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Full Length Research Paper

Effects of *Lonicera japonica* Thunb. on dextran sulfate sodium-induced experimental colitis in mice

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We investigated the effects of buthanol (BuOH) extracts of *Lonicera japonica* on dextran sulfate sodium (DSS)-induced mice colitis. Body weight, histological indices such as crypt injury and inflammation score, biochemical factor such as serum amyloid (SAA) and MPO level data were evaluated. BuOH extracts of *L. japonica* reduced the crypt injury and inflammation score and showed markedly greater more decreases of the SAA and MPO levels relative to the 5-ASA group. In addition, BuOH extracts will reduce the level of IL-6 which is stimulated by a lipopolysaccharide (LPS) in the HT-29 cell line *in vitro*. Therefore, BuOH extracts of *L. japonica* may be useful as a potential inhibitor for preventing inflammatory bowel disease in humans.

Key words: *Lonicera japonica*, dextran sulfate sodium, inflammatory bowel disease.

INTRODUCTION

Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease, is a severe intestinal inflammation, the pathogenesis of which remains unclear. Because intestinal inflammation was absent from various IBD models reared under germ-free conditions, it is suspected that the disease is due to complex mucosal immune responses to resident enteric bacteria, because intestinal inflammation was absent from various IBD models reared under germ-free conditions (Duchmann et al., 1995, 1996, 1999; Kawaguchi-Miyashita et al., 2001; Matsumoto et al., 1998; Sadlack et al., 1993; Taurog et al., 1999). The diverse of animal models identified thus far are consonant with the view that many types of imbalance in the gastrointestinal immune system can lead to mucosal inflammation, and by extension, that human IBD is likely a common denominator for a group of clinically related disorders with multiple etiologies and distinct clinical characteristics. Interleukin (IL)-6 is one of the major cytokines secreted by lamina propria cells in patients with IBD (Fuss et al., 1996; Gross et al., 1992; Podolsky, 1991). Strong expression of IL-6 has also been reported in murine acute bowel inflammation (Rogler and Andus, 1998). Recent studies using antisoluble-IL-6 receptor antibodies demonstrated that IL-6 plays a critical role in the development of chronic colitis (Atreya et al., 2000; Yamamoto et al., 2000). *Lonicera japonica* Thunb. (Caprifoliaceae) are species of honeysuckle native to north eastern Asia, including Japan, Korea, northern and eastern China, and Taiwan. *L. japonica* has traditionally been used as a medicinal plant (Peng et al., 2000), and many pharmacological studies and clinical practices have demonstrated that *L. japonica* exerts many biological effects, including hepatoprotective, cytoprotective, antimicrobial, antioxidative, antiviral, and anti-inflammatory activities (Chang et al., 1995). The constituents of this plant have been investigated and were found to contain iridoid glucosides and polyphenolic compounds (Kakuda et al., 2000). The primary polyphenolic components in *L. japonica* are hyperoside, chlorogenic acid, luteolin, and caffeic acid (Chang et al., 1995). Research has shown that hyperoside, chlorogenic acid, and other flavones can be used to scavenge free radicals in addition to having anti-inflammatory activities. The primary components of *L. japonica* have medicinal

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properties: flower buds have anticancer, anti-microbial, and anti-inflammatory properties (Zhang et al., 2008); the leaf has antioxidant properties and inhibitors of tyrosinase (Byun et al., 2004); the stem also has inhibitory activities against tyrosinase and xanthine oxidase in addition to nitrite scavenging potential (Wang and Helliwell, 2001). However, few studies on the antibacterial properties and activities of *L. japonica* have been reported (Cai et al., 2004). Despite its various medicinal properties, no reports to date have been published on the chemical composition and antibacterial properties derived from the flower of *L. japonica*.

In our previous research, we found that a buthanol (BuOH) extract of *L. japonica* exhibited antimicrobial effects against Gram positive and negative anaerobic bacteria, such as *Bacteroides fragilis, Clostridium difficile, Clostridium perfringenes* and *Propionibacterium acnes* (Rhee and Lee, 2011).

The purpose of the present study is to investigate the effects of the extract of *L. japonica* on dextran sulfate sodium (DSS)-induced mice model.

