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Protective Effect of *Atractylodes macrocephala* and *Taraxacum* spp. Combination Treatment in Balb/c Mice with Dextran Sulfate Sodium-Induced Ulcerative Colitis

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ABSTRACT

Objectives : This study aimed to investigate the protective effects of an herbal mixture of *Atractylodes macrocephala* and *Taraxacum* spp. (ATC) on ulcerative colitis. We have previously screened traditional medicinal herbs to discover the effective candidate by the animal model. *A. macrocephala* and *T.* spp were identified as one of the effective herbs in the screening process.

Methods : Experimental colitis was induced in male Balb/c mice by administering drinking water containing dextran sulfate sodium, which mimics the clinical and histological features of ulcerative colitis in human. ATC at doses of 30, 100 or 300 ㎎/㎏ were orally administered to mice twice per day for 10 consecutive days. To evaluate the damage from experimentla ulcerative colitis, body weight, colon length, disease activity index, myeloperoxidase and histological changes were measured and analyzed.

Results : The administration of dextran sulfate sodium with drinking water resulted in markedly reduced colon length, severe body weight loss, increased levels of myeloperoxidase activity and histological damages in mice. ATC treatment significantly ameliorated the colon shortening, histological damage, body weight loss and disease activity index score in a dose-dependent manner. ATC also attenuated the colonic myeloperoxidase activity which reflects the severity and extent of inflammatory damage of colon.

Conclusions : ATC exerts protective effects against inflammatory colonic structural damage induced by epithelial barrier integrity impairment. ATC also inhibits weight loss and related symptoms of UC which can be considered as the functional recovery of colon.

Key words : *Atractylodes macrocephala*, *Taraxacum* spp., ulcerative colitis

Ⅰ. Introduction

Ulcerative colitis (UC) is the major form of chronic inflammatory bowel disease, with a high cancer risk and is characterized by fever, fatigue, abdominal pain, diarrhoea, rectal bleeding and body weight loss[1]. Although the prevalence and incidence of UC have reached a plateau in Europe and North America, they continue to increase in countries adopting a Western lifestyle[2]. The most widely held hypothesis about the pathogenesis of UC involves excessive immune responses against intestinal bacteria, leading to damage of the epithelial barrier via abnormal pro-inflammatory signals[3]. It is commonly accepted that the overexpression of inflammatory markers, such as interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and myeloperoxidase (MPO), plays a crucial role in the pathogenesis of UC[4-5].

Conventional treatments for UC include corticosteroids and aminosalicylates as the mainstay of therapy. Immunosuppressive agents, such as methotrexate or

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azathioprine, are used for steroid-dependent or -resistant patients[6]. Aminosalicylates are well tolerated but cramps, diarrhoea and abdominal pain are occasional side effects and are accompanied by liver or kidney problems. Corticosteroids have well-known side effects including facial rounding, acne, diabetes and high blood pressure[7]; hence, UC patients are refractory to these drugs or are unable to use current drugs for prolonged periods owing to the side effects. Therefore, there is a pressing need for developing effective and safe therapeutic drugs for UC.

ATC is an herbal complex of *Atractylodes macrocephala* rhizome and *Taraxacum* spp. herb. *A. macrocephala* is a medicinal herb with a long history of use for conditions including gastritis, diarrhoea, indigestion, abdominal pain and fatigue[8]. *A. macrocephala* exerts anti-inflammatory, anti-oxidant, anti-tumour, hepatoprotective and gastric protective effects[9-11]. The major components of *A. macrocephala* are volatile oils, sesquiterpenoids, polysaccharides and amino acids, and they are responsible for its diverse biological activities[12]. The plants of the genus *Taraxacum*, namely dandelions, have long been used as medicinal herbs for various diseases such as dyspepsia, heart burn, spleen and liver complaints and anorexia[13]. These herbs are a good source of vitamins, minerals and oligoelements[14]. *T.* spp. and its congeners have been reported to exhibit various activities, including anti-microbial, hepatoprotective, anti-inflammatory and anti-oxidative activities. In particular, *A. macrocephala* extracts are reported to downregulate IL-6 and IL-17 in a TNBS-induced colitis rat model[15]. *T.* spp. contains inulin, which is reported to be an effective prebiotic for dextran sodium sulfate-induced experimental colitis[16]. However, protective effects of this combination treatment against UC have not yet been studied.

In the present study, we assessed the protective effects of ATC on DSS-induced ulcerative colitis in male Balb/c mice. Many symptoms of DSS-experimental model are similar to those observed in human UC, for example diarrhoea, bloody faeces, body weight loss and shortening of the colon[17-18]. The mechanism by which DSS causes colitis is a direct toxic effect on the intestinal epithelium that allows bacteria to penetrate the inner mucus layer and cause ulceration in the colon[19-20]. The effect of ATC was evaluated by body weight loss, colon length, disease activity index (DAI) score, myeloperoxidase (MPO) activity and histological score.

