) was added before the solution was mixed and incubated at room temperature for 30 min. Absorbance was then measured at 750 nm using a UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan).

Animal studies

The animal protocol used in this study was reviewed by the Pusan National University Institutional Animal Care and Use Committee (PNU-IACUC; approval number PNU-2011-000408). Female C57BL/6J mice (6 weeks old, 16–18 g) were purchased from Korea Central Lab Animal, Inc. (Seoul, South Korea). The mice were housed in environmentally controlled conditions with a standard 12-h light/12-h dark cycle at room temperature and had free access to food and water. The animals were randomly divided into eight groups of seven mice each: group 1, the normal control treated with 0.9% normal saline; group 2, DSS-treated mice; and groups 3–8, DSS-treated animals given low (4 mL/kg) or high (8 mL/kg) doses of the different sauce samples. Sauce samples and vehicle were administered daily through an intragastric route starting 7 days before DSS treatment and continued until sacrifice. Colitis was induced in the mice by administration of 2% DSS in the drinking water for 7 days.

Histological observations

Four samples of the distal colon from each animal were subjected to histological examination. The colon tissues were fixed in 10% (v/v) neutral buffered formalin, dehydrated in ethanol, and embedded in paraffin. Sections (4 μm thick) were then prepared and stained with hematoxylin and eosin (H & E). Images were acquired using a Zeiss Axioskop 2 Plus microscope (Carl Zeiss MicroImaging, Thornwood, NY, USA) equipped with an AxioCam MRc5 CCD camera (Carl Zeiss).

Measurement of serum proinflammation cytokine levels

For the serum proinflammatory cytokine assay, blood collected from the inferior vena cava was transferred to a tube and centrifuged (3000 *g* for 10 min at 4°C). Serum levels of TNF-α, IFN-γ, IL-6, and IL-17α were measured

Table 1. Chemical Composition of the Sauce Samples

Group	*pH*	*Salinity (%)*	*Reducing sugars (g/L)*	*Amino type nitrogen (g/100 mL)*	*Total polyphenols (mg/mL)*
AHSS	4.9 ± 0.1^{b}	20.3 ± 0.3^{a}	1.6 ± 0.1^{c}	1.4 ± 0.1^{a}	48.0 ± 3.5^{b}
FSS	4.7 ± 0.1^{c}	19.2 ± 0.2^{b}	3.7 ± 0.1^{b}	0.7 ± 0.1^{c}	118.4 ± 3.6^{a}
FSeS	5.1 ± 0.1^{a}	18.6 ± 0.3^{c}	4.4 ± 0.2^{a}	0.8 ± 0.1^{b}	122.6 ± 4.5^{a}

Data are presented as the mean ± SD.

[abc]Mean values in the same column with different superscript letters are significantly different ($P < .05$) according to Duncan's multiple range test.

AHSS, acid hydrolyzed soy sauce; FSS, fermented soy sauce; FSeS, fermented sesame sauce.

with a commercial ELISA kit (ELISA MAX; Biolegend, San Diego, CA, USA) according to the manufacturer's protocol.

Semiquantitative reverse transcription-PCR assay

mRNA expression of TNF-α, IL-6, IL-17α, IFN-γ, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) in the colon mucosa was measured with a reverse transcription (RT)-PCR assay. Total RNA was isolated from the colonic tissue (100 mg) using the Trizol reagent according to the manufacturer's recommendations and centrifuged at 12,000 *g* for 15 min at 25°C after the addition of chloroform. Isopropanol was added to the supernatant at a 1: 1 ratio, and the RNA was pelleted by centrifugation (12,000 *g* for 15 min at 4°C). After washing the pellet with ethanol, the RNA was solubilized in diethyl pyrocarbonate-treated RNase-free water and quantified by measuring the absorbance at 260 nm using a UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). Equal amounts of RNA (1 μg) were reverse transcribed in a master mix containing 1 × reverse transcriptase buffer, 1 mM dNTPs, 500 ng of oligodT_{18} primers, 140 U of MMLV reverse transcriptase, and 40 U of RNase inhibitor for 45 min at 42°C. PCR was then carried out in an automatic thermocycler (Bioneer, Daejeon, South Korea) for 25 cycles (94°C for 30 s, 55°C for 30 s, and 72°C for 40 s) followed by an 8-min extension at 72°C. The PCR products were separated in 2% agarose gels and visualized by EtBr staining. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization. Gene expression was quantified using ImageJ software (http://rsbweb.nih.gov/ij/).

