Brusatol-enriched Brucea javanica oil ameliorated dextran sulfate sodium-induced colitis in mice: involvement of NF-κB and RhoA/ROCK signaling pathways

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Abstract

Background: Our previous study indicates that *Bracea javanica* oil (BJO) is beneficial for treatment of ulcerative colitis (UC), and that quassinoids in particular brusatol are bioactive components. However, it is still uncertain whether or not other components in BJO, such as oleic acid and fatty acids, have anti-UC effect.

Purpose: The present study aimed to compare the anti-UC effects between brusatol-enriched BJO (BE-BJO) and brusatol-free BJO (BF-BJO), and to explore the effects and mechanisms of BE-BJO on colon inflammation and intestinal epithelial barrier function.

Methods: Balb/C mice received 3% (wt/vol) DSS for one weeks to establish the UC model. Different doses of BE-BJO, BF-BJO or BJO were treated. Body weight and colon length were measured. Disease activity index (DAI) and histological analysis were evaluated. The levels of pro-inflammatory cytokines in the colon tissues were measured by enzyme linked immunosorbent assay (ELISA). The expressions of tight junction proteins were tested to investigate the intestinal epithelial barrier function. The effects of BE-BJO on NF-κB and RhoA/ROCK pathways were studied.

Results: BE-BJO alleviated DSS-induced loss of body weight, increase of DAI and shortening of colon, whereas BF-BJO did not have these protective effects. BE-BJO treatment improved the morphology of colon tissue, inhibited the production and release of pro-inflammatory cytokines including TNF-α, IFN-γ, IL-6 and IL-1β in the colon tissue, as well as reversed the decreased expressions of ZO-1, Occludin, Claudin-1 and E-cadherin induced by DSS, but augmented Claudin-2 expression.
Mechanistically, BE-BJO repressed phosphorylation of NF-κB subunit p65, suppressed RhoA activation, downregulated ROCK, and prevented phosphorylation of myosin light chain (MLC) in DSS-treated mice.

**Conclusions:** This work demonstrated that BE-BJO could ameliorate DSS-induced UC by preventing colon inflammation and enhancing intestinal epithelial barrier function, probably via suppression of NF-κB and RhoA/ROCK signaling pathways. These findings confirm that quassinoids are active compounds from BJO and suggest the therapeutic potential of quassinoids and BE-BJO in the treatment of UC.

**Key words:** *Brucea javanica* oil; Brusatol; Ulcerative colitis; RhoA/ROCK pathway
1. Background

Ulcerative colitis (UC) is a chronic bowel disorder characterized by diffuse inflammation of the mucosa of the colon and rectum (Zong et al., 2017; Szandruk et al., 2018). The hallmark clinical symptoms of UC include abdominal pain, rectal bleeding and diarrhea (Zhang et al., 2012). Persistent UC leads to the development of colorectal cancer (Seril et al., 2007). Currently, the etiopathogenesis of UC has not been completely understood. Although several categories of therapeutic drugs, such as anti-inflammatory drugs and immunosuppressants, have improved the life expectancy of patients, available effective therapies are still limited and the resurgence of diseases often occur (Christian et al., 2010; Citalan-Madrid et al., 2017; Yu et al., 2017). Therefore, development of effective therapeutic drugs for the management of UC would have important clinical implications.

*Brueca javanica* (L.) Merr. (Ya-dan-zi in Chinese, Simaroubaceae) is a crucial Chinese folk medicine widely used for the treatment of dysentery, which was also known as inflammatory bowel disease (IBD) (J et al., 2013; Qiu et al., 2019). *Brueca javanica* oil (BJO), an extract of the desiccative ripe fruit of the *Brueca javanica* (L.) Merr, exhibits various bioactivities, including anti-inflammatory, antimalarial, and antitumor activities (Shao et al., 2013). Our previous studies have shown that BJO protects against IBD in both 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced Crohn’s disease model and dextran sulfate sodium (DSS)-induced UC model, via suppression of NF-κB activation (Huang et al., 2017b; Huang et al., 2019b). Chemically, BJO is composed of flavonoids, alkaloids, volatile oils, sterols, fatty acids, and
olyphenolic acids. Among all these components, quassinoids are vital bioactive compounds that display various types of biological activity. Indeed, we have reported that quassinoids, in particular brusatol and bruceine D, had potential therapeutic effects against UC (Dou et al., 2018; Zhou et al., 2018). However, it is still uncertain whether or not other components such as oleic acid and fatty acids have anti-UC effect. Thus, we prepared BJO enriched with quassinoids like brusatol, and brusatol-free BJO which mainly consists of oleic acid and fatty acids. The present study aimed to compare the anti-UC effects between brusatol-enriched BJO and brusatol-free BJO.