Ⅱ. Materials and Methods

1. Materials

The dried root of *A. macrocephala* and dried whole plant of *T.* spp. were purchased from Daewoo Hanyak Co. (Seoul, Korea). They were identified by Professor Hocheol Kim and voucher specimens (No. 12112306 and No. 12112304) were deposited in Department of Herbal Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul, Korea.

2. Sample preparation

The dried root of *A. macrocephala* and dried whole plant of *T.* spp. were extracted separately with distilled water for 3 h at 100℃ in a reflux apparatus. The extracts were filtered and concentrated under reduced pressure and samples were lyophilized to yield powders. The yields of individual extracts were 38.8% and 17.0%, respectively. The powders were then mixed in a ratio of 1:1.

3. HPLC analysis

Quantitative authentication of ATC was performed with a high-performance liquid chromatography (HPLC) system equipped with a Waters 1525 pump, a 2707 autosampler and 2998 PDA detector. Chromatographic separation was achieved at 35℃ on a Waters Sunfire C18 (4.6 ㎜ × 250 ㎜ i.d., 5 ㎛ particle size) column. The mobile phase consisted of 0.5% phosphoric acid (A) and acetonitrile (B) eluted by the following program for separation: 0-5 min, 10%; 5-10 min, 10%-20%; 10-15 min, 20%-20%; 15-20 min, 20%-50%; 20-30 min, 50%-50%; 30-35 min, 50%-35%; 35-45 min, 35%-35%; 45-50 min, 35%-10% solvent B. The flow rate was 1 ㎖/min. The injection volume was 10 ㎕ and the eluate was monitored at 220 ㎚ and 348 ㎚ for atractylenolide III and luteolin glucoside, respectively. *T.* spp. and *A. macrocephala* extracts were monitored at 348 ㎚ for luteolin glucoside and 220 ㎚ for atractylenolide III. The concentrations of luteolin glucoside and atractylenolide III were 165.6 and 56.1 ㎍/g, respectively (Fig. 1).

4. Animals

Male Balb/c mice (7 weeks old, 20-24 g) were supplied by Samtako. Mice were housed at 20-22℃ under a 12 h light-12 h dark cycle and were provided food and water *ad libitum*. All procedures were conducted according to the animal welfare guidelines of the National Institute of Health (KISTEM-IACUC-2013-001).

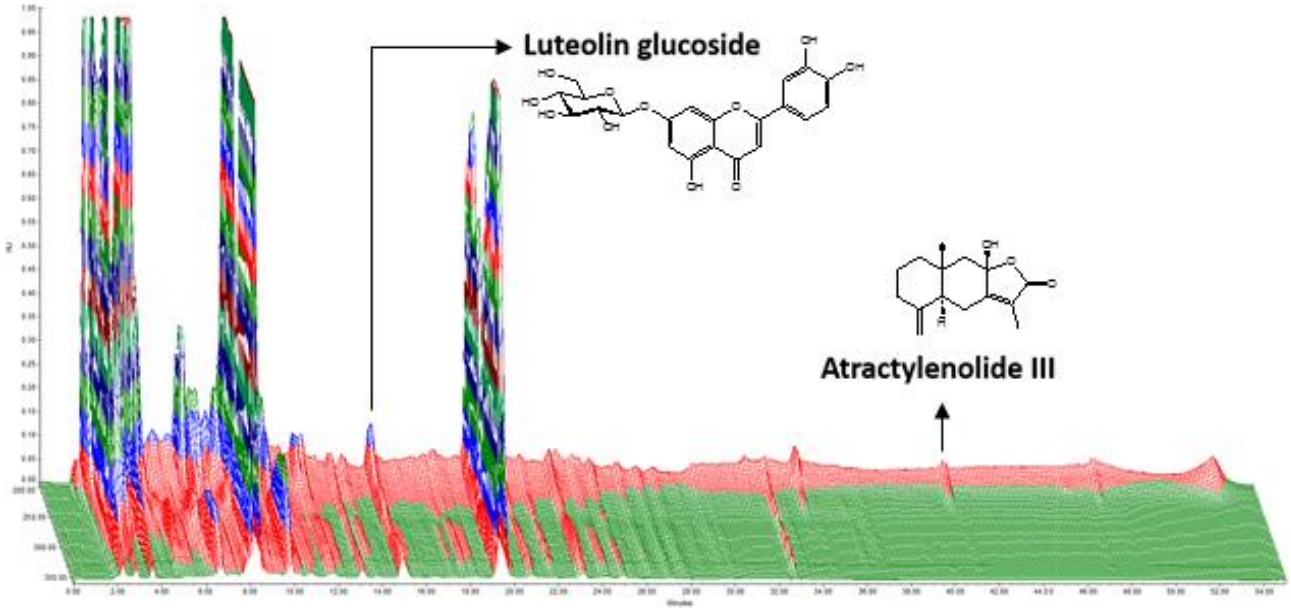

Figure 1. Three-dimensional high-performance liquid chromatogram of ATC. X-axis shows retention time; Y-axis shows wavelength and Z-axis shows absorbance units. Analytical conditions were as follows: column, C_{18}; mobilephase, solvent A(0.5%H_3PO_4) and solvent B(CH_3CN); flowrate, 1 mℓ/min.

