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Original Research

The Mechanism of Polygonum Hydropiper L-Coptis Chinensis in the Treatment of Ulcerative Colitis Based on Network Pharmacology and Experimental Validation

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Abstract

Background: *Polygonum hydropiper L* (PH) was widely used to treat dysentery, gastroenteritis, diarrhea and other diseases. *Coptis chinensis* (CC) had the effects of clearing dampness-heat, purging fire, and detoxifying. Study confirmed that flavonoids in PH and alkaloids in CC alleviated inflammation to inhibit the development of intestinal inflammation. However, how PH-CC affects UC was unclear. Therefore, the aim of this study is to analyze the mechanism of PH-CC on ulcerative colitis (UC) through network pharmacology and *in vivo* experiments. **Methods**: The active ingredients and targets of PH-CC and targets of UC were screened based on related databases. The core targets of PH-CC on UC was predicted by protein-protein interaction network (PPI), and then the Gene Ontology-biological processes (GO-BP) function enrichment analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) database. The binding activity between pyroptosis proteins, core targets and effective ingredients were verified based on molecular docking technology. Finally, combined with the results of network pharmacology and literature research, the mechanism of PH-CC against UC was verified by *in vivo* experiments. **Results**: There were 23 active components and 191 potential targets in PH-CC, 5275 targets in UC, and 141 co-targets. GO-BP functional analysis of 141 co-targets showed that the first 20 biological processes were closely related to inflammation and lipopolysaccharide (LPS) stimulation. Furthermore, core targets had good binding activity with the corresponding compounds. Animal experiment indicated that PH-CC effectively prevented weight loss in UC mice, reduced the disease activity index (DAI) score, maintained colon length, suppressed myeloperoxidase (MPO) activity, inhibited pyroptosis protein expression, and downregulated the levels of IL-18 and IL-1β to alleviate intestinal inflammation. **Conclusions**: The results of network pharmacology and animal experiments showed that PH-CC suppressed the inflammatory response, restored colon morphology, and inhibited pyroptosis in UC mice. Thus, PH-CC may improve UC by regulating the NOD-like receptor protein domain 3 (NLRP3)/Caspase-1 signaling pathway.

Keywords: *Polygonum hydropiper L-Coptis chinensis*; ulcerative colitis; inflammatory response; NLRP3/Caspase-1 signal pathway; proptosis; network pharmacology

1. Introduction

Ulcerative colitis (UC) is a subtype of inflammatory bowel disease, presenting with bloody stool, fever, weight loss, abdominal pain, and diarrhea [1]. More and more people are suffering from UC continuously worldwide continuously worldwide, indicating that UC is gradually becoming a global public health concern [2]. However, the pathogenesis of UC is complex, and some researchers believe that immune dysregulation, genetics, bacterial infection, and eating habits might be the main risk factors for UC [3]. The inflammatory response damages the intestinal mucosa and is involved in the development of UC. In the absence of sufficient treatment, long-term inflammatory response can lead to colon cancer. Pyroptosis, different from cell apoptosis, cell death, and autophagy, can lead to cell expansion and rupture, exacerbating the inflammatory response [4]. Previous studies illuminated that the protein expression of NOD-like receptor protein domain 3 (NLRP3), Caspase-1, and Gasdermin D are upregulated in the colon tissue and induce pyroptosis in UC [5].

Accumulating evidence indicates that *Polygonum hydropiper L* (PH) is commonly used in the treatment of dysentery, gastroenteritis, diarrhea, rheumatic joint pain, swelling and other diseases [6,7]. In addition, the main ingredients of flavonoids, such as rutin, quercitrin, and quercetin, have certain anti-inflammatory effects [8]. *Coptis chinensis* (CC) has anti-inflammatory, blood sugar- and lipid-lowering properties. Alkaloids in CC have a certain therapeutic effect on intestinal mucosal injury in colitis rats and inhibit the p38/NF-κB pathway by suppressing inflammation [9].

The flavonoids in PH and the alkaloids in CC have anti-inflammatory effects and can effectively treat inflammation. Fengliao Changweikang showed a certain thera-

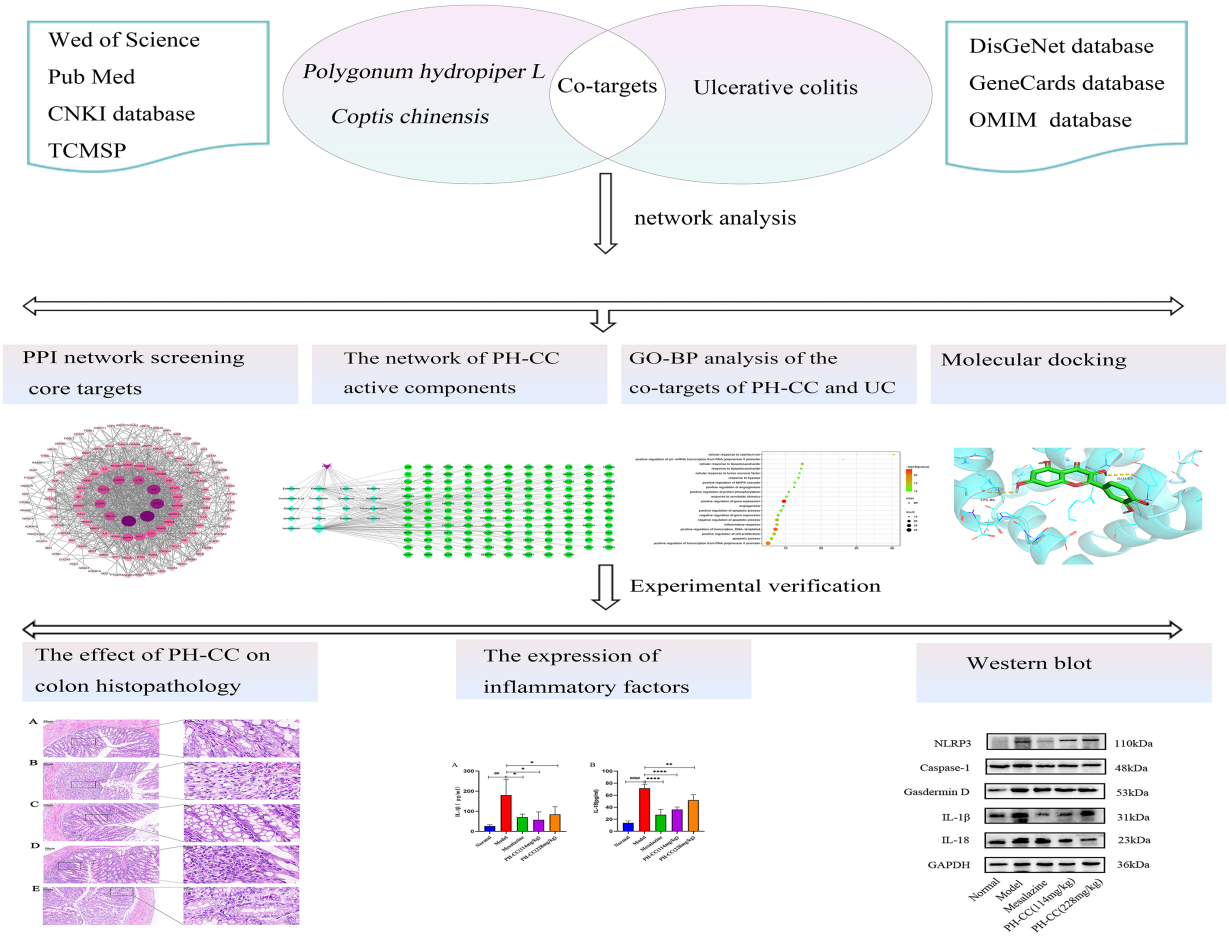

Fig. 1. Analysis strategy of Polygonum hydropiper *L-Coptis chinensis* (PH-CC) against *ulcerative colitis* (UC). CNKI, China National Knowledge Infrastructure; TCMSP, traditional Chinese medicine systems pharmacology; OMIM, Online Mendelian Inheritance in Man; PPI, Protein-Protein Interaction; GO-BP, Gene Ontology-biological process; DSS, Dextran Sulfate Sodium Salt.

