

Portulaca oleracea L. Aqueous Extracts and Portulaca oleracea L. Juice Suppresses Inflammation in Ulcerative Colitis Mice

Yimeng Fan

China Agricultural University

Yuanyuan Wei

China Agricultural University

Huiru Wang

China Agricultural University

Yu Ga

China Agricultural University

Yannan Zhang

China Agricultural University

Zhihui Hao (✉ haozhihui@cau.edu.cn)

China Agricultural University <https://orcid.org/0000-0001-9516-6270>

Research Article

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Abstract

Portulaca oleracea L. (PO) is an edible and medicinal plant for treating gastrointestinal diseases. However, the effects and mechanism of PO on ulcerative colitis (UC) are still unclear. This study evaluated the impact of *Portulaca oleracea* L. aqueous extract (POE) and *Portulaca oleracea* L. juice (PJ) on DSS-induced UC in mice and revealed its mechanisms. The results showed PJ had more bioactive compounds and overlapping targets with UC than POE. Both POE and PJ reduced DAI scores and the infiltration of inflammatory cells in UC mice model while PJ had a better effect. In addition, PJ decreased the expression of NLRP3 inflammasome thus inhibiting pyroptosis. The expression of tight junction proteins was upregulated by PJ and the dysfunction of the intestinal barrier was repaired. In conclusion, PJ improves the colitis caused by DSS and may suppress pyroptosis by interfering with the activation of the NLRP3 inflammasome.

1 Introduction

Ulcerative colitis (UC) is a long-term condition which contributes to the inflammation and ulcers of the colon and rectum (Ford et al. 2013). It is defined by the World Health Organization as one of the modern intractable diseases, which has developed into a global disease due to its repeated recurrence and long-term treatment (Kaplan 2015). Approximately 11.2 million people were affected as of 2015 (Disease et al. 2016) and it often begins in young people aged 15 to 30 years (Ford et al. 2013). UC is mainly manifested by intestinal tissue inflammation, intestinal mucosal damage and intestinal barrier dysfunction (Wang et al. 2019). The patients may exhibit symptoms of bloody stools or abdominal pain. UC not only causes physical and psychological problems for patients but also increases the global economic burden. Ulcerative colitis can affect the mucosa and submucosa of the colon and rectum, and increase the microbes in lamina propria. Macrophages could send out inflammatory signals when they engulf the microbes and increase the expression of pro-inflammatory cytokines. As an idiopathic and chronic inflammatory disease, UC may be led by genetic factors, unhealthy lifestyle and gut microbiota. At present, many drugs are discovered to relieve colitis, such as corticosteroids, aminosalicylates and some biological regents which come with larger side effects (Ooi & Sands 1999). Relapse is common after treatment discontinuation, and there are many adverse reactions. Therefore, new therapeutic drugs with fewer side effects need to be developed (Wang et al. 2021).

Portulaca oleracea L. (PO), also known as Purslane, belongs to the family Portulacaceae. It is a widely distributed traditional folk medicine with insect repellent, febrifuge and antiseptic effects. Its leaves are also frequently used in salad and cooked or baked in pies, soups and omelets in the Mediterranean diet. This edible property makes it become a kind of safe, non-toxic and promising medicinal plant resource for research and development. In 1990, the World Health Organization (WHO) listed PO as a global panacea. As an edible and medicinal plant, PO has a wide range of pharmacological effects, such as antimicrobial, bronchodilatory and anti-inflammatory (Boskabady et al. 2019). Recently, PO was reported to exert a protective effect on joint inflammation mice by suppressing the expression of Nrf2 and

NLRP3(He et al. 2021). Additionally, it has been proved to reduce the inflammatory response in irritable bowel syndrome (IBS) by suppressing the expression of pro-inflammatory cytokines(Kim et al. 2018).

The dried PO and fresh juice of PO may have different uses. In traditional Chinese medicine, the dried plants were decocted for plant extracts and prepared as Chinese herbal medicine pieces and used for the treatment of dysentery with bloody stools and hemorrhoid bleeding, and external application to reduce swellings, eczema, sores, erysipelas as well as snake- and insect-bite (Chen et al. 2009). While the juice of the plant can be used to alleviate the burning sensation. Leaf juice has been recommended to relieve inflammation of the male genitalia (Belcheff 2012). In Pakistan, fresh aboveground parts of PO are considered valuable in the treatment of urinary and digestive problems. It's worth mentioning that PO exerts excellent anti-inflammatory potential in the form of both Chinese herbal medicine pieces and fresh juice. However, the effect of PO on DSS-induced UC mice remains largely undefined.

Pyroptosis is a highly inflammatory form of lytic programmed cell death(Rashidi et al. 2019). Different from apoptosis, pyroptosis is caspase-1 dependence, leading to DNA fragmentation and inflammatory factors release(Wu et al. 2016). It has been proved that pyroptosis of epithelial cells contributes to barrier dysfunction. In addition, tight barrier proteins can maintain the integrity of the intestinal barrier, reduce the possibility of bacterium and toxin invasion. Therefore, reducing the pyroptosis level in macrophages and remodeling the intestinal barrier can be an effective way to alleviate UC.

To promote the application of traditional Chinese medicine in UC treatment, we explored whether PO exerts therapeutic effects on DSS-induced UC in mice and elucidated its underlying molecular mechanisms. First, we compared the therapeutic effects of *Portulaca oleracea* L. juice (PJ) and *Portulaca oleracea* L. aqueous extract (POE) on ulcerative colitis in mice. Then, the anti-inflammatory and anti-pyroptosis effects of PJ were explored in DSS-induced ulcerative colitis mice. Moreover, the protein and mRNA expressions of the intestine barrier were performed to evaluate the intestinal barrier repair effect of PO.

2 Materials And Methods

2.1 Chemicals and reagents

Methanol and formic acid were purchased from Thermo Fisher. Deionized water was prepared using Milli Q Advantage A10 ultrapure water machine. Centrifuge tubes of sizes 1.5 mL and 15 mL and injection vials were purchased from Axygen. All other chemicals or reagents used are of analytical grade.

2.2 Preparation of the *Portulaca oleracea* L.

Dried *Portulaca oleracea* L. was purchased from Tongrentang (Beijing). 100 g *Portulaca oleracea* L. were decocted twice with water for 30 min each time. Then, the extracts were mixed, boiled to 70°C and concentrated to 100 mL (1 g/mL).

Fresh *Portulaca oleracea* L. was collected in September 2020 from the Guangdong region (China). 100 g aerial parts of Fresh *Portulaca oleracea* L. were washed with water, cut into small pieces and blended with a Joyang juicer. The juice was collected from *Portulaca oleracea* L. puree by centrifugation (10,000× g, 20 min, 4°C) and concentrated to 100 mL (1 g/mL). Then sterilized by filtration on 0.22 µm membrane filters.