MATERIALS AND METHODS

Preparation of a BuOH fraction of L. japonica

L. japonica was obtained from an oriental drug store (Chungju, Korea). The chopped material was refluxed with distilled water; the water extract was then partitioned with n-butanol (BuOH) and filtered. The filtrate was evaporated in vacuo to dryness. The yield based on the dry weight of *L. japonica* was 5.7% w/w. In the BuOH fraction, loniceroside A, loniceroside B, lonicerin, and loganin were found to be the primary constituents.

HPLC analysis

Analysis of each component among the fraction was carried out by Agilent 1100 series (Agilent Technologies, USA) system. The chromatographic separation was carried out on a 2.1 mm × 50 mm, 2 µm particle, Innertsil ODS-4 C18 column (GL sciences, Japan) maintained at 40°C. The mobile phase consisting a mixture 0.05% aqueous phosphoric acid and acetonitrile in the ratio of 34:66 (v/v) with flow rate of 0.5 mL/min was employed. The detector wavelength was monitored at 254 nm. The content percentages in this fraction were determined by HPLC and are as follows: 2.5% for loniceroside A, 2.2% for loniceroside B, 2.1% for lonicerin and 4.7% for loganin.

Animals

Male Balb/c mice (8 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan). They were maintained under specific pathogen-free (SPF) conditions during the experiments.

Cell culture

HT-29 human colon epithelial cells (American Type Culture Collection (ATCC) HTB 38) were grown in DME (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS, 2 mM glutamine and 25 mM HEPES.

Measurement of IL-6

The amount of IL-6 in the culture supernatant from LPS-stimulated HT-29 cells was measured by an IL-6-specific sandwich ELISA.

Induction of chronic colitis

Acute colitis was induced by 4% DSS (MW 25,000, TCI, Japan) dissolved in drinking water. Briefly, a total of 37 mice were randomly placed into six groups and were housed individually. The normal group (n=5) was given distilled water, and the other groups were fed 4% DSS for 7 days.

BuOH extracts and 5-aminosalicylic acids (5-ASA) as a positive control were orally administered daily from day 0 and day 11. The IBD scoring system assigns severity scores of 0 to 4 for three parameters: body weight, stool consistency and intestinal bleeding (Cooper et al., 1993).

Histological grading of colitis

Colonic tissues were removed and embedded into paraffin for histological analysis with hematoxylin and eosin staining. Crypt injury was scored as follows: grade 0, intact crypts; grade 1, loss of the bottom third of crypts; grade 2, loss of the bottom two thirds of crypts; grade 3, loss of the entire crypt with the surface epithelium remaining intact; grade 4, loss of the entire crypt and surface epithelium.

Inflammation was scored from 0 to 4 as follows by the same pathologist blinded to the treatment conditions: 0, no inflammation; 1, low leukocyte infiltration; 2, moderate leukocyte infiltration; 3, high leukocyte infiltration, moderate fibrosis and goblet loss; 4, massive loss of goblet cells, extensive fibrosis, and thickening of the colon wall (Erichsen et al., 2005).

Measurement of serum amyloid A

Serum samples were collected for the detection of serum amyloid A (SAA) levels by a murine ELISA kit (Life Diagnostics, Inc, West Chester, PA, USA) 3 and 10 days after induction of colitis according to the manufacturer's recommendations and A450 nm was measured (Uhlar and Whitehead, 1999).

Myeloperoxidase assay

MPO activity was assayed using Krawisz's method (Krawisz et al., 1984). In brief, colonic tissues were homogenized in ice-cold potassium phosphate buffer (pH 6.0) and centrifuged for 10 min at 6000 x *g* at 4°C. The suspension was sonicated on ice and then centrifuged at 1000 x *g* for 30 min. The supernatant was mixed with an enzyme substrate buffer containing 0.167 mg/ml of *O*-dianisidine hydrochloride (Sigma) and 0.0005% hydrogen peroxide. The changes in the absorbance at 405 nm were measured.

