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Protective effects of berberine hydrochloride on DSS-induced ulcerative colitis in rats

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ABSTRACT

Background: Berberine hydrochloride is one the effective compound among Rhizoma Coptidis, Cortex Phellodendri, and other plants. There are several clinical functions of berberine hydrochloride including anti-inflammation, antitumor and immunoregulatory. However, the anti-inflammatory of berberine hydrochloride in ulcerative colitis is barely understood. In this study, we aimed to explore the effects of berberine hydrochloride on dextran sulfate sodium (DSS)-induced rats model of ulcerative colitis.

Methods: The severity of colitis were measured by body weight, survial rate, colon length and disease activity index (DAI) score. The cytokines expression include IL-1, IL-1β, IL-4, IL-6, IL-10, IL-12, TNF-α, TGF-β and IFN-γ were performed by RT-PCR and ELISA. Signaling pathway proteins such as p-STAT3, STAT3, p-NF-κB p65 and NF-κB p65 were analyzed by western blot and immunofluorescence. The proteins expression of tight junction were explored using western blotting and immunohistochemistry.

Result: Rats were administered berberine hydrochloride showed less weight loss and longer colon length than the DSS-induced group. The expression of IL-1, IL-1β, IL-6, IL-12, TNF-α, TGF-β and IFN-γ were suppressed, yet the expression of IL-4 and IL-10 were up-regulated by berberine hydrochloride and sulphasalazine treatment compared to the model group. Meanwhile, treatment with berberine hydrochloride effectively increased the expression of SIgA and decreased the expression of iNOS, MPO, MDA. In terms of the biochemical analyses, the results showed that the expression of p-STAT3 was signifcantly increased, while the expression of p-NF-κB (p65) was suppressed compared to the model group via western blot and immunofluorescence analysis.

Conclusions: Berberine hydrochloride has beneficial effects in UC. The possible mechanism of anti-inflammatory response by berberine hydrochloride may involve in the blocking of the IL-6/STAT3/NF-κB signaling pathway. Collectively, these fndings provide evidence that berberine hydrochloride might be a useful herb medicine and serve as a promising novel therapy in the treatment of UC in humans.

1. Introduction

Ulcerative colitis (UC), one of the two major forms of inflammatory bowel disease (IBD) [1], is an inflammatory disease of the colon connected with recurring inflammation and the formation of ulcers [2]. It is a chronic and relapsing inflammatory disease characterized by dysregulation immune response and imbalanced of cytokines and unresolved inflammation associated with intestinal mucosa [3]. UC is an important public health problem, which counld lead to peritonitis and increase the risk of colorectal cancer. Epidemiological studies have shown that the incidence and prevalence of UC are increasing throughout the world, and tends to afflict young people, which indicates that it is a global disease [4]. Meanwhile, the pathogenic mechanism of UC is barely understood, which is the main reason for the limited choice of current therapeutic.

Berberine hydrochloride is one the effective compound among Rhizoma Coptidis, Cortex Phellodendri, and other plants [5]. Increasing evidence suggests that berberine hydrochloride have several biological activitys such as immunoregulatory, anti-inflammatory, antitumor, hypoglycemic and lipidlowering [6–9]. Even if the anti-inflammatory effect and mechanism of berberine hydrochloride in the intestinal tract are still largely unknown.

NF-κB is considered to be one of the most important regulators of inflammatory process, which is a widely expressed nuclear transcription factor. The downstream NF-κB signaling pathway can be activated through p65 translocation into the nucleus to change the expression of related inflammatory genes, when the phosphorylation and degradation of IκB-α occur [10,11]. The inflammation reaction may be caused by proinflammatory cytokines. Previous studies have suggested that patients

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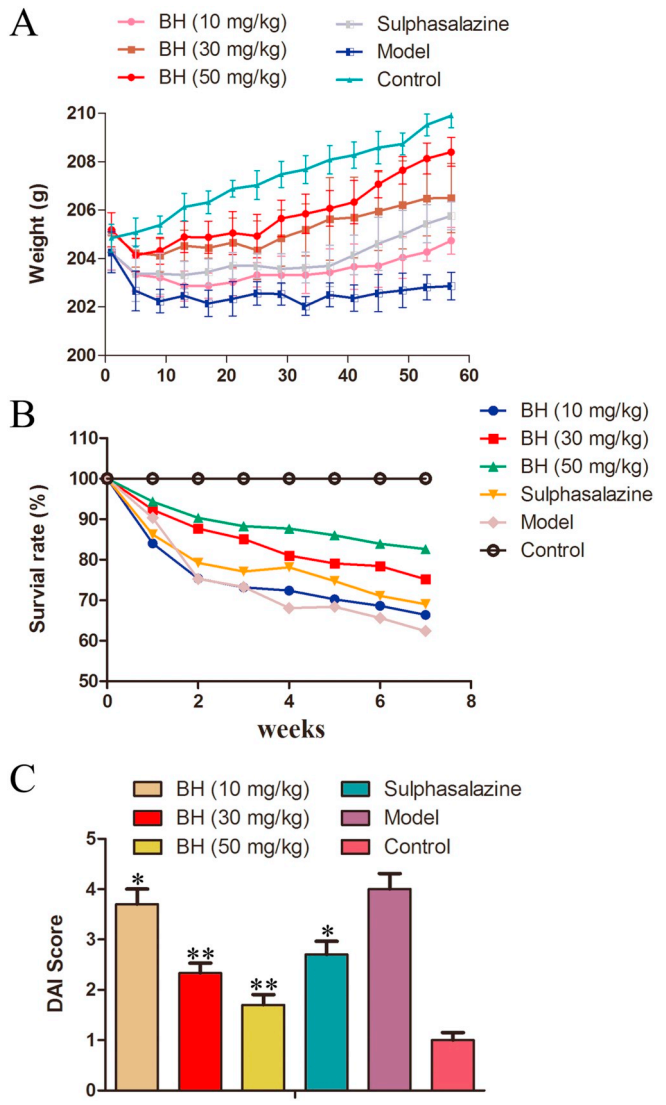

Fig. 1. Effect of berberine hydrochloride (BH) on clinical signs in DSS-induced colonitis. (A) DSS-induced ulcerative colitis caused the loss of body weight in rat. (B) Effect of BH on survival rate in rat. (C) Disease activity index score in the six groups. *P < 0.05, **P < 0.01 vs model group.

with bowel disease have increased the expression of interferon-gamma (IFN-γ), interleukin (IL)-1, IL-6 and tumor necrosis factor-alpha (TNF-α), which regulate the inflammatory response in the blood and colon tissue of UC. Elevated levels of cytokines are also associated with the pathogenesis of bowel disease. IL-6 is a major proinflammatory cytokine that plays an important role in UC. IL-6 is up-regulated and contributes to the occurrence of colonic tumorigenesis in patients with ulcerative colitis [12–14]. IL-6 binds to its membrane-bound receptor to activate signal transducer and activator of transcription 3 (STAT3) [15].

