Protective effect of myricetin in dextran sulphate sodium-induced murine ulcerative colitis

JING ZHAO[1], TIE HONG[1], MAN DONG[1], YIXIAO MENG[2] and JIAYE MU[2]

[1]Department of Pharmacology, School of Pharmacy, Jilin University, Changchun 130021;
[2]College of Pharmacy, Yanbian University, Yanji 133002, P.R. China

Abstract. In the present study, the protective effect of myricetin administered orally at 200, 100 or 50 mg/kg for 10 days was evaluated in a murine model of acute experimental colitis induced by dextran sulphate sodium (DSS). Macroscopic analysis was carried out to determine the effect of myricetin on pro-inflammatory cytokines, inflammatory markers and oxidative damage in biopsies of colonic tissues. The results showed that treatment with myricetin ameliorated body weight loss in a dose-dependent manner and significantly reduced histology scores. Myricetin decreased the production of nitric oxide (NO), myeloperoxidase (MPO) and malondialdehyde (MDA), while increasing the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Furthermore, the levels of the cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) were significantly decreased. Taken together, the results suggest that the anticolitis effects of myricetin may be attributed to anti-inflammatory and antioxidant actions. Additional investigation is required to determine the detailed mechanisms of action.

Introduction

Inflammatory bowel diseases (IBDs), such as Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing inflammatory disorders of the gastrointestinal tract (1). Although their precise aetiology is unknown, they are likely to be correlated with an abnormal exacerbated immune response to otherwise innocuous stimuli, which is not properly abrogated by the feedback system that normally downregulates the mucosal response to luminal factors (2). Similar to other inflammatory processes, IBD is characterised by an upregulation of the synthesis and release of a variety of pro-inflammatory mediators, such as reactive oxygen species and cytokines, thus influencing mucosal integrity and leading to excessive tissue injury (3,4). Currently, a specific causal treatment of IBD is not available, and the best regimen is the regulation of the oxidant/antioxidant balance.

Oral administration of dextran sulphate sodium (DSS) in mice induces colitis that resembles human UC (5). This model corresponds well to the clinical signs of human UC, thus may serve as a reliable model for studies on this disease. In this model, leukocytes, including neutrophils, lymphocytes and macrophages, have been reported to infiltrate inflamed tissues (6). Simultaneously, there are numerous reactive oxygen species in the colonic mucosa. Oxidative stress, with its dual effect of free radical generation and enhanced lipid peroxidation, is the mainstay of disease evolution (7). The production and release of reactive oxygen species by immune cells appear to play a crucial role in the pathophsyiology of UC (8).

Flavonoids have been suggested to exert human health benefits, which may be mediated by their antioxidant and anti-inflammatory activities. Among the known flavonoids, myricetin (3,3',4',5,5',7-hexahydroxyflavone) is one of the major flavonoids found in several foods, including onions, berries, grapes and red wine (9). Myricetin has several beneficial effects, including anti-inflammatory (10), antioxidant (11), analgesic and anticarcinogenic effects (12,13). These pleiotropic effects render myricetin a suitable candidate for study. The main objective of the present study was to evaluate the anticolitis effects and possible mechanisms of action of myricetin in a model of DSS-induced colitis.

Materials and methods

Reagents. Myricetin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and DSS was purchased from Wako Pure Chemical Industries Inc. (Tokyo, Japan).

Animals. Forty specific pathogen-free female BALB/c mice were housed in standard cages with wood shavings. Eight animals/cage were maintained in a room with a carefully controlled ambient temperature (25°C) and artificial illumination (12 h of light from 8:00 a.m. to 8:00 p.m.). The experiments were approved by the ethics committee of Jilin University Changchun, China.

Correspondence to: Professor Tie Hong, Department of Pharmacology, School of Pharmacy, Jilin University, Fujin Road No. 1266, Jilin, Changchun 130021, P.R. China
E-mail: hongtie@jlu.edu.cn

Table I. Degree of inflammation in microscopic cross-sections of the colon.