Statistical analyses

All data are presented as the mean ± standard deviation (SD). Differences between the mean values for individual groups were assessed with a one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered significant when $P < .05$. SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA) was used to perform these analyses.

RESULTS

Chemical composition of the sauce samples

The chemical compositions of AHSS, FSS, and FSeS are shown in Table 1. The pH (4.9) and salinity (20.3%) of AHSS were similar to that of FSS (4.7% and 19.2%, respectively) and FSeS (5.1% and 18.6%, respectively). Reducing sugar contents of AHSS, FSS, and FSeS were 1.6, 3.7, and 4.4 g/L, respectively. Amino type nitrogen levels of the AHSS (1.4 g/100 mL) were higher than those in FSS (0.7 g/100 mL) and FSeS (0.8 g/100 mL). FSeS contained the highest polyphenol contents (122.6 mg/mL) compared to FSS (118.4 mg/mL) and AHSS (48.0 mg/mL).

Anticolitic effect of the sauces in mice

As shown in Table 2, body weights of the DSS-treated mice were significantly decreased by day 15 (the end of the experiment). The fermented sauces more effectively prevented body weight loss compared to AHSS. Among the fermented sauces, FSeS had a greater ability to decrease DSS-induced body weight loss than the FSS. It is worth

Table 2. Effects of FSeS, FSS, and AHSS on Body, Liver, Kidney, and Spleen Weight in C57BL/6J Mice with DSS-Induced Colitis

			Relative organ weight (g/100 g body weight)		
Group	*Treatment*	*Final body weight (g)*	*Liver*	*Kidney*	*Spleen*
1	Normal	17.8 ± 0.6^{a}	5.0 ± 0.3^{ab}	1.5 ± 0.2^{a}	0.6 ± 01^{c}
2	DSS + saline	12.7 ± 0.7^{f}	5.2 ± 1.0^{ab}	1.1 ± 0.4^{b}	0.9 ± 0.2^{b}
3	DSS + AHSS (4 mL/kg)	13.5 ± 0.3^{de}	5.3 ± 0.3^{ab}	1.3 ± 0.1^{ab}	0.8 ± 0.1^{bc}
4	DSS + FSS (4 mL/kg)	13.9 ± 0.5^{d}	5.4 ± 0.5^{a}	1.4 ± 0.2^{ab}	1.1 ± 0.4^{a}
5	DSS + FSeS (4 mL/kg)	15.4 ± 0.5^{b}	4.9 ± 0.3^{ab}	1.3 ± 0.2^{ab}	1.1 ± 0.4^{a}
6	DSS + AHSS (8 mL/kg)	13.2 ± 0.5^{ef}	4.7 ± 0.2^{b}	1.3 ± 0.1^{ab}	0.6 ± 0.1^{c}
7	DSS + FSS (8 mL/kg)	13.7 ± 0.5^{de}	4.9 ± 0.7^{ab}	1.4 ± 0.2^{ab}	0.7 ± 0.1^{bc}
8	DSS + FSeS (8 mL/kg)	14.8 ± 0.2^{c}	5.0 ± 0.7^{ab}	1.3 ± 0.4^{ab}	0.7 ± 0.1^{bc}

Data are presented as the mean ± SD.

[abcdef]Mean values with the different superscript letters are significantly different ($P < .05$) according to Duncan's multiple range tests.

DSS, dextran sulfate sodium.