2.3 Analysis of the main bioactive components of POE and PJ

Chromatographic separation was performed on an ACQUITY UPLC HSS T3 2.1×100mm, 1.8µm column. The column temperature was 35°C. The injection volume was 10 µL. The mobile phase contained (A) aqueous formic acid (0.1%, v/v) and (B) acetonitrile (containing 0.1% formic acid) at a flow rate of 0.3mL/min. The gradient elution was 0–30% B at 0–10 min, 30–40% B at 10–20 min, 40–50% B at 25–30 min, 50–70% B at 30–40 min, 70–100% B at 40–45 min and the re-equilibration time of gradient elution was 15 minutes. Q Exactive Orbitrap high-resolution mass spectrometry was used for mass spectrometry data acquisition, and the detection mode was Full MS-ddMS2 with simultaneous scanning of positive and negative ion modes. The scan range was m/z 100–1200, MS1 resolution was set to 70000, and MS2 resolution was set to 17500. The ion source voltage was 3.2kV. Capillary temperature was 320°C and aux gas heater temperature was 350°C. Sheath gas flow rate was 40 L/min and aux gas flow rate was 15 L/min. AGC Target was set to 1e6, TopN was set to 5, and the collision energy for triggering MS2 scan was set to 30, 40, and 50 using a stepped fragmentation voltage NCE. The data were analyzed using Compound discoverer 3.3 software, and the compounds were structurally identified using local database and mzcloud online database.

2.4 Network pharmacology analysis

The targets of PO were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) (<http://tcmspnw.com>). The OMIM (<https://omim.org/>), Genecards (<https://www.genecards.org/>), and TTD (<http://db.idrblab.net/ttd/>) databases were used to identify UC-related genes. We established a set of UC-associated genes by uniting the search results. The STRING database (<https://string-db.org/>) was used to construct the protein–protein interaction (PPI) network. Targets of UC, PJ, POE, and common composition were imported into Cytoscape 3.9.0 to investigate the critical subnetwork. Based on the scores obtained by Cytoscape, we filtered the genes with scores above the median value and constructed a network using these genes. The CytoNCA plugin in Cytoscape was used to analyze the top 10 genes. All selected targets were entered into the Database for Annotation, Visualization, and Integrated Discovery (DAVID, <http://www.david.niaid.nih.gov>), which could provide systematic annotations of pathways and biological processes.

2.5 Animals

Kunming mice (male, 7–8 weeks old) were purchased from Speyford (Beijing) Experimental Animal Science and Technology Co. Ltd, Ltd. (Beijing, China) (Production license number was SCXK 2019-0010). The experimental protocols were approved by the Institutional Animal Care and Use Committee of China

Agricultural University. All works were conducted according to the guidelines of the National Institute of Health (<https://olaw.nih.gov/>) for laboratory animal care and use.

2.6 Modeling and grouping

Experiment 1 After acclimatization for seven days, twenty-four mice (30–42 g) were randomly divided into four groups: control group, DSS group, POE group (1 g/kg), and PJ group (12 g/kg), with 6 mice per group. The details of the experiment including modeling and grouping were shown in Fig. 3A.

Experiment 2 Thirty mice (30–42 g) were randomly divided into the control group, DSS group, PJ-H group (24 g/kg), PJ-M group (12 g/kg), PJ-L group (6 g/kg), with 6 mice per group. The details of the experiment including modeling and grouping were shown in Fig. 3A.

5% DSS (DSS, M.W. 36000–50000; MP Biomedicals, UK) was dissolved in drinking water for 7 days to induce acute colitis (Shin et al. 2017). The mice in the POE group and PJ group were administered with POE or PJ for one week based on the conversion rate of clinical administration dose. Mice in DSS and controls were gavaged with the same volume of saline. On day 15, all mice were sacrificed and we collected the colon tissues for further analysis after measuring the lengths. Blood samples were collected and then centrifuged at 1500 g for 10 min to obtain serum. Assays were performed following standard procedures.

2.7 Disease Activity Index Assessment

The body weight and stool condition of the mice were monitored to calculate the disease activity index (DAI). The DAI was scored by the well-established methods which were shown in Supplementary Table S1 (Bang & Lichtenberger 2016).

2.8 Histologic and AB-PAS staining

The colonic tissues were fixed in Neutral buffered Formalin (10%) and stained with hematoxylin-eosin (H&E) and Alcian Blue Periodic acid Schiff (AB-PAS). The apoptosis in the colon was assessed by TUNEL staining (Boster, Wuhan, China). The digital images were captured using light microscopy (DS-Ri2, Nikon, Japan). The expression of colon mucin was calculated according to the mucin density, the apoptotic rate is the ratio of the apoptotic cells to the total cells, determined with image analysis using image-pro-plus.

2.9 Enzyme-Linked Immunosorbent Assay

One hundred mg of colon tissues were homogenated into 900ul normal saline, then centrifugation at 3500 g for 15 min to obtain 10% colon tissue homogenate. The levels of (IL)-1 β (ColorfulGene, Wuhan, Cat. No. JYM0543Mo), IL-18 (ColorfulGene, Wuhan, Cat. No. JYM0531Mo) in colons and serum samples were measured by corresponding ELISA kits following the manufacturer's instructions.

2.10 Expression of inflammatory cytokines using antibody array membrane

The expression of inflammatory cytokine was evaluated by the array membrane following the manufacturer's instructions. The protein was isolated from the colon samples with a total protein extraction kit (Solarbio, Beijing, China), then, the concentration of the supernatants was measured by BCA (Solarbio, Beijing, China) and the concentration was adjusted to 100 µg/mL. First, the membranes were placed in the 8-well tray and incubated with the blocking buffer for 30 min, then, the protein supernatants of each group were added to the membrane for 24 h at 4°C. The membranes were washed and incubated with biotin-conjugated anti-cytokines. Two hours later, the membranes were washed and horseradish peroxidase-conjugated streptavidin was added to each well for 2 h at room temperature. After washing, the expression of proteins was detected by enhanced chemiluminescence and visualized by Amersham ImageQuant 800 imaging system (Cytiva, USA, Japan). The measurements of each signal intensity were determined by ImageJ. Data were normalized to the positive control signals and the relative cytokine expression was determined.

2.11 Immunohistochemistry (IHC)

The paraffin sections were incubated with 3% hydrogen peroxide to deactivate endogenous peroxidase. After 5% BSA blocking, the primary antibodies were added to the sections for 10 h at 4°C. The primary antibodies included ASC/TMS1 (1:100, Abcam), Caspase-1/p20/p10 (1:100, Origen), MUC-2 (1:1000), and Occludin (1:50). Then the sections were washed in TBS for three times and incubated with AP-conjugated anti-rabbit secondary antibody at 37°C. The images were selected using a light microscopy (DS-Ri2, Nikon, Japan). The results were quantified by Image-Pro Plus 6.0 software.

2.12 Quantitative Real-Time PCR Analysis

Total RNA was extracted from colon tissues using an RNA extraction kit (Promega, China) followed by reverse transcription with GoScript™ Reverse Transcription System (Promega, USA). Quantitative real-time polymerase chain reaction (qPCR) was performed for quantitative analysis of the genes. Primers of the related genes were listed in Table 1. The relative expression of the target genes was calculated by the $2^{-\Delta\Delta CT}$ method.