5. Induction of colitis and experimental design

Colitis was induced by DSS administration. Mice were provided with drinking water containing 5% DSS (MP Biomedicals) *ad libitum* from day 4 to day 10 for 7 days. To investigate the effects of ATC, mice were randomly allocated into following groups: normal, DSS treated control and DSS treated with ATC (30, 100 and 300 ㎎/㎏). Mice were administered distilled water (10 mℓ/㎏) in the DSS group or each dose of ATC in the DSS + ATC group via feeding needle twice per day from days 1 to 10 for 10 days. The animals were sacrificed on day 10 and tissue samples were collected for additional observations.

6. Assessment of body weight and colon length

Body weight was measured daily from day 1 to 10. Colon was isolated promptly after the last check of body weight. Colon length was measured from anus to caecum using a ruler.

7. Assessment of disease activity index (DAI) score

DAI score was recorded by an investigator blinded to treatment groups, as follows: spontaneous behaviour and posture (0, motionless with hunching [+++]; 1, motion but lethargic with hunching [++]; 2, walking with hunching [+]; 3, running with hunching [−]; 4, running), piloerection (0, piloerection [+++]; 1, piloerection [++]; 2, piloerection [+]; 3, piloerection [−]; 4, normal state [no piloerection]), cleanliness of anal orifice (0, with watery diarrhoea [+++]; anal prolapsed and bleeding [+++]; 1, with loose faeces and bleeding [++]; 2, with slightly loose faeces and bleeding [+]; 3, with faeces and bleeding [−]; 4, healthy state) and edema (0, edema [+++, >0.35 mm in colon thickness]; 1, edema [++, 0.3-0.35 mm]; 2, edema [+, 0.25-0.30 mm]; 3, edema [±, 0.2-0.25 mm]; 4, normal [0.1-0.2 mm]). The DAI score was calculated as the average point score for each test.

8. Assessment of myeloperoxidase (MPO) activity

Colonic tissue specimens (50 mg) were thawed and homogenized on ice in 50 mM potassium phosphate buffer pH 6.5 with 0.5% (w/v) hexadecyltrimethylammonium bromide (HETAB) (Sigma) using a homogenizer (50 mg tissue to 1 mL of buffer). The homogenized samples were then frozen and thawed, sonicated three times and centrifuged at 20,000 × *g* for 30 min at 4℃. Supernatant (10 ㎕) was added to 190 ㎕ phosphate buffer (pH 6.5) containing O-dianisidine (Sigma) (0.167 ㎎/mℓ) and 0.0005% (v/v) H_2O_2 in 96-well plates. The reaction was terminated after 3 min by addition of 20 ㎍/mℓ catalase and 0.2 M sodium acetate. Absorbance was measured at 470 nm using a microplate reader.

$$Unit = \frac{\Delta A}{\Delta t} * \frac{1}{\varepsilon} * V_f * \frac{10^6 \mu M}{M} * 1 \quad c$$

ε: Mallextinction coefficient ($M^{-1}cm^{-1}$)O-dianisidine=7.5
V_f: finalvolume(L) A: absorbance t: time (min)

9. Assessment of histological score

Colons were fixed in 10% buffered paraformaldehyde and embedded in paraffin. Histological sections cut from the paraffin blocks were stained with haematoxylin and eosin (H&E). The scoring of histological damage was divided into three parameters in a blind fashion: severity of inflammation, crypt damage and ulceration. Severity of inflammation was graded on a scale of 0-3 (0, rare; 1, mild; 2, moderate; 3, severe), obtained from each layer of the colon, including surface epithelium, cryptal glands, stroma, submucosa and transmural layer. The crypt damage was graded on a scale of 0-5 (0, none; 1, loss of the basal one-third; 2, loss of the basal two-thirds; 3, entire crypt loss; 4, change in epithelial surface with erosion; 5, confluent erosion). Severity of ulceration was graded histologically on a scale of 0-3 (0, none; 1, 1 or 2 foci of ulceration; 2, 3 or 4 foci of ulceration; 3, confluent or extensive ulceration). The overall score was calculated as the sum of all scores.

10. Statistical analysis

All results were expressed as mean values with their standard errors for each group. Data were analysed statistically using Student's *t* test. $p < 0.05$ was considered statistically significant.

