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Effect of Aqueous Extract of *Schizandra chinensis* and *Evodia rutaecarpa* Fruits on Experimental Mouse Colitis Induced by Dextran Sulfate Sodium

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ABSTRACT

Effect of Aqueous Extract of *Schizandra chinensis* and *Evodia rutaecarpa* Fruits on Experimental Mouse Colitis Induced by Dextran Sulfate Sodium

The aqueous extract of *Schisandra chinensis*, *Evodia rutaecarpa* and meal (SEM-Ex) has been traditionally used in the Oriental countries as an astringent. However, little is known about the effects of aqueous extract of SEM-Ex on dextran-sulfate sodium (DSS)-induced colitis in mice. In this study, we investigated the protective effects of SEM-Ex on DSS-induced colitis in mice. An experimental colitis was induced by daily treatment with 5% DSS. SEM-Ex was orally administered from day 2 of DSS treatment in the different dose (10-50 mg/kg body weight). SEM-Ex reduced significantly clinical sign of DSS-induced colitis, including body weight loss,

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shorten colon length, increased disease activity index (DAI), and histological colon injury. Moreover, SEM-Ex suppressed significantly not only the serum haptoglobin levels and the activities of myeloperoxidase (MPO), but also the colon tissue expression levels of monocyte chemoattractant protein-1 (MCP-1) in DSS-induced mice. In contrast, SEM-Ex increased significantly the colon tissue expression levels of granular colony stimulating factor (G-CSF) well known as anti-inflammatory cytokine. These results suggest that SEM-Ex administration could reduce significantly the clinical signs and regulate of chemokine and anti-inflammatory cytokine in DSS-induced model mice. Therefore, these properties may contribute to the strong anti-ulcerative colitis (UC) response effect of SEM-Ex.

Key word : *Schisandra chinensis, Evodia rutaecarpa,* ulcerative colitis, colon injury, dextran sulfate sodium

Ⅰ. Introduction

Ulcerative colitis (UC) is a worldwide, chronic, idiopathic, inflammatory bowel disease (IBD) of the colon characterized by rectal bleeding, diarrhea, abdominal pain, weight loss and fever[1]. Histological examination of biopsy specimens reveals the presence of infiltrated white blood cells such as neutrophils, monocytes and lymphocytes in the colonic interstitium[2]. In the past, this disease was thought to occur infrequently in the Asia Pacific region. However, new evidence is showing that IBD is on the rise in the region, including in Korea and China[3-5]. Although glucocorticoid and salicylazosulfapyridine have been mainly used for the treatment of this disease, their side effects remain a major clinical problem. Therefore, there is an increasing interest in using traditional Korean medicines as alternative therapy in addition to the conventional therapies that are used to treat UC[6].

Fruits of *Schisandra chinensis* have been traditionally used in China for the treatment of dyspnea, cough, mouth dryness, spontaneous diaphoresis, nocturnal diaphoresis, nocturnal emission, dysentery, insomnia and amnesia[7]. Chemical investigations on the seed extract of *S. chinensis* revealed the presence of lignans including schizandrin A, B and C, schizandrol A and B, schizandrer A and B, etc. These compounds have been found to have activities in suppressing lipid peroxidation[8] and in potentiating glutathione-mediated antioxidation[9,10]. The unripe fruits of *Evodia rutaecarpa* also have been used in Korea, Japan and China as a traditional herbal medicine. *E. rutaecarpa*, which contains quninzoline alkaloids, such as ruatecarpine and

evodiamine, as main components, is reported to have many biological properties including stimulation of vasodilation, positive cardiotonic effects, inhibition of pain, and inhibition of prostaglandin production[11-13] Nevertheless, there were no studies about effect of the aqueous extract from fruits of *S. chinensis* and *E. rutaecarpa* on experimental dextran-sulfate sodium -induced colitis in mice.

In this study, an experimental model of UC was established in BALB/c mice induced by dextran-sulfate sodium (DSS). The aqueous extract of *S. chinensis, E. rutaecarpa* and meal (SEM-Ex) on DSS-induced colitis in mice was evaluated using macroscopic, histological, and immunological assessments.