Table 1
Primers and probes for qPCR

Gene	Primer sequence (5'–3')	GenBank accession no	PCR product T _m (°C)	PCR product GC%	Product size (bp)
<i>Casp-1</i>	F: ACTCGTACACGTCTTGCCCTCA R: CTGGGCAGGCAGCAAATTC	NM_009807.2	76.2	47.4	190
<i>Asc</i>	F: CTGAGCAGCTGCAAACGACTAAAG R: TGCAAAGCATCCAGCACTCC	NM_023258.4	79.0	55.6	160
<i>Nlrp3</i>	F: ACTGAAGCACCTGCTCTGCAAC R: AACCAATGCGAGATCCTGACAAC	NM_001359638.1	75.0	53.4	88
<i>Gsdmd</i>	F: CGTTATTCATGTGTCAACCTGTCAA R: TCCCATCGACGACATCAGAGA	NM_026960.4	75.1	50.5	109
<i>Muc-2</i>	F: GCTGCACCAAGAACATCTCCA R: TTCCAGGGTAACACTTCGGACAC	NM_023566.3	77.5	53.1	143
<i>Ocln</i>	F: GGCAAGCGATCATACCCAGAG R: AGGCTGCCTGAAGTCATCCAC	NM_001360536.1	75.7	52.4	105
<i>Gapdh</i>	F: TGTGTCCGTCGTGGATCTGA R: TTGCTGTTGAAGTCGCAGGAG	NM_001289726	78.6	55.3	150

2.13 Statistical Analysis

All data were presented as the means $\pm$ standard deviation (SD), one-way analysis of variance (ANOVA) was used to analyze the results. Multiple comparisons were performed with Dunnett's t test-post-hoc analysis test, and $p < 0.05$ was considered statistically significant. The statistical analysis was performed by SPSS 26.0 (SPSS Inc., NY, United States).

3 Results

3.1 Major components of *Portulaca oleracea* L. by HPLC-MS/MS

In order to identify the major components of POE and PJ, we used HPLC-MS/MS for the component analysis. A total of 155 components from POE and PJ were identified (Fig. 1). PJ and POE have 63 identical components including 14 organic acids, 1 phenol, 2 flavonoids, 2 alkaloids, etc., and 35 other

substances that are mainly amino acids and plant metabolites. Thirty-nine unique components were identified from POE, containing 7 organic acids, 6 flavonoids and 4 terpenoids. We also identified 51 unique components including 6 organic acids, 6 flavonoids, 11 terpenoids and 2 coumarins.

3.2 Network Pharmacology Predicts the Effect of *Portulaca oleracea* L. on Ulcerative Colitis

To explore potential pharmacological targets and mechanisms of PO involved in UC, we performed network pharmacology analysis on PJ, POE, and common compositions mentioned above. The overlapping targets of UC with common compositions, POE and PJ were 47, 48 and 74, respectively. To further reveal the drug-disease-pathway connections, we built a PPI network, in which the overlapping targets were imported and GO and KEGG enrichment analyses were performed. The PPI network revealed that PJ and POE could both act therapeutic effects through targets including MAPK14, PPARG, and ESR1. GO and KEGG results suggested that POE and PJ could treat UC through pathways associated with platelet activation as well as lipid and atherosclerosis (Fig. 2A). Among the overlapping targets of POE and UC, the targets with higher node degrees contained TP53, IL-6, and CAT. The PI3K-Akt signaling pathway and TNF signaling pathway may be the key pathways in POE for treating ulcerative colitis (Fig. 2B). We also found that IL-1 β , IL-6, TP53, and PTGS2 could be the more important targets of the PJ and UC overlapping targets. In addition to cancer-related pathways, receptor activation and reactive oxygen species accumulation are also relevant and important pathways (Fig. 2C). We therefore hypothesized that PO may improve the degree of inflammation and symptoms of UC by modulating inflammatory factors and antioxidant systems.

3.3 *Portulaca oleracea* L. Improved the Symptoms and Reduced the Colon Injury of DSS-Induced Colitis in Mice

The effects of PO on UC were evaluated through the UC mice induced by 5% DSS solution for 7 days (Fig. 3A). Compared with DSS group, POE (1 g/kg) or PJ (12 g/kg) treatment significantly improved the body weights of DSS mice ($P < 0.01$), reduced the DAI scores ($P < 0.01$) and increased the colon length ($P < 0.01$) (Fig. 3B-D).

To further explore the effects of POE and PJ on DSS mice, we accessed the pathological changes in colon tissue. DSS could lead to severe colonic damage including erosion of lamina propria mucosa, decreasing of glandular cells, and infiltration of numerous neutrophils. Administrate with POE and PJ dramatically decreased the pathological changes and HE scores. In addition, PO could significantly reduce the apoptosis in colon tissue (Fig. 3E). These results confirmed the therapeutic effect of POE and PJ on DSS-induced mice, while PJ had a better effect compared with POE ($P < 0.01$).

3.4 PJ Suppressed DSS-Induced Expression of Inflammatory Responses in Colon

The expression of pro-inflammatory cytokines in colon tissues was detected using an antibody array membrane. DSS-induced significantly increased the concentrations of BLC ($P < 0.01$), IL-1 α ($P < 0.01$),

TNF- α ($P < 0.01$), IL-1 β , IL-6, IL-8, IL-17, and INF- γ . However, these cytokines were markedly decreased after treatment with PJ. The cytokines which were especially affected by DSS and PJ were expressed as fold change (Fig. 4C).

3.5 PJ attenuates clinical colitis parameters in DSS-induced UC mice

The effects of different dose of PJ on DSS-induced UC mice were evaluated (Fig. 5A). In the DSS group, the colonic length was significantly shortened compared with that of the other groups, and diarrhea and weight loss were notably worsened; thus, the DAI rose sharply at days 3, 6 and 9 ($P < 0.05$). PJ (12 g/kg) treatment significantly improved the body weights of DSS mice ($P < 0.01$), reduced the DAI scores ($P < 0.01$) and increased the colon length ($P < 0.01$) (Fig. 5B-D). The medium dose of PJ had the best therapeutic effect, with no remarkable difference compared to the control group. HE staining showed that the intestinal mucosa in the control and PJ group was normal and no obvious abnormalities were found. The number of goblet cells in the model group decreased obviously, and a large number of inflammatory cell infiltration could be seen in these two groups (Fig. 5E).

3.6 PJ repair the intestinal barrier in UC Mice

As shown in Fig. 6, AB-PAS indicated that mucus secretion was reduced in DSS groups, which could be improved by PJ ($P < 0.01$). Then, the expressions of MUC-2 and Occludin were tested by immunohistochemistry (IHC). The results showed that high and middle dose of PJ can significantly increase the expression of MUC-2 and Occludin compared with the DSS group ($P < 0.01$).