Moreover, inflammation and epithelial barrier defects in the alimentary system can damage the normal function of colon and promote the pathogenesis of UC. Since the anti-inflammatory effects of BJO and brusatol have been proven, the present study focused on investigating the potential effect of BJO on improving intestinal epithelial barrier function and repairing mucosal epithelium. This study will also try to elucidate the possible mechanisms of BJO, by investigating the involvement of RhoA/Rho-associated serine-threonine protein kinases (ROCK) signaling pathway, which is pivotal in the process of intestinal inflammation and epithelial barrier dysfunction (Zou et al., 2018).

2. Materials and Methods

2.1 Materials

*Brucea javanica* oil emulsion was provided by Ming Xing Pharmaceutical Co. Ltd.
(Guangzhou, Guangdong, China). Mesalazine (5-aminosalicylic acid, 5-ASA) was purchased from Germany Losan Pharma GmbH Co., Ltd. DSS (molecular weight: 36000–50000) was bought from MP Biomedicals (Canada). Primary antibodies (ZO-1, MLC, PMLC, Occludin, Claudin-1, P65, p-P65, RhoA, p-RhoA, E-cadherin and ROCK-1) were purchased from Affinity Biosciences (CA, USA). β-Actin was purchased from Abcam (Shanghai, China). The enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-6, IL-1β, IFN-γ and IL-10 were obtained from eBioscience (MA, USA).

2.2 Animals

Healthy male Balb/C mice, weighing 22-25 g, were purchased from Laboratory Animal Center of Guangzhou University of Chinese Medicine (Guangzhou, China). The animals were housed under standard environmental conditions where the temperature (23 ± 1 °C), lighting condition (12 h light/ dark cycles) and humidity (40–60%) are tightly controlled. The animals had free access to standard diet and water ad libitum. Animal procedures used in this study were in line with institutional guidelines for the Care and Use of Laboratory Animals (NIH Publication No 85-23, revised 1996).

2.3 Enrichment of quassinoids in BJO and emulsion preparation

Enrichment: 200 g of BJO was mixed with 1000 mL of 95% ethanol. Extraction was performed with ultrasonication for 1 h. The supernatant was collected after the mixture rested for 1 h. The procedure was repeated 3 times to combine the supernatant,
and recovering the lower liquid. The supernatant and lower liquid were concentrated under reduced pressure at 80 °C respectively. The concentrated supernatant was called brusatol-enriched BJO (BE-BJO) and the concentrated lower liquid was called brusatol-free BJO (BF-BJO).

Emulsion preparation: 7.5 g soybean lecithin was weighed and dissolved in 500 mL water, and homogenized at high pressure to obtain a soybean lecithin emulsion. 5.6 g BJO was weighed and added with a small amount of heat soy lecithin emulsion to obtain crude emulsion. After that, the crude emulsion was added with soy lecithin emulsion to the total volume of 28 mL, following high-pressure homogeneous emulsification to obtain BJO emulsion (BJO). Similarly, 6.1 g BE-BJO was weighed and mixed with soy lecithin emulsion to the total volume of 100 mL, and was homogeneously emulsified at high-pressure to obtain BE-BJO emulsion (BJOE); 5.6 g BF-BJO was weighed to prepare 28 mL BF-BJO emulsion (BF-BJO).

