Paeoniflorin improves ulcerative colitis via regulation of PI3K-AKT based on network pharmacology analysis

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Abstract. Paeoniflorin (PF) is the primary component derived from *Paeonia lactiflora* and white peony root and has been used widely for the treatment of ulcerative colitis (UC) in China. UC primarily manifests as a chronic inflammatory response in the intestine. In the present study, a network pharmacology approach was used to explore the specific effects and underlying mechanisms of action of PF in the treatment of UC. A research strategy based on network pharmacology, combining target prediction, network construction, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, and molecular docking simulation was used to predict the targets of PF. A total of 288 potential targets of PF and 599 UC-related targets were identified. A total of 60 therapeutic targets of PF against UC were identified. Of these, 20 core targets were obtained by protein-protein interaction network construction. GO and KEGG pathway analyses showed that PF alleviated UC through EGFR tyrosine kinase inhibitor resistance, the IL-17 signaling pathway, and the PI3K/AKT signaling pathway. Molecular docking simulation showed that AKT1 and EGFR had good binding energy with PF. Animal-based experiments revealed that the administration of PF ameliorated the colonic pathological damage in a dextran sulfate sodium-induced mouse model, resulting in lower levels of proinflammatory cytokines including IL-1β, IL-6, and TNF-α, and higher levels of IL-10 and TGF-β. PF decreased the mRNA and protein expression levels of AKT1, EGFR, mTOR, and PI3K. These findings suggested that PF plays a therapeutic protective role in the treatment of UC by regulating the PI3K/AKT signaling pathway.

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that occurs in the mucosa and submucosa of the large intestine. The primary clinical manifestations are bloody diarrhea, mucopurulent bloody stool and abdominal pain, fecal urgency, and tenesmus, and in severe cases, weight loss and fever (1-3). The overall incidence and prevalence of UC are 1.2/20.3 and 7.6/245 cases per 100,000 individuals per year, respectively (4,5). Epidemiological studies have shown a bimodal distribution of age at UC onset, with a peak incidence between 20-30 years, followed by another peak at 50-80 years (6). The pathogenesis of UC is complex, and environmental factors, genetic susceptibility, intestinal microbiology, and a dysregulated immune response are major risk factors (7,8). In recent years, 5-aminosalicylic acid, corticosteroid drugs, Janus kinase inhibitors, and anti-TNF-α antibodies have been widely applied for the treatment of UC (9).

At present, the incidence of UC is high, and the mortality rate is low. UC not only is incurable, but it is also a challenge to manage. Patients suffer from intractable or intolerable side effects of UC therapeutics following administration, which leads to a high recurrence rate, and UC is closely associated with colorectal cancer (CRC) (10-12). The high incidence of UC in developed and developing countries has evolved into a global burden, and several treatments for UC also induce resistance to said treatments in patients. Thus, there is an urgent need to identify novel drugs that will be effective in achieving optimal disease control (13).

At present, several traditional Chinese formulas, such as *Shen-Ling-Bai-Zhu-San* (14), *Tongxie Yaofang* (15), *Pulsatilla decoction* (16), *Huangkui Lianchang decoction* (17), and *Gegen Qinlian decoction* (18), amongst others, are used for the treatment of UC in China (19). *Tongxie Yaofang* consists of four drugs *Rhizoma Atractylodis Macrocephalae*, *Radix Paeoniae Alba*, *Pericarpium Citri Reticulatae*, and *Radix Saposhnikovia Divaricata* (20) and exhibited efficacy for the treatment of UC in our previously reported work (21). Paeoniflorin (PF) is the primary bioactive component isolated from *Radix Paeoniae Alba*, and *Paeonia suffruticosa Andr.* has been used in several other traditional Chinese formulas for

the treatment of inflammatory bowel diseases, UC, and other gastric and splenic diseases for >2,000 years (22,23).

Several studies have revealed that PF exhibits anti-tumor, anti-inflammatory, anti-oxidative, and immunoregulatory effects (24-26). It has been shown that PF reduces TNF-α, IL-6, and IL-1β levels to protect against inflammatory pain (27). However, the molecular mechanisms underlying the protective effects of PF against dextran sulfate sodium (DSS)-induced UC remain unclear.

Based on the advances in the fields of molecular biology, systems biology, polypharmacology, and bioinformatics, network pharmacology has been developed as a means to reveal the mechanisms of drugs and is considered a cost-effective method of drug development (28). In recent years, network pharmacology has been applied to determine the actions of active compounds and to analyze the mechanisms of action of natural products in the treatment of several diseases (29).

To further analyze the therapeutic mechanism of action of PF in UC, network pharmacology was used to predict the targets and pathways by which PF exerted its effects in UC. Then, the relevant pathways were selected for validation *in vivo* to confirm the therapeutic mechanisms of PF in animal models.

Materials and methods

Screening of PF and UC prediction targets. The Structure Data File (SDF) of PF (C23H28O11) was downloaded from the PubChem database (PubChem CID: 442534; https://pubchem.ncbi.nlm.nih.gov/) and then imported into the PharmMapper database (http://www.lilab-ecust.cn/pharmmapper/) to obtain prediction targets with the conformation generation was set to 'generate conformers' and the pharmacophore mapping was set to 'druggable pharmacophore models (v2017, 16159)'. UC-related human genes were collected from three databases; namely, the Therapeutic Target Database (TTD) (http://bidd.nus.edu.sg/group/cjttd/), Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/), and GeneCards (https://www.genecards.org/). These disease-target network databases were used to verify whether the genes were related to UC. The official gene symbols of the targets were normalized in the UniProt (http://www.UniProt.org) database. The common terms amongst the disease-related targets and drug component targets were obtained using Venny 2.1.0 (http://bioinfo.cnb.csic.es/tools/venny/index.html).

Protein-protein interaction (PPI) analyses. A PPI network was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/; version 10.5). The selection criteria were '*Homo sapiens*' for species and a confidence score >0.4. Then, the PPI network was imported into Cytoscape (30), and the plugin cytoHubba was used to find the clusters (highly interconnected regions) within the PPI network (31). The top-ranked genes were selected as hub targets based on the degree levels. Four topological features (degree, betweenness centrality, average shortest path length and closeness centrality) were analyzed using Network Analyzer (32). The major hub network comprising putative major components and major targets was extracted by defining nodes with degrees higher than the average number of neighbors.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. After obtaining the core target, clusterProfiler (33) in R (34,35) version 3.6.1 was used to perform the GO (36,37) and KEGG (38) enrichment analyses in the RStudio platform 2022.12.0-353. GO enrichment primarily analyzes the biological processes (BPs), cellular composition (CC), and molecular functions (MFs) of the common targets. KEGG pathway enrichment (www.kegg.jp/kegg/kegg1.html) was used to identify the critical pathways that were closely related to the treatment of UC by PF.

