

Dynamic Evaluation of Compound Ento-PB for the Repair of Mucosal Ulcer in Dogs With Acetic Acid-induced Experimental Colitis

Jin-hu Chen

Dali University

Jian-ting Zhao

Dali University

Zheng-yong Yu

Dali University

Yi-hao Che

Dali University

Yu-jia Wang

Dali University

Qian Lu

Dali University

Fu-neng Geng

key lab of periplaneta americana in sichuan province

Heng Liu (✉ lheng125@dali.edu.cn)

Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D, Dali, Yunnan, 671000, China. <https://orcid.org/0000-0003-3937-4985>

Cheng-gui Zhang

Dali University

Xiu-mei Wu

Dali University

Yu Zhao

Dali University

Miao He

Dali University

Research

Keywords: ulcerative colitis, acetic acid, cross-bred dogs, colonoscopy, mucosal healing, Anti-inflammatory

Posted Date: December 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-127468/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Mucosal inflammation and ulcer play important roles in the pathogenesis of ulcerative colitis. As a traditional Chinese medicine compound composed of *Periplaneta americana* and *Taraxacum mongolicum*, Ento-PB is always prescribed for the treatment of ulcer and inflammatory diseases. As for the significant role of *P. americana* in terms of promoting mucosal healing, the compatibility of the anti-inflammatory drug *T. mongolicum* may enable Ento-PB to simultaneously play anti-inflammatory and promote mucosal healing effects on the treatment of UC. Therefore, this study aimed to evaluate the therapeutic potential and possible mechanism of Ento-PB for UC by establishing an acetic acid-induced colitis model in dogs.

Methods: Preliminary identification to the chemical components of compound Ento-PB was carried out through high performance liquid chromatography. A cross-bred dogs model of acetic acid-induced ulcerative colitis was established to evaluate the efficacy of compound Ento-PB. The expression levels of inflammatory cytokines C-reactive protein (CRP), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-10 (IL-10) in plasma were measured by carrying out enzyme-linked immunosorbent assay (ELISA).

Results: With the extension of treatment time, Ento-PB could effectively improve clinical symptoms of UC cross-bred dogs. Colonoscopy displayed that mucosal redness, swelling and congestion decreased gradually, and obviously repaired after mucosal injury. The intestinal texture was gradually clear, and the colonoscopy score gradually reduced. Histopathological examination revealed that the structure of colon was restored significantly, the infiltration of inflammatory cells was reduced, and the histological score was remarkably reduced. At the same time, the results of dynamic monitoring of inflammatory cytokines in plasma proved that Ento-PB can gradually down-regulate the activity of CRP, iNOS and COX-2, reduce the expression levels of inflammatory cytokines TNF- α and IL-1 β , and gradually restore anti-inflammatory and the expression level of cytokine IL-10.

Conclusions: Ento-PB reduces the level of pro-inflammatory cytokines in a dose- and time-dependent manner and inflammation, improves colon tissue lesions and the repair of intestinal mucosa after injury, and effectively increases acetic acid-induced colon inflammation in UC cross-bred dogs.

Background

Characterized by intestinal mucosal damage, recurrence and remission of alternating mucosal inflammation, ulcerative colitis is an intestinal disease [1,2]. In addition to typical intestinal manifestations such as abdominal pain, diarrhea, mucus, pus, and bloody stool, it can also accompany with multiorgan and multisystem extraintestinal manifestations, thus seriously affecting the life and work of patients [3]. So far, the pathogenesis of UC is still unclear, but studies exhibited that multiple factors such as overproduction of inflammatory mediators that include reactive oxygen mediators, proinflammatory cytokines, arachidonate metabolites, and neutrophil infiltration were implicated in the

pathogenesis of colitis [4]. The damage to the intestinal mucosa causes microorganisms enter the lamina propria from lumen, which triggers an inflammatory response, thus leading to the overproduction of inflammatory cytokines, including TNF- α , IL-1 β , and COX-2. These cytokines further exacerbate the damage of intestinal mucosa [2]. At present, UC clinical first-line treatment drugs such as 5-ASA and steroid hormones can relieve clinical symptoms by inhibiting the production of pro-inflammatory cytokines and nitric oxide. With the availability of multiple treatment options in efficacy and safety profiles, there are considerable practices variability in the application of these drugs in the treatment of outpatients and inpatients with moderate-severe UC [5]. Variations in practices may have unintended negative consequences in patient outcomes. The disadvantages of traditional anti-inflammatory drugs such as 5-ASA in colonic mucosal healing become increasingly prominent. As a result, the treatment goal of UC changed from clinical symptom relief to endoscopic remission, namely endoscopic mucosal healing [6]. However, so far, none of the approved IBD drugs directly target this process. In fact, some drugs may also inhibit the repair of intestinal mucosal damage [7].

Previous clinical and experimental studies proved that adjuvant Traditional Chinese Medicine (TCM) treatments such as herbal medicine and acupuncture are beneficial to the relief of UC symptoms, with reliable efficacy, few side effects, and low recurrence rate [8]. Among them, the compound Ento-PB composed of *Periplaneta Americana* L. and *Taraxacum mongolicum* Hand.-Mazz. is a common prescription for the treatment of ulcer and inflammatory diseases in Yunnan folk. Modern pharmacological studies confirmed that *P. Americana* extract has significant advantages in wound repair and ulcer healing. As a raw material, Kangfuxin Liquid can improve the immune barrier of the gastrointestinal mucosa of patients and relieve inflammation in clinical treatment of gastrointestinal diseases in China, increase the gastric mucosa hexose and prostaglandin E2 levels, and promote the repair of gastrointestinal mucosa [9,10]. Li et al. found that *P. Americana* extract can improve colitis in rats with acetic acid-induced ulcerative colitis by reducing inflammation and enhancing the activity of fibroblasts [11]. *T. mongolicum* is the key component of Ento-PB and can exert its anti-inflammatory effect. *T. mongolicum* is commonly applied alone or combined with other Chinese herbal medicine to treat gastrointestinal inflammation in Chinese clinics [12]. It was discovered that dandelion root extract can protect NCM460 colonic cells and alleviate experimental colitis by blocking NF- κ B signal transduction, inhibiting inflammation, and reducing oxidative stress [13]. The active ingredient, namely chicory acid, can regulate the inflammatory response of intestinal epithelial cells that are induced by LPS through regulating the inflammatory signal of NF- κ B p65 and inflammatory factors such as COX-2 and IL-1 β [14]. We speculated that Ento-PB may have anti-inflammatory and promote mucosal healing effects on the treatment of UC, and hope to prove it through experiments, because *P. Americana* and *T. mongolicum* has significant efficacy respectively in the clinical treatment of colitis in China. With reference to the clinical diagnosis and treatment requirements for UC mucosal healing, conventional small animals, including mice, rats and rabbits, cannot meet the requirements of colonoscopy. Therefore, this study selected the canine colitis model, evaluated the repairing effect of compound Ento-PB on the intestinal mucosal injury of colitis dogs through dynamic observation of colonoscopy, and

comprehensively evaluated its efficacy based on the dynamic changes of serum inflammatory factors and histopathological examination.

Materials And Methods

Medicinal materials

Dandelion was purchased from the Dali Traditional Chinese Medicine Market in Yunnan (Yunnan,China), batch number 170306, and was identified as *Taraxacum mongolicum* Hand.-Mazz. by assistant research fellow Miao He, majoring in pharmacognosy at Dali University. Dried adult cockroach was purchased from Good Doctor Pharmaceutical Co.,Ltd d. (Sichuan, China), batch number YF1807014, and was identified as *Periplaneta americana* (L.) by Professor Zizhong Yang, majoring in zoology at Dali University. The research samples are sealed and stored in Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D.

