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Cirsimaritin Alleviates Dextran Sodium Sulfate-Induced Acute Colitis in Experimental Animals: A Therapeutic Approach for Inflammatory Bowel Disease

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ABSTRACT: Inflammatory bowel disease (IBD) is a chronic disease that affects the entire digestive tract. IBD can be classified as ulcerative colitis or Crohn's disease. The key symptoms of IBD include the emergence of abscesses or pustules, pronounced abdominal discomfort, diarrhea, fistulas, and intestinal narrowing, all of which can greatly affect a patient's daily well-being. Several factors, including bacterial infections, immune response irregularities, and changes in the intestinal milieu, can contribute to the onset of IBD. The aim of this study was investigating the role of cirsimaritin in reducing the severity of colitis in animal model. To induce colitis in laboratory Swiss albino mice, a 4% dextran sulfate sodium (DSS) concoction was provided in their hydration source for a duration of six days. Before the onset of colitis, mice were treated with cirsimaritin (10 mg/kg) once daily to evaluate its potential treatment effects against DSS-induced inflammation. The results showed that 10 mg/kg of cirsimaritin decreased colitis severity ($P<0.05$). Moreover, cirsimaritin successfully reversed the detrimental effects induced by DSS, including weight reduction, colon truncation, tissue-related damage, increased levels of inflammatory cells in the affected region, and secretion of proinflammatory cytokines. Our findings suggest that cirsimaritin can effectively alleviate acute colitis triggered by DSS.

Keywords: cirsimaritin, colitis, colon, dextran sulfate, inflammation

INTRODUCTION

Inflammatory bowel disease (IBD) affects the entire digestive system, encompassing the large intestine. IBD is divided into ulcerative colitis (UC), which involves inflammation localized to the colon, and Crohn's disease (CD), which leads to inflammation throughout the digestive system. The predominant symptoms of IBD include abscesses or pustules, acute abdominal discomfort, diarrhea, fistulas, and intestinal narrowing, which greatly affect patients' daily well-being. Recently, IBD cases have increased in Europe, North America, and Asia, increasing from 3.6 million in 1990 to 6.8 million in 2017 (M'Koma, 2013; Alatab et al., 2020; Perri et al., 2021). The typical manifestations of IBD include abdominal discomfort, bloody stools, fever, decreased appetite, and weight loss (Torres et al., 2017; Hastings, 2020). However, the exact genesis and fundamental processes associated with the emergence of IBD remain to be elucidated. Current evidence points toward disruptions in immune functioning, environmental elements, and genetic factors as potential factors that accelerate these inflammatory gastrointestinal disorders (Bernstein et al., 2008; Betteridge et al., 2013; Alavala et al., 2019).

Diseases marked by inflammation in the colon and digestive system are evolving conditions that complicate clinical treatment (Mowat et al., 2011). Various elements, including microbial invasion, dysfunctional immune reactions, and intestinal milieu, can act as catalysts for these disorders. However, the exact triggers and intricate processes underpinning IBD remain unknown (Jang et al., 2019). Regulatory T cells (Tregs) are instrumental in

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preserving immunological equilibrium. Previous studies found that Tregs are key to suppressing the inflammatory burst seen in colitis, mainly by curtailing the surge of inflammatory cytokines (Eastaff-Leung et al., 2010). They also modulate the functions of cells that trigger inflammation. A shift in the equilibrium between $CD4^+$ forkhead box $P3^+$ effector cells and Tregs in the intestine is a key marker in the progression and emergence of inflammatory disorders (Mirlekar et al., 2015). Currently, the treatments for gastro-inflammatory conditions include anti-inflammatory agents, immunosuppressants, and biologically derived treatments (Patel et al., 2009; Ramakrishna et al., 2015; Kedia and Ahuja, 2017). However, several medications and strategies fall short for many patients, resulting in unsatisfactory outcomes, undesirable reactions, and an increased likelihood of colitis relapse. Hence, current studies are exploring potential herbal medications that offer efficacy and safety against IBD.

Cirsimaritin is a dimethoxyflavone (Fig. 1) that has been identified in various plants including *Lithocarpus dealbatus*, *Artemisia judaica*, *Microtea debilis*, *Cirsium japonicum*, and *Ocimum sanctum* (Park et al., 2017). According to literature, cirsimaritin has many biological properties, including antimicrobial, antispasmodic, antidiabetic, and antiproliferative activities (Rijo et al., 2009; Pathak et al., 2021). Rosemary leaf extracts that are rich in cirsimaritin have been found to have potent antioxidant capabilities (Ibañez et al., 2003; Jipa et al., 2009). In addition, cirsimaritin can suppress inflammation, which is marked by the restriction of nitric oxide synthesis, inhibition of nitric oxide synthase expression, and decreased expression of cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α (Shin et al., 2017; Alqudah et al., 2023). To the best of our knowledge, no study has comprehensively investigated the therapeutic effects of cirsimaritin on colitis and related inflammation. Therefore, the present study investigated the potential of cirsimaritin in combating dextran sulfate sodium (DSS)-induced colitis in animal models.

