

# Baitouweng Decoction Alleviates Dextran Sulfate Sodium–Induced Ulcerative Colitis Through Nrf2/HO-1 Pathway Activation and HMGB1 Downregulation

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## Research Article

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# Abstract

## Background

The phrase “baitouweng (BTW) decoction” was first recorded in the ancient Chinese medical text *Shang Han Za Bing Lun*. BTW decoction has been widely used by practitioners of (traditional) Chinese medicine. [VN1] It has been used to treat ulcerative colitis (UC) for hundreds of years. In this study, we investigated the antioxidative properties of BTW and the intestinal immunity of mice with dextran sulfate sodium (DSS)–induced UC and further investigated the mechanism by which BTW alleviates UC.

## Methods

UC was induced in mice by using DSS. The mice were randomly divided into the following five groups: control, DSS, BTW (5, 10, and 20 g/kg [VN2]), berberine (BBR), and 5-aminosalicylic acid (5-ASA). Except for the control group, 3% DSS was administered in drinking water to all groups for 7 days, and the other groups were intragastrically administered with BTW, BBR and 5-ASA independently. [VN3] After gavaging for 12 days, the mice were killed. Subsequently, body weight loss, colon length, colon histopathology, inflammatory cytokine expression, and intestinal protein expression were measured.

## Results

BTW effectively reduced the symptoms and histopathological scores of UC mice. Additionally, it downregulated the inflammatory factors interleukin (IL)-6 and IL-1 $\beta$ , the immunoglobulins vascular cell adhesion molecule 1 and intercellular adhesion molecule 1, and metalloprotease matrix metalloproteinase 9. Moreover, it downregulated high mobility group box 1 protein. Furthermore, it inhibited the nuclear factor erythroid 2–related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway.

## Conclusion

BTW considerably alleviated the inflammatory symptoms of mice with acute colitis, and the latent mechanism of BTW may be related to various signaling pathways, including the modulation of antioxidant signaling pathways such as the Nrf2/HO-1 pathway.

## Background

Ulcerative colitis (UC) is a nonspecific chronic inflammatory disease of colonic mucosa with unknown etiology. Its treatment is difficult owing to its chronic nature, varying degrees of lesions, and recurrence. It is listed as a modern refractory disease by the World Health Organization. Routine drugs for UC treatment in modern medicine are mainly aminosalicylic acids, immunosuppressants, and adrenal glucocorticoids. However, the long-term use of these drugs leads to toxicity and side effects, with disease recurrence after drug withdrawal. Furthermore, these drugs are expensive, which makes them unaffordable for ordinary people. Chinese medicine has unique advantages in the prevention and treatment of complex and chronic

diseases owing to its characteristics of multisystem, multilink, and multitarget regulation. In recent years, the clinical application of traditional Chinese medicine in UC treatment has shown obvious curative effects, low recurrence rates, low toxicity and tolerable side effects, and other advantages that making it a hot spot in UC treatment.

Baitouweng (BTW) decoction originated from Zhang Zhongjing's book *Shanghan Lun*. BTW is composed of four herbs, namely *Radix Pulsatilla* (Bai Tou Weng), *Cortex Phellodendri* (Huang Bai), *Rhizoma Coptidis* (Huang Lian), and *Cortex Fraxini* (Qin Pi). They cool and detoxify the body, thereby cooling the blood, and they cure dysentery. Therefore, BTW is widely used to treat UC in traditional Chinese medicine, and its clinical effect is remarkable. BTW decoction has been the treatment of choice for damp-heat dysentery[1] and has shown a favorable effect on UC in recent years.

BTW decoction regulates various immune cytokines and promotes immune regulation. Modern pharmacological studies have revealed that BTW decoction can reduce the humoral immunity of hyperactivity and thus reduce the immune response mediated by cytokines[2]. Studies have suggested that the transforming growth factor- $\beta$ 1/SMAD family member 3 signaling pathway plays an anti-inflammatory role in inflammatory bowel disease (IBD)[3], maintains intestinal epithelial barrier function through nuclear factor kappa B signaling pathway inhibition[4], modulates colitis through the regulation of intestinal microbiota and interleukin (IL)-6/signal transducer and activator of transcription 3 signaling pathway[5], and ameliorates UC through the regulation of Th17/Treg balance and restoration of the intestinal epithelial barrier[6]. Our previous study found a significant difference in the serum high mobility group box 1 (HMGB1) expression between patients with UC and healthy individuals[7], making HMGB1 a crucial proinflammatory mediator or early warning protein. Furthermore, HMGB1 acts as an indicator of UC in patients diagnosed as having rheumatoid arthritis and periodontitis. The present in vivo experiment revealed that BTW decoction could downregulate intracellular and extracellular HMGB1 levels. Because BTW decoction is a classic prescription for treating UC, this study verified the potential of HMGB1 as a new inflammatory cytokine.

This study aimed to explore the possible role of HMGB1 in treating an enteropathy model with BTW decoction and to explore the anti-inflammatory effect of BTW decoction through the nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) oxidative stress pathway.

## Methods

### Animals and reagents

Healthy male C57BL/6 mice aged 7–8 weeks were purchased from the Experimental Animal Center of Nanjing Medical University; they weighed 22–25 g at the time of the experiment. In the center, the mice were kept in a specific-pathogen-free environment at 18°C–23°C with good ventilation and a 12-h light and dark cycle. Five mice were kept in one cage. The mice were provided food and water ad libitum, and their bedding was replaced regularly. All mice had adapted to these conditions at least 7 days before the experiment began. All animal treatments were approved by the Animal Ethics Committee of Nanjing

Medical University. Investigations were accomplished in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Every animal care and protocols of experiments were all approved by the Nanjing Medical University Committee on Animal Care (Permit Number: IACUC 2004006). Mice were sacrificed through cervical dislocation. BTW was provided by Nanjing Hospital of Chinese Medicine, which is affiliated with Nanjing University of Chinese Medicine. Berberine (BBR) was purchased from MedChemExpress (Stockholm, Sweden), and 5-aminosalicylic acid (5-ASA) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

