The anti-oxidative and anti-inflammatory effect of *Psoralea corylifolia* on Ulcerative Colitis Induced by Dextran Sulfate Sodium in Mice

Ahn Sang Hyun¹, Kim Ki Bong²

¹Department of Anatomy, college of Korean Medicine, Semyung University
²Department of Pediatrics, Korean Medicine Hospital, Pusan National University

**Objectives:** This study was to investigate the anti-oxidative and anti-inflammatory effect of *Psoralea corylifolia* water extract (PE) on ulcerative colitis which was induced by dextran sulfate sodium (DSS) in mice.

**Methods:** Ulcerative colitis was induced by DSS in male BALB/c mice. The mice were divided into 3 groups. The control group (Ctrl) was not induced ulcerative colitis. The pathological group (CE) was induced the colitis. The experimental group (PT) was administered PE after inducing the colitis. The effects of the PE on ulcerative colitis were evaluated by morphological change in the colon tissue and cells, substance P production, activity of tumor necrosis factor (TNF)-α and nuclear factor (NF)-κB, cyclooxygenase (COX)-2 production, and anti-oxidative activity.

**Results:** In the PT group, PE alleviated hemorrhagic erosion in colon mucosa and infiltration of inflammatory cells in lamina propria mucosae. In the colon of the PT group, COX-2 production was inhibited via regulating the activity of TNF-α and NF-κB p65. PE also had an anti-oxidative effect via activating nuclear factor (erythroid-derived 2)-like 2 (Nrf2).

**Conclusions:** In this study, we found the utility of treatment with PE and the potential of developing a medicine for ulcerative colitis by applying our results. Further investigations for the anti-inflammatory mechanism of PE may be needed.

**Key Words**: *Psoralea corylifolia*, ulcerative colitis, anti-inflammatory, anti-oxidative.

**Introduction**

Ulcerative colitis (UC) is a chronic cryptogenic inflammatory bowel disease (IBD) which was localized in the mucosa or submucosa of the colon. It accompanies with recurrent bloody diarrhea and the patient complains of rectal urgency and abdominal pain. UC is caused by the complex effect of genetic and environmental factors. Although the patients are distributed worldwide, UC is the most frequent in the North America and Northern Europe. Ethnically, UC more occurs in the Jewish and the Caucasian but relatively rare in the Asian. However, UC patient is increasing as the incidence increases in the Asian countries including Korea.

The objective of UC treatment is inducing remission by alleviating symptoms and inflammation in mucosa and improving the quality of life by maintaining the remission as long as possible. About 15% of the UC patients arrive at the remission by...
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just a placebo⁵. However, adequate treatment is advisable because most patients continue bloody stool and diarrhea if not treated⁶. 5-aminosalicylic acid (5-ASA), steroids, or immunosuppressants have been used for the standard medication of UC. But 20-40% of the patients fail to conventional medication or have colectomy due to side effects⁷. Recently, there are some efforts to find candidate drugs which is effective for UC and have less side effects from natural products including Korean medicine⁸. There have been studies on herbal medication for IBD and experimental effectiveness of herbal medicine for colitis animal model induced with dextran sulfate sodium (DSS) in Korea⁹. However, the medicine have not been developed because studies on the mechanism of herbal medicine are insufficient.

*Psoralea semen*, which is originated from *Psoralea corylifolia* L. (Leguminosae), has been used as external medicine for vitiligo in Korean medicine and it has melanogenesis activity⁹. According to Dongeuibogam, it invigorates yang by tonifying the kidney, secures essence, and warm the spleen. Thus, it cures impotence, ganacratia, urinary frequency, and diarrhea.

Based on its known effectiveness, *Psoralea corylifolia* may be effective for IBD which has main symptoms such as chronic abdominal pain and diarrhea. However, studies on the anti-oxidative activity and inhibitory effect for cyclooxgenase (COX)-2 expression of *Psoralea corylifolia* are insufficient yet. To provide the basis of utilizing it for UC, we investigated the anti-oxidative and anti-inflammatory effect of *Psoralea corylifolia* water extract (PE). Thus, we found the anti-oxidative activity, inhibitory effect for COX-2 expression, and anti-inflammatory effect in UC mice induced by DSS.