3.7 PJ Suppresses the NF- κ B/NLRP3 Inflammasome Axis Activation in UC Mice

TUNEL was applied to detect the level of DNA damage in the colon. PJ administration reduced the apoptosis rate of colon tissue in UC mice (Fig. 7A). The NLRP3 inflammasome is a cytoplasmic multimolecular platform that is composed of NLRP3, ASC, and procaspase-1. We measured the expression level of the NLRP3 inflammasome to evaluate whether NLRP3 was involved in the DSS-induced colon injury. The results of IHC showed that the expression of ASC and Casp-1 in PJ-treated mice was lower than that in DSS mice ($P < 0.01$) (Fig. 7A). The results of qPCR showed that treatment with PJ could reduce the level of NLRP3 inflammasome and GSDMD ($P < 0.01$) (Fig. 7B). The levels of IL-18 and IL-1 β were dramatically increased in the DSS group while decreased in the PJ group ($P < 0.01$) (Fig. 7C).

4 Discussion

UC is an inflammatory disease of the colon which would lead to colon ulcers and inflammation. Clinical symptoms include diarrhea, intermittent bloody diarrhea, abdominal pain and tenesmus (Feuerstein & Cheifetz 2014). Colon injuries induced by DSS are mainly due to permeability and proinflammatory cytokines, which lead to epithelial damage, a common pathological process of UC (Wang et al. 2020). More and more evidence proved that the inflammatory response plays an important role in the

progression of DSS-induced UC(Wang et al. 2021). *Portulaca oleracea* L., a traditional Chinese herbal, has shown the potential therapeutic effects of intestinal inflammation(Di Cagno et al. 2019, Kong et al. 2018).

In this study, we first performed the component analysis to identify major components of POE and PJ. The results revealed that there were rich flavonoids, terpenoids, organic acids, and alkaloids in POE and PJ. These compounds have various biological activities such as anti-oxidative, anti-inflammatory, antitumor, antiviral and antibacterial. We also found PJ had more bioactive compounds than POE, which indicated that PJ may have better pharmacological effects. Then we used network pharmacology to elucidate the mechanism of PO in treating ulcerative colitis. The key targets including PPARG, IL-1 β , IL-6, TP53, ESR1, MAPK14, CAT, and PTGS2 are directly related to anti-inflammatory effects. In the classical inflammatory signaling pathways, target proteins such as PPARG, TNF, IL-1B, IL-6, and MAPK play a crucial role, which can inhibit inflammation, reduce inflammatory pathological changes, promote cell differentiation, cell growth and apoptosis, as well as activate oxidative stress and inflammatory response(Miao et al. 2019). Most of the therapeutic targets of PO are enriched in the cancer pathway, suggesting that Amaranthus may prevent colon cancer by inhibiting the cancer pathway. Interestingly, we found that compared with POE, PJ had more overlapping targets with PE, which suggested PJ may perform better in the treatment of UC.

In order to confirm the network pharmacology results, we established a UC model to explore the effect of POE and PJ, the results showed that both POE and PJ can improve body weight and colon length, which were concerned as indirect indexes in the assessment of the severity of UC. Moreover, DAI scores were reported as an important parameter in the development of UC(Cooper et al. 1993), and the results indicated that PO could decrease the DAI scores. The results of the histological analysis showed that DSS administration influenced the submucosa and cryptal glands, and the submucosa was infiltrated by numerous inflammatory cells. However, the above changes were improved by the treatment of PO while the PJ had a better effect compared with POE. The reason might be that the steaming process decreased the phenolic composition and antioxidant activity of row PO, which was consistent with the previous findings(Fernandez-Poyatos et al. 2021).

As we all know, one of the most important characteristics of UC is the uncontrolled production of proinflammatory cytokines which lead to severe injury of the intestinal mucosa(Park et al. 2017). The major inflammatory cytokines including tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and IL-6, can activate NF- κ B and promote the secretion of other cytokines(Kuno & Matsushima 1994, Liu & Malik 2006, Paik et al. 2003). The results of the antibody array membrane showed that BLC, IL-1 α , TNF- α , IL-1 β , IL-6, IL-8, IL-17, and INF- γ , which were considered the targets of inflammatory diseases (Yamamoto-Furusho 2018), were significantly suppressed by PJ. Among these cytokines, the upregulation of IL-1 β was associated with pyroptosis(Eder 2009, Ran et al. 2021) which is considered a unique form of cell death.

Moreover, activation of the NLRP3 inflammasome can lead to pyroptosis. NLRP3 inflammasomes have been proven to be associated with colitis pathogenesis(Shao et al. 2019, Zhen & Zhang 2019). Once the

NLRP3 inflammasomes are activated, they will provide a platform for the activation of caspase-1 (Kim et al. 2019), then, the pro-forms of interleukin 1 β (pro-IL-1 β) and IL-18 (pro-IL-18) will be converted to the active forms mediated by caspase-1. The amino-terminal gasdermin-N and carboxy-terminal gasdermin-C domains in GSDMD were cleaved by caspase-1. GSDMD is an executioner mediating cell lysis and inflammatory factor release, thereby inducing pyroptosis (Mulvihill et al. 2018). So, the expression of NLRP3, caspase-1, and GSDMD was measured both in vivo and in vitro. The results showed that the expression of NLRP3, caspase-1, and GSDMD was increased by DSS treatment, suggesting that pyroptosis induced by the NLRP3 inflammasome might be the potential mechanism underlying DSS-induced colon injury. PJ administration significantly downregulated the expression of the NLRP3 inflammasome. This result was consistent with the previous study indicating that the inhibition of NLRP3 inflammasome activation alleviates colon injury (Marinho et al. 2021).

The increased secretion of inflammatory cytokines can also lead to intestinal epithelial necrosis, and tight junction protein decreased. The epithelial barrier plays a very important role in the mucosal immune system, isolating the external stimuli and host immune cells (Camilleri et al. 2012, Ford et al. 2013). The defective epithelial barrier allows the intestinal microbiota to migrate to the cell side and stimulates the immune system and further leads to inflammation (Merga et al. 2014, Vindigni et al. 2016). Clinical trials proved that epithelial barrier dysfunction is associated with the occurrence and development of UC (McCole 2014, Schmitz et al. 1999). The major tight junction proteins and the mucus layer of the colon constitute the basic structure of the barrier (Kimura et al. 1997). Our results showed that PJ can upregulate the expression levels of MUC-2 and Occludin proteins and mRNAs in the intestinal tissues compared with the DSS group, which was similar to the previous results (Ibrahim et al. 2021, Kang et al. 2021). These results indicated that the restoration of barrier function may be part of the mechanism of PJ in inhibiting UC.

Therefore, reducing the secretion of inflammatory factors and repairing the intestinal barrier are the treatments for UC. However, this study also has some limitations, due to the complexity of herbal components, the effective ingredients and other effective factors in the pathway need to be further studied. In short, inhibiting NLRP3 inflammasome and pyroptosis to improve the epithelial barrier may be a mechanism in the treatment of PJ for UC.

In conclusion, we demonstrated that PJ can alleviate the symptoms of UC in mice induced by DSS. The underlying therapeutic mechanism may be that PJ repairs colonic barrier integrity via suppressing the activation of NLRP3 inflammasome, providing a novel insight into the protective effect of PJ on UC.