**2.4 Induction of colitis and drug administration**

Acute colitis was induced by 3% (wt/vol) DSS dissolved in drinking water given ad libitum in Balb/C mice for 7 days. All mice were randomly assigned into seven groups: (1) Normal group, received drinking water without DSS; (2) DSS group, given drinking water with 3% DSS and received soybean lecithin suspension throughout the experimental period; (3) 5-ASA group and BJO group, receiving 3% DSS and administrated with 30 mg/kg 5-ASA and 2000 mg/kg BJOE, respectively; (4) BE-BJO groups, receiving 3% DSS and administrated with BE-BJO 152.5 mg/kg/day (low dose,
BE-BJOL), 305 mg/kg/day (medium dose, BE-BJOM) or 610 mg/kg/day (high dose, BE-BJOH) respectively for 7 days.

Body weight was measured daily by using a digital weight scale. After 7 days, all mice were sacrificed after being fasted for 12 h and anesthetized by carbon dioxide (CO₂) inhalation, then the blood were collected immediately. Colon tissues were quickly removed and its length was measured. Then the colon tissues were collected for morphological observation, Western Blot, ELISA and quantitative real-time polymerase chain reaction (qRT-PCR).

2.5 Evaluation of disease activity index (DAI)

The index of disease activity was evaluated according to a previously reported procedure (Huang et al., 2019a). Briefly, DAI was scored as following: DAI= (score of body weight loss + score of stool properties + score of hematochezia)/3. The criteria for evaluation were listed in Table1.

2.6 Cytokine analysis by ELISA

The levels of TNF-α, IL-6, IL-1β, IFN-γ and IL-10 in colon tissues were measured quantitatively by using ELISA kits in accordance with the manufacturer’s protocol. The levels of cytokine were quantified by standard curves.

2.7 Histological analysis

The colon tissues were immediately fixed with 10% buffered formalin following
harvest, then embedded in paraffin and cut into 5μm thick sections. Finally, sections were stained with hematoxylin and eosin (H&E) for histopathological examination. Macroscopic score was evaluated according to the established histological grade (Kim et al., 2012) and was shown in Table 2.

2.8 Western Blotting analysis

The colon segments from each group were homogenized and lysed using cold Pro-prep protein lysis buffer (Intron Biotechnology, Seoul, Republic of Korea) with addition of protease inhibitor cocktail and phosphatase inhibitors, for 30 min on ice. The tissue lysates were centrifuged and the supernatant was collected. BCA assay kit (Thermo Scientific Pierce, IL, USA) was used to determine the protein concentrations of the supernatant. Equal amounts of protein extract for each sample were separated in 8% or 10% of sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to a polyvinylidene difluoride (PVDF) membrane. The PVDF membranes were blocked with 5% skimmed milk for 1 h at room temperature. After that, the membranes were incubated overnight at 4 °C with primary antibodies (dilution, 1:1000) against ZO-1, MLC, PMLC, Claudin-1, Claudin-2, P65, p-P65, RhoA, p-RhoA, E-cadherin, ROCK-1 and β-actin. The membranes were washed with TBS-T, followed by incubation with appropriate horseradish peroxidase (HPR) conjugated secondary antibody (dilution, 1:5000) for 1h at room temperature. Next, the membranes were rinsed 3 times with TBS-T buffer and the signals of protein level were visualized using Western imaging system. The intensity of the protein bands was quantified using Image J software. β-
actin was used as an internal control for total proteins.

2.9 Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCR was implemented as described previously (Hang Thi Thu et al., 2014). Briefly, the colon samples were homogenized with Trizol reagent. And then total RNA was extracted according to the manufacturer's instructions. Quantitated RNA was reverse-transcribed into cDNA and quantitative real-time PCR amplification was performed using the SYBR Green reagent to examine the mRNA expressions of ZO-1, Occludin and Claudin-1. The primer sequences were listed in Table 3.

2.10 Statistical analysis

The results were analyzed by using Graph Pad Prism software 8.0 (GraphPad Software, La Jolla, CA, USA) and presented as mean ± standard error of mean (SEM). The numbers of mice in each group were shown as “n” number. Statistical significance was identified by using one-way analysis of variance (ANOVA). $P < 0.05$ was considered to be statistically significant different.

