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Calming the inflammatory storm: 4-methylcatechol ameliorates DSS-induced colitis by suppressing the NLRP3 inflammasome signaling pathway

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Abstract

Introduction: *The natural polyphenol metabolite 4-methylcatechol (4-MC) has demonstrated anti-inflammatory and antioxidant properties. However, its effectiveness in ulcerative colitis (UC) and the molecular mechanisms involved are still poorly understood. This study aimed to evaluate the protective effects of 4-MC in a dextran sulfate sodium (DSS)-induced murine colitis model, with a particular focus on its potential role in modulating the NLRP3 inflammasome pathway.*

Material nad methods: *Colitis was induced in mice by administering 5% DSS in drinking water. Mice were divided into the Control, Model (DSS), Model + sulfasalazine (SASP), and Model + 4-MC groups. Disease severity was monitored through body weight changes, disease activity index (DAI) scoring, and colon length measurement. Histological injury was evaluated by H&E and TUNEL staining. Intestinal barrier integrity was examined via immunofluorescence staining of occludin and E-cadherin. Inflammatory markers (TNF-α, IL-6), myeloperoxidase (MPO) activity, oxidative stress markers (SOD, MDA, GSH), and key proteins in the NF-κB and NLRP3 pathways were measured by ELISA, biochemical kits, Western blot and immunofluorescence.*

Results: *4-MC treatment significantly ameliorated the disease manifestations of DSS-induced colitis, including weight loss, increased DAI, and colon shortening. It attenuated colonic histopathological damage, reduced apoptosis, and increased the levels of the tight junction proteins occludin and E-cadherin. Furthermore, 4-MC suppressed the inflammatory response by decreasing TNF-α, IL-6, and MPO levels. Mechanistically, 4-MC inhibited the activation of the NF-κB pathway, alleviated oxidative stress, and suppressed the NLRP3 inflammasome pathway.*

Conclusions: *4-MC may protect against DSS-induced colitis by enhancing intestinal barrier function, attenuating inflammatory and oxidative stress responses, and suppressing NLRP3 inflammasome activation.*

Key words: *4-methylcatechol, NF-κB, NLRP3 inflammasome, oxidative stress, ulcerative colitis.*

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Introduction

Ulcerative colitis (UC), a chronic inflammatory bowel disease, manifests as persistent and recurrent inflammation of the colonic mucosa driven by dysregulated immune reactivity [1]. Current management relies on aminosalicylates, corticosteroids, immunosuppressants, and biologics, yet a significant number of patients experience suboptimal outcomes owing to insufficient effectiveness, acquired drug re-

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sistance, and undesirable side effects [2]. These limitations highlight the pressing demand for innovative therapeutic targets and safer, more efficient treatment options.

The development of UC involves a complex interplay of factors, such as genetic predisposition, impaired epithelial barrier function, alterations in the gut microbiome, and dysregulated innate immunity [3]. In particular, the NLRP3 inflammasome – a central innate immune signaling platform – has emerged as a pivotal regulator of intestinal inflammation [4, 5]. This complex, composed of NLRP3, adapter apoptosis-associated speck-like protein containing a CARD (ASC), and caspase-1, is activated in response to damage signals. Active caspase-1 then processes pro-IL-1β and pro-IL-18 into their mature, inflammatory forms and cleaves gasdermin D to induce pyroptosis, thereby amplifying the inflammatory cascade [6, 7]. Accumulating evidence demonstrates that the NLRP3 inflammasome is markedly activated in the colonic tissues of UC patients and experimental colitis models, with its activity correlating with disease severity [8-10]. Dextran sodium sulfate (DSS)-induced intestinal damage is closely linked to activation of the NLRP3 inflammasome. The NLRP3 inflammasome was identified as a critical mechanism of intestinal inflammation in the DSS colitis model [11]. Notably, in the DSS-induced colitis model, NLRP3 inflammasome activation drives the release of interleukin (IL)-1β and IL-18, thereby contributing to mucosal injury [12]. Inhibiting or knocking down the NLRP3 inflammasome can alleviate the severity of DSS-induced colitis, reduce the release of IL-1β and IL-18, and inhibit the inflammatory response of UC [13, 14]. NLRP3 inhibition attenuated the NLRP3/ASC/caspase-1/IL-1β signaling pathway in the colon, which was over-activated by DSS [15]. Importantly, pharmacological inhibition of NLRP3 using specific inhibitors such as MCC950 has been shown to ameliorate colitis in mice, validating NLRP3 as a promising therapeutic target for UC [16]. However, the clinical translation of many existing NLRP3 inhibitors is hampered by issues such as poor pharmacokinetic properties or potential hepatotoxicity [17]. Thus, the discovery of novel NLRP3 inhibitors of natural origin, with high efficacy and low toxicity, is of great interest.

Plant-derived polyphenols have garnered attention for their broad anti-inflammatory and antioxidant activities [18]. 4-Methylcatechol (4-MC), a metabolite of the widely consumed polyphenol quercetin, exhibits multiple bioactivities, including modulation of oxidative stress and inflammatory pathways [19, 20]. Our previous data indicated that 4-MC effectively suppresses NLRP3 inflammasome activation in macrophages [21]. However, its potential therapeutic effect against UC and the underlying mechanism, particularly whether it involves modulation of the NLRP3 inflammasome pathway *in vivo*, remain unclear.

To address this gap, the present study employed the well-established DSS-induced murine colitis model, which recapitulates key features of human UC (including epithelial barrier disruption, inflammatory cell infiltration, and cytokine production) [22, 23] and is characterized by robust NLRP3-driven inflammation. We hypothesized that 4-MC would alleviate DSS-induced colitis by targeting this pathway. Therefore, this study was designed to evaluate, for the first time, the protective efficacy of 4-MC in a DSS-induced colitis model and to investigate its potential mechanism of action, with a specific focus on the NLRP3 inflammasome signaling axis and associated inflammatory and oxidative stress responses.