Ⅲ. Results

1. Effects of ATC on weight loss in DSS-induced colitis

Body weight changes in the experimental groups are shown in Fig. 2. Compared with the sham group, which showed slow weight gain, the control group showed significant weight reduction beginning on day 8. This reduction in body weight continued for the remaining days. ATC treatment significantly inhibited weight loss compared to the control group at all three doses, with maximal effect at a dose of 100 ㎎/㎏ at day 10 ($p < 0.05$, 30 ㎎/㎏ group; $p < 0.001$, 100 ㎎/㎏ group; $p < 0.01$, 300 ㎎/㎏ group).

2. Effects of ATC on colon shortening in DSS-induced colitis

To determine whether ATC has a beneficial effect on DSS-induced colon shortening, colon length was measured. As shown in Fig. 3, DSS significantly reduced colon length in the DSS-treated control group (5.9 ± 0.1 ㎝), compared with that in the normal sham group (7.8 ± 0.1 ㎝). When mice were given ATC orally for 10 days, there was a dose-dependent inhibition of colon shortening due to DSS that reached maximal levels at 300 ㎎/㎏ (6.4 ± 0.1 ㎝, $p < 0.01$).

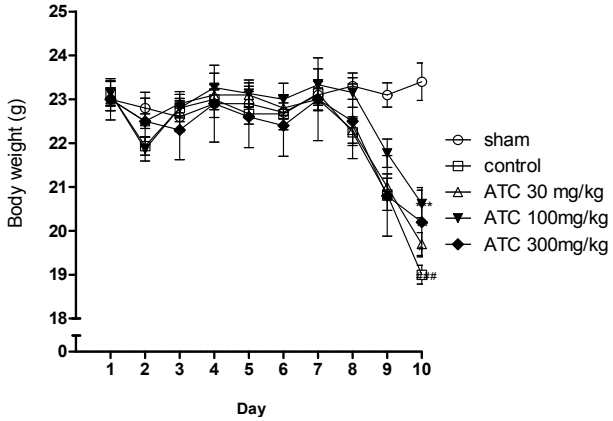

Figure 2. Effects of ATC on body weight in DSS-induced colitis. Each group comprised 10-15 mice. Values are means with their standard errors represented by vertical bars. ###, $p < 0.001$ compared with sham group; *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$ compared with control group.

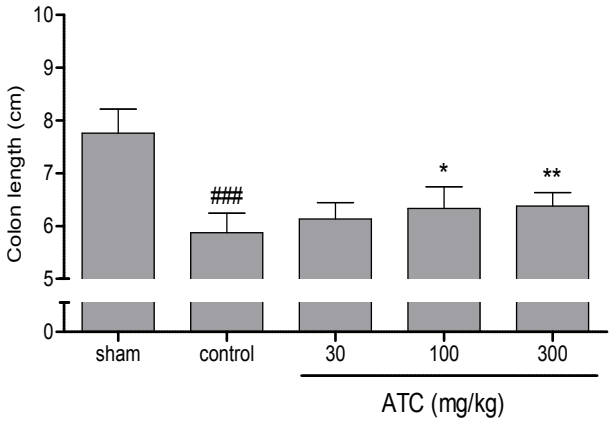

Figure 3. Effects of ATC on colon shortening in DSS-induced colitis. ###, $p < 0.001$ compared with sham group; *, $p < 0.05$ and **, $p < 0.01$ compared with control group.

3. Effects of ATC on DAI score in DSS-induced colitis

To determine whether the protective effects of ATC against DSS-induced colitis were associated with any functional recovery, the DAI score, a composite score reflecting clinical signs of the disease including spontaneous behaviour, posture, rectal bleeding, diarrhoea and piloerection, was measured at day 10. Compared with the control group (2.9 ± 0.3), ATC 30, 100 and 300 ㎎/㎏ treatment significantly improved DAI scoring in a dose-dependent manner by 10.3% (3.2 ± 0.2 score, $p < 0.05$), 20.7% (3.5 ± 0.3 score, $p < 0.001$) and 27.6% (3.7 ± 0.3 score, $p < 0.001$), respectively.

