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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Research

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Abstract

Background: Our previous study indicates that Brucea 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-1and 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.

Full Text

This preprint is available for download as a PDF.

Figures

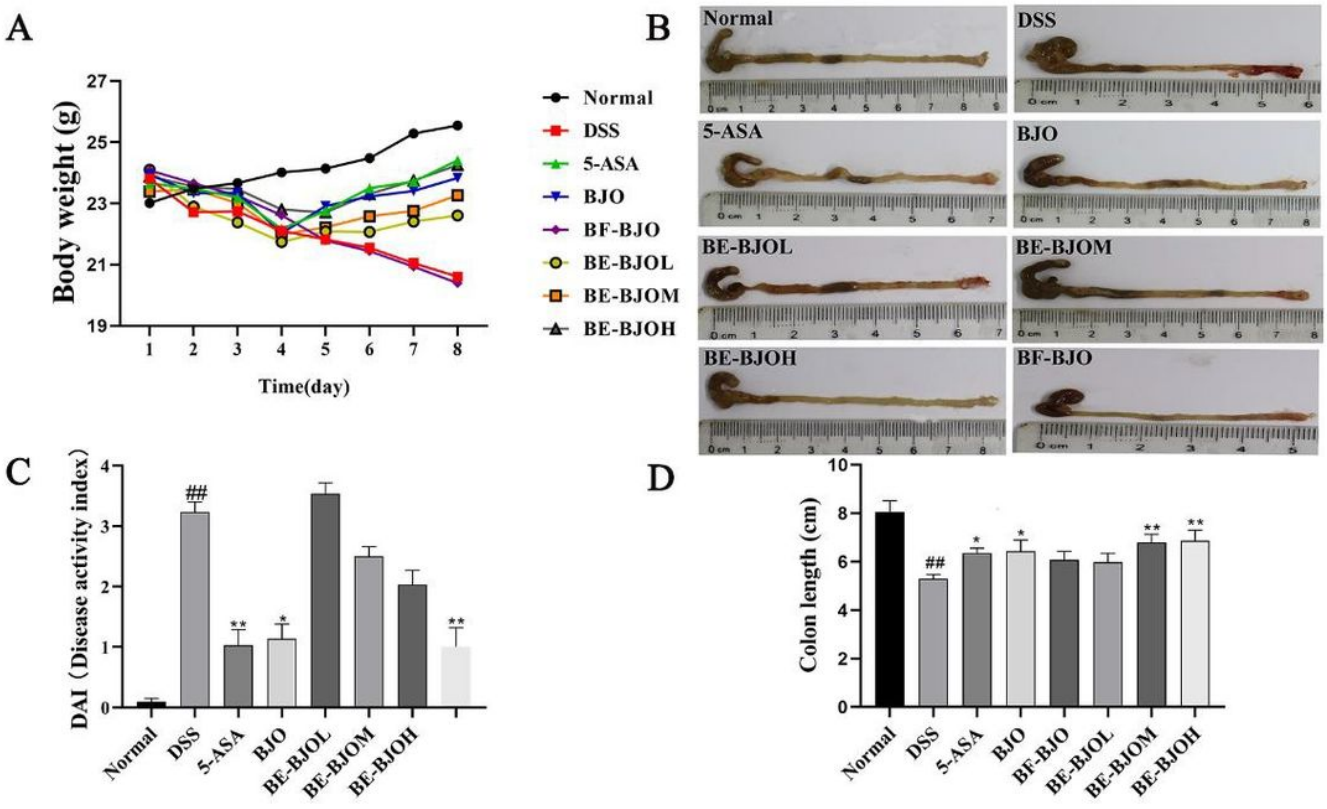

Figure 1

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.

