Therapeutic effects of *Ligularia stenocephala* against inflammatory bowel disease by regulating antioxidant and inflammatory mediators

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**ABSTRACT**

In this report, we studied the effect of leaf extracts of *Ligularia stenocephala* (LS) on inflammatory bowel disease (IBD) by regulating antioxidant and inflammatory mediators. The water extracts (LSW) exhibited more antioxidant activity than that of ethanol extracts (LSE). The extracts suppress the formation of nitric oxide by down-regulating the inducible nitric oxide synthase and pro-inflammatory cytokines (e.g., tumor necrosis factor alpha, interleukin (IL)-6, IL-10 and IL-1β expression) through the suppression of nuclear factor-κB activation and mitogen-activated protein kinases phosphorylation in lipopolysaccharide-stimulated macrophage cells. Our *in vivo* study reveals that the extracts could suppress tissue damage significantly in mice colon. The expression regarding immune-related cytokines was also down-regulated by the extracts of LS leaf. Thus, it is concluded that the extracts could be used as a functional food, which could reduce the formation of oxidants, inflammation and IBD effectively.

**ARTICLE HISTORY**

Received 6 April 2017
Accepted 15 May 2017

**KEYWORDS**

Antioxidant; colitis; dextran sodium sulfate; MAPKs

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Introduction

Inflammatory bowel disease (IBD) (e.g., Crohn’s disease and ulcerative colitis) are intestinal disorders, caused by various factors, such as genetic predisposition and environmental triggers (Bae, Yoo, Lee, Joo Han, & Kim, 2008; Hendrickson, Gokhale, & Cho, 2002). Conventional therapies, such as the use of sulfalsalazine, glucocorticoids or cyclosporine, are reported to have harmful side effects (Oz, Chen, & de Villiers, 2013). Therefore, there is great demand for an alternative therapy having high efficacy with minimum or no harmful effects. The formation of oxidants is considered as one of factors involved in several signs and symptoms of inflammatory diseases. The systematic consumption of functional foods may reduce the risk of oxidative stress-associated diseases including IBD (Gupta & Prakash, 2009; Moura, de Andrade, dos Santos, Araújo, & Goulart, 2015). With this regards, natural products could be of great interest for IBD treatment.

Nuclear factor-κB (NF-κB) has an important role in the pathogenesis of chronic inflammatory diseases by regulating the genes related to immune responses (Abdullah, Abdulghani, Ismail, & Abidin, 2017; Kim et al., 2008; Senthil Kumar & Wang, 2009). In addition, NF-κB mediates the expression of pro-inflammatory mediators and cytokines such as inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)-α, interleukin-1β (IL-1β), interleukin-6 and cyclooxygenase-2 (COX-2). Further, the activation of NF-κB is related to the rapid phosphorylation of inhibitor of kappa B (IκBα) (Karin & Delhase, 2000). The mitogen-activated protein kinases (MAPKs) such as c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinase (ERK) are the important modulators of inflammatory responses. Further, the phosphorylation of MAPKs is related to the activation of NF-κB (Zhou et al., 2008).

The leaves of Ligularia stenocephala (LS) are consumed as an edible vegetable and known as gondalbi in Korea. The genus Ligularia is usually treated as folk medicine to cure influenza, asthma, cough, ulcers, arthritis and tuberculosis (Lee et al., 2010) in some region of eastern and western Asia. Yoon et al. (2008) reported the antithrombotic activity of the leaf extract of LS. However, the anti-IBD effects of the LS have not been scientifically verified. Therefore, this report intended to evaluate the anti-IBD effects for the LS in combination with its antioxidant and anti-inflammatory activities.

Materials and methods

Materials

Folin-Ciocalteu (FC) reagent, gallic acid (GA), sodium bicarbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid and ascorbic acid (AA) were obtained from Sigma (St. Louis, MO, USA). Dextran sodium sulfate (DSS) was purchased from Sigma–Aldrich (St. Louis, MO, USA). FeCl₃ and NaOH were purchased from Wako Pure Chemical Industries Ltd (Tokyo, Japan).

