**Basic Study**

# Berberine prevents stress-induced gut inflammation and visceral hypersensitivity and reduces intestinal motility in rats

Zhi-Chao Yu, Yong-Xin Cen, Ben-Hua Wu, Cheng Wei, Feng Xiong, De-Feng Li, Ting-Ting Liu, Ming-Han Luo, Li-Liangzi Guo, Ying-Xue Li, Li-Sheng Wang, Jian-Yao Wang, Jun Yao

**ORCID number:** Zhi-Chao Yu (0000-0001-5123-9191); Yong-Xin Cen (0000-0003-0793-7261); Ben-Hua Wu (0000-0002-9392-9470); Cheng Wei (0000-0002-6373-1826); Feng Xiong (0000-0002-4021-0817); De-Feng Li (0000-0003-3118-6840); Ting-Ting Liu (0000-0002-1533-5220); Ming-Han Luo (0000-0002-5056-3421); Li-Liangzi Guo (0000-0003-1521-6245); Ying-Xue Li (0000-0001-6350-612X); Li-Sheng Wang (0000-0002-7418-6114); Jian-Yao Wang (0000-0003-1568-4479); Jun Yao (0000-0002-3472-1602).

**Zhi-Chao Yu, Ben-Hua Wu, Cheng Wei, Feng Xiong, De-Feng Li, Ting-Ting Liu, Ming-Han Luo, Li-Liangzi Guo, Ying-Xue Li, Li-Sheng Wang, Jun Yao,** Department of Gastroenterology, Jinan University of Second Clinical Medical Sciences, Shenzhen Municipal People's Hospital, Shenzhen 518020, Guangdong Province, China

**Yong-Xin Cen,** Department of Gastroenterology, Foshan Gaoming Affiliated Hospital of Guangdong Medical University, Foshan 528500, Guangdong Province, China

**Jian-Yao Wang,** Department of General Surgery, Shenzhen Children's Hospital, Shenzhen 518026, Guangdong Province, China

**Corresponding author:** Jun Yao, MD, PhD, Doctor, Department of Gastroenterology, Jinan University of Second Clinical Medical Sciences, Shenzhen Municipal People's Hospital, 1017 East Gate Road, Shenzhen 518020, Guangdong Province, China. yj_1108@126.com
**Telephone:** +86-755-25533497
**Fax:** +86-755-25533497

## Abstract

*BACKGROUND*
Irritable bowel syndrome (IBS) is a common chronic non-organic disease of the digestive system. Berberine (BBR) has been used to treat patients with IBS, but the underlying therapeutic mechanism is little understood. We believe that BBR achieves its therapeutic effect on IBS by preventing stress intestinal inflammation and visceral hypersensitivity and reducing bowel motility.

*AIM*
To test the hypothesis that BBR achieves its therapeutic effect on IBS by preventing subclinical inflammation of the intestinal mucosa and reducing visceral hypersensitivity and intestinal motility.

*METHODS*
IBS was induced in rats *via* water avoidance stress (WAS). qRT-PCR and histological analyses were used to evaluate the levels of cytokines and mucosal inflammation, respectively. Modified ELISA and qRT-PCR were used to evaluate the nuclear factor kappa-B (NF-κB) signal transduction pathway. Colorectal distention test, gastrointestinal transit measurement, Western blot, and qRT-PCR were used to analyze visceral sensitivity, intestinal motility, the expression of C-kit (marker of Cajal mesenchymal cells), and the expression of brain derived neurotrophic factor (BDNF) and its receptor TrkB.

## RESULTS

WAS led to mucosal inflammation, visceral hyperalgesia, and high intestinal motility. Oral administration of BBR inhibited the NF-κB signal transduction pathway, reduced the expression of pro-inflammatory cytokines [interleukin (IL)-1β, IL-6, interferon-γ, and tumor necrosis factor-α], promoted the expression of anti-inflammatory cytokines (IL-10 and transforming growth factor-β), and improved the terminal ileum tissue inflammation. BBR inhibited the expression of BDNF, TrkB, and C-kit in IBS rats, leading to the reduction of intestinal motility and visceral hypersensitivity. The therapeutic effect of BBR at a high dose (100 mg/kg) was superior to than that of the low-dose (25 mg/kg) group.

## CONCLUSION

BBR reduces intestinal mucosal inflammation by inhibiting the intestinal NF-κB signal pathway in the IBS rats. BBR reduces the expression of BDNF, its receptor TrkB, and C-kit. BBR also reduces intestinal motility and visceral sensitivity to achieve its therapeutic effect on IBS.

**Key words:** Irritable bowel syndrome; Visceral hypersensitivity; Berberine; Rifampicin; Nuclear factor kappa-B; Brain-derived neurotrophic factor; Cajal mesenchymal cells; C-kit

**Core tip:** Irritable bowel syndrome (IBS) is a common chronic non-organic disease of the digestive system and the pathophysiology of IBS is still not completely understood. Berberine has been used to treat patients with IBS, but little is known regarding to its therapeutic mechanism. This study aimed to determine the therapeutic effect of berberine on IBS and its underlying mechanisms. The results demonstrated that the therapeutic efficacy of berberine was dose-dependent and may be associated with the inhibition of the intestinal nuclear factor kappa-B signal pathway, the expression of brain derived neurotrophic factor and its receptor TrkB, and the expression of C-kit to reduce intestinal motility and visceral sensitivity.

## INTRODUCTION

Berberine (BBR) is an isoquinoline alkaloid extracted from various Chinese medicinal herbs, *e.g.*, Huanglian and scutellaria. BBR has abundant medicinal value, such as biological effects on the central nervous system, anti-tumor, anti-inflammatory, and anti-Alzheimer's disease effects, and reducing blood fat. A large number of basic and clinical studies have also shown the efficacy of BBR in the treatment of irritable bowel syndrome (IBS). The common dosage of BBR for treating diarrhea in adults is 100-300 mg, three times a day[1-4].

The pathophysiology of IBS is still not completely understood. In the past decade, there has been increasing focuses on the possible connection of IBS with increased intestinal mucosal permeability, inflammation, intestinal bacterial overgrowth, dysfunction of the cerebral intestinal axis, and visceral hypersensitivity[5]. At present, the pathogenesis of IBS is explained by the mechanism of environment-psycho-neuroendocrine-immunity[6,7].

The immunologic disorder of the intestinal tract is closely associated with the pathogenesis of IBS, and the nuclear factor kappa-B (NF-κB) signal pathway plays a very important role in the immune response[8,9]. NF-κB can regulate the transcription of the genes related to inflammation and pain, activate the transcription of inflammatory factors, affect intestinal inflammation, and lead to abdominal pain[10].

Cytokines are an essential part of intestinal immune regulation. According to the performance of cytokines in the immune response, they can be divided into two categories: (1) Th1 cell-secreted pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and interferon γ (IFN-γ); and (2) Th2 cell-secreted anti-inflammatory cytokines, including IL-10 and transforming growth factor-β (TGF-β). The immune response in IBS is not just limited to the intestine. Systemic immune activation characterized by the elevation of pro-inflammatory cytokines and the decrease of anti-inflammatory cytokines is also involved in IBS[11-14].

BBR can reduce visceral hypersensitivity in IBS rats and regulate intestinal motility, but the underlying mechanism is not yet fully understood[1]. Brain derived neurotrophic factor (BDNF) plays an important role in the visceral hypersensitivity and intestinal hyperdynamics of IBS through the brain-gut axis. The increased expression of BDNF in the colonic mucosa and central nervous system may contribute to the visceral hyperalgesia in IBS[15,16]. Higher expression of BDNF in colonic mucosa and central nervous system would indicate the greater degree of abdominal pain in patients[15].