Material and methods

Animals and experimental grouping

A total of 40 male C57BL/6 mice (6 weeks old, 20-22 g) were obtained from Hangzhou Medical College. All animals were kept under specific pathogen-free (SPF) conditions (22 ±2°C, 50 ±5% humidity, 12 h light/dark cycle) with free access to standard chow and water. The animal experiments were approved by the Animal Ethics Committee of Zhejiang Baiyue Biotech Co., Ltd for welfare of experimental animals (No. ZJBYLA-IACUC-20250205) and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

After acclimatizing for one week, mice were randomly divided into 4 groups (n = 10 per group): the Control group, where mice received normal drinking water; the Model group, where mice were given DSS (HY-116282, MedChemExpress, China) dissolved in distilled water to prepare a 5% (w/v) DSS solution, allowed to freely drink this solution for 7 days to induce experimental colitis as previously described, and then received normal drinking water for 3 days [23]; the Model + sulfasalazine (SASP) group, in which the mice were subjected to UC modeling, and at the same time, they received intragastric administration of sulfasalazine (SASP, HY-14655, MedChemExpress, China) at 100 mg/kg/d for 10 days [23]; and the Model + 4-MC group, in which the mice were subjected to DSS-induced colitis and they received intragastric administration of 4-MC (HY-W012814, MedChemExpress, China) at 20 mg/kg/d [21]. No mortality was observed in any of the experimental groups during the modeling and treatment period. Each time point of drug administration is shown in Supplementary Figure 1.

Monitoring of general physiological indicators

Body weight, stool characteristics, and blood in the stool of mice were measured daily at the same time (9:00-10:00 AM) throughout the experiment.

The disease activity index (DAI) was evaluated daily using an established scoring system (weight loss, fecal property and fecal occult blood) as previously described [24].

Upon completion of the study, all mice underwent euthanasia by cervical dislocation. The entire colon (from cecum to anus) was dissected, rinsed with cold physiological saline to remove feces, and measured using a vernier caliper.

Hematoxylin-eosin (H&E) staining

Colon tissue samples underwent fixation in 4% paraformaldehyde (20010, Biolite, China) at 4°C for 24 h. Subsequent processing included sequential dehydration, paraffin embedding, and microtome sectioning at 5 µm thickness. Following deparaffinization with two xylene washes (10 min per wash), sections were progressively rehydrated through a descending ethanol series (100% to 70%) with 5-min intervals at each concentration. Histological staining was performed using a commercial H&E kit (PH0516, PHYGENE, China) according to the manufacturer's protocol. Finally, stained sections underwent dehydration, xylene clearing, and mounting with neutral balsam prior to microscopic examination at 400× magnification (N300M, Yongxin, China).

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining

TUNEL staining was performed on colonic tissue sections using a dedicated kit (C1091, Beyotime, China) to detect apoptotic cells. Following deparaffinization and rehydration, sections were permeabilized with 20 µg/ml proteinase K (ST532, Beyotime, China) and endogenous peroxidases were blocked with 3% H_2O_2. Subsequently, the sections were exposed to the TUNEL reaction mixture followed by streptavidin-HRP, with both steps carried out at 37°C. 3,3′-diaminobenzidine (DAB) was used as the chromogenic substrate, and hematoxylin was appl$_2$ied for nuclear counterstaining. Finally, all samples were visualized and imaged under a light microscope at 400× magnification.

Immunofluorescence staining

The localization and expression of occludin, E-cadherin, NLRP3 and ASC were analyzed by immunofluorescence. Deparaffinized tissue sections underwent heat-induced antigen retrieval in citrate buffer (pH 6.0) using a microwave, followed by gradual cooling to room temperature. Non-specific binding sites were blocked with 5% bovine serum albumin (BSA, AR0004, Boster, China) for 1 h at 37°C. The sections were then probed with the following primary antibodies at 4°C overnight: Occludin (DF7504, Affinity, USA), E-cadherin (AF0131, Affinity, USA), NLRP3 (DF7438, Affinity, USA), and ASC (DF6304, Affinity, USA). After PBS washing the next day, the sections were treated with fluorescent secondary antibodies (ab6717, FITC-conjugated goat anti-rabbit IgG; ab186696, Alexa Fluor 680-conjugated goat anti-rabbit IgG, Abcam, UK) for 1 h at 37°C under light-protected conditions. Nuclei were labeled with DAPI for 5 min, and coverslips were applied using anti-fade mounting medium (36307ES08, YEASEN, China). Images were acquired at 400× magnification on a fluorescence microscope. For each sample and target protein, images from at least five random non-overlapping fields were captured. Specific filter sets for DAPI (excitation 358 nm, emission 461 nm), FITC (excitation 490 nm, emission 525 nm), and Alexa Fluor 680 (excitation 679 nm, emission 702 nm) were used. For quantitative analysis, mean fluorescence intensity values were determined using ImageJ software (v1.8.0, NIH, USA). The final normalized fluorescence intensity for each sample was expressed as the ratio of the target protein's mean fluorescence intensity to the DAPI-stained nuclear area in the same field.

Enzyme-linked immunosorbent assay (ELISA)

First, colonic tissues (100 mg) were homogenized in a nine-fold volume of cold PBS and centrifuged to obtain the supernatant. Tumor necrosis factor α (TNF-α), IL-6, and myeloperoxidase (MPO) levels in the superna-

Table 1. Antibodies used for western blot in this study

Name	Catalog	Dilution	Manufacturer
p-p65	AF3387	1 : 1000	Affinity, USA
p65	AF5006	1 : 1000	Affinity, USA
NLRP3	DF15549	1 : 1000	Affinity, USA
Caspase1	AF5418	1 : 1000	Affinity, USA
Active caspase1	AF4005	1 : 1000	Affinity, USA
GSDMD	AF4012	1 : 1000	Affinity, USA
N-GSDMD	DF13758	1 : 1000	Affinity, USA
GAPDH	ab8245	1 : 10,000	Abcam, UK
Goat anti-rabbit	ab205718	1 : 2000	Abcam, UK
Goat anti-mouse	ab205719	1 : 2000	Abcam, UK

tant were determined using mouse-specific ELISA kits (E-EL-M3063/E-EL-M0044/E-EL-M3125, Elabscience, China) according to the manufacturer's instructions.

Detection of oxidative stress indicators

The levels of oxidative stress in colonic tissues were evaluated by measuring the activities/contents of total superoxide dismutase (T-SOD), malondialdehyde (MDA), and glutathione (GSH) using kits (E-BC-K019-S/E-BC-K025-M/ E-BC-K030-S, Elabscience, China).