Molecular docking simulation. The binding of the potential target and its corresponding components were evaluated by molecular docking simulation. Molecular docking simulations of potential targets and their corresponding components were performed using AutoDock version 4.2 (39) and AutoDock Vina software (Scripps Research Institute) (40) according to published methods. The macromolecular protein target receptors were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank database (https://www.rcsb.org), and the 2D structures of the small-molecule compound components were obtained from the PubChem Database (https://pubchem.ncbi.nlm.nih.gov).

Chemicals and reagents. PF (CAS:23180-57-6) was obtained from Chengdu Herbpuritfy Co., Ltd. and its identity was confirmed by high-performance liquid chromatography with a purity of 99.71%. ELISA kits for IL-6 (cat. no. EK206), IL-10 (cat. no. EK210), TNF-a (cat. no. EK282), TGF-β (cat. no. EK981), and IL-1β (cat. no. EK201B) were purchased from Multisciences (Lianke) Biotech, Co., Ltd. The primers for reverse transcription-quantitative (RT-q) PCR and the Promega M-MLV vector were purchased from Shanghai GeneChem Co., Ltd. All antibodies were purchased from Affinity Biosciences Co., Ltd.

Establishment of the UC mouse model and dosing. A total of 27 specific pathogen-free male Balb/c mice (6 weeks old, from Jinan Peng Yue Experimental Animal Technology Co., Ltd.), weighing 18±2 g, were kept in the Animal Laboratory of Jining Medical University. The animal experiments were approved by the Animal Ethics Committee of Jining Medical University (approval no. JNMC-2020-DW-ZXY-001). If weight loss reached 15-20% of the original weight within 72 h, then the mice were sacrificed by cervical dislocation. After 3 days of adaptive feeding, mice were randomly divided into the following three groups (n=9 per group): Control group, model group, and treatment group. For DSS-induced colitis, 5% (w/v) DSS (Meilun Biotech Co., Ltd.) was added to the drinking water in the model group and treatment group for 7 days. After the establishment of the DSS-colitis model, mice in the control and model groups were administered normal saline, while mice in the treatment group were administered PF (20 mg/kg/day). All administration methods were by gavage, and the mice were dosed continuously for 7 days. Mice from each group were sacrificed by cervical dislocation after 14 days.

Disease activity index (DAI) score. The DAI score in each group was taken during the experimental period. The DAI score was calculated according to loss of body weight, stool

consistency and gross bleeding, as follows: DAI score=(weight loss score + stool shape score + stool blood score)/3 (41,42).

Hematoxylin and eosin staining. Colonic tissue was obtained from the mice and fixed in 10% formalin for 24 h at room temperature and then dehydrated using an increasing alcohol gradient, embedded in paraffin and sectioned in 5-μm thick slices. Then, the sections were stained with hematoxylin and eosin (H&E) for histopathological examination.

ELISA. ELISA kits were used to detect the serum levels of IL-10, TGF-β, IL-6, IL-1β, and TNF-α according to the manufacturers' instructions. The optical density value was measured at 450 nm using a microplate reader (Model 680; Bio-Rad Laboratories, Inc.), and the concentration was calculated using a standard curve.

RT-qPCR. Total RNA was isolated from the tissues using TRIzol® Reagent (Shanghai Pufei Biotechnology Co., Ltd.) according to the manufacturer's protocol. For RT-qPCR analysis, first-strand cDNA synthesis from 1 μg total RNA was performed using an M-MLV Reverse Transcriptase kit according to the manufacturer's protocol (Promega Corporation). The resulting cDNA was analyzed by qPCR using the SYBR Green PCR kit (Takara Bio, Inc.) and the 7500 Fast Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). β-actin was used as the loading control. Comparative quantification was performed using the $2^{-\Delta\Delta Cq}$ method (43). The primers sequences are listed in Table I.

Western blot analysis. Total proteins were obtained from colon tissues using RIPA lysate and then quantified using the BCA method. Equal amounts of protein (50 μg/lane) were separated by 10% SDS-PAGE gel using 100 V for 2 h and then subsequently transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk for 2 h at room temperature and washed and then incubated with primary antibodies (1:500 dilution) against EGFR (cat. no. AF6043), p-PI3K (cat. no. AF3242), p-mTOR (cat. no. AF3308), Bcl-2 (cat. no. AF6139), p-AKT1 (cat. no. AF0016), PI3K (cat. no. AF6241), mTOR (cat. no. AF6308), and AKT1 (cat. no. AF0836) overnight at 4˚C. Then, the membranes were incubated with horseradish peroxidase-labeled secondary antibody (cat. no. S0002, 1:5,000 dilution) for 2 h at room temperature after washing with Tris-buffered saline containing Tween-20 (0.1%) three times. Signals were visualized using enhanced chemiluminescence reagent and analyzed using ImageJ (1.53 h; National Institutes of Health).

Statistical analysis. Data are presented as the mean ± SD. An unpaired Student's t-test was used to assess differences between the two groups. A one-way ANOVA followed by a Dunnett's post hoc test was used for comparisons between multiple groups. GraphPad Prism version 8.0.0 (GraphPad Software, Inc.) was used for statistical analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Screening of PF and UC prediction targets. The chemical structure of PF was obtained from the PubChem database.

Table I. Sequences of the primers.

Gene	Sequence, 5'-3'
PI3K	
Forward	TCATTGACAGTAGGAGGAGGTT
Reverse	TATTTTCATTCCCCAGCCAC
AKT1	
Forward	TGGACTCAAGAGGCAGGAAG
Reverse	GGTTCTCAGTAAGCGTGTGG
mTOR	
Forward	AGGTCATGCCCACATTCCTT
Reverse	AGCCACCACAATCTGCTCAA
BCL2 F	
Forward	ATGCCTTTGTGGAACTATATGGC
Reverse	GGTATGCACCCAGAGTGATGC
EGFR	
Forward	AACTCTTCGGGACACCCAATCA
Reverse	CAGCCTTCCGAGGAGCATAAA
Actin	
Forward	GCACCACACCTTCTACAATG
Reverse	GTGAGGGAGAGCATAGCC

Then the SDF file of PF was imported into the PharmMapper server (http://www.lilab-ecust.cn/pharmmapper/index.html). A total of 288 potable targets were obtained in PharmMapper with NormFit scores >0. Additionally, a total of 599 potential gene targets were obtained from the OMIM, TTD, and GeneCards databases. Common targets identified from both the active ingredient and UC-related searches were considered potential targets. A total of 60 common potential genes were screened by Venny (Fig. 1).

