Dietary Fenugreek Attenuates Dextran Sodium Sulfate-Induced Ulcerative Colitis: Role of Inflammation

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1. Abstract

1.1. Aim: Ulcerative colitis, a chronic relapsing form of inflammatory bowel disease, is often neglected at initial stages due to its difficulty in detection leading to delayed clinical evaluation and treatment. Fenugreek, an annual plant and spice, is known to possess anti-inflammatory property. We examined the effect of dietary fenugreek on inflammatory cells related cytokine-mediated inflammation in dextran sulfate sodium (DSS)-induced colitis model.

1.2. Methods: To investigate the anti-inflammatory effect of fenugreek on DSS-induced ulcerative colitis, DSS (3% w/v) was administered in drinking water for 7 days to induce ulcerative colitis in C57BL/6 mice. Fenugreek (5% and 10%) was fed in diet throughout the experiment for 14 days.

1.3. Results: Fenugreek improved disease activity index, stool consistency, and occult blood. In addition, it increased colon length and decreased colon weight/length ratio, and spleen weight. Additionally, it significantly decreased circulating immune cells and mast cell recruitment and improved hematological parameters. Further, it altered the acidic and neutral mucin in the colon and attenuated inflammation by decreasing tumor necrosis factor-α, interleukin (IL)-1β, and IL-6, but increasing IL-10.

1.4. Conclusion: Fenugreek decreased inflammatory cytokines, thereby protecting the colon against DSS-induced inflammation and injury.

2. Keywords: Fenugreek; Ulcerative colitis; Cytokines; Circulating immune cells; Inflammation

3 Introduction

Fenugreek (Trigonella foenum-graecum L.) belongs to the Leguminosae family, cultivated mainly in Asia, the Mediterranean, and North African regions. Foods of medicinal values are proved to be effective, therefore fenugreek was widely used as food in the form of medicine [1]. Fenugreek is classified as “Generally Recognized as Safe” by the U.S. Food and Drug Administration [2]. The nutrient composition of fenugreek seeds: moisture 9%; protein 26%; fat 7%; saponins 8-10%; total dietary fiber 48% [3]. Its seeds are usually used as a condiment and seasoning in food preparations. It also possesses nutritive and restorative properties [4]. Fenugreek seeds have strong aromatic properties, and are used as one of the spices in the curry preparation. Fenugreek phytochemicals are involved in molecular mechanisms resulting in modulating inflammation related cellular function that is crucial for the development of targeted therapeutic interventions for inflammatory diseases [5]. Fenugreek is used in traditional medicine that includes Ayurvedic and Chinese medicine for centuries against a variety of diseases and morbidity, including labor induction, indigestion and as a general tonic to improve metabolism and health, diabetes, fever, abdominal pain, abscesses, boils, and carbuncles [6, 7]. It is consumed during pregnancy and lactation as a traditional medicine and a traditional food [3]. As an herbal medicine, its effect is carminative, tonic, and aphrodisiac [1]. It is one of the historically oldest medicinal plants, used as olfactory, laxative, and...
galactogogue effects. Recently, immunostimulatory, anti-diabetic, anti-hypertensive, and cholesterol-lowering activities have been investigated. It improves diabetes [8], hypercholesterolemia [9], gastric ulcer [10], hyperthyroidism [11], neoplasia[12], and inflammation [13]. It helps in reducing blood sugar and possess stimulatory effects on macrophages. It contains a good amount of mucilage (28%) and are responsible for inducing macrophages. It also contains substantial amounts of organic iron, which is readily absorbed. Thus, it facilitates haematopoietic stimulation in bone marrow [14].

