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Research article

Therapeutic effect and mechanism of Ento-PB on ulcerative colitis in BALB/c mice induced by sodium dextran sulfate

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ABSTRACT

The traditional Chinese medicine (TCM) formula Ento-PB containing *Periplaneta americana* (Linnaeus) (Blattidae) and *Taraxacum mongolicum* Hand.-Mazz. (Compositae) has great potential for treating inflammation. Thus, this study aimed to explore the pharmacodynamic effect of Ento-PB on DSS-induced ulcerative colitis in BALB/c mice, and its effects on immune function, JAK2/STAT3-related signaling pathways and intestinal flora in UC mice. It was identified that the extract Ento-PB mainly contained 20 compounds, accounting for 78.50 % of the total peak area. Compared with the model group, each dose group of Ento-PB could reduce the DAI score, colon index, CMDI score and colon HS score of mice to varying degrees ($P < 0.05$ or $P < 0.01$). Ento-PB can reduce the content of IL-1β, TNF-α, IFN-γ in serum and IL-7 and IL-17 in colonic tissue, and increase IL-2, IL-10 in serum and EGF in colonic mucosa, TGF-β1 expression level ($P < 0.05$ or $P < 0.01$). In conclusion, Ento-PB has a good therapeutic effect on DSS-induced UC mice. Its mechanism of action may be to up-regulate the levels of IL-2, IL-10, EGF, IL-22 and TGF-β1, and down-regulate the levels of TNF-α,IFN-γ, IL-7 and IL-17 in UC mice. This provides sufficient experimental basis for the clinical treatment of UC with Ento-PB.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are both types of inflammatory bowel disease (IBD), which is a prevalent chronic non-specific intestinal disease. These conditions primarily affect the colon, rectal mucosa, and submucosa, and have a widespread and progressive distribution. The clinical manifestations are abdominal pain, diarrhea, tenesmus, mucus and bloody stools, etc., and may be accompanied by serious complications [1]. UC has the characteristics of high acute fulminant mortality rate, high chronic persistent cancer rate, and easy recurrence. It has been defined as a precancerous disease and is listed as a refractory disease in the world by the World Health Organization [2].

The incidence of UC is increasing year by year due to significant changes in people's lifestyle, eating habits, and environment. This represents a reflection of the overall societal shift. According to data from various Chinese hospitals, the prevalence rate in China is 11.6/100,000, while in Western countries, the incidence rate ranges from 10/100,000 to 20/100,000, with a prevalence rate of 100/100,000 to 200/100,000. UC typically manifests between the ages of 15 and 40, with a possible second peak occurring between 50 and

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80 years old. The incidence does not show a significant difference between men and women, and there is a tendency for the condition to run in families [3–5]. The etiology of UC is complex, and its pathogenesis is still unclear. It is generally believed that its pathogenesis is closely related to factors such as genetics, environment, infection, and immunity [6–9]. At present, the western medicines used clinically to treat UC are mainly divided into 8 categories, including 5-aminosalicylic acid, adrenal cortex hormones, and immunosuppressants [10]. Although there are many types of clinical treatment drugs, there are no specific treatment options and drugs, and long-term use of non-steroidal anti-inflammatory drugs and hormone drugs will cause many adverse reactions. Therefore, it is particularly important to find specific methods and effective drugs for the treatment of this disease.

Chinese medicine is the treasure of the Chinese nation and the crystallization of the wisdom of Chinese medical practitioners. Traditional Chinese medicine treatment methods are mostly oral Chinese medicine, supplemented by enema decoction; and Chinese medicine can be taken for a long time, with less side effects, and Chinese medicine is used to prevent and treat UC. The total clinical effective rate is 92.1 %, which is significantly higher than that of chemical drugs [11]. Therefore, traditional Chinese medicine treatment of UC has important clinical application value. Ento-PB (Extract of *Periplaneta americana* and *Taraxacum mongolicum* Hand.-Mazz.) is an extract prepared from a traditional Chinese medicine compound prepared by the National and Local Joint Engineering Research Center for the Development of Medicinal Special Insects in Yunnan Province, China, with *Periplaneta americana* as the main drug [12]. Ento-PB is an extract derived from a traditional Chinese medicine compound, with the American cockroach as the main ingredient. It was developed by the National Local Joint Engineering Research Center for Medicinal Special Insects in Yunnan Province, China. The use of the American cockroach in medicinal practices can be traced back to "Shennong's Classic of Materia Medica". Folk medicine in Yunnan Province has long recognized its diuretic, detoxifying, and anti-inflammatory properties. Research has confirmed that the extract of the American cockroach is safe and possesses strong tissue repair acceleration, antibacterial, anti-gastric ulcer, anti-inflammatory and anti-edema effects. It also enhances immune function. Previous experiments conducted by our research team have demonstrated that the extract effectively alleviates symptoms such as diarrhea and bloody stools in UC rats, improves inflammation cell infiltration in colonic tissues, and shows promising results in relieving UC symptoms [13–19]. Among them, the mechanism of DSS-induced UC model may be related to the negative charge of DSS affecting the DNA synthesis of colonic epithelial cells, destroying the intestinal mucosal barrier function, and finally causing the intestinal flora imbalance and immune function disorder [20–25]. This model is simple and easy to implement, has a high success rate and good reproducibility, and is similar to human UC lesions. It is an ideal human UC model and can be used for the study of acute and remission stages of UC and carcinogenesis of colitis. Therefore, this study chooses this model to investigate the efficacy of Ento-PB, and provides a certain theoretical basis for its application in clinical treatment.