However, protective effect of berberine hydrochloride on UC is still unclear. Base on these theories mentioned above, we suppose that berberine hydrochloride may inhibit the activation of NF-κB and contributes to the treatment of UC.

2. Material and methods

2.1. Animals and grouping

A total of 60 health male wistar rats (200–230 g) were purchased from the laboratory animal center of yangzhou university. Animals were randomly divided into six groups, each consisting of ten animals, including the model (the dextran sulfate sodium; DSS), low-dose berberine hydrochloride group (10 mg/kg), medial-dose berberine hydrochloride group (30 mg/kg), high-dose berberine hydrochloride group (50 mg/kg), the sulphasalazine group and control group. Ulcerative colitis were induced in rat by administering 5% DSS in the drinking water for 7 days, except for the control group that had access to distilled water only. From the two week to the eight week, rat in the experimental group were gastric with drugs daily. Body weight was measured at the same time on the experimental days. On the eighth weeks, the rats were sacrificed by cervical dislocation and then the colon specimens were collected. Berberine hydrochloride was obtained from Sigma-Aldrich (Merck Millipore, Darmstadt, Germany).

2.2. Morphology and histological evaluation

Colon tissues with severe damage were removed and fixed in 10% neutral formalin. Then they were embedded in paraffin and stained with hematoxylin and eosin (HE). The criteria used to assess colitis using a standard histological scoring system [16]. Briefly, 0 = no damage; 1 = mucosal congestion; 2 = ulceration area < 25% of the damaged area; 3 = ulceration area equal to 25–50% of the damaged area; 4 = ulceration area > 50% of the damaged area.

2.3. Evaluation of UC severity based on the disease activity index (DAI) score

The DAI score has been widely used to evaluate the severity of UC in animal models. Briefly, an investigator complying the protocol recorded and scored the changes in weight, hemoccult positivity or gross bleeding, and stool consistency according to the previous report. The DAI score was a combination of scores of all these parameters mentioned.

2.4. Quantitative real-time RT-PCR (RT-qPCR)

The total RNA in colon tissue was extracted using Trizol (Invitrogen, USA) and RNA was reverse-transcribed into cDNA using RNA reverse transcription kits. mRNA expression levels were examined on a Bio-Rad Q5 instrument (Bio-Rad, CA, USA) using a SYBR Premix EX Taq Real-time PCR Master Mix (TaKaRa). The $2^{-\Delta\Delta Ct}$ method was used to normalize target gene transcription to β-actin expression (internal control) to calculate fold induction of target mRNA.

2.5. Enzyme linked immunosorbent assay (ELISA)

After treated with berberine hydrochloride, whole bloods samples were collected from blood circum after the reperfusion. Immediately, blood samples were centrifuged at 12,000*g* for 10 min at 4 °C and then the supernatant were collected for measurement at −20 °C. Levels of IL-1, IL-1β, IL-4, IL-6, IL-10, IL-12, TNF-α, TGF-β and IFN-γ were measured using the Quantikine ELISA kit. The absorbance was measured at 450 nm.

2.6. Western blotting

Cells were lysed with RIPA lysis buffer and proteins were harvested. Total cell protein extracts were separated by 10% SDS polyacrylamide gel electrophoresis, and then transferred onto a nitrocellulose membrane which block with 5% non-fat milk. Whereafter, the blots were incubated with primary antibodies at 4 °C overnight. The specific primary antibodies are as follows: p-NF-κB/p65 (3033; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), NF-κB/p65 (sc-372; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), p-STAT3 (4113; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), STAT3 (9139; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), Occludin (5506; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), Claudin-1