This study was designed to investigate the effect of cirsimaritin in reducing the severity of inflammation in the colons of animal models after the induction of inflammation by DSS. DSS has been frequently employed to simulate colitis in animal test subjects, causing epithelial disarray, immune cell infiltration, and colon inflammation (Randhawa et al., 2014). This study aimed to determine the impact of cirsimaritin ingestion on the clinical outcomes induced by DSS, including weight reduction, colon truncation, tissue-related damage, increased levels of inflammatory cells in the affected region, and secretion of proinflammatory cytokines.

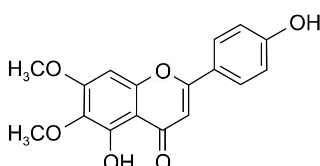

Fig. 1. Chemical structure of cirsimaritin.

MATERIALS AND METHODS

Materials

Various substances were used in this study, including cirsimaritin, DSS, sulfasalazine, and enzyme-linked immunosorbent assay (ELISA) kits. All items were obtained from Sigma-Aldrich.

Animal model

Male Swiss albino mice, specifically chosen at 7 weeks of age weighing 22∼25 g each were used in this study. The mice were accommodated in specially designed polypropylene cages, which were placed in a meticulously controlled laboratory environment. The set conditions included an average temperature of approximately 26°C, with fluctuations of no more than ±1°C. The ambient humidity was consistently maintained between 60% and 70%. Furthermore, we employed a 12-h light-dark cycle for their well-being. The animals were fed with standard rat pellets and given unrestricted access to water. As an essential part of our procedure, the mice were allowed to become accustomed to their new surroundings for 1 week before the core experiments began.

Ethics approval

The animal experimental procedures were approved by the Animal Ethics Committee at The Hashemite University (IRB number: 14/4/2021/2022, April 14, 2022) and were in accordance with the guidelines of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.

Study design

The mice were systematically divided into four distinct groups, with each group having six mice. The mice in Group I served as control subjects and continued their standard diet (negative control). The mice in Group II were induced with UC (positive control). Induction was achieved by providing mice with a concoction containing 4% DSS in their drinking water for 6 days (Sahu et al., 2016). The mice in Group III were given a regimen of cirsimaritin, which was administered at a daily dose of 10 mg/kg body weight (BW) through gavage, before they were exposed to DSS. The dose of cirsimaritin was chosen on the basis of an initial pilot study that evaluated varying cirsimaritin doses: 5, 10, 15, and 20 mg/kg BW. We

observed that 10, 15, and 20 mg/kg BW doses of cirsimaritin showed a notable difference compared with the control group. A smaller dose was selected to reduce the incidence of toxicity (data not shown). By contrast, the mice in Group IV received a reference drug, sulfasalazine (50 mg/kg/d), via gavage before being exposed to DSS. Once all the experimental steps were complete, each mouse was anesthetized, and their colons were extracted for more in-depth analysis.

Determination of disease severity

The cornerstone of our disease severity assessment was a methodology described in detail by Medicherla et al. (2016). Using their approach, we formed a disease activity index (DAI). This index was constructed by closely observing and documenting particular symptoms, including variations in the BW of subjects, changes in the nature of their feces, any instances of bloody feces, and results from a guaiac test to identify occult blood. Each of these symptoms was assigned a score. The DAI was calculated as the sum of BW loss (scored as follows: 0, none; 1, 1%～5%; 2, 5%～10%; 3, 10%～20%; 4, over 20%), presence or absence of fecal blood (scored as follows: 0, negative hemoccult; 2, positive hemoccult; 4, gross bleeding), and stool consistency (scored as follows: 0, well-formed pellets; 2, loose stools; 4, diarrhea).

Assessment of myeloperoxidase (MPO) activity

The MPO activity is essential in understanding the extent of neutrophil infiltration. To determine the MPO activity, we adopted a procedure detailed by Sahu et al. (2016). Colon tissue samples were homogenized in 0.1 M potassium phosphate buffer (pH 6.5) containing 0.5% hexadecyltrimethylammonium bromide using a polytron homogenizer. The homogenates were subjected to a brief sonication for 10 s and then centrifuged at 13,100 *g* for 20 min at 4°C. The supernatant (0.1 mL) was mixed in a reaction solution (2.9 mL) containing 0.167 mg/mL of *o*-dianisidine hydrochloride and 0.0005% hydrogen peroxide, and the rate of absorbance change over 5 min was measured spectrophotometrically at 460 nm.