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD). UC is characterized by chronic and relapsing colon inflammation, resulting in diarrhea, pain, vomiting, weight loss, and bloody stool [15]. Approximately, 1-2 million people in the United States suffer from IBD, almost half were UC. UC is predominant in North America and Europe. However, UC incidences are rapidly increasing in East Asian countries, including China, Japan, and Korea. The exact cause of UC is unknown, but it appears to be related to a combination of genetic and environmental factors [16, 17]. Etiopathology of IBD comprises of the multifaceted interaction of genetic, environmental, microbial, and immune factors, which result in altered mucosal barrier nature and epithelial layers. Mucosal alterations allow luminal toxins and pathogens to penetrate the intestinal mucosa and provoke production of pro-inflammatory cytokines and chemokines for leukocyte infiltration into the intestinal mucosa. Ultimately, it is leading to intestinal inflammation [18]. Infiltration of neutrophils, monocytes, and macrophages to gut is a major contributor to the gastrointestinal inflammation and tissue injury in IBD [19]. At present conventional pharmacological compounds, such as salicylazosulfapyridine, glucocorticoid, corticosteroids, 5-aminosalicylic acid, and mesalazine have been used for the treatment of UC, but these drugs have adverse side effects, especially steroid therapy, which can cause infertility and developmental disability, loss of response or intolerance, and infection due to immunosuppression. In addition, these drugs pose a substantial burden associated with costs, patients functioning and health-related quality of life [16, 17].

Despite advances in modern medical science, traditional herbal medicines are commonly used for thousands of years to maintain health, disease prevention, and even disease treatment in China, Japan, and Korea [17]. Scientifically verified natural products are potential candidates for preventive medicine with complementary and alternative medicines. That natural product usage has rapidly grown over the last decades in both the Eastern and Western world [20]. Adverse effects of conventional therapy and cost issues have turned the focus on alternative methods for the prevention and treatment of UC using natural products such as herbal medicines in the dietary form [16]. Therefore, in this study, we investigated the effect of dietary fenugreek on inflammatory cells related cytokine-mediated inflammation in DSS-induced colitis model.

4. Materials & Methods

4.1. EC-6 Cell Viability Test for Fenugreek

IEC-6 cells at a concentration of 1.2x10^6 cells were seeded in a 96-well plate and allowed to attach for 24 h. The cells were then treated with 7% DSS in the media. Cells were incubated for 24 h, then DSS was removed and added 0, 0.5, 1, and 2 µg/mL of fenugreek in the media. Incubated for 48 h with one change of fenugreek after 24 h. Fenugreek was removed and added a new medium containing 100 µL/well of MTT 0.5 mg/mL and incubated at 37 °C in the dark for 4 h. Dimethyl sulfoxide 100 µL/well was added to IEC-6 cell culture and incubated at 25 °C in a shaker at 50 rpm for 20 min and measured spectrophotometrically in an ELISA reader at 570 nm.

4.2. Diet

LabDiet 5010 was used to prepare the experimental diet. The fenugreek seeds were purchased from an Indian grocery store. To prepare 5% (w/w) and 10% (w/w) fenugreek feed, 5 g and 10 g of fenugreek seeds, were added to 95 g and 90 g animal pellet feed, respectively, and ground in a mechanical grinder to form a uniform powder. The powder was sieved and stored at 4 °C until use.

4.3. Animals and Experimental Protocol

C57BL/6 mice weighing 17-20 g were obtained from and housed in the Institutional Laboratory Animal Center of National Cheng Kung University, in a room 12 h light/dark cycle and with central air conditioning (25 °C, 70% humidity). They were given pellet feed (Richmond Standard; PMI Feeds, Inc., St. Louis, MO) and water ad libitum. The animal care and experimental protocols were in accordance with nationally approved guidelines (IACUC No.104068).