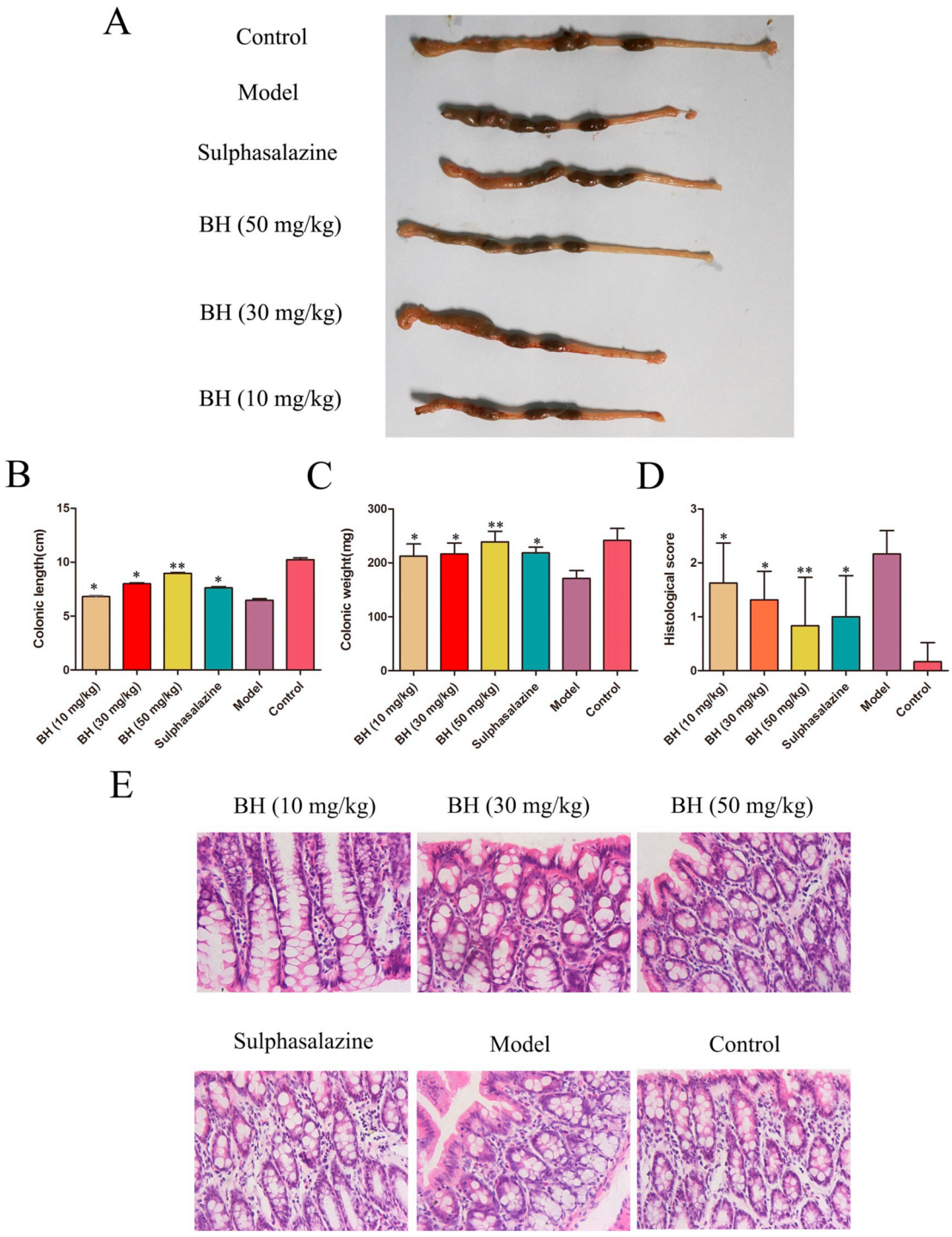

Fig. 2. Berberine hydrochloride (BH) pretreatment alleviated DSS-induced colon inflammation. (A,B) Effect of BH on DSS-induced colon shortening. (C) Colon weights were measured. (D) Effects of BH on microscopic damage scores. (E) Histopathological changes after DSS stimulation in colon (×400). *P < 0.05, **P < 0.01 vs model group.

(4933; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), ZO-1 (5406; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA), VCAM-1 (32,653; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA) and GAPDH (3683; dilution, 1:1000; Cell Signaling Technologies; Beverly, MA, USA). Following three washes with TBST, the membranes were incubated with an horseradish peroxidase-conjugated goat anti-rabbit secondary antibody diluted with 5% BSA at room temperature for 1 h. Immunoblots were visualized using the ECL detection system and the protein levels were quantified using ImageJ software.

2.7. Immunohistochemistry

Frozen colon tissue sections were fxed on glass slides by incubating in methanal for 10 min at 4 °C. Microwave irradiation was performed on the sections and the slides were incubated with 3% H_2O_2 for 20 min at room temperature. This was followed by incubation with primary antibodies at room temperature of 2 h. Then, sections were washed with PBS and incubated with secondary antibodies at room temperature for 1 h. After that, DAB reagents were used for chromogenic reaction. Counter staining was performed with hematoxylin. All images