Statistics

All data were expressed as the mean ± s.e. and evaluated by a

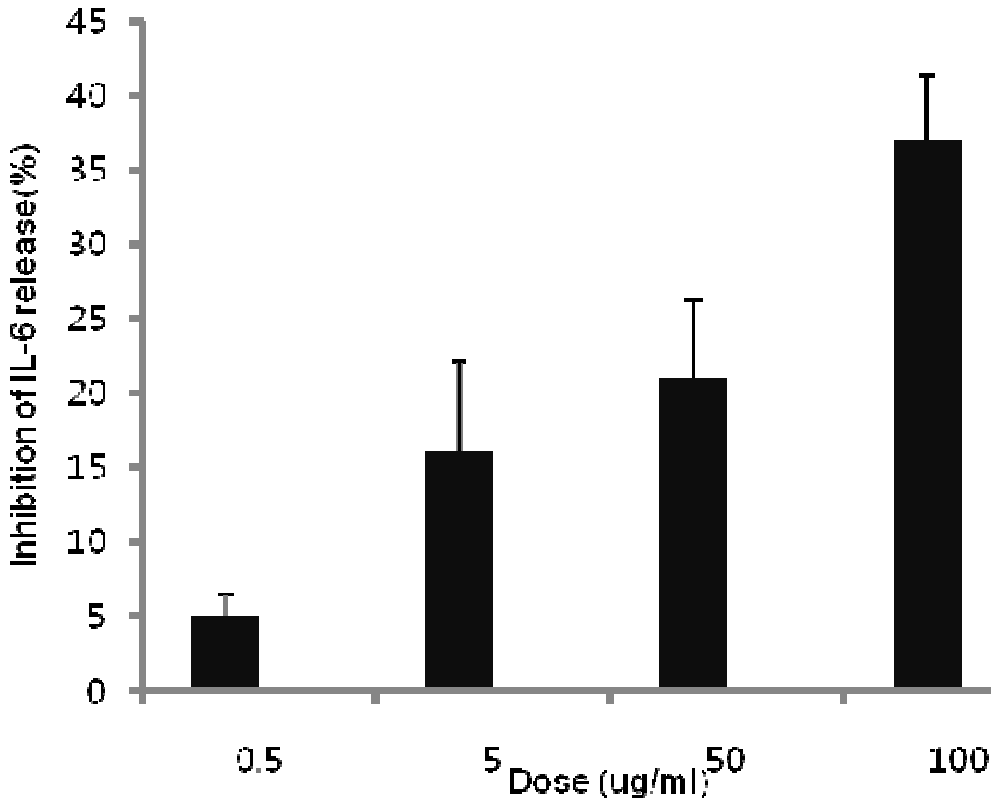

Figure 1. Inhibitory effect of fucoidan and lutein on IL-6 synthesis in LPS-stimulated HT-29 cells. HT-29 was cultured with various doses of the extracts of *L. japonica* in the presence of LPS for 72 h. Data are mean ± S.D.

Tukey or Tukey–Kramer test for multiple comparisons. P-values of less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

HPLC analysis

The content percentages in this fraction were determined by HPLC and were as follows: 2.5% for loniceroside A, 2.2% for loniceroside B, 2.1% for lonicerin, and 4.7% for loganin.

Inhibition of IL-6 production on LPS-stimulated HT-29 cells

BuOH extracts showed inhibition of IL-6 synthesis in LPS-stimulated HT-29. The inhibitory activity on IL-6 release in LPS-stimulated HT-29 cells by BuOH extracts was dose-dependent (Figure 1). We did not detect any up-regulation in the release of IL-6 from HT-29 cells cultured in the presence of single 5-ASA (data not shown).

Change of body weight

After administration of DSS, a dramatic and fast decrease in body weight was observed as a result of colitis and was maintained during the 14-day period (Figure 2). When compared with the vehicle group, the body weight of the only DSS-induced mice decreased. However, when compared with the vehicle, the body weight of mice administered with BuOH extracts showed significant recovery. As shown in Figure 2, at the end of the experiment, the final weight of the group that was treated with 100 mg/kg of BuOH extracts from *L. japonica* closely approached at the weight of the normal group. By the treatment-dose of BuOH extracts, the effects on body weight recovery were displayed in a dose dependent manner and the effect of 10 mg/kg of BuOH extracts was similar to the change of 5-ASA (100 mg/kg) as a positive control. According to these results, the ameliorating effect of BuOH extracts of *L. japonica* was superior to the effects of 5-ASA on DSS-induced colitis.