### Materials and Methods

#### 1. Materials

1) Animal Model

Male BALB/c mice with 6 weeks of gestational age were obtained from Orient (Seongnam, Gyeonggi-do, Republic of Korea). These mice were adapted to the aseptic rearing area for 2 weeks and then mice who have 20 g of body weight were selected. The mice were divided into 3 groups: the control group (Ctrl), the DSS-treated group (CE), and both DDS and PE-treated group (PT). 10 mice were allocated to each group. This study was approved by Institutional Animal Care and Use Committee (IACUC) of Dongguk University (IACUC number: DGU-2015-0006). The management and use of experimental animal were executed according to the guideline of the National Institutes of Health (NIH).

2) Preparing *Psoralea corylifolia* Water Extract

100 g of *Psoralea corylifolia* was decocted in 500 mL of distilled water for 2 hours. After filtration, the filtrate was concentrated under low pressure by rotary evaporator and then freeze-dried. The yield of PE was 13.1%.

#### 2. Methods

1) Inducing UC by DSS and PE administration

To induce UC, we voluntarily administered 5% (weight/volume) DSS (molecular weight: 40,000; ICN, Aurora, OH, USA) to the CE and PT group for 5 days. For the PT group, 20 mg/kg/day of PE was orally administered for 5 days after inducing UC. For the Ctrl and the CE group, 100 μL/day of saline solution was orally administered during the same period.

2) Preparing Tissue Sample

After 5 days from DSS treatment, the mice were anesthetized by sodium pentobarbital solution.
Cardiac perfusion fixation were conducted with vascular rinse and 10% neutral buffered formalin (NBF). Descending colon was separated and fixed by 10% NBF for 24 hours at room temperature. Fixed tissue was embedded in the paraffin and made into 5 μm-thick serial sections.

3) Histochemistry

Phloxine-tartrazine staining was used to observe the change in apical surface of mucous epithelium caused by hemorrhagic abrasion. After nuclear staining with Mayer's hematoxylin for 5 minutes, the tissue reacted with phloxine solution for 30 minutes. Then, the tissue was differential-stained by tartrazine solution. Observation was performed with optical microscope (BX60, Olympus, Tokyo, Japan).

Masson trichrome staining was used to observe the change in mucus secreting cell. At first, the tissue was mordanted by 50-60 °C Bouin solution for 1 hour and then picric acid was removed by 70% ethanol. Next, the tissue reacted with Weigert iron hematoxylin to stain nucleus for 10 minutes. Then, the tissue reacted with Biebrich scarlet-acid fuchsin and phosphomolybdic-phosphotungstic acid for 15 minutes respectively. After reacting it with aniline blue for 5 minutes, we observed the change in mucus secreting cell.

4) Immunohistochemistry

Colon tissue was proteolyzed by 20 μg/mL proteinase K for 5 minutes and then reacted with 10% normal goat serum, a blocking serum, for 4 hours at room temperature. Next, the tissue was reacted with primary antibodies including goat anti-substance P (1:100, Santa Cruz Biotechnology, USA), goat anti-nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (1:50, Santa Cruz Biotechnology, USA), goat anti-tumor necrosis factor (TNF)-α (1:200, Santa Cruz Biotechnology, USA), goat anti-nuclear factor (NF)-κ B p65 (1:500, Santa Cruz Biotechnology, USA), goat anti-p-1 κ B (1:250, Santa Cruz Biotechnology, USA), and goat anti-COX-2 (1:100, Santa Cruz Biotechnology, USA) for 72 hours in the 4 °C humidified chamber. Then, the tissue was reacted with biotinylated rabbit anti-goat immunoglobulin (Ig) G (1:100, Santa Cruz Biotechnology, USA) for 24 hours at room temperature. After that, the tissue was reacted with avidin-biotin complex kit (Vector Lab, Burlingame, CA, USA) for 1 hour at room temperature. Prepared tissue was developed in 0.05 M tris-HCl buffer solution (pH 7.4) composed of 0.05% 3,3'-diaminobenzidine and 0.01% HCl. Hematoxylin was used for counter-staining.