II. Materials and Methods

1. Chemicals and reagents

DSS (mol wt, 36,000-50,000) was purchased from MP biomedicals (France, LLC). Paraformaldehyde, hematoxylin and eosin were purchased from Sigma-Aldrich Chemical Co. (ST Louis, MO). Granulocyte colony-stimulating factor (G-CSF) and monocyte chemoattractant protein-1 (MCP-1) immunoassay kits (QuantikineTM) were purchased from R&D System (Minneapolis, MN). Ninety-six well tissue culture plate and other tissue culture reagents were purchased from Life Technologies (Gaithersburg, MD). Myeloperoxidase assay kit was from CytoStore (Calgary, Canada). Haptoglobin ELISA kit was purchased from Life Diagnostics Inc. (West Chester, PA). All other reagents were purchased from Sigma-Aldrich.

2. Plant materials

The fruits of *S. chinensis* and *E. rutaecarpa* were purchased from the herbal medicine cooperative association of Jeonbuk Province, Republic of Korea. A voucher specimen (No. SC 0777: *S. chinensis*; No. ER 0778: *E. rutaecarpa*) was deposited at the Herbarium of the College of Oriental Medicine, Wonkwang University (Republic of Korea).

3. Preparation of the extract

The dried fruits (*S. chinensis*: 250 g and *E. rutaecarpa*: 100 g) and rice (250 g) were extracted with distilled water (5,000 ml) at 100°C for 7 h. The extract was filtered through 0.45 μm filter, freeze-dried (yield, 25.5 g/600) and kept at -20°C. The dried extract was dissolved in phosphate buffered saline (PBS) and orally administrated using feeding needle.

4. Animals

Male BALB/c mice weighting 18-20 g (7 weeks old) were obtained from Central Lab. Animal Inc. (Seoul, Korea). The animals were housed in cages up to five mice each and acclimated for 1 wk under conditions of controlled temperature (20±2°C), humidity (55±10%), and lighting (7:00 AM - 7:00 PM). Sterilized diet pellets (Central Lab. Animal Inc., Seoul, Korea) and water were supplied *ad libitum* during acclimatization and experimental sessions.

5. Acute DSS colitis model and extract administration

Acute colitis was induced by providing water *ad libitum* containing 5% DSS for 7 days, as

previously reported[14]. Control group received water during all the experimental period. Colitis mice were randomized into 5 groups receiving SEM-Ex (10, 25 and 50 mg/kg body weight of mouse). SEM-Ex diluted with saline (200 μl) was orally administrated twice a day from day 2 of DSS treatment. Mice were checked daily for loss of body weight, stool consistency and the presence of gross bleeding. Mice finally were sacrificed at day 24 after DSS treatment, and their colons were excised to assess the intestinal manifestations.

6. Clinical evaluation of DSS-induced colitis

Disease progression was determined at crucial time points by assessment of disease activity index (DAI), which ranged from 0 to 10[14]. DAI score was calculated accordingly to previous reports[15]. DAI was obtained from score of tree major clinical sign such as weight loss, diarrhea, and rectal bleeding. Loss of body weight was calculated as the difference between the initial and actual weight. Diarrhea was defined by the absence of fecal pellet formation in the colon and the presence of continuos fluid fecal pellet formation in the colon. The appearance of rectal bleeding was separated as diarrhea containing visible blood and gross rectal bleeding and scored as described for diarrhea. DAI index = (weight loss score + diarrhea score + rectal bleeding score).

7. Histopathological evaluation

Colon from the ileocecal valve to the anus was removed, washed in ice-cold saline, fixed in 4% paraformaldehyde (pH 7.4), embedded in paraffin and stained with hematoxylin and eosin. All specimens were visualized under a microscope (Olymphus LK2, Japan). Histopathological score was assigned by two blinded pathologists. Histopathological score was calculated as sum of single scores (ranged from 0 to 15), taking into account crypt distortion, loss of muco-secretive capability, percentage of severe ulcers, amount of inflammatory infiltrate in the severe ulcers expressed as granulocytes/severe ulcer thickness ratio (G/U ratio), and extension of tissue damage. The amount of inflammatory infiltrate in severe ulcers (expressed as G/U ratio) was singularly considered and defined as inflammatory score ranging from 0 to 5.

8. Myeloperoxidase (MPO) activity

Colon was rinsed with chilled PBS and homogenized in 0·5% HTAB solution (1 ml per 50 mg of tissue) by the Polytron PT1200C (Kinematica AG, Littau/Luzern, Swizerland). The homogenate was centrifuged at 11 000 g for 15 min at 4°C and supernatant was stored at -80°C until assayed. The MPO activity was determined using a myeloperoxidase assay kit according to the manufacturer''s instructions.