Cajal mesenchymal cells (ICC) can affect the intestinal motility and visceral sensitivity in IBS patients through the brain-gut axis. The corresponding receptors of many neurotransmitters are expressed on ICC, which are an important intermediary for the central nervous system to regulate visceral sensitivity and intestinal dynamics[17]. At the same time, some studies suggest that abnormalities in the structure and number of ICC in the intestinal tract of IBS patients can lead to abnormal electrophysiological activity in the intestinal tract[18]. C-Kit signaling plays a vital role in the development and maintenance of ICC. Thus, C-kit has also been used as a cell marker of ICC[19,20].

Rifaximin has achieved a good therapeutic effect for IBS patients without constipation[21]. It has been shown that rifaximin relieves symptoms of IBS by reducing visceral hypersensitivity in rats[22]. However, rifaximin has disadvantages, *e.g.*, high prices. Furthermore, long-term oral administration may lead to cross-resistance to similar antibiotics such as rifampicin and rifabutin[23,24]. This study aimed to explore the possible mechanism of BBR in the treatment of IBS at the level of brain and intestinal axis through the study of the high visceral sensitivity and intestinal dynamic mechanism in rats with experimental IBS.

## MATERIALS AND METHODS

### Animals
All experiments were performed on adult male Wistar rats weighing 200-225 g. Rats were obtained from the Guangdong experimental animal center. Animals were housed in plastic cages, with three rats per cage at room temperature (22 ± 1 °C) and 65%-70% humidity. Animals were maintained on a 12 h light/12 h dark cycle, with free access to water and feed. External oblique muscle electrode implantation was implemented after 5-7 d. Surgical preparations involved anesthetization with a xylazine/ketamine mixture. The period of postoperative recovery of rats was 4-6 d. Experimental animals were maintained in accordance with internationally accepted principles for laboratory animal use.

### Chronic water avoidance stress protocol
Chronic exposure of adult rats to water avoidance stress (WAS) was conducted as described previously[25]. Briefly, animals were placed on a block in the middle of a Plexiglas tank filled with sterile water (25 °C; 1 cm below the platform height). They were maintained on the block for 1 h daily for 10 consecutive days (Figure 1). Control rats were placed similarly in a tank without water for 1 h daily for 10 d. In separate studies, rats were treated by oral gavage of 3 mL rifaximin suspension (150 mg/kg, twice daily 6 h apart), 3 mL water once a day, 3 mL low dose BBR suspension (25 mg/kg, once a day), or 3 mL high dose BBR suspension (100 mg/kg, once a day) for 10 consecutive days. The rats were then submitted to daily sessions of WAS or sham WAS 3 h after each AM gavage for 10 d. Specific rifaximin and BBR doses were based on previous studies[22,26,27].

### Visceromotor response to colorectal distention
The protocol for measuring electromyogram (EMG) in response to colorectal distention (CRD) has been previously described[28]. Briefly, rats with a surgery had 5 d for recovery, and were fasted for 24h before intracolonic infusion, CRD, and EMG. EMG of rats were detected on day 0 and day 11 under different pressures. The baseline was the average of EMG amplitudes measured in the control group at the 0-
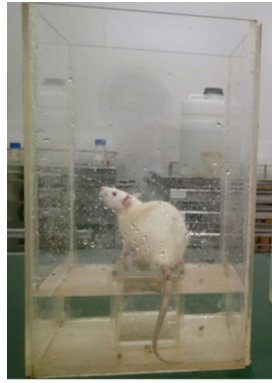
**Figure 1  A rat model of irritable bowel syndrome was established by using water avoidance stress.**

day CRD pressure of 60 mmHg. The amplitudes of EMG under different CRD pressures were compared with the baseline (% of control). We took the average EMG amplitude measured on day 11 subtracted by the average EMG amplitude detected on day 0, and express it as EMG.

### Determination of gastrointestinal motility
The protocol for detecting the gastrointestinal transit has been previously described[29]. For total gastrointestinal transit, after the animals were fasted overnight, activated carbon ink was orally administered at a dose of 10 mL/kg. The time that animals first defecated black feces was recorded. For small intestinal transit, after an overnight fast, activated carbon ink was orally administered at a dose of 10 mL/kg to each animal. After 30 min, the rats were sacrificed by cervical dislocation. The small intestine was immediately excised carefully without stretching and the distance traveled by ink was measured as well as the total length of the small intestine. Data are expressed as the proportion (%) of the distance traveled by the ink along the entire length of the small intestine.

### Evaluation of intestinal inflammatory response in rats
Hematoxylin and eosin (HE) staining was carried out in rat distal ileum tissue, and the inflammatory changes were observed under a microscope.

### Enzyme-linked immunosorbent assay
Distal ileum tissue (50 mg) was homogenized in a glass homogenizer containing 2 mL cold saline. The homogenates were centrifuged at low temperature for 20 min at 3000 rpm. The protein concentration in the supernatant was quantified on a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, United States). The concentrations of IL-1β, IL-6, TNF-α, IFN-γ, IL-10, and TGF-β were determined according to the manufacturer's instructions.

### Modified enzyme-linked immunosorbent assay
Cytoplasmic protein and nuclear protein were extracted from the rat ileum. Expression of NF-κB (P65) DNA-binding protein was measured with a commercially available modified ELISA kit [Cayman NF-κB (P65) Transcription Factor Assay Kit].

### Western blot analysis
Cytoplasmic protein and nuclear protein were extracted and quantified. Protein (20 μg) was separated on an SDS-PAGE gel and then electro-transferred onto a nitrocellulose membrane (0.2μm pore; WHATMAN, England). The membrane was incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibodies labeled with horseradish peroxidase. Signals were quantified using ImageJ software. Antibodies used included anti-NF-kappa B P65 antibody (mouse, 1:1000, Santa Cruz, United States), anti-C-kit antibody (mouse, 1:1000, Santa Cruz, United States), anti-Trkb monoclonal antibody (rabbit 1:1000, Cell signaling Technology, United States), anti-GAPDH antibody (1:1000, Proteintech, United States), and horseradish peroxidase-conjugated anti-rabbit/mouse secondary antibodies (1:10000, Zhongshan Gold Bridge, Beijing, China).

### Quantitative real-time polymerase chain reaction
Total RNA was extracted from distal ileum samples using TRIzol reagent (Thermo Scientific, United States). cDNA was synthesized using a reverse transcription kit (Thermo Scientific, United States). Quantitative PCR for inflammatory cytokines and

C-kit mRNA was performed with an iCycler IQ real-time detection system (Bio-Rad Laboratories, Hercules, CA, United States) and detected with SYBR Green in a fluorescence thermocycler (LightCycler; Roche Diagnostics, Mannheim, Germany). Primer sequences used for PCR are listed in Table 1. The double standard curve method was used to calculate the results.

### Statistical analysis

Changes of EMG under different CRD pressures before and after experiments were analyzed using SPSS13.0. The statistical data are represented by the mean with standard deviation. We conducted normal test and variance homogeneity test for each group of experimental data. If the variance is homogeneous, the one-factor ANOVA was adopted, and the Bonferroni test was used to compare the two groups for the overall difference. If the variance was not homogenous, Kruskal Wallis $H$ test was adopted and the Mann Whitney $U$ test was used to compare each group for the overall difference. $P < 0.05$ was considered statistically significant.

## RESULTS

### WAS induces intestinal inflammation in rats

Compared with the control group, the WAS group showed a low inflammatory response in the intestinal tract (Figure 2). The expression levels of P65 DNA binding protein and NF-κB (P65) were significantly increased in the WAS group ($P < 0.05$) (Figure 3). The levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and IFN-γ were increased, while the levels of anti-inflammatory cytokines IL-10 and TGF-β were decreased ($P < 0.05$) in the WAS group compared with the control group (Figures 4 and 5).