5) Image Analysis

The result of immunohistochemistry were quantified as ‘mean ± standard error’ by Image Pro Plus (Media cybernetics, Rockville, MD, USA). Mucosa samples were randomly selected from each groups were taken as 400×-magnified photos and then analyzed as positive pixels/20 million pixels.

6) Statistical Analysis

Statistical analysis was performed by SPSS software (SPSS 20, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for significance test (P<0.05) and Duncan’s multiple range test was used for follow-up test.

**Results**

1. Alleviating UC in the Colon Mucosa

1) Morphological Change in the Cross Section of Colon

In the CE group, hemorrhagic erosion accompanying loss of intestinal cells and glands was observed in many areas of the mucous superficial epithelium. Infiltration of many inflammatory cells such as lymphocyte, fibroblast, or granulocyte was found in the lamina propria mucosae. In some areas
of the colon, hemorrhagic erosion started from colon mucosa spread to the submucosa through the muscularis mucosae. Hemorrhagic erosion of the PT group was more alleviated than that of the CE group. In the lamina propria mucosae, the infiltration of inflammatory cells also decreased in the PT group compared with the CE group (Fig. 1).

2) Morphological Change in the Colon Tissue

In the CE group, colon cells located at the apical surface of superficial epithelium was almost damaged and brush border was not observed. Except the apical surface of the superficial epithelium, normal cell arrangement was observed in the PT group. Brush border was also observed in the PT group (Fig. 1).

3) Decrease in Substance P Production

To observe the change in substance P distribution, which provokes pain, in the lamina propria mucosae, immunohistochemical staining with goat anti-mouse substance P was used. In the CE group, the distribution of substance P-positive cells more increased in the lamina propria mucosae than that of the Ctrl group. Strong positive reaction was found at the margin of cytoplasm in the positive cell. Positive reaction of the CE group more increased by 881% than that of the Ctrl group. On the other hand, positive reaction of the PT group more decreased by 83% than that of the CE group (Fig. 2).
2. Regulating Pro-inflammatory Cytokines

1) TNF-α Inhibitory Effect

To observe the change in TNF-α activity, which is a pro-inflammatory cytokine, in the lamina propria mucosae, immunohistochemical staining with goat anti-mouse TNF-α was used. In the CE group, the distribution of TNF-α-positive cells more increased in the lamina propria mucosae than that of the Ctrl group. Strong positive reaction was found at the cytoplasm of the positive cell. Positive reaction of the CE group more increased by 639% than that of the Ctrl group. On the other hand, positive reaction of the PT group more decreased by 73% than that of the CE group (Fig. 3).

2) NF-κB Inhibitory Effect

To observe the change in NF-κB activity, which is a transcription factor, in the lamina propria mucosae, immunohistochemical staining with goat anti-mouse NF-κB p65 and anti-mouse p-IκB was used. In the CE group, the distribution of NF-κB p65-positive cells more increased in the lamina propria mucosae than that of the Ctrl group. Strong positive reaction was found at the cytoplasm around the nuclear membrane of the positive cell. Positive reaction of the CE group more increased by 530% than that of the Ctrl group. On the other hand, positive reaction of the PT group more decreased by 71% than that of the CE group (Fig. 4).

For p-IκB, the distribution of the positive cells in the CE group more increased in the lamina propria mucosae than that of the Ctrl group. Strong positive
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3. Inhibitory Effect on COX–2

To observe the change in COX-2 production, which is an inflammatory enzyme, in the lamina propria mucosae, immunohistochemical staining with goat anti-mouse COX-2 was used. In the CE group, COX-2-positive cells more increased in the lamina propria mucosae than that of the Ctrl group. Strong positive reaction was found at the margin of cytoplasm in the positive cell. Positive reaction of the CE group more increased by 375% than that of the Ctrl group. On the other hand, positive reaction of the PT group more decreased by 27% than that of the CE group (Fig. 5).