9. Haptoglobin concentration in mouse serum

The haptoglobin concentrations in sera were determined as previously described[16]. Haptoglobin concentration in mouse serum blood was collected from mice via the abdominal aorta under anaesthesia. The haptoglobin concentrations in the sera were determined by mouse haptoglobin ELISA kit according to the manufacturer''s protocol.

10. Cytokine and chemokine assay in colon tissue

To examine chemokine concentrations, colon

was rinsed with chilled PBS and homogenized (1 ml per 50 mg of tissue). After centrifugation, the supernatant was assayed for granular colony stimulating factor (G-CSF) and monocyte chemoattractant protein-1 (MCP-1) (Quantikine ELISA kits; R&D System).

11. Statistical analysis

All values are expressed as the mean S.D. of three independent determinations. Statistical analysis was performed with analysis of variance (ANOVA) and Student's *t*-test. A confidence level ($P<0.05$) was considered significant.

III. Results

1. Effect of SEM-Ex on the clinical signs in DSS-induced colitis

We first assessed the clinical signs in DSS-induced acute colitis mice that were administered orally with or without SEM-Ex (10, 25, and 50 mg/kg body weight). Untreated mice were also analysed as controls. We observed that groups administrated with SEM-Ex showed the signification attenuation of body loss (Fig. 1A) and colon shortening (Fig. 2B) caused by DSS treatment in a dose-dependent manner. In addition, we demonstrated clearly that groups administrated with SEM-Ex showed the inhibition of DAI (Fig. 2A) and the increase in survival rate (Fig. 1B). These results indicated that SEM-Ex could inhibit effectively the symptoms of colitis caused by DSS.

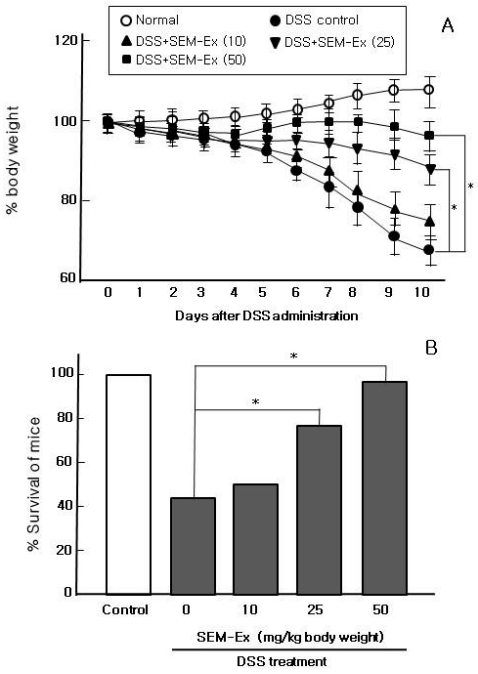

Fig. 1. Effect of SEM-Ex on body weight and survival rate in DSS-induced colitis mice.

Mice were given 5% DSS for 7 days. At day 2 of DSS treatment, SEM-Ex was administrated orally at various doses(10, 25 and 50 mg/kg). (A) Body weight (BW) was measured daily and means ± SD of percent BW are plotted. (B) Survival rates of the mice are shown. $*p < 0.05$ on day 10 indicate significant differences with SEM-Ex untreated control group (DSS alone).

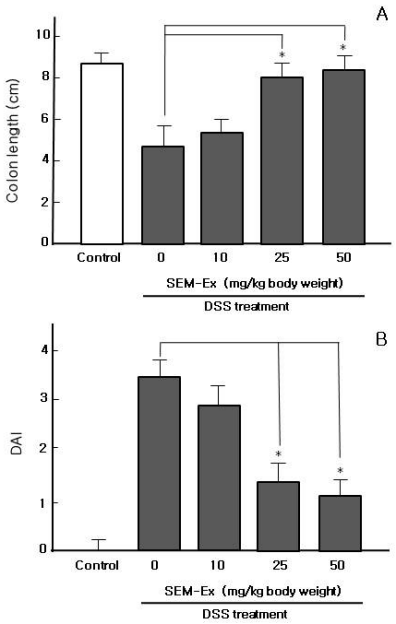

Fig. 2. Effect of SEM-Ex on colon length and Disease activity index (DAI) rate in DSS-induced colitis mice.

