International Immunopharmacology 70 (2019) 235–240

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

# Peanut shell extract inhibits the development of dextran sulfate sodium (DSS)-induced colitis

Ae Sin Lee[1], Kwang Min Lee[1], Jin-Ah Lee, InWook Choi*

*Division of Functional Food Research, Korea Food Research Institute, Wanju-gun, Republic of Korea*

ARTICLE INFO

*Keywords:*
Inflammatory bowel diseases (IBD)
Peanut shell extract (PSE)
Luteolin
Inflammation
Macrophages

ABSTRACT

Inflammatory bowel diseases (IBD) induce inflammation in the colon and small intestine. IBD include ulcerative colitis and Crohn's disease, with such common symptoms as severe diarrhea, fever, and blood in the stool. In the current study, we explored the ability of peanut shell extract (PSE) to alleviate IBD in an experimental colonic inflammation model. Colitis was induced by orally administered dextran sulfate sodium (DSS) in mice. Peanut shell extract was prepared using a method of aqueous ethanol. DSS treatment reduced the colon length and mouse body weight, and aggravated disease condition compared with untreated control mice. Oral administration of 400 mg/kg PSE alleviated colon shortening, body weight loss, DAI, and colon injury score in DSS-induced colitis. These physiological improvements were validated by reduced levels of proinflammatory cytokines and infiltrating macrophage accumulation in the inflamed colon in the PSE administered group. These observations suggest that PSE may be developed as an alternative natural extract for the prevention or treatment of IBD.

## 1. Introduction

Inflammatory bowel diseases (IBD) include inflammatory diseases, e.g., ulcerative colitis (UC). Several anti-inflammatory drugs, including sulfasalazine, steroids, and nonsteroid anti-inflammatory agents, are used to treat UC [1]. However, their use is limited by various side effects. Experimental colitis models have been used to identify potential therapeutic agents and elucidate the underlying physiologic mechanisms of UC [2]. Numerous animal models of colonic inflammation with several features of human UC have been developed. Animal models of colitis require the administration of specific concentrations of colitis-inducing chemicals, such as dextran sulfate sodium (DSS) [3].

Several investigators proposed natural products as treatment of many diseases, including IBD. The therapeutic efficacy of these natural products relies on their ability to reduce the levels of inflammatory cytokines or inflammatory mediators (e.g., TNF-α, iNOS, and COX-2) [4]. Many natural products exert putative antioxidative effects [5], possess anti-inflammatory properties [6], and exert protective effects against external antigenic substances. These effects are closely associated with the prevention of oxidative stress [7].

Numerous bioactive substances (i.e., flavonoids, resveratrol, and plant sterols) are present in peanuts [8]. Peanuts are a rich source of unsaturated oleic and linolenic acids that lower cholesterol levels, maintain clean blood vessels, and prevent atherosclerosis and heart disease [9,10]. Peanuts are also effective in diabetes, preventing the rise of blood glucose levels [11]. Peanut shell extract (PSE) is widely thought to play an important role in the human body as an antioxidant, free radical scavenger, inflammation prevention agent, and immune system modulator. Peanut shell contains abundant functional ingredients. Among these, luteolin is a potent anti-vascular inflammation agent [12]. It also protects retinal epithelial cells from oxidative stress-induced cell death [13]. However, the ability of PSE to control the effects of DSS-induced colitis in a mouse model has not been investigated. In the current study, we investigated the ability of PSE to alleviate UC symptoms in the DSS-induced mouse model of colitis.

## 2. Materials and methods

### 2.1. Preparation of PSE

Peanuts were purchased from Hongchun-Kun (Kwangwon-Do, Republic of Korea) in October 2016. PSE was prepared by shelling by hand, followed by washing, grinding, drying at 45 °C, and pulverizing to a < 1-mm diameter powder. For the extract, 500 g of pulverized powder was suspended in 10 L of 70% aqueous ethanol and ultrasonicated for 1 h in a 750-W ultrasonic processor (VCX 750, Sonics and

* Corresponding author at: 245, Nongsaengmyeong-ro, Iseo-myeon, Wanju-gun, Jeollabuk-do 55365, Republic of Korea.
*E-mail address:* choiw@kfri.re.kr (I. Choi).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.intimp.2019.02.040
Received 4 September 2018; Received in revised form 15 February 2019; Accepted 25 February 2019
Available online 06 March 2019