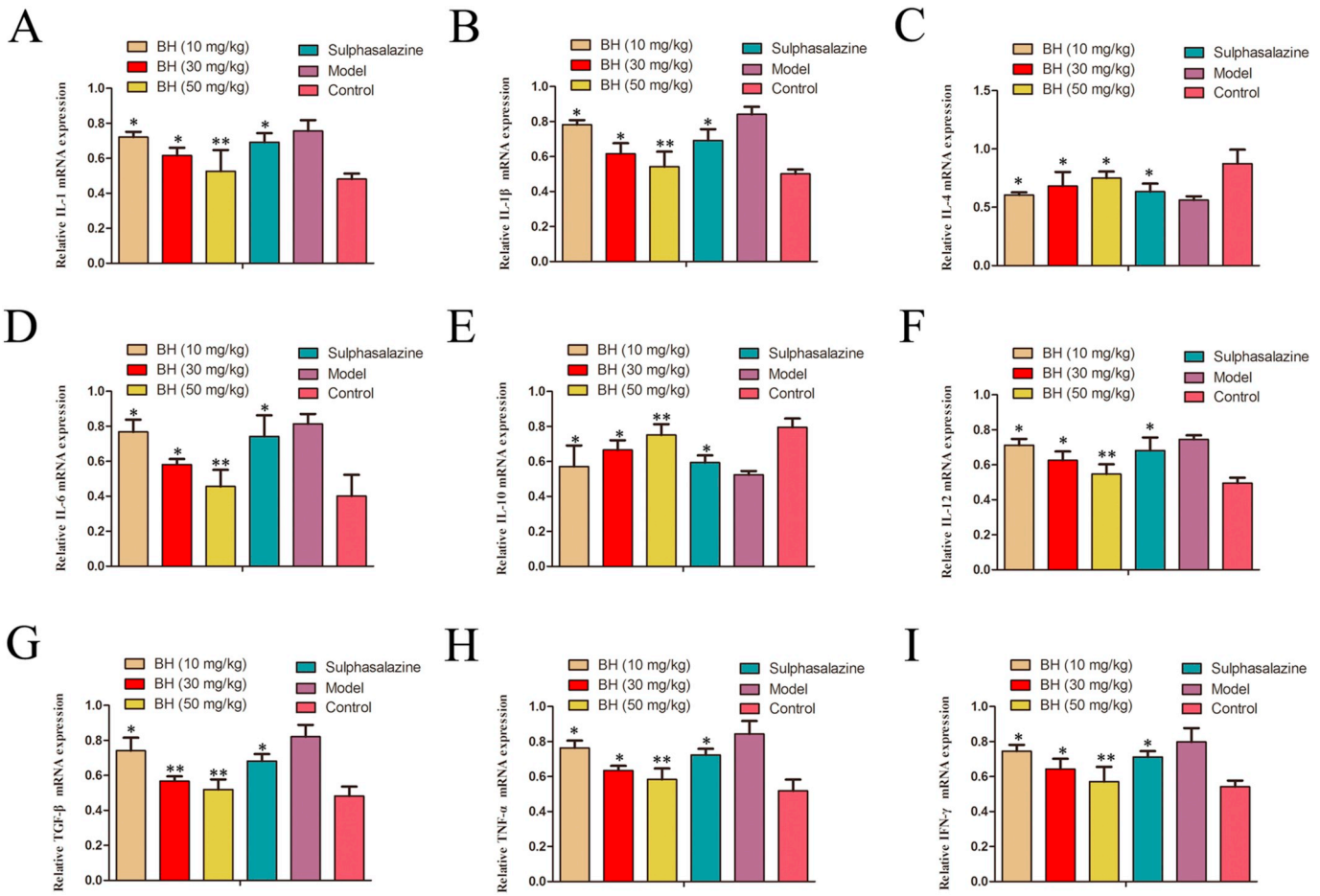

Fig. 3. Effects of berberine hydrochloride (BH) on the mRNA expression of cytokines. (A-I) The mRNA expression of IL-1, IL-1β, IL-4, IL-6, IL-10, IL-12, TNF-α, TGF-β and IFN-γ was detected by RT-PCR and datas were generated from colon tissue. Data are expressed as the mean ± SD. *P < 0.05, **P < 0.01 vs model group.

represented at least three separate experiments.

2.8. Flow cytometry

The DCs isolated from rat colon whole tissues and measured by flow cytometry. Then the DCs were positively sorting by major histocompatibility class II (MHCII) and CD11c. We used FACS analyses and gated on live CD11c high cells and MHCII high cells. MHCII high/CD11c high double-positive cells were used to determine percentages of DCs.

2.9. Statistical analysis

Results were analyzed by SPSS 17.0 software and expressed as the mean ± standard derivation for at least three separate experiments. One-way analysis of variance (ANOVA) was used to determine statistically significant differences. *P < 0.05, **P < 0.01. P < 0.05 was considered significant differences.

3. Results

3.1. Effects of berberine hydrochloride on DSS-induced colitis clinical symptoms of rat

Rats in the control group exhibited normal activity, diet, stool consistency, along with normal hair color and weight gain. However, the rats showed a noteworthy decrease in body weight (Fig. 1A), survial rate (Fig. 1B), colon length and colon weight (Fig. 2A–C) in the model group compared to the control group, respectively. Moreover, as expected, the rats receiving DSS administration showed significantly increased DAI scores (Fig. 1C). These rats exhibited a reduction in daily activity, anorexia, drab hair color and weight loss with a loose stool. These rats had dramatically lower DAI and noteworthy weight loss compared to the control group. In the berberine hydrochloride and sulphasalazine pretreated groups the symptoms were milder than the model group.

3.2. Histological observation and evaluation

The histological analysis were performed to estimate the extent of damage of colon tissues. As shown in Fig. 2E, HE staining of control group showed no evidence of damage in the colonic mucosal epithelium. In contrast, in DSS group had extensive inflammatory cell infltration in the submucosa. However, berberine hydrochloride and sulphasalazine pretreated groups showed reduction in the levels of congestion and edema compared with those of the model group. As shown in Fig. 2D, there was signifcant difference between the microscopic damage scores of the control group and model group. There was no difference between the microscopic damage scores of 10 mg/kg berberine hydrochloride treated group and the model group. On the other hand, treatment with 50 mg/kg berberine hydrochloride caused signifcant reduction compared to the model group.

3.3. Effect of berberine hydrochloride on cytokine levels in DSS-induced UC

The expressions of cytokines were determined by ELISA and PCR assays, respectively. PCR results showed higher IL-1, IL-1β, IL-6, IL-12, TNF-α and IFN-γ levels was found in the model group compared with the control group, but they were dramatically decreased by treatment

