**Pulsatilla koreana Ameliorates Dextran Sulfate Sodium-induced Colitis in Mice**

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Ulcerative colitis (UC) is an inflammatory bowel disease, which is a chronic gastrointestinal disorder. *Pulsatilla koreana* (*P. koreana*) is a perennial plant that grows around Korea and it has various pharmacological effects such as anti-cancer and anti-inflammatory activity. However, the regulatory effects of *P. koreana* in intestinal inflammation are not yet understood. This study attempted to determine the effect of *P. koreana* in dextran sulfate sodium (DSS)-induced colitis in mice. The colitis mice were induced by drinking water containing 5% DSS for 7 days. The results showed that mice treated with DSS showed remarkable clinical signs, including weight loss, and reduced colon length. Administration of *P. koreana* attenuated DSS-induced weight loss, colon shortening and Disease activity index in mice. Additionally, *P. koreana* inhibited the cyclooxygenase-2 and prostaglandin E2 levels in DSS-treated colon tissues. These results provide experimental evidence that *P. koreana* might be a useful therapeutic medicine for patients with UC.

**Key Words:** *Pulsatilla koreana*; Ulcerative colitis; Dextran sulfate sodium; Cyclooxygenase-2

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**INTRODUCTION**

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn's disease, which is the chronic and relapsing inflammatory disease of the intestinal tract. Especially, UC is characterized by bloody diarrhea, colonic mucosal ulceration and in severe cases, systemic symptoms. During the last decades, the incidence of UC in the Korea has been increasing rapidly (Yang, 2002). Most therapies for UC include glucocorticosteroids, sulfasalazine and other such drugs (Sandborn and Targan, 2002; Ishiguro et al., 2006). However, these treatments cause serious side effects and there is a pressing need for developing effective therapeutic approaches for UC.

Recent studies have reported that inflammatory mediators are involved in the initiation of the inflammatory response in colitis (Papadakis and Targan, 2000). Cyclooxygenase (COX) generates a variety of prostaglandins (PGs), which have been implicated in a number of physiological events, including the progression of inflammation, immunomodulation and transmission of pain. Two COX isoenzymes have been recognized: COX-1, a constitutive enzyme, which generates PGs that protect the stomach and kidney against damage, and COX-2, an inducible enzyme induced by inflammatory stimuli, such as cytokines, and capable of generating PGs that contribute to the inflammation and cancer. It was reported that COX-2 is important inflammatory biomarkers involved in the development of UC (Agoff et al., 2000). Prostaglandin E2 (PGE2) is the important prostaglandin in the colon and is associated with colonic inflammation (Montrose et al., 2015).
Traditional herbal medicines have garnered much interest for their potential to treat inflammation (Talhouk et al., 2007; Lin et al., 2009). *Pulsatilla koreana* (*P. koreana*) is an important herb in traditional medicine used to treat various diseases including heart disease, amoebic dysentery and malaria (Kim et al., 2004). It was revealed that roots of *P. koreana* possess anti-tumor, anti-biotic and anti-inflammatory activities (Martín et al., 1990; Cuong et al., 2009; Yang et al., 2010). However, there has been no information on whether *P. koreana* regulates intestinal inflammation.

In this study, the author were interested in determining whether *P. koreana* has the effect in inflammatory diseases and chose the dextran sulfate sodium (DSS)-induced mouse colitis model as the subject for this study. This model resembles human IBD, and is used for pharmacological analysis of potentially effective anti-inflammatory agents (Camuesco et al., 2005; Ramakers et al., 2007). Here, the author investigated the effect of *P. koreana* on a murine model of DSS-induced colitis, in order to provide experimental evidence that *P. koreana* serves as a possible treatment for patients with UC.

**MATERIALS AND METHODS**

**Reagents**

DSS (mol wt; 36,000~50,000) was purchased from MP Biomedicals (Solon, OH, USA). The antibody (Ab) for COX-2 and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Sulfasalazine and other chemical reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

**Animals**

Male Balb/c mice (6 weeks old) were obtained from SamTaco animal facility (Gyeonggi, Korea). Animals were housed 6 heads per cage, allowed spontaneous take in food and water. Animals were kept under a 12-h light/dark cycle (light on 08:00~20:00) at room temperature (23 ± 2°C) and humidity (55 ± 10%). All animal procedures and experiments were approved by the Daegu Haany University of Animal Ethics Committee (approval number DHU2013-086).