3. Results

3.1 BE-BJO alleviated DSS-induced colitis in mice

To investigate the potential therapeutic effect of BE-BJO on UC, the mice were treated with 3% DSS for 7 days to induce colitis. The body weights of the mice were tested; DAI including body weight loss, stool properties and hematochezia were
evaluated; and the length of the colon was measured. As shown in Fig. 1, the DSS model group demonstrated a significant decrease in body weight, an increase in DAI scores, as well as a shortening of colon length. Treatment with BE-BJO alleviated DSS-induced colitis in a dose-dependent manner, as implied by the rebound of body weight and colon length, and the improvement of DAI (Fig. 1). The effects of BE-BJO were similar as that of the positive control drug 5-ASA and BJO (Fig. 1). By contrast, treatment with BF-BJO did not display protective effects against DSS-induced colitis (Fig. 1). Thus, these observations suggest that BE-BJO, but not BF-BJO, is the active constituent from BJO that alleviates DSS-induced colitis in mice.

3.2 BE-BJO prevented histopathological change in the colon of DSS-treated mice

The colon tissues were examined microscopically to observe the morphological changes of structure and integrity. The histopathologic features and scores were evaluated. DSS induced a destruction of the colon architecture, which was exhibited by obvious necrosis, epithelial damage or shedding, submucosal infiltration of inflammatory cells, accompanied by crypt hyperplasia (Fig. 2A). Treatment of BJO, 5-ASA and BE-BJO (305, 610 mg/kg/day), but not BF-BJO, significantly improved the collapse of the colon structure and infiltration of inflammatory cells (Fig. 2A). As shown in Fig.2B, the histopathological score of DSS group was significantly higher than that of normal group; treatment with BJO and 5-ASA significantly reduced the histopathological score as compared to the DSS group; treatment of BE-BJO (305, 610 mg/kg/day) significantly reversed DSS-induced increase of histopathology score, with
a similar trend as BJO and 5-ASA. These results confirm that BE-BJO prevents colon histopathological change induced by DSS.

3.3 BE-BJO attenuated the levels of pro-inflammatory cytokines in the colon of DSS-treated mice

Colon inflammation plays a key role in the development of UC (Camuesco et al., 2005; Larrosa et al., 2009; Wang et al., 2016; Rabelo Andrade et al., 2019). The effects of BE-BJO on the production of pro-inflammatory cytokines, including TNF-α, IL-1β, IFN-γ, and IL-6, were examined in the colon tissue of DSS-treated mice. As displayed in Fig. 3, the levels of these pro-inflammatory cytokines were significantly upregulated in colorectums of DSS group; treatment of BE-BJO dose-dependently reversed DSS-induced production of pro-inflammatory cytokines. In addition, the anti-inflammatory effect of BE-BJO at high dose (610 mg/kg/day) was equivalent to that of BJO and 5-ASA (Fig. 3). Taken together, these data indicate that BE-BJO could ameliorate colon inflammation in the DSS model.

3.4 BE-BJO restored the impairment of intestinal epithelial barrier function in DSS-induced colitis

Since UC is characterized by damage of intestinal mucosal barrier function, therapeutics that could enhance intestinal mucosal healing is a major goal of UC therapy (Hang Thi Thu et al., 2014; Atreya and Neurath, 2017). The intestinal epithelial barrier function depends on the integrity of mucus layer, which is determined by the expression
and assembly of tight junction proteins (Putt et al., 2017; Zeisel et al., 2019). The tight junction complex is constituted by transmembrane proteins, like occludin and the claudin family, and by linker proteins, like zonula occludens-1 (ZO-1), that affiliate with the actin cytoskeleton (Mennigen et al., 2009). To examine the effect of BE-BJO on intestinal mucosal healing, the expressions of tight junction proteins were tested. As shown in Fig. 4, treatment of DSS for 7 days resulted in a remarkable decrease in the protein expressions of ZO-1, Occludin, Claudin-1 and E-cadherin, but augmented the expression of Claudin-2 as compared to the control group. Treatment of BE-BJO reversed the effect of DSS on expressions of these tight junction proteins in a dose-dependent manner. High dose of BE-BJO share a parallel effect with BJO and the positive drug 5-ASA. These observations imply that BE-BJO could enhance intestinal epithelial barrier function by altering the expression levels of tight junction proteins.