peutic effect on gastrointestinal diseases in clinics [10], but *Daphniphyllum Calycinum Benth*, one of its constituents, is a toxic traditional Chinese medicine with scarce resources. The properties and flavors of CC are similar to that of *Daphniphyllum Calycinum Benth*. At the same time, it is safe, non-toxic, widely distributed, easily cultivated, and simply processed. Therefore, *Daphniphyllum Calycinum Benth* was substituted with CC and combined with PH to form a herb-pair. The systematic pharmacological research of *Polygonum hydropiper L-Coptis chinensis* (PH-CC) on UC with inflammation as the major symptom provides an experimental basis for developing new drugs for UC. The multi-component, multi-target, and multi-link properties of traditional Chinese medicine help the body to produce a comprehensive effect. Thus, this study used network pharmacology to predict the effective components and key targets of PH-CC against UC, and the potential mechanisms of PH-CC against UC was verified *in vivo*. The analysis method is shown in Fig. 1.

2. Materials and Methods

2.1 Network Pharmacology Analysis

2.1.1 Target Prediction of PH-CC and UC

The chemical components of PH-CC were searched using Wed of Science (https://www.webofscience.com/wos/), PubMed (https://pubmed.ncbi.nlm.nih.gov/), and China National Knowledge Infrastructure (CNKI, https://www.cnki.net/) databases. All chemical components, based on the traditional Chinese medicine systems pharmacology (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php), were retrieved one by one to get the active components and potential targets of PH-CC (oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18). After deleting the repeated targets, the "drug-ingredient-target" network diagram was constructed using the UniProt database (https://sparql.uniprot.org/) and Cytoscape 3.9.0 software (https://cytoscape.org/).

DisGeNet database (https://www.disgenet.org/), GeneCards database (https://www.genecards.org/), and Online Mendelian Inheritance in Man (OMIM) database

(https://www.omim.org/) were used to screen related targets of UC. After deleting the repeated targets of UC, the co-targets of PH-CC against UC and the Venn diagram were obtained were obtained through Venny2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/). Subsequently, the co-targets were used to construct Protein-Protein Interaction (PPI) networks in the search tool for recurring instances of neighboring genes (STRING, https://string-db.org/), where free nodes were hidden. Genes with scores greater than 0.4 were input into Cytoscape 3.9.0 software for visualization, and the key targets of PH-CC against UC were screened with a degree value greater than 2 times the median.

The key targets of PH-CC regulating UC were analyzed for biological process (BP) in the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/). Finally, the first 20 biological processes were selected to draw a bubble diagram, and the results were analyzed using the bioinformatics database (http://www.bioinformatics.com.cn/).

2.1.2 Molecular Docking

The abnormal activation of NLRP3 pyroptosis pathway may be an important reason for inducing UC through literature review [11]. Therefore, we selected the key proteins (NLRP3, Caspase-1, Gasdermin D) on this pathway for molecular docking verification with the active ingredients (quercetin and kaempferol) with the largest degree value. The small molecule ligands are obtained as follows: the 3D structure of the active ingredient was downloaded from TCMSP and Pubchem (https://pubchem.ncbi.nlm.nih.gov/) databases, and the above results was saved in Mol2 format through OpenBabel 3.1.1 software (https://github.com/openbabel/openbabel/releases). The macromolecular receptor proteins are obtained as follows: The 3D structure of the core target was obtained through the UniProt (https://www.uniprot.org/) and RCSB Protein Data Bank (RCSB PDB, https://www.rcsb.org/) databases and saved in PDB format. Subsequently, the above results were removed water molecules and protein residues and saved in PDBQT format by the Pymol. Finally, the ligands and receptors were docked by AutoDockTools1.5.7 software (https://autodock.scripps.edu/). The binding activity of components and targets was analyzed by calculating the binding energy.

2.2 Experimental Validation

2.2.1 Drug and Reagents

Dextran sulfate sodium (DSS, 160110) was obtained from MP Biomedicals (molecular weight: 36,000–50,000, Aurora, OH, USA). Fecal occult blood qualitative test kit (ml095013) was obtained from Shanghai Enzyme Linked Biotechnology Co., Ltd. China. Myeloperoxidase (MPO, A044-1-1) activity assay kit was purchased from Jiancheng Bio-engineering Institute (Nanjing, China). IL-1β (D721017) and IL-18 (D721113) mouse enzyme-linked immunosorbent assay (ELISA) kits were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). NLRP3 antibody (15101S), Caspase-1 (24232S), Gasdermin D (39754S) and IL-1β (31202S) were obtained from Cell Signaling Technology, Inc. (Boston, USA). IL-18 (AF5207), GAPDH Mouse Monoclonal Antibody (AF0006), HRP-labeled Goat Anti-Mouse lgG (H+L) (A0216) and HRP-labeled Goat Anti-Rabbit lgG (H+L) (A0208) were purchased from Biyuntian Biotechnology Co., Ltd. (Shanghai, China). ECL (BL520A) and BCA Protein Assay Kit (BL521A) were provided from Beijing Labgic Technology Co., Ltd. (Beijing, China). Mesalazine Enteric-coated Tablets (H19980148) was purchased from Sunflower Pharmaceutical Group Jiamusi Luling Pharmaceutical Co., Ltd.

2.2.2 Preparation of Main Components of PH-CC

Polygonum hydropiper L was collected from Wuzhishan City (Hainan, China) and identified by Prof. Niankai Zeng, Hainan Medical University. The voucher specimen (No. 20191016) was deposited in the Laboratory of Traditional Chinese Medicine, School of Pharmacy, Hainan Medical University. *Coptis chinensis* (No: 220201) was provided by Shijiazhuang Chengxin Traditional Chinese Medicine Co., Ltd. (Hebei, China) and identified by Prof. Niankai Zeng, Hainan Medical University.

After weighing the medicinal materials of Stems and leaves of PH and Rhizome of CC (mixed at 2:1), 60% alcohol with 9 times the volume of the total mass of PH-CC was used as the extracting solution to soak the medicinal materials of PH-CC overnight. Medicinal materials of PH-CC were extracted for 1 h and filtered. Subsequently, the residue was added with 8 times the volume of 60% alcohol to extract for 1 h, and the two filtrates were combined. The filtrate was concentrated under reduced pressure and purified by AB-8 macroporous resin. The 60% alcohol eluent was retained, and the eluent was concentrated under reduced pressure to obtain thick liquid medicine, which was dried into a powder using the freeze dryer and stored.