Construction of the PPI network. The 60 common targets were submitted to STRING (https://string-db.org/) to construct a PPI network. Then, the PPI network was constructed and visualized using Cytoscape. The network contained 60 nodes and 481 edges, and the average node degree was 16 (Fig. 2A). Two key topological parameters (degree and betweenness centrality) were used to characterize the most important nodes in the network; higher quantitative values of the topological parameters indicated greater importance of the node. A Cytoscape plug-in, Network Analyzer, was used to identify the network topology attributes to identify the core genes of the PPI network (32). Based on this principle, the top 20 nodes were screened out as key components, and the top 10 included the following: ALB (albumin, degree=51), AKT1 (AKT serine/threonine kinase 1, degree=41), MMP9 (matrix metallopeptidase 9, degree=40), CASP3 (degree=36), SRC (degree=36), EGFR (degree=35), IGF1 (degree=33), MMP2 (degree=29), ESR1 (estrogen receptor 1, degree=28), IL-2 (degree=28) and MAPK14 (mitogen-activated protein kinase 14, degree=25) (Fig. 2B).

GO and KEGG enrichment analysis. GO and KEGG pathway enrichment analyses were performed using the Metascape database and R for visualization of the 60 common target

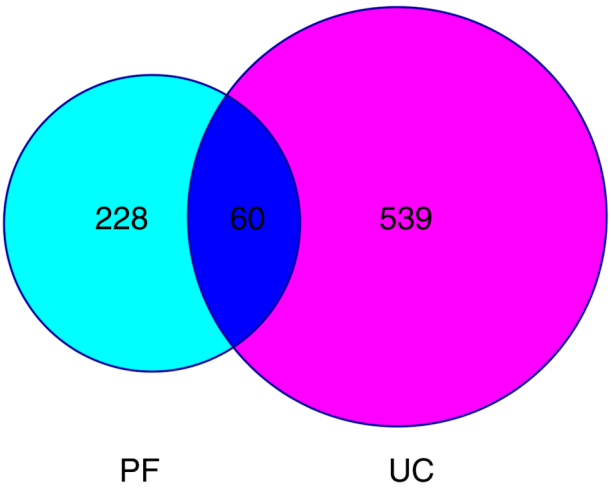

Figure 1. Venn diagram of the target genes of PF and UC. PF, paeoniflorin; UC, ulcerative colitis.

genes. In terms of MFs, treatment of UC with PF primarily involved endopeptidase activity, serine-type peptidase activity, serine hydrolase activity, nuclear receptor activity, transcription factor activity, and direct ligand-regulated sequence-specific DNA binding (Fig. 3A). The BPs primarily involved regulation of the inflammatory response, neutrophil-mediated immunity, response to reactive oxygen species, response to lipopolysaccharide, and response to molecules of bacterial origin (Fig. 3B). The CC primarily involved vesicle lumen, secretory granule lumen, cytoplasmic vesicle lumen, collagen-containing extracellular matrix, and membrane raft (Fig. 3C).

The top 20 functionally enriched processes were selected to plot a bubble diagram following KEGG enrichment analysis (Fig. 3D). The primary signaling pathways involved in the treatment of UC were identified, and the top five pathways included proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, endocrine resistance, prostate cancer, fluid shear stress, and atherosclerosis. The IL-17, PI3K/AKT, and HIF-1 signaling pathways were also included in the top 20 pathways. Among them, 12, 7, and 7 genes were enriched in the PI3K/AKT (AKT1, BCL2L1, EGFR, FGFR2, IGF1, IGF1R, IL2, JAK2, KDR, MET, NOS3, and SYK), IL-17 (CASP3, LCN2, MAPK14, MMP13, MMP3, MMP9, S100A9), and HIF-1 (AKT1, EGFR, HMOX1, IGF1, IGF1R, NOS2, NOS3) signaling pathways, respectively.

Molecular docking simulation analysis. Docking analysis was conducted between PF and the core genes related to the PI3K/AKT signaling pathway (Fig. 4). According to the molecular docking results, docking of PF with AKT1 and EGFR proteins had a good binding energy of -2.44 kcal/mole and -2.19 kcal/mole respectively, and AKT1-PF had the highest binding affinity.

PF reduces DSS-induced experimental colitis. The control group showed a steady weight increase. After administration of DSS, a significant loss in the body weight of the animal was observed, and this continued until the end of the experiment in the PF group and model group compared with the control

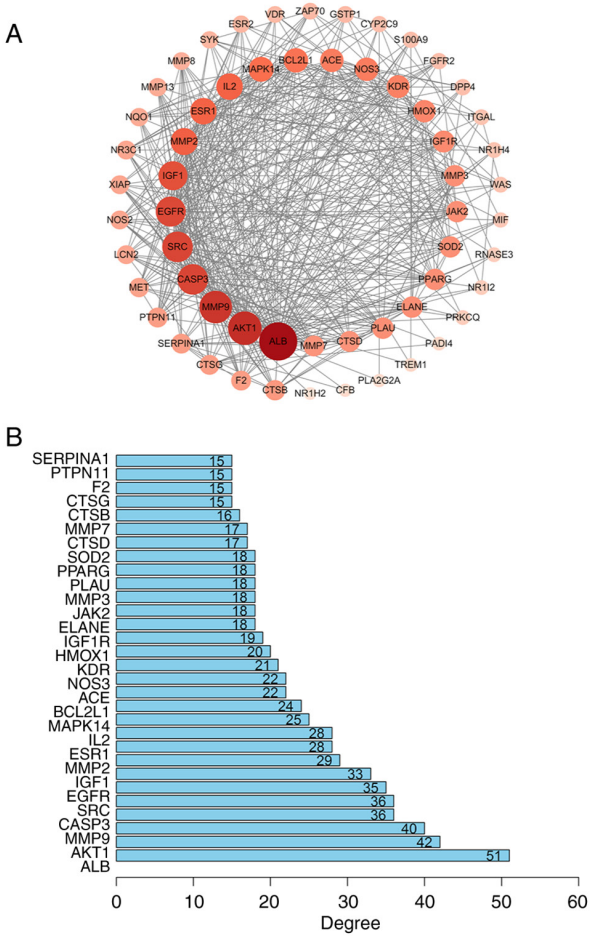

Figure 2. PPI network of the 60 common genes between PF and UC. (A) PPI network of the common genes. The node size and color represent the degree of each node. The bigger and deeper color the node is, the more important the 'hub' is in the network. (B) The degree of the top 30 targets in the PPI network. PPI, Protein-protein interaction.

group (P<0.01, Fig. 5A). Compared with the model group, the PF-treated mice showed a significant decrease in DAI scores (P<0.01, Fig. 5B). The colonic tissue was observed by light microscopy. The histomorphology of the colonic tissue of mice in the control group appeared normal. In the model group, the colonic tissue showed colonic mucosal injury with a decrease in the number of glands and substantial inflammatory cell infiltration in the mucosa and submucosa. In the PF treatment group, these abnormalities were alleviated, the colonic mucosa was restored to the levels of the control group, and inflammatory cell infiltration and congestion were also reduced in the PF group (Fig. 5C-E).

