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ALTERNATIVE MEDICINE PRODUCTS AS A NOVEL TREATMENT STRATEGY FOR INFLAMMATORY BOWEL DISEASE

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Abstract

Inflammatory bowel disease (IBD) affects the mucosal lining of the gastrointestinal tract; the etiology is unknown and treatment is directed at systemic immunosuppression. Natural products, including medicinal herbs, have provided approximately half of the drugs developed for clinical use over the past 20 years. The purpose of our current study was to determine the effects of a novel combination of herbal extracts on intestinal inflammation using a murine model of IBD. Female Swiss-Webster mice were randomized to receive normal water or 5% dextran sulfate sodium (DSS) drinking water to induce colitis. Mice were treated with either a novel combination of herbal aqueous extracts or vehicle control *per os* (po) or *per rectum* (pr) every 24h for 7-8d. Disease activity index score (DAI) was determined daily; mice were sacrificed and colons analyzed by H&E staining, MPO assay, and cytokine (TNF- α , IL-6) ELISAs. Mice treated with the combination of herbal extracts, either po or pr, had significantly less rectal bleeding and lower DAI scores when compared to the vehicle-treated group. Moreover, colonic ulceration, leukocytic infiltration, and cytokine levels (TNF- α and IL-6) were decreased in the colons of herbal-treated mice, reflected by H&E staining, MPO assay, and cytokine ELISA. Treatment with the combination of medicinal herbs decreases leukocyte infiltration and mucosal ulceration, ameliorating the course of acute colonic inflammation. This herbal remedy may prove to be a novel and safe therapeutic alternative in the treatment of IBD.

Keywords

inflammatory bowel disease; herb; natural remedy; traditional medicine

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease, collectively known as inflammatory bowel disease (IBD), are chronic inflammatory conditions of the gastrointestinal tract in which the etiology is unknown and there are no effective long-term treatments. IBD is thought to arise from an inappropriate or exaggerated mucosal immune response to normal enteric flora in a genetically susceptible individual (Ahmad, et al., 2004, Monteleone, et al., 2006, Swidsinski, et al., 2002); defects in both the intestinal epithelial barrier function and the mucosal immune system contribute to the pathogenesis of the disease (Monteleone, Fina, Caruso and Pallone, 2006,

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Podolsky, 2002). Infiltration of leukocytes to the site of inflammation leads to an elevation in the local synthesis of non-specific inflammatory mediators, such as cytokines, chemokines, eicosanoids, nitric oxide (NO), and reactive oxygen species (ROS), enhancing tissue destruction and leading to clinical manifestations of disease (Jackson and Evers, 2006, Podolsky, 2002). Current treatment strategies, consisting predominantly of agents which produce systemic immunosuppression, are fraught with significant clinical sequelae which limit their long-term usage. Treatment strategies which effectively attenuate the mucosal inflammation associated with less side effects are needed.

Naturally occurring compounds have given rise to the development of approximately half of all pharmaceuticals introduced to the market over the past 20 years (Vuorelaa, et al., 2004). The development of acetylsalicylic acid, or aspirin, is a prototypic example. Over 4000 years ago, Babylonians prescribed willow tree (*Salix* sp.) extracts to treat fever, pain, and inflammation (Mahdi, et al., 2006). In the early 1800's, salicin was isolated from willow tree extracts and used in the treatment of rheumatism. By 1897, several chemical modifications were made to the salicin compound, giving rise to acetylsalicylic acid, one of the most commonly used modern medications. Other important medications that have originated from natural sources include simvastatin, enalapril, ciprofloxacin, cyclosporine, taxol, and camptothecin, among others (Harvey, 2000). Epidemiologic studies examining dietary influence on the development of disease, as well as obtaining information about plants used in healing from traditional local healers, have led to the identification of target plants for use in pharmaceutical development (Vuorelaa, Leinonenb, Saikkuc, Tammela, Rauhad, Wennberge and Vuorela, 2004). However, Western medicine has oftentimes resisted the introduction of natural medicine products due to inherent biases and reluctance to accept alternatives to current therapies.