### Chronic WAS induces visceral hyperalgesia and intestinal hyperdynamics

After 10 d of WAS or sham WAS (control group), the rats showed a pressure-dependent increase in EMG amplitude in response to CRD. On day 11, chronic WAS induced a greater increase in EMG amplitude in response to CRD compared to sham WAS. Such increase was significantly different at 40 mmHg (ΔEMG response after WAS over baseline: 46.14 ± 11.1 *vs* 3.8 ± 13.8 after sham WAS over baseline, $P < 0.05$), and 60 mmHg (ΔEMG response after WAS over baseline: 59.58 ± 17.8 *vs* 0.45 ± 9.6 after sham WAS over baseline, $P < 0.05$) (Figure 6). Gastrointestinal motility assay showed that the time to the first black feces in the WAS group was significantly shorter than that in the control group (457.47 ± 25.99 min *vs* 580.40 ± 40.44 min, $P < 0.05$). The proportion (%) of the distance traveled by the ink along the entire length of the small intestine in the WAS group was significantly lower than that in the control group (63.77 ± 2.77% *vs* 49.03 ± 4.60%, $P < 0.05$) (Figure 7). Compared with the control group, the expression of BDNF and its receptor Trkb was significantly increased in the WAS group ($P < 0.05$) (Figure 8). C-kit expression in the WAS group was also significantly increased in the WAS group compared to the control group ($P < 0.05$) (Figure 9).

### BBR prevents mucosal inflammation

Compared with the WAS group, after oral administration of rifaximin, 25 mg/kg BBR, or 100 mg/kg BBR, the tissues were intact, and there were no significant neutrophils at the distal ileum (Figure 2). In the treatment group, the expression level of DNA-binding protein and NF-κB (P65) in distal ileum tissues was significantly reduced compared to the WAS group ($P < 0.05$). The therapeutic effect of oral administration of rifaximin or 100 mg/kg BBR was superior to that of 25 mg/kg BBR ($P < 0.05$) (Figure 3). The expression levels of pro-inflammatory cytokines in the treatment groups were lower than those in the WAS group ($P < 0.05$), and the therapeutic effect of rifaximin or 100 mg/kg BBR was superior to that of 25 mg/kg BBR ($P < 0.05$). The expression levels of anti-inflammatory cytokines IL-10 and TGF-β in the treatment groups were higher than those in the WAS group ($P < 0.05$) (Figures 4 and 5).

### BBR regulates intestinal motility and visceral hypersensitivity

On day 11, rifaximin, 25 mg/kg BBR, or 100 mg/kg BBR treatment reduced the increased level of visceromotor response to CRD induced by both forms of stress at 40 and 60 mmHg ($P < 0.05$). Furthermore, rifaximin or 100 mg/kg BBR resulted in smaller EMG compared to 25 mg/kg BBR ($P < 0.05$) (Figure 6). The time to the first black feces was significantly shorter in the rifaximin and 100 mg/kg BBR groups than that in the WAS and 25 mg/kg BBR groups ($P < 0.05$). There was no significant difference between the rifaximin group and 100 mg/kg BBR groups. The proportion

| Table 1 Sequences of primers used for qRT-PCR | | |
| --- | --- | --- |
| **Gene** | **Forward sequence** | **Reverse sequence** |
| *IL-1β* | AGTCTGCACAGTTCCCCAAC | TTAGGAAGACACGGGTTCCA |
| *IL-6* | CCAACTTCCAATGCTCTCCT | GGTTTGCCGAGTAGACCTCA |
| *IL-10* | GACTGCTATCTTGCCTGCTCTTAC | GGGTCTGGCTGACTGGGAAG |
| *TGF-β* | ATTCCTGGCGTTACCTTGG | AGCCCTGTATTCCGTCTCCT |
| *IFN-γ* | TCTGTGGGTTGTTCACCTCG | TATGGAAGGAAAGAGCCTCC |
| *TNF-a* | CCTCCTCTCTGCCATCAACA | GCAATGACTCCAAAGTAGACCTG |
| *BDNF* | CTTGGAGAAGGAAACCGCCT | GTCCACACAAAGCTCTCGGA |
| *Trkb* | AGAGCTTCCCTGTCCCTCAG | TTGGGAAGGTAACCAGATCG |
| *C-kit* | AATCCGACAACCAAAGCAAC | TGTCACGGAAGCACTGACAT |
| *Rat B-actin* | TGTCACCAACTGGGACGATA | GGGGTGTTGAAGGTCTCAAA |

(%) of the distance traveled by the ink along the entire length of the small intestine in the rifaximin and 100 mg/kg BBR groups was significantly lower than that in the WAS group and 25 mg/kg BBR groups ($P < 0.05$). There was no significant difference between the rifaximin and 100 mg/kg BBR groups (Figure 7).

Compared with the WAS group, the expression levels of BDNF and its receptor Trkb showed no significant difference in the 25 mg/kg BBR treatment group ($P > 0.05$). Compared with the WAS group, the expression levels of BDNF and its receptor Trkb were significantly decreased in the rifaximin group and 100 mg/kg BBR treatment group ($P < 0.05$). Compared with the 25 mg/kg BBR group, the expression levels of BDNF and its receptor Trkb were significantly reduced in the rifaximin group and 100 mg/kg BBR group ($P < 0.05$). The expression levels of BDNF and its receptor Trkb were not significantly different between the rifaximin group and 100 mg/kg BBR group ($P > 0.05$) (Figure 8).

Compared with the WAS group, C-kit expression was not significantly different in the 25 mg/kg BBR group ($P > 0.05$). Compared with the WAS group, the expression levels of C-kit were significantly decreased in the rifaximin and 100 mg/kg BBR groups ($P < 0.05$). Compared with the 25 mg/kg BBR group, the expression levels of C-kit were significantly decreased in the rifaximin and 100 mg/kg BBR groups ($P < 0.05$). There was no significant difference in C-kit expression between the rifaximin group and 100 mg/kg BBR group ($P > 0.05$) (Figure 9).

## DISCUSSION

We exposed adult rats to chronic WAS to study the effects of BBR on mucosal inflammation, visceral hypersensitivity, and intestinal motility. Similar to previous studies, chronic exposure of adult rats to WAS induced mucosal inflammation, and the amplitude of EMG induced by CRD in rats of the WAS group was significantly enhanced, suggesting that the visceral sensitivity was enhanced. Further analysis also showed that intestinal motility was enhanced[22,30,31].