No.	Ulceration	Epithelium	Infiltration	Lymphoid follicles
0	No ulcers	Normal morphology	No infiltrate	No lymphoid follicles
1	1 ulcer	Loss of goblet cells	Infiltrate around crypt bases	1 lymphoid follicle
2	2 ulcers	Loss of goblet cells in large areas	Infiltrate reaching to muscularis mucosae	2 lymphoid follicles
3	3 ulcers	Loss of crypts	Extensive infiltration reaching the muscularis	3 lymphoid follicles
4	>3 ulcers	Loss of crypts in large areas	Infiltration of the submucosa	>3 lymphoid follicles

Induction of colitis. Mice were given drinking water containing 5% (wt/vol) DSS (mol wt 5,000 dissolved in drinking water) *ad libitum* for 10 days. The normal group was given water only. Previous results have shown that there is acute inflammation at this stage, as indicated by the increased number of polymorphonuclear leukocytes, multiple erosive lesions and the loss of crypts (14).

Treatment with myricetin. The mice were treated with myricetin at 200, 100 or 50 mg/kg body weight daily, beginning on the day on which oral DSS water was given. The normal group was administered an equal volume of water orally.

Morphological analysis. At the end of the experiment, the body weight and haemoglobin content were measured. After measuring the colonic length, colon samples were fixed in 10% formalin solution (pH 7.2).

Grading of histological changes. Colon samples were embedded in paraffin. Serial sections 4 μm thick were prepared and stained with haematoxylin and eosin (H&E) for histological grading and evaluation. The degree of inflammation in microscopic cross-sections of the colon was evaluated as shown in Table I.

Assessment of myeloperoxidase (MPO) activity in colonic tissue. MPO activity was determined by a modified method described by Suzuki *et al* (15). In brief, colonic tissue was weighed and homogenised with a homogeniser for 40 sec in ice-cold 50 mmol/l phosphate-buffered saline (PBS; pH 6.0) containing 0.5% hexadecyl-trimethylammonium bromide. The homogenate was thawed three times, followed by repeated sonication for 30 sec each time. The final MPO activity was represented as U/mg tissue.

Assessment of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) content in colonic tissue. After thawing, colon samples were weighed and homogenised in 0.3 ml PBS (pH 7.2) at 4°C. The protein concentration was determined quantitatively using the BAC 100 Protein Determination kit (Shanghai Bio-Technology Co., Ltd., Shanghai, China). GSH-Px activity and the MDA content in colonic tissue were measured by chemical chromatometry using related assay kits (Jiancheng Biotech, Nanjing, China). The final value of GSH-Px is presented as U/mg protein, and MDA is presented as nmol/mg protein. The SOD activity in colonic tissue was measured using the method

of Ohkawa *et al* (16) to evaluate the ability of the xanthine-xanthine oxidase system to inhibit the oxidation of oxymine. SOD is presented as U/mg protein.

Assessment of colonic nitric oxide (NO) content. NO content was determined by measuring its stable metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), based on the method described by Miranda *et al* (17). In brief, 0.1 ml of colonic homogenate (20%) was added to 0.1 ml of methylalcohol and centrifuged at 12,000 x g for 10 min. An aliquot of the resultant supernatant (0.1 ml) was aspirated and mixed with 0.1 ml of vanadium (III) chloride. Then, 50 μl of sulphanilamide solution and 50 μl of *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEDD) were added and incubated at 37°C for 30 min. The optical density was measured at 540 nm against a blank using the spectrophotometer UVWin 5.

Assessment of interleukin (IL)-1β and IL-6. Colon samples were weighed and homogenised in 0.3 ml PBS (pH 7.2) containing 1% bovine serum albumin (BSA) at 4°C. The homogenate was thawed three times and centrifuged at 12,000 x g for 10 min. Cytokine levels were assayed twice with quantitative IL-1β and IL-6 enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Inc., San Diego, CA, USA). IL-1β and IL-6 values are expressed as pg/mg tissues.

Statistical analyses. The data are expressed as the mean ± SEM. The statistical significance of the difference in each parameter among the groups was evaluated using a one-way analysis of variance (ANOVA) followed by the Fisher's protected least significant difference (PLSD) comparison tests for post hoc t-tests. $P < 0.05$ was considered to indicate a statistically significant difference. Scores were analysed using the Wilcoxon's test.

Results

Effects on the general condition of mice with colitis. We found that BALB/c mice subjected to the oral administration of 5% DSS regularly developed pancolitis with severe diarrhoea and rectal prolapse accompanied by extensive wasting disease. In severe cases, gross blood adhering to the anus was noted. Beginning on the 4th day subsequent to DSS administration, the body weight began to decrease, and remained significantly decreased compared with normal mice until the 10th day. However, the administration of myricetin significantly reversed the loss of body weight (Fig. 1).

Table II. Effect of myricetin on damage score and blood haemoglobin.