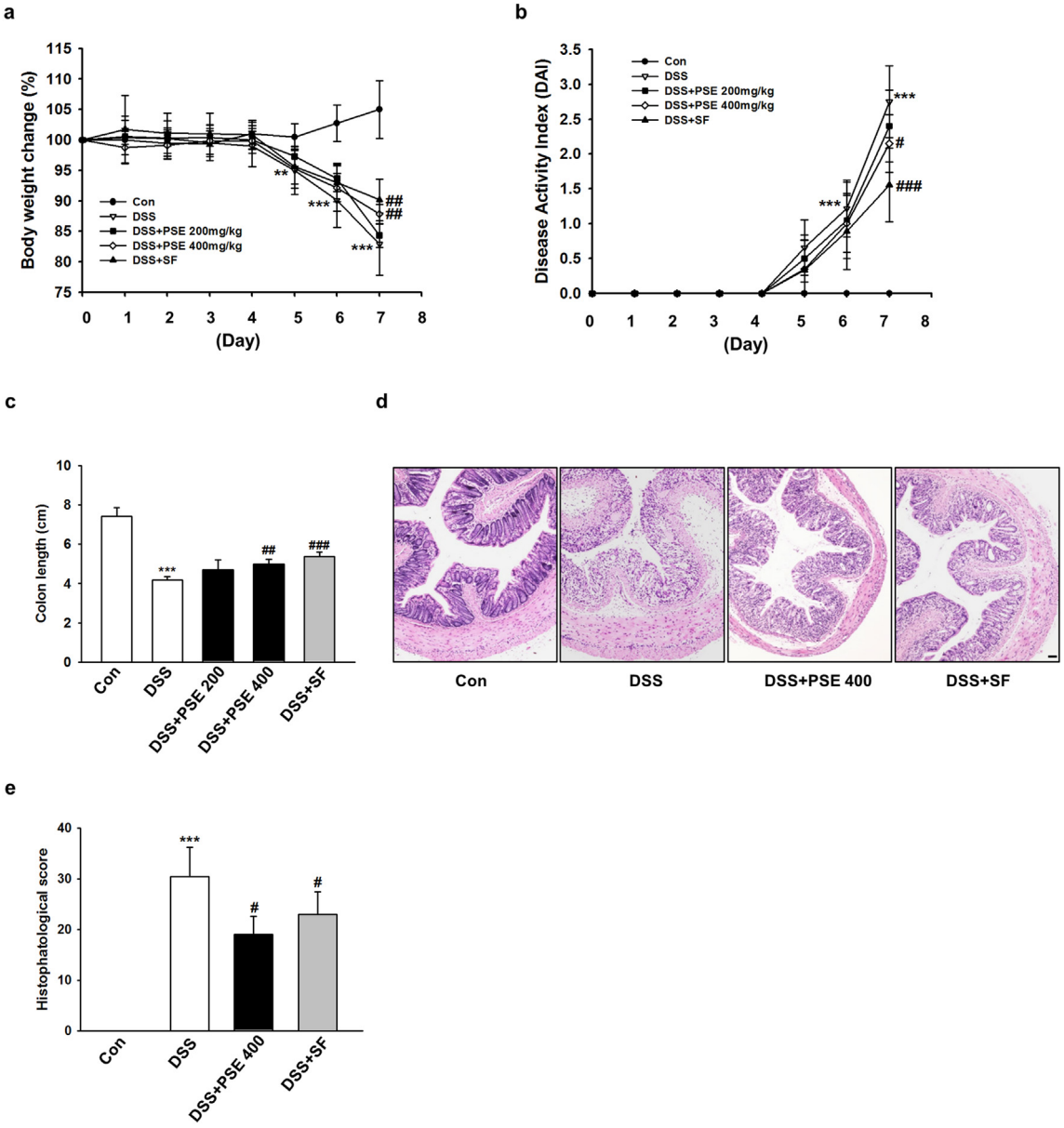


**Fig. 1.** Peanut shell extract (PSE) ameliorates clinical signs of disease in mice with DSS-induced colitis. a Changes in the body weight and b scores of disease activity index (DAI) in PSE-treated mice and control mice administered 3% DSS for 7 d, monitored every day. Body weight values are expressed as a percentage of body weight on a particular day. c Length of the mouse colon after 7 d of DSS administration. d Hematoxylin and eosin staining of the colons of mice administered 400 mg/kg PSE on 7 d of DSS administration. Data shown are from three independent experiments and are expressed as the mean ± SEM ($n$ = 10 mice/group). e Histopathological scores of the analyzed slides. Scale bar, 10 μm. **, $P < 0.01$ vs. con; ***, $P < 0.001$ vs. con; #, $P < 0.05$ vs. DSS; ##, $P < 0.01$ vs. DSS; ###, $P < 0.001$ vs. DSS.