Preparation of Ento-PB

According to the Yunnan folk Yi nationality prescription, take 70 g of *P. americana* and 30 g of *T. mongolicum*, add 10 times the amount of pure water, boil and extract twice, 2 hours each time, filter the extract with gauze. The twice filtrates were combined, placed in a rotary evaporator, and the filtrate was concentrated by rotating at 50 °C to a density of 1.15 g/mL. Add 95% ethanol to the concentrate, stir well, let it stand overnight, centrifuge at 3500 rpm/min for 10 min, take the supernatant and spin and concentrate to dryness at 50°C to obtain the compound Ento-PB extract, which was sealed and stored at -20 °C until required.

HPLC Analysis of Ento-PB

High performance liquid chromatography (HPLC) was used to further characterize the potentially biologically active components in Ento-PB. Firstly, the mixed standard solution containing uracil 1.0 mg/mL, hypoxanthine 1.0 mg/mL, uridine 1.0 mg/mL, adenosine 1.0 mg/mL, inosine 1.0 mg/mL, caftaric acid 1.0 mg/mL, caffeic acid 1.0 mg/mL, cichoric acid 1.0 mg/mL was diluted with pure water into a standard mixed solution with concentration of 0.1 mg/mL. Accurately weigh 200.0 mg of Ento-PB sample, dissolve in an appropriate amount of ultrapure water, sonicate for 15 minutes, add ultrapure water to make Ento-PB sample solution with a final concentration of 20.0 mg/mL. 0.5 mL of the standard mixture or Ento-PB sample solution is filtered through a 0.45 µm microporous membrane for chromatographic analysis. HPLC analysis was performed on Agilent 1260 machine (Agilent technologies, USA), equipped with autosampler and UV DAD detector. Samples were separated using an Agilent Reversed-phase C18 alkyl silica gel column (4.6×250 mm, 5 µm) (Agilent technologies, USA) with a pre-connected column in-line filter at 25 °C. Mobile phase A was 0.1% trifluoroacetic acid-water solution, while mobile phase B was methanol. The gradient elution condition was set as follows: 0-10 min (0-0% B); 10-12 min (0-8% B); 12-19 min (8-8% B); 19-24 min (8-10% B); 24-28 min (10-35% B); 28-38 min (35-35% B);

38-60 min (35-95% B). Ento-PB was analyzed with flow rate at 0.5 mL/min, injection volume of 10 μ L and UV detection wavelength at 254 nm.

Animal

Sixteen male small cross-bred dogs aged 1-2 years old were provided by the Experimental Animal Center of Dali University, weighing about 8-12kg. All dogs were raised separately in the IVC observation room of the Experimental Animal Center of Dali University (temperature 22 ± 2 °C, relative humidity $55 \pm 5\%$, light / dark cycle 12 minutes 12 hours). In the course of the experiment, they were allowed to eat and drink freely and were allowed to adapt to the environment for two weeks. All animal operations, including anesthesia, surgery, postoperative care and sacrifice, have been approved by the Committee on Animal Care and use of Dali University of China ((IACUC)). The ethical qualification number is 2017-0741.

Establishment of acetic acid-induced UC cross-bred dogs model

Before 16 male cross-bred dogs were used for induction of UC by 10% acetic acid, colonoscopy was evaluated by electronic endoscope. For this purpose, animals were kept fasting for 1 day and bowel preparation was performed on the day of induction in cross-bred dogs. All cross-bred dogs underwent enemas that consisted of 10 %, acetic acid (210 mg/kg, in distilled water) administered with a No.15 feeding tube to induce UC. Dynamic examination of colonoscopy and histopathological examination of colon to observe the repair of intestinal mucosa after injury.

Grouping and treatment

After 24 hours of acetic acid induction, colonoscopy was performed on all cross-bred dogs, and the degree of inflammatory damage was scored. According to the colonoscopy score, the cross-bred dogs were randomly divided into 4 groups: model group, Jiechangning group (178 mg/kg), Ento-PB low-dose group (Ento-PB-L, 35.6 mg/kg), Ento-PB high-dose group (Ento-PB-H, 71.2 mg/kg), 4 animals/group. Blood samples were collected from veins and then the drugs were administered by enema, once a day for 10 consecutive days.

Colonoscopy

As shown in the experimental design flow chart in Fig. 1a, dogs were subjected to colonoscopy within a certain period of time to dynamically evaluate the repair effect of Ento-PB on intestinal mucosa injury. The specific scoring standards refer to the Baron endoscopy scoring and Mayo colonoscopy scoring standards commonly used in clinical UC patients [15, 16], with certain modifications (Table 1).

Detection of inflammatory cytokines in plasma

As shown in the flow chart of the experimental design in Fig. 1a, during the specific time of the experiment, the venous blood samples were collected and placed in the EP tube containing anticoagulants, acentrifuged at 4°C, 3500 rpm/min for 10 minutes, and plasma was taken. According to

the instructions of the manufacturer, the ELISA kits were used to detect the levels of IL-1 β , IL-10, COX-2, CRP, TNF- α and INOS in dog plasma.

Histopathology

24 hours after the last administration, the dogs were anesthetized with serazine hydrochloride injection (0.02 mL/kg) and the colon segment was removed. The surrounding mesenteric adipose tissue was removed, the colon cavity was longitudinally opened, and the contents were slowly washed with ice normal saline. samples were provided from mucosa in 10 cm proximal to the anal verge and moved to 4% paraformaldehyde (4% PFA) for histological studies. After fixing the colon tissue (4% PFA, 12-24 h), it was dehydrated by an automatic dehydrator and embedded in paraffin. The coronal section of colon (5 μ m) was prepared. The sections were subsequently stained with hematoxylin and eosin (H&E). According to the criteria of Mehrabani et al [16, 17]. (Table 2), a professional pathologist who is not clear about the grouping of this study scored the pathological changes of the canine colon.

Statistical analysis

The results of inflammatory cytokines were statistically analyzed by the difference before and after modeling (difference = measured value at each time point after modeling - measured value before modeling). The experimental data is expressed as the mean \pm standard deviation, and SPSS 24.0 software was used for statistical analysis. The inflammatory factor difference analysis and colonoscopy score are performed by repeated measures analysis of variance. The histopathology score is performed by one-way ANOVA. The comparison between groups was performed by Dunnett t test. $P < 0.05$ was considered statistically significant.

Results

Identification of chemical constituents by HPLC in Ento-PB

The HPLC chromatogram of Ento-PB and mixed standard compounds at 254 nm is shown in additional file 1: Fig. s1. By comparing with the HPLC chromatograms of the mixed standard, eight compounds were preliminarily identified from Ento-PB: uracil, hypoxanthine, uridine, adenosine, inosine, caftaric acid, caffeic acid and chicoric acid from A to H, respectively. This is consistent with the results reported in the literatures [18, 19].