Western blot

Total protein was extracted from colonic tissues using RIPA lysis buffer (C0045, TargetMol, China), with protein concentration quantified by a BCA assay. Equivalent amounts of protein (30 μg per lane) were resolved electrophoretically on 10% SDS-PAGE gels (P1200, Solarbio, China) and electrotransferred onto PVDF membranes (36125ES03, Yeasen, China) using a wet transfer system. The transfer was conducted at a constant current of 250 mA for 90 minutes in a transfer buffer containing 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol. Subsequently,

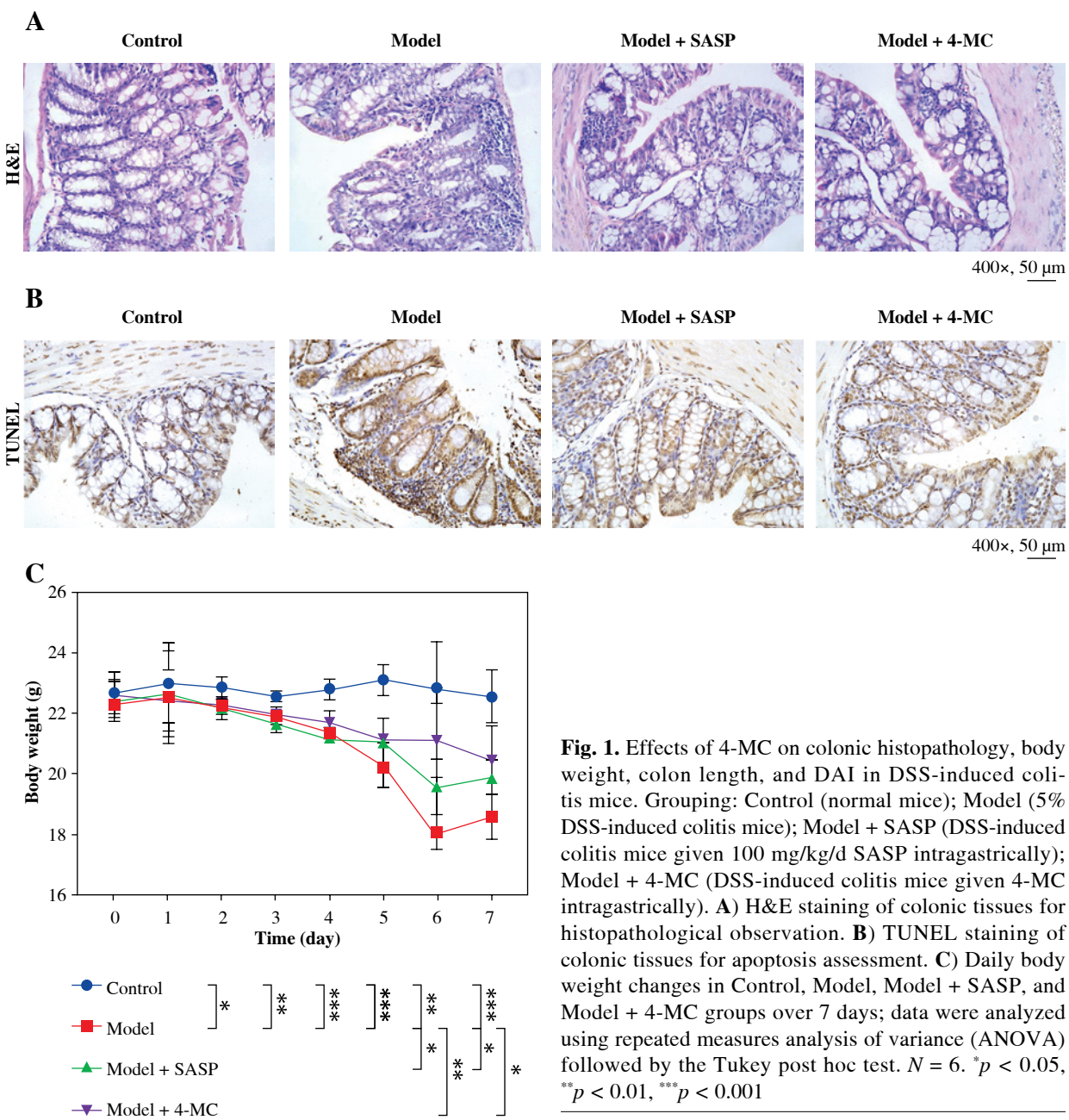

Fig. 1. Effects of 4-MC on colonic histopathology, body weight, colon length, and DAI in DSS-induced colitis mice. Grouping: Control (normal mice); Model (5% DSS-induced colitis mice); Model + SASP (DSS-induced colitis mice given 100 mg/kg/d SASP intragastrically); Model + 4-MC (DSS-induced colitis mice given 4-MC intragastrically). **A**) H&E staining of colonic tissues for histopathological observation. **B**) TUNEL staining of colonic tissues for apoptosis assessment. **C**) Daily body weight changes in Control, Model, Model + SASP, and Model + 4-MC groups over 7 days; data were analyzed using repeated measures analysis of variance (ANOVA) followed by the Tukey post hoc test. $N = 6$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