3.5 BE-BJO suppressed NF-κB activation in DSS-induced colitis

The phosphorylation of p65 (p-p65) is a hallmark of NF-κB activation and leads to colon inflammation during the pathogenesis of UC (Murano et al., 2000; Hegazy and El-Bedewy, 2010). Thus, the levels of p-p65 and total p65 in the colon tissues of mice were determined by Western Blot. The results demonstrated that DSS treatment significantly upregulated the expression of p-p65, without altering the expression of total p65 (Fig. 5). Treatment with 5-ASA, BJO and BE-BJO (152.5, 305, 610 mg/kg/day) significantly reduced the level of p-p65 and the ratio of p-p65/p65 (Fig. 5). Thus, the results indicate that the BE-BJO could repress NF-κB activation through inhibiting the
phosphorylation of p65.

3.6 BE-BJO inhibited the activation of the RhoA/ROCK signaling pathway in DSS-induced colitis

RhoA/ROCK is closely associated with the regulation of adhesion, migration and proliferation of intestinal crypt cells, thus playing a pivotal role in the development of UC (Citalan-Madrid et al., 2017). We therefore investigated the effect of BE-BJO on RhoA/ROCK signaling pathway. Firstly, the activation of RhoA was determined by RhoA activity assay. As shown in Fig. 6 A&B, DSS facilitated the activation of RhoA, as implied by the increased ratio of GTP-RhoA/total RhoA. BE-BJO, BJO and 5-ASA significantly repressed RhoA activation. Secondly, the protein expression of ROCK-1 was augmented by DSS, but was recovered to the normal level in the presence of BE-BJO (610 mg/kg/day), BJO or 5-ASA (Fig. 6 A&C). Thirdly, the phosphorylation level of myosin light chain (MLC), which serves as a substrate of ROCK-1, was determined. BE-BJO (305, 610 mg/kg/day), BJO and 5-ASA significantly reversed DSS-induced phosphorylation of MLC (Fig. 6 A&D). Therefore, these data confirm that BE-BJO could suppress the RhoA/ROCK signaling pathway.

4. Discussion

BJO is well-accepted to have antidiarrheal properties and has been reported to have anti-inflammatory effect in UC disease models, thus suggesting therapeutic potential in the treatment of UC (Huang et al., 2019a). Besides quassinoids including
brusatol and bruceine D, BJO also contains oleic acid, linoleic acid, stearic acid, palmitic acid, arachidonic acid, and other unsaturated fatty acids (Ma et al., 2013). In order to elucidate whether or not these components contribute to the anti-UC properties of BJO, the present study compared the effects of BE-BJO and BF-BJO in DSS-induced UC model. According to our observations, BE-BJO alleviated DSS-induced loss of body weight, increase of DAI and shortening of colon, whereas BF-BJO did not have these protective effects. The effect of BE-BJO is equivalent to that of BJO. It thus suggests that quassinoids in particular brusatol, but not oleic acid or other unsaturated fatty acids, serve as the active components for the anti-UC properties of BJO.

UC is a progressive alimentary system disorder that is characterized by dysregulated immune response, chronic inflammation, unbalanced gut microbiota (dysbiosis), and defective mucosal barrier function (Ungaro et al., 2017; Ma et al., 2018). The present study further explored the effects and mechanisms of BE-BJO on colon inflammation and intestinal epithelial barrier function.