Colonoscopy score of dog colitis was improved by Ento-PB

As shown in fig. 1b, before acetic acid induction, the colon surface of each group was smooth, the vascular structure was clear, and there were no pathological manifestations such as hyperemia, edema, ulcer and so on. On the first day of administration, colonoscopy showed that the mucosal surface was rough, blood vessel structure disappeared, edema, redness, bleeding, and ulcers were obvious. On the 4th day of administration, colonoscopy showed large areas of ulcers, mucosal congestion and redness in the model group; small areas of ulcers and mucosal congestion and redness were found in the

administration group. On the 7th day of administration, colonoscopy showed obvious congestion, edema, roughness, a few spontaneous bleeding points and small area ulcers in the mucosa of the model group; swelling, roughness, and small area ulcers in the mucosa of the Jiechangning group and the low-dose Ento-PB group; Ento-PB high-dose group mucosal redness and swelling subsided significantly and the surface was smoother. 24 hours after the last administration, colonoscopy showed that the mucosal surface of the model group was still rough, small areas of ulcers and redness; the Jiechangning group and the low-dose Ento-PB group had mucosal redness and swelling, the hyperemia subsided significantly, and the surface was smoother; In the Ento-PB high-dose group, the mucosal surface was smooth, the structure of part of the intestine was restored, a small area was hyperemia, and no obvious ulcers were observed.

Repeated measurement analysis of variance was performed on colonoscopy scores of dogs with acetic acid-induced experimental colitis at each time point. Therefore, the Greenhouse-Geisser method is used to correct the degrees of freedom and analyze the results. The results showed that there was statistical significance between different time points ($F=305.130$, $P=0.000<0.01$), and there was an interaction effect between time and grouping ($F=4.435$, $P=0.001<0.01$); the difference between groups was statistically significant ($F=7.209$, $P=0.005<0.01$). As shown in fig. 1c, compared with the model group, after controlling for the influence of different measurement points, the colonoscopy scores of the Jiechangning group and the Ento-PB low and high dose groups were significantly reduced ($P<0.05$ or $P<0.01$).

The results of colon anatomy (Fig. 1d) showed that the model group's colon was obviously widened and thickened, with different widths, and the mucosa showed roughness, redness and swelling, scattered bleeding spots, small area ulcer, and disordered structure; In Jiechangning group and Ento-PB low-dose group, a small amount of scattered bleeding spots and punctate ulcers were found, and the colon was widened; the colon of the Ento-PB high-dose group had clear structure, uniform thickness and width, and no obvious bleeding spots and ulcers.

Ento-PB treatment alleviated the histopathological symptoms of acetic acid-induced colitis in dogs.

As shown in fig. 2a, the model group showed epithelial necrosis and shedding of mucosal tissue, large-scale ulcer formation, enlarged glands surrounding the ulcer, proliferation of glandular epithelial cells, disappearance of goblet cells and crypt cells in the mucosal layer, and obvious inflammatory cell infiltration in the submucosa. And there is extensive edema; In Jiechangning group, the structure of mucosal layer was disordered, some goblet cells and crypt cells were lost, small area ulcers and epithelial hyperplasia could be observed, and there were obvious inflammatory cell infiltration and tissue edema in submucosa; In Ento-PB high dose group, goblet cells and crypt cells were partially lost, but the basic structure of colon was still found. there was a small amount of ulcer surface, and epithelial hyperplasia could be seen on the ulcer surface. In addition, a small number of inflammatory cells infiltrated into the submucosa, and the submucosal edema was obviously alleviated; In the low dose Ento-PB group, a small amount of mucosal epithelial cells fell off, forming a small area ulcer. Besides, partial loss of goblet cells

and crypt cells, obvious inflammatory cell infiltration and tissue edema could be observed in the submucosa. It could be found from fig. 3b-h that compared with the model group, ulcers, mucosal atrophy, inflammatory cell infiltration, and total HS scores were significantly decreased after treatment with Jiechangning and Ento-PB ($P<0.05$ or $P<0.01$). In dog colon tissue, the ulcer was significantly improved, mucosal atrophy and submucosal edema were effectively relieved, and the degree of inflammatory cell infiltration was significantly reduced.

Ento-PB treatment reduced the contents of CRP, COX-2 and iNOS in plasma of dogs with acetic acid-induced colitis

Repeated measurement analysis of variance was performed on the difference between the level of CRP in plasma at each time point after modeling and the level of CRP in plasma before modeling. The results of Mauchly sphericity test showed that the data conformed to the sphere hypothesis ($P=0.244>0.05$). The analysis of the results showed that the difference between different time points was statistically significant ($F=121.174$, $P=0.000<0.01$), there was an interaction effect between time and grouping ($F=6.052$, $P=0.000<0.01$), and the difference between groups was statistically significant ($F=4.084$, $P=0.033<0.05$). As shown in fig. 3a, compared with the model group, after controlling for the effects of different measurement points, the levels of CRP in the Ento-PB low and high dose groups were significantly reduced ($P<0.05$), and the levels of CRP in the Jiechangning group had a decreasing trend, but there was no statistical significance ($P>0.05$).

Repeated measurement analysis of variance was performed on the difference between the content of COX-2 in plasma at each time point after modeling and the level of COX-2 in plasma before modeling. The Mauchly sphericity test results showed that the data did not conform to the sphericity hypothesis ($P=0.006<0.05$), so the Greenhouse-Geisser method is used to correct the degrees of freedom and analyze the results. The analysis of the results showed that the difference between different time points was statistically significant ($F=132.147$, $P=0.000<0.01$), there was an interaction effect between time and grouping ($F=3.977$, $P=0.007<0.01$), and the difference between groups was statistically significant ($F=5.064$, $P=0.017<0.05$). As shown in fig. 3b, compared with the model group, after controlling for the influence of different measurement points, the content of COX-2 in the Jiechangning group and the Ento-PB high-dose group was significantly reduced ($P<0.05$ or $P<0.01$), the content of COX-2 in the Ento-PB low-dose group had a decreasing trend, but it was not statistically significant ($P>0.05$).

Repeated measurement analysis of variance was performed on the difference between the level of iNOS in plasma at each time point after modeling and the level of iNOS in plasma before modeling. The Mauchly sphericity test results showed that the data did not conform to the sphericity hypothesis ($P=0.001<0.05$), so the Greenhouse-Geisser method is used to correct the degrees of freedom and analyze the results. The analysis of the results showed that the difference between different time points was statistically significant ($F=44.892$, $P=0.000<0.01$). There was no interaction effect between time and grouping ($F=0.196$, $P=0.973>0.05$), and there was no statistical difference between groups ($F=0.052$, $P=0.983>0.05$); However, it can be found from fig. 3c, compared with the model group, the Jiechangning

group and Ento-PB low and high doses group could reduce the level of iNOS in plasma of colitis dogs in different degrees. Although the inhibitory effect mediated by Ento-PB is not statistically significant, it at least shows the trend of suppression.

Ento-PB regulated the expression of inflammatory cytokines in plasma of dogs with acetic acid-induced colitis

Repeated measurement analysis of variance was performed on the difference between the level of TNF- α in plasma at each time point after modeling and the level of TNF- α in plasma before modeling. The Mauchly sphericity test results showed that the data did not conform to the sphericity hypothesis ($P=0.000<0.05$), so the Greenhouse-Geisser method is used to correct the degrees of freedom and analyze the results. The analysis of the results showed that the difference between different time points was statistically significant ($F=47.960$, $P=0.000<0.01$), there was an interaction effect between time and grouping ($F=0.589$, $P=0.005<0.01$), and the difference between groups was statistically significant ($F=6.026$, $P=0.010<0.05$). As shown in fig. 4a, compared with the model group, after controlling the influence of different measurement points, the level of TNF- α in the high dose group of Ento-PB decreased significantly ($P<0.01$), while the level of TNF- α in the Jiechangning group and the low dose group of Ento-PB decreased, but there was no statistical significance ($P>0.05$).