The investigation of alternative therapies, including traditional Chinese medicine, for IBD is ideal given a lack of effective treatment strategies for the disease and the relative safety of these compounds. There are several well-known remedies routinely employed in Chinese culture for the treatment of inflammatory diseases, including Liu-Shen-Wan, used with success in the treatment of patients with inflammatory diseases and systemic inflammatory response syndrome; the clinical effects were recently corroborated by the amelioration of inflammation in an *in vivo* mouse sepsis model (Ma, 2006). In addition, Chinese physicians have been treating IBD using herbal concoctions for years with primarily anecdotal evidence of success. For example, Chinese patients with IBD have been treated with a novel herbal remedy, consisting of a combination of seven herbs, including *Forsythia koreana*, *Corydalis saxicola*, *Semiaquilegia adoxoides*, *Taraxacum officinale*, *Chrysanthemum coronarium*, *Glycyrrhiza inflata*, and *Lonicera japonica*. These herbs were individually selected on the basis of known anti-inflammatory properties, which have been characterized on a molecular level (Aly, et al., 2005, Bor, et al., 2006, Cheng, et al., 2005, Hu and Kitts, 2005, Jung, et al., 2006, Kang, et al., 2005, Kim and Lee, 2005, Kim, et al., 2005, Kim, et al., 2006, Kim, et al., 2006, Ko, et al., 2006, Leu, et al., 2005, Leung, et al., 2006, Li, et al., 2006, Li, et al., 2006, Ling, et al., 2006, Makino, et al., 2006, Niu, et al., 2006, Niu, et al., 2006, Rusu, et al., 2005, Seo, et al., 2005, Son, et al., 2006, Strzelecka, et al., 2005, Su, 2004, Suh, et al., 2006, Thanabhorn, et al., 2006, Zhan and Yang, 2006). The purpose of our current study was to validate the use of this herbal remedy in the treatment of IBD using an established mouse model of colitis induced by dextran sulfate sodium (DSS).

MATERIALS AND METHODS

Materials

Forsythia koreana, *Corydalis saxicola*, *Semiaquilegia adoxoides*, *Taraxacum officinale*, *Chrysanthemum coronarium*, *Glycyrrhiza inflata*, and *Lonicera japonica* were obtained from

the Chinese Medical Hospital (Gansu province, Tianshui, China); the quality of the raw herbs was controlled according to the requirements of Pharmacopoeia of the People's Republic of China. Dextran sulfate sodium salt (DSS; MW 40-50,000) was obtained from USB Corporation (Cleveland, OH). Exel Safelet angiocatheters (20G × 1 1/4 in.) were from Exelint International (Los Angeles, CA). Phosphate buffered saline (PBS) was from Life Technologies, Inc. (Grand Island, NY). TNF- α and IL-6 cytokine ELISAs were from R&D Systems (Minneapolis, MN). Complete Mini was from Roche Applied Science (Indianapolis, IN). All other reagents, including Igepal CA-630, were of molecular biology grade and purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of herbal extracts

Dehydrated flowers from *L. japonica* and *C. coronarium*, dehydrated root from *S. adoxoides* and *G. inflata*, dehydrated fruit from *F. koreana*, and dehydrated *C. saxicola* and *T. officinale* plants were extracted 2 times with boiling water. The extracts were then combined, and concentrated by boiling to a final volume of 150-200ml.

Murine colitis model and assessment

Female Swiss-Webster mice (6-8 weeks old) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed in clean pathogen-free rooms in an environment with controlled temperature (22°C), humidity, and a 12 h light/dark cycle. The mice were fed standard chow (Formula Chow 5008; Purina Mills, St. Louis, MO) and tap water *ad libitum* and allowed to acclimate for one week. All studies were approved by the Institutional Animal Care and Use Committee of UTMB. Colitis was induced by the addition of DSS (5% weight to volume ratio dissolved in distilled water) to the drinking water, as previously described (Cooper, et al., 1993). This is a well-characterized model of IBD, resulting in colon ulceration, leukocytic infiltration, hematochezia, and diarrhea reminiscent of human IBD (Cooper, Murthy, Shah and Sedergran, 1993). (i) Mice (n=20) were randomized into 4 groups of 5 mice to receive 5% DSS drinking water to induce colitis. Mice were treated with the combination of herbal compounds or the vehicle control (PBS) every 24h for 8d either *per os* (po) by gavage of 0.5ml of solution, or *per rectum* (pr) by passing a soft 20G angiocatheter 3cm into the rectum and infusing 0.2ml of solution. (ii) Female Swiss-Webster mice (n=40) were randomized into 8 groups of 5 mice to receive normal or 5% DSS drinking water to induce colitis. Mice were treated with either PBS or herbal aqueous extracts as above every 24h for 7d.