4. Anti-oxidative Effect via Increasing Nrf2

To observe the change in Nrf2, which has an anti-oxidative activity, in the lamina propria mucosae, immunohistochemical staining with goat anti-mouse Nrf2 was used. In the CE group, Nrf2-positive cells decreased in the lamina propria mucosae. Strong positive reaction was found at the cytoplasm of the positive cell. Positive reaction of the CE group more decreased by 64% than that of the Ctrl group. On the other hand, positive reaction
Fig. 4. The inhibitory effect of PE on NF-κB p65 and p-IκB in the colon mucosa. Abbreviations: Ctrl, the mice which was not treated anything; CE, the mice which was treated DSS only; PT, the mice which was treated both DSS and PE; MU, colonic mucosa; SM, submucosa. Bar size: 100 μm. Arrow: immunohistochemically positive reaction. *: P<0.05 compared with the CE group.

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Discussion

Proliferation of westernized diet increased the prevalence of IBD such as UC and Crohn's disease. These diseases are recently recognized as one of the causes for increase in the colon cancer mortality of Korean people. Many studies for the pathogenesis of IBD are ongoing. Most of all, therapies such as regulating pro-inflammatory cytokines, transcription factors, arachidonic acid metabolites, and reactive oxygen and nitrogen species (RONS) are attracting
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Fig. 5. The inhibitory effect of PE on COX-2 in the colon mucosa. Abbreviations: Ctrl, the mice which was not treated anything; CE, the mice which was treated DSS only; PT, the mice which was treated both DSS and PE; MU, colonic mucosa; SM, submucosa. Bar size: 100 μm. Arrow: COX-2-positive reaction, *: P<0.05 compared with the CE group.

much attention\(^{10-12}\).

The clinical symptoms of US are inflammation, ulcer, shortness of rectum, and infiltration of immune cells to wounds. For UC, aminosalicylates such as sulfasalazine and mesalazine and steroids are currently in use but they are not able to anticipate complete recovery. Long-term administration of these drugs can cause various side effects such as nausea, vomiting, dyspepsia, anorexia, and headache or tolerance\(^{13}\). Thus, developing novel medicine which has high efficacy and safety is desperately needed. For the UC medicine, attention to Korean medicine has been recently increasing\(^{14}\).

Psoralea semen is the dried seeds of *Psoralea corylifolia* L. (Leguminosae)\(^9\). According to Dongeuibogam, it invigorates yang by tonifying the kidney, secures essence, and warm the spleen. Based on such literature, this study was to find the potential of PE for UC.

5% DSS was used to induce UC in this study. To identify protective effect of PE on the colon, we observed the change in the colon mucosa and cells. Mucous epithelium of gastrointestinal tract acts as a protective barrier against various stimulation. DSS treatment decreases the distribution of zonula occudin (ZO)-1, one of the occluding junction proteins. This is because of the damage of zonula occludens located at the top of the junctional complex between mucous epithelial cells. Increase in intestinal permeability caused by the damage of zonula occludens induces intestinal inflammation and provokes UC\(^{15}\). Also, mice colon treated with DSS

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Fig. 6. The promotive effect of PE on Nrf2 in the colon mucosa. Abbreviations: Ctrl, the mice which was not treated anything; CE, the mice which was treated DSS only; PT, the mice which was treated both DSS and PE; MU, colonic mucosa; SM, submucosa. Bar size: 100 μm, Arrow: Nrf2-positive reaction, *: P<0.05 compared with the CE group.

has pathological characteristics including epidermal ulcer, severe edema of tissue wall, hypertrophy of mucous tissue, and excessive infiltration of granulocytes such as eosinophil\(^\text{16}\).

In the lamina propria mucosae of the CE group, as the observation result of morphological change in colon mucosa, there was hemorrhagic erosion and infiltration of immune cells in many areas of the mucous superficial epithelium. However, the PT group showed alleviated hemorrhagic erosion compared with the CE group. Decrease in the infiltration of inflammatory cells were also observed in the lamina propria mucosae (Fig. 1). These results means that PE decreased intestinal permeability, hemorrhagic erosion, and infiltration of immune cells by alleviating the damage of zonula occludens.