Preparation of extracts

Dried LS leaves were ground to a fine powder, and 500 g aliquots of each sample were then extracted either with distilled water (DW) at 85°C for 3 h or with 94% ethanol at room temperature (RT) for 7 d to obtain the LS water extract (LSW) and LS ethanol extract.
(LSE), respectively. The extracts were then filtered through a filter paper followed by evaporation on a rotary vacuum evaporator at 40°C under reduced pressure. Finally, the extracts were lyophilized for 2 d and kept in a refrigerator at 4°C before further use. The percentage of yields (w/w) were determined using the equation: yield (%) = (total extracted sample mass/total dry sample mass) × 100.

**Measurement of total phenolic compounds**

The total amount of phenolic compounds was measured by the reported FC method (Im et al., 2016). The GA was used as the standard. Briefly, 10 mg extract was mixed with 1 mL of 75% ethanol. The standard solutions (0.02–0.2 mg/mL) were prepared in DW. A 40-μL aliquot of each sample or the standard was mixed with 20 μL of 1 N FC reagent. Then, the mixture was added to 60 μL of 20% NaCO₃ aqueous solution followed by the incubation at ambient temperature for 30 min in the dark. Finally, the absorbance was measured by using a UV–visible spectrophotometer at a wavelength of 700 nm.

**Measurement of total flavonoid compounds**

Total flavonoid content was measured by the reported colorimetric assay described by Samad et al. (2014). Briefly, 10 mg extract was mixed with 1 mL of DW. Catechin (as a standard) was dissolved in ethanol at different concentrations (0–25 mg/mL). A 25-μL aliquot of the sample or standard was added to 125 μL DW. Then, 8 μL of 5% NaNO₃ was added to the mixture. It was incubated at ambient temperature for 5 min followed by the addition of 10% AlCl₃ solution. Then, the total flavonoid content was calculated by measuring the absorbance at 517 nm.

**Activity for DPPH radical scavenging**

The activities for DPPH radical of water and ethanol extracts of LS were measured according to the report of Debnath et al. (2011). The butylated hydroxytoluene (BHT) with different contents (0.1–3 mg/mL) was used as a standard. Then, 80 μL solution of sample or standard was added to 80 μL DPPH. It was incubated at ambient temperature for 30 min under dark. Then, the activity was determined using the equation: activity (%) = \{(a – b) – (x – y)/(a – b)\}×100, where x is the absorbance of DPPH + sample/standard at 517 nm, y is the absorbance of sample/standard + methanol at 517 nm, a is the absorbance of DPPH + DW (or ethanol)/methanol at 517 nm and b is the absorbance of methanol + DW (or ethanol) at 517 nm.

**2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity**

Seven millimolar aqueous ABTS solution was mixed with potassium persulfate solution to make a solution of 2.45 mM. It was then left undisturbed at RT for 12–16 h under dark before use (Debnath et al., 2011). The absorbance of ABTS solution was adjusted to 0.70 ± 0.02 at 734 nm wavelength by diluting with 0.01 M phosphate buffer saline (PBS, pH 7.4). Then, 0.9 mL of ABTS solution was mixed with 0.1 mL of each sample (0.12–2.00 mg/mL) and BHT (0.025–0.125 mg/mL) followed by the
incubation at ambient temperature for 5 min. The activity for ABTS scavenging was determined by using a UV–visible spectrometer at 734 nm as reported before (Debnath et al., 2011).

**Scavenging activities for hydroxyl and superoxide radicals**

The activities for hydroxyl and superoxide radicals were determined by using electron spin resonance (ESR) spectra as reported elsewhere (Debnath et al., 2011). Briefly, 20 μL of each 0.3 M 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 10 mM H2O2 were mixed with PBS of pH 7.4. Then, 20 μL of each 10 mM iron sulfate and that of sample or standard were added to the above mixture followed by the measurement after 2.5 min by ESR. The reaction mixture was prepared by adding 20 μL of various concentrations of sample or standard to 20 μL of each 0.8 M DMPO, 1.6 mM EDTA and 0.8 mM riboflavin. Before the ESR spectrum was recorded, the mixture was illuminated under UV lamp (λ = 365 nm) for 1 min.

**In vitro cell culture**

RAW 264.7 mouse macrophage cells were obtained from the Korean Cell Line Bank, Seoul, Korea. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin in an incubator (5% CO2, 37°C).