Daily clinical evaluations, including the assessment of body weight, stool consistency, and rectal bleeding, were made in a blinded fashion, and disease activity index (DAI) score, graded on a scale of 0 to 4, assigned according to a previously validated scoring system assessing weight loss, stool consistency, and rectal bleeding (Table 1) (Cooper, Murthy, Shah and Sedergran, 1993). Upon sacrifice, the colon was isolated, and the length from cecum to anus measured. The colon was then opened longitudinally, the distal 1/3 isolated and snap-frozen for later analysis, and the remainder prepared in a "swiss roll" (Park, et al., 1987), placed into 10% neutral buffered formalin for 24h, then into 70% ethanol for an additional 24h.

Histology

After formalin fixation, samples were paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E). Histologic examination was then performed in a blinded fashion by a pathologist, using a scoring system previously described, with modification (Dieleman, et al., 1998, Morteau, et al., 2000). Briefly, two independent parameters were determined: extent of crypt damage (0-4 with 0 = none, 1 = basal 1/3 damaged, 2 = basal 2/3 damaged, 3 = only surface epithelium intact, 4 = entire crypt and epithelium lost), and extent of mucosal ulceration (1-4 with 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%). The entire length of

colon was evaluated for each sample. Scores were then multiplied, with a maximum possible score of 16.

Myeloperoxidase (MPO) assay

To quantify the degree of inflammation and estimate the accumulation of neutrophils in tissues, MPO assay was performed as previously described (Bradley, et al., 1982, Stucchi, et al., 2000). Briefly, tissue (approximately 10mg) was homogenized in 20mM $\text{NaH}_2\text{PO}_4\text{:Na}_2\text{HPO}_4$ buffer, 4:19 ratio (pH 7.4), then centrifuged for 12 min at 4°C. The pellet was resuspended in 50mM $\text{NaH}_2\text{PO}_4\text{:Na}_2\text{HPO}_4$ buffer, 2.3:1 ratio (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB). Samples were frozen and thawed once, then sonicated for 40 sec in an ice bath. The sample was centrifuged for 10 min at 4°C, and supernatant was collected for analysis. Protein concentrations were determined using the method of Bradford (Bradford, 1976). Approximately 50 μL of each sample was added to a 96-well plate, and 50 μL tetramethylbenzidine (TMB) substrate was added. After 2 min of incubation at 37°C, absorbance was measured at 655 nm.

Tissue cytokine ELISA

Frozen colon samples were pulverized by mortar and pestle in liquid nitrogen. Protein was then extracted using a method previously described (Matalaka, et al., 2005, Rosengren, et al., 2003). Ice-cold protein extraction buffer, consisting of 0.1% Igepal CA-630 nonionic detergent in PBS with a protease inhibitor cocktail tablet (Complete Mini), was added to pulverized tissue (50 μL to 10 mg of tissue), and vortexed every 10 min for 30 min. The resultant homogenate was transferred to a microcentrifuge tube and centrifuged at 4°C for 10 min at 12 $\times g$, and supernatant was collected for use in colorimetric ELISA for TNF- α or IL-6. Briefly, 50 μL of supernatant was added to 50 μL of assay diluent (1:1 dilution) in a 96-well plate. After a 2 h incubation, wells were aspirated and washed, conjugate was added, and wells were again aspirated and washed after a 2 h incubation. Substrate solution was added to each well, and the plates were incubated in the dark for 30 min. Stop solution was then added, and optical density determined at 450 nm (with a correction wavelength set at 570 nm). Experiments were performed in duplicate to assure reproducibility.

Statistical analysis

All data were expressed as the mean $\pm$ SD. Groups (PBS po vs. herb po, PBS pr vs. herb pr) were analyzed using one-way classification analysis of variance at the 0.05 level of significance. A *P* value of 0.05 was considered significant.