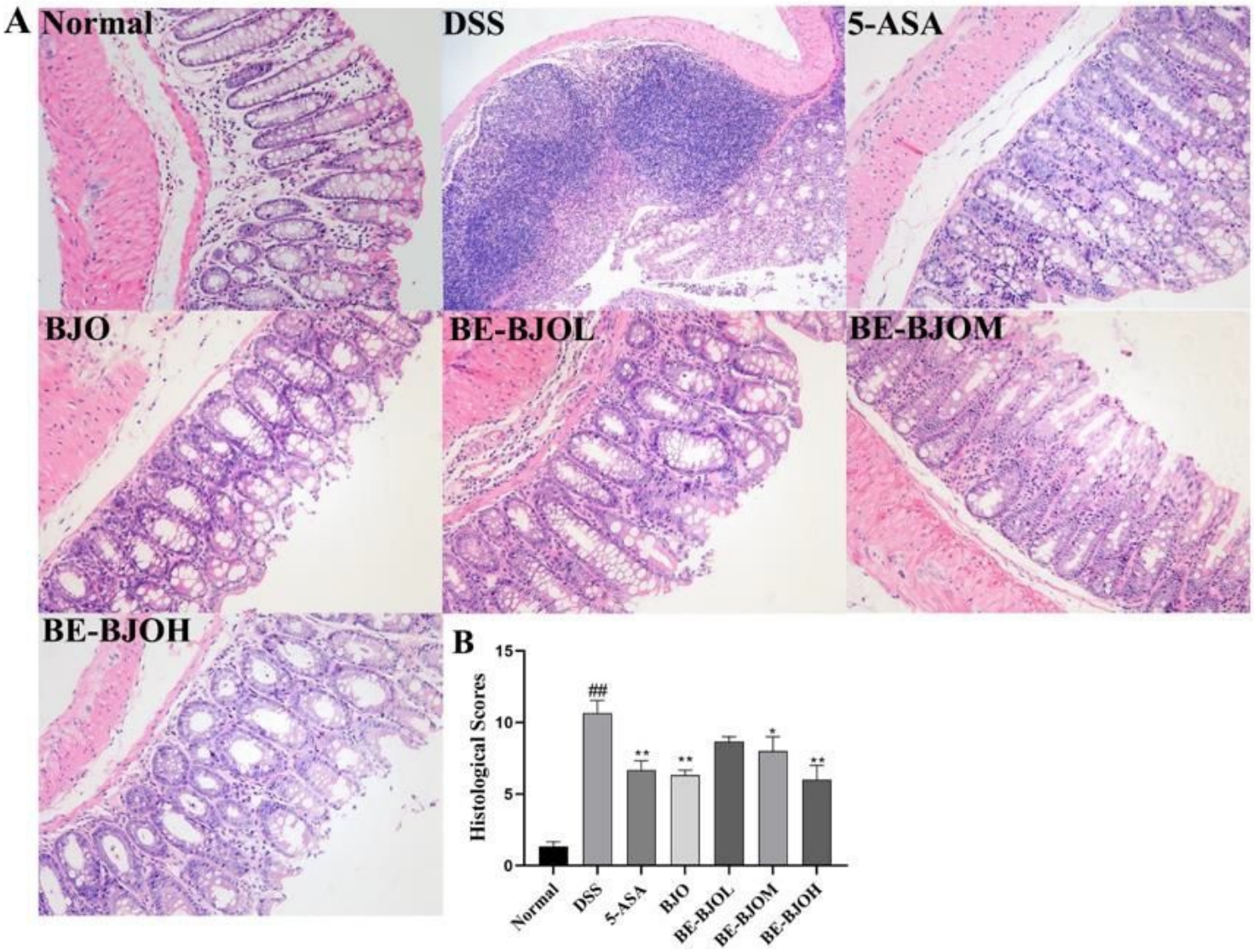

Figure 2

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.