Materials Inc., Newtown, CT, USA). Extraction in an ultrasonic processor was repeated three times. The undissolved debris was removed by a quantitative Whatman no. 1 filter paper (Whatman, Kent, UK) and centrifugation.

### 2.2. HPLC analysis of PSE

PSE flavonoids were analyzed by reversed-phase HPLC using a Waters HPLC system (Waters Corp., Milford, MA, USA) with a photodiode array detector (Waters model 2998) and an Agilent C18 column (4.6 mm × 250 mm; 5 μm, Waters Corp.). The solvent system was a gradient of solvent A (water:tetrahydrofuran:trifluoroacetic acid, 98:2:0.1, v/v/v) and solvent B (acetonitrile), as follows: initially, 17% B; 0–2 min, isocratic at 17% B; 2–7 min, linear gradient to 25% B; 7–15 min, linear gradient to 35% B; 15–20 min, linear gradient to 50% B; 20–30 min, linear gradient to 17% B; 30–35 min, isocratic to 17% B; washing and reconditioning of the column for xx min. The elution was conducted at a flow rate of 1 mL/min, and the UV spectra were monitored at 350 nm. The injection volume was 20 μL. The luteolin content of PSE was calculated using the linear segment of luteolin standard curve (2.5–200 μM). As determined, PSE contained 3.1% of luteolin.

### 2.3. Animal experiments

Mice used in the current study were 7-week-old male C57BL/6 mice ($n$ = 50, Charles River Korea, Seoul, South Korea). Experimental colitis was induced in mice by supplementing drinking water with filter-purified 3% (w/v) DSS (MW 1/4 36–50 kDa; MP biochmicals, Aurora, OH, USA), to which the animals had free access. Mice in the control group received tap water without DSS for 7 d. Mice were randomly divided