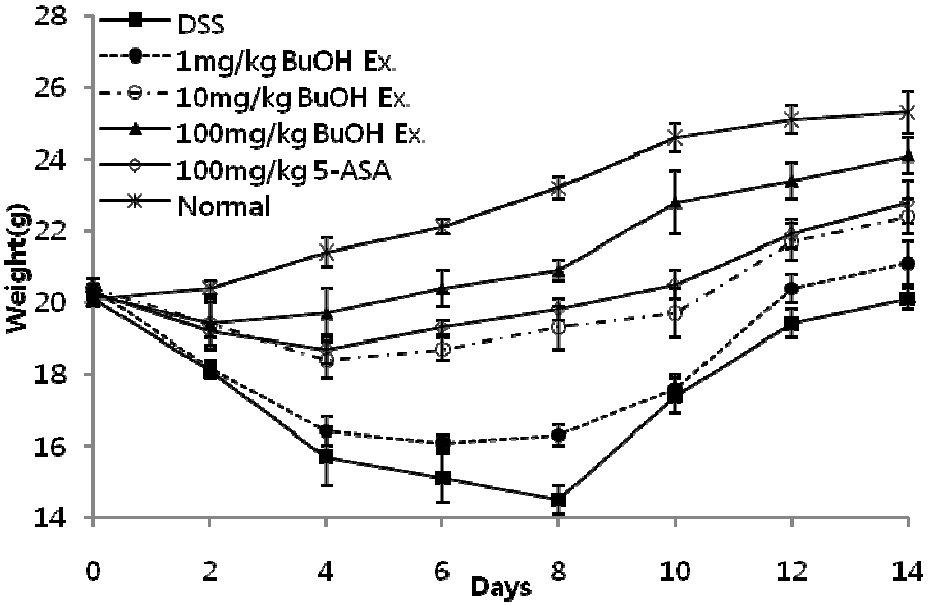

Figure 2. Weight change of DSS-induced mice with BuOH extracts of *L. japonica*.

Table 1. Crypt injury and inflammation score and related peptide level in colitis model treated with BuOH extracts of *L. japonica*

	Crypt injury score	Inflammation score	SAA (μg/ml)
Normal	0 ± 0	0.0 ± 0.0	0.07 ± 0.02
DSS	4 ± 0	4.0 ± 0.1	402.5 ± 5.9
1 mg/kg BuOH extracts	2 ± 0	1.9 ± 0.4	105.2 ± 4.6
10 mg/kg BuOH extracts	2 ± 0	2.0 ± 0.3	99.1 ± 5.7
100 mg/kg BuOH extracts	1 ± 0	1.0 ± 0.3	2.4 ± 0.5
5-ASA	2 ± 0	1.7 ± 0.2	50.3 ± 3.9

Histopathology

Histologic changes in the cecum and colon in DSS-treated Balb/c mice were examined by hematoxylin and eosin staining to evaluate the effectiveness of BuOH extracts of *L. japonica* against tissue damage. Histopathological evaluation showed indications of colitis in all groups receiving DSS. Changes were most prominent in the distal colon with areas of erosions, crypt distortion, and inflammatory infiltration. In the case of BuOH extracts, mean crypt score was significantly reduced by 1.0 and the mean inflammation score was similarly decreased by 1.0 ± 0.3. However, the no treatment group showed a decreased mean crypt score of 4.0 and reduced the mean inflammation score by 4.0 ± 0.1. The histopathologic results correlated well with clinical signs of colitis. Regarding histologic scores, the crypt injury score in the tissue was significantly reduced from 4.0 to 1.0 by treatment of 100 mg/kg of BuOH extracts and the score was reduced from 4.0 to 2.0 by the treatment of 1 and 10 mg/kg of BuOH extracts and 100 mg/kg of 5-ASA as a positive control, respectively (Table 1). The histopathologic analysis using the inflammation score after induction of colitis showed infiltration of neutrophils and macrophages into the colonic mucosa and submucosa layers. In the no treatment group, transmural inflammation, characterized by massive infiltration of lymphocytes, was associated with a thickening of the colon wall, ulcerations and loss of goblet cells through the colon. Treatment of BuOH extracts (100 mg/kg) improved these signs, restoring the histological appearance of the mucosa and submucosa compared with untreated mice, although the other experimental doses and 5-ASA-treated mice showed minor infiltration of lymphocytes as a result of a mild inflammation (Figure 3). As another important index of inflammation, SAA was measured in the serum. Finally, one of the most intensively studied systemic responses against an