Group	Damage score	Blood haemoglobin (g/dl)
Normal	0	12.12±0.87
DSS-induced colitis	11 (13-9)[d]	9.04±0.61[d]
Myricetin (mg/kg)		
200	6 (8-4)[b]	10.01±0.26[c]
100	8 (10-7)[a]	9.88±0.32[b]
50	10 (13-8)	9.40±0.15[b]

Damage score is expressed as median (range). Blood haemoglobin is expressed as the mean ± SEM; n=8. [a]P<0.05, [b]P<0.01, [c]P<0.001 vs. the DSS model. [d]P<0.001 vs. the normal group. DSS, dextran sulphate sodium.
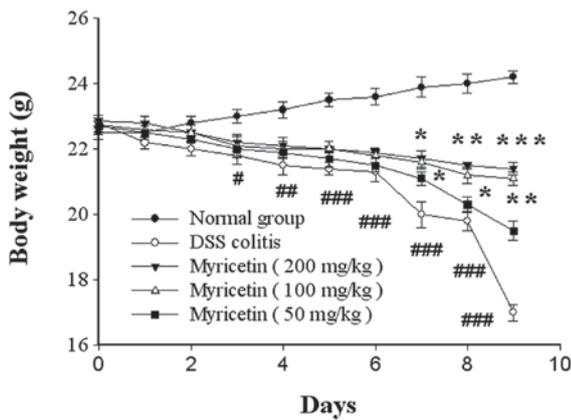
Figure 1. Effect of myricetin administration on the time-course changes in the mouse body weight. [*]P<0.05, [**]P<0.01 vs. the DSS model; [#]P<0.05, [##]P<0.01, [###]P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. DSS, dextran sulphate sodium.

Effect on damage score and histological evaluation. The severity of UC-like lesions was most marked in the colon on the 10th day. Compared with normal mice, the distal colon of DSS-treated mice showed intense inflammatory cellular infiltration in all the layers, with polymorphonuclear leukocytes and multiple erosions. Crypt abscesses and regenerating epithelium were observed in the colonic mucosa. Myricetin treatment attenuated morphological damage but showed mild cellular infiltration (Table II, Fig. 2).

Compared with the DSS-induced colitis groups, the damage score and the content of blood haemoglobin in the myricetin groups were significantly lower. Mice treated with 200 and 100 mg/kg myricetin showed a significantly lower damage score and blood haemoglobin content (P<0.01 and P<0.05, respectively), although those treated with 50 mg/kg showed no significant differences. The results showed a concentration-response correlation (Table II).

Effect on the length of the colon. The colon and caecum of the DSS-treated mice were significantly shorter compared with those of the normal group. The administration of myric-
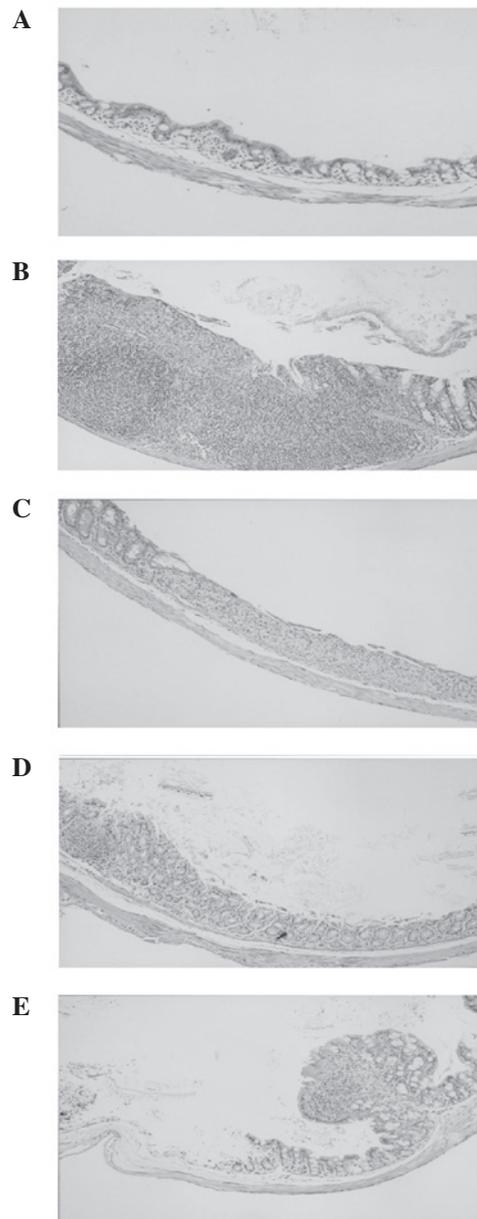
Figure 2. Effect of myricetin on histological changes of the colon [haematoxylin and eosin (H&E) staining; magnification, x100]. (A) Normal group; (B) DSS colitis; (C) Myricetin (200 mg/kg); (D) Myricetin (100 mg/kg) and (E) Myricetin (50 mg/kg). DSS, dextran sulphate sodium.

etin significantly increased the length of the shortened colon induced by DSS (Fig. 3).