2.2.3 Animals

Sixty male SPF BALB/C mice (6–8 weeks,18–22 g) were supplied by Changsha Tianqin Biotechnology Co., Ltd., China (SCXK (xiang) 2022-0011). All mice were kept in rooms under controlled laboratory conditions (22 $\pm$ 1 °C, 12 h light/dark cycles, 40%–60% humidity) and allowed free access to food and water. The animal experiment project was conducted in strict accordance with the guidelines for the care and use of laboratory animals and approved by the Ethics Committee of Hainan Medical University (HYLL-2022-365).

2.2.4 Model Establishment and Treatment

After adaptive feeding for one week, sixty mice were randomly divided into 6 groups (n = 10): normal group,

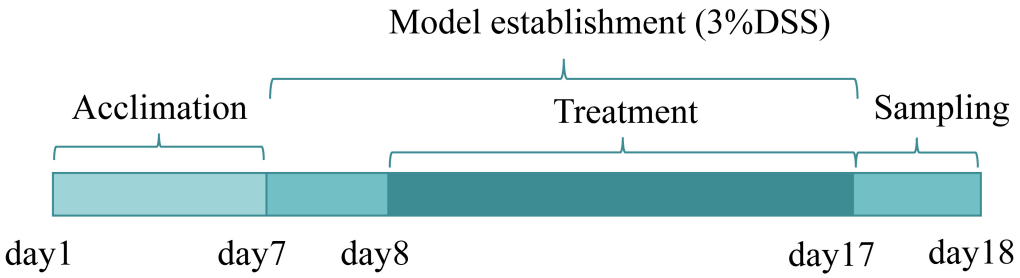

Fig. 2. The experimental design diagram.

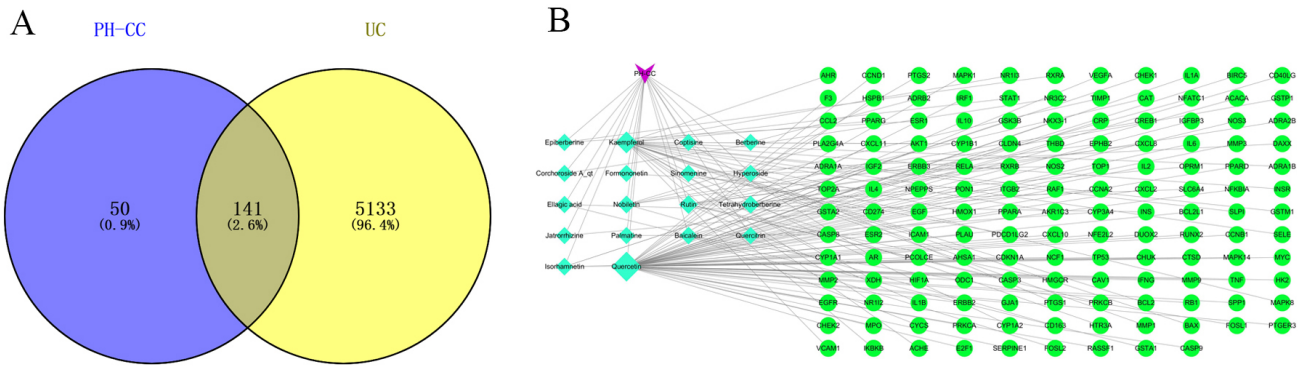

Fig. 3. The screening of active components and targets. (A) The co-targets of PH-CC and UC. (B) The network of PH-CC active component targets.

model group, mesalazine group (800 mg/kg), and PH-CC group (114 mg/kg, 228 mg/kg). Except for the normal group, mice in other groups were free to drink 3% DSS solution for 10 days, and 3% DSS solution was replaced every day. After drinking the 3% DSS solution for one day, the mesalazine group (800 mg/kg) and PH-CC group (114 mg/kg, 228 mg/kg) orally received treatment once a day for 9 days, and meanwhile, the other groups were given deionized water by gavage (Fig. 2). On the 11th day, mice were anesthetized with 1% pentobarbital sodium, and orbital blood and colonic tissues were collected.

2.2.5 Assessment of the Disease Activity Index (DAI)

Fecal occult blood: Using fecal occult blood qualitative test kit. Percentage of weight loss: The score was calculated according to the percentage of weight loss, no weight loss (0), weight loss 1%–5% (1), weight loss 5%–10% (2), weight loss 10%–15% (3), weight loss >15% (4). Stool consistency: Normal (0), loose stools (1–3), diarrhea (4). The DAI was considered the average score of the sum of the three parameters [12].

2.2.6 Histological Analysis of the Colon

The colon tissue of all groups was fixed in 4% paraformaldehyde solution. After conventional dehydration, transparency, paraffin embedding, slicing, baking, dewaxing, and H&E staining, the slices were sealed. The pathological changes of colon tissue were assessed using an optical microscope.

2.2.7 MPO Activity

Colon tissues were weighed and cut into small pieces. According to the weight-to-volume ratio (1:19), 5% homogenate suspensions were prepared by adding a homogenate medium. Following the manufacturer's instructions, MPO activity was detected.

2.2.8 The Determination of Inflammatory Factor

The expression of IL-18 and IL-1β were detected following the instructions of the Enzyme-Linked Immunosorbent Assay (ELISA) kit. The absorbance value was determined by enzyme calibration (450 nm), and the standard curve was drawn. Finally, the serum levels of inflammatory factors were calculated according to the absorbance value.

2.2.9 Western Blotting

Target proteins were subjected to electrophoresis, membrane transfer and blocking (1 h). Subsequently, they were incubated with the corresponding primary antibodies at 4 °C overnight. The primary antibodies were as follows: NLRP3 (1:1000), Caspase-1 (1:1000), Gasdermin D (1:1000), IL-1β (1:2000), IL-18 (1:1000) and GAPDH (1:5000). The next morning, the primary antibodies were recovered and the membranes were washed. They were incubated with the secondary antibodies for 2 h. Finally, the enhanced chemiluminescence reagents were used for the development exposure, saving the strips and analyzing the gray value.

Table 1. The active ingredients of PH-CC.

Number	Mol ID	Molecule name	Molecule weight	OB (%)	DL
1	MOL001454	berberine	336.39	36.86	0.78
2	MOL002897	epiberberine	336.39	43.09	0.78
3	MOL001458	coptisine	320.34	30.67	0.86
4	MOL000785	palmatine	352.44	64.60	0.65
5	MOL00789	jatrorrhizine	338.41	19.65	0.59
6	MOL001455	Tetrahydroberberine	339.42	53.83	0.77
7	MOL000098	quercetin	302.25	46.43	0.28
8	MOL001554	Scopolamine	303.39	67.97	0.27
9	MOL002907	Corchoroside A_qt	404.55	104.95	0.78
10	MOL000622	Magnograndiolide	266.37	63.71	0.19
11	MOL001457	columbamine	338.41	26.94	0.59
12	MOL002668	Worenine	334.37	45.83	0.87
13	MOL002904	8-Oxyberberine	351.38	36.68	0.82
14	MOL004368	Hyperoside	464.41	6.94	0.77
15	MOL000354	isorhamnetin	316.28	49.60	0.31
16	MOL000422	kaempferol	286.25	41.88	0.24
17	MOL000701	quercitrin	448.41	4.04	0.74
18	MOL000415	rutin	610.57	3.20	0.68
19	MOL002714	baicalein	270.25	33.52	0.21
20	MOL000392	formononetin	268.28	69.67	0.21
21	MOL005828	nobiletin	402.43	61.67	0.52
22	MOL001002	ellagic acid	302.20	43.06	0.43
23	MOL012920	sinomenine	329.43	30.98	0.46

OB, oral bioavailability; DL, drug-likeness.