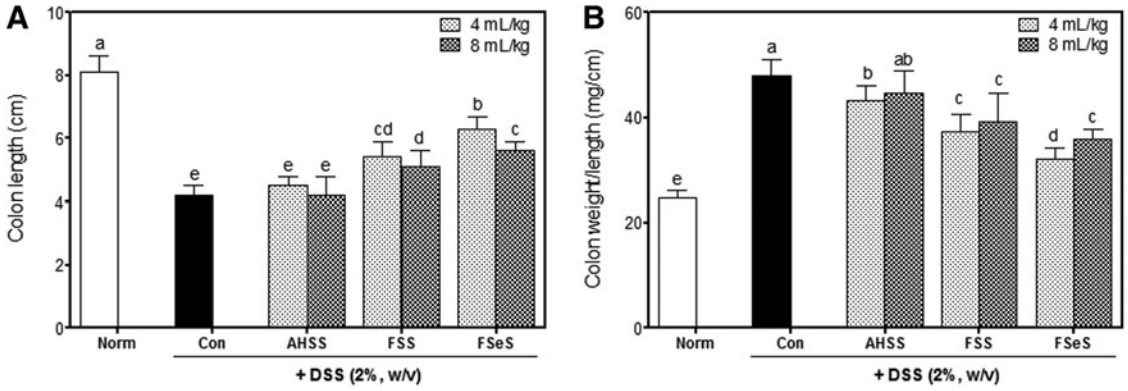

FIG. 1. Sauce samples attenuate clinical signs of dextran sulfate sodium (DSS)-induced colitis in mice. **(A)** Colon length and **(B)** colon weight/length ratio were evaluated. Data are expressed as the mean ± SD. [abcde]Columns with different letters are significantly different ($P < .05$) according to Duncan's multiple range test. AHSS, acid hydrolyzed soy sauce; FSS, fermented soy sauce; FSeS, fermented sesame sauce.

noting that the low dose (4 mL/kg) of the fermented sauces more effectively prevented DSS-induced body weight loss than the high dose (8 mL/kg).

Shortening of the colon is a typical symptom of UC. As shown in Figure 1A, total colon length of the DSS-treated mice (4.2 ± 0.3 cm) was significantly shorter compared with the normal control animals (8.1 ± 0.5 cm). At doses of 4 and 8 mL/kg, the fermented sauces, especially FSeS (6.3 ± 0.4 and 5.6 ± 0.3 cm, respectively), more effectively mitigated colon length shortening induced by DSS than FSS (5.4 ± 0.5 and 5.1 ± 0.5 cm, respectively) and AHSS (4.5 ± 0.3 and 4.2 ± 0.6 cm, respectively).

The ratio of colon weight to colon length was significantly elevated in the DSS-treated mice, indicating that DSS promoted edematous changes associated with colitis. Mice treated with DSS had a significantly increased colon weight/length ratio (48.0 ± 3.1 mg/cm) compared with that of the normal mice (24.6 ± 1.6 mg/cm). These DSS-associated changes were ameliorated by administering the fermented sauce samples at both low and high doses (Fig. 1B). Among the animals treated with 4 mL/kg of sauce, FSeS (31.9 ± 2.2 mg/cm) significantly reduced edematous changes in the colon compared to FSS (37.2 ± 3.4 mg/cm) and AHSS (43.1 ± 2.9 mg/cm). Similar results were also observed for the mice treated 8 mL/kg of the sauces (35.9 ± 1.9, 39.1 ± 5.5, and 44.5 ± 4.4 mg/cm for FSeS, FSS, and AHSS, respectively). In addition, all of the sauce samples were well tolerated by the mice and no obvious signs of systemic toxicity were observed during the entire treatment period. Toxicity was monitored by evaluating the general appearance of the animals along with differences in organ weight of the liver, kidney and spleen between normal mice and ones with DSS-induced colitis (Table 2).

Histological observations

Colon tissues were histologically analyzed to evaluate DSS-induced intestinal injury. As shown in Figure 2, colon