PF treatment reduces inflammatory cytokine release. The expression levels of IL-10 and TGF-β in the model group were significantly decreased (P<0.01), while the expression levels of IL-6, TNF-α, and IL-1β were significantly increased compared with those in the control group (P<0.01). The expression levels of IL-10 and TGF-β in the PF group were significantly increased compared with those in the model group (P<0.01). The expression levels of IL-6, TNF-α, and IL-1β were significantly decreased in the PF group compared with the model group (P<0.01, Fig. 6).

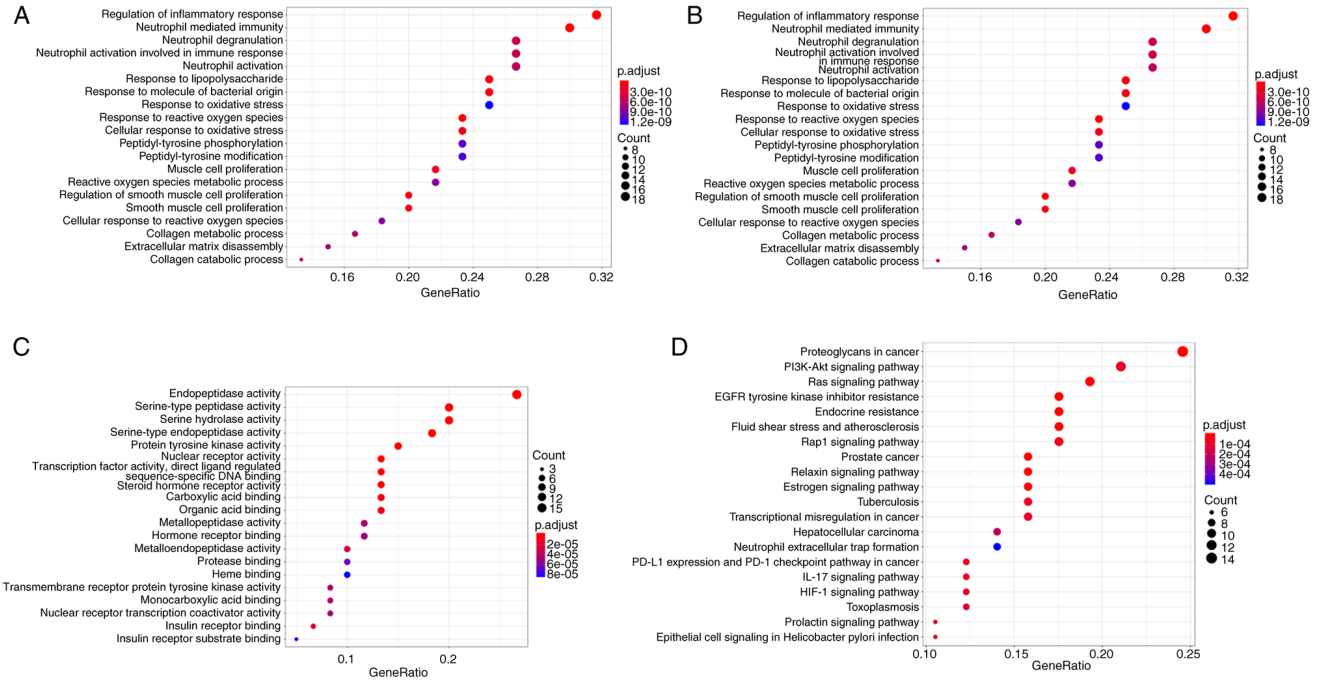

Figure 3. GO and KEGG enrichment analyses of the common targets and the PI3K/AKT signaling pathway. Results of GO enrichment analysis; (A) biological process, (B) cellular composition, and (C) molecular function. (D) KEGG pathway enrichment analysis. The color represents the significance of the-log10(P) value, which is shown as a gradient from green to red, while the size of the bubble represents the counts of the potential active targets involved in the pathways. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

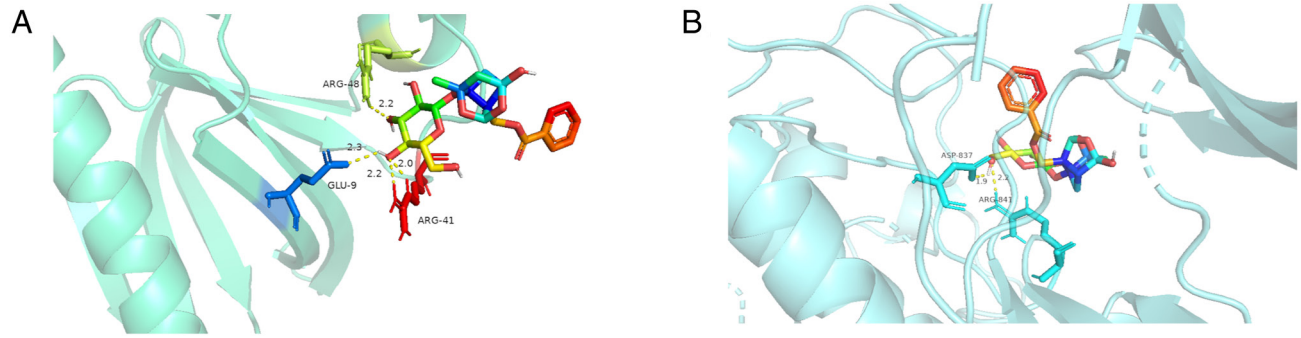

Figure 4. Molecular docking simulation. Simulation of the docking between paeoniflorin and (A) the AKT1 protein and (B) the epidermal growth factor receptor.

PF reduces DSS-induced inflammation via regulation of the PI3K/AKT signaling pathway. The mRNA expression levels of PI3K, AKT1, mTOR, BCL2, and EGFR were significantly increased in the model group compared with those in the control group (all P<0.05). After treatment with PF, the mRNA expression levels of PI3K, AKT1, mTOR, BCL2, and EGFR were significantly decreased compared with those in the model group (P<0.05, Fig. 7).