Table 1. Criteria for disease activity index

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight loss (%)</th>
<th>Stool consistency</th>
<th>Bloodstain or gross bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non</td>
<td>Normal</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>1~5</td>
<td>Loose stool</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>5~10</td>
<td>Loose stool</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>10~15</td>
<td>Diarrhea</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>15&lt;</td>
<td>Diarrhea</td>
<td>Gross bleeding</td>
</tr>
</tbody>
</table>

**Preparation of *P. koreana***

Dried of *P. koreana* were purchased from the Human herb (Gyeongbuk, Korea). The roots (100 g) were chopped using a blender with 1 L of 70% aqueous ethanol solution under room temperature for 24 h and then concentrated under a vacuum. Then the extract solution obtained was filtered, concentrated on a water bath under vacuo, frozen and lyophilized to yield ethanol extracts (yield: 10.2%).

**Induction of colitis by DSS and experimental procedures**

Acute colitis in mice was induced by providing 5% (w/v) DSS drinking water ad libitum for seven days. The mice (n=6) were examined daily for weight loss, stool consistency, and the presence of gross bleeding. The mice were randomized into groups (n=6/group) receiving *P. koreana* (100 mg/kg), sulfasalazine (150 mg/kg) as a positive control, or water as a negative control. *P. koreana* and sulfasalazine diluted with water (200 μl) were administered orally once a day from day 0 of DSS treatment. The mice were euthanized and assessed after seven days of DSS treatment.

**Disease activity index (DAI)**

Intestinal disease activity was assessed based on weight loss, the presence of diarrhea accompanied by blood and mucus, and colonic shortening (Hendrickson et al., 2002). DAIs were calculated by scoring weight loss, diarrhea, and rectal bleeding, based on a previous scoring system (Table 1) as described by Murthy et al. (1993) Weight loss was defined as the difference between initial and final weights, and diarrhea as the absence of fecal pellet formation and the presence of continuous fluid fecal material in the colon.
Rectal bleeding was assessed based on the presence of diarrhea containing visible blood and on the presence of gross rectal bleeding. DAI values were calculated using the following formula: DAI = (weight loss score) + (diarrhea score) + (rectal bleeding score). The clinical parameters used in the present study were chosen to represent the subjective clinical symptoms observed in human ulcerative colitis.

**Western blot analysis**

Distal colons were homogenized in lysis buffer (iNtRON Biotech, South Korea), and centrifuged at 13,000 rpm for 5 min. The supernatants were transferred to fresh tubes and protein concentrations were determined using BCA protein assay reagent (Sigma). Lysates (50 μg of protein) were separated by 10% SDS-PAGE and transferred to membranes (Amersham Pharmacia Biotech, Piscataway, NJ), which were then blocked with 5% skim milk in phosphate-buffered saline (PBS)-Tween-20 (PBST) for 1 hour at room temperature. They were then incubated overnight with primary Abs against COX-2, and washed 3 times with PBST. Blots were incubated with secondary Abs for 1 hour at room temperature and antibody-specific proteins were visualized using an enhanced chemiluminescence detection system (Amersham Corp. Newark, NJ, USA). Protein band densities were quantified by densitometry.

**PGE2 assay**

The PGE2 concentration in colon tissue was measured by enzyme-linked immunosorbent assay (ELISA) using a PGE2 assay kit (Stressgen Biotechnologies, USA) according to the manufacturer's directions. Duplicate aliquots of supernatant were measured for each sample.

**Histological processing**

All trimmed rectums were fixed in 10% neutral buffered formalin. After paraffin embedding, 4 μm sections were prepared. Representative sections were stained with hematoxylin and eosin (H&E) for examination under light microscopy (∼100).

**Statistical analysis**

The results are presented as the mean ± S.D. of at least three experiments. The results were examined using an independent t-tests and ANOVA with a Tukey post hoc test. A P value < 0.05 of was considered significant.