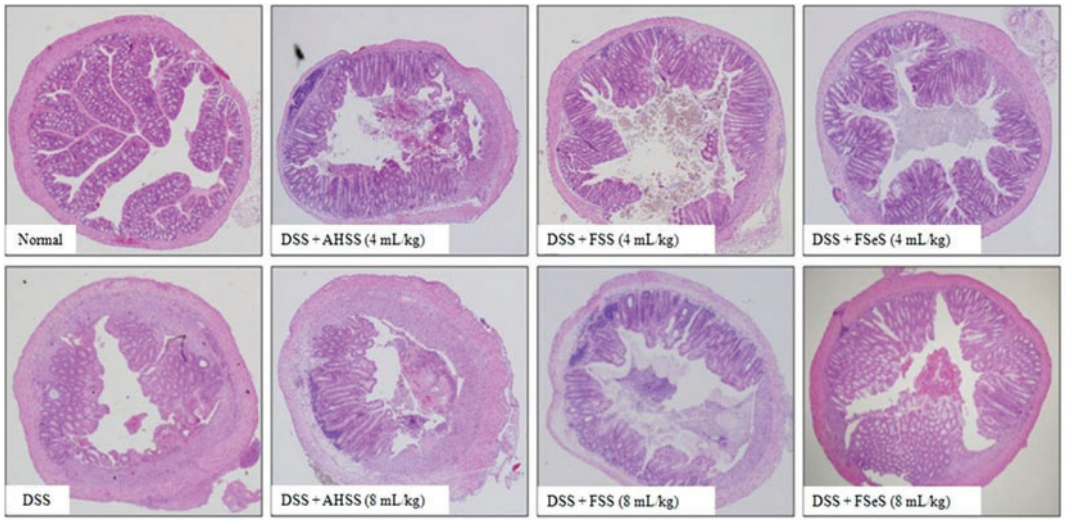

FIG. 2. Histological evidence of DSS-induced colitis in mice treated with different sauce samples. Colon samples were obtained 7 days after DSS administration, sectioned, stained with H&E, and observed with a light microscope (original magnification, 40×). Color images available online at www.liebertpub.com/jmf

tissue from the DSS-treated mice showed typical acute inflammatory changes in the colonic architecture such as ulceration, crypt dilation, and goblet cell depletion. Infiltration of inflammatory cells was also observed. Conversely, colons from DSS-treated mice given the fermented sauces showed greatly reduced numbers of infiltrating cells and a lesser degree of mucosal injury and edema, particularly in the FSeS-treated groups. AHSS had a weaker ability to prevent DSS-induced inflammatory injury in the mice.

Effects of the fermented sauce samples on serum proinflammatory cytokine levels

Increased serum proinflammatory cytokine levels are associated with UC pathogenesis.[22,23] Therefore, effects of the fermented sauce samples on serum levels of TNF-α, IL-6, IL-17α, and IFN-γ were evaluated using an ELISA assay. As shown in Figure 3, DSS significantly increased the serum levels of TNF-α, IL-6, IL-17α, and IFN-γ. Fermented sauces, specifically FSeS, significantly decreased the levels of TNF-α (47%), IL-6 (67%), IL-17α (47%), and IFN-γ (35%) in serum compared to those found in mice treated with DSS alone (control group). At a concentration of 4 mL/kg, FSeS and FSS also decreased the serum levels of TNF-α (62% and 48%, respectively), IL-6 (32% and 18%, respectively), IL-17α (23% and 8%, respectively), and IFN-γ (40% and 35%, respectively) more effectively than AHSS.

Effect of the sauces (4 mL/kg) on proinflammatory cytokine expression levels in the colonic mucosa

To further investigate the anti-inflammatory effects of fermented sauces on DSS-induced colitis in C57BL/6J mice, mRNA expression of TNF-α, IL-6, IL-17α, and IFN-γ in the colonic mucosa was analyzed by RT-PCR. As presented in Figure 4, colon inflammation induced by DSS resulted in elevated expression of all of the proinflammatory cytokines. FSeS and FSS significantly reduced the colonic mRNA levels of TNF-α (83% and 66%, respectively), IL-6 (73% and 58%, respectively), IL-17α (58% and 31%, respectively), and IFN-γ (28% and 21%, respectively) compared to the levels in mice treated with DSS alone. Our findings also indicated that the administration of FSeS and FSS more effectively reduced the mRNA expression of TNF-α (82% and 63%, respectively), IL-6 (61% and 43%, respectively), IL-17α (56% and 28%, respectively), and IFN-γ (30% and 24%, respectively) than AHSS in the mice with DSS-induced colitis.

Effect of the sauces (4 mL/kg) on iNOS and COX-2 expression in the colonic mucosa

DSS significantly increased the mRNA expressions of iNOS and COX-2 in the colonic mucosa of the mice (Fig. 5). Subsequent treatment with 4 mL/kg of the fermented sauces caused a marked decrease in iNOS and COX-2 mRNA