In line with our previous observations (Huang et al., 2017a; Huang et al., 2019a), the present results showed that treatment of BE-BJO suppressed the production and release of pro-inflammatory cytokines including TNF-α, IFN-γ, IL-6 and IL-1β in the colon tissue. TNF-α is a pro-inflammatory molecule that mediates multiple physiological and pathological processes of UC. Excess production of TNF-α leads to secretion of chemokines by colonic epithelial cells and damage of epithelial barrier (Akdis et al., 2016; Ungar et al., 2016). IL-1β emerges as a requisite role in the development of inflammatory reaction, and the increase of IL-1β level can cause autoimmune process
and then impairs colon tissue (Karmakar et al., 2015; Dror et al., 2017). IL-6 is an immunomodulatory cytokine that participates in the progression of UC (Grivennikov et al., 2009; Gupta et al., 2018). IL-6 and IL-1β are team players in the development of UC. INF-γ is an activator of phagocytes and neutrophil with immunomodulatory activity (Tenger et al., 2005). Thus, inhibiting the release of these pro-inflammatory mediators contributes to the anti-inflammatory properties of BJO.

NF-κB is pivotal in the inflammatory cascades during the pathogenesis of colitis (Zhang et al., 2017). The p65 subunit is the major functional subunit in NF-κB. In response to stimulation, p65 undergoes phosphorylation and is transferred to the nucleus, where it binds to promotors of target genes to enhance the inflammatory response (Sandur et al., 2006; Christian et al., 2010). Our results demonstrated that BE-BJO dose-dependently reversed DSS-induced p65 phosphorylation, thus suggesting that BE-BJO could repress NF-κB activation.

Moreover, the present study indicates that the anti-UC effect of BE-BJO is attributed to improvement of intestinal stability and structural integrity by enhancing tight junctions. The primary structure of intestinal barrier is the apical junctional complex, which is consists of the tight junctions and the subjacent adherent junctions that contribute to apical-basal cell polarity maintenance and to cell signaling events (Gehren et al., 2015). The tight junction complex is constituted by transmembrane proteins such as occludin and the claudin family, and by linker proteins like ZO-1 (Mennigen et al., 2009). These proteins regulate the permeability and integrity of intestinal mucosal barrier (Coopman and Djiane, 2016; Ruebsam et al., 2017). In this
study, BE-BJO rebounded the decreased expressions of ZO-1, Occludin, Claudin-1 and E-cadherin induced by DSS, but augmented Claudin-2 expression, thus indicating that BE-BJO could regulate the expressions of colonic epithelial barrier-associated proteins, thereby repairing the damaged colonic mucosa during UC. Taken into considerations that therapeutics targeting enhancing intestinal mucosal healing is a major goal of UC therapy (Brown and Mayer, 2007; Atreya and Neurath, 2017), the present findings provide important evidence for the therapeutic potential of BJO in treatment of UC.

To elucidate the mechanisms underlying the effect of BJO on intestinal epithelial barrier function, we tested the possible involvement of RhoA/ROCK signaling. ROCK and myosin light chain kinases (MLCK) are involved in the regulation of tight junctions during the intestinal inflammatory response (Walsh et al., 2001; Cetin et al., 2004). Activated RhoA/ROCK signals deactivate myosin phosphatase (MLCP), leading to failure of dephosphorylation of MLC, resulting in increased levels of intracellular phosphorylation and increased actin-myosin cross-linking, finally promoting the polymerization of the actin microfilaments (Tang et al., 2016; Kang et al., 2017). In addition, RhoA/ROCK elevates MLC phosphorylation levels. The present study indicates that BE-BJO inhibits RhoA/ROCK signaling pathway. This conclusion is supported by the following observations: (1) BE-BJO repressed DSS-induced activation of RhoA; (2) BE-BJO treatment downregulated ROCK expression; and (3) BE-BJO prevented phosphorylation of MCL in DSS-treated mice. Since RhoA/ROCK signaling pathway is associated with colon inflammation and regulation of intestinal epithelial barrier function, it is probably that BE-BJO ameliorated UC by repressing
RhoA/ROCK. Intriguingly, there is a crosstalk between NF-κB and RhoA/ROCK which work together to accelerate the progression of UC (Elamin et al., 2014; Ma et al., 2018), the inhibitory effect of BE-BJO on both signaling pathways might help to explain the remarkable anti-UC effect of BE-BJO.

5. Conclusion

In conclusion, the present study has shown that BJO enriched with quassinoids could ameliorate DSS-induced UC by preventing colon inflammation and enhancing intestinal epithelial barrier function. Mechanistically, these anti-UC effects of BE-BJO are probably associated with repression of NF-κB and RhoA/ROCK signaling pathways. These findings confirm that quassinoids are active compounds from BJO and suggest the therapeutic potential of quassinoids and BE-BJO in the treatment of UC.