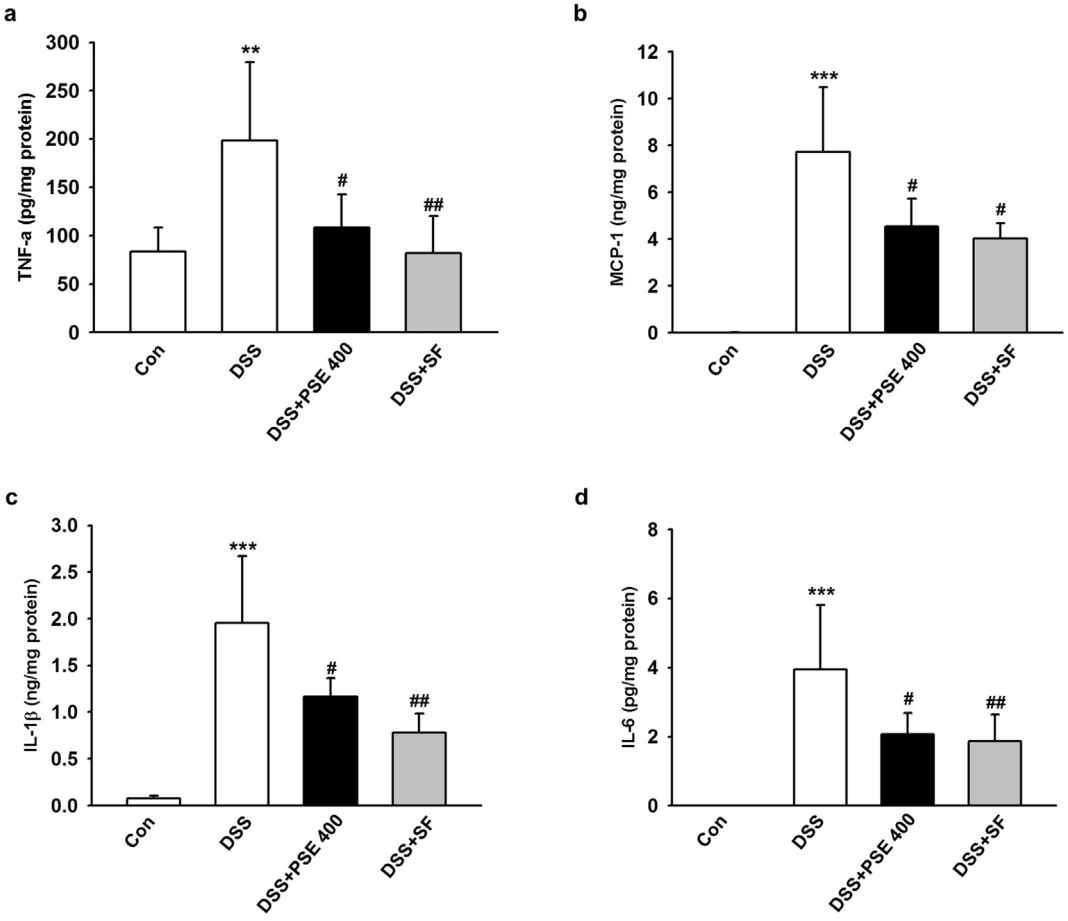


**Fig. 2.** PSE reduces the expression of inflammatory cytokines in the DSS-induced colitis model. The colonic expression of inflammatory cytokines TNF-α (a), MCP-1 (b), IL-1β (c), and IL-6 (d) was examined by ELISA and normalized by total protein. Administration of 400 mg/kg PSE reduced DSS-induced inflammatory cytokine levels in colitis. Data represent the means ± SD from three independent experiments. **, $P < 0.01$ vs. con; ***, $P < 0.001$ vs. con; #, $P < 0.05$ vs. DSS; ##, $P < 0.01$ vs. DSS.

into the following groups ($n = 10$ mice/group): group 1, control mice (Con group); group 2, mice administered 3% DSS solution (DSS group); groups 3 and 4, DSS-treated mice administered PSE (200 and 400 mg/kg, respectively), daily, via an intragastric route (DSS + PSE 200 mg/kg and DSS + PSE 400 mg/kg groups, respectively) for 14 d; group 5, DSS-treated mice given 50 mg/kg sulfasalazine (DSS + SF). Mouse body weight, water intake, stools, occult blood, and survival were monitored daily. The DAI was determined by averaging the scores for weight loss, stool form, and occult blood. The following scores were given: (1) for changes in body weight: 0, < 1%; 1, 1–5%; 2, 5–10%; 3, 10–15%; and 4, > 15%; (2) for occult blood occurrence: 0, negative; 2, positive; and 4, gross bleeding; and (3) for stool form: 0, normal; 2, loose stools; and 4, diarrhea, as previously described. On day 7, the mice were sacrificed by anesthesia with ketamine and xylazine, following which the colon tissue was collected.

### 2.4. Histopathological analysis and immunohistochemistry

The distal colon sections were fixed in 4% paraformaldehyde and embedded in paraffin. The paraffin blocks were sliced into 4-μm thin sections, mounted on glass slides, and evaluated histologically after hematoxylin and eosin staining (H&E). For immunohistochemistry, the colon sections were first deparaffinized in xylene and rehydrated in ethanol. After blocking for 1 h, the slides were incubated overnight at 4 °C with anti-ER-HR3 antibodies (BMA, Augst, Switzerland). The sections were then treated with AEC substrate-chromogen solution (DakoCytomation, Glostrup, Denmark) to visualize the immunocomplexes. The results of immunohistochemical staining were visualized using a Nikon Eclipse 80*i* light microscope (Nikon Instruments Inc., Melville, NY, USA). The number of ER-HR3–positive macrophages was counted in 10 randomly chosen fields of view under ×400 magnifications using a digital image analysis program (Image J, Soft Imaging System, Münster, Germany).

### 2.5. Enzyme-linked immunosorbent assay (ELISA)

Colon was cut into pieced and homogenated in lysis buffer and extracted total protein. Protein concentration was performed using the Bradford reagent (Sigma-Aldrich, St. Louise, MO, USA) and a certain amount of protein was loaded. Levels of TNF-α, MCP-1, IL-1β, and IL-6 in the colon tissue and lysate from cells were determined using a DuoSet sandwich ELISA (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's recommendations. The absorbance was measured at 450 nm in VERSA Max Microplate Reader (Molecular Device, Concord, Canada).

### 2.6. Statistics

Data are expressed as the means ± standard deviation (SD). Significant differences between the means of two groups were examined by the analysis of variance (one-way ANOVA), followed by individual comparisons using Tukey's post-hoc test, with $P < 0.05$ considered to indicate a statistically significant difference.