### **BTW decoction preparation**

The four medicinal materials were accurately weighed and boiled twice in boiling water at a volume ratio of 1:10 for 1 h. The aqueous solution was passed through five layers of medical gauze, and the concentrated solution was combined into 1 g/ml liquid containing crude drugs followed by storage at 4 °C. (Table 1)

### **High-performance liquid chromatography–mass spectrometry analysis**

Esculin, aesculetin, jatrorrhizine, coptisine, palmatine hydrochloride, BBR, pulchinoside A3, and pulchinoside B4 were obtained from Shanghai Yuanye Biological Co. Ltd. (Shanghai, China). Because of the detection limit, two instruments were used for content determination. Ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, Agilent Technologies, Santa Clara CA, USA) was performed to detect the amounts of esculin, aesculetin, jatrorrhizine, coptisine, palmatine hydrochloride, and BBR. Furthermore, a Thermo Fisher UHPLC-Q-Orbitrap HRMS was used to detect the amounts of pulchinoside A3 and pulchinoside B4.

The sample (1 mL) was precisely measured in a 10-mL volumetric flask, and methanol was added to 10 mL. The flask was plugged, and ultrasonic dispersion was conducted for 30 min. It was then cooled and shaken well. Subsequently, 1.0 mL of the sample–methanol solution was extracted and added to 1.0 mL of water, mixed well, and filtered through a 0.22- $\mu$ m microporous filter membrane. The filtrate was collected and used as the test product solution for the study. The top layer was used for UPLC-MS/MS analysis. Separation was achieved using an Agilent ZORBAX Eclipse Plus C18 (2.1 mm  $\times$  50 mm, 1.8  $\mu$ m). The column temperature was set to 30°C. The mobile phase was composed of methanol (phase A) and 0.1% formic acid aqueous solution (phase B), with the flow rate of 0.4 mL/min. The injection volume was 5  $\mu$ L. The raw MS and tandem MS (MS/MS) data were collected using Agilent 6460 triple quadrupole mass spectrometer. The analytes were ionized using electrospray ionization (ESI) at positive and negative modes. The drying temperature was set to 350°C, the drying gas flow rate was 10 L $\cdot$ min<sup>-1</sup>, and the capillary voltages were 4000 V(+) and 3500 V(-) (Tables 2, and 3).

A sufficient amount of standard substance was accurately weighed, and pure methanol that can completely dissolve into 2.00 mg/mL reserve solution was added and reserved for use. Pulchinoside A3 and B4 reserve solutions were diluted with pure methanol, and a series of standard solutions with

concentration gradients were prepared for injection analysis. A T3 column (2.1 mm × 150 mm, 3 μm) was used. The mobile phases of 0.10% formic acid in acetonitrile and 0.10% formic acid in water were used for gradient elution (0–0.5 min, 2% A; 0.5–4 min, 2% A; 0.5–4 min, 98% A; 4–9 min, 98% A; 9–9.3 min, 2% A; and 9.3–10 min, 2% A); flow rate: 0.300 mL/min; injection volume: 5.0 μL; and the temperature of the Ultra-High Performance Liquid Chromatography(UHPLC) column was fixed at 45°C. The ion source was ESI, the carrier gas was nitrogen, the spray voltage was 3.2 kV(+), the capillary temperature was 300°C, the auxiliary heating temperature was 300°C, the resolution was 17 500, and the scanning range was 50.0–500.0 m/z (Tables 2 and 3).

### **Establishment of the dextran sulfate sodium–induced UC mouse model**

Acute UC was induced in the mice through the administration of dextran sulfate sodium (DSS) in drinking water (3%, w/v) after 1 week of living in the Experimental Animal Center.. The mice were divided into the following five groups: control, DSS, 5-ASA (800 mg/kg), BBR (100 mg/kg), and BTW (5, 10, and 20 g/kg), 6 mice/group . Mice in the treatment groups were administered 5-ASA and BTW through gavage starting from the day of DSS treatment. The control and DSS-treated groups were provided water and 3% DSS, respectively. Mice were sacrificed after 12 days of DSS administration. After the mice were killed, colon segments from the anus to the cecum were removed, and the colon lengths were measured. Colons were rinsed with phosphate buffered saline (PBS), placed in cryopreservation tubes, rapidly frozen in liquid nitrogen for 24 h, and then stored at –80°C until analysis.

### **Histological analysis**

Colon sections (approximately 1 cm long) containing the lesion site were fixed with 4% neutral formalin. Then, conventional paraffin embedding, sectioning, and hematoxylin and eosin staining were performed. Histological changes in pathological sections were observed under a light microscope. The evaluation criteria were previously described[8].

### **Enzyme-linked immunosorbent assay**

Serum was obtained from mice. The concentrations of inflammatory cytokines (IL-6 and IL-1β) were quantified using enzyme-linked immunosorbent assay (ELISA) kits from Elabscience (China). Furthermore, HMGB1 was quantified using ELISA kits from IBL (USA).

### **Immunohistochemistry**

Colon tissue sections were dewaxed in water and washed thrice with 0.01 M PBS (5 min each time). Sections were treated with a 0.3% H<sub>2</sub>O<sub>2</sub> and 0.5% Triton X-100 mixture at room temperature for 30 min. For antigen repair, the sections were treated with 0.05 M PBS solution, cooled to room temperature, and heated to 100°C for 20 min in a microwave oven. Nonspecific antigens were blocked with normal sheep serum and kept in a 37°C water bath for 30 min. The sections were incubated with primary antibody incubated at 4°C overnight. They were then washed thrice with a 0.01 M PBS solution (5 min each) and

subsequently incubated with secondary antibody for 1 h in at 25°C, washed thrice with a 0.01 M PBS solution (5 min each), and subjected to diaminobezidin(DAB) color rendering. They were restained with hematoxylin for 3–5 min. Then, they were examined under a microscope, and images were acquired and analyzed. Nuclei stained with hematoxylin were blue, and the positive expression of DAB was indicated in brown{Xuan-Qing, 2020 #2591}.