Mice were given 5% DSS for 7 days. At day 2 of DSS treatment, SEM-Ex was administrated orally at various doses(10, 25 and 50 mg/kg). Colon length (A) and DIA (B) was measured on day 10. Each column represents the mean ± S.D. from 10 mice. $*p < 0.05$ on day 10 indicate significant differences with SEM-Ex untreated control group (DSS alone).

2. Effect of SEM-Ex on the histological damages in colon tissue of DSS-induced colitis mice

We next evaluated the inhibitory effect of SEM-Ex on the histological damages of colon resultant from DSS treatment. As shown in Fig. 3, there was a typical lesion of colon in DSS-treated group manifested by multifocal areas, mucosal erosion, a loss of epitherial and goblet cells, the shortening and collapse of crypts, and submucosal edema. SEM-Ex administration reduced remarkably lesions of colon in DSS-induced colitis in a dose-dependant manner (Fig. 3). Especially, the protective and healing effects of SEM-Ex on the colon damages were more prominent at over 25 mg/kg. These result indicate that SEM-Ex might inhibit effectively the inflammation-related damage of colon in this model.

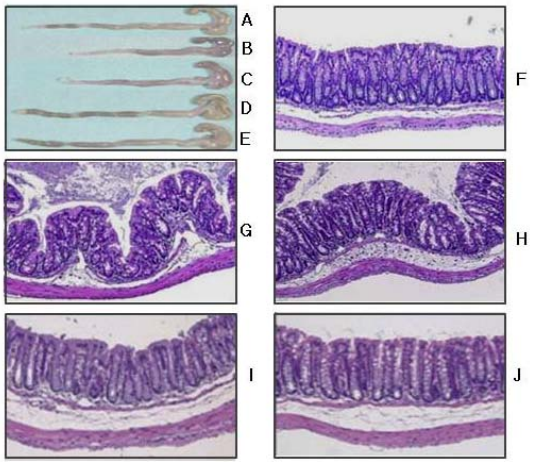

Fig. 3. Macroscopic and microscopic findings of SEM-Ex effect on the histological damages in colon tissue of DSS-induced colitis mice.

Mice were administered with or without dextran sodium sulphate (DSS) for 7 days. At day 2 of DSS treatment, SEM-Ex was administrated orally at various doses(10, 25 and 50 mg/kg). Shown are the representative gross appearance (a: normal; b: DSS alone; c: DSS plus 10 mg/SEM-Ex; d: DSS plus 25 mg/SEM-Ex; e: DSS plus 50 mg/SEM-Ex) and microscopic views of haematoxylin and eosin-stained sections (f: normal; g: DSS alone; h: DSS plus 10 mg/SEM-Ex; i: DSS plus 25 mg/SEM-Ex; j: DSS plus 50 mg/SEM-Ex) of the colons. Original magnification in ×100.

3. Effect of SEM-Ex on serum haptoglobin level in DSS-induced colitis

We then measured the effect of SEM-Ex on serum haptoglobin levels, which is a typical

acute-phase protein with a diagnostic significance for the progression of systemic inflammation[17]. As shown in Fig. 4A, the haptoglobin was almost undetectable in the sera of SEM-Ex alone treated mice without DSS, while the serum concentration of the protein was remarkably high in mice that received DSS administrations. However, SEM-Ex administrated groups significantly inhibited the serum haptoglobin levels in a dose-dependent manner (Fig. 4A). These results indicated that SEM-Ex could inhibit effectively the systemic inflammation of colitis caused by DSS.

4. Effect of SEM-Ex on MPO activity in DSS-induced colitis

Because MPO is a useful indicator of the extent of neutrophil infiltration, the colon was homogenized and MPO activity in the supernatant was measured. As shown in Fig. 4B, mice treated with DSS showed drastically higher MPO activity compared with the mice treated with SEM-Ex alone, consistent with the severe intestinal inflammation in these animals. In contrast, SEM-Ex administrated groups reduced significantly the MPO activity elevated by DSS treatment in a dose-dependent manner. These results clearly support the evidence that SEM-Ex inhibited the infiltration and activation of inflammatory cells in this model.