5 Additional Requirements List Of Abbreviations

UC: ulcerative colitis; PO: *Portulaca oleracea* L.; POE: *Portulaca oleracea* L. Extract; PJ: *Portulaca oleracea* L. juice, DSS: Dextran sulfate sodium; DAI: disease activity index; IL-1 β : interleukin-1beta; IL-6: interleukin-6; IFN- γ : interferon-gamma; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; qPCR: quantitative real-time PCR

Declarations

Acknowledgments

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Ethical Approval All Institutional and National Guidelines for the care and use of animals were followed, and approved by the Institutional Animal Care and Use Committee of China Agricultural University (No. Aw20801202-2-2).

Consent to Participate and Publish Not applicable.

Author Contributions The study was designed by Z.-H.H. and Y.-M.F., Y.-M.F. drafted the manuscript and Z.-H.H., Y.-Y.W., and H.-R.W. revised the manuscript. Y.-M.F., Y.-Y.W., and Y.G. performed most of the experiments and analyzed the data. Y.-N.Z. assisted in data interpretation and manuscript revision. All authors read and approved the final manuscript.

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Conflict of Interest The authors declare that there is no conflict of interest regarding the publication of this paper.

Availability of data and materials All data generated or analyzed during this study are included in this published article [and its supplementary information files]

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Figures

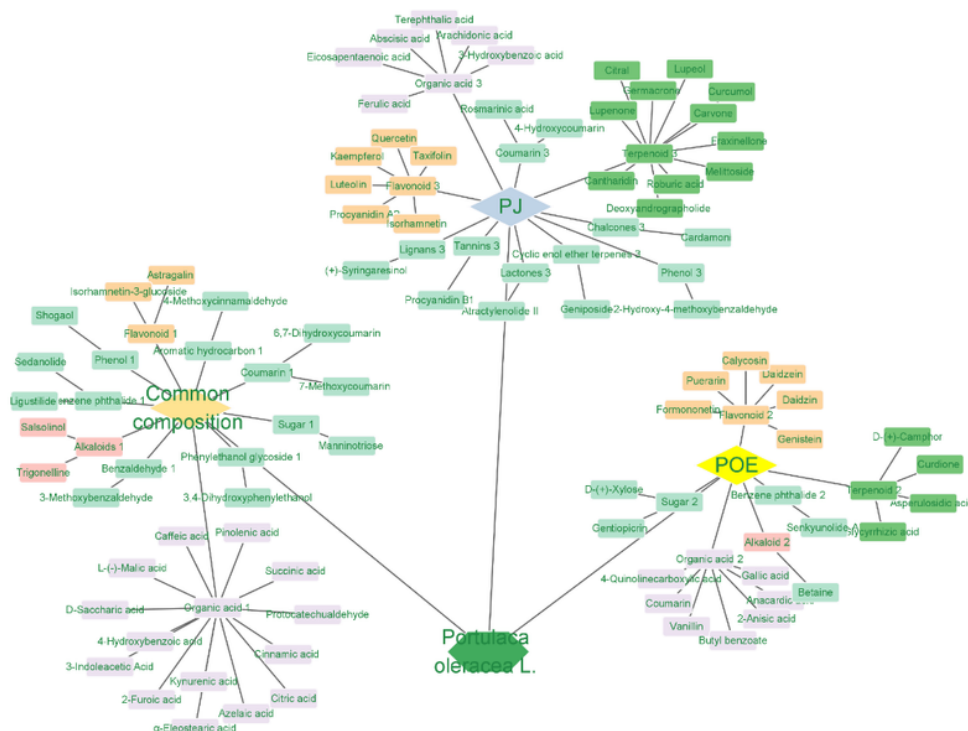


Figure 1

Major components of PO by HPLC-MS/MS.

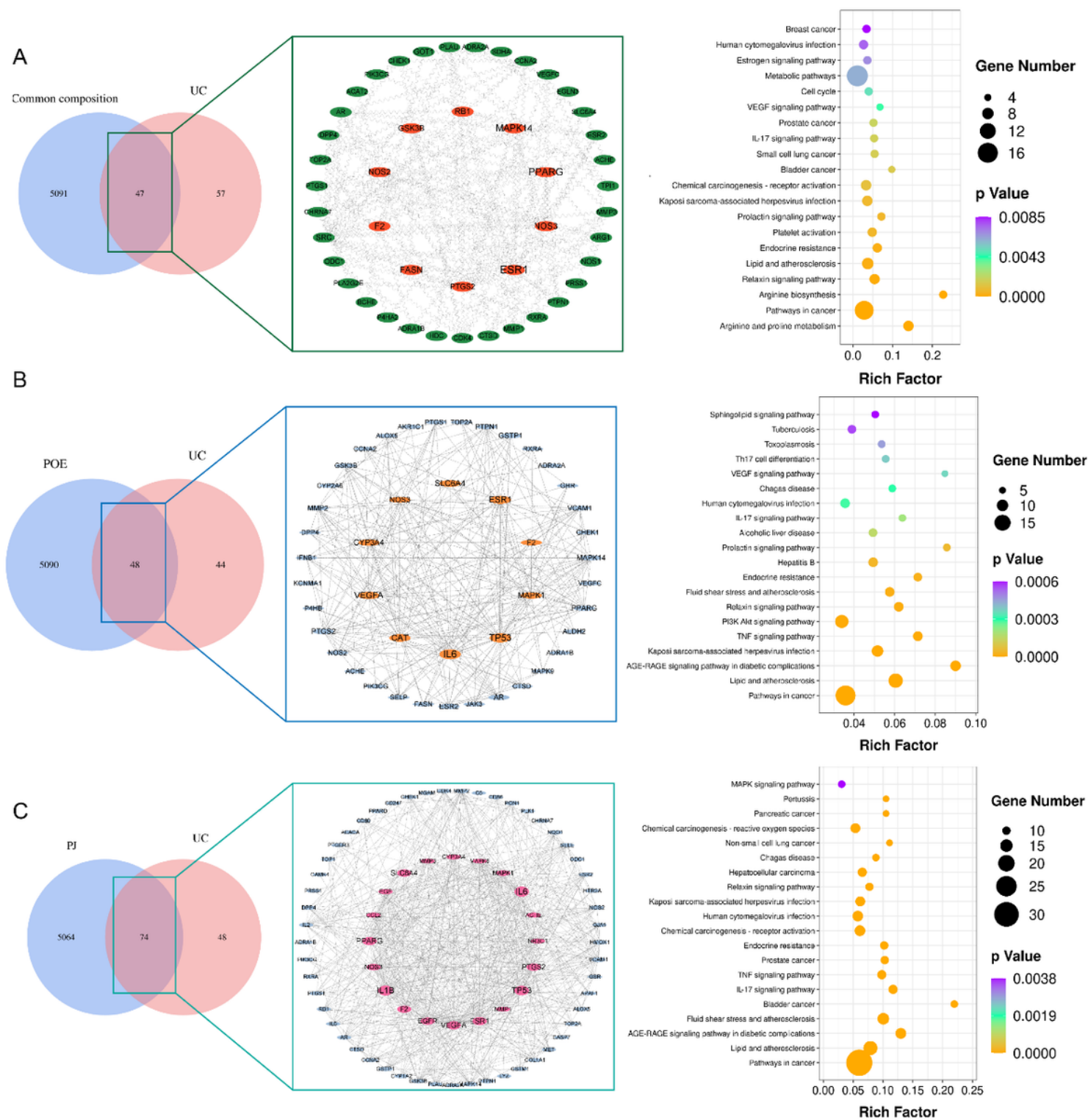


Figure 2

Network pharmacology predicts the mechanism of action of POE, PJ and common components separately. (A) Common components. (B)POE. (C)PJ.