Quantification of proinflammatory cytokines

The colon samples were finely homogenized using a unique phosphate buffer. This buffer was enhanced with a protease enzyme inhibitor cocktail. Once processed, the blend was centrifuged at 16,000 *g* for 30 min at 4°C and segregated into phases. We used the superior phase to determine the levels of critical proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, by employing mouse-specific ELISA kits for accuracy in accordance with the manufacturer's instructions.

Western blot assay for the evaluation of protein expression

To determine protein expression in colon tissue samples, a series of steps was performed. Initially, the tissues were washed in buffered saline twice. Thereafter, the tissue suspensions were centrifuged at 490 *g* for 10 min. After centrifugation, the gathered pellet was combined with radioimmunoprecipitation assay buffer augmented with protease inhibitors to facilitate cell lysis. The resultant lysed suspensions were again subjected to centrifugation at 20,000 *g* for 20 min. The upper phase after centrifugation was designated for protein quantification, in accordance with the method of Lowry et al. (1951). Next, 50 μg of the isolated proteins were placed onto a 10% sodium dodecyl sulfate gel for separation. After separation, the proteins were transitioned onto polyvinylidene difluoride membranes. Before any treatment, the membranes were blocked using a 5% solution of bovine serum albumin (BSA) for 1 h. The subsequent step involved incubating these membranes with target-specific primary antibodies, specifically matrix metalloproteinase-9 (MMP-9, ab76003, Abcam; dilution 1:1,000), nuclear factor-κB (NF-κB, ab16502, Abcam; dilution 1:1,000), cyclooxygenase-2 (COX-2, ab15191, Abcam; dilution 1:1,000), and inducible nitric oxide synthase (iNOS, ab3523, Abcam; dilution 1:1,000). This antibody solution was prepared using 5% BSA in a base of Tris-buffered saline, complemented with 0.05% Tween-20, and was allowed to react overnight. After treatment with the primary antibodies, the membranes were exposed to a secondary antibody conjugated with horseradish peroxidase enzyme. The incubation lasted 2 h and was conducted at 37°C. In the final phase, the membranes were exposed to an advanced chemiluminescence technique to detect horseradish peroxidase. Quantitative analysis of these blots was accomplished using ImageJ software (National Institutes of Health).

Histopathological examination of the colon

After the experimental phase, the colon of each mouse was extracted, carefully cleaned, and segmented. The preserved segment was then processed, sectioned, and stained with hematoxylin and eosin. Under a microscope, we meticulously examined the sections for indications of tissue anomalies or signs of inflammation.

Statistical analysis

All gathered data were subjected to rigorous statistical evaluation. The results, represented as mean values accompanied by standard error of the mean, were subjected to one-way analysis of variance. Subsequently, the Tukey-Kramer *post hoc* test was used to further scrutinize the data. For all our results, a significance benchmark of $P < 0.05$ was set as the criterion for drawing meaningful conclusions.

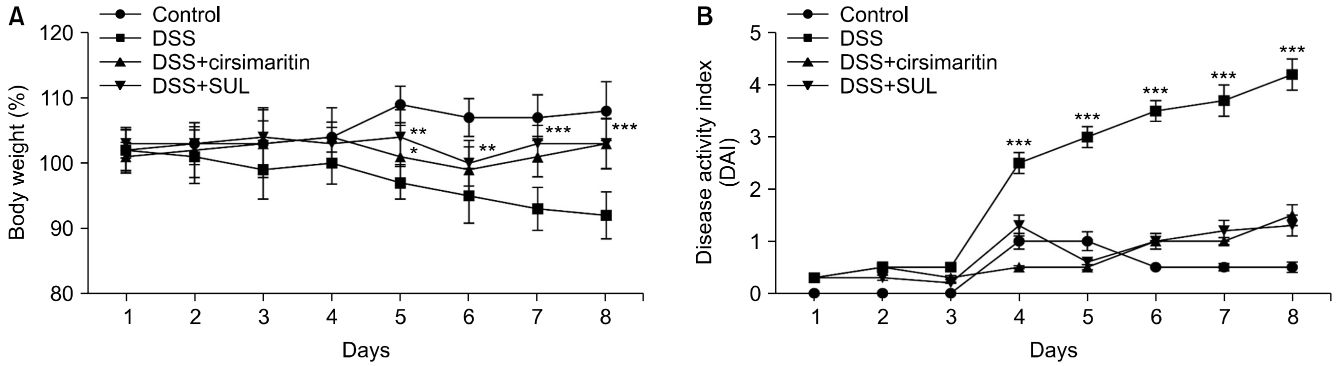

Fig. 2. Effects of cirsimaritin on body weight (A) and disease activity index (B) in a mouse model of induced colitis. The data present the mean value±SEM from a group of six animals (n=6). Significant variances between the control group and other groups at significance levels of *P<0.05, **P<0.01, and ***P<0.001, respectively, as assessed by Tukey-Kramer *post hoc* test. DSS, dextran sulfate sodium; SUL, sulfasalazine.