Our experiments showed that the mucosal inflammation occurred in the distal ileum in WAS group rats. Previous studies have shown that there is positive feedback regulation between the NF-κB signaling pathway and inflammatory cytokines. On one hand, activated NF-κB can promote the expression of pro-inflammatory cytokines. On the other hand, the released pro-inflammatory cytokines react to the activation state of the NF-κB, leading to a cascade of inflammatory response in the intestinal tract, and pro-inflammatory cytokines are further released[10,32-34]. Thus, it is reasonable to consider that the activation of the NF-κB signaling pathway and the activation of inflammatory cytokines in IBS rats lead to low mucous mucosal inflammation in the distal ileum. Our experiments showed that oral administration of high-dose BBR or rifaximin can decrease the expression levels of NF-κB (P65) DNA-binding protein and NF-κB (P65) in IBS rats, which suggests that BBR or rifaximin may restrain the NF-κB signaling pathway, inhibit the activation of pro-inflammatory cytokines, increase the expression of anti-inflammatory cytokines, and reduce the inflammatory response in the distal ileum of rats. As mentioned above, high-dose BBR effectively inhibited mucosal inflammation by interdicting the positive feedback between the NF-κB signal pathway and inflammatory factors in IBS rats (Figure 10). Studies have shown that mucosal inflammation can lead to visceral hyper-sensitivity[35]. Therefore, oral high-dose BBR reduced visceral sensitivity possibly by
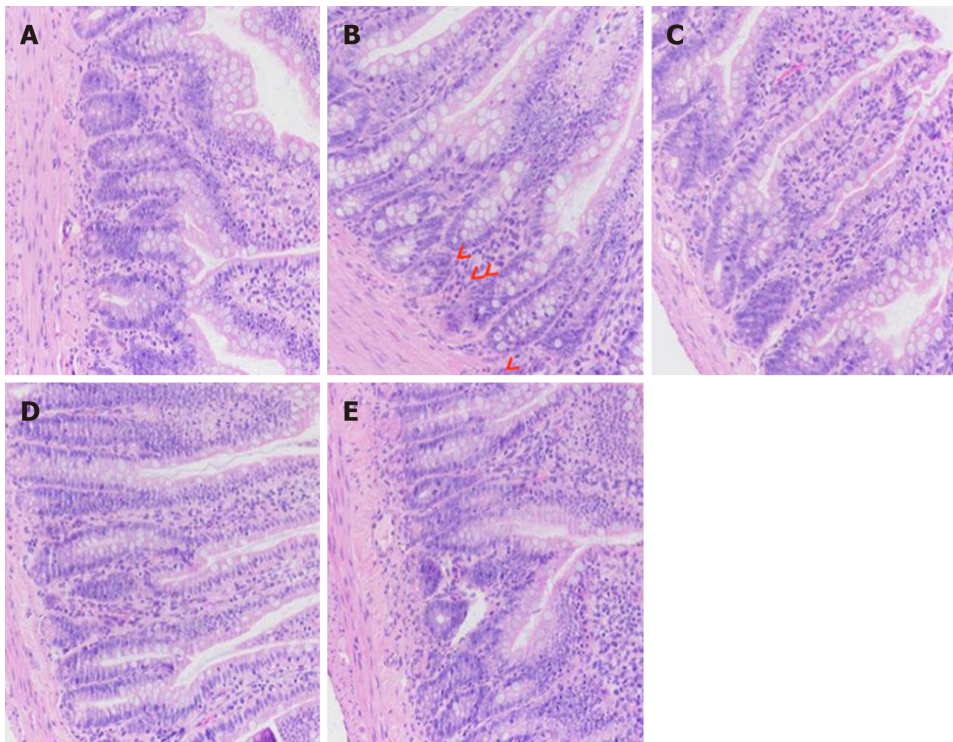
**Figure 2 Effects of berberine on histological score of the distal intestine in rats (×200).** A: Control group; B: Water avoidance stress group; C: Rifaximin group; D: 25 mg/kg berberine (BBR) group; E: 100 mg/kg BBR group (the labeled cells are neutrophils). Compared with the control group (A), rats in the water avoidance stress (WAS) group (B) showed low-grade intestinal inflammatory reaction. Microscopically, the tissues of the terminal ileum were intact, the mucosal structure and epithelium were intact, and the crypt was intact. Compared with the WAS group, after treatment with rifaximin (C), 25 mg/kg BBR (D), or 100 mg/kg BBR (E), the tissues of the terminal ileum of rats were intact, the mucosal structure and epithelium were intact, the crypt was intact, and no obvious neutrophil infiltration was observed. WAS: Water avoidance stress; BBR: Berberine.

controlling mucosal inflammation in the intestine.

The expression levels of BDNF and its receptor TrkB were increased in WAS group rats. Previous studies have shown that BDNF plays an important regulatory role in IBS patients' visceral sensitivity and intestinal motility through the braingut axis. BDNF activates the signal transduction pathways of CaMK and MAPK after binding the high affinity receptor protein Trkb[36,37]. High-dose BBR or rifaximin can reduce the visceral hypersensitivity and intestinal motility of IBS rats and decrease the expression levels of BDNF mRNA, Trkb mRNA, and Trkb protein in the distal ileum. BBR reduces visceral hypersensitivity and intestinal dynamics by reducing BDNF and its receptor Trkb expression perhaps *via* the following mechanisms: (1) The increased expression of BDNF may damage the ultrastructure of intestinal nerve fibers, and result in a higher density of nerve fibers in the intestinal tract and the release of excitatory neurotransmitters, leading to the allergy of intestinal neurons[38]. BBR inhibits the expression of BDNF in the IBS rats to maintain the normal form of the enteric nervous system, keeps normal operation of the enteric nervous system, and eventually prevents the visceral hypersensitivity; (2) BDNF promotes the release of pain mediators in the intestinal tract[38], and BBR alleviates the pain of the IBS rats by reducing the expression of BDNF; and (3) BBR reduces the activity of serine protease by reducing the expression of BDNF in the intestinal tract, thereby improving the symptoms of diarrhea in IBS patients, as well as the severity and frequency of abdominal pain.

C-kit mRNA and protein expression levels increased in the distal ileum of WAS group rats, suggesting that the C-kit signaling pathway in rats was activated. C-kit activation may affect the regulatory effect of ICC cells on intestinal sensitivity and intestinal motility in rats. ICC can spontaneously produce rhythmic slow wave, transmit the intestinal nerve signals to the smooth muscle cells to ensure the normal movement of the intestinal tract, and regulate the intestinal motility and visceral sensitivity of IBS patients through the brain-gut axis[20,39,40]. The C-kit signal pathway plays an important role in the proliferation and development of ICC cells. Thus, C-kit protein expression is an important marker for ICC cells[41,42]. Stem cell factor (SCF), an important ligand of C-kit, is one of the important cytokines that induce IBS[19,43]. High-dose BBR or rifaximin can reduce the visceral hypersensitivity and intestinal motility of IBS rats, and decrease the expression levels of C-kit in the distal ileum. BBR reduces
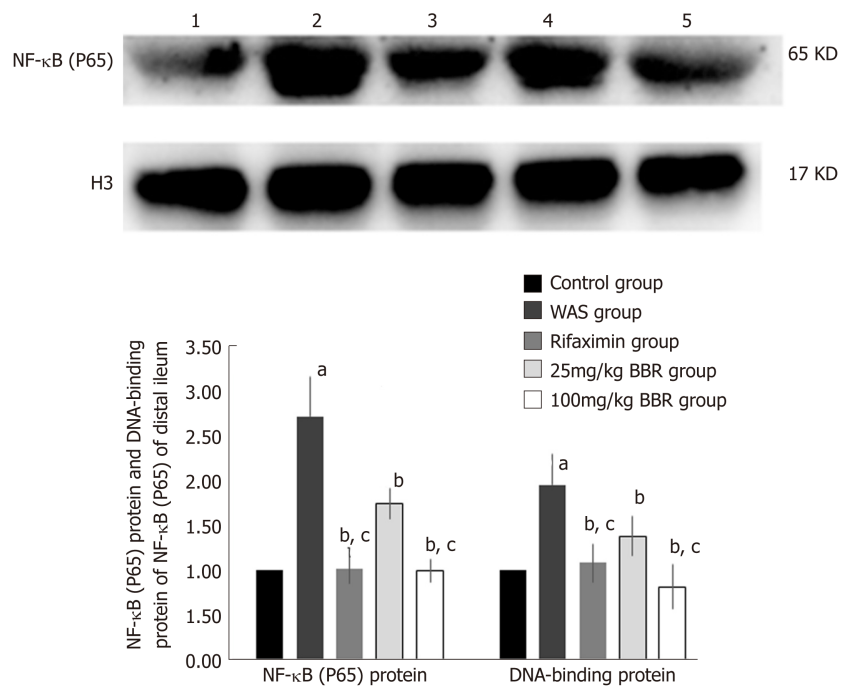
**Figure 3 Effects of berberine on NF-κB (P65) protein and NF-κB (P65) DNA-binding protein expression.** Expression of NF-κB (P65) protein and DNA-binding protein of NF-κB in 1: Control group; 2: Water avoidance stress (WAS) group; 3: Rifaximin group; 4: 25 mg/kg berberine (BBR) group; 5: 100 mg/kg BBR group. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine.