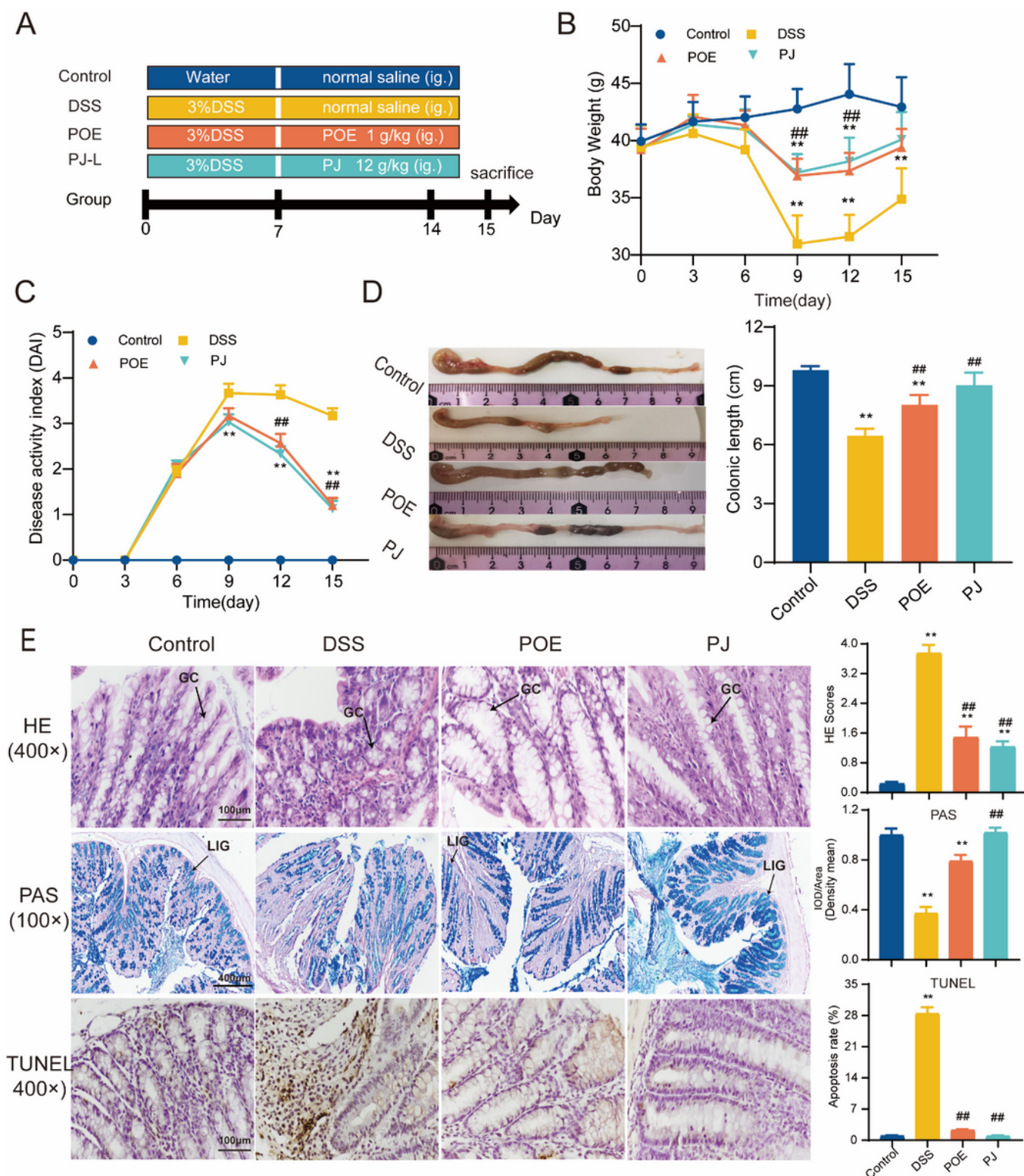


Figure 3

Portulaca oleracea L. relieved the symptoms in DSS-induced UC mice. (A) Experimental procedure and grouping of the research. (B) The body weight of mice was weighed during the study. (C) Disease activity index of mice was calculated from day 1 to day 15. (D) The length of the colon tissues. (E) The images of colon tissues stained with HE, PAS, and TUNEL. * $P < 0.05$, ** $P < 0.01$ vs. control group, # $P < 0.05$, ## $P < 0.01$ vs. DSS group. LIG: large intestinal gland, GC: goblet cell