Effect on MPO, GSH-Px and SOD activity, and MDA and NO content in colonic tissue. The MPO levels were 0.106±0.012 U/mg colonic tissue in normal mice and 0.260±0.018 U/mg colonic tissue in mice with DSS-induced colitis. Myricetin at 50, 100 and 200 mg/kg decreased the MPO levels to 0.180±0.013, 0.166±0.01 and 0.159±0.018 U/mg colonic tissue, respectively (Fig. 4).

The GSH-Px activity was 39.0±6.0 U/mg colonic tissue in normal mice and 29.0±5.0 U/mg colonic tissue in mice with DSS-induced colitis. Myricetin at doses of 50, 100 and 200 mg/kg increased the GSH-Px activity to 30.1±3.1, 35.0±4.2 and 40.1±6.3 U/mg colonic tissue, respectively (Fig. 5).
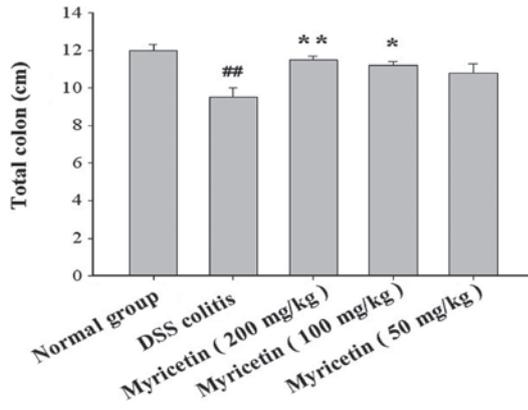
Figure 3. Effect of the administration of myricetin on colonic length. *P<0.05, **P<0.01 vs. the DSS model; ##P<0.01 vs. normal. Data are presented as the mean ± SEM; n=8. DSS, dextran sulphate sodium.
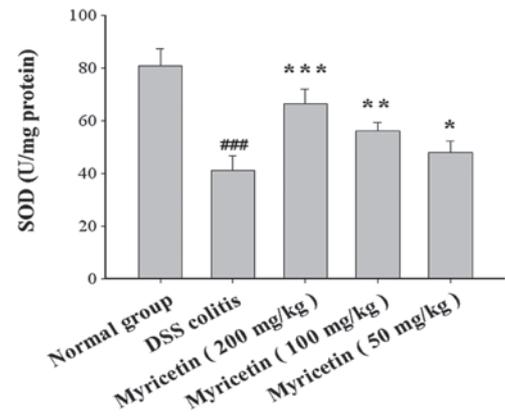
Figure 6. Effect of myricetin administration on SOD activity in colonic tissue. *P<0.05, **P<0.01, ***P<0.001 vs. the DSS model; ###P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. SOD, superoxide dismutase; DSS, dextran sulphate sodium.
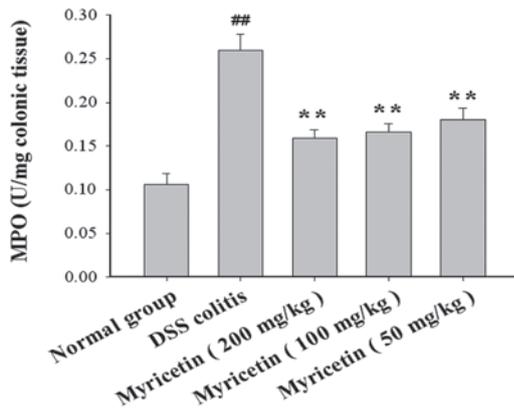
Figure 4. Effect of myricetin administration on MPO levels in colonic tissue. **P<0.01 vs. the DSS model; ###P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. MPO, myeloperoxidase; DSS, dextran sulphate sodium.
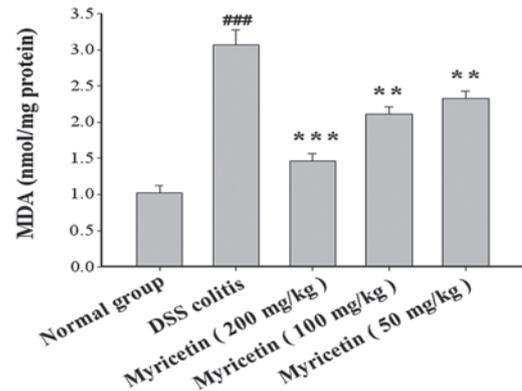
Figure 7. Effect of myricetin administration on MDA content in colonic tissue. **P<0.01, ***P<0.001 vs. the DSS model; ###P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. MDA, malondialdehyde; DSS, dextran sulphate sodium.
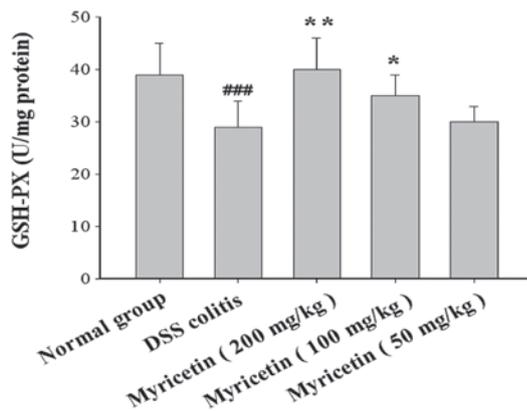
Figure 5. Effect of myricetin administration on GSH-Px activity in colonic tissue. *P<0.05, **P<0.01 vs. the DSS model; ###P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. GSH-Px, glutathione peroxidase; DSS, dextran sulphate sodium.
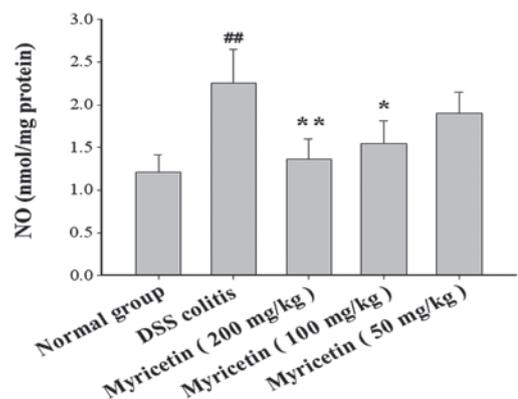
Figure 8 Effect of myricetin administration on NO content in colonic tissue. *P<0.05, **P<0.01 vs. the DSS model; ##P<0.05 vs. normal. Data are presented as the mean ± SEM; n=8. NO, nitric oxide; DSS, dextran sulphate sodium.