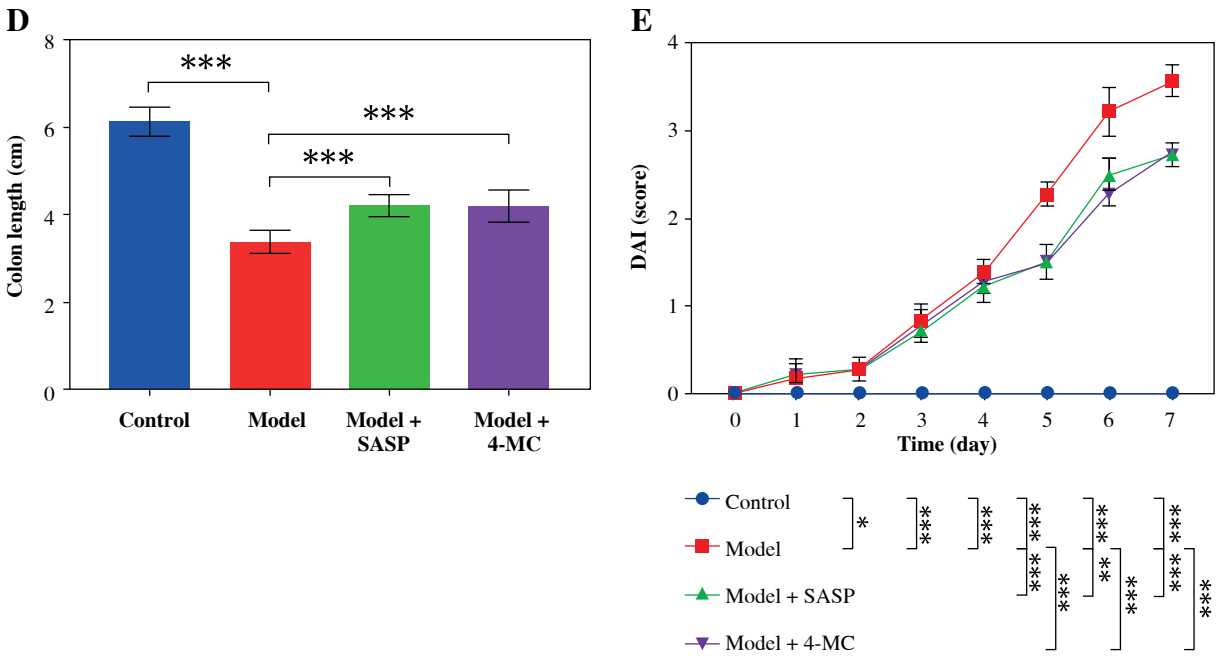

Fig. 1. Cont. **D**) Colon length in each group at end of experiment; data were analyzed using one-way ANOVA followed by the Tukey post hoc test. **E**) Daily DAI score evaluation, and data were analyzed using repeated measures ANOVA followed by the Tukey post hoc test. $N = 6$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

membranes were blocked for 1 h at room temperature with 5% non-fat dry milk (36120ES60, Yeasen, China), followed by overnight incubation at 4°C with the primary antibodies specified in Table 1. After thorough washing, membranes were exposed to secondary antibodies for 1 h at room temperature. Protein bands were visualized with an ECL substrate (AP34L024, Life-ilab, China) and imaged using a chemiluminescence system (eBLOT, China). GAPDH was used as the loading control, and band intensity was analyzed quantitatively with ImageJ software (National Institutes of Health, USA).

Statistical analysis

All statistical analyses were performed using GraphPad Prism software (v8.0, GraphPad Software, USA), with data presented as mean ± standard deviation. The comparisons among different groups at different time points were conducted using repeated measures analysis of variance (ANOVA), and other multiple groups were analyzed using one-way ANOVA, followed by the Tukey post hoc test. A p-value below 0.05 was considered statistically significant.

Results

4-MC ameliorates general phenotypes, colonic histopathology, and apoptosis in DSS-induced colitis mice

H&E staining revealed severe inflammatory cell infiltration and mucosal structure destruction in the colonic tissues of the Model group; after intervention with SASP or 4-MC, the histopathological damage was significantly alleviated (Fig. 1A). TUNEL staining results indicated that colonic cell apoptosis was notably increased in the Model group, while SASP and 4-MC treatment reduced the level of cell apoptosis (Fig. 1B). DSS-induced colitis mice showed significant body weight loss (Fig. 1C, $p < 0.05$), shortened colon length (Fig. 1D, $p < 0.001$), and a continuous increase in DAI (Fig. 1E, $p < 0.05$), confirming successful modeling of UC. Notably, 4-MC treatment reversed these alterations: body weight was restored, colon length was increased, and DAI scores were decreased (Fig. 1C-E, $p < 0.05$). These results indicate that 4-MC can reduce disease severity and colonic tissue damage in DSS-induced colitis mice.

4-MC enhances intestinal barrier function and inhibit inflammatory response in DSS-induced colitis mice

Figure 2A-C revealed a marked reduction in the protein abundance of the tight junction components occludin and E-cadherin in colonic tissues from the model mice (Fig. 2A-C, $p < 0.01$); this decrease was reversed following administration of either SASP or 4-MC (Fig. 2A-C, $p < 0.05$). Colonic tissues from the model mice exhibited significantly elevated concentrations of the pro-inflammatory cytokines TNF-α and IL-6 as well as MPO activity (Fig. 2D-F, $p < 0.05$). In contrast, treatment with SASP or 4-MC effectively suppressed the levels of these inflammatory mediators (Fig. 2D-F, $p < 0.05$). These data indicate