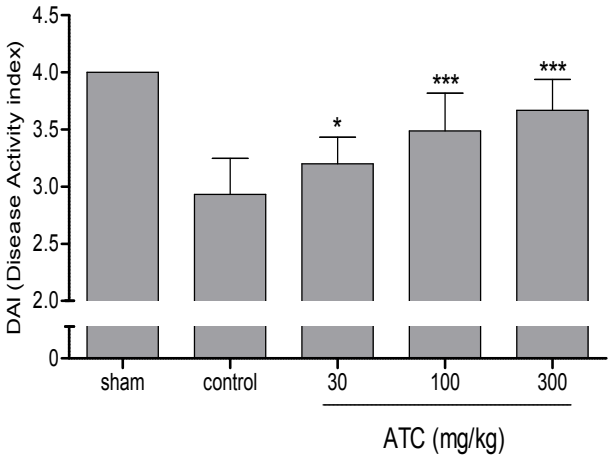

Figure 4. Effects of ATC on DAI scores in DSS-induced colitis. *, p < 0.05; **, p < 0.01 and ***, p < 0.001 compared with control group

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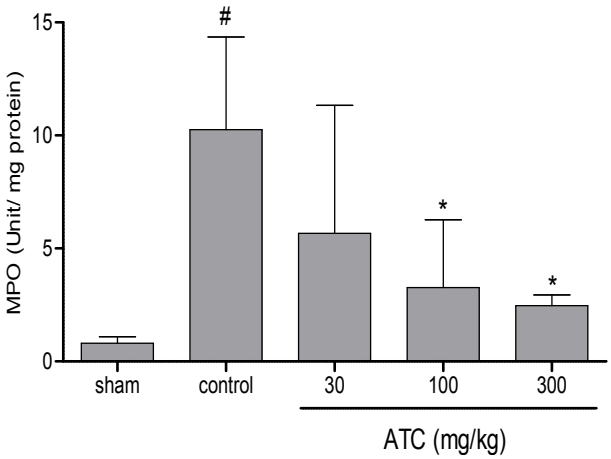
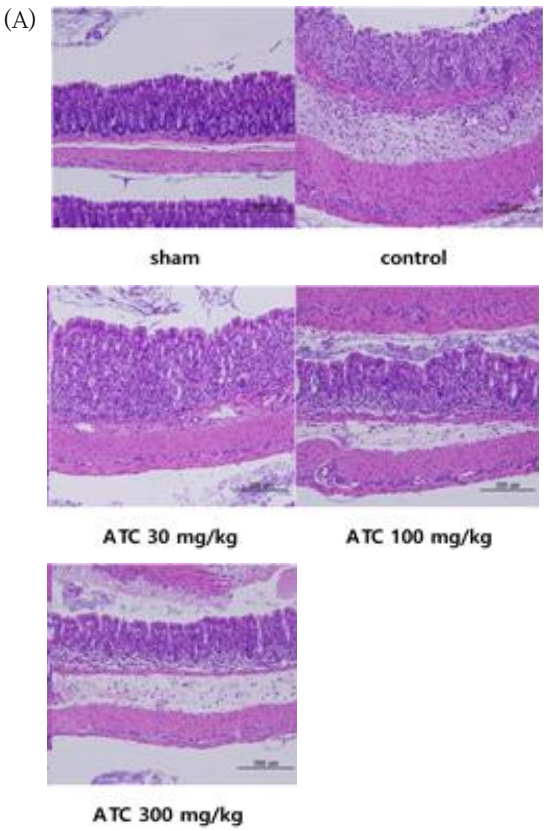


Figure 5. Effects of ATC on MPO scores in DSS-induced colitis. #, p < 0.05 compared with sham group; *, p < 0.05 compared with control group.

5. Effects of ATC on histological damage to colonic mucosa in DSS-induced colitis

Pathological examinations of colons were performed after H&E staining and representative results are shown in Fig. 6. Administration of DSS resulted in marked histopathological changes in the colon appearing as focal loss of surface epithelium, disruption of the cryptal glands and infiltration of the inflammatory cells. However, treatment with ATC attenuated DSS-induced histopathological changes in the colon. ATC-treated groups showed protective effects against histological damage of the colonic mucosal layer induced by DSS. The histological damage observed in the ATC 30 mg/kg group was similar to that in the control group. Treatment with ATC at 100 or 300 mg/kg reduced infiltration of the inflammatory cells and showed more intact cryptal glands and surface epithelium than those of the control group (Fig. 6A).

The histological score of the control group was significantly increased compared to the sham group (0.7 ± 0.6 vs. 8.0 ± 0.8, $p < 0.001$). Compared with the control group, the ATC 30, 100 and 300 mg/kg treatments significantly reduced the histological score in a dose-dependent manner by 12.5% (7.0 ± 1.0, $p = 0.2031$), 27.5% (5.8 ± 0.8, $p < 0.01$) and 31.3% (5.5 ± 0.6, $p < 0.01$), respectively (Fig. 6B)



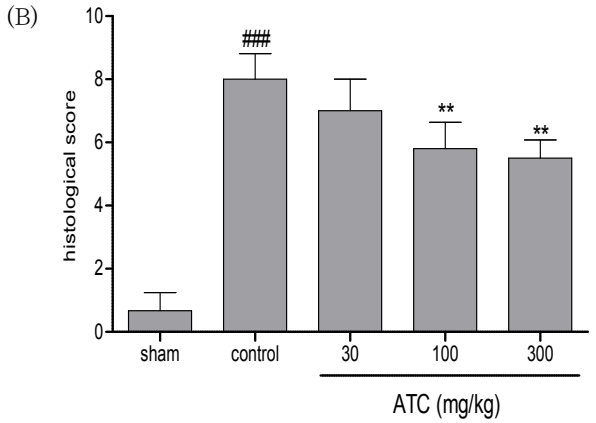


Figure 6. Effects of ATC on histological characterization in DSS-induced mouse colitis.
(A) Representative H&E-stained histological sections.
(B) Overall histological scores in these different groups.
###, $p < 0.001$ compared with sham group; **, $p < 0.01$ compared with control group.