RESULTS

Cirsimaritin maintains BW and reduces DAI in mice with DSS-induced colitis

Mice exposed to DSS exhibited significantly lower BW compared with the control group (P<0.05). In parallel, mice treated with cirsimaritin (10 mg/kg) showed significantly higher BW compared with the DSS-treated group (P<0.05; Fig. 2). This finding was similar to that in the sulfasalazine group (50 mg/kg), which showed efficacy against DSS-triggered colitis, reflecting a notable increase in BW (P<0.05). The colitis severity score (DAI), which included changes in BW, stool consistency, and the presence of blood in stools, was assessed to evaluate disease severity. In mice exposed to DSS, the clinical signs of colitis markedly increased after day 4 (DAI score: 2.46±0.19) and reached maximum severity (DAI score: 4.30±0.11) on day 8. Mice treated with cirsimaritin had lower DAI score (1.66±0.08) compared with the DSS-treated group (Fig. 2B).

Cirsimaritin improves colon dimension and mass in mice with DSS-induced colitis

DSS exposure led to a considerable decline in colon weight and length compared with untreated mice (P<0.05; Fig. 3). However, the colon weight and length were markedly improved in mice treated with cirsimaritin (10 mg/kg) (P<0.05) compared with the DSS-treated group. Similarly, sulfasalazine treatment revealed a prominent therapeutic action against DSS-induced colitis, resulting in a discernible growth in colon dimensions (P<0.05; Fig. 3).

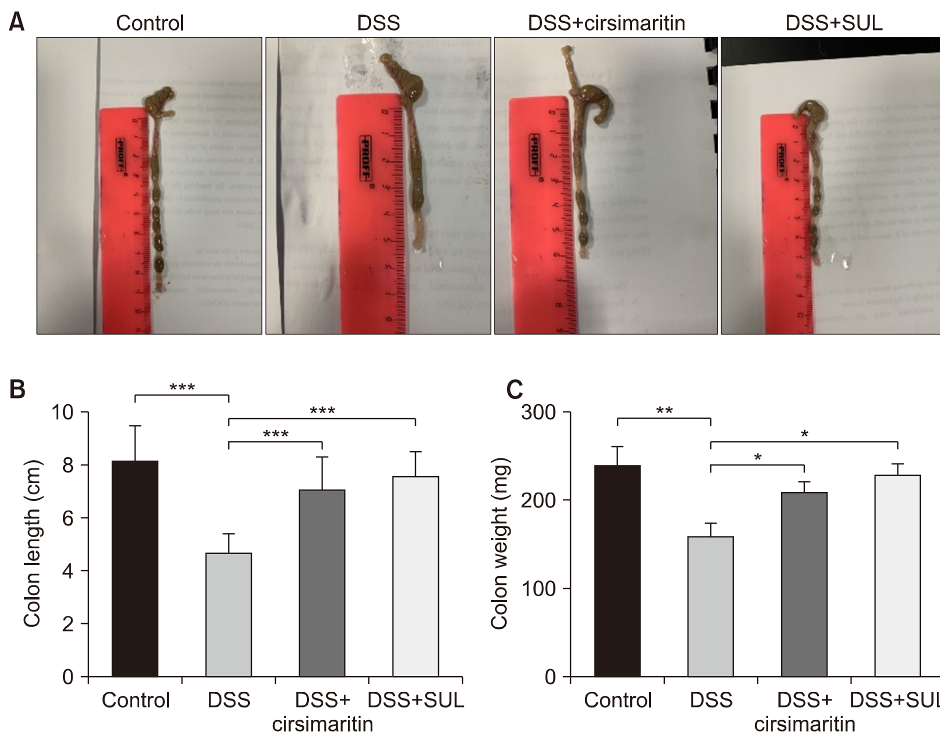

Fig. 3. The effects of cirsimaritin on colon length (A and B) and weight (C) within a colitis experimental model. The data is presented as mean value±SEM from a group of six animals (n=6). Notable differences between the control and other groups at significance levels of *P<0.05, **P<0.01, and ***P<0.001, respectively, based on Tukey-Kramer *post hoc* test. DSS, dextran sulfate sodium; SUL, sulfasalazine.