In this study, DSS was used to induce acute ulcerative colitis in BALB/c mice, and the UC mice were treated with Ento-PB by intragastric administration. To observe changes in the general condition of mice, body weight changes, DAI scores, organ indexes, colon CMDI scores, colon pathology and biochemical indicators. This study aims to explore the effect of Ento-PB on UC mice and the mechanism of Ento-PB on DSS-induced UC in BALB/c mice, aiming to provide sufficient experimental basis for the development of Ento-PB as a new anti-UC drug.

2. Materials and methods

2.1. Experimental animals

240 healthy SPF grade BALB/c male mice, 6–8 weeks old, body weight 18–22g, provided by Hunan Slack Jingda Experimental Animal Co., Ltd., the license number of the experimental unit: SCXK (Xiang) 2016-0002. They were raised in the barrier system of the Animal Experiment Center of Dali University, with a temperature of 20–26 °C, a humidity of 40–70 %, a pressure difference of 25–50 Pa, and alternating light and dark for 12 h. The feed and corn cob litter for mice were purchased from Chengdu Dashuo Experimental Animal Co., Ltd., batch number: 20170601. All experimental studies complied with the guidelines of the Chinese Ethics Committee on animal research.

2.2. Experimental drugs and Reagents

Dextran sulfate sodium salt, Shenzhen Regent, batch number: SLBP0889V. Olsalazine sodium, Tianjin Lisheng Pharmaceutical Co., Ltd., batch number: 1608005. Ento-PB, provided by the national and local joint engineering research center for the development of medicinal special insects, batch number: 20170623.

2.3. Laboratory apparatus

Medical Image Analysis System (BI-2000; Chengdu Taimeng Technology Co., Ltd.); Flow Cytometry (FACS Calibur; American BD Company). Biological tissue embedding machine, BM-VIII, Xiaogan Hongye Medical Instrument Co., Ltd.

2.4. Preparation of Guinea pig serum

Blood was collected from three guinea pigs and centrifuged at 3000 rpm for 10 min to separate the serum. The serum was stored in EP tubes and kept in a refrigerator at −20 °C. When used, it was diluted 1:10 with normal saline.

2.5. Establishment of UC model

Male healthy BALB/c mice were adaptively fed for one week. Except for the normal group, which was given autoclaved ultrapure water, the remaining groups were provided with a 3 % (w/v) DSS aqueous solution for the mice to drink freely for a duration of seven days. After the modeling was completed, they were fed with autoclaved ultrapure water for 10 days, a total of 17 days [19].

2.6. Group abbreviation

Simplify the complex English in the corresponding group and replace it with easy-to-understand letters or numbers (Table 1).

2.7. LC-MS analysis of Ento-PB

2.7.1. Sample handling

To prepare the sample, 20 mg of Ento-PB was added to a centrifuge tube. Then, 500 μL of extract solution (methanol:water, volume ratio 3:1) that was pre-cooled at −40 °C and contained the internal standard was added. The mixture was vortexed for 30 s, homogenized at 35 Hz for 4 min, and sonicated in an ice-water bath for 5 min. Next, it was centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected and filtered through a 0.22 μm microporous membrane before being tested on the machine.

2.7.2. Parameter setting of Sciex Qtrap 6500 mass spectrometry instrument

In this study, we utilized a SCIEX 6500 QTRAP + triple quadrupole mass spectrometer equipped with an IonDrive Turbo V ESI ion source to perform mass spectrometry analysis in multiple reaction monitoring (MRM) mode. The ion spray voltage was set at +5500/-4500 V, the curtain gas pressure was maintained at 35 psi, the temperature was maintained at 400 °C, and the ion source gas pressures were set at 1:60 psi and 2:60 psi respectively. Additionally, the DP (declustering potential) was adjusted to ±100 V.

2.7.3. Processing of data

All mass spectrometry data acquisition and quantitative analysis of target compounds were performed using SCIEX Analyst Work Station Software (Version 1.6.3). The original mass spectrum was converted to TXT format using Msconventer software. Peak extraction and annotation were then carried out using a self-written R program package combined with a self-built database. Cluster analysis of the measured components was performed using SPSS 25.0 software.

2.8. Effects of Ento-PB on inflammatory cytokines in UC mice

2.8.1. Grouping and administration

The acute UC model in mice was constructed as described in section 2.5. After modeling on the 7th day, all mice were randomly divided into 8 groups according to the severity of inflammation (DAI score), 10 in each group, respectively normal group, model group, western medicine Olsalazine group (600 mg/kg), Chinese patent medicine Xianglianzhixiepian group (1210 mg/kg), Ento-PB four dosage groups (T400, T200, T100, T50). Each treatment group was intragastrically administered the corresponding drugs at 0.1 mL/kg/d, and the model group and the normal group were intragastrically administered with normal saline 0.1 mL/kg/d for 7 consecutive days, once a day.

2.8.2. Methods of observation of general indicators

Observation of the state of the mouse: The experiment involved observing and recording changes in body weight, drinking water and diet, and feces properties of mice in each group.

Disease activity index (DAI) score: Referring to Hamamoto's standard, mice were weighed, fecal properties and fecal occult blood were observed [26]. The scores of weight loss, fecal properties, and fecal occult blood were summed to calculate the disease activity index of each mouse to evaluate the disease activity (Table 2 and Appendix 1.1).

Colonmucosa damage index (CMDI) score: The removed colon was spread flat on white paper, and the colonic mucosal injury was visually observed according to the standards of Ekström GM et al. and Luk et al. (Table 3) [27,28].