IV. Discussion

Administration of 5% DSS in drinking water for 7 days to BALB/c mice resulted in marked reduction in colon length, weight loss, increased levels of MPO activity and histological damage mimicking the clinical and histological signs of UC. ATC treatment ameliorated colon shortening, weight loss and DAI score after DSS administration in mice. ATC also attenuated colonic MPO activity and histological damages induced by DSS.

Oral administration of ATC significantly inhibited colon shortening in DSS-treated mice in a dose-dependent manner, with maximal effect at a dose of 300 ㎎/㎏. DSS administration induces inflammatory structural changes of colon, initiated by direct damage to gut epithelial cells of basal crypts and subsequent damage of the mucosal barrier integrity, which mimics the effects of UC[21]. At the damage site, inflammatory cytokines including IL-6 and TNF-α are produced by intestinal bacteria, leading to the inflammation of colon mucosa, ulceration and consequently shortening of the colon[19]. It is generally accepted that colon length is inversely correlated with the severity of colonic structural damage induced by DSS[22]. The histological findings in this study showed that DSS administration destroyed the integrity of the mucosal barrier, leading to the disruption of the intestinal epithelial layer, mucosal and submucosal ulceration and infiltration of inflammatory cells that mimics symptoms of UC. Treatment with ATC inhibited the loss of cryptal glands, epithelial damage, infiltration of the inflammatory cells and shortening of the colon. This result suggests that ATC has protective effects against inflammatory structural damage induced by UC.

Treatment with ATC at doses of 100 and 300 ㎎/㎏ significantly reduced MPO activity. In the DSS-induced colitis model, DSS leads to a decrease in mucus barrier thickness and integrity. This damaged mucus barrier allows luminal antigens to migrate into the submucosa. We found that an elevated MPO level was correlated with the development of colonic inflammation and that administration of ATC significantly suppressed MPO accumulation in the colonic tissues of mice. MPO, a member of the haem peroxidase-cyclooxygenases, constitutes the major component of azurophilic granules in neutrophils and MPO activity reflects the degree of neutrophil infiltration, a marker of acute inflammation[23]. The reduced MPO activity in this study suggests that ATC inhibits neutrophil infiltration into the colonic mucosa and protects colonic tissue from damage induced by ulcerative colitis.

In the DSS-induced colitis model, neutrophils infiltrate into the intestinal epithelial cells and expression of TNF-α, IL-1β, IL-6, IL-10 and IL-12 is increased, leading to chronic inflammation [24]. Atractylenolide III, a sesquiterpenoid from *A. macrocephala*, exerts an anti-inflammatory effect by blocking the IL-6 secretion pathway[25]. Taraxasterol, a pentacylic triterpene from *T.* spp., exerts an anti-inflammatory response by suppressing the production of TNF-, IFN-g, IL-1b, IL-6 and NO, and eudesmanolides from *T.* spp. can inhibit the production of NO[26-27]. In a previous study, orally fed inulin, a compound of dandelion, showed anti-inflammatory potential as evidenced by lower tissue MPO fewer mucosal lesions and increased counts of lactobacilli[28]. Anti-inflammatory effects of compounds from *T.* spp. and *A. macrocephala* may contribute to the mechanism of the protective effect of ATC against ulcerative colitis.

DSS administration not only leads to histological damage in the colon but also results in colonic functional disorder. DSS-induced inflammatory structural changes lead to colonic functional disorders such as diarrhoea, blood loss and poor intestinal absorption capacity, which are necessarily accompanied by weight loss[29-31]. Consequently, weight loss is considered one of the major systemic symptoms induced by DSS administration owing to colonic structural damage and functional insufficiency[32-33]. We too observed that mice fed DSS showed marked weight loss compared with mice receiving regular drinking water. Oral administration of ATC at doses of 30, 100 and 300 ㎎/㎏ for 10 days significantly inhibited the weight loss of DSS administered mice and achieved the maximal effect at a dose of 100 ㎎/㎏. This outcome was consistent with the morphological result, and the dosages that protected against structural

damage were similar to those associated with functional insufficiency. This result is consistent with those previously reported. Atractylenolide III exerted a protective effect on the mucosal barrier and promoted intestinal nutrient absorption of intestinal epithelial cells[34]. A polysaccharide of *A. macrocephala* improved weight loss, loss of appetite and watery stool caused by disordered intestinal flora[35]. An N-butanol fraction of *T.* spp. increased gastric emptying and gastrointestinal smooth muscle motility in mice[36]. Our results indicate that protective effects of ATC against inflammatory structural damage are associated with restoration of colitis-induced weight loss, suggesting that ATC provides functional restoration after colitis.