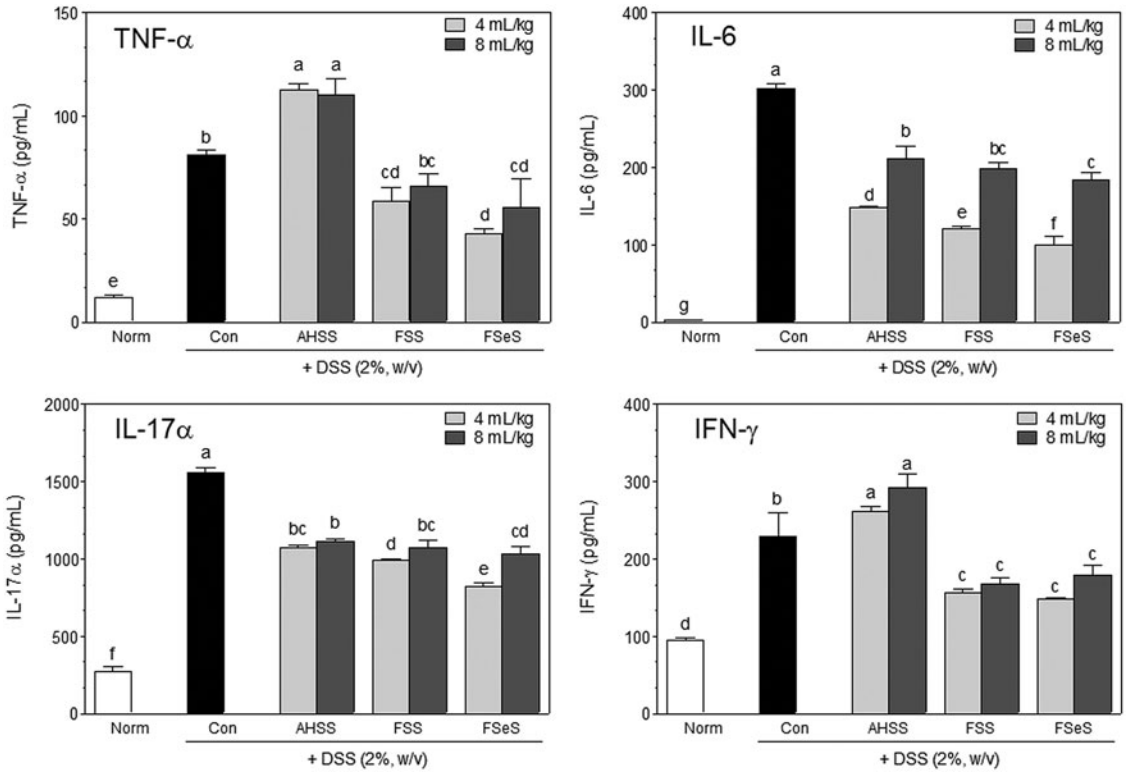

FIG. 3. Effects of the sauce samples on serum levels of proinflammatory cytokines (TNF-α, IL-6, IL-17α, and IFN-γ) in mice treated with 2% DSS. Data are expressed as the mean ± SD. [abcdefg]Columns with different superscript letters are significantly different ($P < .05$) according to Duncan's multiple range test.