Histopathological score (HS) score: The longitudinally dissected colon was cut in half, wrapped and rolled up, put in 10 % neutral formalin solution for fixed storage, stained with HE, and observed pathological sections under an optical microscope. Referring

Table 1
Abbreviated list of groups.

normal group	N
Model group	M
Olsalazine group	OL
XiangLianZhiXiePian group	XL
Ento-PB-1(400 mg/kg) group	PB-1 or T400
Ento-PB-2(200 mg/kg) group	PB-2 or T200
Ento-PB-3(100 mg/kg) group	PB-3 or T100
Ento-PB-4(50 mg/kg) group	PB-4 or T50

Table 2
Evaluation of disease activity index (DIA).

Score of DIA	Stool consistency	Occult blood test	Weight lossa (%)
0	Normal	Negative(−)	<1
1	Normal-Sparse stool	Weak positive(+)	1–5
2	Sparse stool	positive(++)	5–10
3	Sparse stool'Diarrhea	Strong positive(+++)	10–15
4	Diarrhea	Bloody stool	≧15

Table 3
CMDI scoring criteria.

Score	The mucous membrane of the colon
0	No damage
1	Mild hyperemia and edema, smooth surface, no erosion or ulcer
2	Hyperemia and edema, rough mucous membrane, granular sensation, erosion or intestinal adhesion.
3	Severe hyperemia and edema, necrosis and ulcer formation on the surface, thickening of intestinal wall or necrosis and inflammatory polyps on the surface.
4	Severe hyperemia and edema, mucosal necrosis and ulcer formation, whole intestinal wall necrosis, death caused by toxic megacolon.

to the criteria of Ram and Waxman (Table 4 and Appendix 1.2) [29]. Histopathological scores were diagnosed by attending physician Yan Changbao of the People's Hospital of Dali Bai Autonomous Prefecture in Yunnan, China, and reviewed by chief physician Li-ping Dai.

2.8.3. Detection of biochemical factors

Mice were taken from eyeballs and left to stand at 4 °C for 4 h, then centrifuged at 3000 rpm (4 °C) for 10 min, and finally the supernatant was taken. The other half of the colon that was longitudinally dissected was taken to make a 10 % homogenate in an ice bath, centrifuged at 3000 rpm (4 °C) for 10 min, and finally the supernatant was taken. According to the manual, ELISA method was used to measure the content of related biochemical factors in serum and colonic mucosa, and ELISA Calc was used to calculate the data.

2.8.4. Statistical method

Statistical description and statistical analysis of data were performed using SPSS 21.0 and GraphPad Prism 5.0. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), and *t*-test and one-way analysis of variance were used for data that conformed to normal distribution and homogeneous variance, and data that did not conform to normal distribution were used rank sum test. Repeated measures analysis of variance and one-way analysis of variance were used for continuous data, and LSD test was used for pairwise comparison between groups, and $P < 0.05$ was used as the standard for statistically significant differences.

3. Results

3.1. LC-MS analysis of Ento-PB

The obtained mass spectrum data of each component were searched by computer to the standard spectral library. The relative percentage content of each component was calculated by the peak area normalization method. The top 20 compounds with the highest content were identified from Ento-PB, and their components accounted for 78.50 % of the total peak area. The chromatographic peak positions and corresponding chemical structural formulas of the main components are shown in Figures (Figs. 1 and 2).

Table 4
Scoring criteria of histopathology.

Score	Epithelial cell	Degree of inflammatory cell infiltration
0	Normal form	No infiltration.
1	Loss of a small number of goblet cells	Infiltrate into the basal layer of the crypt
2	Massive loss of goblet cells	Infiltrate into the muscular layer of the mucosa
3	Loss of a small number of crypt cells	The infiltration penetrated into the muscular layer of the mucosa, accompanied by mucosal thickening and obvious edema.
4	Massive loss of crypt cells	Infiltrate into the submucosa

Note: Colonic HS score = "epithelial cell" score + "inflammatory cell infiltration" score.

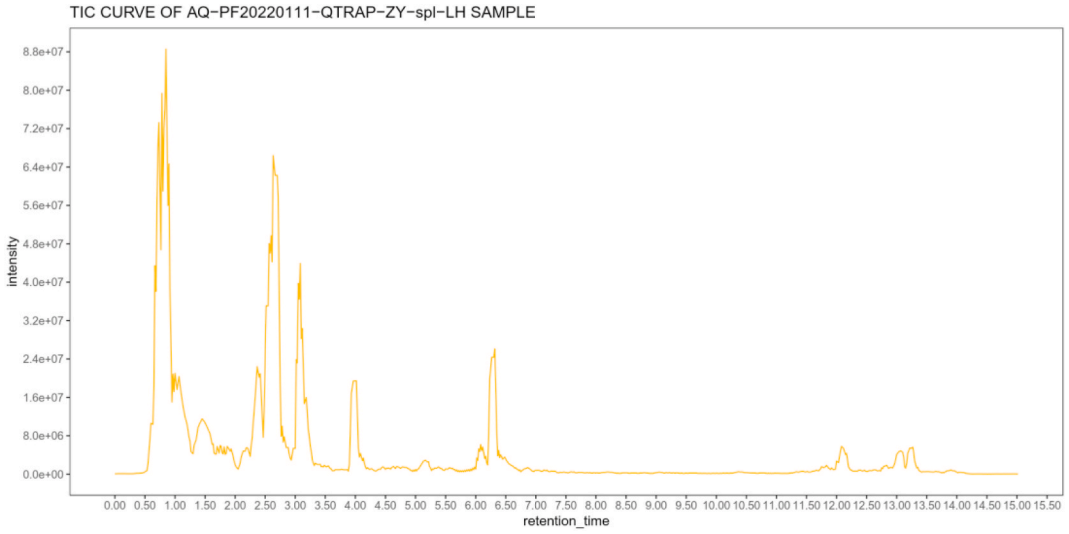

Fig. 1. Total ion chromatogram of sample of Ento-PB.