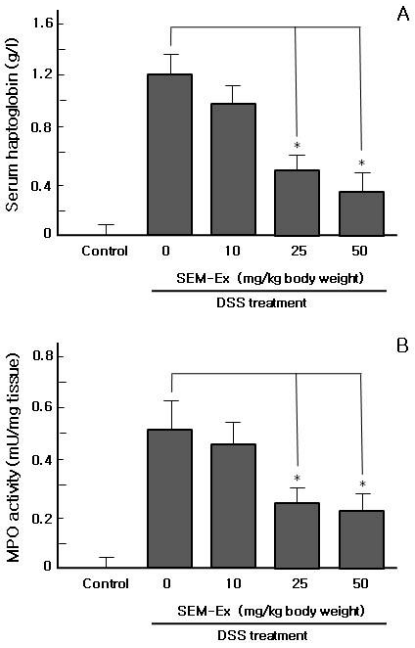

Fig. 4. Effect of SEM-Ex on serum haptoglobin level and myeloperoxidase (MPO) activity in DSS-induced colitis mice.

Mice were given 5% DSS for 7 days. At day 2 of DSS treatment, SEM-Ex was administrated orally at various doses(10, 25 and 50 mg/kg). Serum haptoglobin levels (a) were determined on day 7. Colon length (A) and DIA (B) was measured on day 10. Each column represents the mean ± S.D. from 10 mice. $*p < 0.05$ on day 10 indicate significant differences with SEM-Ex untreated control group (DSS alone). The colons were homogenized and MPO activities in the lysates were measured as described in Materials and methods. Each column represents the mean ± S.D. from 10 mice. $*p < 0.05$ on day 10 indicate significant differences with SEM-Ex untreated control group (DSS alone).

5. Effect of SEM-Ex on MCP-1 and G-CSF expression levels in DSS-induced colitis

To determine whether SEM-Ex could inhibit the production of inflammatory cytokine and chemokine, we investigated the expression profiles of the chemokine MCP-1 and the cytokine

G-CSF using ELISA methods in the homogenized colon supernatant of DSS-treated mice. As shown in Fig. 6, mice treated with DSS showed drastically higher MCP-1 expression compared with control mice. But SEM-Ex administrated groups reduced significantly the MCP-1 (Fig. 5A) expression elevated by DSS treatment in a dose-dependent manner. In contrast, SEM-Ex administrated groups increased significantly the G-CSF (Fig.5B) expression compared with control and DSS treated mice. These results strongly support the evidence that SEM-Ex inhibited the expression of MCP-1 and increased level of G-CSF for homeostasis from damage by DSS.

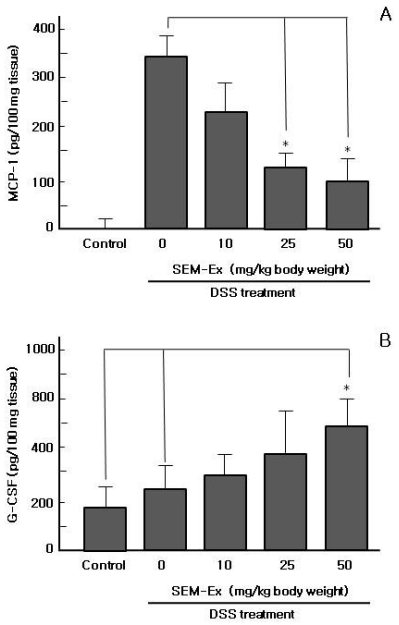

Fig. 5. Effect of SEM-Ex on serum haptoglobin level and myeloperoxidase (MPO) activity in DSS-induced colitis mice.

Mice were given 5% DSS for 7 days. At day 2 of DSS treatment, SEM-Ex was administrated orally at various doses(10, 25 and 50 mg/kg). The colons were homogenized on day 7 and the concentrations of G-CSF (a) and MCP-1 (b) levels in the lysates were measured by enzyme-linked immunosorbent assay (ELISA). Each column represents the mean ± S.D. from 10 mice. $*p < 0.05$ on day 10 indicate significant differences with SEM-Ex untreated control group (DSS alone).

IV. Discussion

UC is a chronic relapsing inflammatory disease that is primarily driven by an underlying inflammatory response leading to the pathophysiological manifestations of the disease[1-3]. DSS treatment of mice promotes a chronic experimental UC, which possesses certain pathophysiological features of UC[15-18]. These features include extensive ulceration of the epithelial layer, massive bowel wall edema, fibrotic thickening of the mucosa, and a dense cellular infiltrate characterized by eosinophils[17,18]. Studies in DSS-induced colitis mice have demonstrated that local release of pro-inflammatory cytokines or chemokines is critical for the generation of UC reaction[16-19].