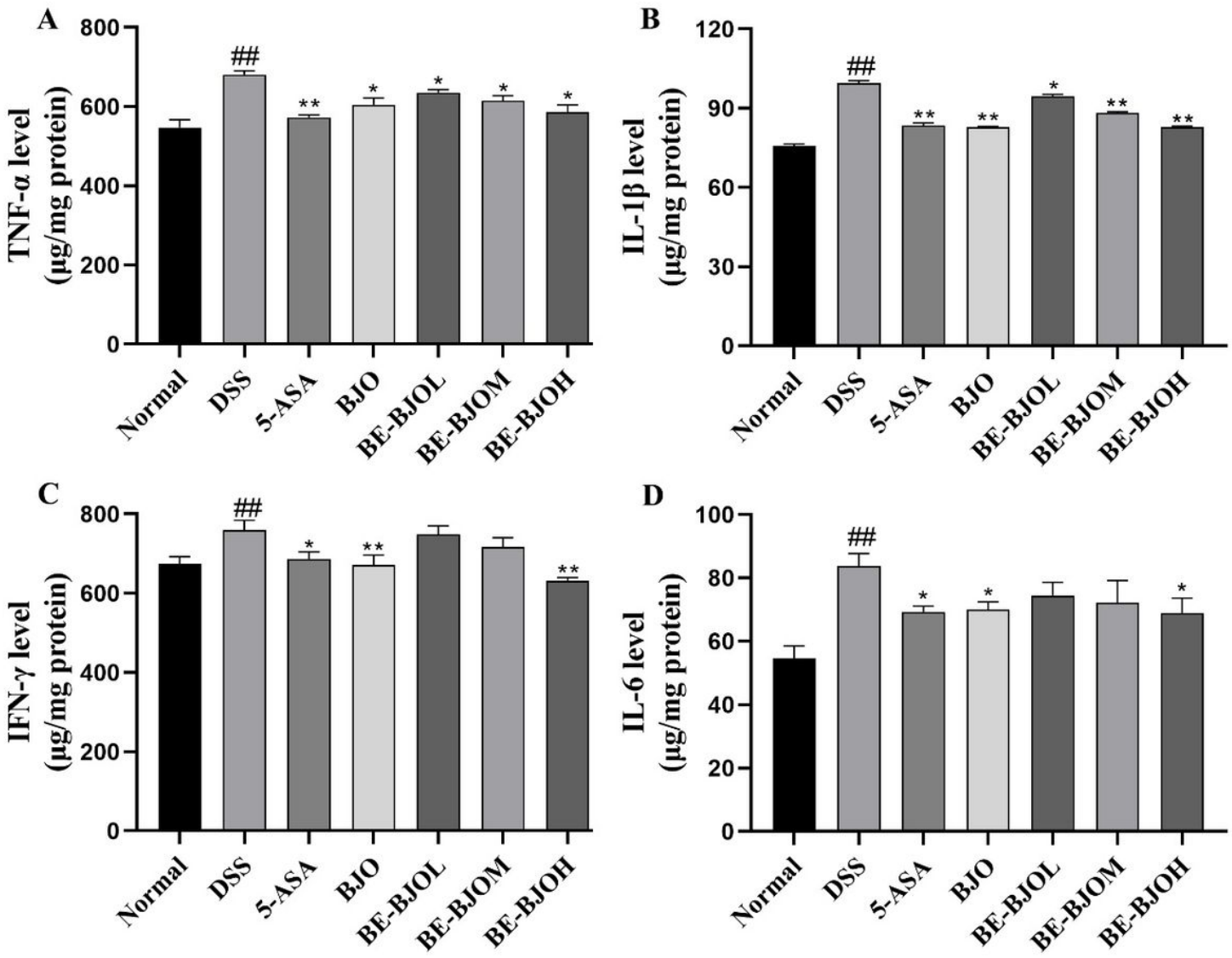

Figure 3

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.