**Cell viability**

Cells were seeded at 5 × 10^5 cells per well in a 96-well plate, containing 100 μL of DMEM media. Following an overnight incubation at 37°C, the cells were mixed with LSW and lipopolysaccharide (LPS) for 18 h to make 1 μg/mL of final concentration. After treatment, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) with 2.5 μg/mL in media was added to each well followed by incubation at 37°C for 4 h. The medium was discarded, and 100 μL of dimethyl sulfoxide was added to the above mixture followed by measuring the absorbance at 570 nm.

**Measurement of nitric oxide (NO)**

Cells were seeded at 5 × 10^5 cells per well in a 96-well plate, containing 100 μL of DMEM media. Following an overnight incubation at 37°C, the cells were mixed with LSW and LPS for 18 h to make 1 μg/mL of final concentration. The supernatant of the cell media was added to the same volume of Griess reagent followed by measuring the absorbance at 520 nm.

**Western blotting**

Cells were seeded in 6-well plates with a density of 1 × 10^6 cells/well. The next day, the cells were treated with LSW with or without LPS. After treatment, the cells were harvested and suspended in a lysis buffer. The same amounts of proteins were added to a 10% acrylamide
gel (SDS-PAGE) and the polypeptides moved to a poly(vinylidene fluoride) membrane. Five percent skim milk blocked the membrane for 1 h and incubated overnight with primary antibodies at 4°C. The membrane was washed with TBS buffer (150 mM NaCl, 10 mM Tris-HCl, pH 8.0) containing 0.05% Tween 20 three times followed by the incubation with anti-rabbit secondary antibody with horseradish peroxidase at RT for 1 h and detected by chemiluminescence reagents.

**In vivo animal study**

**Animals**

Female BALB/c mice at 5–6 weeks of age were purchased from Orient Bio (Seongnam, Korea). The treatment and care of the mice and experimental protocol were performed by the institutional guidelines. For experiment, the animals were acclimatized for 1 week. The mice were kept in a temperature-controlled room with a facility of the 12 h light/dark cycle. The mice were randomly divided into five groups, namely, Group A (control mice), Group B (DSS-induced colitis), Group C (LSW administration), Group D (DSS-induced colitis + LSW administration) and Group E (DSS-induced colitis + sulfasalazine administration). Group A were orally fed a commercial diet. Group C mice were oral dosed daily with the LSW extract for up to 15 d. Group B mice were given the water containing 3% DSS for 7 d to induce colitis and then switched to clean drinking water for the next 15 d. Groups D and E mice were treated orally with doses of extract dissolved in water (50 mg/kg mouse) and sulfasalazine (3 mg/kg), for up to 15 d, after induction of colitis by DSS treatment.

**Western blot analysis**

Cellular proteins were obtained from the extraction of treated and untreated control mice. One-hundred milligram of spleen tissue was homogenized in RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS and 2 mM EDTA) with 1% protease inhibitor cocktail. Then, it was sonicated using a dismembrator followed by centrifugation at 12,000 × g at 4°C for 15 min. The supernatants were collected. Then, the total protein concentration was calculated by the Bio-Rad protein assay.

**Histological score**

Histology was performed on cryosectioned colonic tissue of the mice, embedded in a frozen section compound (FSC 22, Leica Microsystems). The fixed specimens were then sectioned (10 μm). Finally, the colonic sections were stained with hematoxylin/eosin (H&E). Histological activity index was scored according to the report by Obermeier et al. (1999). The scoring was as follows: 0 = no change; 1–2 = low level of inflammation in colonic tissues; 3 = moderate inflammation and wound in colonic tissues; 4 = severe inflammation and wound in colonic tissues.
Statistical analysis

The statistical analysis was assessed using Student’s t test with GraphPad Prism (v5.0, GraphPad Software Inc., La Jolla, CA, USA).