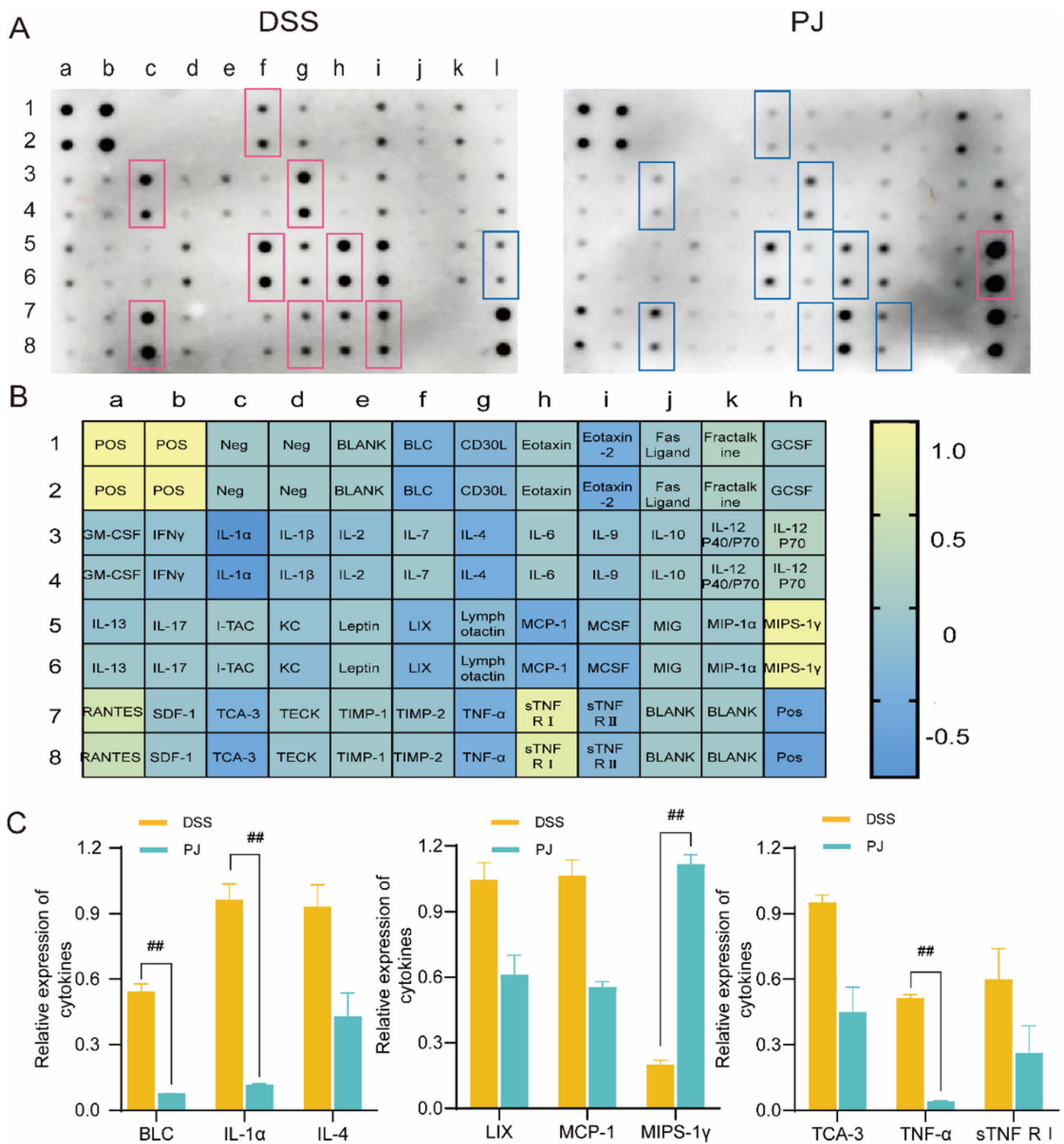


Figure 4

The expression of inflammatory cytokines was detected by antibody array membrane. (A) The images of antibody arrays membrane. Differentially expressed cytokines were marked by squares, red represents high expression and blue represents low expression. (B) Antibody array map with the inflammatory Proteins. (C) Normalized expression of differentially expressed cytokines in DSS and PJ group detected by antibody arrays.

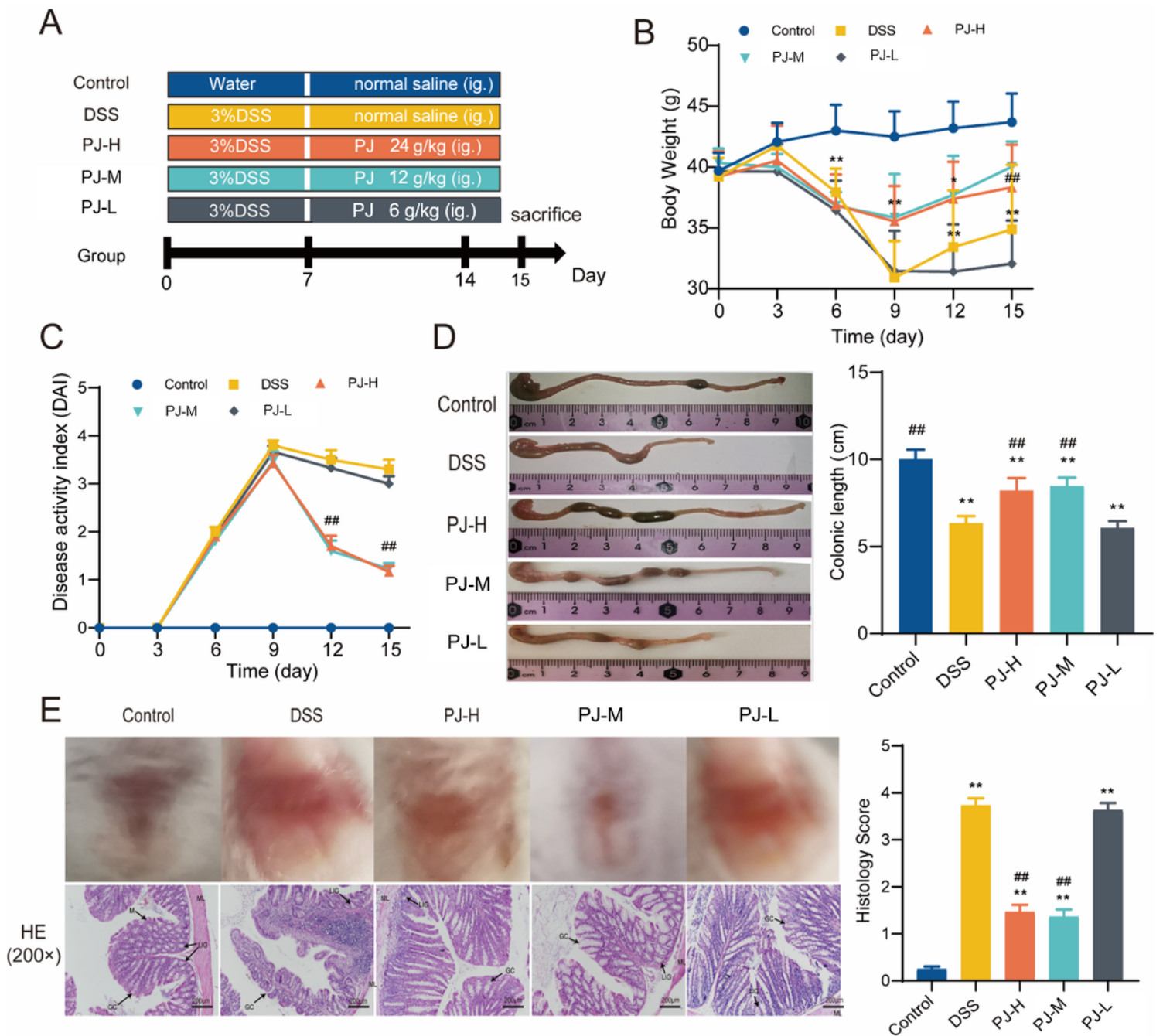


Figure 5

Promotion of apoptosis inhibits the therapeutic effect of PJ on UC mice. (A) Experimental procedure and grouping of the research. (B) Body weight of each mouse was monitored. (C) Disease activity index of mice was detected. (D) The length of colonic tissues was measured. (E) Representative image of the anus and HE staining. LIG: large intestinal gland, GC: goblet cell, ML: muscular layer, M: mucosa.