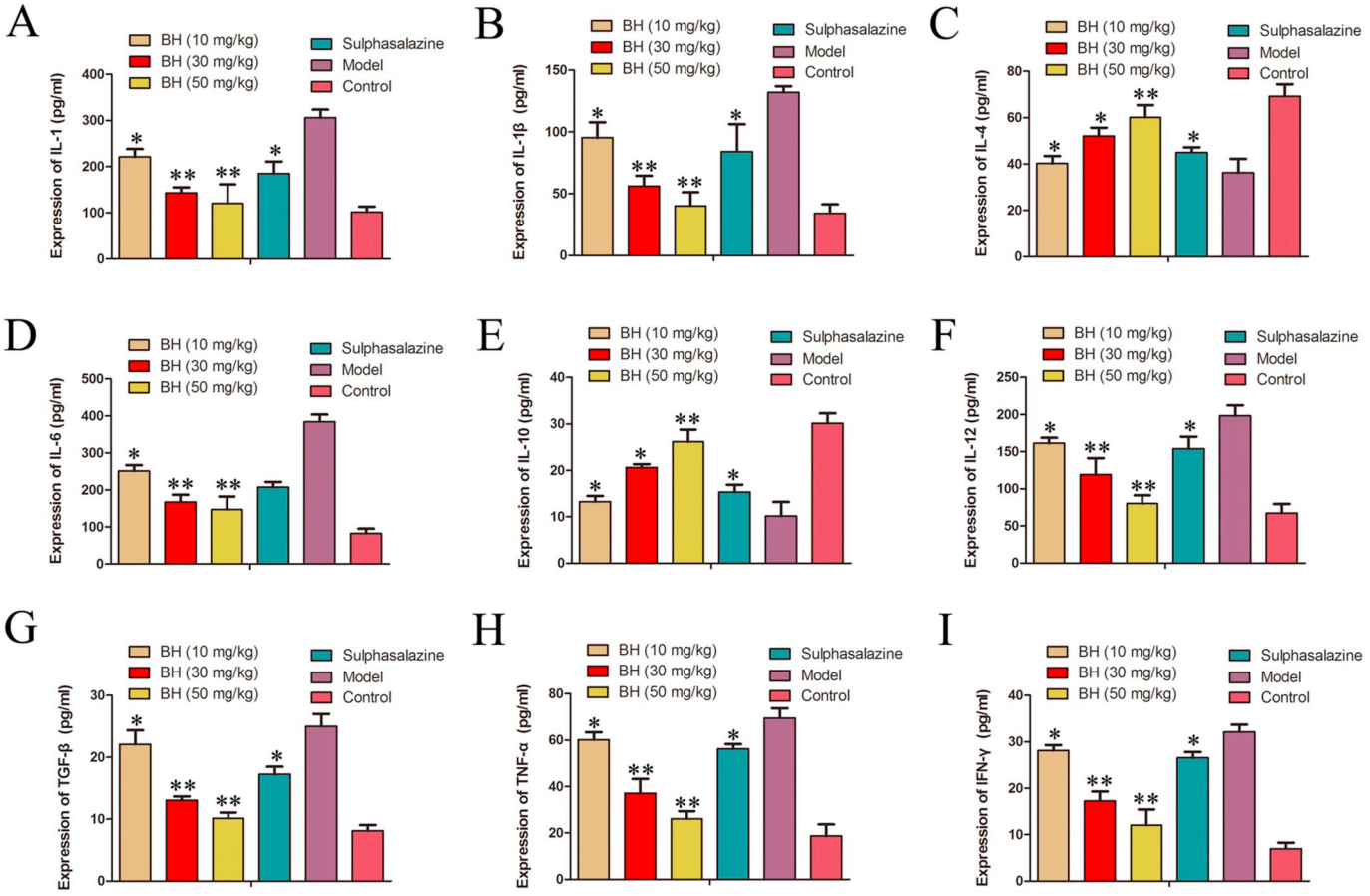

Fig. 4. Effects of berberine hydrochloride (BH) on the production of cytokines. (A-I) The expression of IL-1, IL-1β, IL-4, IL-6, IL-10, IL-12, TNF-α, TGF-β and IFN-γ proteins was detected by ELISA and datas were based on the serum. Data are expressed as the mean ± SD. *P < 0.05, **P < 0.01 vs model group.

of berberine hydrochloride (Fig. 3). However, DSS significantly decreased the mRNA expression of IL-4, IL-10 and compared with the control group. Berberine hydrochloride pretreatment dose-dependently induceed the mRNA expression of IL-4 and IL-10. Similarly, ELISA results suggested that IL-1, IL-1β, IL-6, IL-12, TNF-α, TGF-β and IFN-γ levels were noteworthy higher in berberine hydrochloride and sulphasalazine than in the control group, but was lower in the model group (Fig. 4). On the other hand, berberine hydrochloride pretreatment significantly stimulate the protein expression of IL-4 and IL-10. Collectively, these results demonstrated that berberine hydrochloride inhibited the levels of pro-inflammatory cytokines in UC.

3.4. Effect of berberine hydrochloride on inflammation

In an attempt to identify the effectiveness of berberine hydrochloride on DSS-stimulated colon inflammation, we further analysis the activity of iNOS, MPO, SIgA and MDA. As shown in Fig. 5A–D, the data indicated that the activity of iNOS, MPO and MDA in the model group significantly increased compared with the control group. Berberine hydrochloride pretreatment dramatically decreased the levels of INOS, MPO and MDA. However, SIgA level was noteworthy decreased in model group compared with the control group. On the other hand, treatment with berberine hydrochloride markedly reduced iNOS, MPO and MDA activity and induced SIgA level. In addition, the number - of DC cells were assessed by flow cytometry (Fig. 5E).

3.5. Expressions of signal pathway related genes and proteins

We tested the signal pathway-related genes and proteins to further characterize the anti-inflammatory mechanism of berberine hydrochloride by activated STAT3 via PCR and western blot analysis. As shown in Fig. 6A and B, berberine hydrochloride noteworthy decrease the expressions of IL-6 and p-NF-κB compared with the model group. Conversely, berberine hydrochloride markedly induced the expression of p-STAT3. There were no significant differences in the total amount STAT3 and NF-κB proteins between the model group and other group. In addition, we analyzed these proteins by immunofluorescence analysis, similar results were obtained (Fig. 6C).

3.6. mRNA and protein expressions of tight junction

The expressions of occludin, claudin-1, ZO-1 and VCAM-1 were detected by RT-PCR, western blot and immunohistochemistry, respectively. As shown in Fig. 7A, the mRNA expressions of occludin, claudin-1, ZO-1 and VCAM-1 in berberine hydrochloride and sulphasalazine were clearly increased compared with the model group. Meanwhile, the results shown that administration of berberine hydrochloride significantly increased the protein expression levels of occludin, claudin-1, ZO-1 and VCAM-1 compared to the model group by western blot (Fig. 7B). Immunohistochemistry staining for occludin, claudin-1, ZO-1 and VCAM-1 showed stronger immunoreactivity in the berberine hydrochloride and sulphasalazine group than in the model group (Fig. 7C). (Fig. 8.)