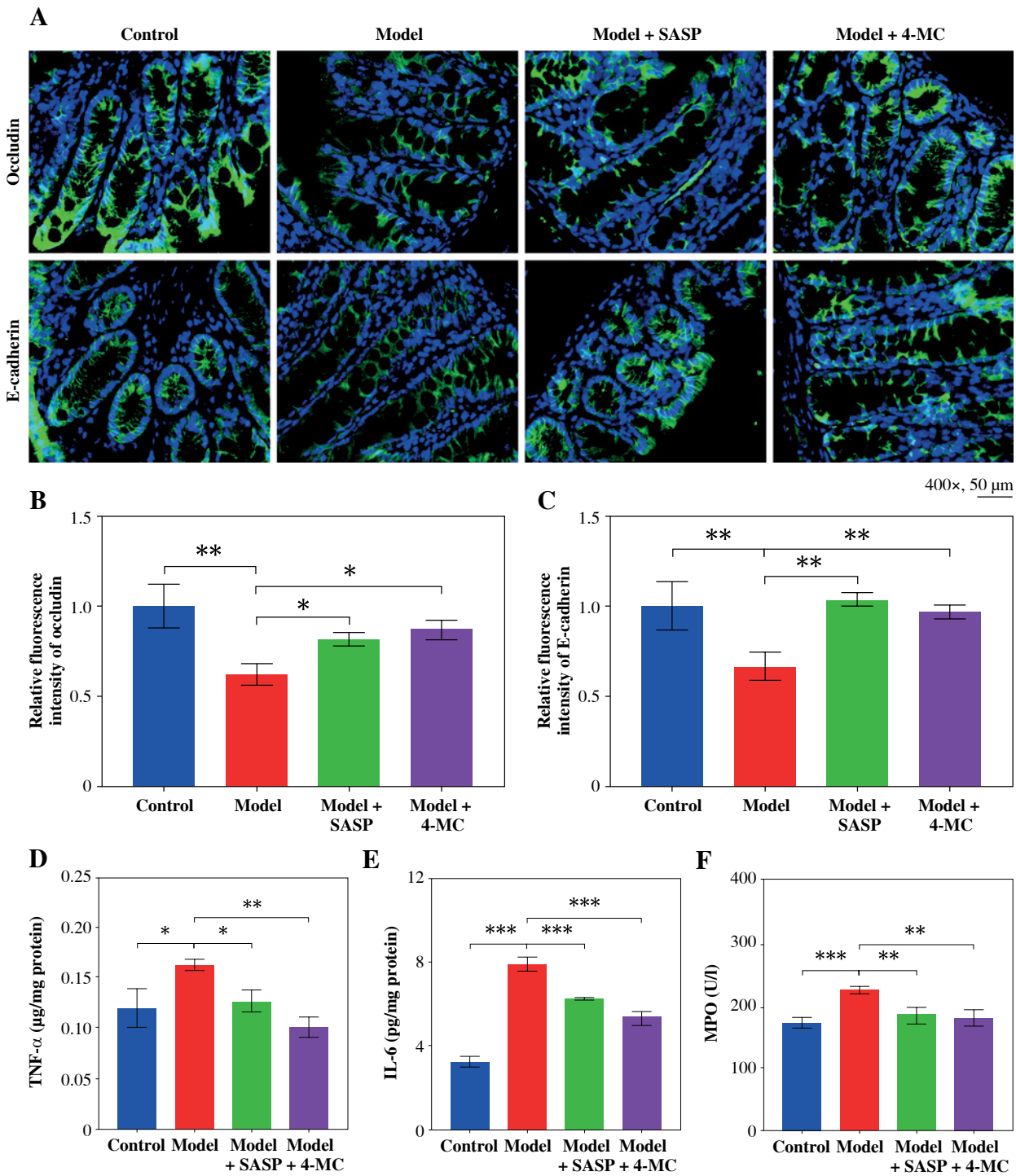

Fig. 2. Effects of 4-MC on intestinal barrier proteins, inflammatory cytokines, and MPO activity in DSS-induced colitis mice. Grouping: Control (normal mice); Model (5% DSS-induced colitis mice); Model + SASP (DSS-induced colitis mice given 100 mg/kg/d SASP intragastrically); Model + 4-MC (DSS-induced colitis mice given 4-MC intragastrically). **A-C**) Immunofluorescence staining of occludin and E-cadherin in colonic tissues. Green represents target proteins, and blue represents cell nuclei (DAPI staining). **D-F**) The levels of TNF-α, IL-6 and MPO in colonic tissues were determined by ELISA. $N = 3$. Data were analyzed using one-way ANOVA followed by the Tukey post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

that 4-MC contributes to the restoration of intestinal barrier integrity and attenuation of colonic inflammation in mice with DSS-induced colitis.

4-MC inhibits NF-κB activation, oxidative stress, NLRP3 inflammasome pathway in DSS-induced colitis mice

Western blot analysis revealed a marked increase in the p-p65/p65 ratio in colonic tissues from the Model mice, which was significantly reduced by SASP or 4-MC treatment (Fig. 3A, B, $p < 0.001$). For oxidative stress indicators, SOD activity was decreased, MDA content was increased, and GSH content was reduced in the Model group; both SASP and 4-MC treatment effectively reversed these changes (Fig. 3C-E, $p < 0.05$). The expression of NLRP3, the active caspase1/caspase1 ratio, and the N-GSDMD/GSDMD ratio were upregulated in the Model group, and SASP and 4-MC treatment downregulated their expression (Fig. 3F-I, $p < 0.05$). Moreover, immunofluorescence staining showed that the fluorescence intensity of NLRP3 (green) and ASC (red) was significantly enhanced in the colonic tissues of the Model group; SASP and 4-MC treatment significantly weakened the fluorescence inten-