Cirsimaritin reduces MPO activity in mice with DSS-induced colitis

Mice with DSS-induced colitis showed increased MPO activity (P<0.05; Fig. 4A) compared with controls. This increase in MPO activity underscored increased inflammation and oxidative stress. However, treatment with cirsimaritin (10 mg/kg) remarkably reduced MPO activity (P<0.05; Fig. 4A). This reduction was more evident in the cirsimaritin-treated group than in the DSS-treated group. Sulfasalazine (50 mg/kg) also effectively curbed MPO activity in mice (P<0.05; Fig. 4A). The data suggest cirsimaritin's potential use in improving DSS-induced inflammation.

Cirsimaritin reduces proinflammatory markers in mice with DSS-induced colitis

DSS-induced colitis increased the levels of inflammatory cytokines in mouse colon cells. The administration of cirsimaritin (10 mg/kg) markedly reduced the levels of cytokines, especially TNF-α, IL-1β, and IL-6 (P<0.05; Fig. 4B~4D). Furthermore, cirsimaritin administration led to a decrease in cytokines compared with the DSS-treated group (Fig. 4B~4D). Likewise, treatment with 50 mg/kg of sulfasalazine also decreased the levels of proinflammatory markers compared with the DSS-treated group (P<0.05; Fig. 4B~4D).

Cirsimaritin reduces MMP-9, NF-κB, COX-2, and iNOS in mice with DSS-induced colitis

In DSS-treated mice, the immunoreactivity of MMP-9, NF-κB, COX-2, and iNOS was increased. This finding shows the proinflammatory effect of DSS on animals (P< 0.05; Fig. 5 and 6) relative to controls. However, treatment with 10 mg/kg of cirsimaritin remarkably decreased the immunoreactivity associated with inflammation markers such as MMP-9, NF-κB, COX-2, and iNOS (P<0.05; Fig. 5 and 6). Similarly, treatment with 50 mg/kg of sulfasalazine also reduced the expression of these inflammation-signaling proteins compared with mice in the DSS-induced colitis group (P<0.05; Fig. 5 and 6).

Cirsimaritin preserves the histological integrity of colon tissues

Histological examination of colonic tissues from DSS-treated mice revealed profound ulceration and inflammatory cell infiltration compared with controls. Treatment with cirsimaritin (10 mg/kg) exhibited a potent protective effect (P<0.05), preventing the harmful tissue alterations induced by DSS (20×, Fig. 7). Similarly, treatment with sulfasalazine (50 mg/kg) also showed protective effects against these tissue changes induced by DSS exposure.

DISCUSSION

IBD is a growing health issue worldwide (Kaplan, 2015; Tontini et al., 2015). In the USA, the incidence of IBD has increased from 0.9% in 1999 to 1.3% in 2015. IBD is more common in colder, developed countries than in warmer regions (Dahlhamer et al., 2016). It includes CD, UC, and other types, causing chronic gastrointestinal inflammation that often requires surgical treatment (Hanauer, 2006; Bewtra et al., 2007). Doctors typically recommend anti-inflammatory and steroid-based therapy, but their extended use may lead to severe side effects (Varga et al., 2018). Our study explored the ther-