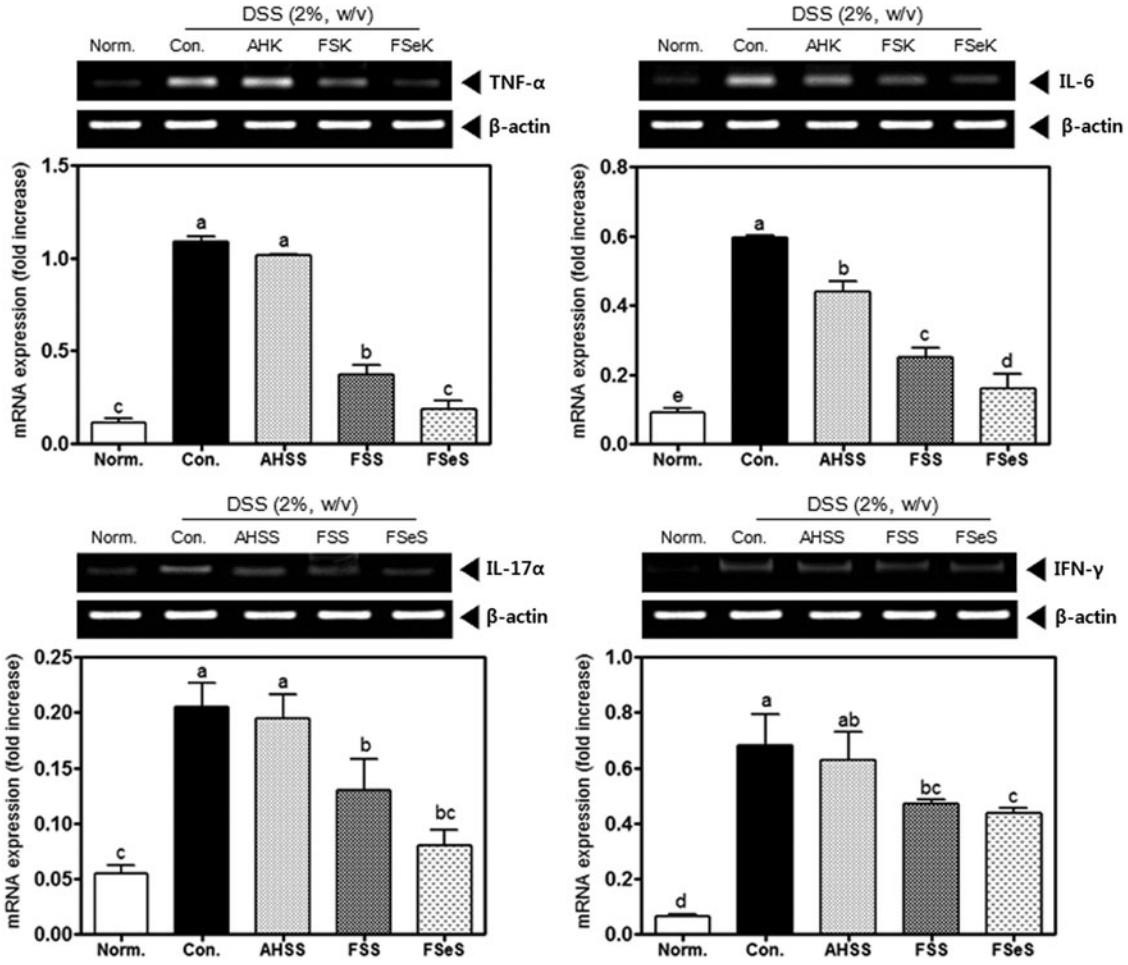

FIG. 4. Effects of the sauce samples (4 mL/kg) on mRNA expression of proinflammatory cytokines (TNF-α, IL-6, IL-17α, and IFN-γ) in the colonic mucosa of mice treated with 2% DSS. The mRNA levels of inflammatory cytokines were measured using a reverse transcription (RT)-PCR assay, as described in the Materials and Methods section. The PCR products were quantified and normalized relative to GAPDH (internal control). Data are expressed as the mean ± SD. [a–d]Bars with different letters are significantly different ($P < .05$) according to Duncan's multiple range test.

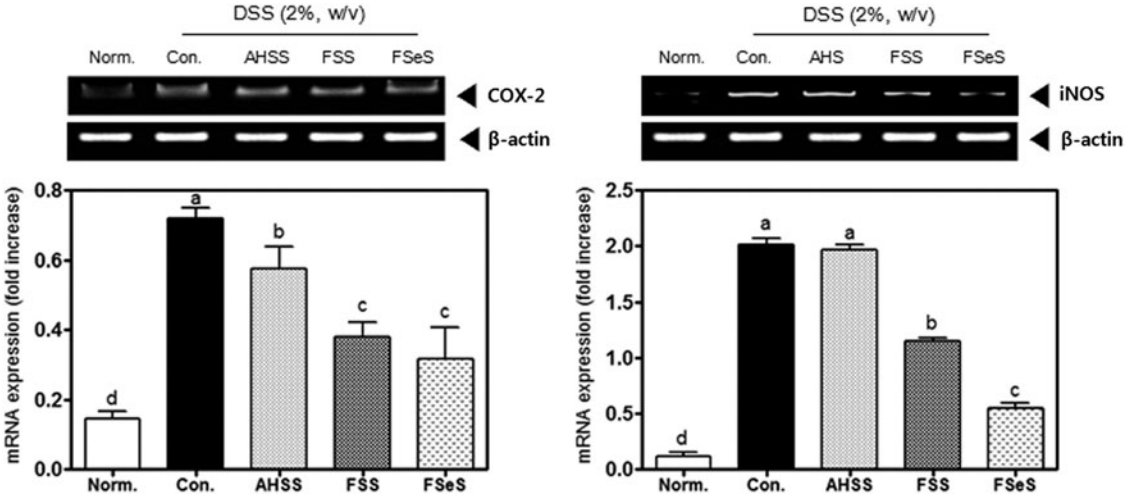

FIG. 5. Effects of the sauce samples (4 mL/kg) on mRNA expression of iNOS and COX-2 in the colonic mucosa of mice treated with 2% DSS. The mRNA levels of iNOS and COX-2 were measured with an RT-PCR assay, as described in the Materials and Methods section. The PCR products were quantified and normalized relative to GAPDH (internal control). Data are expressed as the mean ± SD. [a–d]Bars with different letters are significantly different ($P < .05$) according to Duncan's multiple range test.