Results and discussion

Eating adequate fruits and vegetables regularly is beneficial to health because it contains multi-nutrients and biologically active and non-nutritive compounds (Oomah & Mazza, 2000). In particular, they are good sources of natural antioxidants. It has been reported that natural antioxidants effectively decrease oxidative stress and inflammation, without or with very low cytotoxicity (Laranjinha, Almeida, & Madeira, 1994). Crude plant extracts have raised interest among pharmaceutical and food industries for their medicinal value and food preservation quality (Barros, Calhelha, Josiana, & Estevinho, 2007). In this report, we determined the anti-IBD effects of LS, together with its antioxidant and anti-inflammatory activities. As shown in Table 1, 13.2% and 8.26% yields were obtained for LSW and LSE, respectively. Phenolic compounds have various functions including free radical scavenging activity (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003; Obermeier et al., 2007). LSW and LSE showed phenolic contents of 42.24 and 22.63 mg GAE/100 g (dry basis), respectively, and the corresponding flavonoid contents were 24.30 and 12.63 mg CE/100 g (dry basis) (Table 1). There was an agreement between the total phenolic compounds and flavonoid contents ($r^2 = 1$ and 0.952, respectively, for the LSW and LSE).

Methods involving DPPH and ABTS are commonly used to estimate the free radical scavenging capacities of plant extracts. The DPPH scavenging activities were found to be 92% and 87%, with the IC$_{50}$ values being 1.67 and 1.86 mg/mL for LSW and LSE, respectively (Table 1). Then, the scavenging activity for ABTS was determined by the color reduction method. In this instance, the IC$_{50}$ values of LSW and LSE were found to be 0.26 and 0.48 mg/mL, respectively (Table 1).

Further, the formation of hydroxyl radicals contributes to inflammation and cytotoxicity by causing oxidative cleavage of proteins and lipids (Uauy, Hoffman, Peirano, Birch, & Birch, 2001). In this report, the scavenging activities of LSW and LSE for hydroxyl radical increased with the increase in dose. Furthermore, LSW had a significantly higher activity than that of LSE. Specifically, the hydroxyl radical activity ranged from 3% to 80%

### Table 1. Total phenolic compound and flavonoid contents, yield and DPPH, ABTS, hydroxyl and superoxide radical scavenging activities (IC$_{50}$) of the water (LSW) and ethanol (LSE) extracts obtained from Ligularia stenocephala leaves.

| Sample | Total phenolic content$^a$ (mg GAE/100 g of dry mass) | Total flavonoid content$^a$ (mg CE/100 g of dry mass) | Yield (%) | DPPH radical$^b$ IC$_{50}$ (mg/mL) | ABTS radical$^b$ IC$_{50}$ (mg/mL) | Hydroxyl radical$^b$ IC$_{50}$ (mg/mL) | Superoxide radical$^b$ IC$_{50}$ (mg/mL) |
|--------|-----------------------------------------------------|-----------------------------------------------------|-----------|----------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| LSW    | 42.24 ± 1.53$^a$                                    | 24.30 ± 2.22$^a$                                     | 13.2      | 1.67 ± 0.07                      | 0.26 ± 0.05                       | 0.5 ± 0.05                           | 1.0 ± 0.06                           |
| LSE    | 22.63 ± 1.22                                       | 12.63 ± 0.30                                        | 8.26      | 1.86 ± 0.04                      | 0.48 ± 0.09                       | 1.0 ± 0.10                           | 2.0 ± 0.10                           |
| AA     | N/d                                                | N/d                                                 | N/d       | 0.054 ± 0.00                     | 0.007 ± 0.00                      | 0.12 ± 0.05                          | 0.25 ± 0.02                          |

Notes: AA: ascorbic acid; CE: catechin equivalent; GAE: gallic acid equivalent; N/d: not detected.

$^a$Each value is expressed as mean ± standard deviation ($n = 3$).

$^b$IC$_{50}$ (mg/mL): the concentration at which 50% is inhibited.
Superoxide free radicals are detrimental to cellular components. These radicals cause arthritis, gastric cancer, ischemia and AIDS (Kumpulainen & Salonen, 1999; Sakanaka, Tachibana, & Okada, 2005). Thus, the activity to suppress the radicals is highly desirable. In this study, the LSW and LSE exhibited significant scavenging activities against superoxide radicals. At 2 mg/mL, the activities of LSW and LSE were 54% (IC$_{50}$ = 1.0 mg/mL) and 46% (IC$_{50}$ = 2.0 mg/mL), respectively (Table 1). Also, both extracts showed a concentration-dependent activity, with LSW displaying a higher scavenging activity than LSE. Here, the higher scavenging activities of LSW on DPPH, ABTS, hydroxyl and superoxide free radicals suggest a higher antioxidant activity compared with LSE. Further, the higher phenolic and flavonoid contents of LSW correspond to its higher antioxidant activity compared with LSE. It is in good agreement with the previous comparative studies on antioxidant activity of plant extracts (Debnath et al., 2011; Samad et al., 2014).