The mice were acclimated for one week, and randomly divided into five different experimental groups. Control group CTL (n = 6), mice was fed with regular diet and normal drinking water. DSS, DF5, and DF10 groups (n = 6, each group), mice were given only DSS (3% w/v) in drinking water for 7 days from 7 a
to 14th day, DF5 and DF10 group (n = 6, each group), mice were fed with 5% (w/w) and 10% (w/w) fenugreek feed for 14 days. F10 group (n = 6, each group), mice was fed with 10% (w/w) fenugreek feed for 14 days. During the study disease activity index, diarrhea, and fecal bleeding were assessed. The mice were then sacrificed on the 14th day, the colons length from the cecum to anal verge, and spleen weight was measured.

4.4. Evaluation of Disease Activity Index

The disease activity index (DAI) score was calculated as the sum of scores for weight loss, stool consistency and rectal bleeding as previously described [21].

4.5. Hematological and Circulating Immune Cell Quantification

Hematological examination was done to quantify the red blood cell count, and hemoglobin content. Circulating immune cells were done to quantify the neutrophils, monocytes, lymphocytes, and platelets. The blood samples were collected from the inferior vena cava and 0.2 mL blood sample was added into tube coated with EDTA (0.5 mol/L). The procedure for conducting hematological examination were finished within 4 h by Seil Vet Focus 5 Hematology Analyzer (Seil Animal Care Company, Gurnee, IL, USA) in automatic mode.

4.6. Sample Collection

At the end of the experiments, the animals were sacrificed. Colon tissue and spleen were collected and kept on ice. The colons were excised and the length from the cecum to the anus was measured. The spleen was excised and the weight was measured. One portion of each distal colon was cut and fixed in 10% formalin for histopathological and immunostaining; the remaining portion of the colon was stored at -80 °C for further analysis.

4.7. Histological Examination

Colons were washed in phosphate-buffered saline (PBS) and placed in 10% buffered formalin (pH 7.4) at room temperature for 24 h. The specimens were then paraffin-embedded using the standard procedure. Serial 5 μm sections were stained with hematoxylin and eosin. To assess the degree of inflammation and tissue injury the stained sections were observed under a microscope (OLYMPUS BX51). Briefly, sections were scored based on three criteria; inflammatory cell infiltration (0–3), tissue damage (0–5), and edema (0–2). The degree of intestinal inflammation was scored according to the density and extent of the acute inflammatory infiltrate, loss of goblet cells, and edema.

4.8. Staining Neutral and Acidic Mucins

The colonic neutral and acidic mucins were determined individually using the modified histochemical technique of Periodic Acid-Schiff (PAS) staining and Alcian blue-PAS staining. The colon tissue sections were deparaffinized, rehydrated, and stained in Alcian blue solution for 15 min, rinsed and PAS-stained using the standard protocol using Schiff’s reagent for 10 min; and then counterstained using hematoxylin for 30 sec. The sections were dehydrated, cleared, and mounted using DPX. The stained sections were observed under a microscope and photomicrographs were taken (OLYMPUS BX51).

4.9. Immunohistochemistry Staining of IL-6

The colon tissue sections were deparaffinized, rehydrated, blocked and then incubated with IL-6 (dilution 1:20 overnight at 4 °C and developed (Ultra Vision Detection System Anti-Rabbit, HRP/DAB [Ready-to-Use] Kit; Thermo Fisher). The stained sections were counterstained with hematoxylin. After mounting using DPX, the slides were examined under microscope (magnification, 100X).

4.10. Measurements of TNF-α, IL-1β, IL-6, and IL-10

A 96-well immunoassay plate was coated with 100 µL/well capture antibody overnight at room temperature, followed by a blocking step. Recombinant cytokines ranging from 6.25 to 4000 pg/mL were used as standards. One hundred mL of medium and serial standards diluted in sample buffer (PBS containing 1% (w/v) BSA, pH 7.6) were incubated at room temperature for 2 h. Followed by incubating with 100 µL of biotinylated rabbit anti-mouse TNF-α, IL-1β, IL-6, and IL-10 antibody, streptavidine-conjugated reaction was initiated by the addition of 100 µL of TMB for 30 min, and then stopped by adding 50 µL of 0.5 M H₂SO₄. The absorbance was measured at 450 nm with ELISA reader.