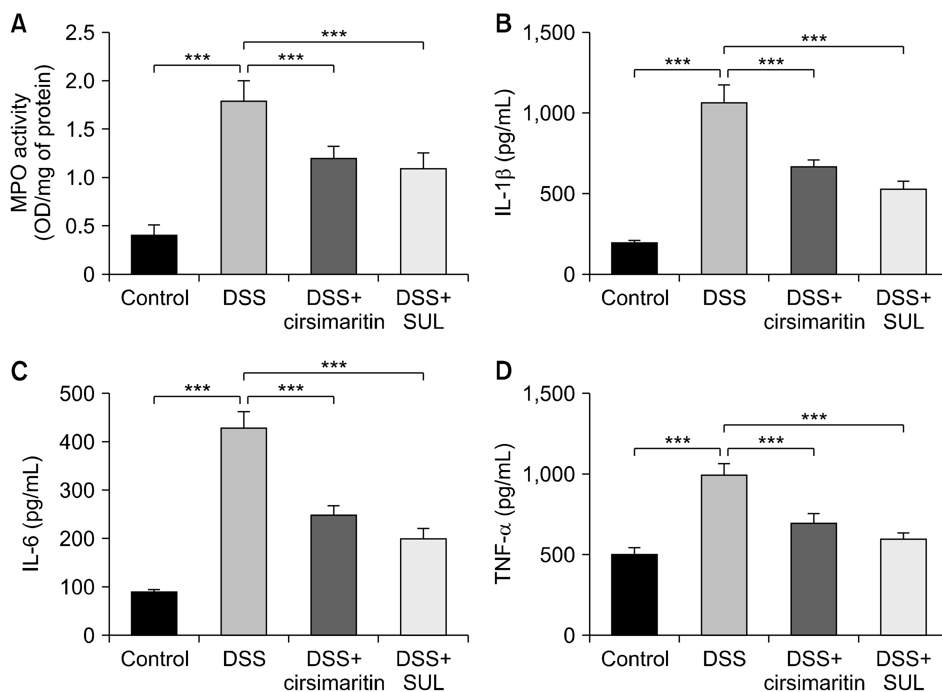

Fig. 4. The effects of cirsimaritin on myeloperoxidase (MPO) (A) activity and specific proinflammatory cytokines [B, interleukin (IL)-1β; C, IL-6; and D, tumor necrosis factor (TNF)-α] in a mouse model of induced colitis. The data is showcased as mean value±SEM from a group of six animals (n=6). A significant difference between the control and the other groups at a significance level of ***P<0.001, as evaluated by Tukey-Kramer *post hoc* test.

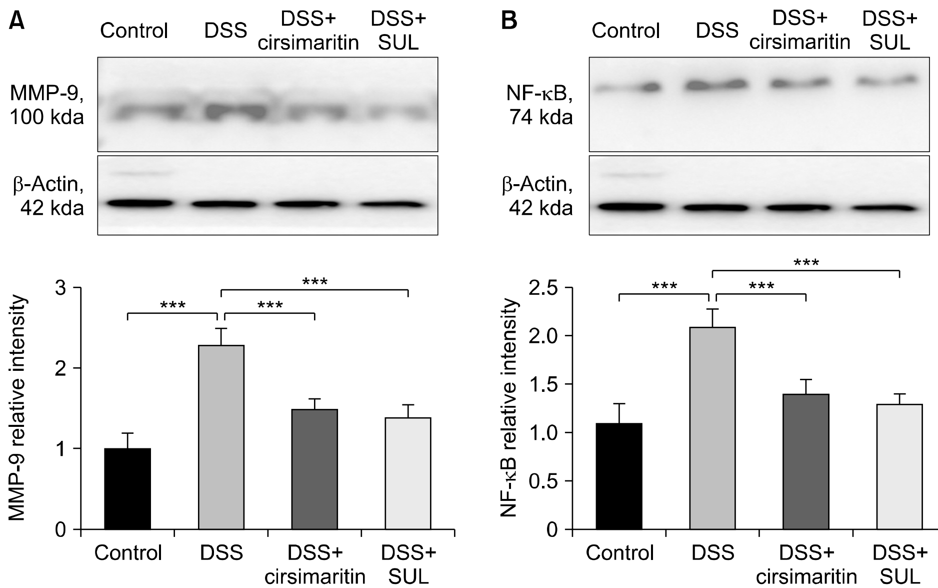

Fig. 5. Effects of cirsimaritin on the expression of prominent inflammatory markers, matrix metalloproteinase-9 (MMP-9) (A) and nuclear factor-κB (NF-κB) (B), in the colon cells of mice with dextran sulfate sodium (DSS)-induced colitis. The data present the mean value±SEM derived from a group of six animals (n=6). A statistically significant difference between the control and experimental groups at a significance level of $^{***}P<0.001$. SUL, sulfasalazine.