Macrophage not only produces inflammatory enzyme as COX-2 but also plays an important role in regulating inflammation and immune response in the early stage of IBD\(^\text{17}\). Furthermore, COX-2 expressed by inflammation causes chronic inflammation by promoting prostaglandin synthesis in the inflammatory cells and central nervous system\(^\text{18}\). Producing inflammatory enzyme as COX-2 are regulated by transcription factors such as nuclear factor interleukin (NF-IL)-6, Fos/Jun complex (activator protein (AP)-1), CCAAT/enhancer-binding protein (C/EBP), and NF-κB\(^\text{19}\). Especially, TNF-α and NF-κB p65 regulate genes related to the inflammation in IBD\(^\text{20,21}\). If NF-κB was activated by intestinal oxidative stress, increase in oxidative stress phosphorylates the serine residue of IκB
protein by activating IκB kinase (IKK: IKKα and IKKβ). Phosphorylated IκB protein is ubiquitinated and then degraded by 26S proteasome. Inactivated NF-κB located in the cytoplasm binds with the NF-κB binding site (consensus sequence: 5'-GGGpuNNPyPyCC-3') of the target gene in the nucleus and then induces expression of inflammatory genes22).

In this study, the distribution of TNF-α-positive cells increased at the lamina propria mucosae of the CE group and the reaction strongly appeared in the cytoplasm of the positive cell. The positive reaction of the CE group more increased by 639% than that of the Ctrl group. On the other hand, the PT group was less positive by 73% than the CE group (Fig. 3). Also, the distribution of NF-κB p65-positive cells increased at the lamina propria mucosae of the CE group and the reaction strongly appeared at the cytoplasm around the nuclear membrane of the positive cell. The positive reaction of the CE group more increased by 530% than that of the Ctrl group. On the other hand, the PT group was less positive by 71% than the CE group (Fig. 4). For p-IκB, the distribution of positive cells increased at the lamina propria mucosae of the CE group and the reaction strongly appeared at the cytoplasm around the nucleus membrane of the positive cell. The positive reaction of the CE group more increased by 1919% than that of the Ctrl group. On the other hand, the PT group was less positive by 56% than the CE group (Fig. 4). These results means that the PE has anti-inflammatory activity via regulating inflammatory enzyme synthesis.

In the UC patient, increase in the synthesis of COX-2 by NF-κB provokes severe inflammation. COX-2, which is an inducible isoform and expressed in various cells including fibroblast and macrophage, causes many kinds of chronic inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, UC, and Helicobacter pylori-induced gastritis via continuous prostaglandin secretion induced by growth factors and mitogens and is related to vasodilation and angiogenesis23,24). Thus, we observed the distribution of COX-2-positive cell. As the result, the COX-2 positive cells increased in the CE group but decreased in the PT group. In the result of COX-2 image analysis, the positive reaction of the CE group more increased by 375% than that of the Ctrl group. On the other hand, the PT group was less positive by 27% than the CE group (Fig. 5). These results means that the PE has anti-inflammatory activity via regulating inflammatory enzyme synthesis.

We had anticipated that the anti-inflammatory activity of the PE is related to the anti-oxidative effect. Thus, the change of the Nrf2 activity in the lamina propria mucosa was observed to find the anti-oxidative effect of the PE. The distribution of Nrf2-positive cell in the CE group decreased in the lamina propria mucosa. The reaction strongly appeared in the cytoplasm of the positive cells. The positive reaction in the CE group more decreased by 64% than that of the Ctrl group. On the other hand, the PT group was more positive by 94% than the CE group (Fig. 6). From these results, we found that the PE has an anti-oxidative activity via activating Nrf2.

From our results, PE has the potential for UC medicine because of anti-inflammatory, anti-oxidative activity, and protective effect on the colon mucosa. Thus, PE is applicable enough for UC. However, because our results were obtained from DSS-treated animal model, there is a limit to apply for human UC directly. Furthermore, further studies on isolation and identifying anti-inflammatory compound of PE and anti-inflammatory mechanism of PE are necessary.

**Conclusion**

This study was conducted to investigate the therapeutic effect of the PE on UC. The PE alleviated hemorrhagic erosion of the colon mucosa
in the UC mice treated with DSS. In the lamina propria mucosa, the PE reduced the infiltration of inflammatory cells. In the colon of the mice which was administrated PE, the PE inhibited COX-2 production via regulating the activity of pro-inflammatory cytokines including TNF-α and NF-κB p65. Furthermore, the PE had an anti-oxidative activity via activating Nrf2. From our results, the PE may alleviate mucosa damage via regulating excessive inflammation in UC.

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Competing Interests

The authors declare that they have no competing interests.

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