4. Discussion

Ulcerative colitis (UC) is an inflammatory disease of the colon associated with recurring inflammation and the formation of ulcers. Approximately 10 individuals per 100,000 people are diagnosed annually with UC [17]. Although the pathogenic mechanism of UC is

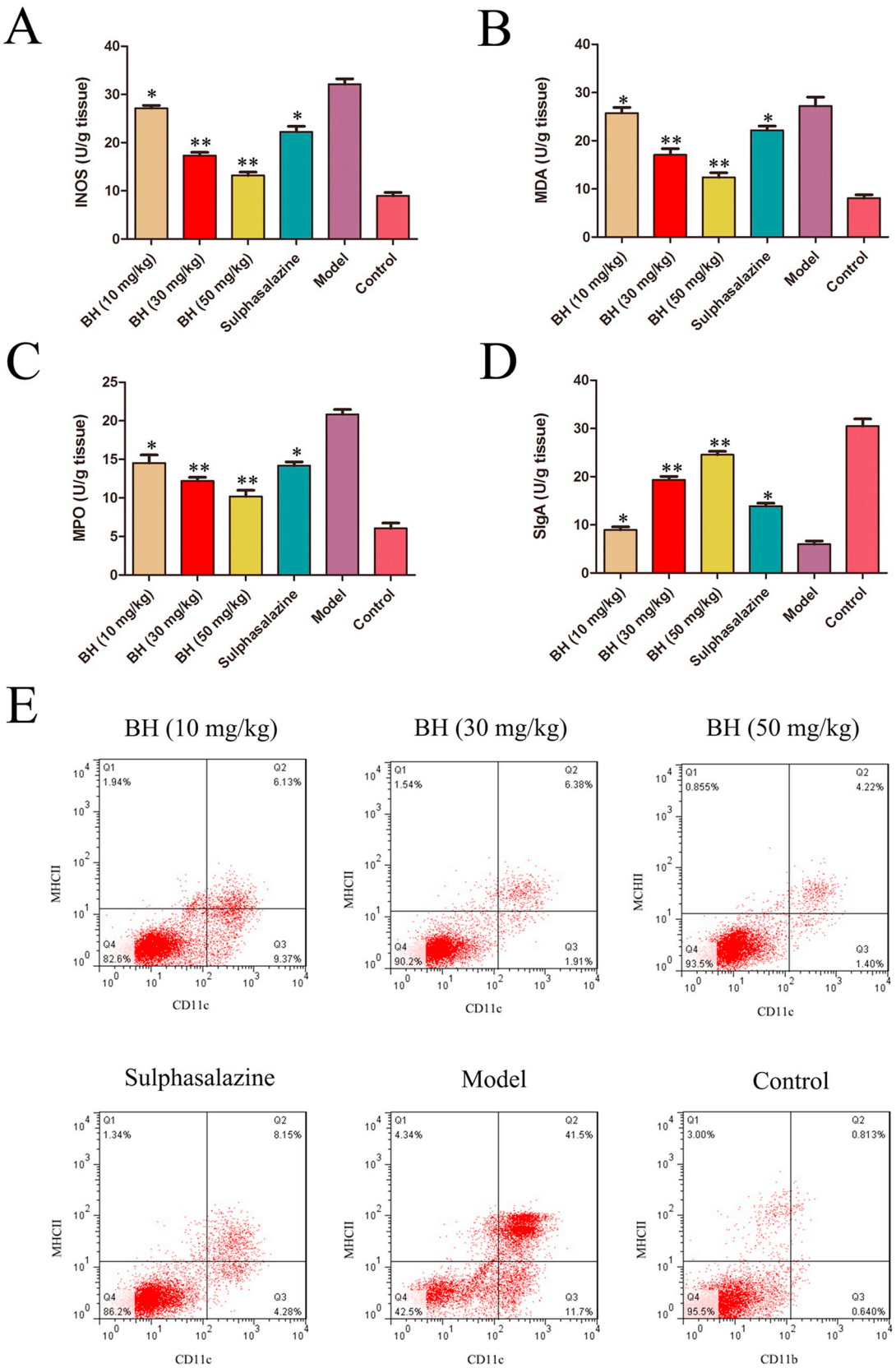

Fig. 5. Effects of berberine hydrochloride (BH) on INOS, MPO, SIgA, MDA activity of serum in rats and DC cells. The datas were based on the serum. Data are presented as means ± SEM. *P < 0.05, **P < 0.01 vs model group.