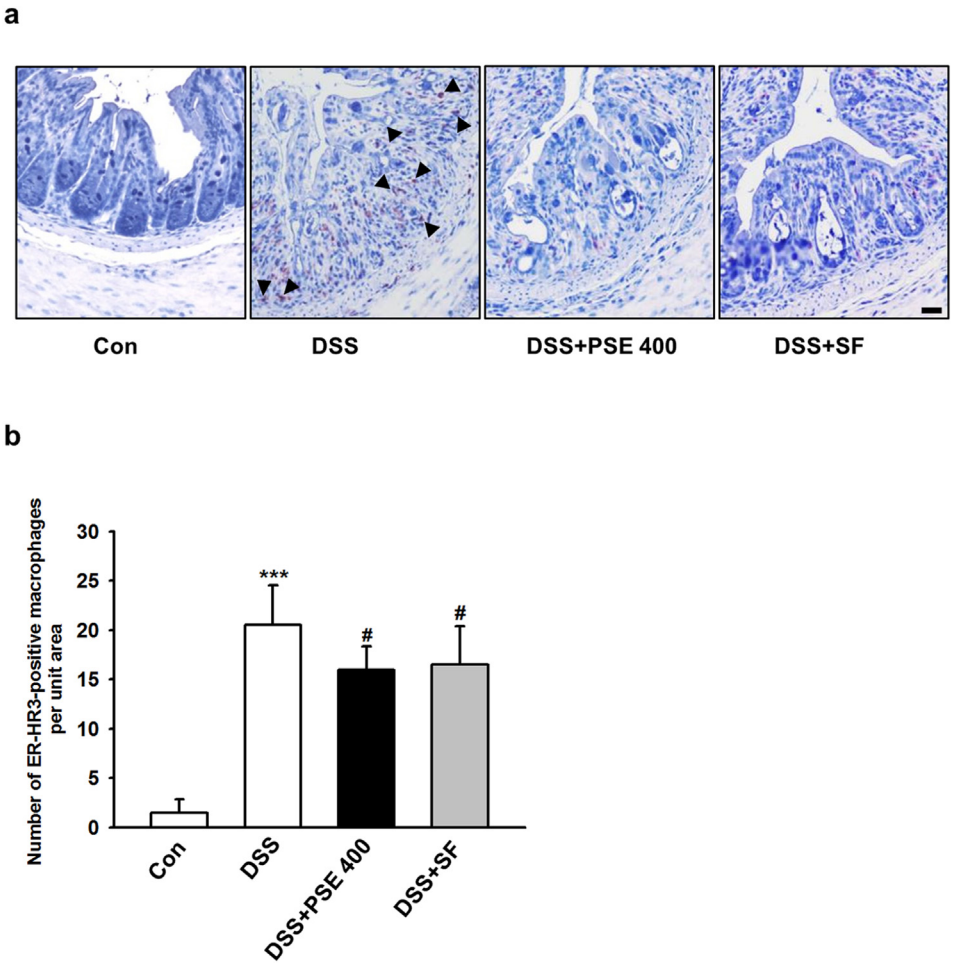


**Fig. 3.** Administration of 400 mg/kg PSE inhibits macrophage infiltration in the DSS-induced colitis model. a Representative images of paraffin-embedded colon sections from mice with DSS-induced colitis stained with anti-ER-HR3 antibodies and b the number of ER-HR3–positive macrophages in each group. Scale bar, 10 μm. Data represent the means ± SD from three independent experiments. ***, $P < 0.001$ vs. con; #, $P < 0.05$ vs. DSS.