The SOD activity was 81.0±6.4 U/mg colonic tissue in normal mice and 41.1±5.6 U/mg colonic tissue in mice with DSS-induced colitis. Myricetin at 50, 100 and 200 mg/kg increased the SOD activity to 48.1±4.2, 56.2±3.4 and 66.4±5.6 U/mg colonic tissue, respectively (Fig. 6).

The MDA content was 1.02±0.10 nmol/mg protein in normal mice and 3.07±0.20 nmol/mg protein in mice with DSS-induced colitis. Myricetin at 50, 100 and 200 mg/kg decreased the MDA content to 2.33±0.10, 2.11±0.11 and 1.46±0.11 nmol/mg protein, respectively (Fig. 7).
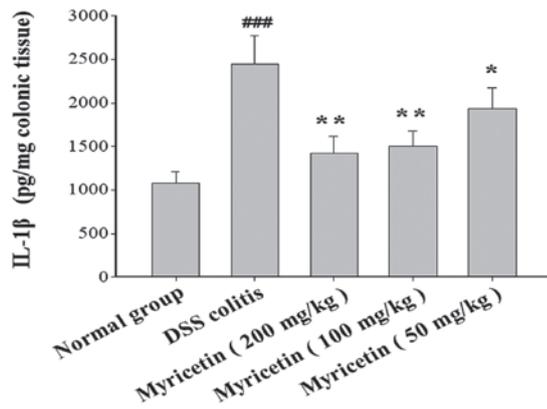
Figure 9. Effect of myricetin administration on IL-1β concentration in colonic tissue. *P<0.05, **P<0.01 vs. the DSS model; ###P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. IL-1β, interleukin-1β; DSS, dextran sulphate sodium.
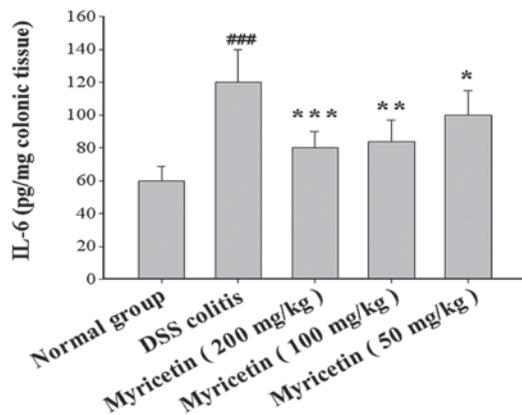
Figure 10. Effect of myricetin administration on IL-6 concentration in colonic tissue. *P<0.05, **P<0.01, ***P<0.001 vs. the DSS model; ###P<0.001 vs. normal. Data are presented as the mean ± SEM; n=8. IL-6, interleukin-6; DSS, dextran sulphate sodium.