2.2.10 Statistical Analysis

All data were expressed as mean ± standard deviation (SD) in the experiments and analysed by one-way ANOVA using GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA, USA). $p < 0.05$ was considered to be statistically significant.

3. Results

3.1 Network Pharmacology Analysis

3.1.1 Active Components and Targets of PH-CC Against UC

37 ingredients of PH-CC were obtained. Previous studies showed that quercitrin [13], hyperoside [14], rutin [15], jatrorrhizine [16], and columbamine [17] have strong anti-inflammatory activity; therefore, they are suitable for further studies. In total, 23 active ingredients and 191 targets were finally obtained by screening and deleting repeated targets (Table 1). In addition, 5275 UC targets were achieved, and 141 co-targets were obtained by intersecting PH-CC and UC targets (Fig. 3A). The network graph of "active ingredients-intersection targets" (Fig. 3B) demonstrate that the effect of PH-CC on UC was achieved through the interaction of "multi-active ingredients and multi-targets", in which the active ingredients were ranked by degree and the top 4 were quercetin, kaempferol, rutin and tetrahydroberberine.

3.1.2 Construction of the PPI Network

The PPI network information of 141 co-targets of PH-CC on UC was obtained in the STRING database, and disconnected nodes were hidden. Result showed that the network contained 140 nodes and 2734 edges. Subsequently, The above results are visualized and analyzed through the Cytoscape 3.9.0 (Fig. 4A,B). In total, 20 core targets were obtained, indicating that these targets may be the core targets of PH-CC in UC.

3.1.3 Gene Ontology Analysis

The first 20 biological processes (BP, $p < 0.01$) were selected to draw the bubble diagram (Fig. 5). Gene Ontology-biological processes (GO-BP) analysis showed that PH-CC against UC was involved in negative regulation of apoptosis, cell response to lipopolysaccharide (LPS), positive regulation of cell proliferation, inflammatory response, response to xenobiotic stimulus, and response to hypoxia. Among them, there were four biological processes related to inflammatory response and LPS stimulation, suggesting that PH-CC may regulate inflammatory response and LPS stimulation to alleviate UC.

3.1.4 Molecular Docking Analysis

The docking results of pyroptosis proteins and active ingredients are shown in Table 2. The absolute value of binding energy >5.0 kcal/mol indicated that the bind-

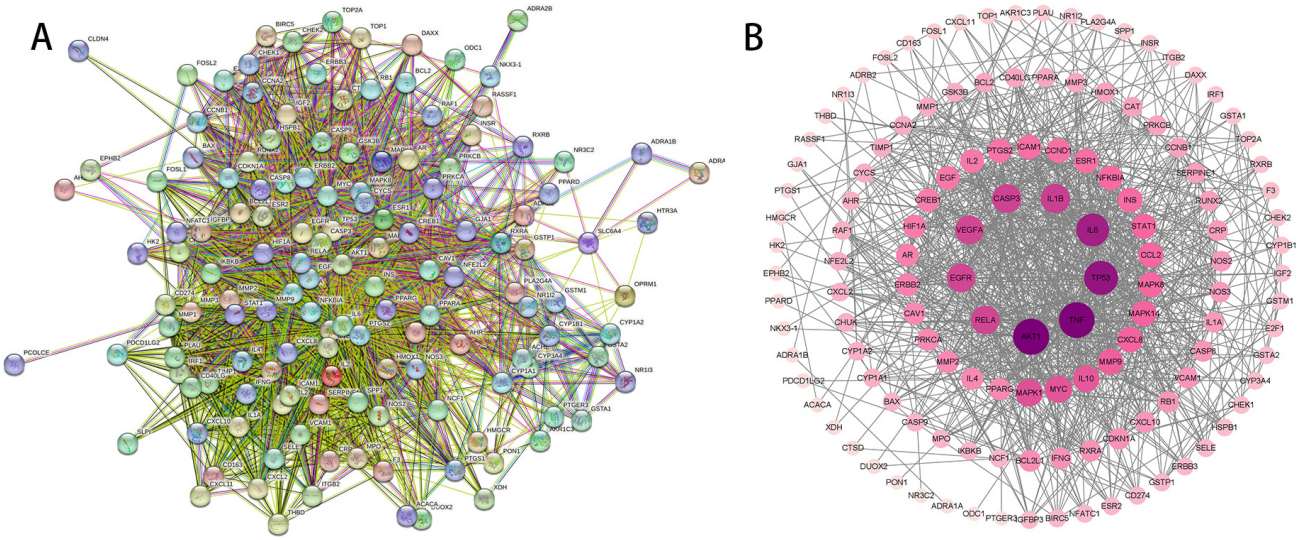

Fig. 4. The analysis of PPI network results. (A) PPI network exported from search tool for recurring instances of neighboring genes (STRING, https://string-db.org/) database. (B) PPI network visualized with Cytoscape 3.9.0.

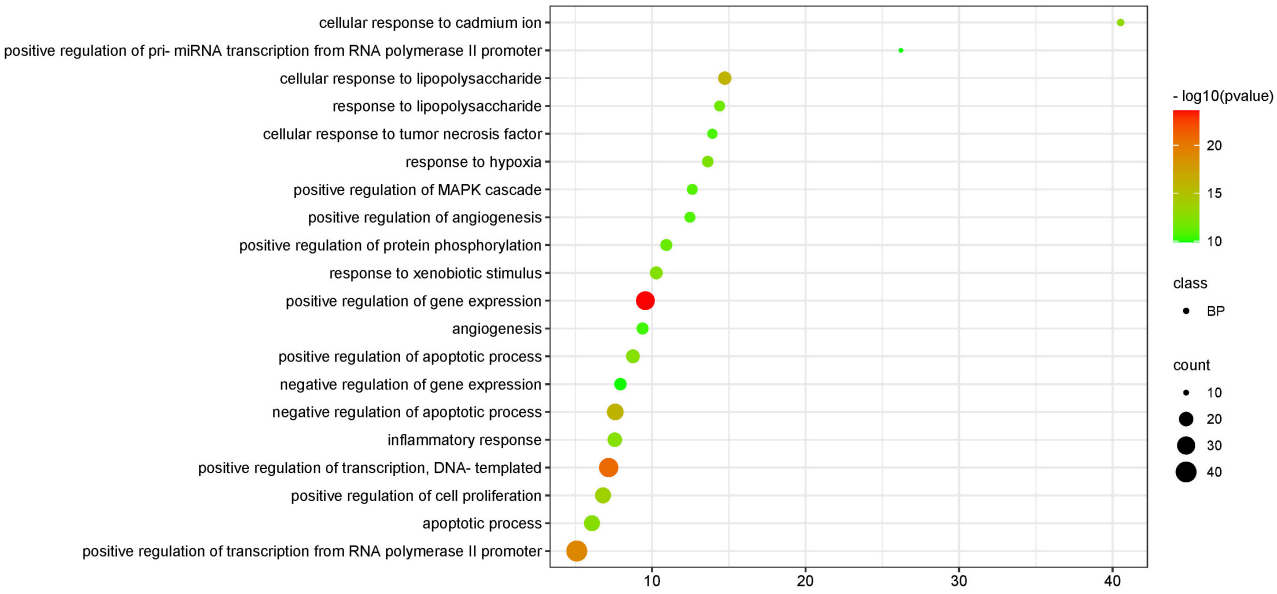

Fig. 5. GO-BP analysis of the co-targets of PH-CC and UC.

ing ability was stronger. Result indicated that the binding energy of components and targets was greater than 5.0 kcal/mol, demonstrating that these two ingredients had a strong binding ability with the core targets and to a certain extent, the binding ability between the components and the targest was verified. Finally, we visualized the docking results through pyMOL (Fig. 6).