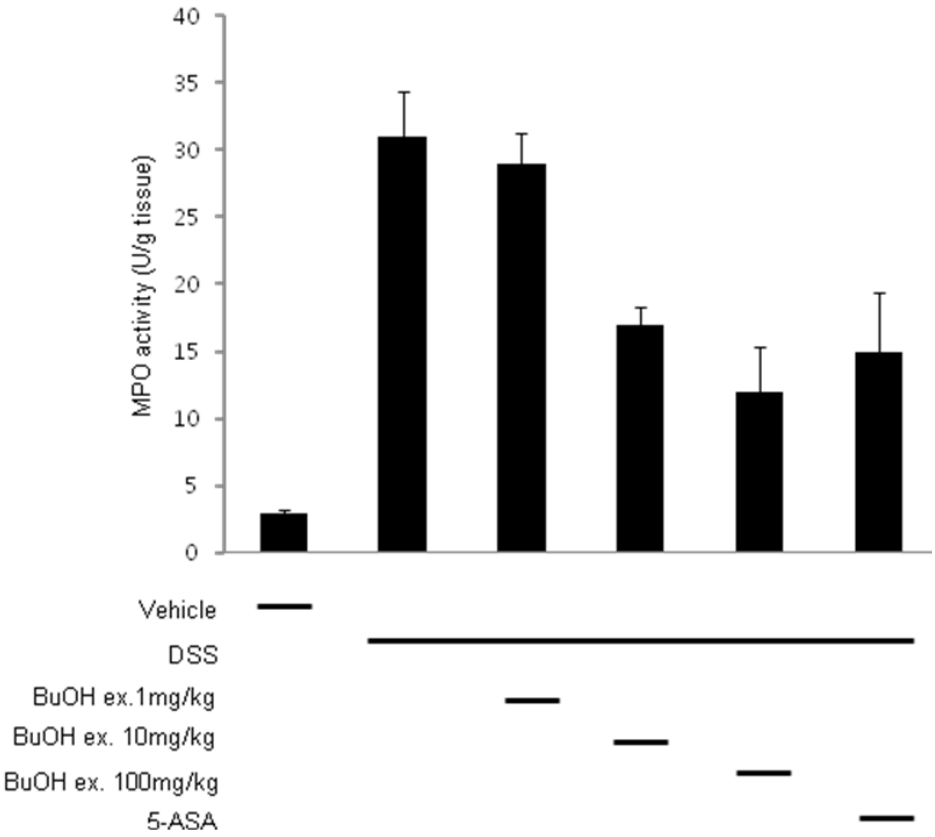

Figure 3. MPO activity of the colonic tissue in DSS-induced mice treated with BuOH extracts of *L. japonica*. Treatment dose was 1 mg/kg, 10 mg/kg and 100 mg/kg, respectively and 5-ASA was 100 mg/kg. Data are mean ± S.D.

inflammatory stimulus is the hepatic synthesis of acute phase proteins, including the family of SAA. As Table 1 shows, the serum SAA levels of DSS-induced group was increased more than approximately 5000-fold (402.5 ± 5.9 ug/ml) compared with the normal group (0.007 ± 0.02 ug/ml). Treatment of 100 mg/kg of BuOH extracts induced a marked decrease in SAA serum levels.

MPO activity measurement

The MPO activity was used as an index of polymorphonuclear (PMN) infiltration. The MPO activity of the vehicle, DSS control and BuOH extracts are shown in Figure 4. Mice from the DSS control group demonstrated the highest MPO activity in the colon, while each treatment of dose of BuOH extracts significantly inhibited the increase in MPO activity.

In this present study, we examined the inhibitory effects of BuOH extracts from *L. japonica* on the production of IL-6 in LPS-stimulated HT-29 cells and also examined the ameliorating effects on DSS-induced inflammation.