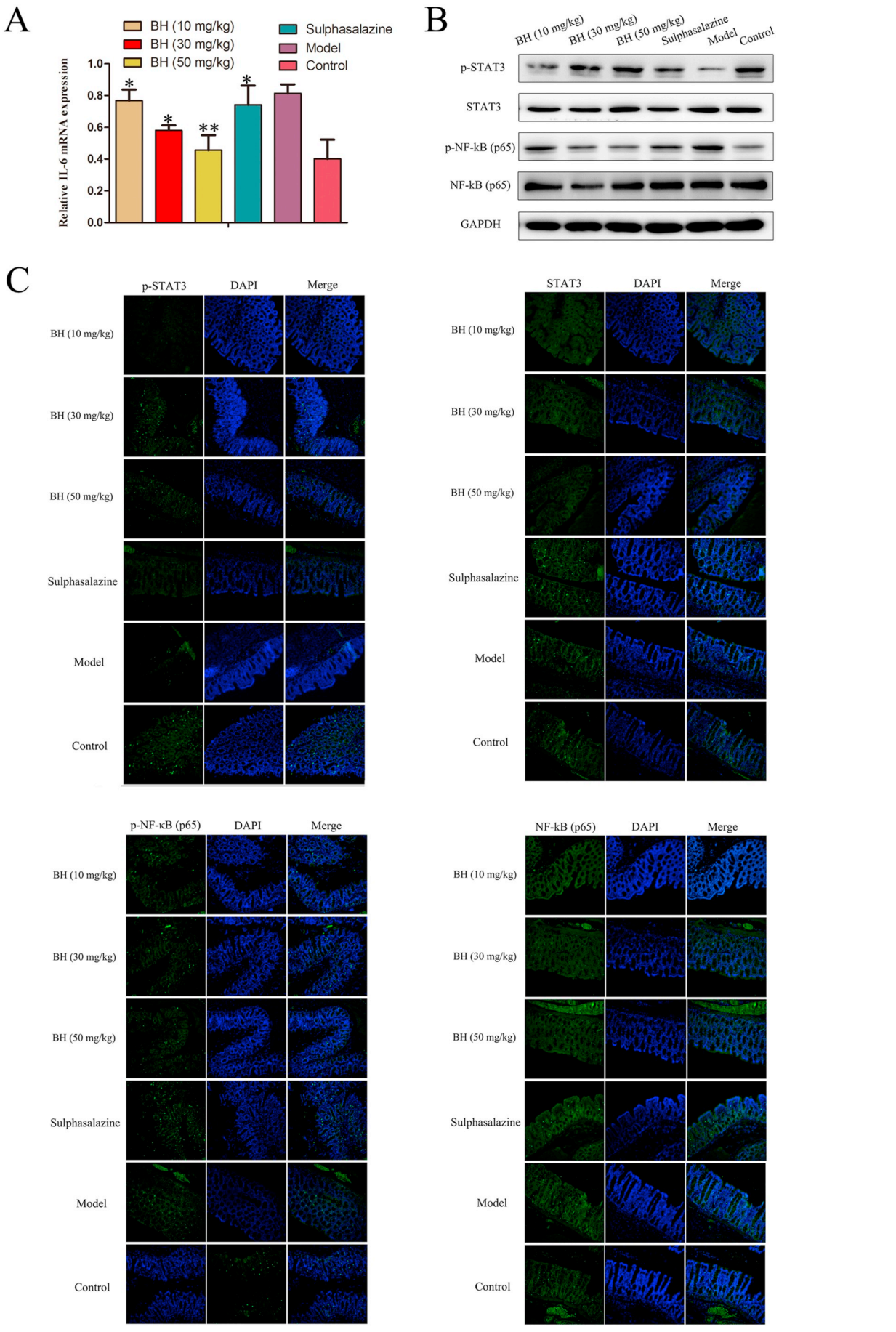

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Fig. 6. Berberine hydrochloride (BH) develop the anti-inflammatory activity though the STAT3 pathway in UC. (A-C) BH regulates the STAT3 pathway activity and expression of some related genes and proteins. *P < 0.05, **P < 0.01 vs model group. *P < 0.05, **P < 0.01 vs model group.

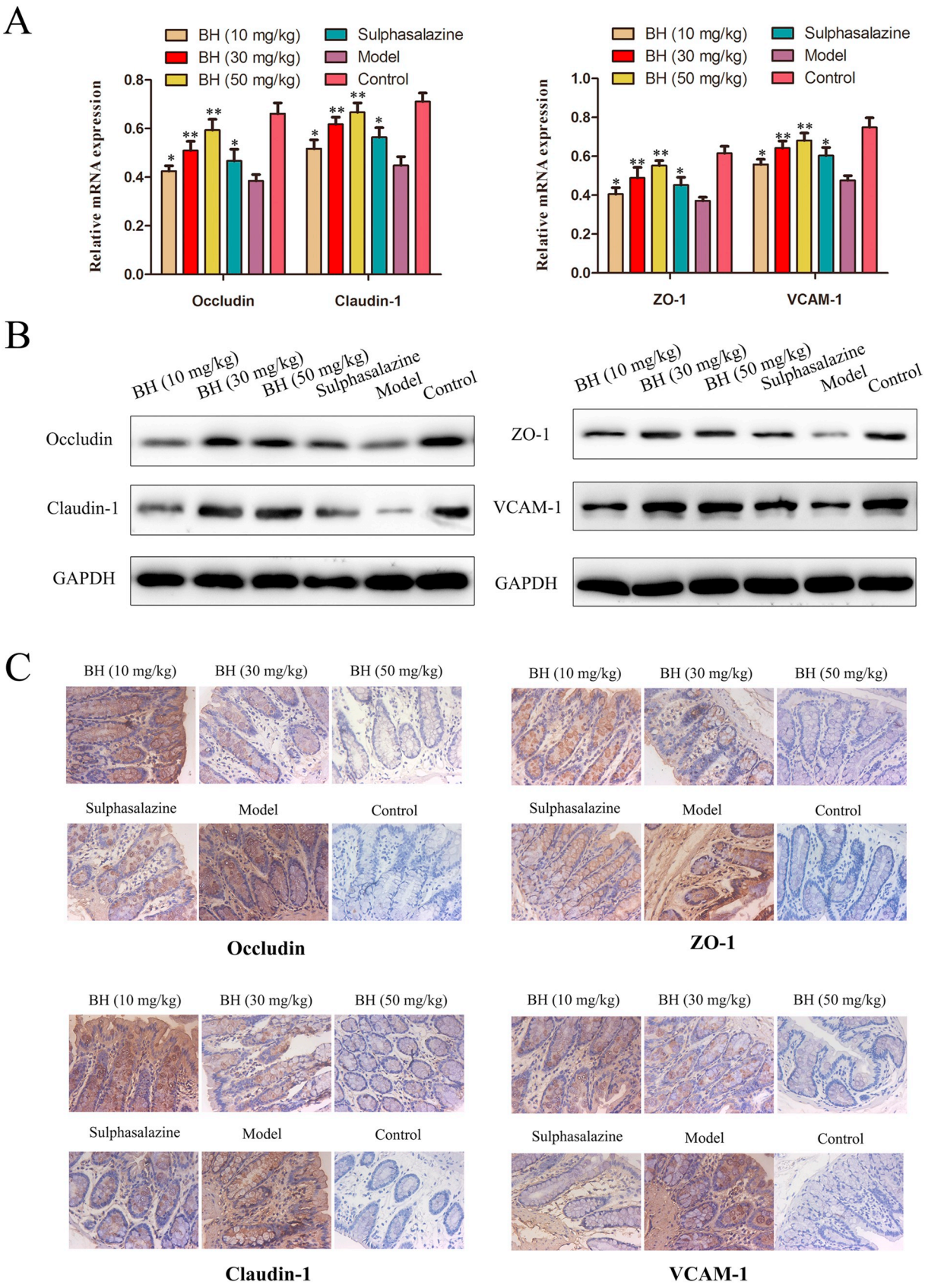

Fig. 7. The mRNA and protein expressions of tight junction. (A) Occludin, claudin-1, ZO-1 and VCAM-1 relative mRNA expression in UC tissues. (B) Occludin, claudin-1, ZO-1 and VCAM-1 relative protein expression in UC tissues. (C) The expressions of these proteins were tested by immunohistochemistry in experimental colitis in rats. *P < 0.05, **P < 0.01 vs model group.