The NO content was 1.21 ± 0.20 μmol/mg protein in normal mice and 2.25 ± 0.40 μmol/mg protein in mice with DSS-induced colitis. Myricetin at 50, 100 and 200 mg/kg decreased the NO content to 1.90 ± 0.25, 1.55 ± 0.26 and 1.36 ± 0.24 μmol/mg protein, respectively (Fig. 8).

Effect on IL-1β and IL-6 in colonic tissue. DSS-induced colitis was accompanied by a disturbance in cytokine levels. The effects of myricetin on IL-1β and IL-6 production in colonic tissue were evaluated. The administration of myricetin to the DSS-induced colitis group resulted in lower levels of IL-1β and IL-6 (Figs. 9 and 10).

Discussion

In this study, the oral administration of myricetin for 10 days markedly prevented the loss of body weight and reduced the damage score in the colonic mucosa of DSS-induced colitis, and histological analysis demonstrated that myricetin ameliorated the pathological manifestations of DSS colitis, including inflammatory cell infiltration, crypt abscesses and regenerating epithelium, in the colonic mucosa. Mucosal

lesions in UC are characterised by a dense inflammatory cell infiltrate that primarily contains neutrophils, macrophages and lymphocytes. During chronic inflammation, whereby the sustained production of reactive oxygen species (ROS) occurs, antioxidant defences may weaken, resulting in oxidative stress. Increased ROS levels in the intestinal epithelial cell membrane may lead to oxidative damage mediated by free radical attacks and lipid peroxidation, which may be an early critical event in the model of experimental IBD. Acute attacks of UC lead to an inflamed colonic mucosa, which contributes to an excess production of reactive oxygen metabolites by lipid peroxidation (18). Inflammatory tissue injury may further stimulate ROS production and reactive oxygen metabolites, thus forming a positive feedback loop and rendering the antioxidant system insufficient (19). DSS-induced colitis is a well-established experimental model with several of the signs and symptoms of human UC. ROS are highly reactive, and their levels are significantly increased in the colonic mucosa (20).

Myricetin treatment was found to significantly lower MPO activity, MDA and NO content and increase the activity of SOD and GSH-Px. MPO and NO are known sources of free radicals and are able to induce the reduction of ferritin (Fe^{3+}) to free Fe^{2+}, contributing to oxidative damage (21,22). Moreover, increased MDA activity leads to lipid peroxidation, which causes the cross-linking of protein and nucleic acid molecules and cell toxicity. MDA is an essential co-factor of GSH-Px and SOD and plays a significant antioxidative role by binding to the active site of GSH-Px. SOD protects cells against ROS-induced damage by removing these molecules. GSH-Px detoxifies organic peroxides and H_2O_2 and permits the regeneration of cellular lipid molecules through reacylation in the cell membrane.

Furthermore, our data showed that myricetin was able to decrease the production of IL-1β and IL-6. Inhibiting the action of endogenous IL-1β or IL-6 attenuates acute and chronic experimental colitis and their systemic complications (23-25). Previous studies have shown that the anti-inflammatory mechanisms of myricetin are most likely associated with the inhibition of antioxidant activity (26). Therefore, myricetin may exert its effects in DSS colitis through its antioxidant effect.

In conclusion, myricetin may ameliorate inflammation in mice treated with DSS. The mechanism is likely due to its antioxidant effect. Myricetin may be used as a novel therapeutic agent of colitis.

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