### **Malondialdehyde and superoxide dismutase analysis**

Colon tissues from five mice in each group were collected and placed in an ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China). The colon tissue were then homogenized in a Polytron homogenizer (Retech GmbH mm400, Germany) followed by centrifugation at 3000 rpm/min for 15 min; supernatant was collected for superoxide dismutase (SOD) and malondialdehyde (MDA) measurement.

An MDA detection kit (A003; Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was selected to determine the MDA level as a marker of lipid peroxidation. The assay was conducted according to the manufacturer's instructions[9]. An SOD detection kit (A001; Nanjing Jiancheng Bioengineering Institute) was used for SOD measurement. The assay was conducted according to the manufacturer's instructions.

### **Western blot analysis**

Colon tissues were prepared using RIPA lysis buffer. The protein concentration was determined using a bicinchoninic acid protein assay, and 20 µg of protein was separated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. Separated proteins were transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). Subsequently, membranes were blocked with 5% BSA in tris-buffered saline with 0.1% Tween 20 detergent and incubated with primary antibodies at 4°C overnight. Then, the membranes were incubated with secondary antibodies for 90 min. Finally, the membranes were chemically exposed and semiquantitatively analyzed with Quantity One software (Bio Rad){Xuan-Qing, 2020 #2591}.

### **Statistical analysis**

All data are expressed as means and standard deviations. GraphPad Prism 5 software was used to analyze all data. Differences between each group were analyzed using one-way analysis of variance. *p* values < 0.05 were considered significant (<sup>#</sup>*p* < 0.05 and <sup>##</sup>*p* < 0.01 vs the control group; \**p* < 0.05 and \*\**p* < 0.01 vs the DSS group).

## **Results**

### **Chemical profile of BTW decoction**

More information was acquired under the anion mode than under the cation mode. The total ion chromatograms of the standard mixture and BTW are shown in Table 3. Eight compounds were identified

in BTW (**Fig. 1**). In 1 g/mL BTW, the proportions of palmatine, coptisine, aesculin, aesculetin, BBR, jatrorrhizine, pulchinoside A3, and pulchinoside B4 were 1.048, 1.46, 30.52, 0.048, 0.038, 1.05, 1.70, and 0.16 µg/mL, respectively.

### **BTW ameliorates DSS-induced colitis**

To determine whether BTW influences inflammation in DSS-induced UC, we recorded the body weight, survival rate, and colon length of the mice. After treatment for 7 days, the body weight of the mice with DSS-induced UC was significantly lower than that of the control mice; mice in the groups treated with BTW (5, 10, and 20 g/kg/day), BBR (100 mg/kg/day), and 5-ASA (800 mg/kg/day) were substantially heavier than those in the DSS-treated group. Moreover, after treatment for 7 days, the survival rates of the BTW and 5-ASA groups were higher than that of the experimental UC group. The oral administration of BTW significantly ameliorated colon shortening (**Fig. 2**). The colon histology for the mice with DSS-induced UC was characterized by inflammatory cell infiltration, crypt loss, goblet cell reduction, and extensive destruction of the mucosal layer. The histological scores of the experimental UC group increased significantly compared with those of the control group, indicating that the colonic mucosa was severely impaired in the DSS-induced UC group. To investigate the effects of BTW on UC, mice in the BTW group were treated with BTW for 7 days before histological scoring. The BTW group exhibited remarkable restoration of goblet cells and crypt architecture and reduction of inflammatory cell infiltration, consistent with the results of the 5-ASA group. Histological scores in the 5, 10, and 20 g/kg BTW groups and 5-ASA group were significantly reduced compared with those of the DSS group (**Fig. 3**). The main proinflammatory cytokines were detected through ELISA. Proinflammatory cytokines, including IL-1 $\beta$  and IL-6, significantly increased in the DSS group, and BTW and 5-ASA treatments notably reduced the levels of all these proinflammatory cytokines (**Fig. 4**).

### **BTW modulated HMGB1 inflammatory cytokines**

HMGB1 expression increased in the DSS groups but decreased in the BTW group as evident in western blotting and immunohistochemical staining. Secretory HMGB1 was determined using an ELISA kit. These results show that DSS remarkably upregulated HMGB1 but BTW downregulated HMGB1 (**Fig. 5**).

### **BTW ameliorated oxidative stress**

To investigate the effect of BTW on the endogenous antioxidant system, the activities of colon tissue SOD and MDA were evaluated. Regarding SOD activity in the control group, BTW and 5-ASA treatment could restore SOD activity to a level comparable with that of the control group, indicating the protective effects of BTW and 5-ASA. The MDA level is an index of lipid peroxidation; therefore, it is a biomarker of oxidative stress. The MDA level significantly increased with BTW and 5-ASA treatment. Furthermore, BTW and 5-ASA treatment significantly reduced oxidative damage (**Fig. 6**).

### **BTW ameliorated DSS-induced UC through Nrf2/HO-1 pathway activation**

A study[10]revealed that Nrf2/HO-1 expression is low in UC. Therefore, we investigated whether BTW plays a therapeutic role through the Nrf2/HO-1 signaling pathway, which involves Nrf2, HO-1, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and matrix metalloproteinase 9 (MMP-9; Fig. 7). Our data suggest that BTW activates the Nrf2/HO-1 signaling pathway in UC mice (Fig. 7).

## Discussion

HMGB1 is an essential endogenous danger-signaling molecule that signals tissue damage. It is a late-onset inflammatory mediator and is secreted into the extracellular system to produce other beneficial substances. HMGB1 may play a role in IBD, and electroacupuncture can effectively reduce the level of HMGB1, thus inhibiting the inflammatory colonic response caused by UC[11]. In addition, anti-HMGB1/HMGB2 antibodies combined with anti-*Saccharomyces cerevisiae* antibodies can be used to distinguish between UC and Crohn disease(CD)[12]. Our research group previously found that the serum HMGB1 content of patients with UC differed from that of healthy people, but the molecular mechanism of HMGB1 in UC remains unclear.