Repeated measurement analysis of variance was performed on the difference between the level of IL-1 β in plasma at each time point after modeling and the level of IL-1 β in plasma before modeling. The results of Mauchly sphericity test showed that the data conformed to the sphere hypothesis ($P=0.238>0.05$). The analysis of the results showed that the difference between different time points was statistically significant ($F=86.332$, $P=0.000<0.01$), there was an interaction effect between time and grouping ($F=3.668$, $P=0.001<0.01$), and the difference between groups was statistically significant ($F=43.653$, $P=0.044<0.05$). As shown in fig. 4b, compared with the model group, after controlling the influence of different measurement points, the level of IL-1 β in the high dose group of Ento-PB decreased significantly ($P<0.05$), while the level of IL-1 β in the Jiechangning group and the low dose group of Ento-PB decreased, but there was no statistical significance ($P>0.05$).

Repeated measurement analysis of variance was performed on the difference between the level of IL-10 in plasma at each time point after modeling and the level of IL-10 in plasma before modeling. The Mauchly sphericity test results showed that the data did not conform to the sphericity hypothesis ($P=0.022<0.05$), so the Greenhouse-Geisser method is used to correct the degrees of freedom and analyze the results. The analysis of the results showed that the difference between different time points was statistically significant ($F=235.087$, $P=0.000<0.01$) there was an interaction effect between time and grouping ($F=8.623$, $P=0.000<0.01$), and the difference between groups was statistically significant ($F=4.012$, $P=0.010<0.05$). As shown in fig. 4c, compared with the model group, after controlling the influence of different measurement points, the level of IL-10 in the high dose group of Ento-PB increased significantly, while the level of IL-10 in the Jiechangning group and the low dose group of Ento-PB increased, but there was no statistical significance ($P>0.05$).

Discussion

P. americana in Ento-PB can invigorate spleen, dispel blood stasis, and promote the subsidence of swelling, wound healing sore muscle and repair. *T. mongolicum* can clear away heat dry dampness, purge intense heat and dsintoxicate. The two drugs are applied together to regulate viscera, replenish qi to invigorate spleen, promote granulation and the removal of necrotic tissues, and eliminate inflammation and edema. Jiechangning (enema) is a Chinese herbal compound and approved by CFDA for the clinical treatment of ulcerative colitis [20]. The main components are Typhae and Polygonum syringae. It was proved that, combined with mesalazine, Jiechangning retention enema can effectively relieve UC symptoms in moderate active stage, improve intestinal mucosal permeability, and is superior to dexamethasone and mesalazine alone when promoting the proliferation of probiotics and restoring the diversity of intestinal flora [21]. Jiechangning could decrease the level of IFN - γ and increase the level of IL-10 in serum and colonic mucosa, regulate the balance of Th1/Th2, improve immunity, and improve DSS-induced acute colitis in rats [20]. Based on the reliability of Jiechangning in clinical and experimental colitis, we selected Jiechangning (enema) as the positive control drug.

Acetic acid-induced colitis can cause severe inflammation of colon and rapid formation of ulcer, and be accompanied by diarrhea, hematochezia, and weight loss, which is similar to the situation of acute ulcerative colon inflammation that caused by abnormal arachidonic acid metabolism in human colitis [22,23]. Mucosal ulcer is an early event in the occurrence mechanism of UC, and repair after mucosal injury is used as an important index to evaluate the efficacy of drugs [24]. Colonoscopy is currently an important method to clinically evaluate the healing of intestinal mucosal injury after the treatment of UC patients [25,26]. In this study, we referred to the clinically common Baron endoscopy score and Mayo enteroscopy score for the pathological manifestations of experimental colitis, focused on mucosal damage and intestinal hemorrhage, redness, and developed a colonoscopy score criterion for experimental colitis animal model, so as to evaluate the repairing effect of Ento-PB after intestinal mucosal injury in experimental colitis dogs. The results illustrated that after the treatment with Ento-PB, the color of the intestinal mucosa of dogs with colitis gradually changed from hyperemia and redness to lighter, the surface changed from granular to smooth, the ulcers significantly reduced or disappeared, and the colonoscopy score gradually decreased. By anatomy visible, the colon morphology was normal. Histopathological examination demonstrated that mucosal epithelial cells were intact, and inflammatory cell infiltration significantly reduced. In addition, CRP is a representative biomarker of acute inflammatory response, and has high sensitivity to human inflammatory response. Meanwhile, it is positively correlated with endoscopic activity of patients, and is frequently adopted to verify the extent of remission and mucosal healing of IBD [27,28]. Our results displayed that after the acetic acid induction, the level of CRP in plasma increased significantly, and Ento-PB could gradually decrease its expression level, which was consistent with the results of colonoscopy and histopathological score. All these suggested that Ento-PB could improve colonic mucosal ulcers in dogs, effectively relieve mucosal atrophy and submucosal edema, reduce the degree of inflammatory cell infiltration, and effectively promote the healing of intestinal mucosa.

The occurrence and development of UC are largely due to chronic inflammation, and massive infiltration of immune cells, and accompanied by increased production of pro-inflammatory mediators, including IL-1 β , TNF- α , and COX-2, and decreased production of anti-inflammatory factors such as IL-10 [29,30]. Many evidences indicated that cytokines TNF- α and IL-1 β play important roles in intestinal inflammation and barrier dysfunction [31]. Elevated levels of TNF- α and IL-1 β increase neutrophil infiltration, damage the intestinal barrier, and drive diarrhea symptoms of UC [2]. The mucosal biopsy of IBD patients and the acetic acid-induced rat colitis model also confirmed that the expression level of TNF- α was positively correlated with the degree of colitis [32]. TNF- α could trigger the phosphorylation of myosin light chain, destroy the intestinal barrier and tight junction proteins, and can up-regulate the expression of iNOS and COX-2, activate the NF- κ B pathway and other inflammatory signals, and initiate the cascade of inflammation reaction, thus resulting in sustained damage to the intestinal mucosa [2]. COX-2 catalyzes the release of many kinds of PGs (such as PGE₄) and leukotrienes by arachidonic acid [33]. These inflammatory mediators can cause redness, swelling, pain, edema and inflammatory cell infiltration of intestinal mucosa, which in turn affect intestinal transport, intestinal activity and immune regulation, thus aggravating the existing inflammation [34]. The high expression of a variety of pro-inflammatory cytokines stimulates the up-regulation of iNOS expression and leads to the synthesis of a large number of NO, thus further aggravating the injury of intestinal mucosa [35]. As an effective anti-inflammatory cytokine, IL-10 can protect the host from excessive inflammation and immune response by exerting the immunosuppressive characteristics of negative feedback regulation that is related to TNF- α and IL-1 β signal transduction [36,37]. Its level is strongly down-regulated in UC. Therefore, the development of colonic inflammation can be inhibited by regulating the balance of inflammatory cytokines. The results of dynamic monitoring of inflammatory cytokines in dogs with acetic acid-induced colitis suggested that Ento-PB treatment could gradually reduce the levels of pro-inflammatory cytokines COX-2, iNOS, TNF- α , and IL-1 β , and gradually increase the level of anti-inflammatory cytokines IL-10, indicating that Ento-PB could reduce the acetic acid-induced colon inflammation in UC dogs by reducing the level of pro-inflammatory cytokines and increasing the level of anti-inflammatory cytokines.