3.2 Experimental Validation

3.2.1 PH-CC Alleviated the Symptoms of UC in Mice

Mice in the normal group had smooth hair, normal eating and drinking, brown-black granular feces, and stable body weight. Mice subjected to DSS-induced UC gradually showed lower food and water intake and obvious weight loss, were curled up and depressed, and had rough hair. Loose stool was noticeable since the third day, and occult blood and hematochezia appeared on the fifth day. After treatment with PH-CC, the symptoms of mice became milder than those in the model group. Colon length was significantly shorter, and the DAI score and MPO activity were significantly upregulated in DSS-induced mice (Fig. 7). Treatment with PH-CC reduced the above indexes.These results demonstrated that PH-CC might mitigate DSS-induced UC.

3.2.2 The Effect of PH-CC on Colon Histopathology

Histological result demonstrated that the colon of normal mice was intact, with normal mucosal epithelium and

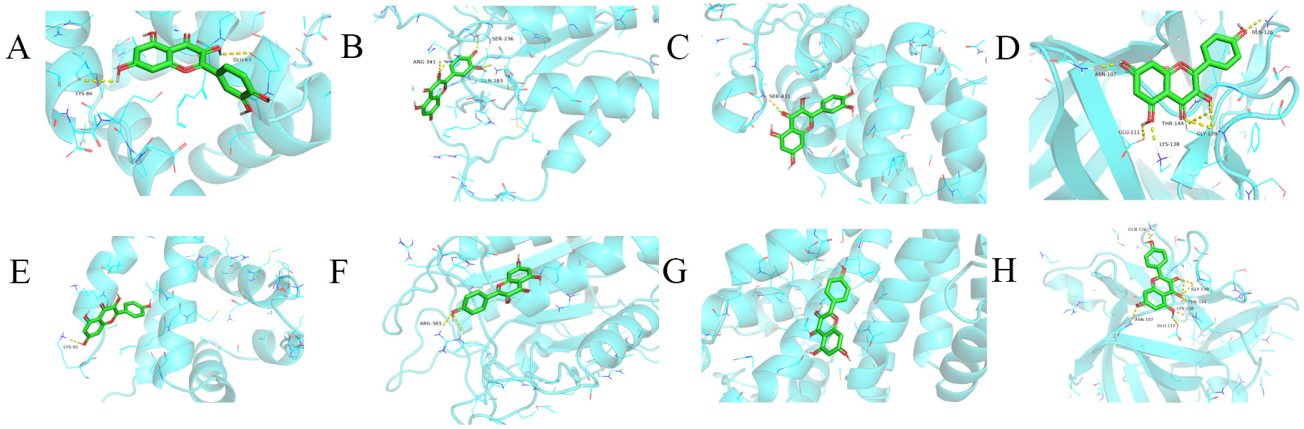

Fig. 6. The results of molecular docking for active components and core targets. (A) Quercetin and NLRP3. (B) Quercetin and Caspase-1. (C) Quercetin and Gasdermin D. (D) Quercetin and IL-1β. (E) Kaempferol and NLRP3. (F) Kaempferol and Caspase-1. (G) Kaempferol and Gasdermin D. (H) Kaempferol and IL-1β. NLRP3, NOD-like receptor protein domain 3.

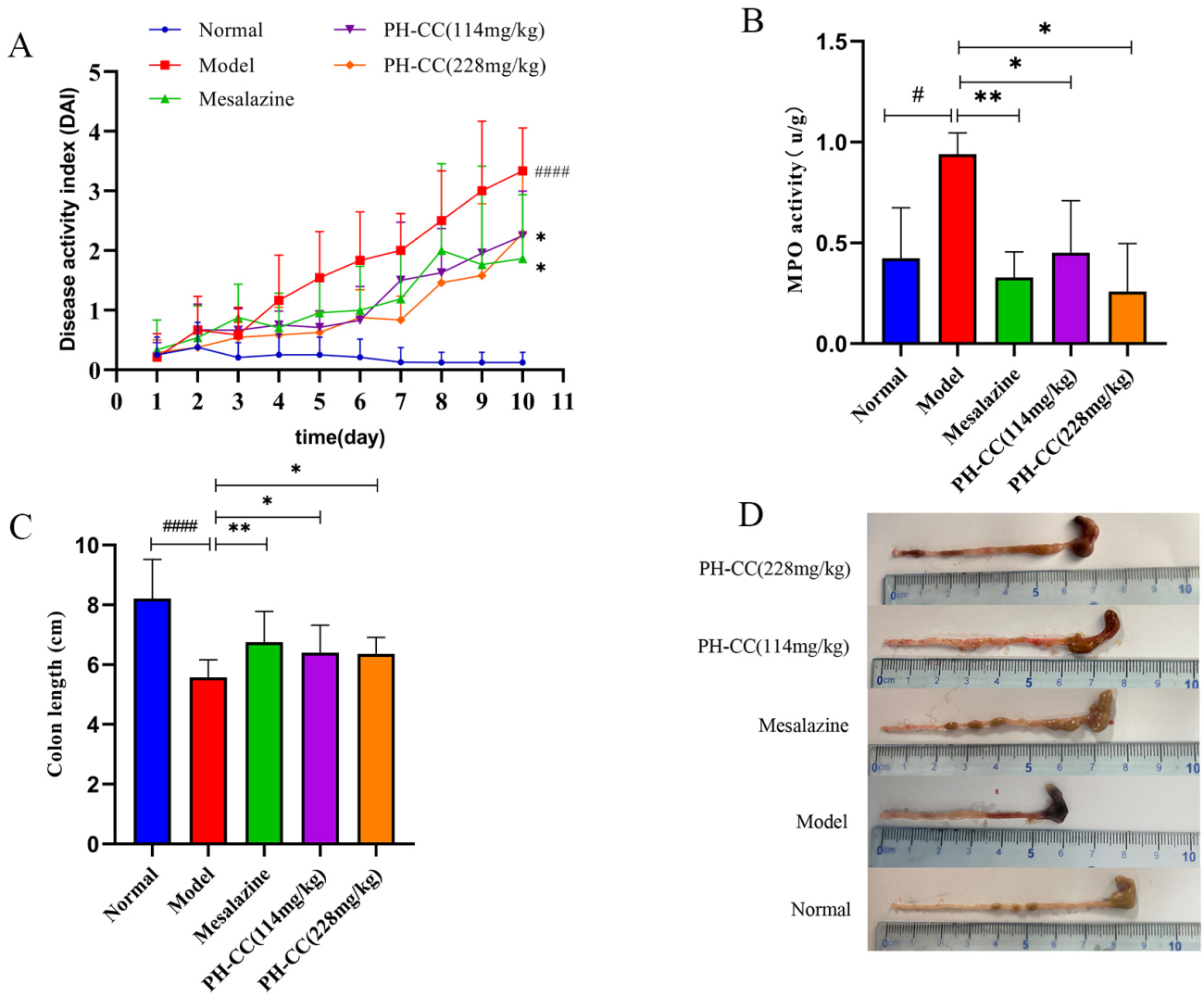

Fig. 7. PH-CC alleviated the symptoms of DSS-induced UC in mice. (A) Disease activity index. (B) Myeloperoxidase (MPO) activity. (C,D) The length of the colon. $^{\#}p < 0.05$, $^{\#\#\#\#}p < 0.0001$, normal *vs.* model; $^{*}p < 0.05$, $^{**}p < 0.01$, treatment groups *vs.* model.