4.11. Immunofluorescence

Tissue sections were deparaffinized, rehydrated, through a series of graded ethanol (100, 95, 70, 50, and 30%) and water, followed by an antigen retrieval step by heating the sections in microwave apparatus using a glass container containing 10 mM sodium citrate buffer, pH 6.0 for 10 min. Sections were then cooled at room temperature, incubated in blocking solution (3% bovine serum albumin (BSA) with 0.2% Tween 20 in PBS for 1 h, followed by incubation overnight at 4 °C with primary antibodies: p47phox (1:500 dilution, Santa Cruz) diluted with 3% blocking solution. Next day, sections were washed and incubated with secondary antibody conjugated to Alexa Fluor 488 (1:400 dilution, Abcam) for 1 h at room temperature in the dark chamber. The sections were washed in PBS and rinsed in distilled water and mounted using Fluoroshield mounting medium with 4’, 6 diamidino-2-phenylindole (DAPI) (Abcam). Images were captured with a microscope (OLYMPUS BX51) under fluorescence setup with
appropriate filters.

4.12. Statistical Analysis

Data were expressed as mean ± SD. Comparison between groups was made by using SPSS statistical software (SPSS, USA) or Microsoft Excel (Microsoft, USA). Significant differences of measurement traits were analyzed using one-way analysis of variance (ANOVA) followed by Least significant difference (LSD) post hoc or Student’s t-test analysis. The significance was set at $P < 0.05$.

5. Results

5.1. Fenugreek Increased the IEC-6 Viability

To investigate the cytoprotective nature of fenugreek on DSS induced cell death, we evaluated the DSS dosage of 50% cell viability. Subsequently, we found 7% of DSS had optimum cell viability of 50%. Therefore, we chose 7% of DSS with different concentration of fenugreek 0.5, 1, and 2 µg/mL for the main experiment. All the doses of fenugreek were found to protect the IEC-6 cells, however, 2 µg/mL fenugreek had similar viability to that of control IEC-6 cells (Figure 1a, 1b).

5.2. Fenugreek Improved the Clinical Manifestations

To evaluate the protective effect of fenugreek against the development of colitis, clinical manifestations, such as changes in stool consistency and fecal blood score were observed and DAI score was calculated. Experimental animals showed no mortality. In DSS group, more than 60% of the mice developed loose stools after day 11, subsequently to diarrhea and gross bleeding in the most of the mice at day 12 and persisted until the end of the experiment. However, DF5 and DF10 groups significantly decreased diarrhea and gross bleeding from day 10 to day 14. Altogether, the DAI score was significantly increased from day 10 until the end of the experiment compared to DSS group (Figure 2a, 2b, 2c). CTL and F10 groups showed no significant difference in body weight, stool consistency, fecal blood, and DAI score.

5.3. Fenugreek Increased the Colon Length and Decreased the Spleen Weight

To investigate the colon protective effect of fenugreek, the colon and spleen of mice from each group were harvested after the sacrifice and the colon length from the cecum to the anus and colon weight was measured. In DSS group, the colon length was significantly shortened, colon weight/length ratio was increased (Figure 3a, 3b, 3c) and spleen weight was significantly increased (Figure 3d, 3e) compared to CTL group. However, DF5 and DF10 groups significantly increased the colon length; colon weight/length ratio was decreased and significantly decreased the spleen weight. The colon length, weight/length ratio of F10 group had no significant difference compared to CTL group.
5.4. Fenugreek Increased Red Blood Cells, Hemoglobin and Decreased Neutrophil, Monocytes, Lymphocytes, and Platelets

The protective effect of fenugreek on red blood cells, hemoglobin, neutrophil, monocytes, lymphocytes, and platelets were analyzed by automated blood-cell analyzer. In DSS group red blood cells hemoglobin decreased and neutrophil, monocytes, lymphocytes, platelets were significantly increased compared to CTL group. RBC parameters increased and inflammatory cells decreased in DF5 and DF10, compared to DSS group (Figure 4a, 4b, 4c, 4d, 4e, 4f). CTL and F10 showed no significant difference in the hematological parameters.

