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Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis

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ABSTRACT

Ethnopharmacological relevance: Patrinia scabiosaefolia Fisch is used in folk medicines to treat intestinal abscesses, acute appendicitis, and dysentery in Asia. Although recent reports indicate that *Patrinia scabiosaefolia* has sedative and anti-tumor effects, its effects on ulcerative colitis have not been previously explored.

Aim of the study: To determine the effects and the mode of action of the methanol extract of the roots of *Patrinia scabiosaefolia* (PME) on a model of colitis in mice induced by dextran sulfate sodium (DSS).

Materials and methods: We induced colitis using DSS in 5-week-ICR mice over 7 days and estimated disease activity index (DAI), which took into account body weight, stool consistency, gross bleeding, and tissue myeloperoxidase (MPO) accumulation. Colon lengths and spleen weights were measured. Histological changes were observed by H&E staining. Pro-inflammatory mediators, namely, nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), were determined using Griess assays, immunoassays, and by quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR), respectively.

Results: PME significantly attenuated DSS-induced DAI scores and tissue MPO accumulation, which implied that it suppressed weight loss, diarrhea, gross bleeding, and the infiltrations of immune cells. PME administration also effectively and dose-dependently prevented shortening of colon length and enlargement of spleen size. Histological examinations indicated that PME suppressed edema, mucosal damage, and the loss of crypts induced by DSS. Furthermore, PME inhibited the abnormal secretions and mRNA expressions of pro-inflammatory cytokines, such as, TNF-α, IL-1β, and IL-6.

Conclusion: These results suggest that PME has an anti-inflammatory effect at colorectal sites that is due to the down-regulations of the productions and expressions of inflammatory mediators, and that it may have therapeutic value in the setting of inflammatory bowel disease (IBD).

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are referred to as inflammatory bowel diseases (IBDs), and are manifestations of numerous immunological disorders associated with cellular and humoral immune response (Owczarek et al., 2009). The pathogenesis of IBD remain unclear, but imbalances between pro-inflammatory cytokines, such as, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-1 (IL-1), IL-6, and IL-12, and anti-inflammatory cytokines, such as, IL-4, IL-10, IL-11, are believed to play a central role in modulating inflammation (Ardizzone and Bianchi Porro, 2005).

Traditional therapeutic agents, like 5-aminosalicylates (5-ASA) and corticosteroids, are still used to treat IBD. In addition, some immunomodulators, such as, azathioprine and 6-mercaptopurine, and antibiotics are becoming important in the setting of steroid resistant and steroid-dependent patients (Hanauer, 1996). However, all of these drugs have shortcomings. 5-ASA is well tolerated but diarrhea, cramps, and abdominal pain are occasional side

Abbreviations: 5-ASA, 5-aminosalicylic acid; CD, Crohn's disease; DAI, disease activity index; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; IL-1β, interleukin-1β; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; MPO, myeloperoxidase; NO, nitric oxide; PME, *Patrinia scabiosaefolia* methanol extract; RT-PCR, reverse-transcriptase polymerase chain reaction; TNF-α, tumor necrosis factor-α; UC, ulcerative colitis.

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effects, and these may be accompanied by a fever, rash, or kidney problems. Corticosteroids have also been used for many years to treat patients with severe CD and UC, and have well known side effects, which include rounding of the face, acne, increased body hair, diabetes, weight gain, and high blood pressure (Xu et al., 2004).

Patrinia scabiosaefolia Fisch belongs to the Valerianaceae family, and is commonly used in herbal medicines in Korea. Traditionally, *Patrinia scabiosaefolia* is used to treat anti-inflammatory diseases, especially colonic inflammations, but has also been used to treat virus infections, hepatitis, and uteritis (Shin, 1998). Previous studies have reported that the water extract of *Patrinia scabiosaefolia* has inhibitory effects on cholecystokinin octapeptide-induced acute pancreatitis in rats (Seo et al., 2006), and the ethyl acetate extract of *Patrinia scabiosaefolia* has been reported to induce the apoptosis of human breast carcinoma MCF-7 cells via the caspase-independent mitochondrial cell death pathway (Chiu et al., 2006). Previous phytochemical investigations of *Patrinia scabiosaefolia* have shown that oleanonic acid, oleanolic acid, and ursolic acid from *Patrinia scabiosaefolia* have anti-inflammatory effects (Giner-Larza et al., 2001; Nakanishi et al., 1993; Tapondjou et al., 2003; Yang et al., 1999). Although *Patrinia scabiosaefolia* has been used to treat inflammatory intestinal diseases, no reports have been issued on its anti-inflammatory effects on intestinal epithelial cells or the mechanism involved. In the present study, we investigated whether the methanol extract of the roots of *Patrinia scabiosaefolia* (PME) suppresses gut inflammation in an *in vivo* inflammatory model of bowl disease (IBD) induced by DSS.