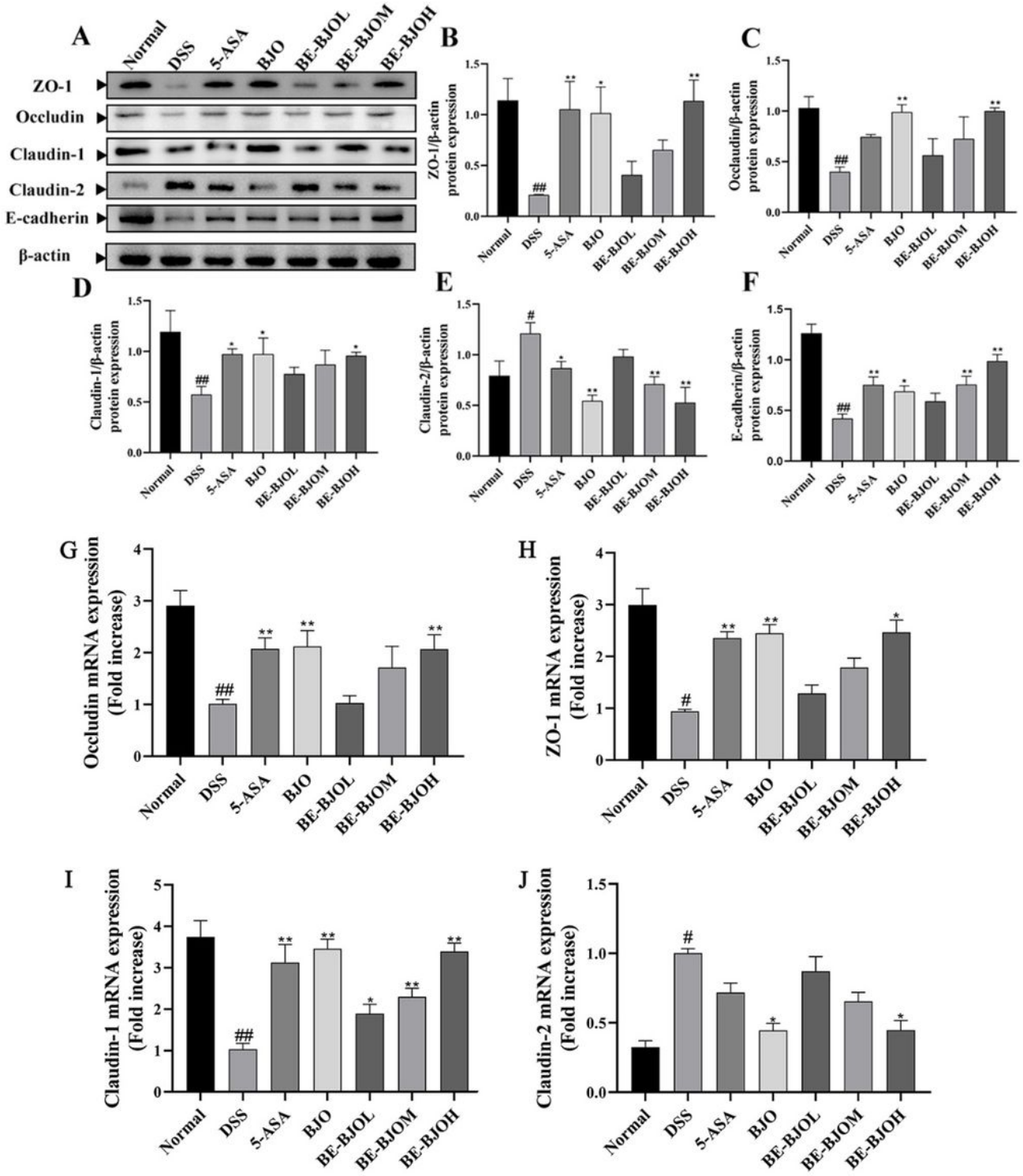

Figure 4

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.

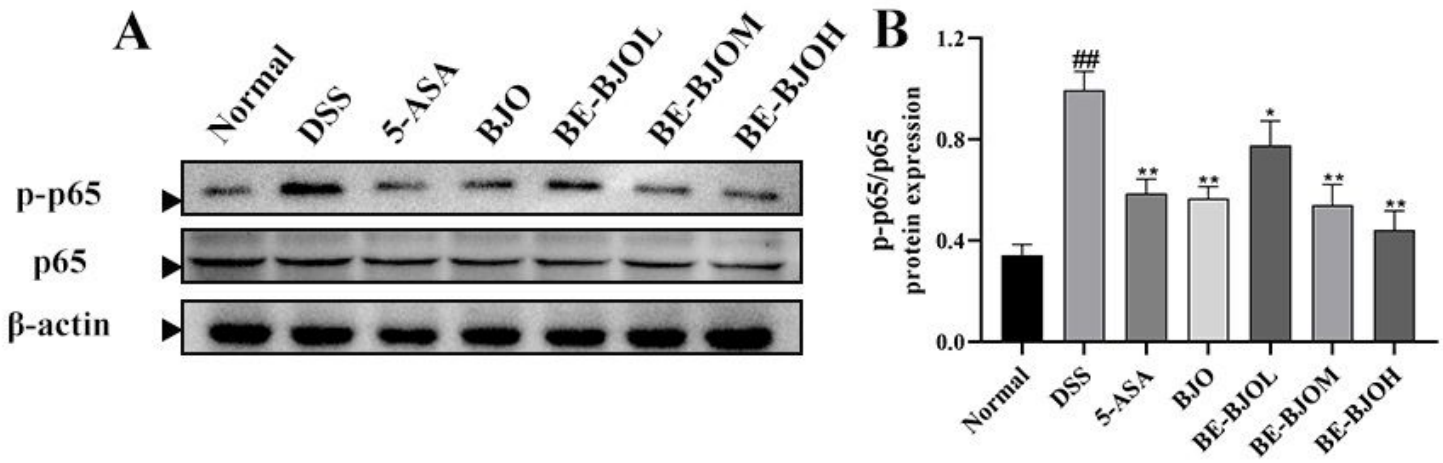

Figure 5

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.