visceral hypersensitivity and intestinal dynamics by reducing C-kit expression, which may be associated with the following mechanisms: (1) Through both SCF and C-kit, the JAK/STAT signaling pathway is activated, and STAT translocates into the nucleus, leading to the proliferation of ICC cells. Pathological changes occur in the new ICC, leading to abnormal intestinal electrophysiological pacing. BBR may inhibit the activation of the JAK/STAT signaling pathway by decreasing the expression of C-kit, reducing the abnormal regeneration of ICC, inhibiting the generation of abnormal electrical signals, and ultimately reducing the intestinal motility; (2) SCF and C-kit activate the P13K-AKT signaling pathway, which increases the expression of gap junction proteins in the intestinal smooth muscle cells. The electrical signals generated by the ICC are more likely to be transmitted to each other, which increases intestinal movement. Reduction in the expression level of C-kit can inhibit the activity of the P13K-AKT signaling pathway, leading to a decline of the expression levels of gap junction proteins in the intestinal smooth muscle cells and transmission efficiency of abnormal electrical signals being reduced, which eventually causes reduction of the intestinal motility; and (3) Activation of the RAS-ERK signaling by SCF and C-kit increases the sensitivity of the neurotransmitter receptors on the ICC surface, making them easier to bind the neurotransmitter secreted by the central nervous system and the enteric nervous system. Thus, intestinal sensitivity and intestinal motility are increased. BBR inhibits the RAS-ERK signaling pathway by reducing the expression of C-kit, decreases the sensitivity of neuron receptors on the surface of ICC cells, and ultimately reduces visceral sensitivity and intestinal motility[6,44,45].

Our results showed that BBR can reduce inflammation of the distal ileum in IBS rats by inhibiting NF-κB signal pathways, and regulate visceral sensitivity and intestinal motility in the treatment of IBS by reducing the expression of BDNF, its receptor TrkB, and C-kit. Recent studies have shown that when patients experience the colon expansion test (CRD), IBS patients not only show increased abdominal withdrawal reflex, but also increased range of brain activity reflex. These results suggest that IBS patients not only have visceral hypersensitivity, but also have increased sensitivity of the central system. This interaction may be achieved through the axis of the brain. First, hormones and neurotransmitters secreted by the neuroendocrine system act on immune cells and mast cells in the intestinal mucosa. Mast cells release SCF and inflammatory media. Subsequently, inflammation reduces intestinal sensitivity and intestinal motivation *via* influencing the intestinal smooth muscle cells and neurons[46-49]. Second, in the stress state, the primary afferent neurons in the gastrointestinal tract are activated and the spinal cord is sensitized[50], which
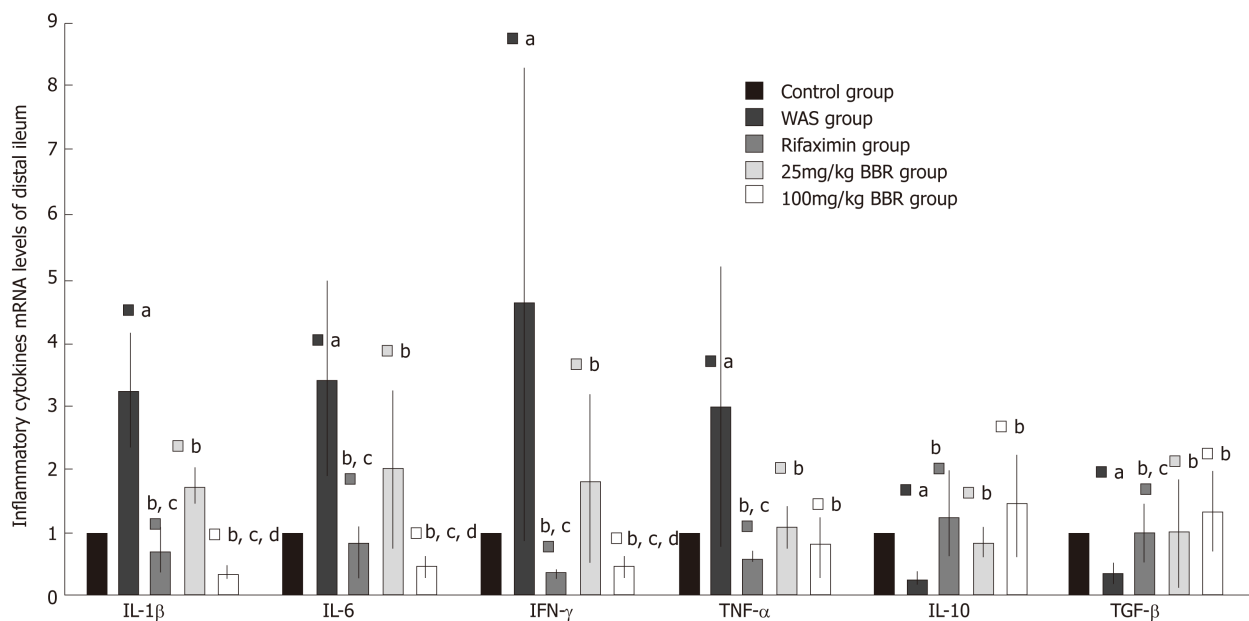
**Figure 4 Effects of berberine on cytokine mRNA levels.** Cytokine mRNA levels in the control group, water avoidance stress (WAS) group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group are shown. Letters a, b, c, and d: $P < 0.05$ compared with those in the control group, WAS group, 25 mg/kg BBR group, and rifaximin group, respectively. WAS: Water avoidance stress; BBR: Berberine; IL: Interleukin; IFN: Interferon; TNF-α: Tumor necrosis factor-α; TGF-β: Transforming growth factor-β.

increases the permeability of the intestine[51,52]. The increase of intestinal permeability leads to the defect of mucosal barrier, and enhances bacterial adhesion and infiltration into the gastrointestinal mucosa[32,48]. DNA of these bacteria can interact with Toll-like receptors[53] to regulate cytokines such as TNF and IFN[54] and activate intestinal mucosal immune response[55]. Third, anatomically, the gut immune cells are closely linked to the axons of the gut neurons. The inflammatory factors may change the structure of the nerve and increase the visceral sensation through the distal end of the afferent nerve and the activated spinal dorsal horn[56,57]. Ultimately, the intestinal motility is enhanced[58]. The three pathways studied in this study were also connected through the brain-gut axis. The immune activation of IBS is not only limited to the intestinal wall, but also the whole body, which is manifested by the increase of the pro-inflammatory cytokines and the reduction of anti-inflammatory cytokines mediated by the NF-κB signal pathway[13,14]. Cytokines are involved in the interaction of the brain-gut axis, and these inflammatory factors can act on smooth muscle cells and neurons in the gut, leading to the changes in intestinal motility and visceral sensitivity[13,59]. Stress stimulates intestinal smooth muscle cells to release SCF. Binding of SCF and C-kit activates C-kit kinase and promotes the secretion of mast cells to release a series of inflammatory mediators and inflammatory factors, leading to low inflammatory response in the intestinal tract. In the central nervous system, BDNF promotes the release of pro-inflammatory cytokines by activating nerve cells such as astrocytes, and the inflammatory response also causes BDNF to increase in dorsal root ganglia. Therefore, BDNF interacts not only with the intestinal nervous system, but also with the intestinal immune system, which can affect the visceral sensitivity and intestinal dynamics of IBS rats[60,61]. These pathways reinforce each other through the brain-gut axis, resulting in intestinal inflammation, visceral hypersensitivity, and increased intestinal motility in IBS patients. BBR can treat IBS patients by regulating these three pathways and blocking the interaction among them.