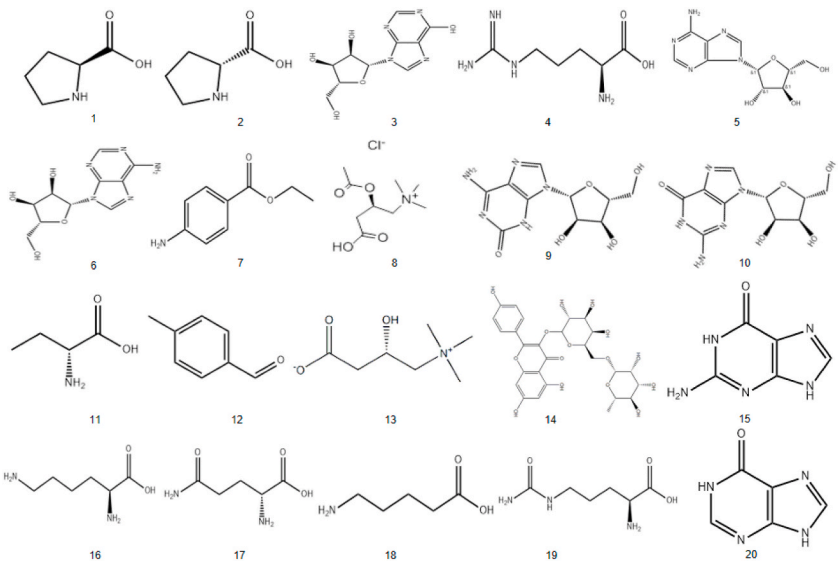

Fig. 2. Structural formulas of the top 20 compounds in Ento-PB.

Table 5
Effect of Ento-PB on the DAI score of UC mice ($n = 10, \bar{x} \pm s$).

Group	Dose (mg/kg)	Administration for 1 day	Administration for 4 days	Administration for 7 days
N	–	1.0 ± 0.0	1.2 ± 0.8	1.2 ± 0.4
M	–	9.5 ± 1.4**	9.2 ± 1.0**	8.7 ± 0.9**
OL	600	9.9 ± 1.3**	6.0 ± 1.5**△△	4.5 ± 1.1**△△
XL	1210	9.4 ± 1.2**	7.5 ± 1.3**△△▲▲	5.9 ± 0.9**△△
T400	400	9.3 ± 1.3**	7.0 ± 1.2**△△▲	5.2 ± 2.0**△△
T200	200	9.4 ± 1.1**	7.4 ± 1.0**△△▲▲	6.3 ± 0.8**△△▲
T100	100	9.6 ± 1.4**	7.2 ± 0.9**△△▲	6.1 ± 1.1**△△
T50	50	9.3 ± 1.3**	7.6 ± 1.2**△△▲▲	6.4 ± 1.1**△△▲

Note: Compared with N group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with M group, $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$. Compared with OL group, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$. Compared with the XL group, $^{\triangledown}P < 0.05$, $^{\triangledown\triangledown}P < 0.01$.

3.2. Effect of Ento-PB on inflammatory cytokines in UC mice

3.2.1. Changes in general signs

The mice in the normal group had smooth hair, white color, quick movements, flexible responses, normal diet and water intake, and normal urine and stool. The mice in the model group were emaciated, with dry and dull hair, emaciated body, and reduced diet. After treatment with Olsalazine and Xianglianzhixiepians, the activity of the mice was enhanced, the body weight recovered quickly, the frequency of defecation decreased, and the feces gradually formed. After treatment with various doses of Ento-PB, the activity and food intake of the mice increased, the symptoms of diarrhea were improved, the luster of the fur was restored, the mental state was good, and the feces gradually formed.

3.2.2. Impact of DAI score

Compared with the normal group, the DAI scores of the model mice were significantly increased on the first day after administration ($P < 0.01$). On the 4th and 7th day of drug treatment, the DAI scores of mice in each drug group were significantly lower than those in the model group ($P < 0.01$). The DAI scores of the mice in the T400 group were similar to those in the Olsalazine group (Table 5).

3.2.3. Effect of Ento-PB on CMDI and colon length in UC mice

Compared with the normal group, the colon length of the model group was significantly shortened, and the colon CMDI score was significantly increased ($P < 0.01$). The colons of mice all grew significantly after administration. Except for the T50 group, the CMDI scores of the mice in the other treatment groups were significantly reduced ($P < 0.01$) (Figs. 3 and 4).

3.2.4. Effect of Ento-PB on organ index of UC mice

Compared to the normal group, the lung index and liver index in the model group exhibited a tendency to increase, although the difference was not statistically significant ($P > 0.05$). On the other hand, the spleen index showed a significant increase ($P < 0.05$). Additionally, the colon index demonstrated a significant increase ($P < 0.01$). Compared with the model group, the colonic index of the Olsalazine group and the Ento-PB four dose groups were significantly reduced ($P < 0.01$) (Table 6).

3.2.5. Effect of Ento-PB on colonic HS score of UC mice

The colonic HS score of the model group was significantly higher than that of the normal group ($P < 0.01$). The colonic HS scores in the Olsalazine group, T400, and T50 groups were all significantly lower compared to the model group ($P < 0.05$). After HE staining of the mouse colon tissue, loss of goblet cells and crypt cells in the colon of the model group mice was observed under an optical microscope, along with a large number of inflammatory cells infiltrating into the muscularis mucosa. Each treatment group showed varying degrees of repair in lost goblet cells and crypt cells, as well as a reduction in the infiltration of inflammatory cell (Fig. 5 amd Table 7).