Abnormal oxidative stress is generally considered a key event in UC. Under normal conditions, SOD can maintain the balance of oxygen free radicals and inhibit MDA production. The MDA level can indirectly indicate the degree of damage caused by oxygen free radicals to tissue cells, and SOD can reduce this damage. Some studies have indicated that UC is often accompanied by a certain degree of oxidative stress damage at onset, this oxidative stress stimulates the excessive generation of oxygen free radicals, leading to a decrease in SOD activity and increase in MDA concentration, aggravating the disease[13]. UC can affect peroxidation, increase mucosa permeability, and reduce SOD activity. Furthermore, MDA can stimulate the production of proinflammatory substances, such as arachidonic acid and cyclooxygenase, and induce tumor necrosis factor (TNF)- $\alpha$  expression. TNF- $\alpha$  can not only fight tumors but also release inflammatory mediators and promote the necrosis of intestinal epithelial cells[14]. In addition, neutrophils produce excessive reactive oxygen species in patients with UC and subsequently attack and destroy key macromolecules, disrupt intracellular homeostasis, and cause oxidative damage in tissues, thus accelerating and prolonging inflammatory response[15]. The present study found that SOD activity decreased and MDA concentration increased in the DSS- treated group. After treatment with BTW decoction, SOD activity increased and MDA concentration decreased. The results show that the decoction could inhibit oxidative stress and reduce intestinal damage, which was consistent with previous report [16].

During active intestinal inflammation, the innate response is manifested as increased leukocyte infiltration in the inflammatory mucosa, leading to oxidative stress injury[17]. VCAM-1 and ICAM-1 are essential inflammatory cytokines in vivo. VCAM-1 and ICAM-1 are adhesion molecules that mediate cell-to-cell and cell-to-extracellular matrix adhesion and binding. ICAM-1 mainly exists between macrophages and lymphocytes, is stimulated by inflammation and trauma, and can promote endothelial cell formation, thus enhancing the adhesion of vascular endothelium and white blood cells and aggravating

inflammatory response development. VCAM-1 is involved in the infiltration of lymphocytes and mononuclear macrophages into the inflammatory region[18]. IL-6 is a crucial regulator of inflammation in the body. IL-6 is closely related to inflammation and immune response in the body. IL-6 overexpression leads to immune disorders and exacerbates inflammation[19]. Studies have shown that the expression levels of VCAM-1 and ICAM-1 proteins increase because of the activity of various inflammatory mediators, such as IL-1 $\beta$ [20]. Furthermore, together with VCAM-1 and ICAM-1 molecules, MMPs are involved in the development of various diseases. VCAM-1 and ICAM-1, as inflammatory factors, cause intestinal tissue fibrosis in UC rats by altering MMP-9 levels. The present study found that in the model group, IL-1 $\beta$  and IL-6 levels increased, ICAM-1, VCAM-1, MMP-9 protein expression level, such as BTW decoction after the intervention, IL-1 $\beta$  and IL-6 levels decreased, ICAM-1, VCAM-1, MMP-9 protein expression, such as BTW decoction by inhibiting the inflammatory reaction, reduced the inflammation factor VCAM-1 and ICAM-1 level, thereby reducing MMP-9 levels, lowering the intercellular adhesion effect, reducing oxidative stress injury, and preventing UC.

Regarding whether Chinese herb decoctions affect the oxidative stress pathway, Nrf2 activation can significantly inhibit reactive oxygen species production, reduce inflammatory response, promote cell survival, and improve the redox and inflammatory status of intestinal epithelial cells. HO-1 is a powerful antioxidant defense enzyme that reduces oxygen free radical damage in vivo[21]. Conversely, a recent study confirmed that HO-1 supplementation had a protective effect on DSS-induced colitis mice[10]. Therefore, we studied Nrf2/HO-1, a crucial oxidative stress pathway, and found that DSS inhibited HO-1 and Nrf2 and activated HMGB1 in UC mice. Therefore, we speculate that BTW decoction downregulates HMGB1 through the Nrf2/HO-1 pathway. Indeed, HMGB1 plays a role in oxidative pathways in ethanol-induced brain inflammation[22]. In addition, studies have shown that the Nrf2 pathway is involved in HMGB1 pathway regulation and inflammatory factor expression[23]. So far, we have confirmed that BTW decoction can activate the Nrf2/HO-1 pathway to downregulate HMGB1, inflammatory factors IL-1 $\beta$ , TNF- $\alpha$ , and IL-6, and adhesion factors VCAM-1, ICAM-1, and MMP-9, thus inhibiting the oxidative stress process, reducing the expression of intestinal inflammatory factors and matrix proteins, and ultimately improving intestinal inflammatory response.

## Conclusion

Baitouweng decoction significantly improved the inflammatory symptoms of mice with acute colitis, and the latent mechanism of BTW may be related to various signaling pathways, including the modulation of antioxidant signaling pathways, such as Nrf2/HO-1.

## Abbreviations

HMGB1 High Mobility Group Box 1

UC ulcerative colitis

DSS dextran sulfate sodium

BBR berberine

BTW baitouweng decoction

5-ASA 5-aminosalicylic acid

Nrf2 erythroid 2-related factor 2

HO-1 heme oxygenase-1

IBD inflammatory bowel disease

SOD superoxide dismutase

MDA malondialdehyde

PBS phosphate buffered saline

VCAM-1 vascular cell adhesion molecule 1

ICAM-1 intercellular adhesion molecule 1

MMP-9 matrix metalloproteinase 9

CD Crohn disease

TNF- $\alpha$  tumor necrosis factor - $\alpha$

## **Declarations**

### ***Ethics approval and consent to participate***

Investigations were accomplished in accordance with the ARRIVE guidelines. Every animal care and protocols of experiments were all approved by the Nanjing Medical University Committee on Animal Care (Permit Number: IACUC 2004006).

### ***Consent for publication***

Not applicable.

### ***Availability of data and materials***

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## ***Competing interests***

The authors declare that they have no competing interests.