In conclusion, BBR inhibits the mucosal inflammation of the intestinal tract by inhibiting the intestinal NF-κB signal pathway in the IBS rats. BBR reduces the expression of BDNF and its receptor TrkB, and the expression of C-kit to reduce intestinal motility and visceral sensitivity and produce a therapeutic effect on IBS. The therapeutic effect of 100 mg/kg BBR is superior to that of 25 mg/kg BBR.
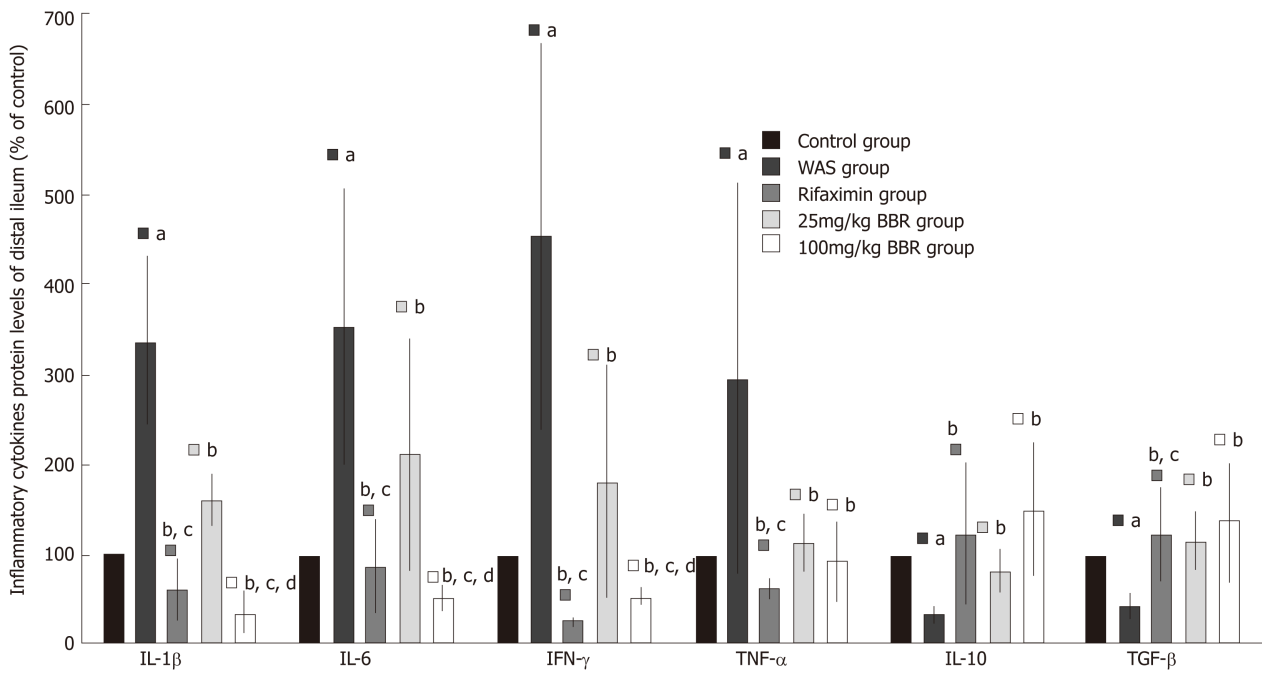
**Figure 5  Effects of berberine on cytokine protein levels.** Cytokine protein levels in the control group, water avoidance stress (WAS) group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group are shown. Letters a, b, c, and d; *P* < 0.05 compared with those in the control group, WAS group, 25 mg/kg BBR group, and rifaximin group, respectively. WAS: Water avoidance stress; BBR: Berberine; IL: Interleukin; IFN: Interferon; TNF-α: Tumor necrosis factor-α; TGF-β: Transforming growth factor-β.
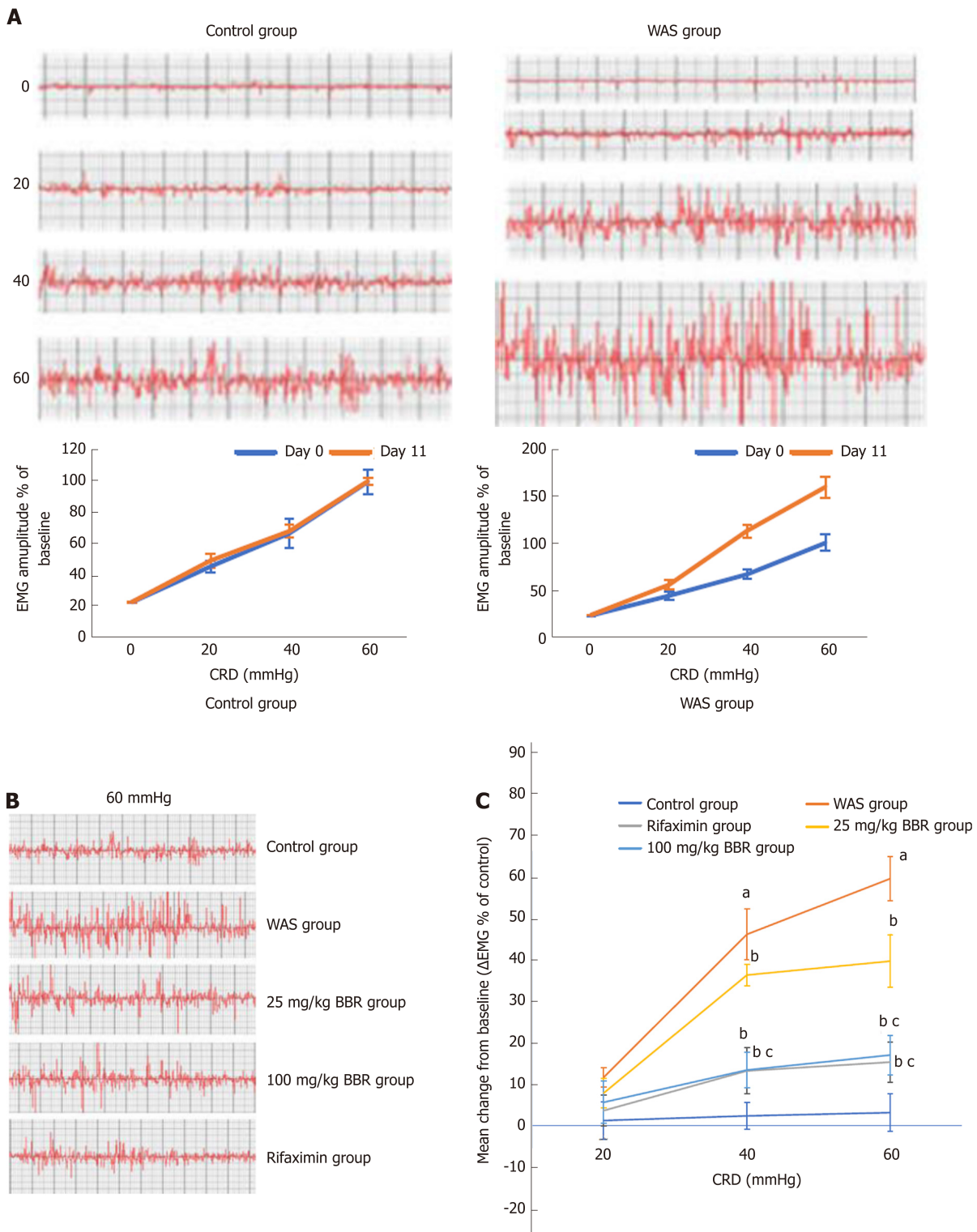
**Figure 6 Effects of berberine on visceromotor response to colorectal distention in rats.** We took the average EMG amplitude measured on day 11 subtracted by the average EMG amplitude detected on day 0, and express it as EMG. A: The amplitude of electromyogram (EMG) was changed in the control group and water avoidance stress (WAS) group under different pressures of colorectal distention (CRD); B: The amplitude of EMG in the control group, WAS group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group under 60 mmHg of CRD on day 11; C: EMG of different group rats under different pressures of CRD. Letters a, b, and c: *P* < 0.05 compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine; CRD: Colorectal distention; EMG: Electromyogram.