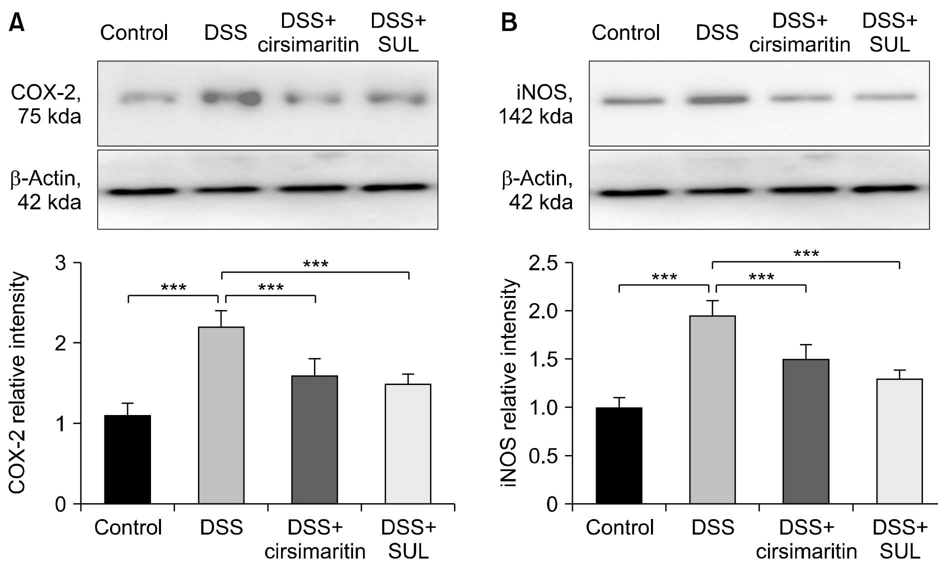

Fig. 6. Effects of cirsimaritin on the expression of critical inflammatory markers, cyclooxygenase-2 (COX-2) (A) and inducible nitric oxide synthase (iNOS) (B), in the colon cells of mice with dextran sulfate sodium (DSS)-induced colitis. The data present the mean value±SEM from a group of six animals (n=6). A statistically significant difference between the control and experimental groups at a significance level of $^{***}P<0.001$. SUL, sulfasalazine.

apeutic potential of cirsimaritin. Combined with certain extracts, cirsimaritin, known for its various biological effects (Pathak et al., 2021; Alqudah et al., 2023), exhibits potent antioxidant capabilities (Ren et al., 2019), which might mitigate the markers of DSS-induced IBD. In this study, cirsimaritin reversed the detrimental effects induced by DSS, including weight reduction, colon truncation, tissue-related damage, increased levels of inflammatory cells in the affected region, and secretion of pro-inflammatory cytokines.

MPO, an enzyme in neutrophils and monocytes, helps produce reactive oxygen species (ROS) (Takeshita et al., 2006; Galijasevic, 2019). High levels of MPO indicate severe colitis in patients with IBD (Sangfelt et al., 2001; Peterson et al., 2002; Wagner et al., 2008). Our data showed that DSS exposure increased MPO levels; however, these effects were reversed following treatment with cirsimaritin, suggesting its protective effects. Immune system imbalances, which are influenced by microbial flora, can initiate IBD (Abraham and Cho, 2009). Different T-helper cells are involved in CD and UC (Papadakis and Targan, 2000; Korn et al., 2009). Bacterial flora induce IBD by disrupting cells and increasing the expression of inflammation-promoting cytokines (Marín et al., 2013; Lee et al., 2018). High TNF-α levels have been reported in patients with IBD (Singh et al., 2016). In the present study, we assessed the levels of these cytokines in DSS-induced IBD mice treated with cirsimaritin. We found that cirsimaritin treatment considerably decreased the levels of these cytokines.

MMPs participate in tissue restructuring (Shapiro, 1998), and their levels are increased in patients with UC (Sykes et al., 1999; Santana et al., 2006; Lakatos et al., 2011). Among them, MMP-9 is associated with CD and UC (Meijer et al., 2007). An increase in MMP-9 expression has been observed in patients with ischemic colitis,