3.2.6. Effect of Ento-PB on TNF-α, IL-1β, IL-2, IL-10, IFN-γ levels in serum of UC mice

Compared with the normal group, the contents of TNF-α, IL-1β and IFN-γ in the serum of the mice in the model group were significantly increased ($P < 0.01$), and the contents of IL-2 and IL-10 in the serum of the mice in the model group were significantly decreased ($P < 0.01$ or $P < 0.05$). After administration, the contents of TNF-α, IL-1β, and IFN-γ in the serum of mice in each administration group decreased to varying degrees, and the contents of IL-2 and IL-10 increased to varying degrees. Among them, the contents of IL-1β and IFN-γ in the serum of mice in each dose group of Ento-PB were significantly lower than those in the model group ($P < 0.01$ or $P < 0.05$). Except for the T50 group, the TNF-α content in the mouse serum of each dose group of Ento-PB was significantly lower than that of the model group ($P < 0.01$). The content of IL-10 in serum of mice in T200 group and T50 group was significantly higher than that in model group ($P < 0.01$ or $P < 0.05$) (Fig. 6).

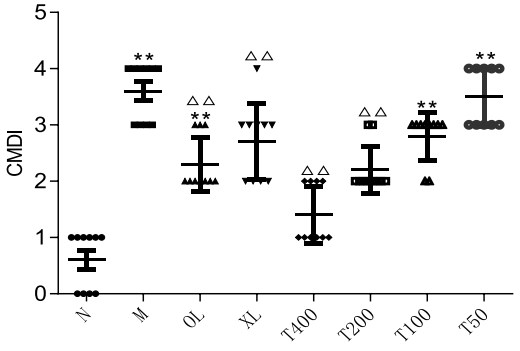

Fig. 3. Effects of Ento-PB on colonic index in UC mice Compared with the normal group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with the model group, $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$. Compared with XiangLianZhiXiePian, $^{\triangledown}P < 0.05$, $^{\triangledown\triangledown}P < 0.01$.

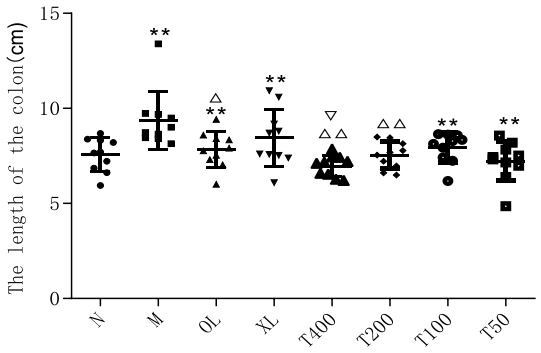

Fig. 4. Effect of Ento-PB on colonic length in UC mice Compared with the normal group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with the model group, $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$. Compared with XiangLianZhiXiePian, $^{\triangledown}P < 0.05$, $^{\triangledown\triangledown}P < 0.01$.

Table 6
Effect of Ento-PB on the organ index of UC mice (unit: mg/g, $n = 10, \overline{x} \pm s$).

Group	Lung index	Liver index	Spleen index	Thymus index	Colon index
N	5.56 ± 0.92	47.64 ± 2.91	3.37 ± 0.70	1.19 ± 0.58	7.56 ± 0.89
M	6.22 ± 0.87	50.52 ± 6.88	6.04 ± 1.61*	1.14 ± 0.60	9.37 ± 1.52**
OL	5.91 ± 1.69	46.65 ± 5.06	3.88 ± 0.58	1.18 ± 0.51	7.83 ± 0.95$^{\triangle\triangle}$
XL	6.58 ± 1.32*	49.70 ± 7.47	5.72 ± 1.64*	1.45 ± 0.48	8.44 ± 1.51
T400	5.85 ± 0.38	49.95 ± 4.08	4.73 ± 0.82*	1.55 ± 0.50	7.00 ± 0.55$^{\triangle\triangle\triangledown}$
T200	5.86 ± 0.87	49.19 ± 4.10	8.88 ± 13.69	3.67 ± 5.94*$^{\triangle\blacktriangle\triangledown}$	7.54 ± 0.71$^{\triangle\triangle}$
T100	5.76 ± 0.38	48.88 ± 5.18	5.38 ± 1.90	1.62 ± 0.58	7.94 ± 0.78$^{\triangle\triangle}$
T50	5.82 ± 1.15	47.68 ± 3.62	4.71 ± 0.84*	1.32 ± 0.45	7.22 ± 1.03$^{\triangle\triangle\triangledown}$

Note: Compared with N group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with M group,$^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$. Compared with OL group, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$. Compared with the XL group, $^{\triangledown}P < 0.05$, $^{\triangledown\triangledown}P < 0.01$.