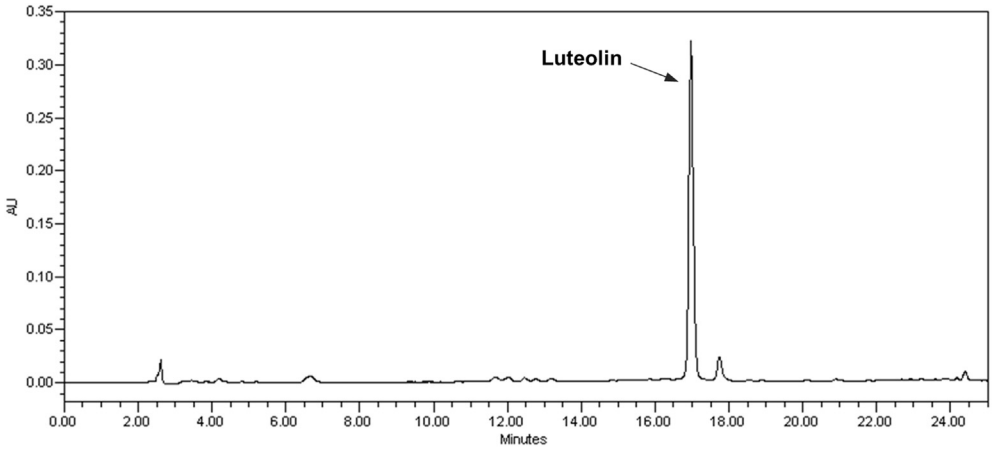


**Fig. 4.** Chromatogram of an HPLC separation of luteolin in the crude peanut shell extracts (PSE). Luteolin was quantified in PSE samples using an HPLC system with a photodiode array detector and an Agilent C18 column.

## 3. Results

### *3.1. PSE alleviates colon shortening, body weight loss, DAI, and the colonic histopathological score in DSS-induced colitis*

We first examined the symptoms of disease, such as colon shortening, body weight loss, DAI, and histopathological score of the colon associated with colitis, 7 d after the start of the administration of 3% DSS in drinking water (Fig. 1). DSS treatment reduced the colon length and body weight, and aggravated disease condition compared with untreated control mice. The water intake not significantly changed despite of DSS administration (Fig. S1). Administration of PSE alleviated the colon shortening and DAI (Fig. 1a and c), and resulted in the recovery of body weight in mice administered 400 mg/kg PSE on day 7

(Fig. 1a). As determined by histopathological analysis, administration of PSE alleviated such DSS-induced inflammatory changes as erosion of the epithelial monolayer, crypt loss, infiltration of immune cells, and submucosa edema (Fig. 1d and e). The extent of alleviation was comparable with the effect of sulfasalazine administration.

### 3.2. PSE reduces the levels of pro-inflammatory cytokines in DSS-treated mice

Elevated secretion of pro-inflammatory cytokines during colitis induces colon inflammation. To investigate the changes in pro-inflammatory cytokine levels in colitis induced inflamed colon, levels of TNF-α, MCP-1, IL-1β, and IL-6 were quantified in the colon tissue using ELISA. The elevation of secretion of all these cytokines observed in DSS-treated mice was markedly reduced in PSE-administered mice (Fig. 2). In addition, the secretion of these cytokines in macrophages, which are known to be the main cells that secrete pro-inflammatory cytokines in the intestinal inflammatory response, also showed that PSE was reduced by as much as luteolin (Fig. S2). These observations suggested that PSE exerts an anti-inflammatory effect in mice with DSS-induced colitis.

### 3.3. PSE reduces macrophage infiltration in DSS-treated mice

Macrophages play a critical role in the regulation of intestinal inflammatory processes [14]. To determine whether the observed anti-inflammatory effect of PSE was correlated with the colonic infiltration of macrophages in DSS-treated mice, colon sections were stained with anti-ER-HR3 antibodies. Immunohistochemical staining revealed the presence of infiltrating macrophages in the interstitial space of the inflamed colon. The numbers of ER-HR3–positive macrophages elevated in DSS-induced colitis were significantly reduced by the administration of PSE (Fig. 3a and b).

### 3.4. Identification of the active components of PSE

Peanut shell contains such functional components, as polyphenols and flavonoids, especially luteolin [15]. Luteolin is a crucial component of the peanut shell (usual content of 2.36 mg/g) [16]. HPLC was used to characterize PSE. The analysis revealed that PSE was mostly composed of luteolin (Fig. 4). The luteolin content of PSE was determined based on the linear segment of an HPLC luteolin standard curve. As determined, PSE contained about 3.1% of luteolin.

## 4. Discussion

DSS-induced colitis is a well-known animal model, with a phenotype resembling human UC, i.e., mucosal infiltration of inflammatory cells, epithelial injury, and ulceration [17]. In the current study, we demonstrated that PSE was able to alleviate the DSS-induced colitis symptoms, e.g., body weight loss, diarrhea, colon shortening, pathological injury, as well as the DSS-induced production of inflammatory cytokines and accumulation of infiltrating macrophages in the inflamed area.