RESULTS

Treatment with the herbal extract, either po or pr, significantly decreased DAI compared with control mice

To determine the effects of our combination of herbal components on the development of murine colitis, female Swiss-Webster mice were given 5% DSS drinking water *ad libitum*, and randomized to receive either PBS or the extract po or pr every 24h for 8 days; daily weight, stool consistency, and presence or absence of rectal bleeding was noted by an investigator blinded as to treatment groups. Mice treated with PBS, either po or pr, demonstrated the expected gradual increase in DAI, attaining an average score of approximately 4. In contrast, mice treated with the herb extract developed disease at a significantly slower rate, attaining an average score of 3.0 for mice treated po, and an average score of 2.8 for mice treated pr (Fig. 1A). One mouse from each group died on the day 7 to day 8 transition; this was a result of colonic perforation in one mouse treated with herb extract pr, but integrity of the esophagus and colon was maintained in the other mice. Upon sacrifice, colons were harvested, and length

from cecum to anus determined (Fig. 1B, C). Significant foreshortening of the colon was noted in mice treated with PBS (4.3cm for mice treated po; 4.9cm for mice treated pr) when compared to mice treated with the herbal extract (5.8cm for mice treated po; 7.2cm for mice treated pr).

To corroborate our findings, female Swiss-Webster mice were given either normal water or 5% DSS drinking water *ad libitum*, and randomized to receive either PBS or herb extract po or pr every 24h for 7d. As in our prior study, mice treated with PBS demonstrated the expected gradual increase in DAI, attaining an average score of 3.4 for mice treated either po or pr. In contrast, mice treated with the herb extract developed disease at a significantly slower rate, attaining an average score of only 2.4 for mice treated po, and an average score of 1.8 for mice treated pr (data not shown). Upon sacrifice, we again noted a significant foreshortening of the colon in mice treated with PBS (5.3cm for mice treated po; 5.2cm for mice treated pr) when compared to mice treated with herb extract (6.1cm for mice treated po; 7.5cm for mice treated pr) (data not shown). Overall, mice treated with the combination of herb extracts pr had consistently lower DAI scores and less colonic foreshortening than mice treated po. Mice given normal drinking water and administered PBS or herb extract did not suffer any adverse effects.

Herbal treatment significantly decreases colonic ulceration

We next determined whether the combination of herbal extracts altered the histologic structure of the colonic mucosa. Slides were blinded and submitted to a pathologist for grading. A two-tiered scoring system was employed as described in “Materials and Methods.” Consistent with our observations of disease activity and colon length determination, mice treated with PBS had significantly more ulcerations (average score 5.4; 6.0 for po treated mice, 4.8 for pr treated mice) compared with mice treated with herb extract (average score 2.5; 2.8 for po treated mice, 2.2 for pr treated mice) (Fig. 2A). In addition, there was significantly less crypt damage and leukocytic infiltration noted in mice treated with herb extract.

Treatment with herbal extracts decreases neutrophil sequestration in the colonic mucosa

To quantify the degree of inflammation and estimate the accumulation of neutrophils in tissues, MPO assays were performed using colonic homogenates. Mice given regular water and treated with PBS or the combination of herbal extracts demonstrated no difference in MPO activity (0.45 and 0.41, respectively). However, consistent with previous findings, MPO activity was higher in mice given 5% DSS and treated with PBS (1.05; 1.06 for po treated, 1.04 for pr treated) than in mice treated with herbal extract (0.65; 0.61 for po treated, 0.68 for pr treated) (Fig. 2B). Thus, treatment with herbal extract leads to decreased inflammation as represented by decreased sequestration of neutrophils within the colon.