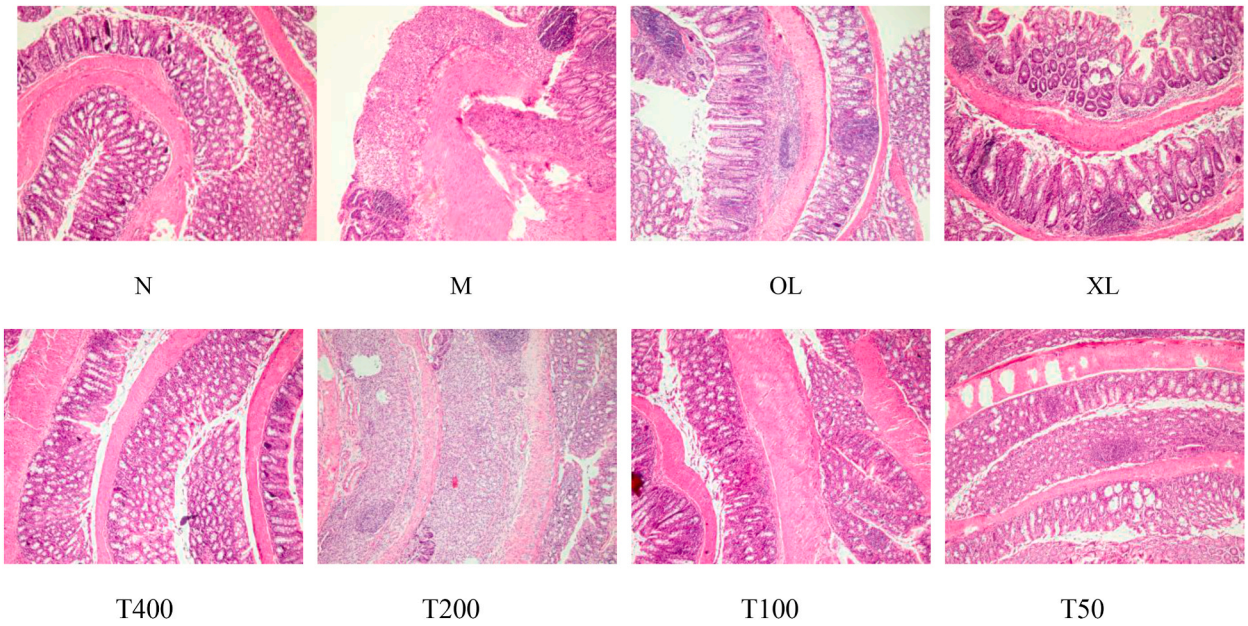

Fig. 5. Effects of Ento-PB on colonic pathological tissue of UC mice.

3.2.7. Effect of Ento-PB on IL-7, IL-17, IL-22, EGF, TGF content in colon homogenate of UC mice

Compared with the normal group, the contents of IL-17 and IL-7 in the colon tissue of mice in the model group were significantly increased ($P < 0.01$), and the contents of IL-22, TGF-β1 and EGF were significantly decreased ($P < 0.01$). Compared with the model group, the IL-7 content in the mouse colon of the Olsalazine group, the Xianglianzhixiepian group, the T400 group, and the T100 group

Table 7
Effect of Ento-PB on the colonic HS score of UC mice.

Group	Quantity	Colonic HS score					
		Scoring of epithelial cells		Score of inflammatory cell infiltration		Total score	
		M	QR	M	QR	M	QR
N	8	1.0	0.0	1.0	0.0	2.0	0.0
M	8	3.0**	0.0	3.0**	0.0	6.0**	0.0
OL	8	1.5△△	1.0	1.5△△	1.0	3.0△△	2.0
XL	8	2.0	2.0	2.0	2.0	4.0	4.0
T400	8	1.0△△	0.0	1.0△	1.5	2.0△	1.5
T200	8	1.5	2.0	1.5	2.0	3.0	4.0
T100	8	2.0*	1.8	2.0*	1.8	4.0*	3.5
T50	8	1.5△△	1.0	1.5△△	1.0	3.0△△	2.0

Note: Compared with N group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with M group, $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$.