Steroids or nonsteroid anti-inflammatory drugs are being used to treat IBD [1]. However, the use of these drugs may be associated with severe side effects. Thus, it has been proposed that alternative medicines and natural products that exhibit similar activity to sulfasalazine might be used to treat IBD [18,19]. E.g., *Aloe vera* contains an abundance of phytochemical substances with pronounced anti-inflammatory effects [20] and aqueous extract of unripe fruit of *Aegle marmelos* is effective in treating IBD [21]. The popularity of herbal therapy for IBD is increasing worldwide. In the current study, we demonstrated that PSE alleviated DSS-induced colitis by suppressing inflammatory mediators and macrophage infiltration.

Previous studies reported that luteolin suppresses experimental colitis via the Nrf2 signaling pathway [22], and the JAK/STAT pathway in a cellular model of intestinal inflammation [23]. Similarly, in the current study, PSE appeared to exert an anti-inflammatory effect to reduced cytokine levels in the colon and improve the phenotype. Further, luteolin inhibits colon cancer that involves progressive disruption of homeostatic mechanism, regulating the progression of colitis [24]. The anti-cancer effect of luteolin, which is abundant in PSE, plays an important role in halting the development of UC into colon cancer. In the current study, we found that luteolin is abundant in PSE and might play an important role in alleviating the DSS-induced colitis. These results warrant further investigation of the effect of PSE in colonic dysplasia, cancer development, and chronic intestinal inflammation. Since herbal compounds and their derivatives are widely available, and are frequently taken by an increasing number of patients without proper medical monitoring, the current study stresses the need for further analysis of their impact on various diseases.

## 5. Conclusion

In the current study, we demonstrated that PSE significantly alleviated the disease symptoms and histopathological changes associated with IBD, and reduced the inflammation in a rodent model of colitis. Moreover, PSE dramatically reduced the levels of proinflammatory cytokines and infiltrating macrophage accumulation in the colon. Therefore, PSE might be a useful anti-inflammatory agent, and may be used as a bioflavonoid to prevent colitis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.02.040.

## Acknowledgments

This research was supported by Main Research Program (E0186700-02) of the Korea Food Research Institute (KFRI) funded by the Ministry of Science and ICT.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

## References

[1] M. Cottone, S. Renna, A. Orlando, F. Mocciaro, Medical management of Crohn's disease, Expert. Opin. Pharmacother. 12 (16) (2011) 2505–2525, https://doi.org/10.1517/14656566.2011.609556.
[2] V. Valatas, M. Vakas, G. Kolios, The value of experimental models of colitis in predicting efficacy of biological therapies for inflammatory bowel diseases, Am. J. Physiol. Gastrointest. Liver Physiol. 305 (11) (2013) G763–G785, https://doi.org/10.1152/ajpgi.00004.2013.
[3] Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Current Protocols in Immunology/edited by John E Coligan [et al]. 2014; vol. 104: Unit 15 25. doi:https://doi.org/10.1002/0471142735.im1525s104.
[4] T. Debnath, D.H. Kim, B.O. Lim, Natural products as a source of anti-inflammatory agents associated with inflammatory bowel disease, Molecules 18 (6) (2013) 7253–7270, https://doi.org/10.3390/molecules18067253.
[5] Kotakadi VS, Jin Y, Hofseth AB, Ying L, Cui X, Volate S et al. Ginkgo biloba extract EGb 761 has anti-inflammatory properties and ameliorates colitis in mice by driving effector T cell apoptosis. Carcinogenesis. 2008; 29(9): 1799–806. doi:https://doi.org/10.1093/carcin/bgn143.
[6] H.S. El-Abhar, L.N. Hammad, H.S. Gawad, Modulating effect of ginger extract on rats with ulcerative colitis, J. Ethnopharmacol. 118 (3) (2008) 367–372, https://doi.org/10.1016/j.jep.2008.04.026.
[7] V.K. Wong, L. Yu, C.H. Cho, Protective effect of polysaccharides from Angelica sinensis on ulcerative colitis in rats, Inflammopharmacology 16 (4) (2008) 162–167, https://doi.org/10.1007/s10787-007-0026-5.
[8] A.E. Griel, B. Eissenstat, V. Juturu, G. Hsieh, P.M. Kris-Etherton, Improved diet quality with peanut consumption, J. Am. Coll. Nutr. 23 (6) (2004) 660–668.
[9] L.M. Alderson, K.C. Hayes, R.J. Nicolosi, Peanut oil reduces diet-induced atherosclerosis in cynomolgus monkeys, Arteriosclerosis 6 (5) (1986) 465–474.
[10] E. Emekli-Alturfan, E. Kasikci, A. Yarat, Peanut (Arachis hypogaea) consumption improves glutathione and HDL-cholesterol levels in experimental diabetes, Phytother. Res. 22 (2) (2008) 180–184, https://doi.org/10.1002/ptr.2281.
[11] M. Wien, K. Oda, J. Sabate, A randomized controlled trial to evaluate the effect of