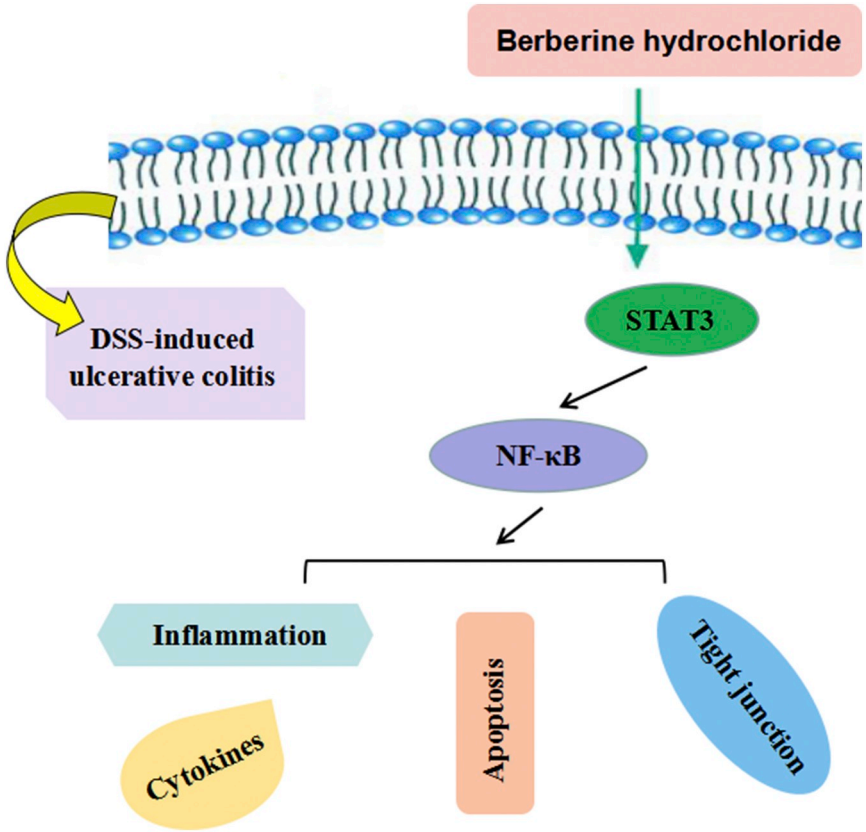

Fig. 8. Schematic presentation of probable protective effect of berberine hydrochloride on ulcerative colitis.

barely understood, accumulating evidence indicates that several factors in the initiation of human colitis, such as genetic, environmental factors, prolonged neutrophil infiltration and increased production of inflammatory mediators are contributes to the pathogenesis of UC [18]. Aberrant immune responses can be observed in the large intestine, in which distinct immune cells and cytokines [19,20]. Immune modulators, nonsteroidal anti-inflammatory drugs, such as sulphasalazine and glucocorticosteroids, have been broadly used for therapy for UC [21,22]. However, these therapies can cause side effects, such as vomiting, toxicity, generalized edema and anemia. Therefore, people are becoming more and more interested in the use of many natural compounds in traditional herbs. These natural compounds have anti-inflammatory effects and can be used to treat chronic inflammatory diseases [23,24].

Berberine hydrochloride is a traditional herbal medicine which have an anti-inflammatory properties [25]. Accumulating evidence suggested that berberine hydrochloride appears to alleviate symptoms of several chronic inflammatory diseases, such as allergic diseases, hepatitis and experimental autoimmune encephalomyelitis. Furthermore, some previous studies have suggested that berberine hydrochloride has obvious protective effects on LPS-induced endometritis in mice [26]. Dendritic cells (DCs) are the only and most powerful antigen presenting cells, which are capable of activating naive T cells. DCs have important pathogenic role in occurrence, progression, and immune regulation of DSS-induced colitis [27–31]. During the inductive phase of colitis, DCs, by producing inflammatory cytokines and chemokines, promote the influx of immune cells in injured colon and induce inflammation [32]. DCs were critical for the generation of immune tolerance to autoimmune disorders. Elaborately, suppressive DNA motifs did not directly disturb the uptake of nucleic acid or competitively bind to TLR9 in DCs, but showed high affinity to highmobility group box proteins, which were critical for the activation of subsequent nuclei acid-mediated innate immune signal cascades [33,34]. Along with IL-6, NF-κB p65 phosphorylation, TNF-α and inflammation related factors affect clinical signs in UC [35–38]. Morever, NF-κB is a critical signaling molecule in inflammatory process and the activation of NF-κB is important in the pathogenesis of UC [39,40].

In the present study, the rats induced colitis by DSS, which has been widely used as an experimental model in the study of the pathogenesis of UC. The clinical parameters such as DAI were remarkably increased in the model group. The symptoms of weight loss and survial rate were noteworthy ameliorated after berberine hydrochloride and sulphasalazine treatment. In addition, the histological change of the intestinal mucosa were close to the control group.

We further verified the expression of the cytokines IL-1, IL-1β, IL-4, IL-6, IL-10, IL-12, TNF-α, TGF-β and IFN-γ which are known mediators of inflammation in UC [41]. The result of the ELISA assay showed that the expression of IL-1, IL-1β, IL-6, IL-12, TNF-α, TGF-β and IFN-γ were dramatically depressed in berberine hydrochloride group, accompany with an increasing levels of IL-4 and IL-10 compared with the model group. Furthermore, our study has demonstrated that berberine hydrochloride were able to suppress the expressions of phosphorylation of STAT3 and phosphorylation of NF-κB p65 which were induced by DSS in colonic tissues. These findings suggested that berberine hydrochloride can efectively inhibit the activation of IL-6/STAT3/NF-κB in an experimental UC model in vivo.

All these results indicated that the berberine hydrochloride may be alleviated inflammatory response in UC via blocking IL-6/STAT3/NF-κB signal pathway. In summary, we have shown for the frst time that berberine hydrochloride can prevent DSS-induced colitis in rats. Taken together, these findings indicated that berberine hydrochloride might be a promising and useful approach for treatment of UC in humans.

Conflict of interest

The authors have no conflict of interest pertaining to this manuscript.

Acknowledgements

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