Table 2. The results of molecular docking.

Targets	Phytochemicals	Binding energy (kcal/mol)
NLRP3	quercetin	–6.0
	kaempferol	–6.1
Caspase-1	quercetin	–6.9
	kaempferol	–6.2
Gasdermin D	quercetin	–7.1
	kaempferol	–6.8
IL-1β	quercetin	–7.6
	kaempferol	–7.6

without inflammatory cell infiltration, congestion, and ulceration. The colonic mucosa and crypt structure of UC mice were severely damaged, showing numerous inflammatory cells, gland disappearance, and deranged structure. The symptoms and colon injury were obviously relieved, crypt destruction and inflammatory cell infiltration were alleviated, and glandular structure was preserved in the PH-CC and mesalazine group (Fig. 8).

3.2.3 PH-CC Alleviated the Expression of Inflammatory Factors

Compared with the normal group, the serum levels of IL-1β and IL-18 in the model group were significantly increased (Fig. 9). Compared with the model group, treatment with PH-CC significantly reduced the serum levels of IL-1β and IL-18.

3.2.4 PH-CC Inhibited the Expression of Pyroptosis Proteins

Compared with the normal group, the protein levels of NLRP3, Caspase-1, Gasdermin D, IL-1β, and IL-18 were significantly upregulated in UC mice (Fig. 10). Compared with the model group, treatment with PH-CC and mesalazine could downregulate the level of the above proteins.

4. Discussion

UC is a complex disease of the gastrointestinal tract, and its incidence continues to increase in China and worldwide. The inflammation-related colon cancer in UC is more challenging to treat than other types of colon cancer, with a higher mortality rate [18,19]. The adverse reactions of glucocorticoids, sulfasalazine, and immunosuppressive agents in clinical practice are becoming more and more prominent, affecting the health status of patients [20]. Consequently, it is extremely vital to find effective and safe medicines for treating UC. PH-CC are traditional Chinese medicines. Previously, it has been reported that the extract of PH significantly improve the colon shortening induced by UC [21]. In addition, studies have found that CC can prevent intestinal barrier damage and alleviate the symptoms of UC by ameliorating gut dysbiosis and inhibiting the inflammatory response in rats [22]. Some active components of PH-CC were shown to have anti-inflammatory activity [13,23–27]. However, the relationship between PH-CC and UC has not yet been systematically elucidated. Moreover, the complexity of traditional Chinese medicines with several components made it difficult to study the potential mechanism. Therefore, the effective ingredients and potential targets of PH-CC against UC were screened through network pharmacology. We also measured the potential mechanisms in animal experiments to provide a reference for future clinical studies.

By analyzing the "drug-active component-target" network, we found that quercetin and kaempferol were the essential components of PH-CC against UC. Quercetin is the active ingredient of PH-CC, and kaempferol is the main active ingredient of PH. Twenty-three active ingredients and 191 targets of PH-CC were examined. Study found that isorhamnetin has a therapeutic effect on mice with inflammatory bowel disease through the metabolism of xenobiotics induced by pregnane X receptor (PXR) was upregulated and nuclear factor kappa-B (NF-κB) signal transduction was downregulated [28]. Kaempferol may play a protective role in colitis mice by regulating intestinal microflora and toll-like receptor 4 (TLR4)-related signaling pathways [29]. The plant rhizome extract with quercetin can attenuate DSS-induced colonic histopathological changes in UC and reduce the expression of malondialdehyde,myeloperoxidase and nitric oxide (NO) to achieve the effect of treating UC [30]. Study showed that berberine attenuated LPS-induced intestinal injury by inhibiting the release of inflammatory factors and the activation of toll-like receptor 4 and NF-κB [31,32]. Jatrorrhizine reduced the expression of myeloperoxidase in DSS-induced UC, and up- regulated the expression of IL-10 and TGF-β to a certain extent [33]. Palmatine, epiberberine and coptisine all have the effect of activating aryl hydrocarbon receptor (AHR), suggesting that these alkaloids can regulate the number of ILC3s cells and increase the release of IL-22 to promote the repair of intestinal mucosal barrier function [34].

In this study, 141 co-targets of PH-CC against UC were obtained, and the top five core targets (TNF-α, IL-6, IL-1β, AKT1, and TP53) were screened by PPI network. TNF-α, IL-6, and IL-1β can regulate intestinal inflammation in UC by activating immune cells [35–37]. As for the docking test, we found that quercetin and kaempferol have higher binding ability to pyroptosis proteins, suggesting that they may be the core targets of PH-CC in treating UC. Furthermore, we further verified how pyroptosis affects UC *in vivo* experiments. In addition, the four biological processes were related to inflammation and LPS stimulation through GO-BP enrichment analysis, indicating that PH-CC may alleviate UC by modulating the inflammatory response and LPS-induced cellular response. Meanwhile, three core targets (TNF-α, IL-6 and IL-1β) were enriched in the aforementioned biological processes. In addition, some