**RESULTS**

**Effects of *P. koreana* on DSS-induced the weight loss and DAI in mice**

DSS-induced colitis in mice has a phenotype similar to that of human acute and chronic UC. The inhibitory effects

![Image](https://example.com/image1.png)

**Fig. 1.** Effects of *P. koreana* on DSS-induced the body weight loss and DAI in mice. Experimental colitis in mice (n=6/group) was induced by a 5% DSS dissolved in the drinking water for 7 days. *P. koreana* was administered orally at doses of 100 mg/kg once a day for 7 days prior 5% DSS supplement. (A) Body weight of mice was measured. (B) DAI was calculated as described in Materials and Methods. SFZ (150 mg/kg) was used as a positive control. Data were represented in the mean ± S.D. from triplicate experiments (#P < 0.05 vs. control, *P < 0.05 vs. DSS alone).
of *P. koreana* on the intestines of mice in DSS-induced experimental colitis were evaluated. Firstly, the weight loss of physiological signs induced by 5% DSS treatment was evaluated in mice. As shown in Fig. 1A, mice treated with DSS showed a significant weight loss compared to the control, and the groups administrated with *P. koreana* showed a significant attenuation of body weight loss caused by DSS. As sulfasalazine has been used as a treatment for colitis, it was used as a positive control in this study. Another common feature of the DSS-induced model of colitis is an increase in DAI. Increased DAI score was remarkably inhibited in the group administered with *P. koreana* compared to the group with DSS (Fig. 1B).

**Fig. 2.** Effect of *P. koreana* on DSS-induced the colon length shortening in mice. Experimental colitis in mice was induced by 5% DSS dissolved in the drinking water for 7 days. *P. koreana* (100 mg/kg) was administered orally once a day for 7 days prior to 5% DSS supplement. (A) The colons were removed at day 7 after DSS treatment, and the colon lengths were measured. (B) Relative colon lengths were represented. SFZ (150 mg/kg) was used as a positive control. Data were represented in the mean ± S.D. (n=6) from triplicate experiments (#P < 0.05 vs. control, *P* < 0.05 vs. DSS alone).

**Fig. 3.** Effect of *P. koreana* on the PGE2 and COX-2 levels in DSS-induced colitis. Experimental colitis was induced by 5% DSS drinking water for seven days in mice. *P. koreana* (100 mg/kg) was administered orally once a day for seven days prior to 5% DSS supplementation. SFZ (150 mg/kg) was used as a positive control. At the end of the experiment, the colon tissues were excised and homogenized. (A) The levels of PGE2 were quantified by ELISA. (B) COX-2 levels in tissues of colitis were measured via western blot analysis. (C) Relative COX-2 levels were represented. All data are expressed as the means ± S.D. of three independent experiments (#P < 0.05 vs. control, *P* < 0.05 vs. DSS alone).
Effects of *P. koreana* on DSS-induced the colon length shortening in mice

The DSS-induced model of colitis is associated with a significant decrease in colon length (Hendrickson et al. 2002) and colon length measurement has often been used as a morphological parameter for the degree of inflammation in DSS colitis. To assess colon length in the present study, mice from each group were killed at days 7. As shown in Fig. 2A, the result shown that colon length in the DSS-administered mice was significantly shorter than that of control. However, *P. koreana* treatment alleviated DSS effects on colon shortening. Relative colon lengths are shown in Fig. 2B.

Effect of *P. koreana* on PGE2 and COX-2 levels in DSS-induced colitis

To determine the effect of *P. koreana* on major inflammatory mediator in mouse tissues of colitis, PGE2 assay was performed. At the end of the experiment, colon tissues were excised and homogenized. As shown in Fig. 3A, the levels of PGE2 were significantly increased in the colon tissues of DSS-treated mice compared to those of the control group. However, administration of *P. koreana* reduced these levels induced by DSS. The rate of inhibition of PGE2 levels by *P. koreana* and sulfasalazine was approximately 31.71% (*P* < 0.05) and 39.02% (*P* < 0.05), respectively. COX-2 is considered important inflammatory mediators that play a key role in pathogenesis of UC. We also evaluated the effects of *P. koreana* on COX-2 levels in tissues of colitis using Western blot analysis. COX-2 levels were significantly increased as a result of DSS exposure. Elevated COX-2 levels were reduced in the *P. koreana* group (Fig. 3B). Relative COX-2 levels in colon tissues are represented in Fig. 3C.

Effects of *P. koreana* on epithelial injury in DSS-induced colitis

Epithelial injury was detected in DSS control as compared with intact control. However, these histopathological changes induced by DSS treatments were inhibited by treatment of *P. koreana* (Fig. 4).