2. Material and methods

2.1. Chemicals and reagents

DSS was purchased from MP biomedicals (MW; 36,000–50,000, MP Biomedicals, Solon, OH). iNOS, β-actin monoclonal antibodies, and peroxidase-conjugated secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The enzyme immunoassay (EIA) kits for TNF-α, IL-1β, and IL-6 were obtained from R&D Systems (Minneapolis, MN, USA). Random oligonucleotide primers and M-MLV reverse transcriptase were purchased from Promega (Madison, WI, USA). dNTP Mix and SYBR green ex Taq were obtained from TaKaRa (Seoul, Korea). TNF-α, IL-1β, IL-6, and β-actin oligonucleotide primers were purchased from Bioneer (Seoul, Korea). 5-Aminosalicylic acid (5-ASA) and all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant material and preparation of the methanol extract

The roots of *Patrinia scabiosaefolia* Fisch (Valerianaceae) were delivered by the Department of Oriental Pharmacy, Kyung Hee Medical Center and identified by Prof. Nam-In Back (Kyung Hee University, Suwon, Korea). A voucher specimen (KHUOPS-08-51) was deposited at the herbarium of the College of Pharmacy, Kyung Hee University (Seoul, Korea). The plant material (206.65 g) was extracted two times with 70% methanol (MeOH) under reflux. The methanol extract was filtered and evaporated under reduced pressure to give a solid PME (22.21 g), which was then stored at −20 °C until required. To set a standard of PME contents, total phenols and flavonoids contents were 26.6 and 5.1 μg/ml, respectively (Tunalier et al., 2007).

2.3. Experimental animals

Male ICR mice weighing 28–30 g were purchased from Daehan Biolink (Eumsung, Korea). All mice were housed 2/cage and fed standard laboratory chow in the animal room with 12 h dark/light cycles and constant temperature of 20 ± 5 °C. All animal experiments were conducted under university guideline and approved by ethical committee for Animal Care and Use of the Kyung Hee University according to an animal protocol (KHP-2009-10-11).

Table 1
Evaluation of disease activity index (DIA).

DIA score	Weight loss (%)	Stool consistency	Occult/gross bleeding
0	None	Normal	Normal
1	1–5		
2	5–10	Loose stools	Hemoccult positive
3	10–20		
4	>20	Diarrhea	Gross bleeding

2.4. Induction of colitis

Experimental colitis was induced by giving mice drinking water *ad libitum* containing 5% (w/v) DSS for 7 days. Mice of each of the groups were monitored carefully every day to confirm that they consumed an approximately equal volume of DSS-containing water. For each experiment, mice were divided into seven experimental groups. The first group was kept as vehicle-treated control and treated with saline as the same route as PME, and the second group was given drinking water with DSS only throughout the experimental period. Other 4 groups consisted of mice receiving 5% DSS were administrated 5-ASA (75 mg/kg/day p.o.) or PME (10, 30, 50 mg/kg/day p.o.) daily for 7 days according to experimental design. And the last group was given PME (50 mg/kg/day p.o.) alone daily for entire experimental period without DSS. Our preliminary toxicity studies in normal mice, which no toxicities were observed with oral administration of PME once day for 7 days up to 1000 mg/kg/day. All materials were dissolved in vehicle (0.9% saline). Control groups were given vehicle daily for 7 days as appropriate. Administration of each drug was started with the DSS treatment at the same time. The experiments were conducted two times with eight mice in each group.

2.5. Evaluation of disease activity index (DAI)

Body weight, stool consistency and gross bleeding were recorded daily. Disease activity index (DAI) was determined by combining scores of (i) body weight loss, (ii) stool consistency and (iii) gross bleeding, divided by 3. Each score was determined as follows: change in body weight loss (0: none, 1: 1–5%, 2: 5–10%, 3: 10–20%, 4: >20%), stool blood (0: negative, 1: +, 2: ++, 3: +++, 4: ++++) and stool consistency (0: normal, 1 and 2: loose stool, 3 and 4: diarrhea). Body weight loss was calculated as the percent difference between the original body weight (day 0) and the body weight on any particular day (Table 1). At the end of the experiment, mice were killed and the colons were separated from the proximal rectum, close to its passage under the pelvisternum. The colon length was measured between the ileo–cecal junction and the proximal rectum. The spleens were also obtained and their weight was measured.

2.6. Histopathology

The resected large intestine was grossly examined for the mucosal defect, hemorrhage, or ulcerative lesions, and then fixed immediately in 4% neutral formalin. For histopathological analysis, tissue sections were made from the representative region of large intestine by the conventional tissue preparation methods, and viewed under the light microscope (10–1000×) after hematoxylin and eosin (H&E) staining.