The chemical constituents of *P. americana* and *T. mongolicum* were reported in many pharmacological studies, and many potential anti-UC bioactive chemical components were described, such as polypeptides [38], and amino acids [11] in *P. americana*, and caffeic acid [39], chicory acid [14], dandelion polysaccharides [40] in *T. mongolicum*. In this study, we preliminarily determined the presence of uracil, hypoxanthine, uridine, adenosine, inosine, caftaric acid, caffeic acid and cichoric acid in Ento-PB by HPLC analysis. Lu et al. proposed that the adenosine extract of *P. americana* can accelerate the remodeling of epithelium after skin injury through macrophage-TGF- β 1-Smad signal, and the repair of skin injury [41]. Caftaric acid, caffeic acid and cichoric acid all have significant antioxidant, anti-inflammatory and anti-tumor activities, among which cichoric acid could also extremely inhibit the growth of colon cancer cells [42,43]. Thus, the combination of all these identified compounds could be directly or indirectly associated with the prevention of ulcerative colitis, and it could be argued that these components contribute, at least in part, to the confirmation of the anti-ulcerative colitis effect on Ento-PB.

Although current studies revealed that Ento-PB has significant intestinal mucosal repair and good anti-inflammatory levels in dogs with acetic acid-induced colitis, there are still certain limitations that need to be considered. We have determined some chemical components in Ento-PB by HPLC, but we cannot determine which components can provide main support for Ento-PB anti-UC. Therefore, the main pharmacodynamic material basis of Ento-PB and the mechanism of anti-UC need to be further explored, so as to provide strong evidence support for the better development of ethnic experience prescription Ento-PB.

Conclusion

In conclusion, this study demonstrated that Ento-PB could promote the healing of colon ulcer in a dose and time-dependent manner, reduce the inflammatory response that is caused by the infiltration of neutrophils and other immune cells, and prevent the development of acetic acid-induced canine colitis. At the same time, the significant repairing effect of Ento-PB on intestinal mucosal injury attracted our great attention. If being used with anti-inflammatory drugs together, it will be possible to make up the low healing rate of mucosal injury after anti-inflammatory drug treatment. Most importantly, this study also proved that the folk TCM compound Ento-PB has the potential to treat ulcerative colitis.

Abbreviations

HPLC: High Performance Liquid Chromatography; IL-1 β : Interleukin-1 β ; IL-10: Interleukin-10; COX-2: Cyclooxygenase-2; CRP: C-reactive protein; TNF- α : Tumor necrosis factor alpha; INOS: Inducible nitric oxide synthase.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Jinhu Chen: Article writing, picture finishing; Jianting Zhao, Zhengyong Yu, Qian Lu: Experimental work, data analysis; Yujia Wang, Yihao Che: Analysis of the chemical composition of the medicine, assisting in the supplement of the article content; Funeng Geng: Provide raw materials for medicine; Heng Liu, Chenggui Zhang: Project funding support, article structure design and revision; Miao He, Xiumei Wu, Yu Zhao: Project funding support.

Funding

This study is supported by several funds as the following: The National Natural Science Fund of China (NO.81660605, 81860742, 81860765, 82060780); Yunnan Fundamental Research

Projects (grant NO.2018FB143, 2019FH001-003, 202001AT070021); Innovation Team of Dali University for Advanced Pharmaceuticals of Entomological Bio-pharmacy R&D (NO.ZKLX2019101); Innovative Team of Dali University for Digital Development of Medicinal Insects and Arachnids Resources (NO.ZKLX2020303); Special Fund for Traditional Chinese Medicine Pieces of Department of Industry and Information Technology of Yunnan Province (NO.2019-YG-067).

Availability of data and materials

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

Ethics approval and consent to participate

All animal research procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of Dali University. This article does not contain any studies with human participants performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

References

- [1] Rasmussen B, Haastrup P, Wehberg S, et al. Predictors of health-related quality of life in patients with moderate to severely active ulcerative colitis receiving biological therapy. *Scand J Gastroenterol.* 2020(9): 1-8.
- [2] Zou QH, Zhang X, Liu XS, et al. Ficus carica polysaccharide attenuates DSS-induced ulcerative colitis in C57BL/6 mice. *Food Funct.* 2020; 11.
- [3] Lu MQ, Zhang T, Lu Z, et al. A comparison of the efficacy and safety of complementary and alternative therapies for ulcerative colitis: A protocol for systematic review and meta-analysis. *.Medicine (Baltimore).* 2020; 99(28): e21219.
- [4] Wang R, Luo YZ, Lu YD, et al. Maggot Extracts Alleviate Inflammation and Oxidative Stress in Acute Experimental Colitis via the Activation of Nrf2. *Oxid Med Cell Longev* 2019; 2019: 1-18.
- [5] Singh S, Allegretti JR, Siddique SM, et al. AGA Technical Review on the Management of Moderate to Severe Ulcerative Colitis. *Gastroenterology.* 2020; 158(5).

- [6] Shin J, Kong SM, Kim TJ, et al. Clinical Significance of Residual Nonrectal Inflammation in Ulcerative Colitis Patients in Clinical Remission. *Gut Liver*. 2020.
- [7] Shin J, Kong SM, Kim TJ, et al. Clinical Significance of Residual Nonrectal Inflammation in Ulcerative Colitis Patients in Clinical Remission. *entific Reports*. 2020.
- [8] Lin J, Wu J, Wang F, et al. QingBai decoction regulates intestinal permeability of dextran sulphate sodium-induced colitis through the modulation of notch and NF- κ B signalling. *Cell Prolif*. 2019; 52(2): e12547.
- [9] Zhang HW, Wei LY, Zhao G, et al. *Periplaneta americana* extract used in patients with systemic inflammatory response syndrome. *World J. Emerg. Med*. 2016; 7(1): 50-54.
- [10] Zhang HW, Wei LY, Zhang ZY, et al. Protective effect of *periplaneta americana* extract on intestinal mucosal barrier function in patients with sepsis. *J TRADIT CHIN MED*. 2013(001): 70-73.
- [11] Li N, Lu RZ, Yu YY, et al. Protective effect of *Periplaneta americana* extract in ulcerative colitis rats induced by dinitrochlorobenzene and acetic acid. *Pharm Biol* 2016; 54(11): 1-8.
- [12] Fang R, Wu R, Zuo Q, et al. *Sophora flavescens* Containing-QYJD Formula Activates Nrf2 Anti-Oxidant Response, Blocks Cellular Transformation and Protects Against DSS-Induced Colitis in Mouse Model. *Am. J. Chin. Med*. 2018: 1-15.
- [13] Ding A, Wen X. Dandelion root extract protects NCM460 colonic cells and relieves experimental mouse colitis. *J Nat Med*. 2018; 72(4): 857-866.
- [14] Xue YS, Zhang SM, Du M, et al. Dandelion extract suppresses reactive oxidative species and inflammasome in intestinal epithelial cells. *J. Funct. Foods*. 2017; 29: 10-18.
- [15] Li K, Marano C, Zhang H, et al. Relationship Between Combined Histologic and Endoscopic Endpoints and Efficacy of Ustekinumab Treatment in Patients With Ulcerative Colitis. *Gastroenterology*. 2020.
- [16] Chen L, Xu ZP, Zhang CG, et al. Establishment of Acute Ulcerative Colitis Model Induced by Acetic Acid in Dog. *China Anim. Husb. Vet. Med*. 2018; 45(05): 1395-1400.
- [17] Mehrabani D, Ziaei M, Hosseini S, et al. The effect of *calendula officinalis* in therapy of acetic Acid induced ulcerative colitis in dog as an animal model. *Iran Red Crescent Med J*. 2011; 13(12): 884-890.
- [18] Fen MD. The Research On Quality Standard Of *Periplaneta Americana*. 2017.
- [19] Liu A, Gou L, Xue Z, et al. Quality evaluation of *Taraxaci Herba* based on fingerprint analysis and quantitative analysis of multi-components. *China J. Chin. Mater. Med*. 2018; 43(18): 3715-3721.
- [20] Peng XQ, LI XY, Wang W, et al. Expression of Th1/Th2 inflammatory cytokines in rat treatment model of ulcerative colitis. *J Cent South Univ (Med Sci)*. 2013; 38(10): 1020-1028.