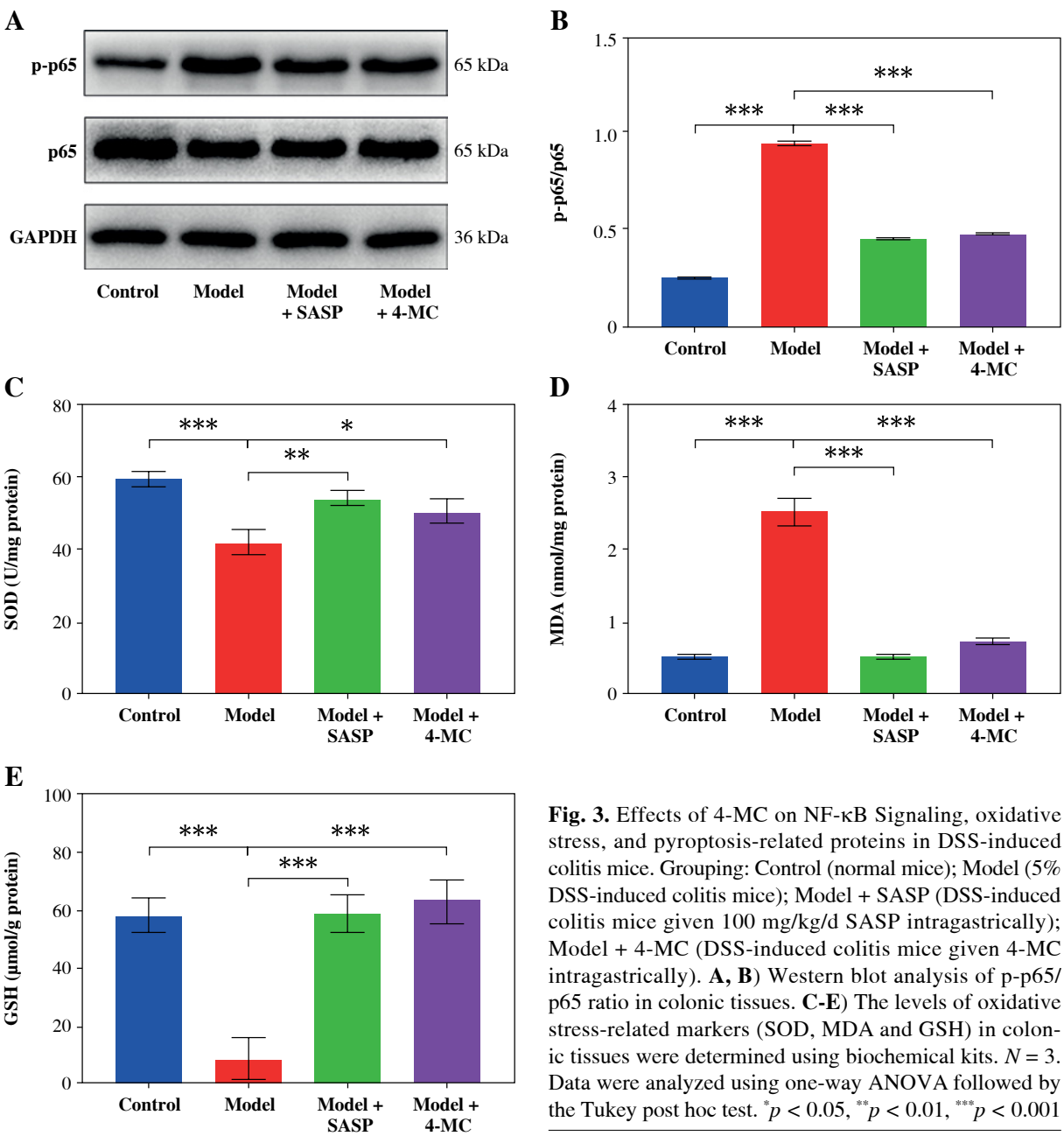

Fig. 3. Effects of 4-MC on NF-κB Signaling, oxidative stress, and pyroptosis-related proteins in DSS-induced colitis mice. Grouping: Control (normal mice); Model (5% DSS-induced colitis mice); Model + SASP (DSS-induced colitis mice given 100 mg/kg/d SASP intragastrically); Model + 4-MC (DSS-induced colitis mice given 4-MC intragastrically). **A, B**) Western blot analysis of p-p65/p65 ratio in colonic tissues. **C-E**) The levels of oxidative stress-related markers (SOD, MDA and GSH) in colonic tissues were determined using biochemical kits. $N = 3$. Data were analyzed using one-way ANOVA followed by the Tukey post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

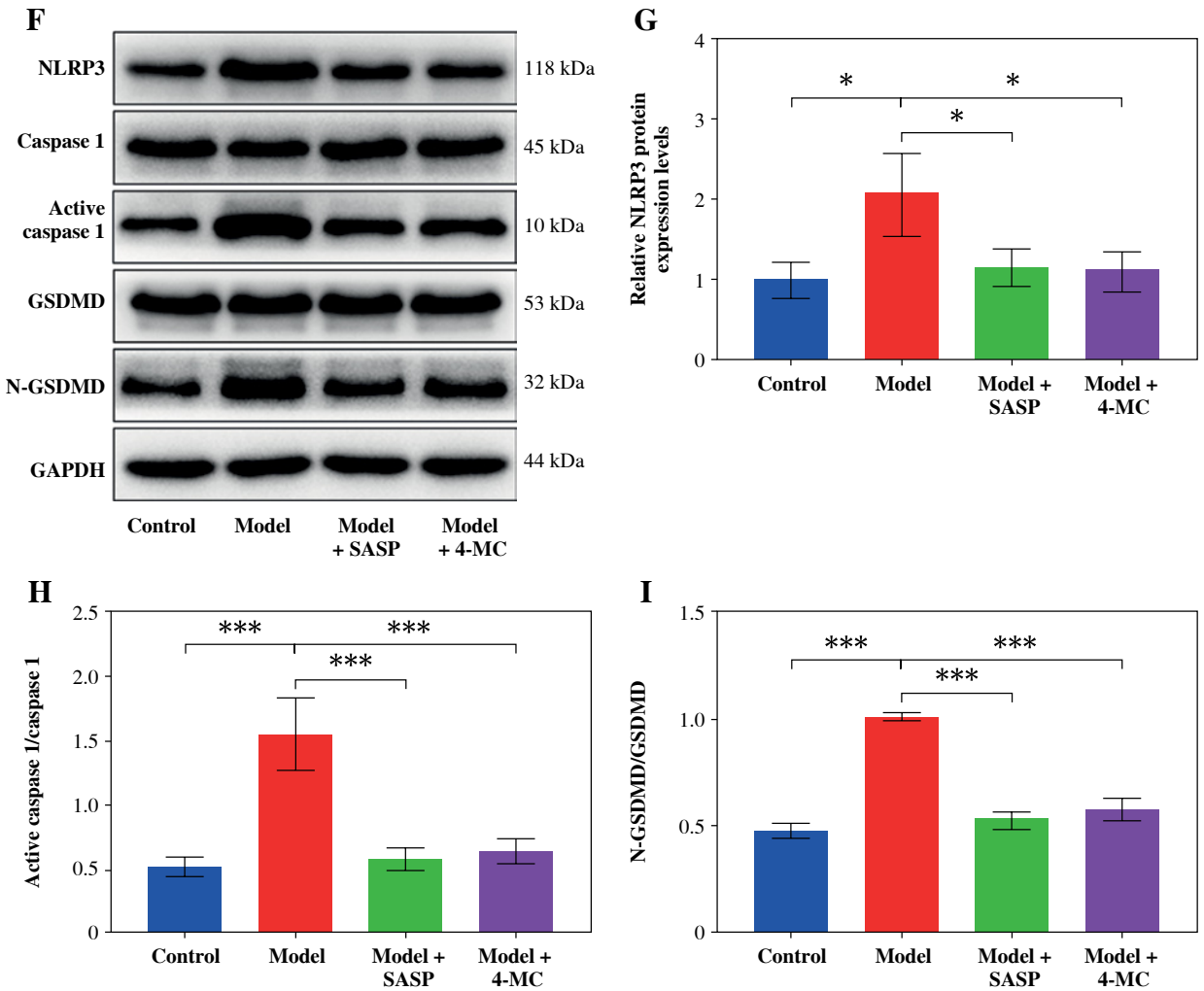

Fig. 3. Cont. **F-I**) Western blot analysis of NLRP3, active caspase1/caspase1 ratio, and N-GSDMD/GSDMD ratio in colonic tissues. $N = 3$. Data were analyzed using one-way ANOVA followed by the Tukey post hoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

sity of both (Fig. 4). Collectively, these results suggest that 4-MC can inhibit NF-κB signaling, reduce oxidative stress, and suppress NLRP3 signaling in colonic tissues of DSS-induced colitis mice.