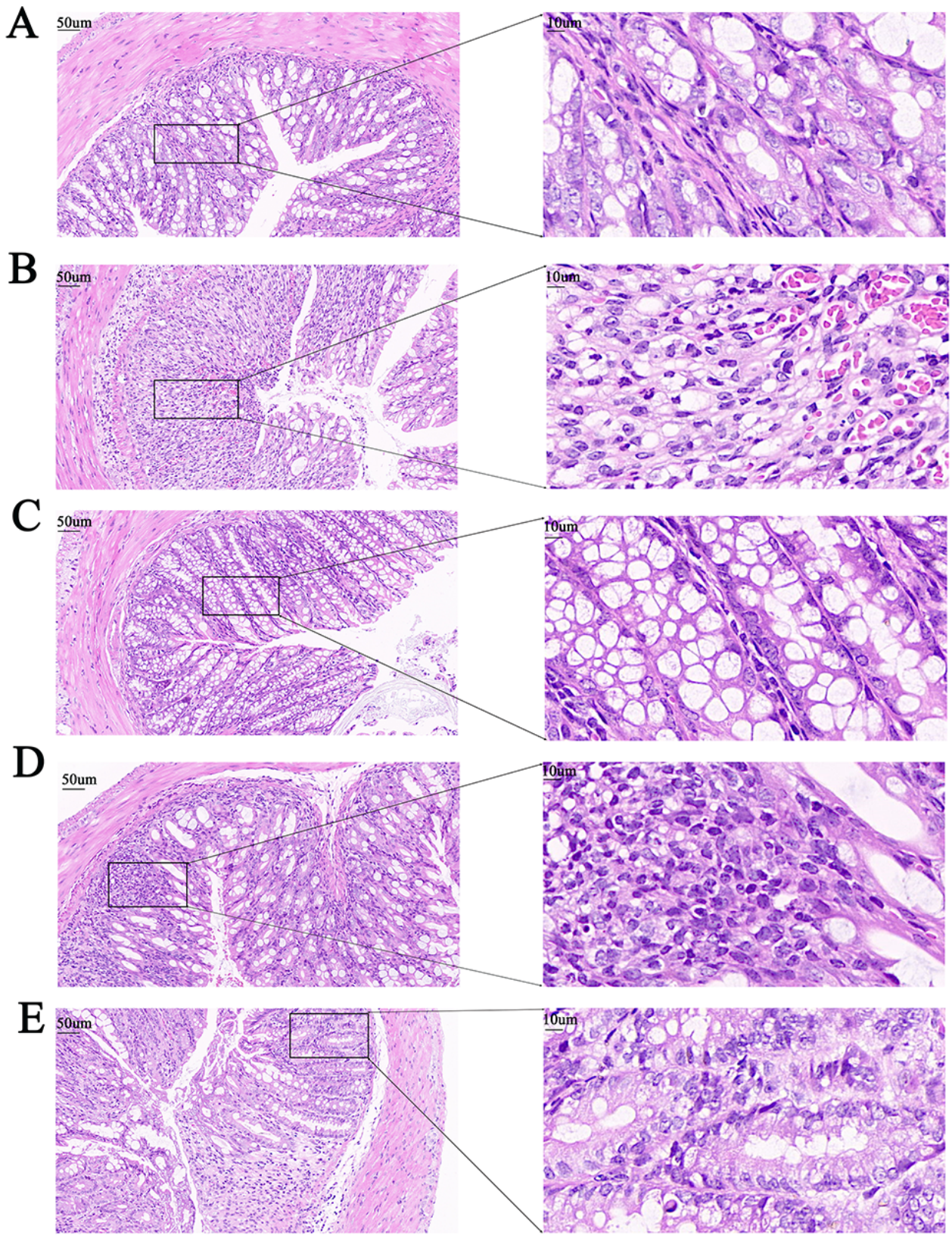

Fig. 8. Effect of PH-CC on histopathological changes of colon of UC mice (HE×20, HE×100). (A) Normal group. (B) Model group. (C) Mesalazine group. (D) PH-CC 114 mg/kg group. (E) PH-CC 228 mg/kg group. HE, hematoxylin-eosin staining.

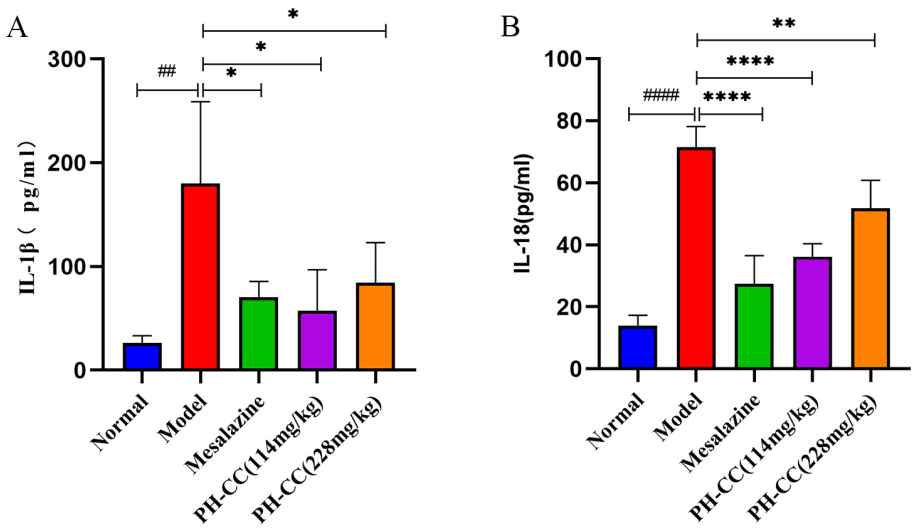

Fig. 9. PH-CC inhibited the inflammatory response in UC mice. The serum levels of IL-1β (A) and IL-18 (B) in each group. $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$, normal *vs.* model; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$, treatment groups *vs.* model.

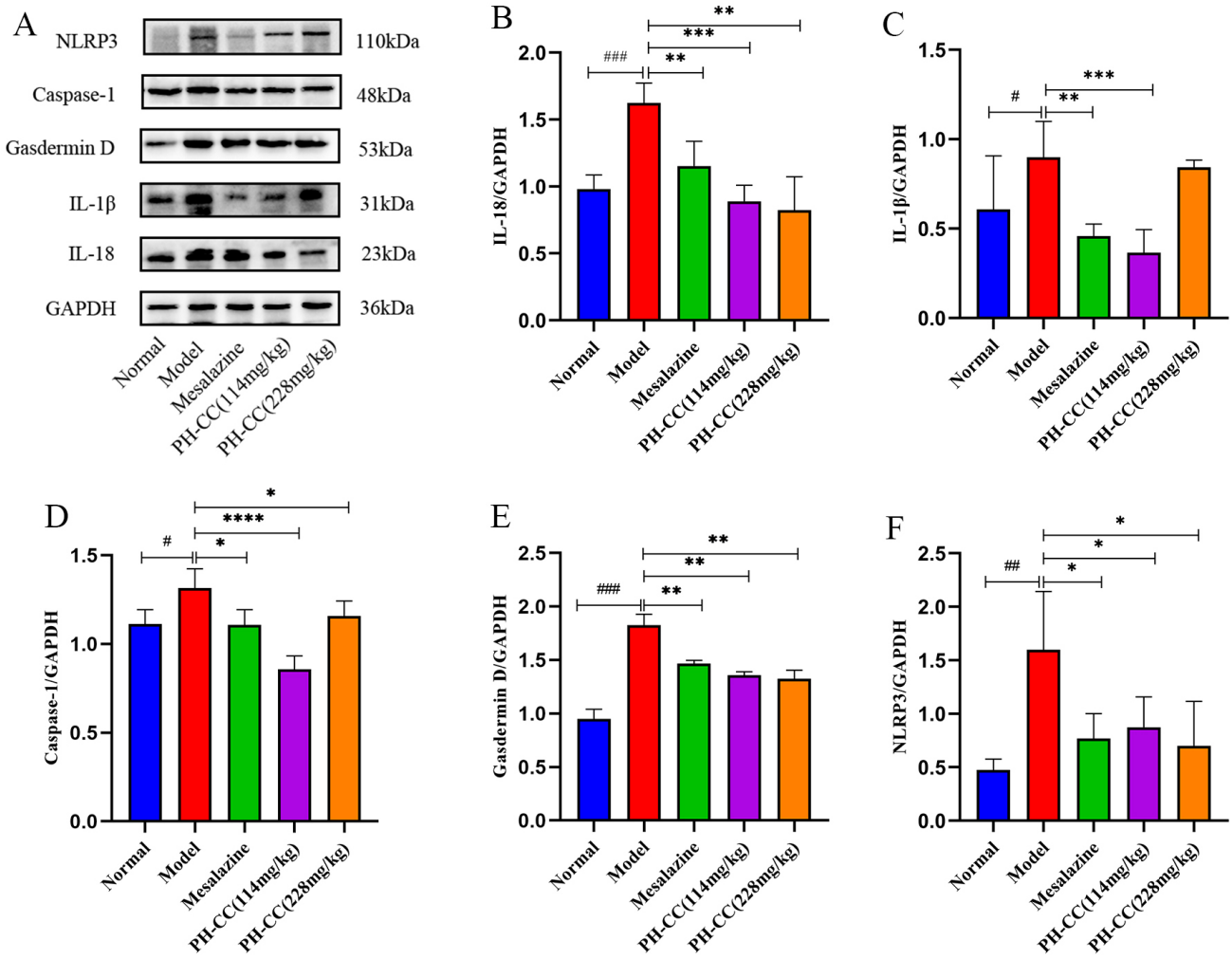

Fig. 10. Effect of PH-CC on the expression of pyroptosis proteins. Effect of PH-CC on pyroptosis proteins electrophoresis in UC mice colon tissue (A), The protein levels of IL-18 (B), IL-1β (C), Caspase-1 (D), Gasdermin D (E), and NLRP3 (F) were normalized to GAPDH. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, normal *vs.* model; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, treatment groups *vs.* model.

studies also confirmed that inflammation and cellular response to LPS were the main factors inducing UC [38,39].