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## ***Authors' contributions***

Weina Zhu performed the research, analysed data, and wrote the paper.

Chunhua Ma and Weina Zhu performed the histological examination of the colon.

Jie Ruan performed Biochemical data.

Fuqiong Zhou analyzed and interpreted the Liquid mass spectrometry data regarding the Baitouweng decoction.

Yajie Zhang designed research, analysed most of the data.

Hongyan Long discussed the results and revised the manuscript.

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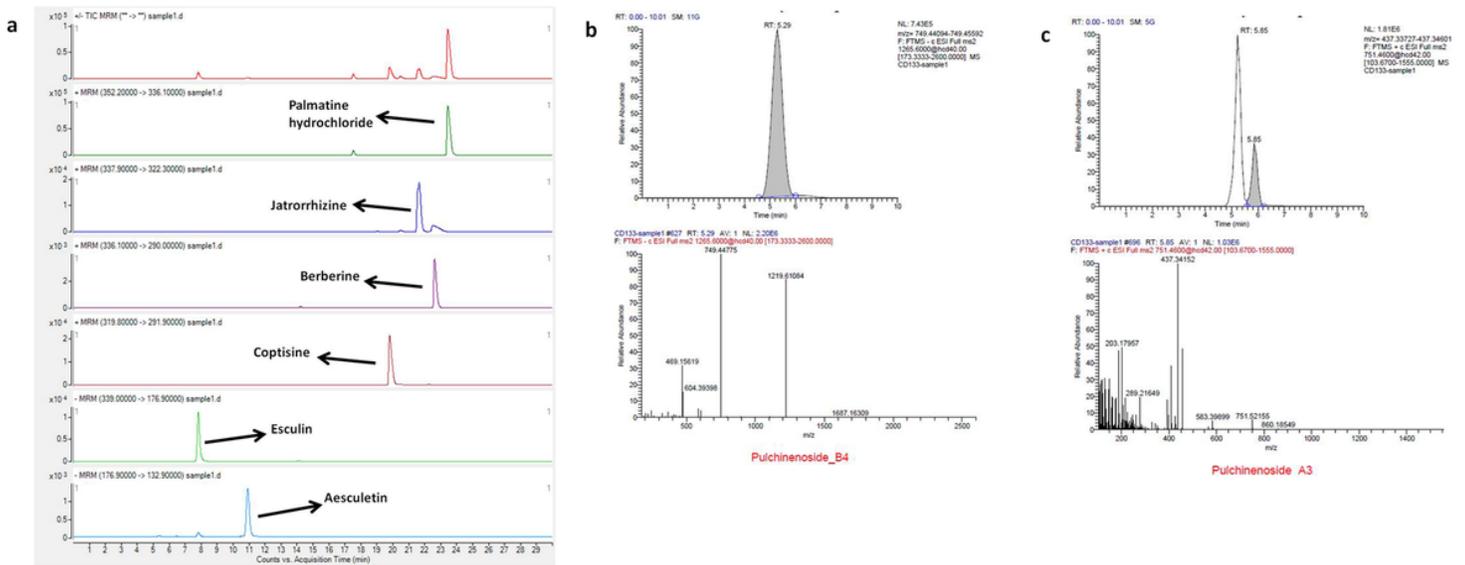
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## Tables

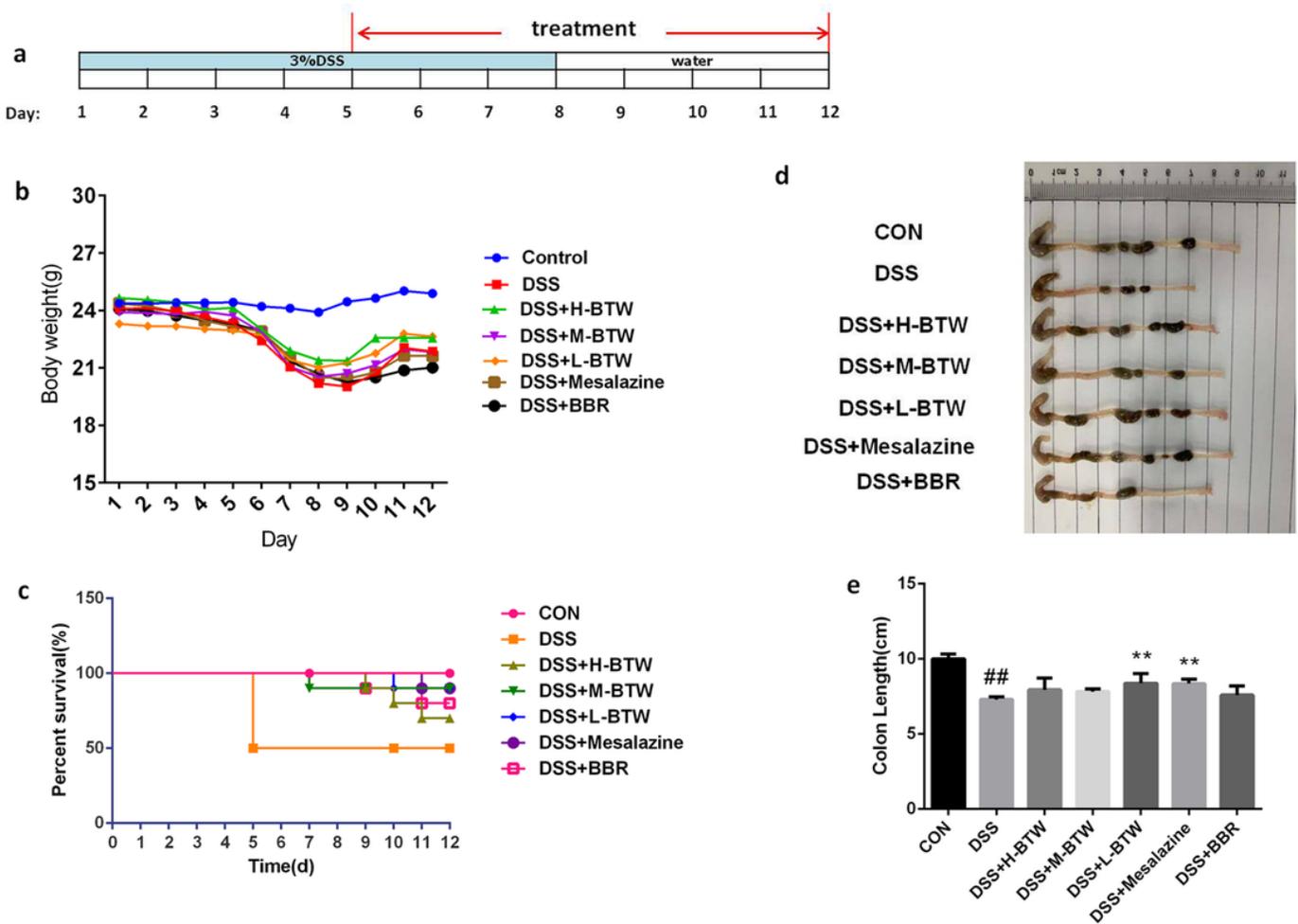
Due to technical limitations, table 1 to 3 is only available as a download in the Supplemental Files section.