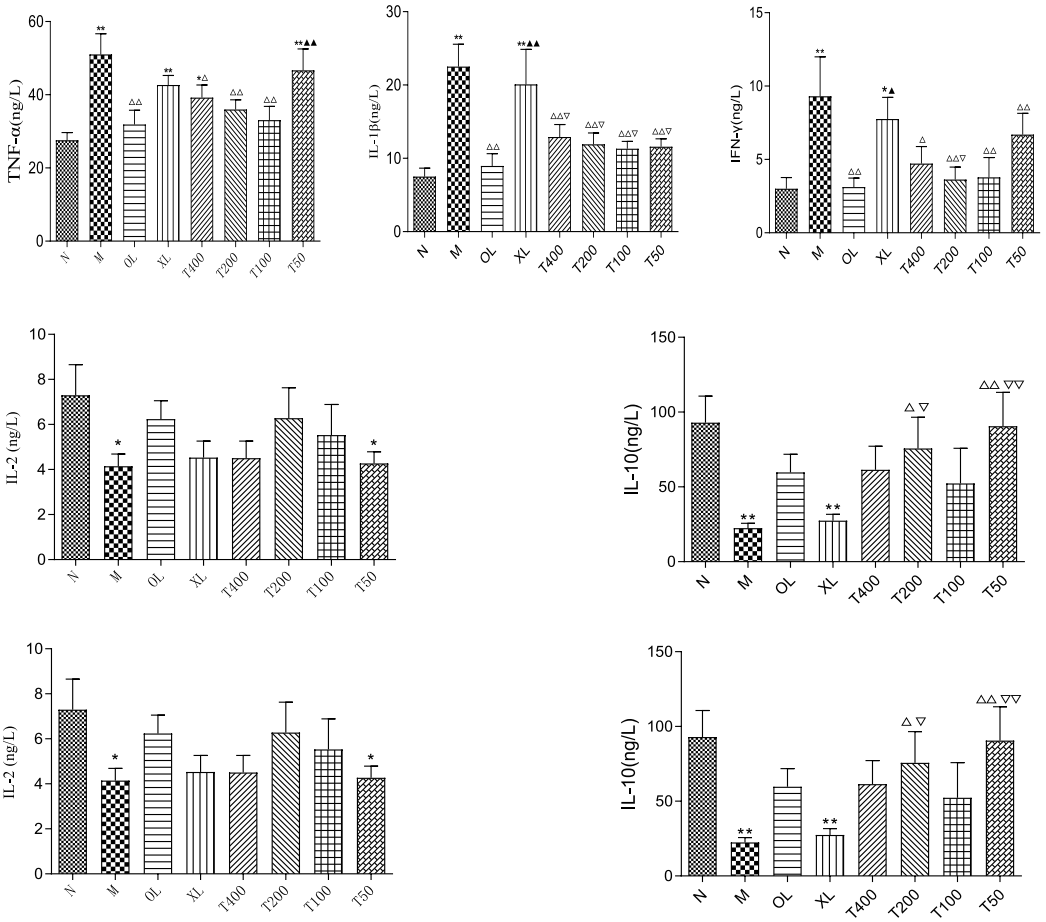

Fig. 6. Effects of Ento-PB on cytokines in serum of UC mice Compared with the normal group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with the model group, $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$. Compared with Osalazine, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$. Compared with XiangLianZhiXiePian, $^{\triangledown}P < 0.05$, $^{\triangledown\triangledown}P < 0.01$.

was significantly lower than that of the model group ($P < 0.01$ or ($P < 0.05$). The content of IL-17 in the colon of mice in the Olsalazine group, T400 group, T200 group, and T100 group was significantly lower than that of the model group ($P < 0.01$ or ($P < 0.05$), and the content of TGF-β1 in the colon of mice in the Olsalazine group was significantly increased ($P < 0.01$), the EGF content in colon tissue of mice in Osalazine group, Xianglianzhixiepian group, T400 group, T100 group was significantly increased ($P < 0.01$ or ($P < 0.05$) (Fig. 7).

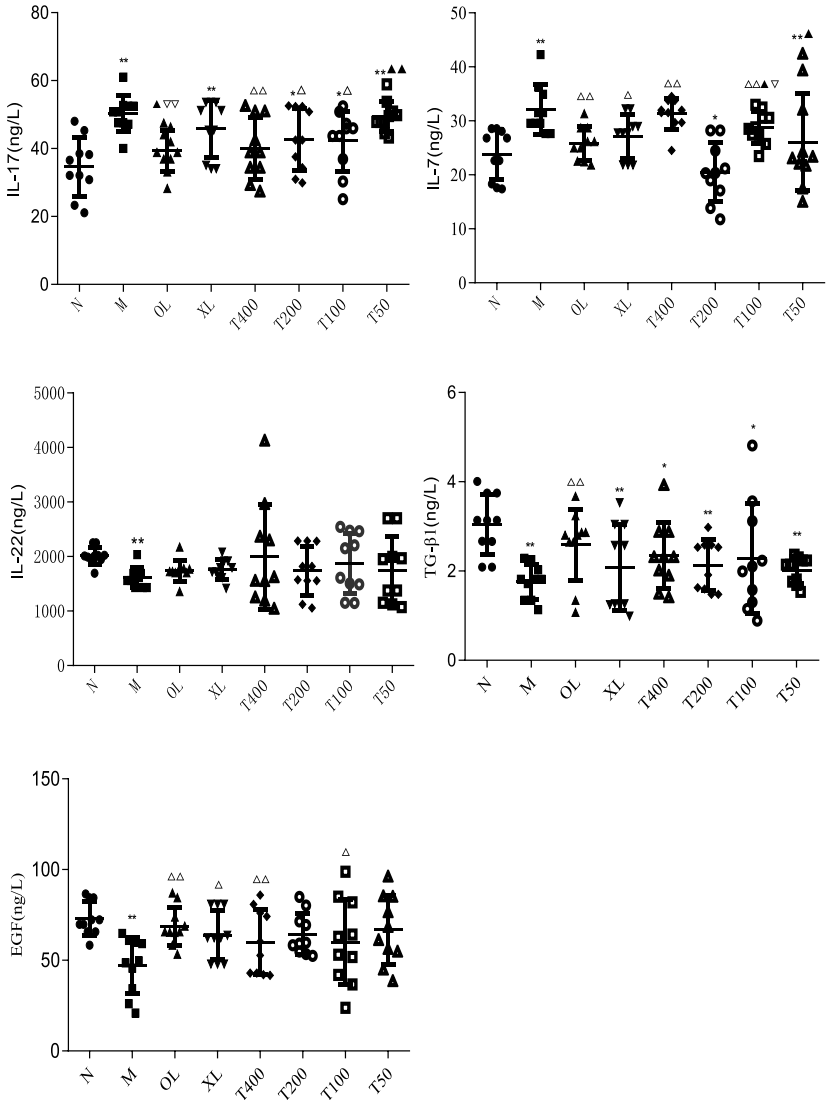

Fig. 7. Effects of Ento-PB on the contents of IL-7, IL-17, IL-22, EGF and TGF-β1 in colonic homogenate of UC mice Compared with the normal group, $^{*}P < 0.05$, $^{**}P < 0.01$. Compared with the model group, $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$. Compared with Osalazine, $^{\blacktriangle}P < 0.05$, $^{\blacktriangle\blacktriangle}P < 0.01$. Compared with XiangLianZhiXiePian, $^{\triangledown}P < 0.05$, $^{\triangledown\triangledown}P < 0.01$.

4. Discussion

The method of letting mice drink DSS freely was used to construct the UC mouse model in this experiment. After the modeling, the mice had bloody stools, weight loss and other symptoms. The colonic mucosa exhibited visible ulcers and erosions, along with a substantial loss of crypts and infiltration of inflammatory cells into the submucosa. Following drug treatment, the CMDI score and colon HS score showed significant reduction in all groups of mice, indicating that the drug can effectively ameliorate the pathological alterations in the colon and promote the healing of colon lesions.