levels in the colonic mucosa. FSeS and FSS effectively decreased the mRNA levels of iNOS (72% and 42%, respectively) and COX-2 (57% and 48%, respectively) compared to the levels found in mice treated with DSS alone. AHSS only weakly inhibited mRNA expression of iNOS and COX-2 in colonic mucosa compared to the fermented sauces.

DISCUSSION

Kanjang (fermented soy sauce) has been traditionally used as both a condiment and a health food with antioxidant, antimutagenic, and antitumor activities.[3–10] Both FSS and FSeS are prepared by fermenting soybeans or sesame seeds as main ingredients, respectively, with *A. oryzae* or *Aspergillus sojae* at 30°C for over 6 months. Soybeans, a raw material of soy sauce, contain high concentrations of soy isoflavones, including daidzin, genistin, daidzein, genistein, and glycitein.[24,25] During the fermentation process, daidzin and genistin are transformed into daidzein and genistein, respectively, by *A. oryzae* and other fungi of this genus.[26,27] It has been reported that FSS is rich in phytochemicals, including free daidzein (0.9–23.5 μg/g) and free genistein (2.8–17.9 μg/g).[28,29] Sesame, an oil-rich crop, has been traditionally considered a health food in Asia. Some studies reported that sesame seeds contain high concentrations of sesamin and sesaminol along with their glycosides.[30,31] During the fermentation of sesame sauce, sesamin is transformed by starters of the genus *Asperillus* into sesaminol monoglucoside (13.7 μg/g), sesaminol triglucoside (0.2 μg/g), sesaminol 6-catechol (1.1 μg/g),[2] and epsisesamin 2, 6-dicatechol.[32] We also previously found that FSeS is enriched with sesamin (13 μg/g).[2]

Unlike fermented sauces, AHSS is a product prepared from defatted soybeans (or other protein-rich materials) and 18% food-grade hydrochloric acid by heating (107°C) for 15–20 h. This sauce mainly contains reducing sugars and amino acids.[1] Acid hydrolysis of defatted soybeans is an important process in AHSS manufacturing.[1] During this procedure, residual fatty acid esters (glycerol) exist in the raw soy sauce materials in the form of chloropropanols.[33] Among these compounds, 1,3-dichloropropan-2-ol (1,3-DCP) and 3-chloropropane-1,2-diol (3-MCPD) are two of the most toxic.[34,35] 1,3-DCP has been deemed a genotoxic carcinogen and has mutagenic effects on bacterial and mammalian cells *in vitro*.[36] 3-MCPD is absorbed by the gastrointestinal tract and metabolized into glycidol, a genotoxic carcinogen that induces mutagenesis in humans and rodents.[37] As shown in Table 1, total phenol contents of FSS and FSeS were higher than those of AHSS. It has been suggested that more free-form and aglycone phenolic compounds can be formed during fermentation. However, the phenolic compounds can exist as complex forms without fermentation in AHSS. Shao[38] reported that the level of phenolic compounds is increased during soy sauce fermentation and showed that *in vitro* antioxidant, anticancer, and antimutagenic activities are increased. Jung *et al.*[39] also reported that *doenjang* (Korean fermented soy paste) exerts stronger antitumor and antimetastatic effects when fermented for longer periods of time. Our data indicated that fermentation is able to increase the production of bioactive phenolic compounds and could play an important role in preventing colitis.

We also observed that 4 mL/kg of fermented sauce had a greater preventive effect against DSS-induced colon shortening compared to 8 mL/kg. In addition, the lower dose of fermented sauces increased the ratios of colon weight to colon length and effectively ameliorated DSS-induced edema, crypt dilation, goblet cell depletion, and infiltration of inflammatory cells in the colonic mucosa.