As shown in Fig. 8, western blot results showed that there was a marked increase in the protein expression levels of p-PI3K/PI3K, p-AKT1/AKT1, and p-mTOR/mTOR in the colonic mucosa of the model group after the administration of DSS in the drinking water (P<0.05). The expression levels of Bcl-2 and EGFR were also increased in the model group, but the differences were not significant (P>0.05). In the PF group, the protein levels of p-PI3K/PI3K, p-AKT1/AKT1, and p-mTOR/mTOR, Bcl-2, and EGFR were decreased compared with those in the model group (P<0.05). Taken together, these results demonstrate that PF could protect against UC partially through inhibition of the PI3K/AKT signaling pathway.

Discussion

UC, a type of inflammatory bowel disease, is characterized by abdominal pain, diarrhea, rectal bleeding, and mucopurulent bloody stools. The incidence and prevalence rates of UC have significantly increased based on hospital-based studies and register studies in China (44,45) with a significant increasingly trend of UC prevalence from 2013 to 2016 of 24.2% reported by another study (46). Several factors are correlated with the occurrence of UC including the gut microbiota, genetic factors, antibiotics use (47,48). The primary treatment options for UC are 5-aminosalicylic acid, corticosteroids, immuno-suppressants, as well as fecal microbiota transplantation,

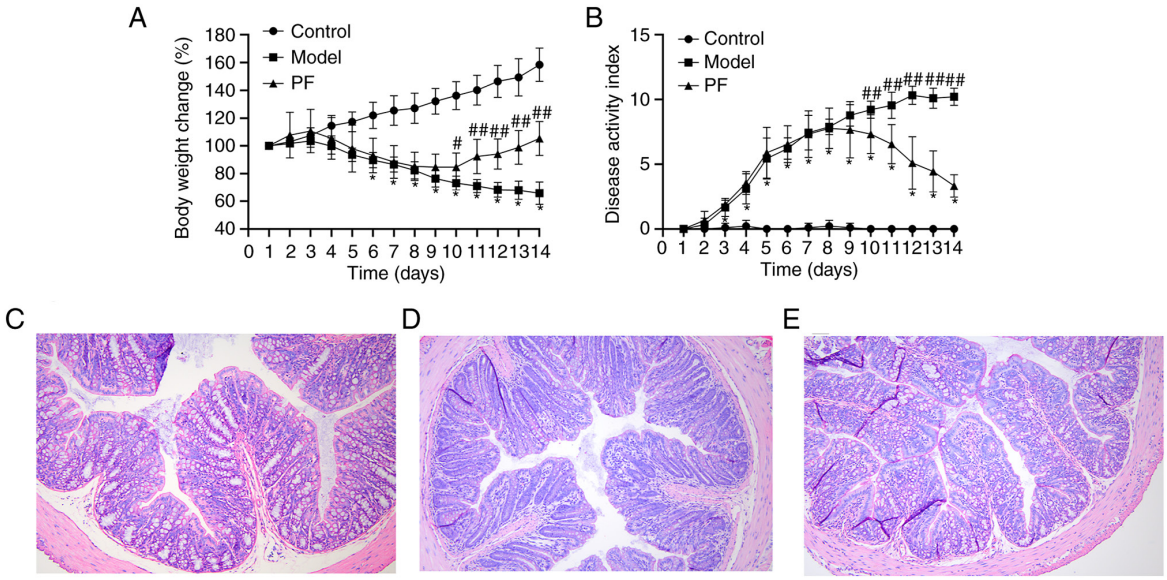

Figure 5. Clinical symptoms of mice with DSS-induced colitis treated with PF. (A) Change in body weight. (B) Disease activity index of the DSS-induced mice. Data are presented as the mean ± SD. (C-E) Histological examination of the colonic tissues using hematoxylin and eosin staining. The black arrows show inflammatory cell infiltration. *P<0.05 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. model group. DSS, dextran sodium sulfate; PF, paeoniflorin.