Preliminary studies had shown that the colon length of rats with UC induced by TNBS was significantly shorter than that of the normal group, and the colonic edema was severe, the intestinal wall became thicker, and irregular ulcers formed, and the symptoms were relieved and the ulcers healed after drug treatment [30]. In a study conducted by Draberova (2020) using a mouse model of experimental colitis in the chronic phase, it was observed that the length of the colon in the model group was significantly reduced. However, following drug intervention, there was an increase in the length of the colon [31]. The results of this study revealed that the colon of the model animals exhibited shortened, congested, and swollen characteristics, along with thickened intestinal walls. In comparison to the normal group, the model group displayed a significantly reduced colon length ($P < 0.01$) and a higher CMDI score. Following administration, the length of the colon in each dose group of Ento-PB showed varying degrees of improvement ($P < 0.01$), leading to a significant decrease in the CMDI score ($P < 0.01$ or $P < 0.05$) when compared to the model group. Each dose group of

Ento-PB could reduce the colonic index to varying degrees. It was suggested that Ento-PB might relieve intestinal injury and promote the repair of intestinal mucosa.

Previous studies have demonstrated that IL-17 possesses a notable capability to induce neutrophil activation, T cell activation, and stimulation of macrophages, fibroblasts, and epithelial cells, resulting in the production of diverse inflammatory factors. Ultimately, this cascade leads to inflammation and tissue damage [32]. Previous studies have shown that tumor necrosis factor TNF-α plays an important role in regulating immune and inflammatory responses, and is recognized as a cytokine that mediates UC [33–35]. A large number of reports have shown that TNF-α in the serum of active UC is elevated. Komatsu et al. measured the concentration of TNF-α in the serum of UC patients, and the serum concentration of TNF-α in active UC patients was 380 times that of normal people [36]. It could be seen that studying the change of the expression level of TNF-α was a hotspot in the mechanism of action of inflammatory bowel disease. Normal human Th1/Th2 cytokines were in a balanced state, and IFN-γ was mainly secreted by Th1 cells, which could promote the proliferation and differentiation of Th1 cells and inhibit the increase of Th2 cells. Th1-type factors are shifted, manifesting as the acute injury pattern [37]. Based on the experimental results, the levels of serum TNF-α, IFN-γ, and IL-17 were found to be increased to varying degrees in the model group compared to the normal group ($P < 0.05$ or $P < 0.01$). However, after administering Ento-PB through intragastric administration, the contents of TNF-α, IFN-γ, and IL-17 in the serum of mice in each drug group were reduced to varying degrees compared to those in the model group ($P < 0.05$ or $P < 0.01$). Previous studies have shown that epidermal growth factor (EGF) can promote the expression of differentiation genes and induce cell growth and migration. By increasing the synthesis of intracellular proteins, it stimulates the growth and repair of tissues, which is of great significance to the restoration and integrity of the intestinal mucosal barrier function [38,39]. TGF-β1 is a pleiotropic cytokine with a strong anti-inflammatory effect. Its main function is to inhibit inflammatory response and cell proliferation, regulate cell growth, differentiation and enhance immunity [40]. The experimental results showed that, compared with the model group, each dose group of Ento-PB could increase the content of TGF-β1 and EGF to varying degrees, and the content of TGF-β1 in the T400 group was significantly increased ($P < 0.01$). The imbalance of IL-1β and IL-1 receptor is an important cause of UC. Evenikoa et al. found that IL-1β mainly acts locally in UC patients.In the past, the content of IL-1β in the intestinal fluid of UC was significantly increased, and it synergized with antigens to activate CD4+T cells, express IL-2R, promote the growth and activation of B cells, and promote the presentation of antigens such as monocytes and macrophages. The expression of antigens on cells attracts the release of inflammatory mediators [41]. The experimental results showed that compared with the model group, each dose group of Ento-PB could reduce the IL-1β content to varying degrees ($P < 0.01$ or $P < 0.05$). IL-2 is a lymphokine with various biological activities, which is mainly synthesized and secreted by helper T lymphocytes stimulated by antigens or mitogens and induced by IL-1. As an important cytokine, it can activate and proliferate T cells after binding to IL-2 receptors on the surface of T cells, B cells, and monocytes, promote the killing effect of cytotoxic T cells, and enhance the activity of NK cells. It promotes cellular immune responses such as B cells secreting Ig, so it plays an important role in immune regulation [42]. Compared with the normal group, the content in the model group decreased significantly ($P < 0.01$ or $P < 0.05$), and compared with the model group, the drug-administered group had a tendency to increase but there was no statistical difference. IL-22 is a member of the IL-10 cytokine family, and IL-10 is an anti-inflammatory cytokine produced by helper T cell subsets Th1 and Th2, B cell nuclei, monocytes, macrophages, and keratinocytes. Its main function is to inhibit the release of inflammatory factors and inflammatory response, regulate the proliferation and differentiation of various immune cells, and is currently recognized as an anti-inflammatory factor. The experimental results showed that IL-22 and IL-10 in the model group were significantly lower than those in the normal group, and the levels increased after drug intervention ($P < 0.01$ or $P < 0.05$).

5. Conclusion

Each dose group of Ento-PB has a certain therapeutic effect on DSS-induced ulcerative colitis in mice. Its mechanism of action may be to up-regulate the levels of IL-2, IL-10, EGF, IL-22 and TGF-β1, and down-regulate the levels of TNF-α,IFN-γ, IL-7 and IL-17 in UC mice.

Additional information

No additional information is available for this paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Xueping Cui: Writing – review & editing. **Chunmei Wu:** Writing – original draft. **Yusheng Xu:** Writing – original draft. **Chunchu Zou:** Methodology, Formal analysis. **Xiayun Jiang:** Writing – original draft, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34539.

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