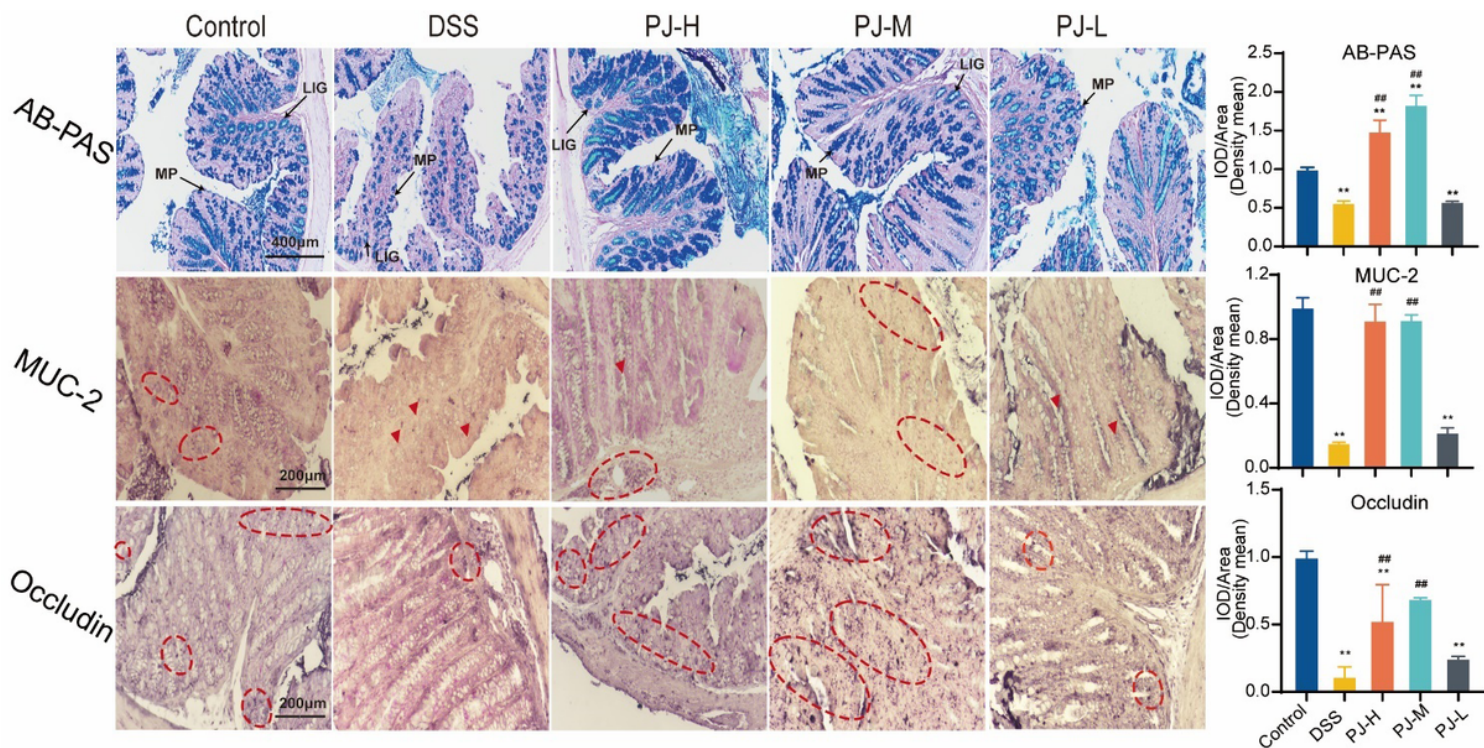


Figure 6

The intestinal barrier repairing effect of PJ on UC mice. The images of AB-PAS staining colon tissues and the expressions of MUC-2 and Occludin were detected by immunohistochemistry.

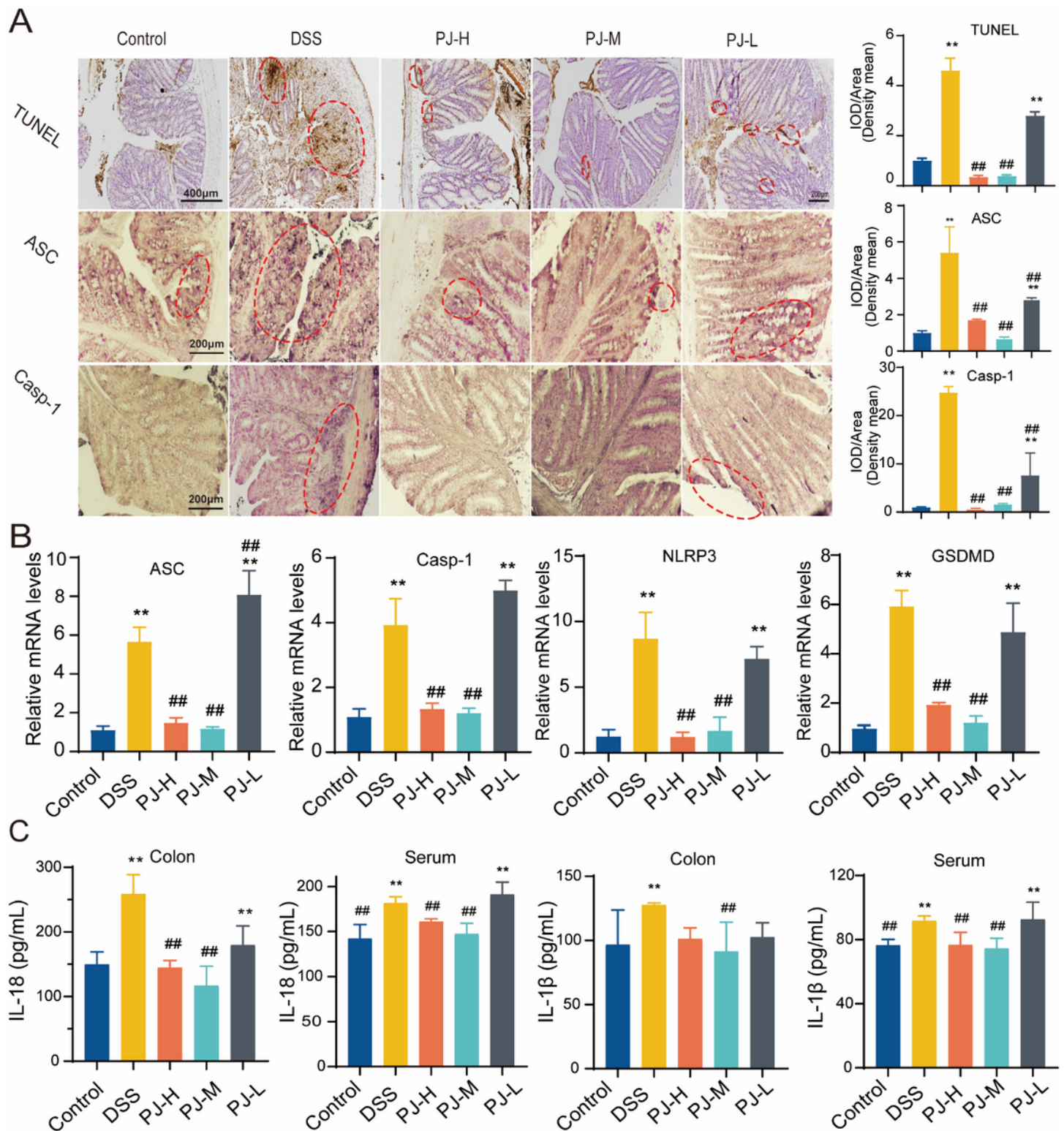


Figure 7

Figure 6 PJ inhibits the activation of NLRP3 inflammasome and thus inhibited pyroptosis in UC mice. (A) The apoptosis of mice colon tissue and the expressions of ASC and caspase-1 were detected by immunohistochemistry. (B) The mRNA level of NLRP3 inflammasome and GSDMD in colon tissues were tested by qPCR. (C) The levels of IL-18 and IL-1 β in colon tissues and serum were analyzed by ELISA.

Supplementary Files

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