ATC reduced DAI scores in a dose-dependent manner, with the maximal effect at a dose of 300 ㎎/㎏. The DAI score describes spontaneous behaviour and posture, piloerection, blood in the anus, diarrhoea and oedema[37]. These are functional parameters that are somewhat analogous to clinical symptoms observed in human UC[38] and there were correlations between DAI and histological findings in DSS-induced colitis in rodents[39]. It is consistent with a previous study showing that *A. macrocephala* suppresed diarrhoea by stimulation of the Th-2 type immune response in mice[40], and that traditional uses of *A. macrocephala* and *T.* spp. included treatment of diarrhoea, indigestion and abdominal pain. These results suggest that ATC might reduce related symptoms of UC, an effect that can be considered as the functional recovery of the colon.

V. Conclusion

This study addressed the protective effects of ATC against dextran sulfate sodium induced ulcerative colitis in rats. The summary of results are as follows:

1. Oral administration of ATC inhibited colon shortening in DSS-treated mice in a dose-dependent manner.

2. ATC reduced MPO activity, which suggests inhibitory effects of ATC against neutrophil infiltration

3. ATC inhibited colitis-induced weight loss and DAI score, which suggest effects of ATC on functional recovery of the colon.

Taken together, ATC exerts protective effects against inflammatory colonic structural damage induced by epithelial barrier integrity impairment with inhibition of weight loss and related symptoms of UC, and these effects can be considered as the functional recovery of the colon. Based on these results, we assume that ATC promotes the recovery of normal colon function and morphology and could be a therapeutic candidate for UC patients.

References

1. Head KA, Jurenka JS. . 2003. Inflammatory bowel disease Part 1: ulcerative colitis--pathophysiology and conventional and alternative treatment options. Alternative medicine review : a journal of clinical therapeutic 8: 247-83.
2. Loftus EV,Jr. . 2004. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology 126: 1504-1517.
3. Campieri M, Gionchetti P. . 2001. Bacteria as the cause of ulcerative colitis. Gut 48: 132-135.
4. Sartor RB. . 2006. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nature clinical practice Gastroenterology & hepatology 3: 390-407.
5. Fournier BM, Parkos CA. . 2012. The role of neutrophils during intestinal inflammation. 5: 354-366.
6. Burger D, Travis S. . 2011. Conventional medical management of inflammatory bowel disease. Gastroenterology 140: 1827-1837.e2.
7. Xu CT, Meng SY, Pan BR. . 2004. Drug therapy for ulcerative colitis. World J Gastroenterol 10: 2311-2317.
8. China. CftPoPsRo. Pharmacopoeia of The People's Republic of China2010.
9. Kiso Y, Tohkin M, Hikino H. . 1983. Antihepatotoxic principles of Atractylodes rhizomes. J Nat Prod 46: 651-654.
10. Wang R, Zhou G, Wang M, Peng Y, Li X. . 2014. The Metabolism of Polysaccharide from Atractylodes macrocephala Koidz and Its Effect on Intestinal Microflora. Evid Based Complement Alternat Med 2014: 926381.
11. Liu H, Zhu Y, Zhang T et al. . 2013. Anti-tumor effects of atractylenolide I isolated from Atractylodes macrocephala in human lung carcinoma cell lines. 18: 13357-68.
12. Zhang J, Cao G, Xia Y, Wen C, Fan Y. . 2014a. Fast analysis of principal volatile compounds in crude and processed Atractylodes macrocephala by an automated static headspace gas chromatography-mass spectrometry. Pharmacognosy magazine 10: 249-53.
13. Schutz K, Carle R, Schieber A. . 2006. Taraxacum-