## Figures



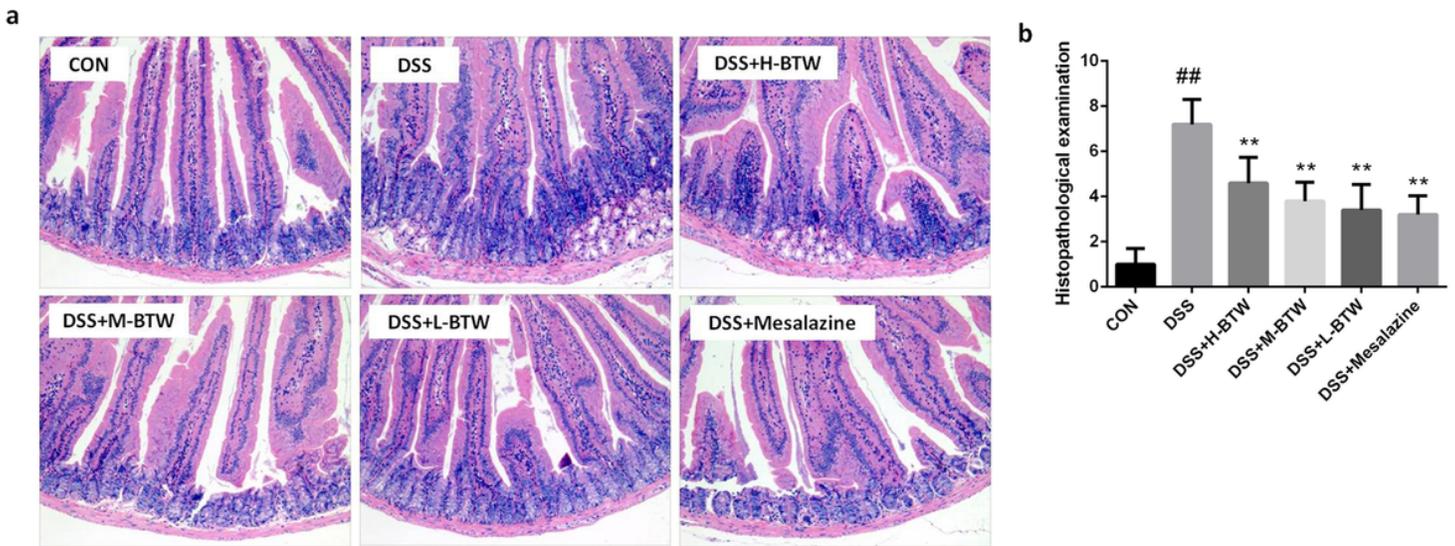
**Figure 1**

Chromatographic profile of BTW by using (a) UPLC-HPLC-MS and (b) UPLC-Q-Exactive-MS in anion and cation mode.