Both fermented sauces as well as AHSS contained high levels of salt (approximately 18–20% of NaCl). High salt intake can result in gastrointestinal damage, increased DNA replication, and cell proliferation.[40] High levels of NaCl also exert a comutagenic effect in the Ames test.[41] In our previous studies, FSS showed an *in vitro* antioxidant activity (approximately 28%); however, the salt used to make soy sauce was not found to have the antioxidant activity ($< 1\%$) at the same concentrations (data not shown). Thus, *kanjang* is a more healthy condiment compared to salt alone when used for cooking and as a food flavoring.

In active cases of UC, the inflamed colonic mucosa has increased electrical conductivity and enhanced permeability to monovalent ions.[42] In the descending/sigmoid colon and rectum, inflammation causes a notable decrease or loss of the lumen-negative transmucosal potential difference, a consequence of both increased epithelial permeability and the virtual absence of electrogenic Na^+ transport.[43,44] We found that 8 mL/kg of the sauces did not possess effective anticolitic activity (compared to 4 mL/kg of the sauces) in the mice treated with DSS. This difference is thought to be associated with the greater NaCl levels in the higher dose of fermented sauces. This finding emphasizes the importance of identifying appropriate concentrations of *kangjang* or NaCl to prevent UC.

Imbalances between pro- and anti-inflammatory cytokines are associated with the development of UC and are dramatically shifted toward the proinflammatory factors.[22,23] Down-regulating the production of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-17α, and IFN-γ in the intestine can inhibit inflammatory responses and successfully ameliorate UC, as indicated by a previous clinical study, in humans and animal models of DSS-induced UC.[45] In the present investigation, we found that FSeS and FSS both reduced the serum levels of proinflammatory cytokines (TNF-α, IL-6, IL-17α, and IFN-γ) and also inhibited colonic mRNA expression of these cytokines compared to AHSS. A number of studies have reported that the main bioactive compounds in soy sauce, including genistein and daidzein, effectively reduce the levels of TNF-α, an important inflammatory mediator, elevated by IBD in experimental rodent models.[46–48] Genistein also fails to decrease T-cell proliferation and reduces the production of IL-1β and IL-6 in lipopolysaccharide (LPS)-treated mice.[49] Sesamin and sesamolin have been shown to inhibit TNF-α, IL-1β, and IL-6 activity *in vitro* and *in vivo*.[50–52]

In addition to proinflammatory cytokines, overexpression of iNOS and COX-2 in the colonic mucosa is associated with UC pathogenesis.[53,54] Following treatment with 4 mL/kg

of fermented sauces (FSeS or FSS), the colonic mRNA expressions of iNOS and COX-2 were reduced compared to the levels found in AHSS-treated mice with DSS-induced colitis. The main bioactive compounds in FSeS and FSS, such as genistein, daidzein, sesamin, and sesamolin, can decrease LPS-induced production of nitric oxide (NO), an important proinflammatory mediator, during IBD pathogenesis *in vitro*[49,55,56] and *in vivo*.[50] Genistein and daidzein can also inhibit LPS-induced iNOS expression by decreasing the activities of nuclear factor-κB (NF-κB) as well as signal transducer and activator of transcription-1 (STAT-1), two important transcription factors, in murine J774 macrophages.[55] In addition, genistein, daidzein, and sesamin reduce COX-2 activity *in vitro*.[57–59] Based on these results, we suggest that fermented sauces have anti-inflammatory effects on DSS-induced UC.

In conclusion, the results from the present study indicated that fermented sauces, FSeS and FSS, administered orally at 4 mL/kg significantly prevent DSS-induced body weight loss, colonic shortening, and intestinal wall thickening in mice. FSeS and FSS administration also decreased the serum and colonic mRNA levels of proinflammatory cytokines (TNF-α, IFN-γ, IL-6, and IL-17α), iNOS, and COX-2. Furthermore, 4 mL/kg of the fermented sauce samples more effectively attenuated DSS-induced colitis than 8 mL/kg of the sauces. FSeS exhibited more potent anticolitic effects than FSS, indicating that raw materials for the fermentation process are important for increasing the functionality of the sauces. AHSS could not confer protection against colitis. Taken together, the results from the current investigation suggest that fermented sauces, particularly FSeS, have a potent anticolitic efficacy. Our findings also indicated that the fermentation process is very important for enhancing the levels of functional compounds and improving the beneficial properties of *kangjang*. Finally, appropriate intake of fermented sauce is important to maximize the anticolitic activity.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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