IL-6 has been shown to play important roles in the pathogenesis of murine Th-1-mediated colitis (Yamamoto et al., 2000). Intestinal epithelial cells and lamina propria lymphocytes are a major source of IL-6, which is released by the cultured intestinal epithelial cell line T84, and induced intracellular (Ca++) flux and degranulation in neutrophils, in inflammatory bowel disease (Hungness et al., 2000; Sitaraman et al., 2001). These results showed that secretion of IL-6 by intestinal epithelial cells plays a major role in the pathogenesis of IBD and that the prevention of this secretion may be useful in the treatment of inflammatory bowel disease. To test this possibility, we examined the inhibitory effect of BuOH extracts on IL-6 secretion in LPS-stimulated HT-29 and our results were consistent with those of previous studies (Jin et al., 2006; Matsumoto et al., 2004). In the inflammation process, one of the most intensively studied systemic responses against an inflammatory stimulus is the amyloid A (SAA) of the family of hepatic synthesis of acute phase proteins. In general, SAA is an acute-phase protein measured for clinical monitoring of Crohn's disease and is potently induced in response to proinflammatory stimuli that synergize with IL-6 cytokines (Preciado-Patt et al., 1996). In our results, SAA was

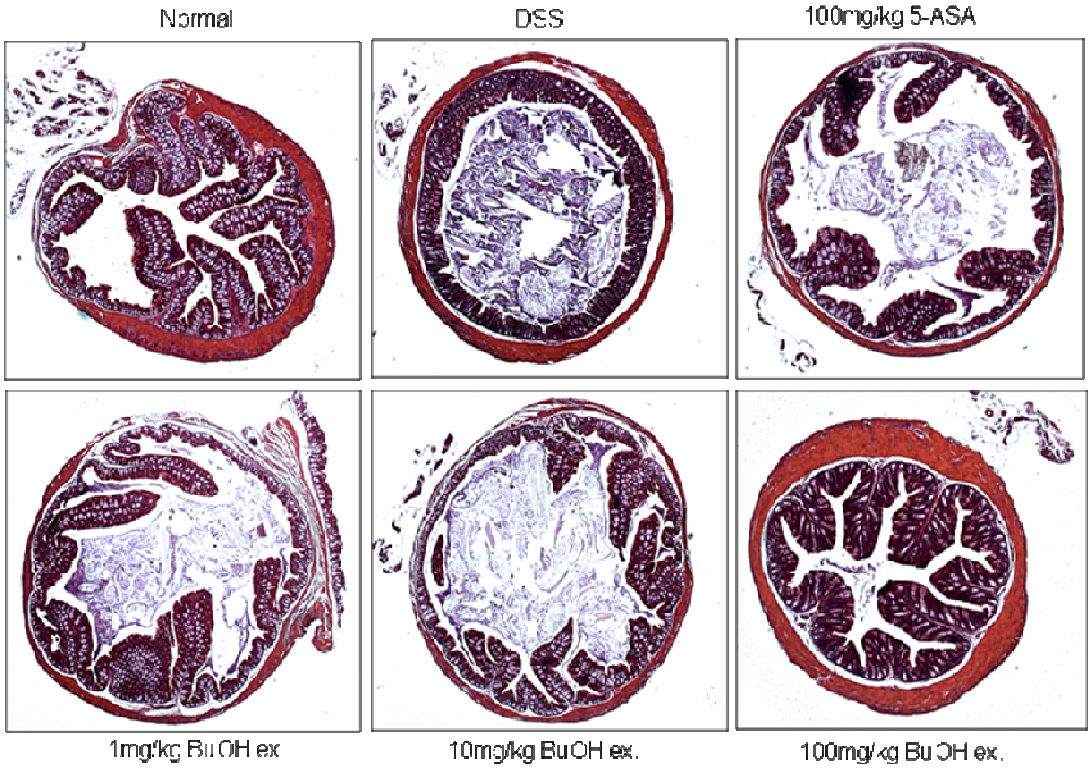

Figure 4. Microscopic study (original magnification 50X) of colons of mice with DSS-induced colitis treated with BuOH extracts of *L. japonica*. Treatment dose was 1 mg/kg, 10 mg/kg and 100 mg/kg, respectively and 5-ASA was 100 mg/kg.

significantly reduced by BuOH extracts on a DSS-induced mice disease model. These change of biochemical factors showed the correlation with the disease parameters such as the disease activity index and MPO activity in colonic tissue that is compared with these parameters in normal mice.

In conclusion, we discovered that BuOH extracts of *L. japonica* inhibited the synthesis of IL-6 in an LPS-stimulated colonic epithelial cell line *in vitro* and DSS-induced colitis *in vivo*. Moreover, we observed a positive effect of BuOH extracts in murine chronic colitis. Therefore, *L. japonica* may be useful as a potential inhibitor for preventing inflammatory bowel disease in humans.

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