Abbreviations

5-ASA, 5-aminosalicylic acid; AJ adherens junction; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma-2; BE-BJO, brusatol-enriched BJO; BJO, *Bracea javanica* oil; CTX, Cyclophosphamide; Cytochrome C, Cytochrome C; DAI, Disease Activity Index; DSS, Dextran sulphate sodium; ELISA, enzyme linked immunosorbent assay; GC, gas chromatography; GC-MS, Gas Chromatography-Mass Spectrometer; HE, hematoxylin-eosin; HPLC, High Performance Liquid Chromatography; IBD Inflammatory bowel disease; IFN, Interferon; IL-1β, Interleukin-1β; IL-6, Interleukin-6; MLC, myosin light chain; MLCK, myosin light chain kinase; NF-κB, nuclear factor
kappa-B; PBS, phosphate buffer saline; PVDF, polyvinylidene fluoride; RT-PCR, Reverse Transcription-Polymerase Chain Reaction; ROCK, Rho-associated kinase; TNF-α, Tumor Necrosis Factor-α; TJ, Tight junction; UC, Ulcerative colitis; ZO, zonula occluden

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Authors’ contributions

XZ, TW and LM designed the study; XZ and LM wrote the main manuscript text; XZ, TW and LM performed the animal experiments; XZ and LM analysed the data and prepared the figures. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal care and experimental procedures used in the current study were approved by the Institutional Animal Care and Use Committee of the Guangzhou University of Chinese Medicine.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
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Figure legends

**Fig. 1.** BE-BJO attenuated the severity of DSS-induced colitis in mice. Mice were monitored daily in terms of reductions of body weight (A), DAI score (B) in DSS-induced UC mice model. Representative photographs of colon length and colon length (C-D). All values are presented as the mean ± SEM. ##p<0.01, and ###p<0.001 versus Normal group; *p<0.05, **p<0.01, and ***p<0.001 versus DSS group.

**Fig. 2.** BE-BJO relieved the colonic injury in DSS-induced UC mice. The images of H&E staining of mice in each treatment group and (A) Histopathological score (B). All values are presented as the mean ± SEM. ###p<0.001 versus Normal group; *p<0.05, and ***p<0.001 versus DSS group.

**Fig. 3.** BE-BJO inhibited DSS-triggered inflammation. The expression of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ and IL-6) (A–D) was detected by ELISA in colon sections. All values are presented as the mean ± SEM. ###p<0.001 versus Normal group; *p<0.05, **p<0.01, and ***p<0.001 versus DSS group.

**Fig. 4.** BE-BJO restored intestinal barrier function in DSS-induced colitis in mice. Protein expression of ZO-1, Occludin, Claudin-1, Claudin-2, E-cadherin (A) in colon tissue. The bar graph of the relative intensities of Western Blotting bands (B-F). The mRNA expression of occludin(G), ZO-1(H), and claudin-1(I) and claudin-2(J) in colon tissue. All values are presented as the mean ± SEM. ###p<0.001 versus Normal group; *p<0.05, **p<0.01, and ***p<0.001 versus DSS group.

**Fig. 5.** BE-BJO inhibited the activation of the NF-κB signaling pathway. The expression of p-65 and p-p65 was detected by western bolt (A-B). All values are presented as the
mean ± SEM. ### $p<0.001$ versus Normal group; * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ versus DSS group.