- [21] Wang ZJ. To Discuss the Influence of Jiechangning on Intestinal Barrier Function and Microecology of Ulcerative Colitis Patients. 2016.
- [22] Dijk A, Wilson J, Zijlstra F. The effect of malotilate, a derivative of malotilate and a flavenoid on eicosanoid production in inflammatory bowel disease in rats. *Mediators Inflamm.* 1993; 2(1): 67-72.
- [23] Oliveira N, Souza B, Moita L, et al. Proteins from *Plumeria pudica* latex exhibit protective effect in acetic acid induced colitis in mice by inhibition of pro-inflammatory mechanisms and oxidative stress. *Life Sci.* 2019; 231: 116535.
- [24] D'Amico F, Bonovas S, Danese S, et al. Review article: faecal calprotectin and histologic remission in ulcerative colitis. *Aliment Pharmacol Ther.* 2020; 51(7): 689-698.
- [25] Belvis Jiménez M, Hergueta-Delgado P, Gómez Rodríguez B, et al. Index of the Mayo Endoscopy and Ulcerative Colitis Endoscopy Index of Severity: are they equally valid? *Rev Esp Enferm Dig.* 2020.
- [26] Fukuda T, Naganuma M, Sugimoto S, et al. Efficacy of Therapeutic Intervention for Patients With an Ulcerative Colitis Mayo Endoscopic Score of 1. *Inflamm Bowel Dis.* 2019; 25(4): 782-788.
- [27] Okada K, Itoh H, Ikemoto M. Circulating S100A8/A9 is potentially a biomarker that could reflect the severity of experimental colitis in rats. *Heliyon.* 2020; 6(2): e03470.
- [28] Orfanoudaki E, Gazouli M, Foteinogiannopoulou K, et al. Infliximab trough levels are decreasing over time in patients with inflammatory bowel disease on maintenance treatment with infliximab. *Eur J Gastroenterol Hepatol.* 2019; 31(2): 187-191.
- [29] Li S, Takasu C, Lau H, et al. Dimethyl Fumarate Alleviates Dextran Sulfate Sodium-Induced Colitis, through the Activation of Nrf2-Mediated Antioxidant and Anti-inflammatory Pathways. *Antioxidants (Basel).* 2020; 9(4).
- [30] Abdallah H, Ammar N, Abdelhameed M, et al. *Acacia saligna* Protective Mechanism of Butanol Extract and Its Nano-Formulations against Ulcerative Colitis in Rats as Revealed via Biochemical and Metabolomic Assays. *Biology (Basel).* 2020; 9(8).
- [31] Yao DD, Dong M, Dai CL, et al. Inflammation and Inflammatory Cytokine Contribute to the Initiation and Development of Ulcerative Colitis and Its Associated Cancer. *Inflamm Bowel Dis.* 2019; 25(10): 1595-1602.
- [32] Rashidian A, Dejbani P, Karami Fard K, et al. Bupropion Ameliorates Acetic Acid-Induced Colitis in Rat: the Involvement of the TLR4/NF- κ B Signaling Pathway. *Inflammation.* 2020; 43(5): 1999-2009.
- [33] Yu L, Yan J, Sun Z. D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. *Mol Med Rep.* 2017; 15(4): 2339-2346.

- [34] Abdallah Hajj Hussein I, Freund J, Reimund J, et al. Enteropathogenic e.coli sustains iodoacetamide-induced ulcerative colitis-like colitis in rats: modulation of IL-1 β , IL-6, TNF- α , COX-2, and apoptosis. *J Biol Regul Homeost Agents*. 2012; 26(3): 515-526.
- [35] He ZQ, Zhou Q, Wen K, et al. Huangkui Lianchang Decoction Ameliorates DSS-Induced Ulcerative Colitis in Mice by Inhibiting the NF-kappaB Signaling Pathway. *Evid Based Complement Alternat Med*. 2019; 2019: 1040847.
- [36] El-Mahdy N, El-Sayad M, El-Kadem A, et al. Targeting IL-10, ZO-1 gene expression and IL-6/STAT-3 trans-signaling by a combination of atorvastatin and mesalazine to enhance anti-inflammatory effects and attenuates progression of oxazolone-induced colitis. *Fundam Clin Pharmacol*. 2020.
- [37] Amirshahrokhi K. Febuxostat attenuates ulcerative colitis by the inhibition of NF- κ B, proinflammatory cytokines, and oxidative stress in mice. *Int Immunopharmacol*. 2019; 76: 105884.
- [38] Chen JY, Li HW, Wu DX, et al. A Study of the Anti-inflammatory Effect and Mechanism of C β -3 Extracted from *Periplaneta americana*
J Dali Univ. 2015; 14(02): 8-11.
- [39] Zhang Z, Wu XY, Cao SY, et al. Caffeic acid ameliorates colitis in association with increased Akkermansia population in the gut microbiota of mice. *Oncotarget*. 2016; 7(22): 31790-31799.
- [40] Wang Q, Bie YL, Wang D, et al. Effects of Dandelion polysaccharide on IL-6/STAT3 signaling pathway in ulcerative colitis rats. *Chin J Appl Phys*. 2017; 33(5): 422-425.
- [41] Lu HQ. The role and mechanism of *Periplaneta Americana* adenosine extract in promoting wound healing. 2019.
- [42] Hussein OE, Hozayen WG, El-Tawab SA, et al. Chicoric acid prevents methotrexate hepatotoxicity via attenuation of oxidative stress and inflammation, and up-regulation of PPAR γ and Nrf2/HO-1 signaling. *Environ. Sci. Pollut. Res*. 2020; 27(8).
- [43] Widurski G, Jabońska-Trypu A, Kalinowska M, et al. Spectroscopic, Theoretical and Antioxidant Study of 3d-Transition Metals (Co(II), Ni(II), Cu(II), Zn(II)) Complexes with Cichoric Acid. *Materials*. 13(14).

Tables

Table 1 The variables used for microscopic scoring.