In this study, 5% DSS in drinking water was administrated for 7 d to induce acute colitis in mice. All DSS-treated mice showed numerous clinical symptoms such as body weight loss, diarrhea, colon shortening and DIA of UC because of damage to the digestive system. In SEM-Ex groups, mice showed a steady increase in body weight throughout the experiment. In comparison, mice exposed to DSS had a lower rate of body weight increase followed by a dramatic decrease from day 6 onwards. Treatment of colitic mice with SEM-Ex showed a less severe weight loss from day 6 to day 10. In DSS-treated group, diarrhea and rectal bleeding

occurred in day 2 and day 3, respectively. The SEM-Ex administration delayed the occurrence of both symptoms, with less severity. Colon shortening is always found in UC patients, which can act as an indirect marker of colonic inflammation. SEM-Ex treatment was similar to the DSS-untreated control group in colon length on day 10 (Fig. 2a). The data suggested that SEM-Ex treatment could induce a decrease in the extent of colitis accompanied by reducing the severity and delaying the occurrence of the associated clinical symptoms.

The current studies have reported that the production of pro-inflammatory cytokines and chemokines by leukocyte infiltration and activation was involved in the initiation of inflammatory response in colitis. In particular, MCP-1 is produced by a variety of cells including dendritic cells, macrophages, endothelial cells, and fibroblasts, and its expression is upregulated after exposure to inflammatory stimuli such as IL-1 and TNF-α[20]. MCP-1 was originally identified as a monocyte -specific chemoattractant but was later shown to act on T cells, mast cells, basophils, and natural killer cells[21,22]. Elevation of MCP-1 is observed in mucosal tissues from patients with CD and UC[23,24] and also in experimental models of colitis[25,26]. Additionally, an imbalance of mucosal chemokines and anti-inflammatory cytokines is crucial in the pathogenesis of inflammatory bowel disease (IBD)[27]. GM-CSF influences the development of hemopoietic cells in DSS-treated mouse model (Ref is required!). The present study demonstrates that G-CSF production elicited by SEM-Ex administration leads to reduced severity of acute DSS colitis by multiple disease parameters (Fig. 6b). These results strongly support that SEM-Ex regulated the expression levels of MCP-1 and G-CSF for homeostasis in the model.

Fruits of *S. chinensis* and *E. rutaecarpa* have been traditionally used in the Oriental countries as an astringent. Studies have shown that Fruits of *S. chinensis* comprised dibenzocyclooctadiene lignans including schizandrin A, B and C, schizandrol A and B, schizandrer A and B[8,9], that these compound significantly reduce the CCl_4-induced hepatotoxicity[28] and the *L*-glutamate -induced neurotoxicity[29] through the inhibition in increase of intracellular Ca^{2+} and the formation of cellular peroxide. Additionally, *E. rutaecarpa*, which contains quninzoline alkaloids is reported to have many biological properties including stimulation of vasodilation, positive cardiotonic effects, inhibition of pain, and inhibition of prostaglandin production[11-13]. However, there were no studies about the potential effect of the aqueous extract from fruits of *S. chinensis* and *E. rutaecarpa* on experimental DSS-induced colitis in mice. Our study is the first *in vivo* report for the elevation of pharmacological effects of SEM-Ex (*S. chinensis* and *E. rutaecarpa* plus meal). We thus considered the protective effect of SEM-Ex on the DSS-induced colon damage. Our data showed that oral administration of SEM-Ex significantly reduced the clinical symptoms (DIA, colon tissue MPO and serum hepatoglobin) and the histological change of colon tissue in DSS-induced mice. However, although cellular mechanism that SEM-Ex led to suppression of DSS-induced colitis is not clearly understood, it is possible that antioxidant activity of SEM-Ex contributes to its therapeutic effects on the pathogenesis of DSS-induced colitis. Natural products that improve the antioxidant defense

systems might offer a useful therapeutic choice in treatment of degenerative disorders caused by oxidative stress[30].

In conclusion, we demonstrated that SEM-Ex administration could reduce significantly the clinical signs and regulate the production of chemokine and anti-inflammatory cytokine in DSS-induced model mice. Therefore, Our data suggest that the SEM-Ex may be a useful therapeutic candidate for ulcerative colitis. However, the further studies must be performed to elucidate the precise mechanism of action of SEM-Ex for the treatment of UC.

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