incorporating peanuts into an American Diabetes Association meal plan on the nutrient profile of the total diet and cardiometabolic parameters of adults with type 2 diabetes, Nutr. J. 13 (2014) 10, , https://doi.org/10.1186/1475-2891-13-10.
[12] Middleton E, Jr., Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 2000; 52(4): 673–751.
[13] M. Hytti, N. Piippo, E. Korhonen, P. Honkakoski, K. Kaarniranta, A. Kauppinen, Fisetin and luteolin protect human retinal pigment epithelial cells from oxidative stress-induced cell death and regulate inflammation, Sci. Rep. 5 (2015) 17645, , https://doi.org/10.1038/srep17645.
[14] Bain CC, Mowat, AM. Macrophages in intestinal homeostasis and inflammation. Immunol. Rev. 2014; 260(1): 102–17. doi:https://doi.org/10.1111/imr.12192.
[15] F. Gao, H. Ye, Y. Yu, T. Zhang, X. Deng, Lack of toxicological effect through mutagenicity test of polyphenol extracts from peanut shells, Food Chem. 129 (3) (2011) 920–924, https://doi.org/10.1016/j.foodchem.2011.05.046.
[16] J. Qiu, L. Chen, Q. Zhu, D. Wang, W. Wang, X. Sun, et al., Screening natural antioxidants in peanut shell using DPPH-HPLC-DAD-TOF/MS methods, Food Chem. 135 (4) (2012) 2366–2371, https://doi.org/10.1016/j.foodchem.2012.07.042.
[17] J.A. Jiminez, T.C. Uwiera, G. Douglas Inglis, R.R. Uwiera, Animal models to study acute and chronic intestinal inflammation in mammals, Gut Pathog 7 (2015) 29, , https://doi.org/10.1186/s13099-015-0076-y.
[18] Cipolla G, Crema F, Sacco S, Moro E, de Ponti F, Frigo G. Nonsteroidal anti-inflammatory drugs and inflammatory bowel disease: current perspectives. Pharmacol. Res. 2002; 46(1): 1–6.
[19] K.L. Wallace, L.B. Zheng, Y. Kanazawa, D.Q. Shih, Immunopathology of inflammatory bowel disease, World J Gastroenterol: WJG 20 (1) (2014) 6–21, https://doi.org/10.3748/wjg.v20.i1.6.
[20] L. Langmead, R.J. Makins, D.S. Rampton, Anti-inflammatory effects of aloe vera gel in human colorectal mucosa in vitro, Aliment. Pharmacol. Ther. 19 (5) (2004) 521–527.
[21] J.P. Behera, B. Mohanty, Y.R. Ramani, B. Rath, S. Pradhan, Effect of aqueous extract of Aegle marmelos unripe fruit on inflammatory bowel disease, Indian J. Pharm. 44 (5) (2012) 614–618, https://doi.org/10.4103/0253-7613.100389.
[22] Y. Li, L. Shen, H. Luo, Luteolin ameliorates dextran sulfate sodium-induced colitis in mice possibly through activation of the Nrf2 signaling pathway, Int. Immunopharmacol. 40 (2016) 24–31, https://doi.org/10.1016/j.intimp.2016.08.020.
[23] C. Nunes, L. Almeida, R.M. Barbosa, J. Laranjinha, Luteolin suppresses the JAK/STAT pathway in a cellular model of intestinal inflammation, Food Funct. 8 (1) (2017) 387–396, https://doi.org/10.1039/c6fo01529h.
[24] Pandurangan AK, Kumar SA, Dharmalingam P, Ganapasam S. Luteolin, a bioflavonoid inhibits azoxymethane-induced colon carcinogenesis: involvement of iNOS and COX-2. Pharmacogn. Mag. 2014; 10 (Suppl 2): S306–10. doi:https://doi.org/10.4103/0973-1296.133285.