Treatment with herbal extracts decreases cytokine elaboration within the colonic mucosa

To determine the expression of cytokines in the colonic mucosa of mice treated with PBS or herbal extracts, TNF- α and IL-6 colorimetric ELISAs were performed on tissue protein extracts. Mice given normal drinking water and treated with PBS or combination of herbal extracts demonstrated little expression of either TNF- α or IL-6. However, there was significantly higher TNF- α expression in the colons of mice given 5% DSS drinking water and treated with PBS (403.3 pg/ml po, 233.9 pg/ml pr) than in mice treated with herbal extract (228.5 pg/ml po, 165.7 pg/ml pr) (Fig. 3A). Similarly, there was significantly higher IL-6 expression in the colons of mice given DSS drinking water and treated with PBS (94.8 pg/ml po, 121.4 pg/ml pr) than in mice treated with herbal extract (28.3 pg/ml po, 48.8 pg/ml pr) (Fig. 3B). Therefore, we conclude that treatment with herbal extract not only decreases leukocyte infiltration into the diseased colon, but decreases cytokine expression in response to an inflammatory stimulus.

DISCUSSION

Using a murine model of DSS colitis, which mimics human IBD in the elaboration of cytokines, chemokines, and upregulation of inflammatory pathways such as COX-2 and NF- κ B (Spiik, et al., 2002), we found that the treatment of mice with a novel herbal concoction led to amelioration of the disease, as determined by lower DAI scores, less colonic foreshortening, less colonic ulceration, and less leukocytic infiltration. There appeared to be increased benefit in mice treated with herb pr relative to mice treated po. While the exact mechanisms contributing to the protective effects of the treatment are not known, much is known about the individual herbal components.

F. koreana, *C. saxicola*, *S. adoxoides*, *T. officinale*, *C. coronarium*, *G. inflata*, and *L. japonica* are commonly employed ethnopharmacological agents used for the treatment of inflammatory diseases in China (Aly, Al-Alousi and Salem, 2005, Bor, Chen and Yen, 2006, Cheng, Li, You and Hu, 2005, Hu and Kitts, 2005, Jung, Richter, Kabrodt, Lucke, Schellenberg and Herrling, 2006, Kang, Yoon, Cho, Han, Lee, Park and Kim, 2005, Kim and Lee, 2005, Kim, Min, Jeong, Lee, Lee and Seo, 2005, Kim, Kim, Baek, Lee, Kim, Kwon and Lee, 2006, Kim, Oh, Kwon, Oh, Lim and Shin, 2006, Ko, Wei and Chiou, 2006, Leu, Wang, Huang and Shi, 2005, Leung, Kuo, Yang, Lin and Lee, 2006, Li, Yuan, Xiong, Lu, Qin, Chen and Liu, 2006, Li, Zhang, Zhang, Liu, Wang, Wang, Zhu and Chen, 2006, Ling, Wu and Li, 2006, Makino, Tsubouchi, Murakami, Haneda and Yoshino, 2006, Niu, Chang, Jiang, Cui, Chen, Yuan and Tu, 2006, Niu, Cui, Li, Chang, Jiang, Qiao and Tu, 2006, Rusu, Tamas, Puica, Roman and Sabadas, 2005, Seo, Koo, An, Kwon, Lim, Seo, Ryu, Moon, Kim, Kim and Hong, 2005, Son, Moon, Lee, Son, Kim, Kang, Son, Lee and Chang, 2006, Strzelecka, Bzowska, Koziel, Szuba, Dubiel, Riviera Nunez, Heinrich and Bereta, 2005, Su, 2004, Suh, Chung, Son, Kim, Moon, Son, Kim, Chang and Kim, 2006, Thanabhorn, Jaijoy, Thamaree, Ingkaninan and Panthong, 2006, Zhan and Yang, 2006). *F. koreana*, used as a single-agent antimicrobial, anti-inflammatory, and diuretic, contains the cyclohexylethanoid compound rengyolone, found to be a potent inhibitor of NO and tissue necrosis factor- α (TNF- α) *in vitro*; this compound inhibits inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) expression in macrophages in response to lipopolysaccharide (LPS) stimulation (Kim, Kim, Baek, Lee, Kim, Kwon and Lee, 2006, Ko, Wei and Chiou, 2006). *Corydalis* species, commonly employed as antimicrobial, antiviral, and anticancer agents, are rich in alkaloids such as berberine, coptisine, and dehydrocavidine; a study by Ling et al (Ling, Wu and Li, 2006) demonstrated the protective effects of this herb in a rat cardiac ischemia/reperfusion model through regulation of Bcl-2. *S. adoxoides* is thought to exert its anti-inflammatory effects through the expression of several diterpenoids, including E- and Z-semiaquilegin (Niu, Chang, Jiang, Cui, Chen, Yuan and Tu, 2006, Niu, Cui, Li, Chang, Jiang, Qiao and Tu, 2006, Su, 2004). *T. officinale*, commonly known as dandelion, and *C. coronarium*, commonly known as chrysanthemum, have impressive intrinsic antioxidant activity attributable to flavonoid expression (Bor, Chen and Yen, 2006, Cheng, Li, You and Hu, 2005, Hu and Kitts, 2005, Jung, Richter, Kabrodt, Lucke, Schellenberg and Herrling, 2006, Kim and Lee, 2005). *T. officinale* has proven to be protective against cholecystokinin-induced pancreatitis in rats, with decreased pancreatic wet weight and decreased expression of TNF- α and IL-6, and has been found to inhibit nitric oxide production from LPS-stimulated mouse macrophages (RAW264.7) (Hu and Kitts, 2005, Seo, Koo, An, Kwon, Lim, Seo, Ryu, Moon, Kim, Kim and Hong, 2005). *C. coronarium* has proven protective against the development of CCl₄-induced liver injury in rats and decreases delayed-type hypersensitivity in mice, as well as inhibiting iNOS expression and NO production from RAW264.7 cells *in vitro* (Bor, Chen and Yen, 2006, Rusu, Tamas, Puica, Roman and Sabadas, 2005). *G. inflata*, also known as licorice, inhibits the production of NO and prostaglandin E₂ (PGE₂) from RAW264.7 cells in response to LPS stimulation, likely through inhibition of nuclear factor κ B (NF- κ B), and confers a survival advantage and decreases cytokine expression in murine LPS-induced septic shock (Aly, Al-Alousi and Salem, 2005, Kang, Yoon, Cho, Han,