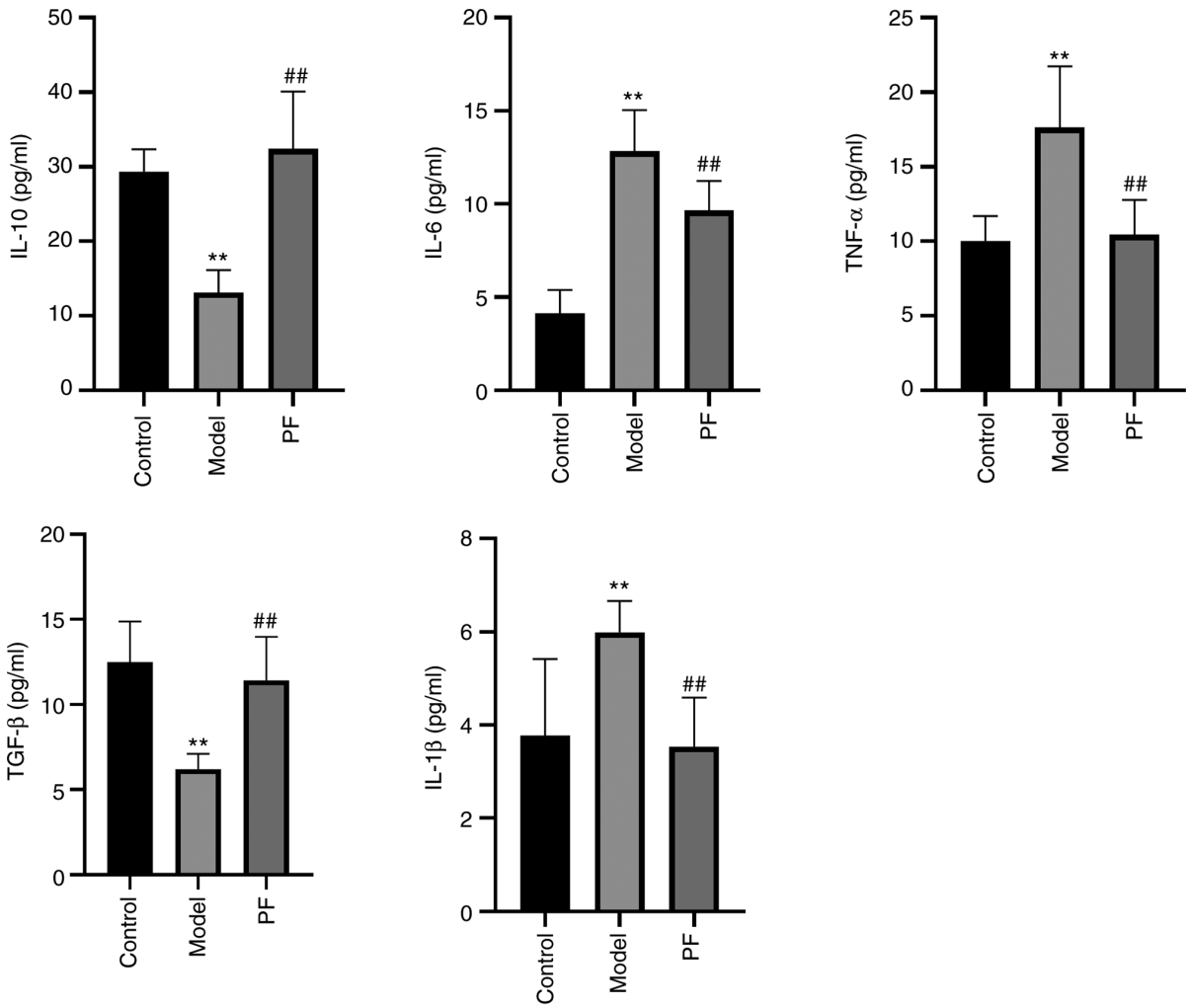

Figure 6. Expression levels of (A) IL-10, (B) IL-6, (C) TNF-α, (D) TGF-β, and (E) IL-1β in the serum. The expression levels of IL-10 and TGF-β in the model group were decreased, while the expression levels of IL-6, TNF-α, and IL-1β were increased when compared with those in the control group (F=37.470, 23.370, 58.343, 19.526, 11.615). The expression levels of IL-10 and TGF-β in the PF group were increased compared with those in the model group (F=37.470 and 23.370, respectively). The expression levels of IL-6, TNF-α, and IL-1β were decreased in the PF group compared with those in the model group (F=58.343, 19.526, and 11.615, respectively). Data are presented as the mean ± SD. **P<0.001 vs. control group; $^{\#\#}$P<0.001 vs. model group.

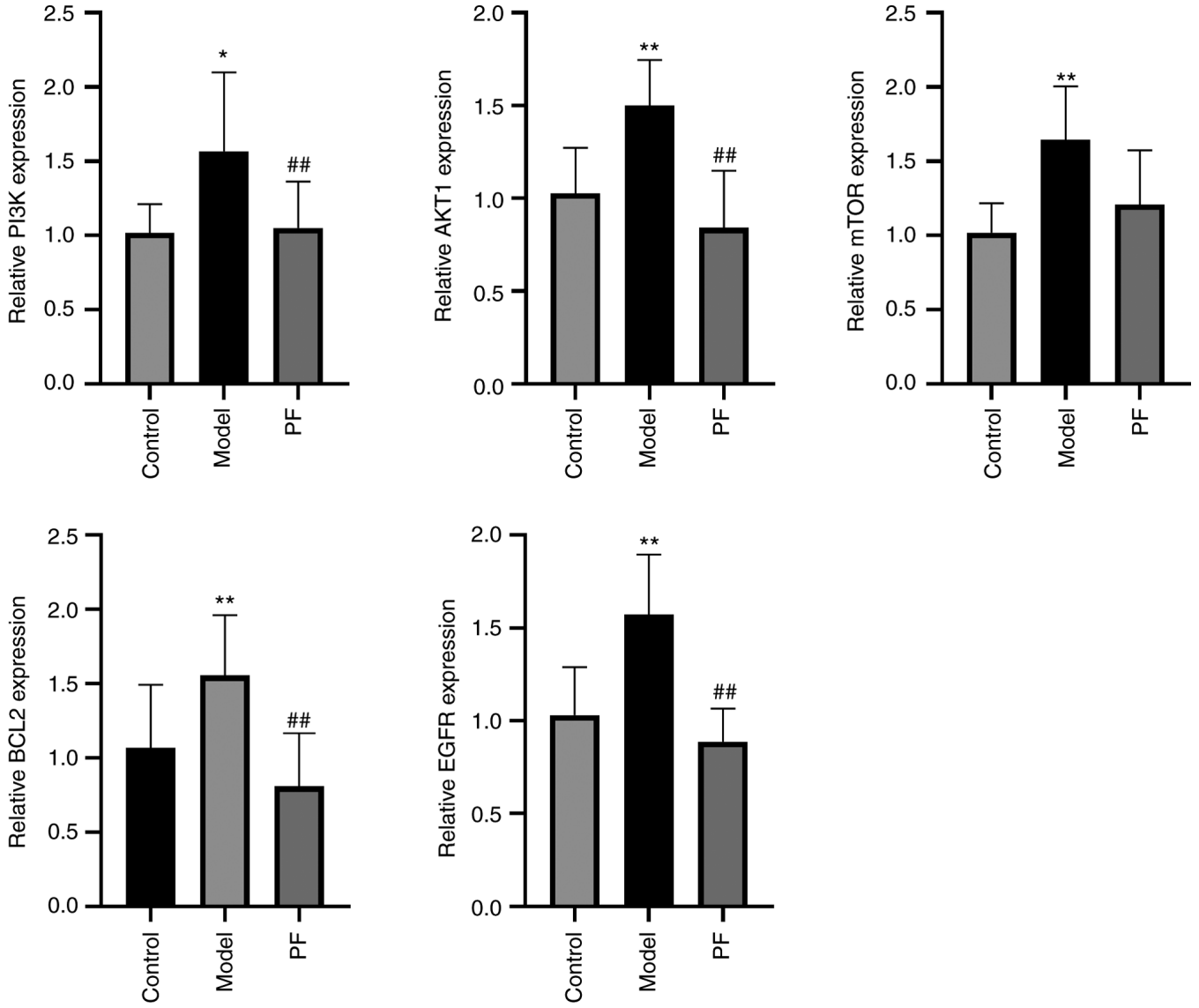

Figure 7. PF downregulated the mRNA expression levels of AKT1, BCL2, EGFR, mTOR, and PI3K in the colonic mucosa. The mRNA expression levels of PI3K, AKT1, mTOR, BCL2, and EGFR were significantly increased in the model group compared with those in the control group (F=8.256, 19.157, 9.787, 10.327, and 15.592, respectively; P=0.001, <0.0001, <0.0001, 0.003, and <0.0001, respectively). The mRNA expression levels of PI3K, AKT1, mTOR, BCL2, and EGFR in the PF group were significantly decreased compared with those in the model group (P=0.002, <0.0001, 0.007, <0.0001, and <0.0001, respectively). Data are presented as the mean ± SD. *P<0.05 and **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. model group. PF, paeoniflorin.

although they are limited by suboptimal clinical efficacy and several side effects (48-50). In recent years, certain natural compounds have received increasing attention given their safety and efficacy, lack of side effects, and wide-ranging pharmacological benefits, and have thus been used for the treatment of a variety of diseases, including cancer, diabetes, and UC (15,51-57).