Overexpression of LPS in intestinal mucosa can cause neutrophil infiltration and subsequent intestinal mucosal cell damage and inflammatory factor release [40,41]. It is noteworthy that LPS is also an important activator of Caspase-1. LPS activates cytoplasmic lipid A, thereby activating Caspase-1 and triggering pyroptosis [42]. The abnormal activation of NLRP3 can cause uncontrollable inflammatory response and induce pyroptosis, and further leads to the occurrence and aggravation of UC. Therefore, blocking the activation of NLRP3 may alleviate UC. Animal experiments have confirmed that inhibiting the activation of Caspase-1 and NLRP3 in colitis mice can improve colon inflammation [43]. Pyroptosis induces cell enlargement and rupture, an inflammatory factor release, intestinal mucosal barrier damage, and infernal circle formation [44]. Sustained inflammation and pyroptosis are positively correlated with the severity of UC. However, whether PH-CC alleviates pyroptosis in intestinal mucosal cells remains unclear. Therefore, this study intends to further analyze the underlying mechanism of PH-CC against UC from the perspective of pyroptosis.

Pyroptosis, a novel mode of inflammatory programmed cell death, became a research hotspot in the pathogenesis of UC [45]. NLRP3, an inflammatory complex associated with inflammation, can be activated by bacterial, viral, fungal infection, K^+ efflux and endogenous damage signals [46,47]. Activated Caspase-1 can convert inactivated precursors of interleukin 1β and interleukin 18 into mature IL-1β and IL-18 to be released. IL-1β and IL-18, two proinflammatory cytokines mainly released by macrophages, can upregulate other chemokines and growth factors, exacerbating inflammation and intestinal mucosal injury [48].

This study adopted a DSS-induced UC model in mice [49]. DSS-induced UC can destroy the colonic mucosal barrier, increase colon permeability, lead to the secretion imbalance of inflammatory and anti-inflammatory factors, Th1/Th2 imbalance, and gut dysbiosis [50–53]. The inflammatory-immune response mechanism in DSS-induced UC is similar to the pathogenesis of human UC. Therefore, the DSS-induced UC model has been recognized and widely used in experimental studies of UC [54–56]. The DAI and colon length can help assess DSS-induced UC model whether it is successfully established [57,58]. Our results showed that DSS-induced UC mice had hair discoloration, slow response, significant weight loss, bloody stool, and anal bleeding. Compared with the normal group, the DAI was significantly elevated, and colon length was shortened in UC mice. The histopathological assessment showed incomplete shedding of colon mucosa, derangement of intestinal glands, and severe crypt damage in the model group.

Immune-mediated inflammatory response is a main pathological feature of UC. Our results indicated that the tissue expression of NLRP3, Caspase-1, and Gasdermin D was increased in UC mice. Concomitantly, the expression of activated IL-1β and IL-18 was also upregulated. On the other hand, treatment with PH-CC suppressed the protein levels of the above proteins. The results demonstrated that PH-CC can inhibit pyroptosis and reduce inflammatory response to improve the symptoms of UC by regulating the NLRP3/Caspase-1 signaling pathway.

MPO is a neutrophil marker released into phagosomes to promote reactive oxygen species (ROS) generation and accelerate local intestinal inflammation [59]. Furthermore, MPO activity has a close relation with intestinal inflammation [60]. In the present study, MPO expression was obviously upregulated in UC mice and significantly decreased after treatment with PH-CC. These results demonstrated that DSS-induced UC mice increased neutrophil infiltration which exacerbated colonic mucosal inflammation. On the other hand, PH-CC inhibited the inflammatory response and improved the repair process by reducing neutrophil infiltration.

IL-1β can induce neutrophils to produce numerous inflammatory substances, which can lead to mucosal hyperemia, edema, necrosis, and ulcers. Clinical research indicated that the serum level of IL-1β in patients with UC is markedly higher than that in normal subjects, and IL-1β level predicts disease severity in these patients [61]. In this study, We found that PH-CC can reduce the serum levels of the above inflammatory factor.

Study proved that activated NLRP3 inflammasome enhances inflammatory factors expression in UC [62]. Research found that curcumin and *Morus macroura* Miq. fruit extract decreased ROS generation, downregulated activated NLRP3, and inhibited the expression of inflammatory factors to relieve intestinal lesions in the mice model of DSS and acetic acid-induced UC [46,63]. Similarly, studies have shown that by inhibiting NLRP3, its downstream molecules, pyroptosis, cardamonin alleviated intestinal inflammation [64]. Our results are basically consistent with previous findings.

5. Conclusions

In conclusion, our study provides preliminary evidence supporting the efficacy of PH-CC against UC through network Pharmacology and *in vivo* experiments, thereby offering a novel perspective for further mechanistic investigations on the therapeutic action of PH-CC in UC. However, there are still certain limitations in this study. Firstly, a more comprehensive analysis of the synergy between the predicted multi-components and multi-targets is lacking. Additionally, the experimental verification method employed here appears relatively singular. Therefore, it is imperative to conduct further in-depth molecular biology

experiments in order to provide a more precise experimental foundation for both the prediction results of this study and their potential clinical applications.

Abbreviations

UC, Ulcerative colitis; PH-CC, *Polygonum hydropiper L-Coptis chinensis*; NLRP3, NOD-like receptor protein domain 3; TCMSP, Traditional Chinese Medicines for Systems Pharmacology; OMIM, Online Mendelian Inheritance in Man; STRING, Search Tool for Recurring Instances of Neighbouring Genes; DAVID, Database for Annotation, Visualization and Integrated Discovery; GO, Gene Ontology; IL-1β, Interleukin-1beta; DSS, Dextran sulfate sodium; DAI, Disease Activity Index; ELISA, Enzyme-Linked Immunosorbent Assay; TBST, Tris Buffered Saline with Tween 20; DL, drug-likeness; OB, oral bioavailability.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

FZ and SR conceived and designed the experiments; FZ conducted the experimental work and analysis; YL analyzed network pharmacological data and assisted in animal experiment; FZ and YZ developed the animal models; FZ drafted the manuscript; HN made colon pathological sections and analyzed the pathological results; SR and HN provided major revisions and comments to the manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

This study was approved by the Animal Ethics Committee of Hainan Medical College to pass the review of animal experiment ethics (HYLL-2022-365). The plants of *Polygonum hydropiper L* and *Coptis chinensis* were used in this study. *Polygonum hydropiper L* (No: 20191016) collected from Wuzhishan City (Wuzhishan, China) was identified by Prof. Niankai Zeng (the School of Pharmacy, Hainan Medical University, Haikou, China). *Coptis chinensis* (No: 220201) was purchased (Shijiazhuang Chengxin Traditional Chinese Medicine Co., Ltd., Shijiazhuang, China) and was identified by Prof. Niankai Zeng (the School of Pharmacy, Hainan Medical University, Haikou, China).

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Conflict of Interest

The authors declare no conflict of interest.

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