Lee, Park and Kim, 2005, Kim, Oh, Kwon, Oh, Lim and Shin, 2006, Makino, Tsubouchi, Murakami, Haneda and Yoshino, 2006). Ochnaflavone, a biflavonoid, has been isolated from *L. japonica*; *in vitro*, this compound inhibits phosphorylation of ERK1/2 and p38 mitogen-activated protein kinase (MAPK) and inhibits NF- κ B activity in RAW264.7 cells, leading to decreased iNOS expression and NO formation in response to LPS (Suh, Chung, Son, Kim, Moon, Son, Kim, Chang and Kim, 2006). Similarly, ochnaflavone inhibits cyclooxygenase-2 (COX-2)-dependent prostaglandin D₂ (PGD₂) generation and leukotriene C₄ (LTC₄) production from bone marrow-derived mast cells (Son, Moon, Lee, Son, Kim, Kang, Son, Lee and Chang, 2006).

The chronic inflammatory response in IBD represents a balance between active inflammation, destruction, and repair that occurs in response to a persistent stimulus over an extended period of time (Jackson and Evers, 2006). While the stimulus is unknown, IBD is characterized by an influx of inflammatory cells into the gut tissue, followed by a marked increase in the local synthesis of non-specific inflammatory mediators, including cytokines, chemokines, eicosanoids, NO, and ROS (Monteleone, Fina, Caruso and Pallone, 2006). Extensive cross-talk between immune and non-immune cells, including mucosal endothelial cells and fibroblasts, leads to the amplification of signals that ultimately leads to tissue damage (Monteleone, Fina, Caruso and Pallone, 2006). Important signaling pathways upregulated in inflamed tissue include COX-2 and NF- κ B (Jackson and Evers, 2006). COX-2 belongs to a class of genes known as early growth response genes; it is induced by inflammatory growth factors and cytokines, including IL-1 and TNF- α , and its products include pro-inflammatory prostaglandins and eicosanoids (Jackson and Evers, 2006, Sheng, et al., 1997). Derivatives of 5-aminosalicylate, inhibitors of COX-2 activity, are mainstays of treatment for patients with IBD (Podolsky, 2002). NF- κ B is a ubiquitously expressed transcription factor that plays a pivotal role in the inflammatory response (Ethridge, et al., 2002). Activated by infectious agents or cytokines, including IL-1, ROS, LPS, and TNF- α , its products include growth factors, cytokines, cell adhesion molecules, immunoreceptors, and cell survival proteins, making it an important contributor to the potentiation of the local inflammatory response (Jackson and Evers, 2006, Schwartz, et al., 1999). Thus, a complex interplay between cytokines, chemokines, and growth factors, as well as cell signaling pathways that augment the inflammatory response, lead to the tissue destruction and clinical manifestations characteristic of IBD.