Figure 1. Effects of the water extract of Ligularia stenocephala leaves (LSW) on (a) the viability of RAW 264.7 cells treated with lipopolysaccharide (LPS) and (b) nitric oxide (NO) production in LPS-induced RAW 267.4 cells.
LPS stimulates macrophages to produce NO and inflammatory cytokines as well (Zhao, Li, Chen, Hu, & Zhao, 2017). The effect of LSW on the viability of RAW 264.7 cells was examined by the MTT method. The cell viability decreased significantly in the treatment of LPS for 18 h compared with the untreated cells. However, LSW (0.15–0.5 mg/mL) tended to increase the viability of the LPS-treated cells (Figure 1(a)). No toxicity was evident when normal cells were treated with up to 2 mg/mL LSW (data not shown).

The excess production of NO can also lead to deleterious consequences, such as malignancy, and inflammatory diseases (Bates & Maxwell, 2005). Therefore, the inhibition of NO has a potential therapeutic value for inflammation (Stichtenoth & Frölich, 1998). LPS-stimulated RAW 264.7 cells are expected to produce NO. As shown in Figure 1(b), LSW treatment decreased NO formation in the stimulated cells with the increase of dose.

It has been reported that the production of pro-inflammatory cytokines were up-regulated by LPS in the RAW cells (Sareila et al., 2008). The protein levels of the cytokines and mediators in LSW-treated cells were estimated (Figure 2(a)). It indicated that the incubation of RAW 264.7 cells with LSW (0.125–0.5 mg/mL) in the presence of LPS for 18 h inhibited iNOS, TNF-α, IL-10, IL-6 and IL-1β protein expressions with the increase of dose.

The transcription factor, NF-κβ has a critical role in the gene regulation in the cells associated with inflammation (Kim et al., 2017; Senthil Kumar & Wang, 2009). The release of inflammatory cytokines is mediated by down-regulation of the NF-κβ pathway (Hsieh, Chang, Teng, Chen, & Yang, 2011). In turn, the activation of NF-κβ is regulated by MAPKs (such as ERK, JNK and p38), which plays a major role in inflammation as a group of signaling molecules (Guha & Mackman, 2001). As shown in

Figure 2. Effects of the water extract of *Ligularia stenocephala* leaves (LSW) on (a) protein expression of TNF-α, IL-10, IL-6 and IL-1β. β-actin expression was used as a control. (b) Mitogen-activated protein kinases (MAPKs) and NF-κβ and IκB expression levels in lipopolysaccharide (LPS)-induced RAW 267.4 cells. β-actin expression was used as a control.
Figure 2(b), the treatment of LPS markedly increased NF-κβ and I-κβ expressions, compared to the control. In contrast, LSW treatment suppressed the expressions in a dose-dependent manner. Further, the western blot was performed to analyze the expression of MAPKs (ERK, JNK and p38). As shown in Figure 2(c), LPS treatment up-regulated the phosphorylation of the MAPKs. However, LSW treatments at 0.125–0.5 mg/mL for 18 h inhibited the LPS-stimulated phosphorylation of MAPKs with the increase in dose. These results indicated that LSW inhibits LPS-stimulated inflammation in RAW 264.7 cells through the NF-κβ and MAPKs pathway.

An animal model of IBD using 3% DSS treatment was established. Mice supplemented with water containing 3% DSS showed similar symptoms to human IBD patients. The

| Group | Body (g)a | Organs weight (g) and length (cm)a |
|-------|-----------|-----------------------------------|
|       | Initial   | Final               | Spleen | Liver | Intestine |
| A     | 18.80 ± 1.30 | 19.75 ± 0.45        | 0.06 ± 0.00 | 0.71 ± 0.24 | 41.48 ± 1.58 |
| B     | 20.57 ± 0.58 | 18.75 ± 0.71        | 0.06 ± 0.01 | 0.80 ± 0.08 | 38.40 ± 2.09 |
| C     | 19.33 ± 0.58 | 19.50 ± 0.58        | 0.06 ± 0.01 | 0.72 ± 0.08 | 41.53 ± 0.25 |
| D     | 18.88 ± 1.29 | 18.22 ± 1.91        | 0.06 ± 0.01 | 0.72 ± 0.14 | 39.64 ± 1.54 |
| E     | 19.43 ± 1.62 | 20.40 ± 0.55        | 0.07 ± 0.04 | 0.70 ± 0.15 | 41.50 ± 1.92 |

Notes: A: control; B: DSS-induced colitis mice; C: mice treated with LSW; D: DSS-induced colitis mice treated with LSW; E: DSS-induced colitis mice treated with sulfasalazine.