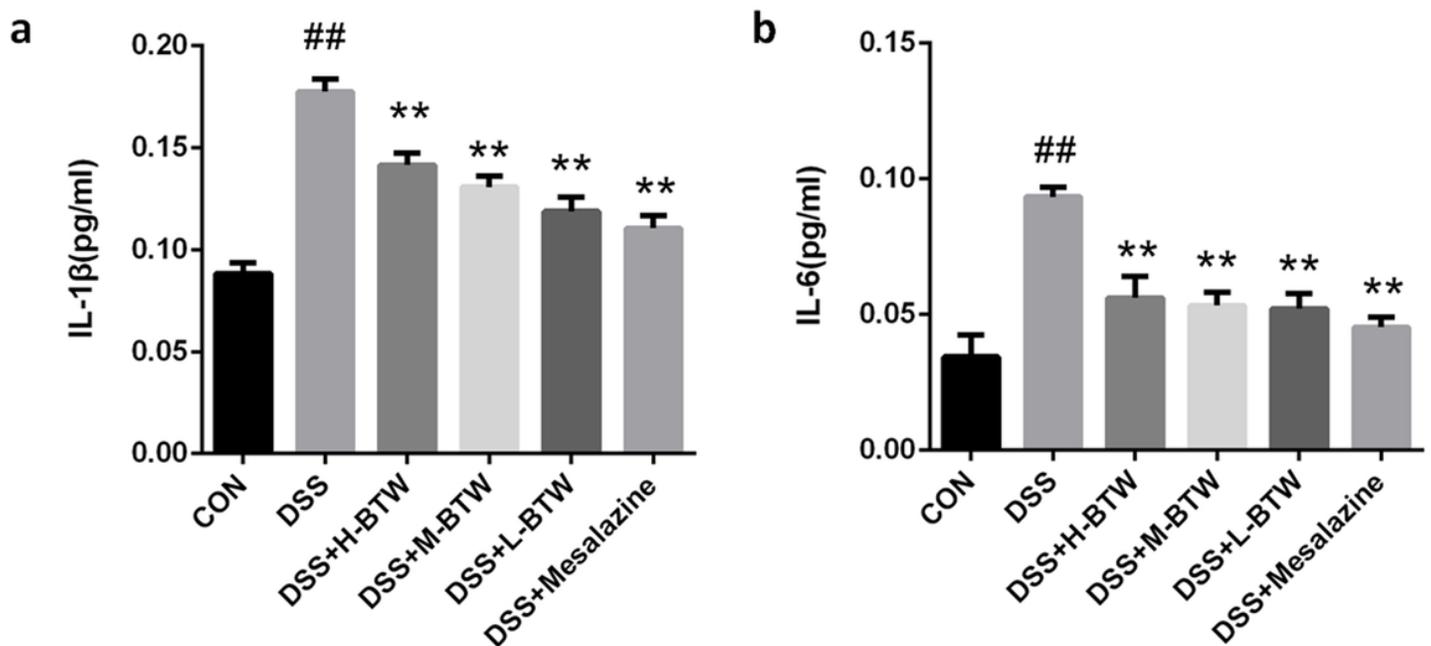
**Figure 2**

BTW ameliorates DSS-induced colitis. (a) The UC model and drug treatment procedure. (b) Body weight changes from day 1 to day 12 during the experiment. (c) Effect of BTW on the survival rate in mice with DSS-induced UC. (d) Photographs of the mice colons. (e) Colon length of mice in the DSS-treated group; n=6/group. \*p < 0.05, \*\*p < 0.01, ##mean values were significantly different from those of the control group; p < 0.01



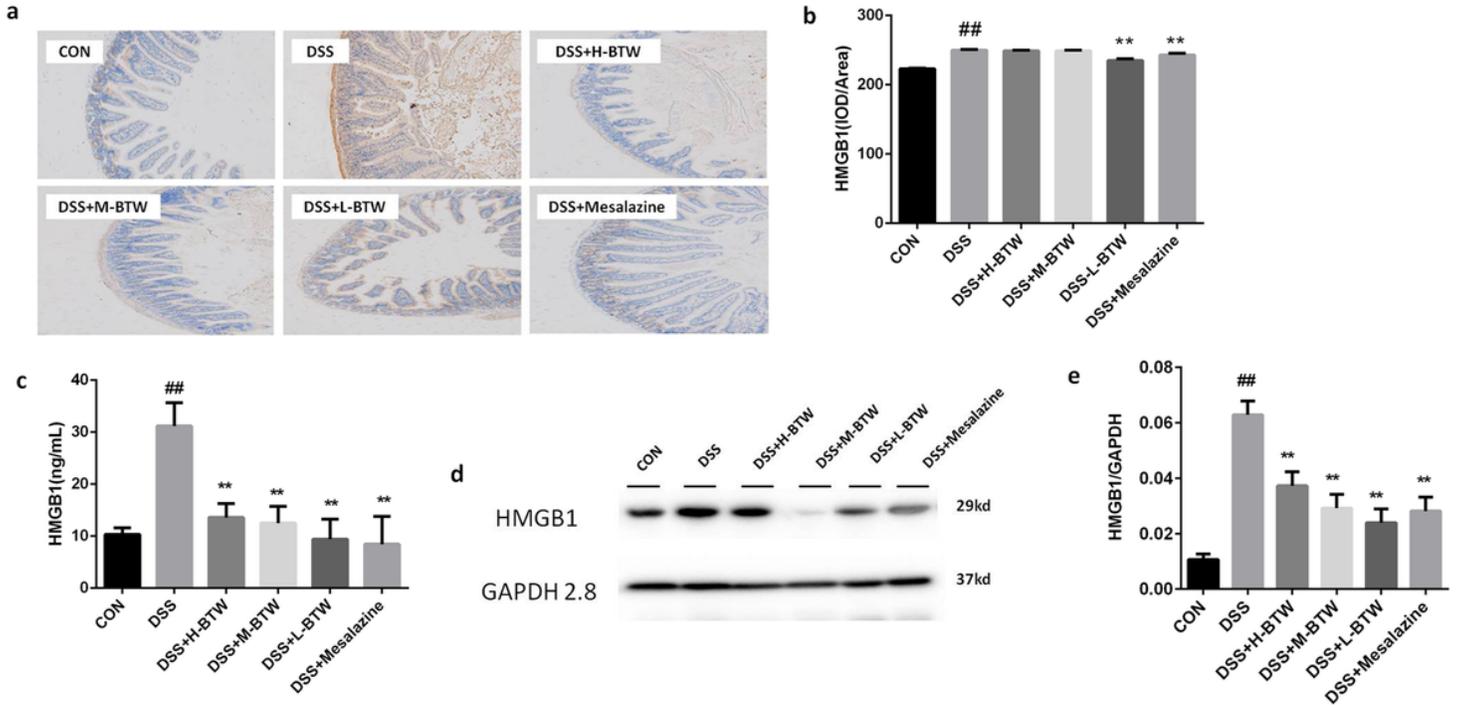
**Figure 3**

Treatment of DSS-induced colitis with BTW was associated with histological changes. (a) Representative histological photos (×200). (b) Histological injury index of the mice colons. (c) myeloperoxidase(MPO) content of the mice colons. Mean values were significantly different from those of the DSS-treated group; n=3group. \*p < 0.05, \*\*p < 0.01. ## Mean values were significantly different from those of the control group; p < 0.01.