−a review on its phytochemical and pharmacological profile. J Ethnopharmacol 107: 313−323.
14. Gonzalez−Castejon M, Visioli F, Rodriguez−Casado A. . 2012. Diverse biological activities of dandelion. Nutrition reviews 70: 534−47.
15. Ye H, Chen C, Zhu S. . 2014. Effects of baizhu decoction on ulcerative colitis model rats and the serum IL − 6, IL − 17. Journal of Shaanxi college of traditional Chinese medicine 1: 69−71.
16. Leenen CH, Dieleman LA. . 2007. Inulin and oligofructose in chronic inflammatory bowel disease. J Nutr 137: 2572S−2575S.
17. Shin HS, Satsu H, Bae MJ, Zhao Z, Ogiwara H, Totsuka M, et al. Anti−inflammatory effect of chlorogenic acid on the IL−8 production in Caco−2 cells and the dextran sulphate sodium−induced colitis symptoms in C57BL/6 mice. Food Chem. 2015;168:167−75.
18. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990;98(3):694−702.
19. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. . 1993. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Laboratory investigation; a journal of technical methods and pathology 69: 238−49.
20. Johansson ME, Gustafsson JK, Sjoberg KE et al. . 2010. Bacteria penetrate the inner mucus layer before inflammation in the dextran sulfate colitis model. PloS one 5: e12238.
21. Kitajima S, Takuma S, Morimoto M. . 2000. Histological analysis of murine colitis induced by dextran sulfate sodium of different molecular weights. Exp Anim 49: 9−15.
22. Jadert C, Phillipson M, Holm L, Lundberg JO, Borniquel S. . 2014. Preventive and therapeutic effects of nitrite supplementation in experimental inflammatory bowel disease. Redox biology 2: 73−81.
23. Bradley PP, Priebat DA, Christensen RD, Rothstein G. . 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. The Journal of investigative dermatology 78: 206−9.
24. Yan Y, Kolachala V, Dalmasso G et al. . 2009. Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis. PloS one 4: e6073.
25. Kang TH, Bang JY, Kim MH, Kang IC, Kim HM, Jeong HJ. . 2011. Atractylenolide III, a sesquiterpenoid, induces apoptosis in human lung carcinoma A549 cells via mitochondria−mediated death pathway. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 49: 514−9.
26. Kim YH, Choo SJ, Ryoo IJ, Ahn JS, Yoo ID. . 2011. Eudesmanolides from Taraxacum mongolicum and their inhibitory effects on the production of nitric oxide. Archives of pharmacal research 34: 37−41.
27. Zhang X, Xiong H, Li H, Cheng Y. . 2014b. Protective effect of taraxasterol against LPS−induced endotoxic shock by modulating inflammatory responses in mice. Immunopharmacology and immunotoxicology 36: 11−6.
28. Videla S, Vilaseca J, Antolin M et al. . 2001. Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat. Am J Gastroenterol 96: 1486−1493.
29. Kitajima S, Takuma S, Morimoto M. . 1999. Tissue distribution of dextran sulfate sodium (DSS) in the acute phase of murine DSS−induced colitis. J Vet Med Sci 61: 67−70.
30. Roediger WE. . 1990. The starved colon−−diminished mucosal nutrition, diminished absorption, and colitis. Diseases of the colon and rectum 33: 858−62.
31. Fakhoury M, Negrulj R, Mooranian A, Al−Salami H. . 2014. Inflammatory bowel disease: clinical aspects and treatments. Journal of inflammation research 7: 113−20.
32. Kawada M, Arihiro A, Mizoguchi E. . 2007. Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. World J Gastroenterol 13: 5581−5593.
33. Egger B, Bajaj−Elliott M, MacDonald TT, Inglin R, Eysselein VE, Buchler MW. . 2000. Characterisation of acute murine dextran sodium sulfate colitis: cytokine profile and dose dependency. Digestion 62: 240−248.
34. Shan GS, Zhang LX, Zhao QM et al. . 2014. Metabolomic study of raw and processed Atractylodes macrocephala Koidz by LC−MS. J Pharm Biomed Anal 98: 74−84.
35. Wang R, Zhou G, Wang M, Peng Y, Li X. . 2014. The Metabolism of Polysaccharide from Atractylodes macrocephala Koidz and Its Effect on Intestinal Microflora. Evid Based Complement Alternat Med 2014: 926381.
36. Jin YR, Jin J, Piao XX, Jin NG. . 2011. The effect of Taraxacum spp. on gastric emptying and smooth muscle motility in Rodents. Neurogastroenterol Motil 23: 766−e333.
37. Gottfries J, Melgar S, Michaelsson E. . 2012. Modelling of mouse experimental colitis by global property

screens: a holistic approach to assess drug effects in inflammatory bowel disease. PloS one 7: e30005.
38. Murthy SN, Cooper HS, Shim H, Shah RS, Ibrahim SA, Sedergran DJ. . 1993. Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin. Digestive diseases and sciences 38: 1722-34.
39. Kumar GK, Dhamotharan R, Kulkarni NM, Honnegowda S, Murugesan S. . 2011. Embelin ameliorates dextran sodium sulfate-induced colitis in mice. International immunopharmacology 11: 724-31.
40. Kim SH, Jung HN, Lee KY, Kim J, Lee JC, Jang YS. . 2005. Suppression of Th2-type immune response-mediated allergic diarrhea following oral administration of traditional Korean medicine: Atractylodes macrocephala Koidz. Immunopharmacology and immunotoxicology 27: 331-43.