Discussion

The core pathological features of the DSS-induced colitis model include inflammatory infiltration of the colonic mucosa, disruption of the epithelial structure, and aberrant apoptosis. These changes closely mirror the classic pathological manifestations of human UC, such as "mucosal erosion and crypt destruction". Meanwhile, weight loss, colon shortening, and an increased DAI serve as established macroscopic indicators for assessing disease severity [22, 25]. In this study, 4-MC significantly reversed these macroscopic and microscopic injuries. A direct comparison with the first-line clinical drug sulfasalazine (SASP) reveals that 4-MC exerted a comparable therapeutic efficacy in ameliorating key disease parameters. Specifically, both treatments similarly mitigated body weight loss, reduced the DAI score, and attenuated colon shortening. These findings suggest that 4-MC has an important role in mucosal repair and disease remission. Notably, 4-MC appeared to exert stronger effects than SASP in certain mechanistic aspects, demonstrating more pronounced upregulation of the tight junction protein occludin and stronger suppression of the NLRP3/caspase-1 axis. This indicates that while sharing overall protective efficacy with SASP, 4-MC may possess a distinct or additional mechanism centered on enhancing barrier integrity and directly targeting the NLRP3 inflammasome axis. Our findings extend the documented anti-inflammatory effects of 4-MC – previously observed in models of STZ-induced acute kidney

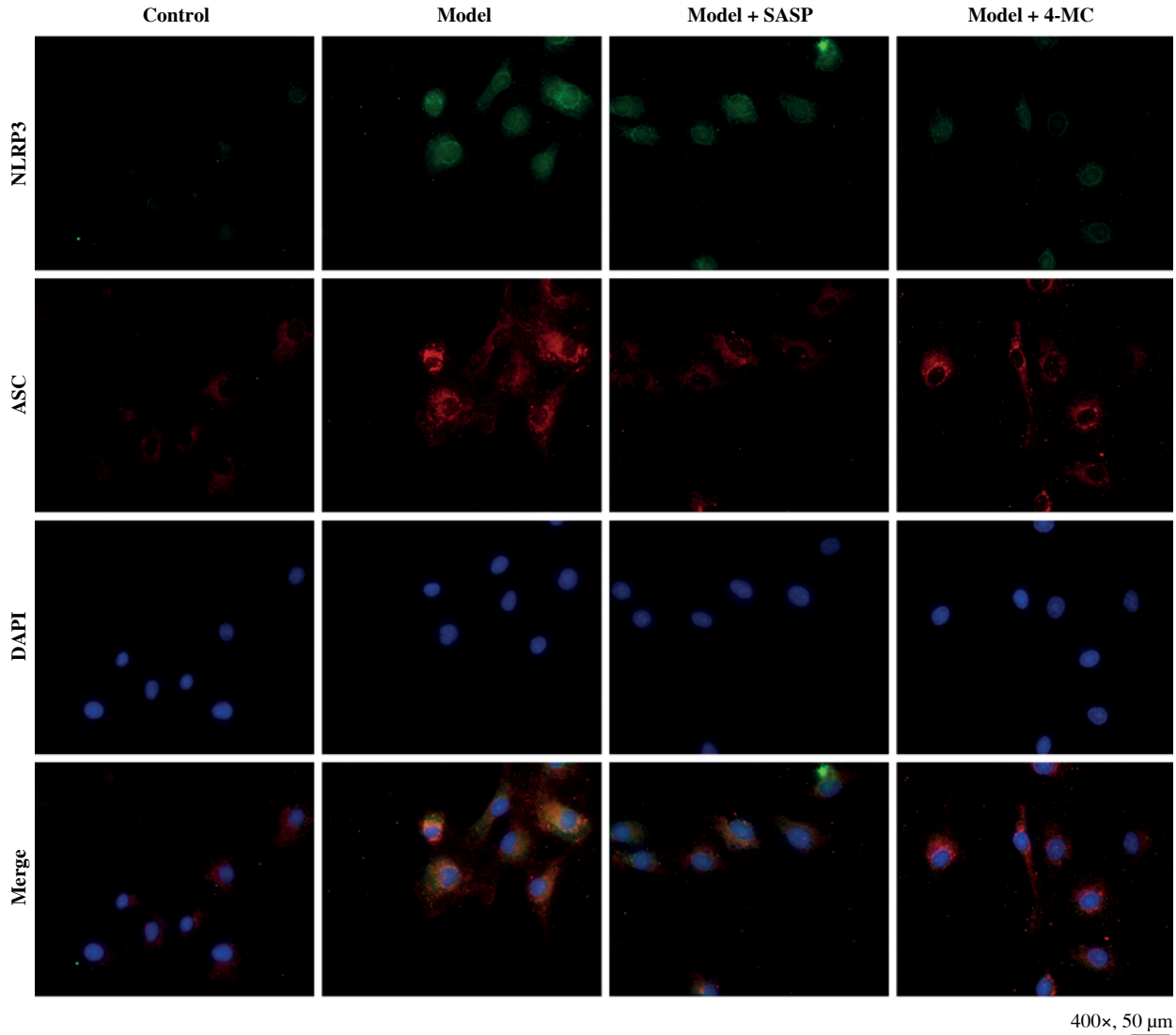

Fig. 4. Effects of 4-MC on NLRP3 and ASC expression in DSS-induced colitis mice. Grouping: Control (normal mice); Model (5% DSS-induced colitis mice); Model + SASP (DSS-induced colitis mice given 100 mg/kg/d SASP intragastrically); Model + 4-MC (DSS-induced colitis mice given 4-MC intragastrically). Immunofluorescence staining for NLRP3 (green), ASC (red), and DAPI (blue, for cell nuclei) in colonic tissues, with merged images (Merge) presented. $N = 3$

injury and collagen-induced arthritis [21, 26] – to the context of DSS-induced colitis, providing further evidence of its anti-inflammatory activity across different tissues and disease models. The comparable effects to those of a standard clinical drug, coupled with its targeted action on the NLRP3 pathway, position 4-MC as a promising candidate for further investigation as a potential alternative or complementary therapeutic agent for UC.