Each value is expressed as mean ± standard deviation (n = 5).

Figure 3. The effects of the water extract of *Ligularia stenocephala* leaves (LSW) on (a) the histopathological changes and (b) the histological scores in the colon of DSS-induced colitis mice. A: Control; B: DSS-induced colitis; C: LSW treatment only; D: DSS-induced colitis mice with LSW treatment; E: DSS-induced colitis mice with sulfasalazine treatment. Statistical comparisons were made between the control and treatment groups vs. the DSS-induced group (⁎p < .05, ⁎⁎p < .01).
Effects of LSW on body weight, spleen and liver weights, and the intestinal length are shown in Table 2. DSS administration caused a small decrease in the body weight of the mice with respect to control mice. Mice with LSW treatment showed improved body weights. DSS and LSW had no effects on the spleen weight. Conversely, liver weight was significantly increased, whereas intestinal length was decreased due to DSS treatment. On the contrary, LSW treatment remarkably lowered liver weight and improved intestinal length in the colitis-induced mice.

The effect of LSW on the histopathological changes in the colon of the mice is shown in Figure 3. Tissue sections from the colons of Group B exhibited severe crypt and epithelial damage due to DSS administration (Figure 3(a)). In addition, the administration of DSS resulted in a remarkably higher histological score than those of other groups (Figure 3(b)). DSS-induced colitis mice treated with LSW (Group D) displayed a significant regeneration of epithelial tissue and crypts in the colon. Moreover, the histological score was substantially lowered by LSW treatment. Similarly, sulfasalazine treatment ameliorated colonic tissue damage and crypt loss as well as the histological score. However, based on the histological evidence, LSW had a comparably greater effect in recovering colonic tissue damage and crypt loss.

The effects of LSW treatment on the expression for inflammatory mediators and cytokines and their relative intensity in spleen are shown in Figure 4(a–f). The expressions of pro-inflammatory cytokines and mediators, such as iNOS, COX-2 and TNF-α, were up-regulated in Group B (Figure 4(a,c,e)). Treatments with LSW and sulfasalazine lowered the expression of iNOS, COX-2 and TNF-α. In particular, Group D showed the highest suppression of iNOS and TNF-α, whereas Group E showed the highest suppression of COX-2 (Figure 4(a,c,e)). Therefore, it can be suggested that LSW had an intense down-regulatory effect on the pro-inflammatory mediators and cytokines.

![Figure 4](image-url)

**Figure 4.** Effects of water extract of *Ligularia stenocephala* leaves (LSW) on (a) iNOS and (b) its relative intensity, (c) COX-2, (d) its relative intensity, (e) TNF-α and (f) its relative intensity. β-actin expression was used as a control. A: Control; B: DSS-induced colitis; C: LSW treatment only; D: DSS-induced colitis mice with LSW treatment; E: DSS-induced colitis mice with sulfasalazine treatment.
**Conclusion**

This study demonstrated that LSW had strong antioxidant and anti-inflammatory effects. Compared to LSE, LSW had higher contents of phenolic compounds and flavonoids as well as higher antioxidant activity. LSW inhibited the expression of pro-inflammatory mediators and cytokines in LPS-stimulated RAW cells. LSW also inhibited the nuclear translocation of NF-κB and MAPKs. Further, LSW ameliorated the DSS-induced colitis in mice by improving body weight, reducing crypt damage and down-regulating the inflammatory cytokines expressions. Therefore, LSW could be used as a potential anti-IBD agent along with antioxidative and anti-inflammatory properties.

**Conflict of interest**

No potential conflict of interest was reported by the authors.

**Funding**

This study was supported by Dongguk University, 2017.

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