Given the mechanisms of action of the various herbs included in our concoction, which include downregulation of COX-2, NF- κ B, TNF- α , IL-1, IL-6, and iNOS, as well as decreased NO, ROS, leukotiene and prostaglandin production, this herbal remedy may be a complex and potent immunomodulator, with the various constituents working in a synergistic fashion to diminish the local immune response. We therefore conclude that this herbal treatment, combining the therapeutic benefits of *F. koreana*, *C. saxicola*, *S. adoxoides*, *T. officinale*, *C. coronarium*, *G. inflata*, and *L. japonica*, may be a novel therapy for IBD. Future studies will better delineate the effective compound responsible for the beneficial effects noted in our study.

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Fig. 1. Treatment of mice with herbal extract ameliorates DSS-induced colitis as reflected by decreased DAI scoring and preserved colon length

Colitis was induced by the addition of DSS (5% weight to volume ratio dissolved in distilled water) to the drinking water; mice were sacrificed after 7 d of DSS exposure. A. Disease activity index (DAI) score, which assesses weight loss, stool consistency, and blood loss, was assigned daily in a blinded fashion. Data is shown for mice treated with DSS; all mice receiving normal water had DAI scores of 0 for the duration of treatment. Upon sacrifice of the mice, colons were harvested for (B) length determination and (C) gross comparison. Four mice receiving normal water and treatment with PBS pr are shown for comparison. † = $p < 0.05$ vs. DSS + PBS po; * = $p < 0.05$ vs. DSS + PBS pr.

Fig. 2. Treatment of mice with herbal extract ameliorates DSS-induced colitis as reflected by decreased ulceration and neutrophils sequestration

DSS colitis was induced as previously described, and mice were sacrificed after 7 d of DSS exposure. A. Histologic scoring, including assessment of crypt damage and extent of mucosal ulceration, was performed in a blinded fashion. All mice receiving normal water had histologic scores of 0 (data not shown). B. MPO assay was performed on colonic protein lysates; briefly, tissue was homogenized in sodium phosphate buffer, pelleted, and resuspended in sodium phosphate buffer containing 0.5% HTAB. Samples were frozen and thawed, then sonicated in an ice bath. Protein concentrations of the supernatant were determined. TMB substrate was added to samples and absorbance determined. † = $p < 0.05$ vs. DSS + PBS po; * = $p < 0.05$ vs. DSS + PBS pr.

Fig. 3. Treatment of mice with herbal extract decreases cytokine elaboration within the inflamed mucosa

DSS colitis was induced as previously described, and mice were sacrificed after 7 d of DSS exposure. Cytokine analysis was performed on colonic lysate; briefly, colon samples were pulverized by mortar and pestle in liquid nitrogen, and protein extracted. Supernatant was added to assay diluent in a 1:1 dilution. After incubation, conjugate was added. After incubation and washing, substrate solution was added, and the plates were incubated in the dark. Stop solution was then added, absorbance measured, and concentration of (A) TNF- α and (B) IL-6 determined. Experiments were performed in duplicate to assure reproducibility. [†] = $p < 0.05$ vs. DSS + PBS po; * = $p < 0.05$ vs. DSS + PBS pr.

Table 1**Disease activity index (DAI) score**

DAI score, graded on a scale of 0 to 4, is a previously validated scoring system assessing weight loss, stool consistency, and rectal bleeding. The sum of scores for each category, divided by 3, yields the DAI.

Score	Weight Loss	Stool	Rectal Bleeding
0	None	Normal	Normal
1	1-5%		
2	6-10%	Loose	Hemoccult positive
3	11-20%		
4	>21%	Diarrhea	Gross Bleeding

$$\text{DAI} = \frac{\text{weight loss} + \text{stool consistency} + \text{rectal bleeding}}{3}$$