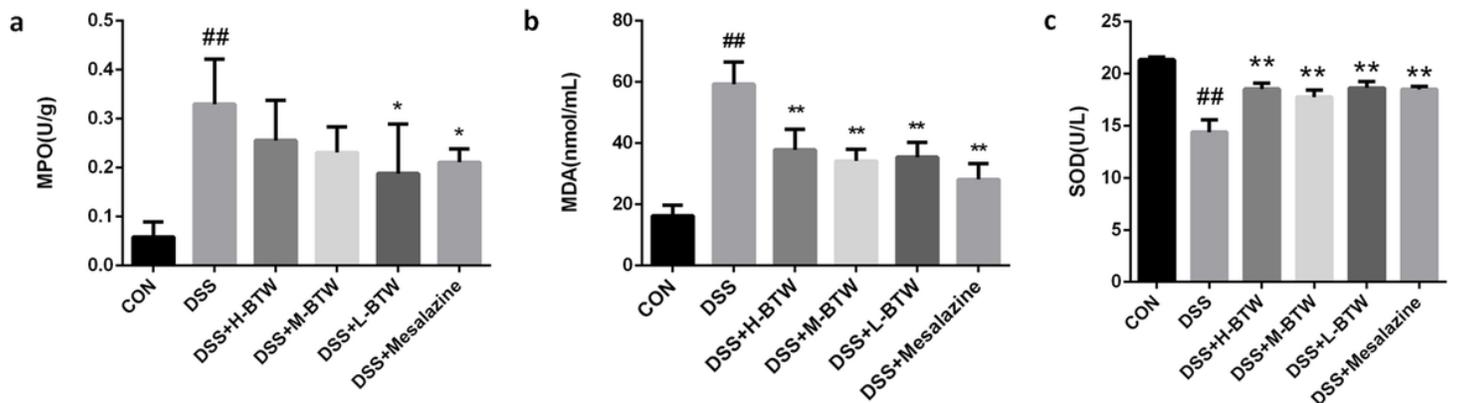
**Figure 4**

BTW reduced the expression of inflammatory cytokines in mice with DSS-induced UC. The inflammatory cytokines, including (a) IL-6 and (b) IL-1 $\beta$  were detected using ELISA kits. Mean values were significantly different from those of the DSS-treated group; n=3/group.\*p < 0.05, \*\*p < 0.01. ## Mean values were significantly different from those of the control group; p < 0.01.



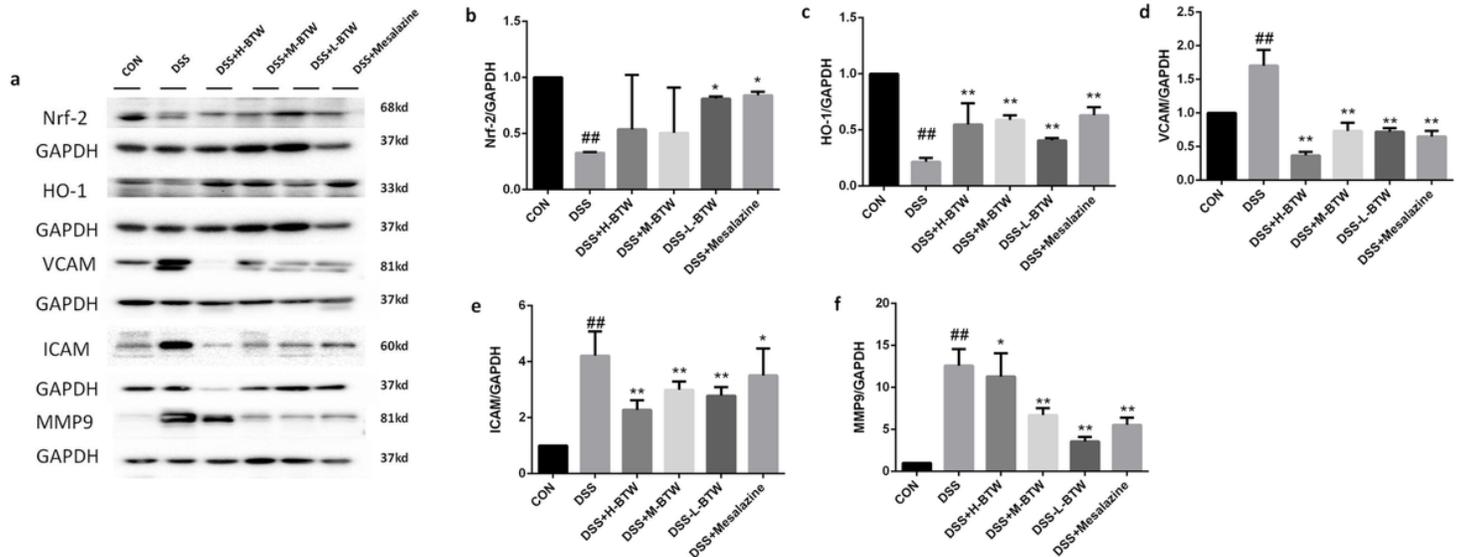
**Figure 5**

BTW modulated intracellular and extracellular HMGB1 inflammatory cytokines. (a) The inflammatory cytokine HMGB1 was determined using ELISA kits. (b) HMGB1 was detected through immunohistochemical staining by using HMGB1 antibody. (c, d) HMGB1 proteins were measured through western blotting, and these protein folds were calculated using Image-ProPlus6.0 software. Mean values were significantly different from those of the DSS-treated group; n=6/group.\*p < 0.05, \*\*p < 0.01. Mean values were significantly different from those of the control group; ##p < 0.01.



## Figure 6

Antioxidation effect of BTW in mice with DSS-induced UC. (a) MPO activity and (b) MDA and SOD levels in the colon tissues of all groups were examined. Mean values were significantly different from those of the DSS-treated group;  $n=3/\text{group}$ . \* $p < 0.05$ , \*\* $p < 0.01$ . Mean values were significantly different compared with those of the control group; ## $p < 0.01$



## Figure 7

BTW ameliorated DSS-induced colitis through Nrf2/HO-1 pathway activation. Western blotting of Nrf2, HO-1, VCAM-1, ICAM-1, and MMP-9 in colonic tissues. Mean values were significantly different from those of the DSS-treated group;  $n=3/\text{group}$ . \* $p < 0.05$ , \*\* $p < 0.01$ . Mean values were significantly different from those of the control group; ## $p < 0.01$

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xlsx](#)
- [Table2a.xlsx](#)
- [Table2b.xlsx](#)
- [Table3.xlsx](#)