Variable	Severity of changes			
	0	1	2	3
Ulceration	No ulcer	Erosion or single ulceration not exceeding lamina muscularis mucosa	Multifocal ulcerations not exceeding the submucosa	Ulcerations exceeding the submucosa
Mucosal cell damage	Preserved mucus cell	Mild depletion in a few cells	Moderate depletion (<50% of cell)	Severe depletion or complete disappearance of mucosa
Inflammation	No Inflammatory cell	Increased mildly	Increased moderately	Increased Severely
Mucosal atrophy	Normal thickness	Mild atrophy (<10%)	Moderate atrophy (10–50%)	Severe atrophy (<50%)
Submucosal edema	Normal thickness	Mild edema (submucosal Expansion(<10%))	Moderate edema (submucosal expansion, 10–100%)	Severe edema (submucosal Expansion>100%)
Inflammatory cell infiltration	No inflammatory cell infiltration	Mild inflammatory cell infiltration	Moderate (distributed but not dense)	Dense inflammatory cell infiltration

Table 2 Criteria for scoring of macroscopic damage

Score	Macroscopic morphology
0	The mucosa is pale with normal vascular pattern and without mucosa hyperemia, edema
1	The mucosa is still smooth, but a small area of hyperemia, redness and the refractive index enhanced
2	The mucosa is hyperemia, edema, and granularity. The mucosa is friability with contact bleeding
3	The mucosa is obviously hyperemic, edema, rough, and a few spontaneous bleeding points or contact bleeding. There are more inflammatory secretions, multiple erosions and small ulcers.
4	The mucosa becomes extensively congested and rough mucus membrane with mucosal edema, marked and spontaneous bleeding or contact bleeding. Multiple punctate erosion and large area ulcer.

Figures

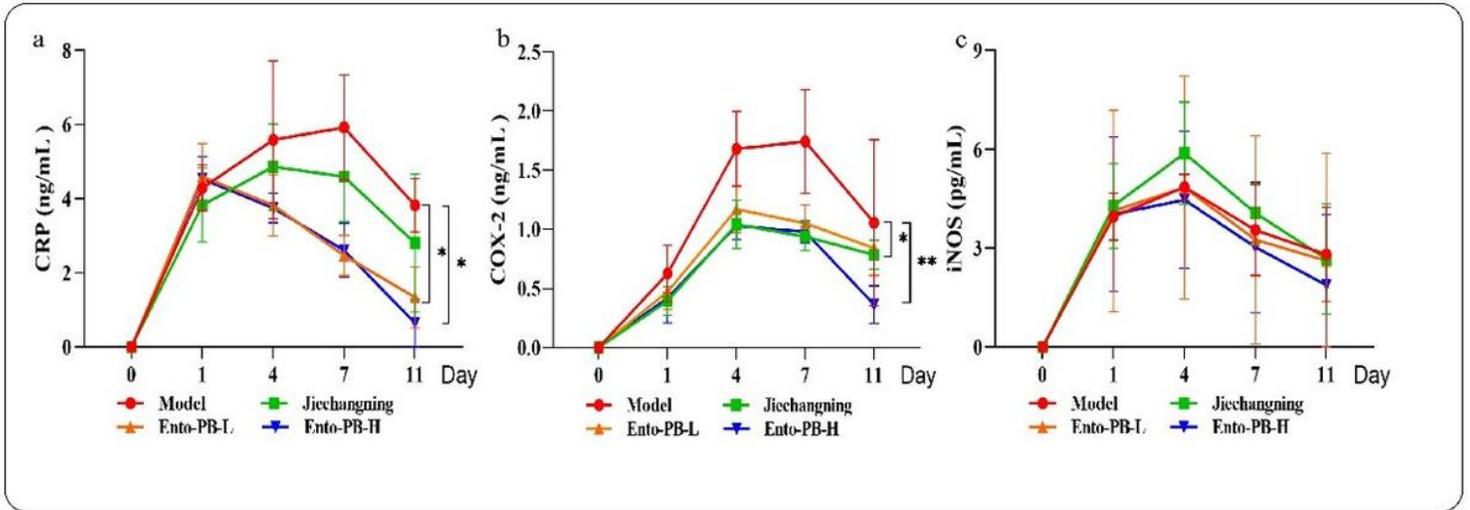


Figure 1

The effect of Ento-PB on plasma inflammatory markers in dogs with colitis. a. CRP; b. COX-2; c. iNOS. *P<0.05, **P<0.01, vs. model group.

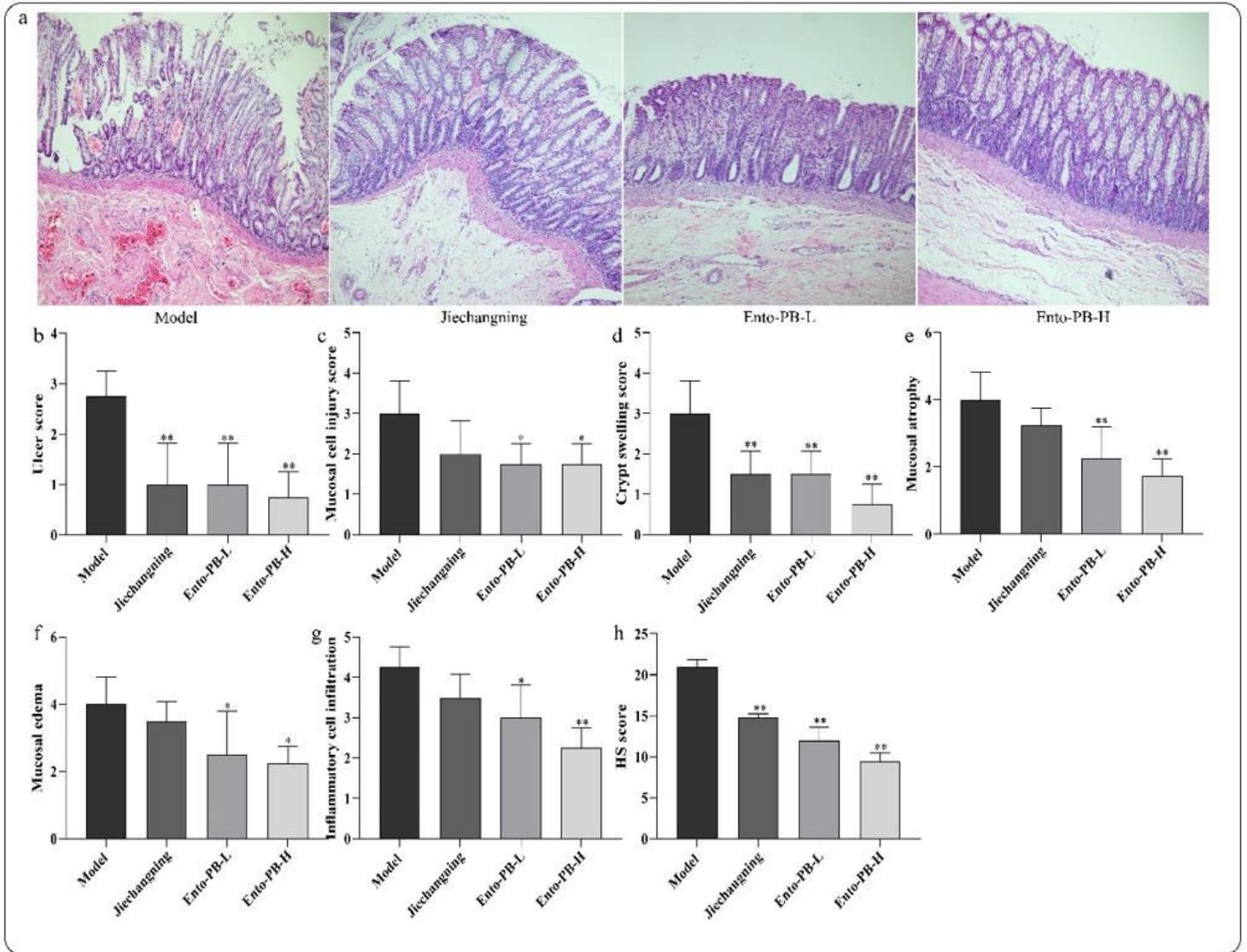


Figure 1

Ento-PB improves the pathological symptoms of colon tissue in dogs with colitis. a. Representative pathological sections of canine colon tissue shown by H&E staining (×200, scale bar = 200 μm); b. Ulcer score; c. Mucosal cell injury score; d. Crypt swelling score; e. Mucosal atrophy; f. Mucosal edema; g. Inflammatory cell infiltration; h. HS score. *P<0.05, **P<0.01, vs. model group.