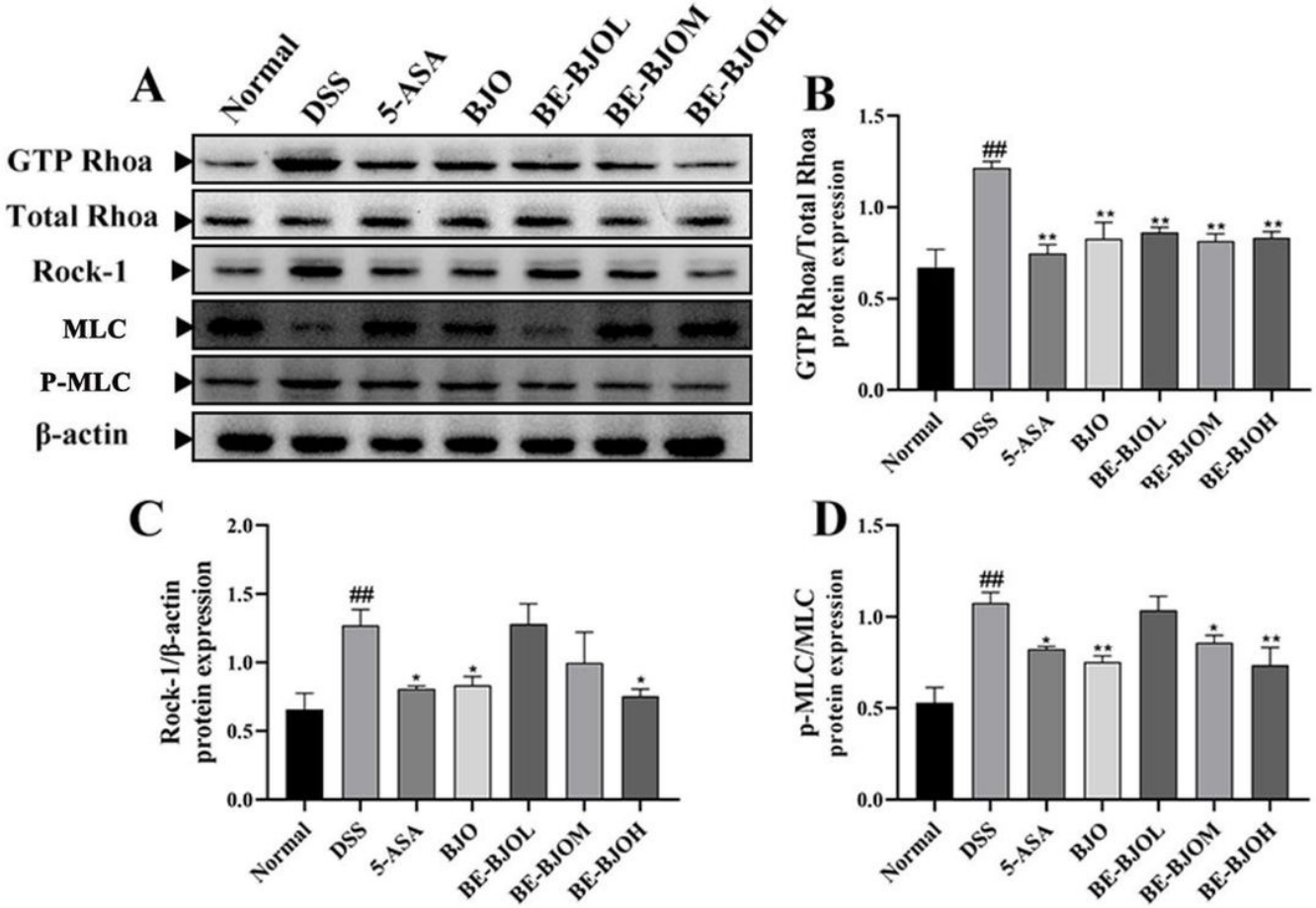

Figure 6

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.