DISCUSSION

Traditional herbal medicine has been the subject of increased interest for its potential in the treatment of inflammation (Talhouk et al., 2007; Lin et al., 2009). However, their pharmacological mechanisms of action have remained largely unresolved. To the best of our knowledge, this study is the first report demonstrating that *P. koreana* ameliorates DSS-induced colitis in mice clinically and the beneficial effect of *P. koreana* treatment might be linked to the inhibition of inflammatory mediators. These findings suggest that *P. koreana* may be a useful therapeutic approach to the treatment of UC.
UC is an idiopathic disease characterized by the development of intestinal inflammation (Bouma and Strober, 2003). Unfortunately, despite many years of extensive research implicating immune dysfunction, genetic susceptibility, and bacterial flora within the intestinal environment as possible factors associated with development of the disease, its pathogenesis is still poorly understood. Several therapies including corticosteroids and sulfasalazine have been used for UC but these treatments cause serious side effects such as hormonal disturbance, peptic ulcers, liver dysfunction, and psychological problems (Sandborn and Targan, 2002; Ishiguro et al., 2006). Consequently, these adverse effects sometimes lead to discontinuation of corticosteroid treatment and result in acute UC exacerbation. An alternative treatment for active UC is therefore needed to help patients avoid these clinical problems. Recently, traditional herbal medicine has been increased interest for the treatment of these disorders. As *P. koreana* has antibiotic and anti-inflammatory activities, the author predicted that *P. koreana* may possess anti-colitis effect and investigated the regulatory effect of *P. koreana* on DSS-induced colitis. The finding showed that *P. koreana* reduced the weight loss and colon shortening caused by DSS. In addition, the DAI, scored using three major clinical signs (weight loss, diarrhea and rectal bleeding), was remarkably inhibited in the group given *P. koreana*. The inhibitory effect of *P. koreana* on colon shortening and DAI was similar to the sulfasalazine group. These results suggest that *P. koreana* effectively inhibits the symptoms of colitis caused by DSS.

Inflammatory mediators are associated in inflammatory response in colitis (Talero et al., 2011). Especially, COX-2 levels increase dramatically, leading to the production of PGs in inflammatory process (Morita, 2002). It was reported that the expression of COX-2 is elevated in the inflamed mucosa of patients with UC. Therefore, research on new biological therapies for UC has focused on blocking components of the inflammatory mediators. Recently, COX-2 inhibitors have been developed as nonsteroidal anti-inflammatory drugs, many of which have been shown to be efficacious in a model of chemically induced colitis (Brune and Hinz, 2004; El-Medany et al., 2003). Additionally, 5-minosalicylates, another drug class used to treat IBD, exert anti-inflammatory effects by inhibiting COX-2 activation. In this study, the result shown that the levels of COX-2 and PGE₂ increased in DSS treated-colon tissues compared with those of the control, and that treatment with *P. koreana* reduced these levels. These results indicated that the anti-inflammatory effect of *P. koreana* is attributable to the regulation of inflammatory mediator in DSS-induced colitis.

Saponin and anemonin are constituents of *P. koreana*. Other studies have demonstrated that saponin and anemonin have anti-inflammatory effects. Especially, saponin inhibited the colonic inflammation through inhibition of COX-2 and inflammatory cytokines in rat (Puangpraphant et al., 2013). It was also reported that anemonin attenuated the LPS-induced iNOS expression and NO production in macrophage (Lee et al., 2008). From this, we predicted that *P. koreana* exert its beneficial effect in UC by the anti-inflammatory effect of saponin and anemonin and so on. Although *P. koreana* attenuated the DSS-induced the clinical signs and inflammatory mediators, pharmacological mechanism of *P. koreana* was not determined in present study. Therefore, further studies will be necessary in order to clarify more precisely the role of *P. koreana* in UC.

In conclusion, the author demonstrated at first that a treatment of *P. koreana* could reduce significantly the clinical signs and the levels of inflammatory mediators in a colitis model caused by DSS treatment. Therefore, these results suggested that *P. koreana* may be a useful therapeutic candidate for colitis. However, the further studies must be performed to elucidate the precise mechanism of *P. koreana* for the treatment of intestinal inflammatory disorders.

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**Conflict of interest**

There is no conflict of interest.

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