5.5. Fenugreek Attenuated Submucosal Ulceration with Inflammation, Crypt Abscesses, and Tissue Injury

To evaluate the protective effect of fenugreek against ulcerative colitis, the histopathological parameters of the colonic mucosa were investigated using microscope. CTL and F10 groups showed a normal colon mucosa with an intact epithelium, and no inflammatory infiltrations. However, the DSS group exhibited marked inflammation, edema in the lamina propria mucosa, disappearance of glandular epithelium, inflammatory cell infiltration, and crypt abscesses compared to CTL group. DF5 and DF10 groups depicted decreased inflammatory cell infiltration, mucosal edema, and abnormal crypt cells (Figure 5a, 5a).

5.6. Fenugreek Maintained Mucin Barrier of the Colon

To investigate the protective effect of fenugreek on DSS-induced neutral and acidic mucin, the neutral and acidic mucin staining was performed. PAS and Alcian blue-PAS staining revealed a significant increase in the acidic mucin and decrease in neutral mucin in the DSS group compared to CTL group. However, in DF5 and DF10 groups the colon section depicted significant decrease in the acidic mucin and increase neutral and compared to DSS group. CTL and F10 groups showed no change in the staining of PAS and Alcian blue-PAS (Figure 5b, 6b).

5.7. Fenugreek Decreased Mast Cell Recruitment

The role of fenugreek on mast cell recruitment was investigated by toluidine blue staining. In DSS group mast cell count was increased significantly increased compared to CTL group. Mast cell count decreased significantly in DF5 and DF10, compared to DSS group (Figure 5c, 6c). CTL and F10 showed no significant alterations in mast cell count.

5.8. Fenugreek Inhibited Pro-Inflammatory Cytokine Production and Stimulated Anti-Inflammatory Cytokine Production

To evaluate the effect of fenugreek on DSS-induced inflammatory cytokine, IL-1β, IL-6, TNF-α and IL-10 levels in colon tissue were assessed. A significant increase in the level of TNF-α, IL-1β, and IL-6 (Figure 7a, 7b, 7c) and IL-10 (Figure 7d) decreased in DSS group compared to CTL group. The level of TNF-α, IL-1β and IL-6 were significantly decreased and IL-10 was significantly increased in DF5 and DF10 groups compared to DSS group. The level of pro- and anti-inflammatory cytokines were unchanged.
between CTL and F10. Immunohistochemical expression of IL-6 was significantly increased in DSS group relative to CTL. A significant decrease was observed in DF5 and DF10 groups compared to DSS group (Figure 6d).

5.9. Fenugreek Attenuated MUC1 Expression

MUC1 immunofluorescence was significantly increased in the DSS group compared with CTL group. MUC1 expression in CTL and F10 groups depicted only on the mucosal villi, because MUC1 is a membrane-associated type of mucin. However, in DSS group the MUC1 expression was found all over the colon, including lamina propria mucosa, glandular epithelium, and the crypt. In DF5 and DF10, MUC1 immunofluorescence (Figure 8) were significantly decreased relative to DSS group.