PF is the primary active compound of *Paeonia suffruticosa*, *Paeonia suffruticosa*, and *Paeonia lactiflora* and has good, anti-tumor, anti-ulcer, immunoregulatory, and neuroprotective effects (25,26). In a network pharmacology and experimental validation study, Cao *et al* (58) revealed that PF can regulate inflammatory factors including IL-6 and IL-10 expression through the p38 MAPK signaling pathway in the treatment of pancreatic cancer. Sun *et al* (59) found that PF downregulated IL-18, TNF-α, and IL-1β expression levels in a rat model of diabetic foot ulcers and was related to NLRP3/ASC/caspase-1 inflammasome formation and NF-κB transcription. Several studies have revealed that PF regulates the activation of immune cells, decreases the release of inflammatory cytokines, and participates in the regulation of the GPCR and PI3K/AKT/mTOR pathways (58-63). PF has also been shown to regulate GPCR signaling by suppressing the expression of β-arrestin 2 and upregulating cAMP-PKA signaling, and this regulation was important for its therapeutic effect in rheumatoid arthritis (60). Another study found that PF could protect against cholestasis by activating Nrf2 via a PI3K/Akt-dependent pathway (64). It was also shown that PF could alleviate liver fibrosis and inhibit the activation of immune cells by inhibiting HIF-1α expression partly through suppressing the mTOR pathway (60).

However, the molecular mechanisms underlying the protective effects of PF against DSS-induced UC remain unknown. To determine the mechanism of action of PF in the treatment of

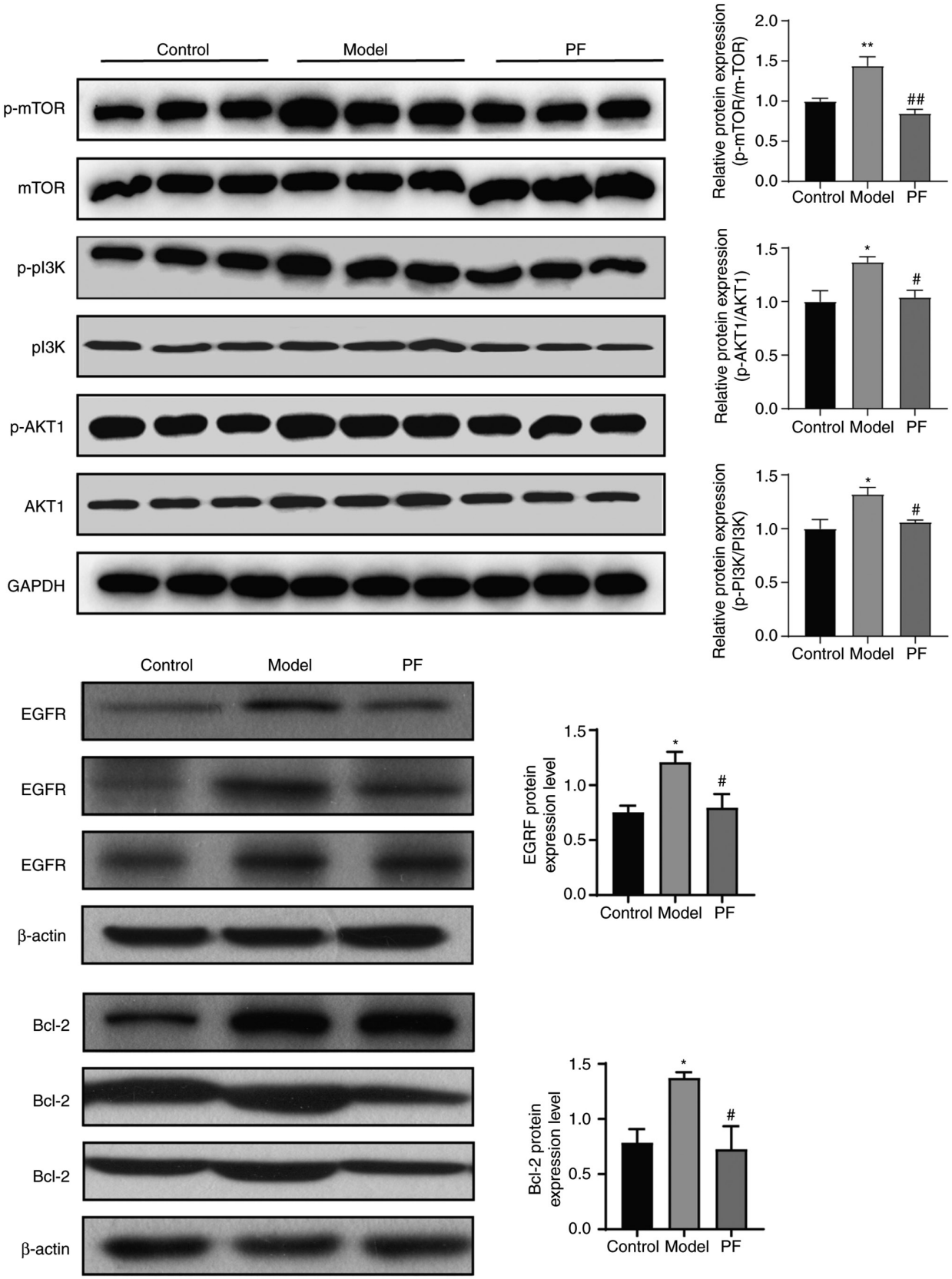

Figure 8. PF downregulated the protein expression levels of p-AKT1/AKT1, Bcl-2, EGFR, p-mTOR/mTOR, and p-PI3K/PI3K in the colonic mucosa from the dextran sodium sulfate-induced mice. Data are presented as the mean ± SD. *P<0.05, **P<0.01 vs. control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. model group. PF, paeoniflorin.

UC, network pharmacology was used to predict the target genes and pathways of its action, followed by experimental validation.

The results showed that PF treatment and the pathogenesis of UC shared 60 common target genes, and the top 10

targets were ALB, AKT1, MMP9, CASP3, SRC, EGFR, IGF1, MMP2, ESR1, IL2 and MAPK14, which are ranked according to degree values. The pathways identified included proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, endocrine resistance, prostate cancer, fluid shear stress and atherosclerosis, IL-17, PI3K/AKT, and HIF-1 signaling pathways. AKT1, BCL2L1, EGFR, FGFR2, IGF1, IGF1R, IL2, JAK2, KDR, MET, NOS3, and SYK were identified as genes that participate in the PI3K/AKT signaling pathway.

The findings of the present study are consistent with those of several previous studies. Multiple pathways have been revealed to be involved in the pathogenesis and progression of UC including the MAPK/AP-1, PPAR-α, PI3K/AKT, and NF-κB signaling pathways (65,66). Studies have found that the PI3K/AKT signaling pathway is related to the production of proinflammatory cytokines, such as TNF-α, NF-κB, IL-6, and IL-17, and regulates inflammation, apoptosis, and cell proliferation by modulating the activity of downstream effector molecules, such as NF-κB, Bcl-2, caspase 9, and mTOR (67,68). Once the PI3K/AKT signaling pathway is activated by Akt, it can regulate the key cellular processes involved in the immune-inflammatory response and induce NF-κB to produce anti-apoptotic and pro-inflammatory effects (69).