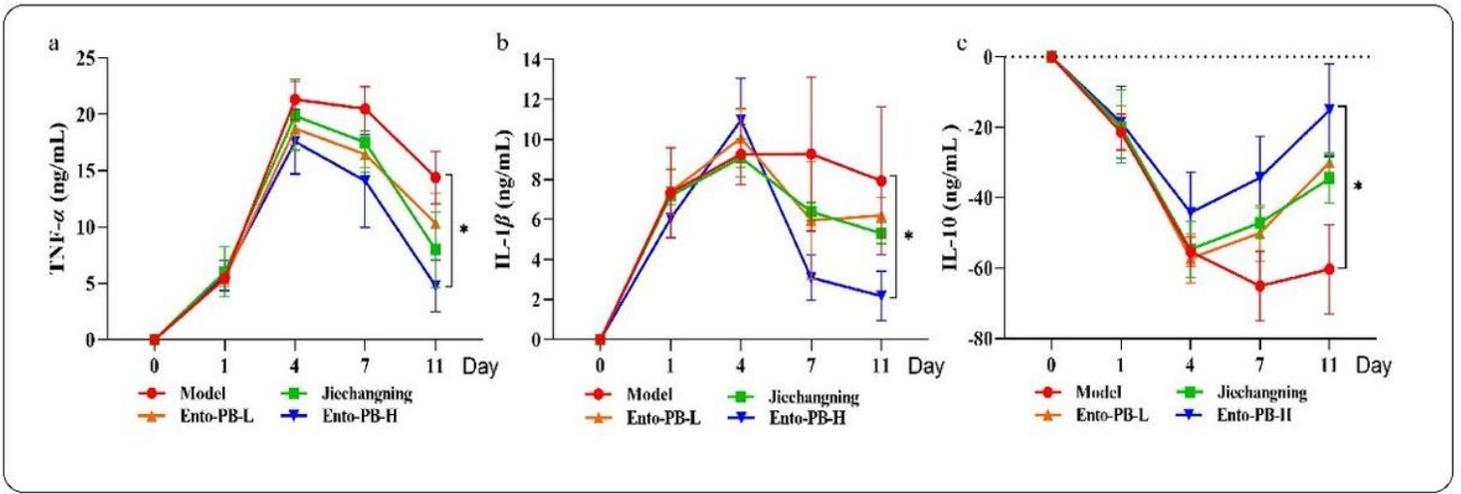


Figure 1

The effect of Ento-PB on the expression of plasma inflammatory cytokines in dogs with colitis. a. TNF-α; b. IL-1β; c. IL-10. *P<0.05, **P<0.01, vs. model group.

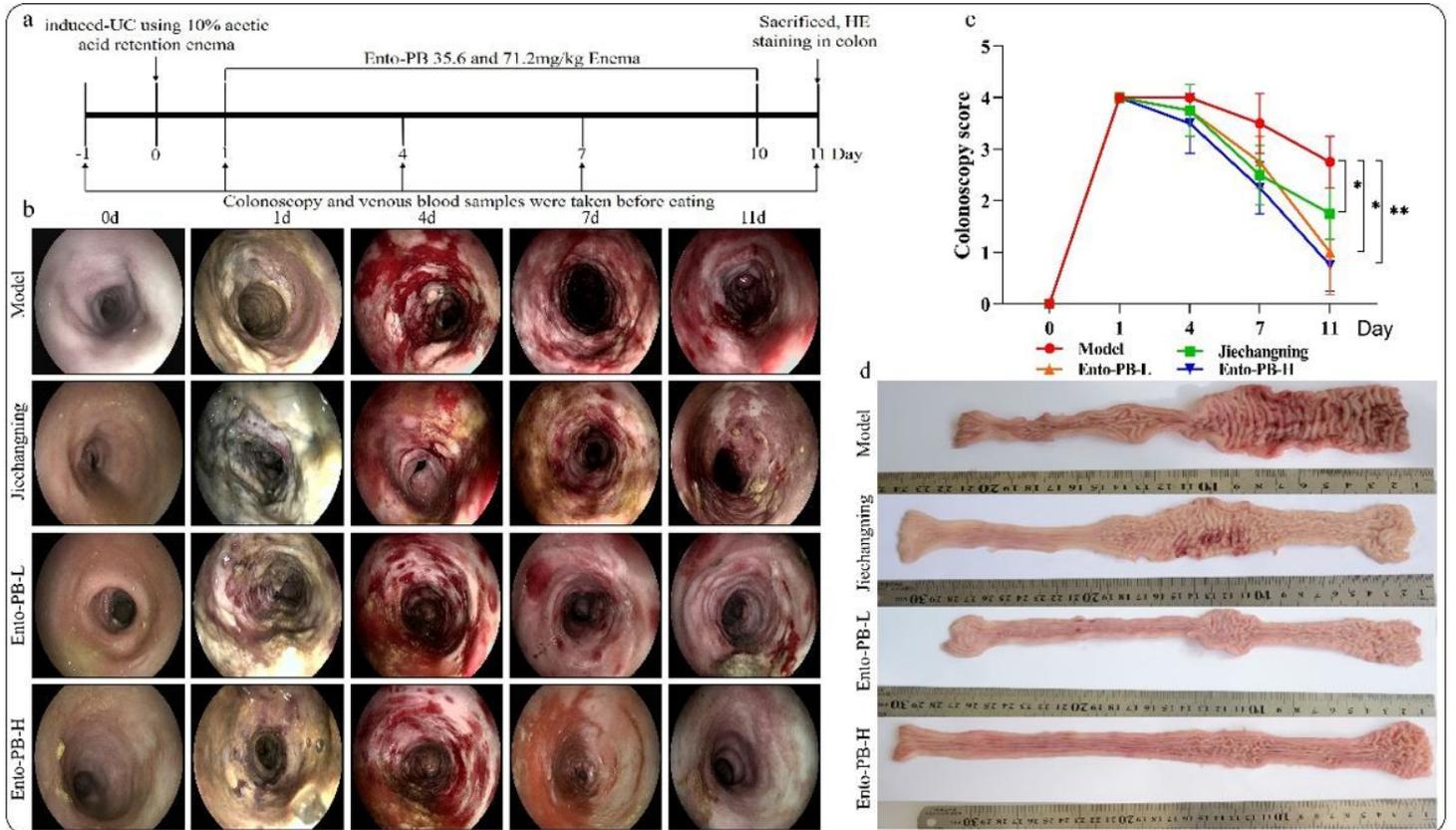


Figure 1

Ento-PB improves colonoscopy scores in dogs with colitis. a. Experimental design of acetic acid-induced colitis in dogs. Give experimental colitis dogs with Enema Jiechang Ning (178 mg/kg), Ento-PB-L (35.6 mg/kg), Ento-PB-H (71.2 mg/kg) and normal saline (model group) for 10 consecutive days. b.

Representative photos of colonoscopy in each group; c. Colonoscopy score; d. Representative photos of colon in each group. *P<0.05, **P<0.01, vs. model group.

Supplementary Files

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

- [Supplementaryinformation.docx](#)