**Fig.6.** BE-BJO inhibited the activation of the RhoA/ROCK signaling pathway in DSS-induced UC in mice. Representative Western Blot images of GTP RhoA, Total RhoA, ROCK-1, p-MLC and MLC (A). The relative protein expressions of GTP RhoA (B), ROCK-1 (C) and p-MLC (D) in colon tissue as detected by Western Blot. All values are presented as the mean ± SEM. ### $p<0.001$ versus Normal group; * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ versus DSS group.
Figure 2

A Normal  DSS  5-ASA
BJO  BE-BJOL  BE-BJOM
BE-BJOH

B

Histological Scores

Normal  5-ASA  BE-BJOM  BE-BJOH  BE-B JOH
Figure 3

**A**

TNF-α level (μg/mg protein)

- Normal
- DSS
- 5-ASA
- BIO
- BE-BIO-L
- BE-BIO-M
- BE-BIO-H

**B**

IL-1β level (μg/mg protein)

- Normal
- DSS
- 5-ASA
- BIO
- BE-BIO-L
- BE-BIO-M
- BE-BIO-H

**C**

IFN-γ level (μg/mg protein)

- Normal
- DSS
- 5-ASA
- BIO
- BE-BIO-L
- BE-BIO-M
- BE-BIO-H

**D**

IL-6 level (μg/mg protein)

- Normal
- DSS
- 5-ASA
- BIO
- BE-BIO-L
- BE-BIO-M
- BE-BIO-H
Figure 4
Figure 6
Table 1

The criteria of DAI

| Body weight loss | Stool consistency | Occult blood or bloody stool | Score |
|------------------|-------------------|------------------------------|-------|
| no change        | Normal            | Negative                     | 0     |
| 1-5%             | Loose Stool       | Negative                     | 1     |
| 5-10%            | Loose Stool       | Occult blood positive        | 2     |
| 10-20%           | Diarrhea          | Occult blood positive        | 3     |
| ≥ 20%            | Diarrhea          | Gross hematochezia           | 4     |
Table 2

The criteria of histological score.

| Categories                  | Feature                                      | Score |
|-----------------------------|----------------------------------------------|-------|
| Architectural changes       | No change                                    | 0     |
|                             | Mild abnormality in mucosa                   | 1     |
|                             | Moderate diffuse or multifocal abnormalities in submucosa | 2     |
|                             | Severe diffuse or multifocal abnormalities   | 3     |
|                             | Peritonitis                                  | 4     |
| Inflammatory infiltrate     | No change                                    | 0     |
|                             | Mild but unequivocal increase                | 1     |
|                             | Moderate change                              | 2     |
|                             | Marked change                                | 3     |
|                             | Severe increase                              | 4     |
| Lamina propria leukocytes   | No changes                                   | 0     |
|                             | Mild increase                                | 1     |
|                             | Moderate increase                            | 2     |
|                             | Marked increase                              | 3     |
|                             | Severe increase                              | 4     |
| Intraepithelial neutrophils | No changes                                   | 0     |
|                             | 25%                                          | 1     |
|                             | 50%                                          | 2     |
|                             | 75%                                          | 3     |
|                             | 100%                                         | 4     |
| Erosion or ulceration       | No change                                    | 0     |
|                             | Erosion focally stripped                     | 1     |
|                             | Marked erosion                               | 2     |
|                             | Severe erosion                               | 3     |
|                             | Ulceration or granulation tissue             | 4     |
| Crypt destruction           | No change                                    | 0     |
|                             | Blunted crypts                               | 1     |
|                             | Marked attenuation                           | 2     |
|                             | Crypt necrosis                               | 3     |
|                             | No architecture                              | 4     |
Table 3

Primer sequences for qRT-PCR.

| Gene      | Primer (5’ to 3’)                  |
|-----------|------------------------------------|
| ZO-1      | Forward: CACCTCGCACGCATCAGC        |
|           | Reverse: GGCAGCAATGGTGCTCTTC       |
| Occludin  | Forward: TTGGCTACGGAGGTGCTATGG     |
|           | Reverse: CCTTTGGCTGCTCTTTGGGTCTG   |
| Claudin-1 | Forward: TGGCTTCTCTGGATGGATCGG    |
|           | Reverse: CCTGAGCGGTACAGATGGTTC    |
| Claudin-2 | Forward: CCGTGTTCTGCAAGTTTCTCG    |
|           | Reverse: AGCCAGGATGCCACCAAGG       |
| GAPDH     | Forward: GGTTCCTCTCGCAGACTTCA     |
|           | Reverse: TGGTCAGGGTTTCTTACTCC     |