**Figure 7 Effects of berberine on total gastrointestinal transit and small intestinal transit.** Total gastrointestinal transit and small intestinal transit in the control group, water avoidance stress (WAS) group, rifaximin group, 25 mg/kg berberine (BBR) group, and 100 mg/kg BBR group are shown. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine.
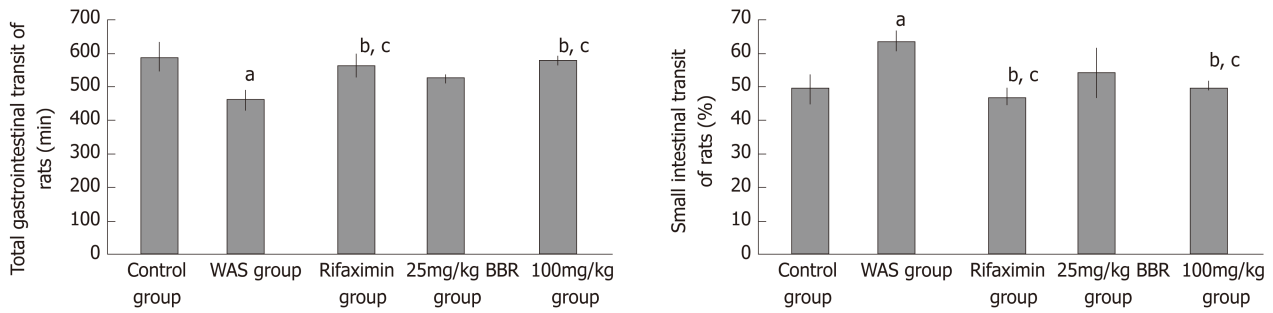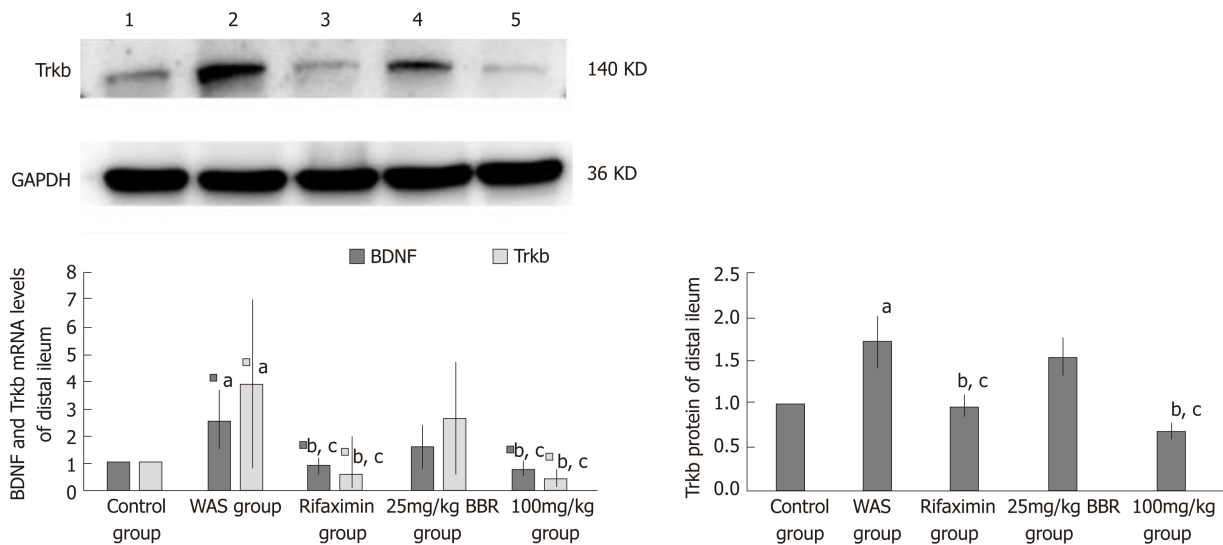


**Figure 8 Effects of berberine on brain derived neurotrophic factor and Trkb mRNA and protein expression.** Expression of brain derived neurotrophic factor and Trkb mRNA and protein in 1: control group; 2: water avoidance stress (WAS) group; 3: rifaximin group; 4: 25 mg/kg berberine (BBR) group; and 5: 100 mg/kg BBR group. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. BDNF: Brain derived neurotrophic factor; WAS: Water avoidance stress; BBR: Berberine.
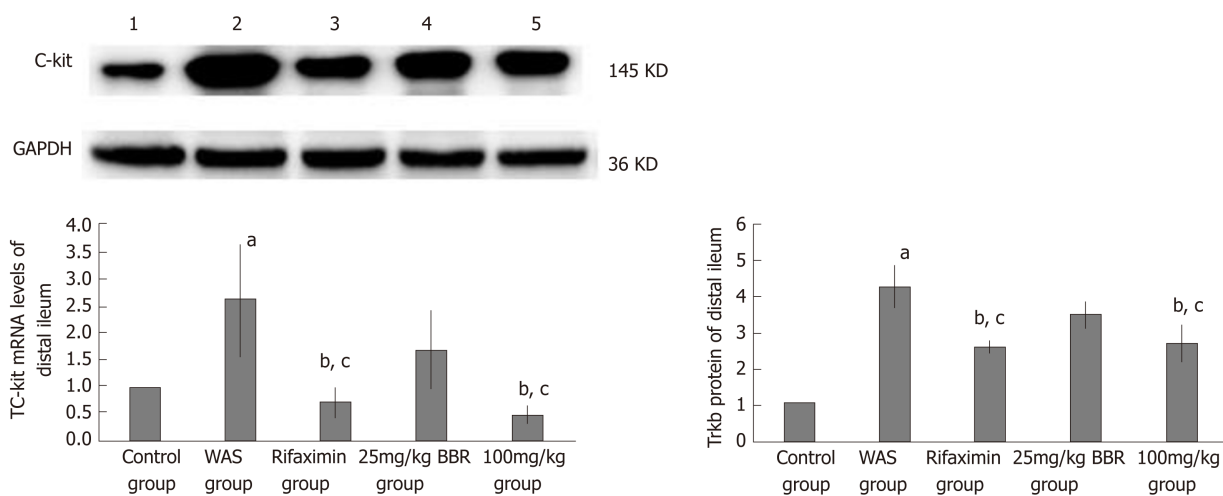