6. Discussion

The aim of conventional UC therapies is primarily to attenuate abnormal immune responses and inflammatory cascades, but the efficacy is still not satisfactory. The use of natural products, such as herbal medicines, for the treatment of UC has steadily increased. Natural products as a source of potential candidates for complementary and alternative medicines that use scientifically verified is rapidly increasing over the last decades [20]. In this study, we focused on the natural product fenugreek in diet, instead of a single isolated active component, because the assortment of active components of fenugreek in food might be a good remedy for a multifaceted disease such as UC. The chemical constituents of fenugreek are fibers, flavonoids, polysaccharides, saponins, flavonoids and polysaccharides, fixed oils, alkaloids, and choline [22]. It contains five different flavonoids, namely, vitexin, tricin, naringenin, quececin, and tricin-7-O-β-D-glucopyranoside [23]. In addition, it contains tannic acid, volatile oils, diosgenin, trigonelline, trigocoumarin, trigomethylcoumarin, and steroidal saponin such as gitogenin and traces of trigogenin and vitamin A [14]. The soluble dietary fiber fraction of fenugreek increased gastrointestinal motility [24]. Therefore, we hypothesize fenugreek is a potential candidate to attenuate inflammation related tissue injury in UC.

Fenugreek diet consumption increased colon length, but decreased colon weight/length ratio, spleen weight, diarrhea, fecal bleeding, and DAI score in DSS-induced UC. Administration of 3% DSS in drinking water induced diarrhea followed by bloody stool and increased DAI score indicating severe UC manifestations. In addition, colon shortening, increased colon weight/length ratio, and spleen enlargement were found. These clinical manifestations of UC might be due to highly negative charged DSS, which is toxic to the colonic epithelia and induces ulceration that ultimately compromise barrier integrity resulting in increased colonic epithelial permeability. In addition, its anticoagulant property exacerbates intestinal bleeding. DSS administration within 3-4 days showed severe rectal bleeding and anemia. Rectal bleeding is associated with thinning and shortening of the colon and enlargement of the spleen [25]. Dietary fenugreek decreased the diarrhea that followed rectal bleeding and bloody stools, thereby decreasing the UC manifestation by DAI score. In addition, dietary fenugreek attenuated colon shortening, decreased colon weight/length ratio and spleen enlargement. Fenugreek is used as antipyretic and diuretic and to treat liver and spleen enlargement [26].

Fenugreek increased red blood cells and hemoglobin in DSS-induced UC. In DSS-induced UC red blood cells and hemoglobin decreased due to rectal bleeding. Significant blood loss also indicates disease severity of UC. UC causes substantial alterations in the marrow and the thymus with substantial reductions in erythrocytic lineages that would contribute to anemia with significantly reduced numbers of red blood cells and blood hemoglobin [19, 27]. Fenugreek seeds are rich source of dietary fiber. About 28% mucilage in fenugreek [14], may partly be responsible for the colon protection. Dietary fiber and mucilage are fermented by gut microbes to produce short chain fatty acids (SCFAs) that include butyric acid, propionic acid and acetic acid; these SCFAs protects, repairs, and maintains colon cells by modulating inflammation [28]. Fenugreek also contains...
Dietary fenugreek decreased neutrophils, monocytes, lymphocytes, platelets and mast cell recruitment in DSS-induced UC. In IBD patients, circulating neutrophils are activated and released more proteases to activate resident immune cells which play an important role in inflammation. In addition, animal model DSS-induced colitis is characterized by a large influx of neutrophils [31]. Colonic infiltration of neutrophils, monocytes, and macrophages are the major contributor of tissue injury, including goblet cell depletion, crypt abscesses and inflammation in IBD. The migration of circulating immune cells from bone marrow to colon in large numbers increases pro-inflammatory cytokine production to exacerbate and extend the ongoing inflammation. Mast cells are involved in the recruitment of neutrophils in DSS-induced colitis [19, 27, 32, 33]. Human IBD is typically manifested by 50-70% increase in circulating platelet count. In DSS-induced colitis increased number of circulating activated platelets along with the formation of aggregate of leukocytes, neutrophils and monocytes with platelets were found [34]. Mast cells were increased in the mucosal biopsies of UC patients. Mast cells can release cytokines, such as TNF-α and IL-10, that contribute to inflammatory damage. It contains histamine, heparin, serine proteases such as chymase and tryptase, and multifunctional cytokines, which are important factors in the wound-healing process [35, 36]. Fenugreek decreased the circulating immune cells count, it might be due to immuno-modulating function of fenugreek saponins. In addition, fenugreek possess antioxidant properties and induce the immunostimulant effect and immunomodulatory properties [14, 37]. Its flavonoid-rich fractions that contain trigonelline content is active anti-inflammatory in nature [38]. Fenugreek seed 100 mg/ kg dose was most pharmacologically effective dose in inducing the immune functions and immunomodulatory effect [14]. Fenugreek regulated the activation of circulating immune cells, thereby regulated the immune functions and immunomodulatory effect; leading to inhibition of inflammation related tissue injury in DSS-induced UC.