Impaired intestinal barrier function is a critical initiating factor in UC pathogenesis. The tight junction proteins occludin and E-cadherin are core molecules maintaining intestinal epithelial integrity; their downregulation increases intestinal permeability, allowing gut microbiota and their metabolites to translocate into the lamina propria, thereby activating immune cells and triggering persistent inflammation [27]. This study found that 4-MC significantly restored the DSS-induced downregulation of occludin and E-cadherin expression. This result is not coincidental: as a metabolite of the dietary polyphenol quercetin, quercetin itself has been confirmed to ameliorate DSS-induced intestinal barrier damage in mice by upregulating tight junction proteins [28]. 4-MC, being a major active metabolite of quercetin *in vivo*, may retain some of its barrier-protective properties, positioning it as a candidate molecule for intestinal barrier repair. Furthermore, the alleviation of oxidative stress by 4-MC observed in this study is highly

consistent with the pathological mechanism of UC. Significant oxidative stress imbalance exists in the colonic tissue of UC patients; excessive ROS generation exacerbates epithelial cell damage and activates pro-inflammatory signaling pathways [29]. The antioxidant activity of 4-MC has previously been validated in OVX-induced osteoporotic mice and in neural stem/progenitor cells [30, 31]. This study is the first to demonstrate that its antioxidant activity may contribute to the attenuation of DSS-induced colitis in an in vivo intestinal inflammation model, suggesting that the combined effects of antioxidant, anti-inflammatory, and barrier-protective activities may be important mechanisms of action for 4-MC.

The NF-κB signaling pathway is a central hub regulating intestinal inflammation and is persistently activated in both UC patients and experimental colitis models. Its downstream target genes encompass various pro-inflammatory mediators, ranging from cytokines such as TNF-α and IL-6 to essential constituents of the NLRP3 inflammasome (e.g., NLRP3, pro-caspase-1). Pathway activation directly exacerbates the initiation and amplification of intestinal inflammation [32]. This study revealed that 4-MC significantly reduced the p-p65/p65 ratio in DSS-induced colonic tissue, indicating inhibition of NF-κB phosphorylation activation – a mechanism consistent with the classic anti-inflammatory pathway of quercetin in UC [33]. Notably, NF-κB not only directly regulates pro-inflammatory cytokine expression but also transcriptionally modulates NLRP3 expression levels, forming an inflammatory amplification loop *via* the "NF-κB-NLRP3" axis [34]. Therefore, the inhibitory effect of 4-MC on NF-κB may concurrently block NLRP3 inflammasome activation at the transcriptional level, laying the groundwork for subsequent NLRP3 regulation.

Further analysis suggests that the antioxidant effect of 4-MC may represent an important link connecting its regulation of the NF-κB and NLRP3 pathways. ROS are common upstream signals involved in the activation of both NF-κB and NLRP3. On one hand, ROS can promote IκBα degradation through oxidative modification, thereby facilitating NF-κB nuclear translocation and activation [35]. On the other hand, ROS can facilitate NLRP3 oligomerization and complex assembly by oxidizing cysteine residues on NLRP3 [36]. In this study, 4-MC was associated with reduced oxidative stress in intestinal tissue, as indicated by increased SOD (a ROS-scavenging enzyme) activity and GSH (non-enzymatic antioxidant) content, together with decreased MDA (a lipid peroxidation product) levels. Consequently, these findings suggest that 4-MC may attenuate ROS-associated activation of both NF-κB and NLRP3, forming a potential regulatory network characterized by antioxidant activity, suppression of inflammatory signaling, and attenuation of inflammation. This mechanism may contribute to the protective effects of 4-MC against experimental colitis. Compared to inhibitors targeting solely NF-κB or NLRP3, 4-MC's antioxidant action may enable modulation of multiple inflammatory pathways, potentially providing advantages over single-target inhibition.

Despite the positive findings, we must acknowledge the limitations of this study. First, the DSS-induced colitis model, while classic and widely used, does not fully recapitulate the chronic and complex pathophysiology of human UC. Furthermore, the present study focused on innate immune and barrier pathways, and did not explore other key aspects of UC pathogenesis such as the gut microbiota. The imbalance of intestinal flora UC is characterized by a decrease in bacterial diversity, especially in regions of active inflammation [37]. Given that polyphenol metabolites are known to interact with microbial communities [38], the therapeutic effects of 4-MC (polyphenol metabolites) may involve modulation of dysbiosis – an important mechanism that remains to be investigated. Future studies should validate the efficacy of 4-MC in more chronic or spontaneous models (such as TNBS-induced chronic colitis or IL-10 knockout mice), assess its long-term safety profile, and explicitly examine its impact on the composition and function of the gut microbiome to provide a more comprehensive understanding of its mechanism of action. Determining whether 4-MC can restore a healthy microbial ecology, increase the production of beneficial metabolites, or decrease the abundance of pro-inflammatory pathobionts would provide a more holistic understanding of its therapeutic mechanism in UC.

In conclusion, our findings provide evidence that the natural small-molecule compound 4-MC exerts protective effects in a DSS-induced murine colitis model. Its protective mechanism is multifaceted, involving improvement of the intestinal barrier, alleviation of oxidative stress, and suppression of inflammatory responses. These effects may involve modulation of two pivotal inflammatory pathways: NF-κB and the NLRP3 inflammasome. These findings support further investigation of 4-MC as a potential candidate for the treatment of UC and highlight the potential advantage of compounds that modulate multiple pathogenic processes in complex diseases such as UC. Future studies should aim to identify the direct molecular targets of 4-MC and assess its efficacy in chronic models that better recapitulate aspects of human disease to advance its clinical translation.

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Disclosures

The study was approved by the Animal Ethics Committee of Zhejiang Baiyue Biotech Co., Ltd for ex-

perimental animals welfare (Approval No. ZJBYLA-IACUC-20250205).

The authors declare no conflict of interest.

Supplementary material is available on the journal's website.

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