**Figure 9 Effects of berberine on C-kit mRNA and protein expression.** Expression of C-kit mRNA and protein in 1: control group; 2: water avoidance stress (WAS) group; 3: rifaximin group; 4: 25 mg/kg berberine (BBR) group; and 5: 100 mg/kg BBR group. Letters a, b, and c: $P < 0.05$ compared with those in the control group, WAS group, and 25 mg/kg BBR group, respectively. WAS: Water avoidance stress; BBR: Berberine.
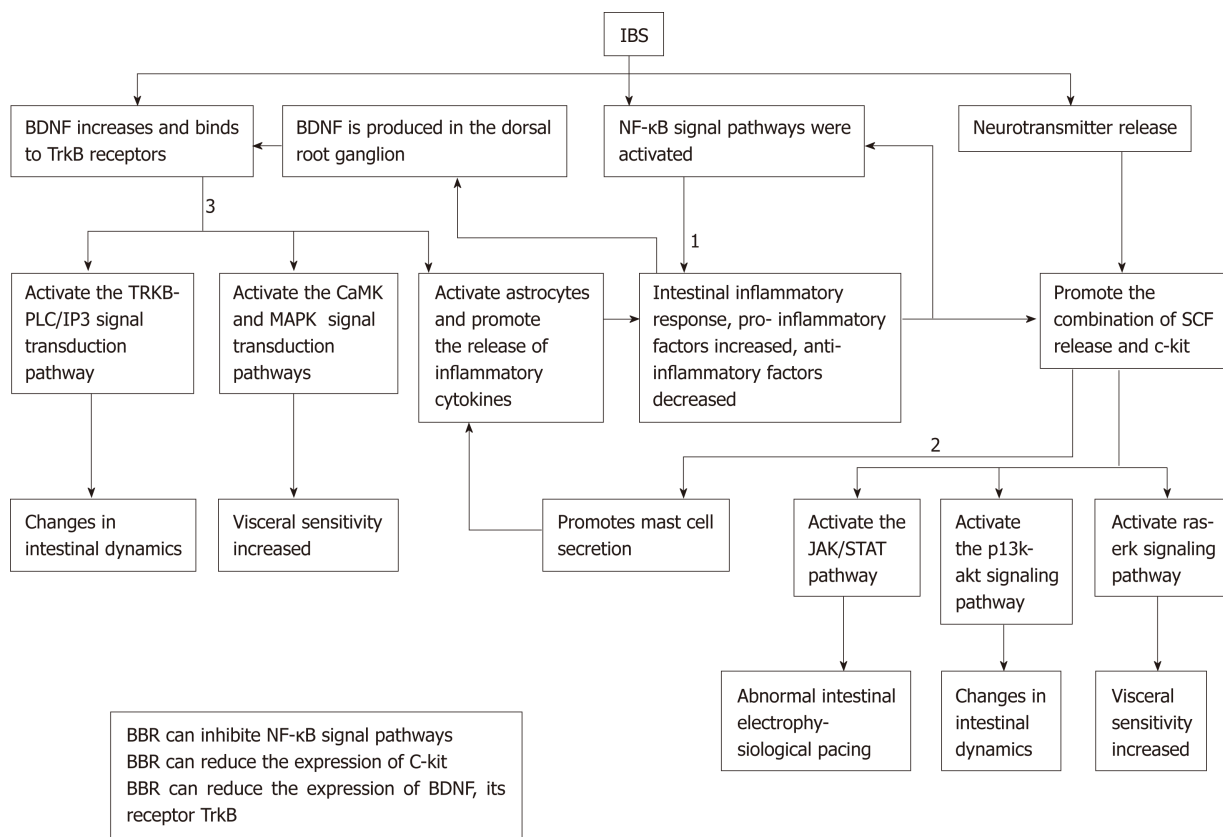
IBS

BDNF increases and binds to TrkB receptors

BDNF is produced in the dorsal root ganglion

NF-κB signal pathways were activated

Neurotransmitter release

3

Activate the TRKB-PLC/IP3 signal transduction pathway

Activate the CaMK and MAPK signal transduction pathways

Activate astrocytes and promote the release of inflammatory cytokines

Intestinal inflammatory response, pro-inflammatory factors increased, anti-inflammatory factors decreased

1

Promote the combination of SCF release and c-kit

Changes in intestinal dynamics

Visceral sensitivity increased

Promotes mast cell secretion

Activate the JAK/STAT pathway

Activate the p13k-akt signaling pathway

Activate ras-erk signaling pathway

2

Abnormal intestinal electrophy-siological pacing

Changes in intestinal dynamics

Visceral sensitivity increased

BBR can inhibit NF-κB signal pathways
BBR can reduce the expression of C-kit
BBR can reduce the expression of BDNF, its receptor TrkB

**Figure 10  Relationship among NF-kB signaling, brain derived neurotrophic factor, and C-kit and the underlying role of berberine.** IBS: Irritable bowel syndrome; BDNF: Brain derived neurotrophic factor; SCF: Stem cell factor.

## ARTICLE HIGHLIGHTS

### Research background

Irritable bowel syndrome (IBS) is a common chronic non-organic disease of the digestive system. Rifaximin has been used in clinical treatment of patients with IBS and has achieved good efficacy. However, rifaximin is expensive, and long-term oral administration may lead to cross-resistance to rifabutin and rifampicin. A large number of basic and clinical studies have also shown the efficacy of berberine in the treatment of IBS.

### Research motivation

Many studies have demonstrated that the pathogenesis of IBS may be related to the disorder of brain-gut axis, visceral hypersensitivity, intestinal immune abnormality, intestinal motility change, increased intestinal mucosal permeability, and intestinal flora disorder. The treatment mechanism of berberine for IBS is still unclear. In this study, we tried to investigate the effect of berberine on intestinal inflammation, intestinal motility, and intestinal sensitivity in rats with IBS and explore the therapeutic mechanism of berberine for IBS.

### Research objectives

The purpose of this study was to investigate the effect of berberine on the NF-κB signaling pathway in rats with IBS, which may improve intestinal inflammation in rats with IBS. And we studied the influence of berberine on the expression levels of brain-derived neurotrophic factor (BDNF) and C-kit in the intestinal tract of rats, which may affect the intestinal motility and visceral sensitivity of rats.

### Research methods

Water avoidance stress (WAS) was used to establish an IBS rat model, and the rats were divided into a control group, a WAS group, a rifaximin group, a 25 mg/kg BBR group, and a 100 mg/kg BBR group. We evaluated the histopathological changes of the terminal ileum in rats by hematoxylin and eosin (HE) staining. We measured the expression levels of NF-Kb (P65) DNA binding protein in the terminal ileum tissues of rats of each group by modified ELISA and Western blot. The mRNA expression levels of IL-1β, IL-6, IFN-γ, TNF-α, IL-10, and TGF-β were determined by qRT-PCR. ELISA was used to detect the inflammatory cytokines. Intestinal motility of rats in each group was detected by total gastrointestinal and small intestinal transit functions. The mRNA expression levels of BDNF and its receptor TrkB were detected by qRT-PCR. Western blot was used to detect the expression level of TrkB protein in the terminal ileum tissues in each group of rats. The mRNA expression levels of C-kit in ileum terminal tissues of

rats in each group were detected by qRT-PCR. The expression level of C-kit protein in ileum terminal tissues of each group of rats was detected by Western blot.

### Research results

We successfully applied the WAS model to induce visceral hypersensitivity in rats, changes in intestinal inflammation, and intestinal motility, which are consistent with the characteristics of IBS. Berberine can effectively improve the inflammation of in terminal tissues. Berberine can inhibit the activated NF-κB signal pathway in the intestinal tract of IBS rats, significantly reduce the expression of inflammatory IL-1β, IL-6, IFN-γ, and TNF-α in the terminal ileum tissues of IBS rats, and significantly increase the expression levels of anti-inflammatory cytokines IL-10 and TGF-β. Berberine can effectively regulate the intestinal motility of IBS rats and inhibit the expression of BDNF and its receptor TrkB as well as C-kit in ileum terminal tissues of IBS rats. The treatment effect of large dose (100 mg/kg) berberine on IBS rats was significantly better than that of small dose (25 mg/kg) berberine.

### Research conclusions

Berberine can inhibit the mucosal inflammation of the intestinal tract by inhibiting the intestinal NF-κB signal pathway in IBS rats. Berberine reduces the expression of BDNF and its receptor TrkB as well as C-kit to reduce intestinal motility and visceral sensitivity and produce a therapeutic effect on IBS. The therapeutic effect of 100 mg/kg berberine is superior to that of 25 mg/kg berberine.

### Research perspectives

This study confirms the exact therapeutic effect of berberine on IBS at the level of animal experiments, discusses the possible mechanism of its therapeutic effect, and provides a theoretical basis for the clinical application of berberine in the treatment of IBS. However, the optimal dosage of berberine in the clinical treatment of IBS still needs further pharmacological and toxicological studies

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