Dietary fenugreek modulated inflammatory cytokines in DSS-induced UC. Inflammatory cytokines, IL-1β, IL-6, and TNF-α were increased and IL-10 was decreased in DSS-induced colitis. UC mucosal barrier properties and epithelial layers were altered. These alterations allow luminal toxins, pathogens, and antigens penetrate the intestinal mucosa and provoke an overproduction of pro-inflammatory cytokines and trafficking of effector leukocytes into the intestinal mucosa, ultimately leading to uncontrolled and exaggerated intestinal inflammation. Thus, in DSS-induced UC, during the course of inflammation, neutrophils accumulate in epithelial crypts and stimulate pro- and anti-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-10 [18, 34, 39]. Dietary fenugreek attenuated the production of TNF-α, IL-1β, and IL-6 and up-regulated IL-10 expression. Fenugreek seed extracts contain steroidal saponins 26-O-β-D-glucopyranosyl-(25 R)-furost-5(6)-en-3β,22β,26-triol-3-O-α-L-rhamno-pyranosyl-(1”→2”)-O-[β-D-glucopyranosyl-(1”→6”)-O]-β-D-glucopyranoside, minutoside B, and pseudoprotodioscin strongly suppressed the production of inflammatory cytokines [40]. In addition, fenugreek administration markedly lowered Th2 cytokines, IL-6, IL-15, and TNF-α and inflammatory cells. Fenugreek up-regulated the Th1 cytokine IL-10. It regulated the balance of Th cells and attenuated the expression of Th2 cytokines may lead to anti-inflammatory effect and modulating the balance of the Th1/Th2 cytokines [7]. Fenugreek seeds and its mucilage decreased IL-1β, IL-6, and TNF-zleivels [29, 30, 41]. Fenugreek regulated the balance of circulating immune cell population, thereby regulated the expression of pro- and anti-inflammatory cytokines; ultimately attenuated the inflammation and tissue injury in DSS-induced UC.

Dietary fenugreek decreased the expression of MUC1 in DSS-induced UC. The toxicity of DSS affects the integrity of the mucosal barrier through gut epithelial cells of the basal crypts in DSS-induced UC [42]. MUC1, membrane-associated mucin expressed in the apical membrane of goblet cells in the colon, responsible for epithelial restitution [43]. MUC1 offers protective and lubricates epithelial cells. Its cytoplasmic tail is an active pivot point for multiple signaling interactions and functions [44, 45]. MUC1 expression is also induced by inflammatory cytokines, including TNF-α and IL-6 [46]. MUC1 is a functional analog of cytokine receptors [47]. In the present study, fenugreek decreased the overall mucins. In addition, it decreased the expression of MUC1 indicating that fenugreek mucilage might be involved in epithelial restitution of the colonic mucosa and attenuates further expression and secretion of MUC1. In addition, MUC1 is a functional analog of cytokine receptors, fenugreek attenuated inflammatory cytokine production; thereby decreasing the further inflammatory process. Thus, fenugreek protects and repairs the mucous barrier and helps healing colon. Together, dietary fenugreek showed the potential to attenuate inflammation to protect against DSS-induced UC.
7. Acknowledgment

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