The pathogenesis of UC may be associated with the abnormal proliferation of colonic epithelial cells, enhanced leukocyte infiltration into the colonic interstitium, elevated mucosal T-cell activation, increased proinflammatory cytokine production, and dysregulation of signal transduction pathways (70-76). Inflammation plays an important role in the progression of UC (77). Anti-TNF agents have been assessed for the treatment of UC in clinical settings and they showed adequacy as a long-term maintenance therapy (78,79). Researchers have found that IL-1β and IL-6 can promote an immune response and that their expression levels are associated with genetic susceptibility and steroid dependence in patients with UC (80,81).

PF has also been shown to have an inhibitory effect on inflammation. A study by Tao *et al* (82) confirmed that the use of PF at 5-20 mg/kg significantly inhibited the levels of inflammatory mediators such as NF-κB, TNF-α, IL-1β, and IL-6 and downregulated caspase-3 expression. Another study showed that *Moutan Cortex Radicis*, a Chinese herb that contains PF as its primary compound, can inhibit the activation of NF-κB and decrease IL-6 and TNF-α expression levels in a DSS-induced colitis model (83). PF has also been shown to significantly reduce the occurrence of liver fibrosis, inhibit the inflammatory response caused by cholestasis primarily by upregulating the PI3K/Akt/Nrf2 pathway (24), and inhibit the expression of HIF-1α through the mTOR pathway (84).

PF is able to ameliorate the inflammatory degree of DSS-induced colitis, primarily by inhibiting NF-κB pathway activation and downregulating the expression of proinflammatory factors; it also inhibits the reduction of eosinophil-related chemokine gene expression and eosinophil infiltration and slightly inhibits COX2 activity, as demonstrated by enzymatic assays (85). PF can inhibit LPS-induced inflammation in Caco-2 cell monolayers, which is correlated with its inhibition of NF-κB activation and reduction of IL-6, iNOS, TNF-α, and COX-2 expression (62). Several studies have demonstrated that PF can suppress the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in inflammatory conditions and have demonstrated that this is related to the NF-κB and JAK2/STAT3 pathways (84-88). The total glucosides of peonies, which consist of PF, has been found to improve the pathological state of a DSS-induced IBD animal model and to downregulate the expression of TNF-α, IL-17A, IL-23, and IFN-γ in colonic tissues to improve mucosal barrier function (89).

The results of the present study showed that PF can reduce the levels of IL-6, TNF-α, and IL-1β, which was consistent with the findings of the abovementioned studies, indicating that PF exerts its therapeutic effect by improving the pathological condition of UC by inhibiting the inflammatory response.

The Bcl-2 family has been found to play an important role in the modulation of apoptosis including DNA cleavage and plasma membrane blebbing and also negatively regulates cell death (90,91). Studies have shown that medical treatment of UC may be accomplished via suppression of apoptosis by modulating BAX and Bcl-2 levels and caspase activity (91). Gu *et al* (92) found that PF blocked the nuclear translocation of NF-κB, downregulated the expression of COX-2 and Bcl-2, and upregulated the expression of BAX by inhibiting the MAPK/NF-κB pathway, thereby regulating intestinal immune abnormalities, inhibiting inflammation and therefore reducing UC. In addition, studies have suggested that the PI3K/AKT signaling pathway may be involved in both inflammatory and cancerous processes in UC and that activation of the EGFR/PI3K/AKT pathway can increase the expression of vascular endothelial growth factor by upregulating HIF-1α, which can lead to slower blood flow and increased interstitial blood pressure, forming tumor vessels (66,93,94).

The EGFR signaling pathway plays a critical role in the regulation of colonic epithelial biology and the response to injury and inflammation and is activated in colonic macrophages in mice with DSS-induced colitis with UC (95). The present study found that PF can decrease the expression levels of Bcl-2 and EGFR in a mouse model of UC. The results revealed that the mechanism of action of PF was related to EGFR and Bcl-2, which was consistent with the findings of a previous study (96).

The results of the present study also showed that PF could decrease the expression levels of p-AKT1, Bcl-2, p-mTOR, and p-PI3K. The animal experiments demonstrated that PF treatment improved the pathological structure of the animals, increased the levels of IL-10 and TGF-β, and decreased the levels of IL-6, TNF-α, and IL-1β. Taken together, it was confirmed that PF could protect against UC through inhibition of the PI3K/AKT signaling pathway. In other studies, wortmannin was used as a PI3K inhibitor and it was found that it could relieve the clinical symptoms, alleviate the inflammatory response, and decrease the secretion of TNF-α in the intestinal mucosa of UC patients and in a mouse model of colitis (66). This suggested that the activation of PI3K resulted in damage to the intestinal mucosa by increasing the secretion of pro-inflammatory cytokines. The application of PI3K/Akt antagonists may be used to treat UC in the future. However, here it was found that PF exerted its effects through affecting multiple pathways, and

the use of a PI3K/AKT agonist or antagonist may lead to other side effects or affect other biological processes. Thus, PI3K/Akt agonists and antagonists were not included in the present study. The present study also confirmed that the mechanism of PF in the treatment of UC was associated with the regulation of the HIF-1 pathway, which is worthy of future study.

In conclusion, 60 common genes were identified between PF- and UC-related targets. The top 10 corresponding genes included ALB, AKT1, MMP9, CASP3, SRC, EGFR, IGF1, MMP2, ESR1, IL2 and MAPK14. GO functional and KEGG pathway enrichment analyses indicated that the molecular mechanisms of action of PF in UC were closely related to important biochemical processes and signaling pathways such as EGFR tyrosine kinase inhibitor resistance, the IL-17 signaling pathway, the PI3K-Akt signaling pathway, and the HIF-1 signaling pathway. PF treatment reduced colonic damage caused by DSS and decreased IL-6, TNF-α, IL-1β, PI3K/Akt pathway-related activity, and EGFR, PI3K, mTOR, and Bcl-2 mRNA and protein expression levels. Thus, it was found that the PI3K/Akt pathway played a critical role in PF-mediated treatment of UC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

BY conceived and designed the study. SZ, QL and KN designed the experiments. YQ, KN, BL and CZ acquired the data. SZ, KN, YL, YZ, BL and BY analyzed and interpreted the data. KN and BY wrote and revised the manuscript. BY, SZ and KN confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent participate

This study was approved by the Ethics Committee of Jining Medical University (approval no. JNMC-2020-DW-ZXY-001).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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