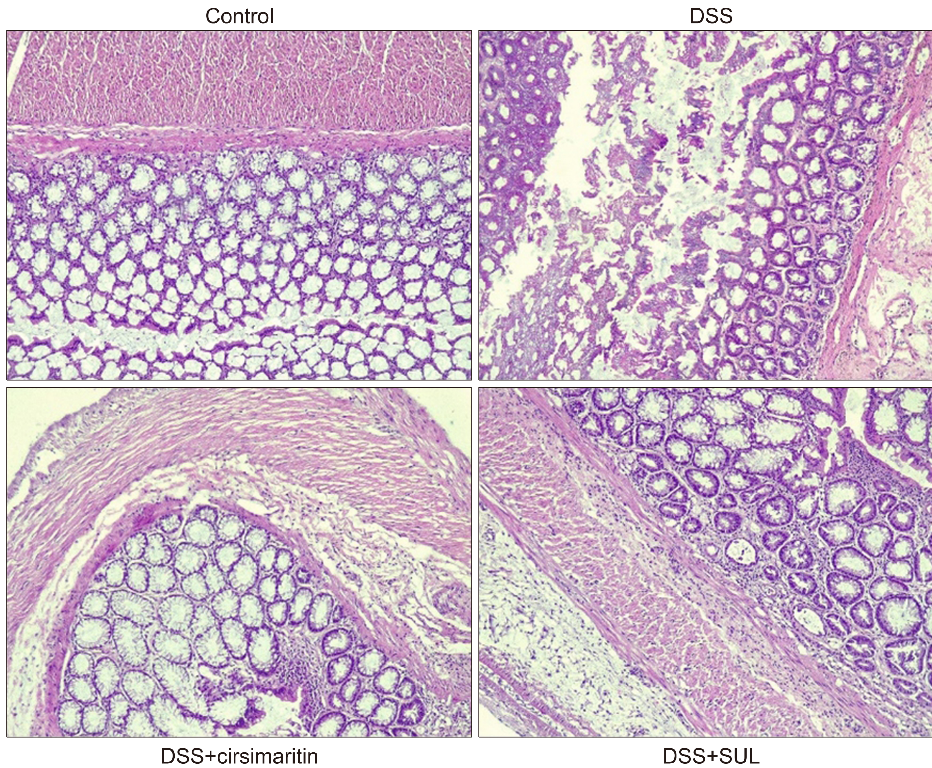

Fig. 7. Effects of cirsimaritin on histological alterations in the colorectal tissues of mice with dextran sulfate sodium (DSS)-induced colitis. The colon tissue sections from DSS-induced mice show the destruction of crypt structure with loss of goblet cells, disruption of the epithelial layer, and massive infiltration of inflammatory cells into the mucosa and submucosa with cryptic abscess. The colon tissue sections from DSS-induced mice treated with cirsimaritin show less evidence of cryptic damage with preserved goblet cells and epithelial lining and mild infiltration of inflammatory cells into the colon tissue. Colon tissues were stained using hematoxylin and eosin and then imaged (20X) using light microscope. SUL, sulfasalazine.

and it has also been reported in murine colitis models induced by toxicants such as DSS (Medina et al., 2006; Garg et al., 2009). In the present study, mice treated with DSS exhibited higher expression levels of MMP-9 protein compared with control mice, indicating the induction of colitis by DSS. Conversely, mice treated with cirsimaritin showed reduced MMP-9 expression. This finding suggests that cirsimaritin may have a protective effect against colitis by modulating MMP-9 levels. NF-κB is a central regulatory factor that plays a dual role in cell apoptosis and carcinogenesis. NF-κB governs various cellular processes, including the regulation of proinflammatory cytokines, chemokines, growth factors, and anti-apoptotic factors (Shen and Tergaonkar, 2009). When activated, NF-κB undergoes IκB phosphorylation, leading to dimer formation and translocation into the nucleus, where it initiates the transcription of proinflammatory mediators such as iNOS, COX-2, TNF-α, and IL-6 (Surh et al., 2001; Lappas et al., 2002). The increased expression of NF-κB induces proinflammatory cytokines, ultimately leading to the generation of ROS, which can trigger apoptosis (Nakano et al., 2006). In conditions such as colorectal cancer, iNOS and COX-2 are overexpressed, and colitis has been linked to ROS production. Therefore, inhibiting the NF-κB protein presents an attractive approach for treating colitis. Western blot analysis of colon extracts showed that cirsimaritin inhibited NF-κB expression. This inhibition, in turn, led to reduced levels of proinflammatory cytokines and decreased expression of iNOS and COX-2, effectively preventing inflammation induced by DSS in the colon. Furthermore, a recent study revealed that cirsimaritin exerts anti-inflammatory activity by inhibiting nitric oxide production, inducing the expression of nitric oxide synthase, and blocking various cytokines, including IL-6 and TNF-α (Benali et al., 2022).

Histopathological analysis of colon extracts from mice treated with DSS revealed a reduction in the length and thickening of the mucosal layer in the colon, along with an increased presence of inflammatory cells in the lamina propria. This phenomenon is likely attributed to the increased levels of MPO, which could have triggered the infiltration of neutrophils into the affected area. However, cirsimaritin demonstrated its effectiveness by inhibiting MPO production. In addition, it decreased the activation of NF-κB and the synthesis of proinflammatory cytokines. As a result, cirsimaritin effectively prevented colon inflammation induced by DSS in mice, mitigating the adverse effects observed in the mucosal layer and inflammatory cell infiltration.

The therapeutic efficacy of cirsimaritin in attenuating IBD inflammation is multifaceted, primarily involving the modulation of critical inflammatory pathways. Our study demonstrated that cirsimaritin significantly downregulated the expression of proinflammatory cytokines, including MMP9, NF-κB, COX-2, and iNOS, suggesting that cirsimaritin has a complex mechanism of action. Among them, the NF-κB signaling pathway is a pivotal regulator of inflammation, orchestrating the transcription of various inflammatory genes. The effect of cirsimaritin on NF-κB, alongside COX-2 (a key enzyme in the inflammatory process), suggests that its anti-inflammatory effects may be mediated through the inhibition of these

pathways. However, the precise mechanism likely involves a synergistic modulation of multiple pathways rather than a single dominant route. To further elucidate the mechanism of cirsimaritin, future investigations should focus on examining the expression levels of upstream, intermediate, and downstream regulatory factors within these pathways. Such studies will provide deeper insights into the molecular underpinnings of the therapeutic potential of cirsimaritin in IBD.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: AA, MW. Analysis and interpretation: EQ, OG. Data collection: YB. Writing the article: AA. Critical revision of the article: AA. Final approval of the article: all authors. Statistical analysis: MA. Obtained funding: AA. Overall responsibility: TH.

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