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Original Article

Mechanisms of Shufeng Jiedu Capsule in treating bacterial pneumonia based on network pharmacology and experimental verification

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ABSTRACT

Objective: The aim of this study was to investigate the underlying mechanism of Shufeng Jiedu Capsule (SFJD) for treating bacterial pneumonia (BP) *in vivo* based on network pharmacology and experimental verification study.

Methods: Network pharmacology was used to screen the active compounds and target genes of SFJD. Then, the multi drug resistance-*Pseudomonas aeruginosa* (MDR-PA) mice lethal model and MDR-PA pneumonia model were established to evaluate the therapeutic effects and underlying mechanisms of SFJD. Western blot and ELISA were used to determinate the protein expression level of the IL-17 signaling pathway and JAK/STAT signaling pathway.

Results: After screening, 172 potential components of SFJD were generated, based on which we constructed an SFJD-component-target-BP interaction network. The Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) enrichment revealed that SFJD could regulate the IL-17 signaling pathway and Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway. Molecular docking showed that the potential target proteins had good combinations with the main active components. SFJD significantly reduced the mortality and prolonged survival days in lethal models. The lung index and pathological changes in the lung were also significantly decreased. SFJD could significantly decrease the expression of interleukin-17A (IL-17A), TNF receptor associated factor 6 (TRAF6), phospho-inhibitor of nuclear factor-kappa B (p-IκB)/inhibitor of NF-κB (IκB), phospho-NF-κB p65 (p-NF-κB p65), phospho-protein kinase B (p-AKT)/AKT, phospho-signal transducer and activator of transcription 3 (p-STAT3)/STAT3, phospho-signal transducer and activator of transcription 1 (p-STAT1)/STAT1, and the protein level of interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and IL-1β.

Conclusion: Combined with network pharmacology and *in vivo* study, it was found that SFJD exerted its therapeutic effects on BP by inhibiting the IL-17 pathway and JAK/STAT signaling pathway. This study provides new evidence for SFJD in treatment of BP.

1. Introduction

Bacterial pneumonia (BP) is a worldwide healthcare challenge (Kaier, Heister, Götting, Wolkewitz, & Mutters, 2019). Pathogenic microbial infections of the lower respiratory tract are estimated to affect 471.8 million people and cause 2.6 million deaths (Carugati et al., 2020). *Pseudomonas aeruginosa* (PA) is a common pathogenic bacterium in clinical BP infection and shows increasing isolation and multi-drug resistance rates (Chen et al., 2022). Antibiotic resistance and multidrug-resistant bacterial strains have become increasingly common (Terreni, Taccani, & Pregnolato, 2021); Multidrug resistance (MDR)-bacterial and Gram-negative strains including PA, are increasing incidences of hospital-acquired pneumonia. With high morbidity and mortality, MDR-BP seriously threatens public health worldwide (Maurice, Bedi, & Sadikot, 2018; Wang et al., 2021). Infections associated with MDR-bacterial pathogens have increased dramatically, limiting the efficacy of traditional antibiotic therapies. A large number of antimicrobial molecules have been obtained over the past nearly 100 years by screening or synthesizing natural products targeting bacteria. However, in the post-antibiotic era, MDR-bacterial infections have become a major global public health threat. Therefore, there is an urgent need for alternative antibiotic intervention

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strategies. More and more researchers are shifting their attention to traditional Chinese medicine (TCM) and natural products. The antibacterial effect of Chinese medicines does not depend on the direct killing of bacteria, so it is not easy to produce bacterial drug resistance. BP in TCM is seated in a theory of "plague", which has a large number of clinical applications. Recent studies have shown that many Chinese medicines protect against lung injury and inhibit various bacteria (Chen et al., 2022; Ding et al., 2020; Mei et al., 2020).

In China, Shufeng Jiedu Capsule (SFJD) is a commercial Chinese herb formula used for aspiratory system infectious disease for more than 30 years (Liao et al., 2021; Tao et al., 2017), which contains eight herbs: *Polygoni Cuspidati Rhizoma* et *Radix* (Polygonaceae, Huzhang in Chinese), *Forsythiae Fructus* (Oleaceae, Lianqiao in Chinese), *Isatidis Radix* (Brassicaceae, Banlangen in Chinese), *Bupleuri Radix* (Apiaceae, Chaihu in Chinese), *Patrinia scabiosaefolia* Fisch. ex Trevir. (Caprifoliaceae, Baijiang in Chinese), *Verbenae Herba* (Verbenaceae, Mabiancao in Chinese), *Phragmitis Rhizoma* (Gramineae, Lugen in Chinese), and *Glycyrrhizae Radix* et *Rhizoma* (Leguminosae, Gancao in Chinese) (Huang et al., 2020; Tao et al., 2020). The herb name has been checked with "World Flora Online" (https://www.worldfloraonline.org) or MPNS (https://mpns.kew.org). SFJD shows a broad-spectrum antibacterial effect (Bao, Gao, & Cui, 2016). In clinal applications, SFJD treats fever, sore throat, headache, and pneumonia (Sawakami, Xia, & Song, 2017; Zhu et al., 2022). SFJD contains various potential components, but its effective chemical components and underlying mechanisms against BP are still unrevealed. This research combined network pharmacology methods and *in vivo* studies to reveal the underlying mechanism of SFJD against BP.

2. Materials and methods

2.1. Reagents

Shufeng Jiedu Capsule (Lot Number: 3201010) was purchased from Anhui Jiren Pharmaceutical Co., Ltd. (Bozhou, China). The related quality control data were shown in Fig. S1 and Table S1. Levofloxacin Tablet (Lot Number: BY098G1) was supplied by Daiichi Sankyo Pharmaceutical Co., Ltd. (Beijing, China). Phosphate buffered saline (PBS) was purchased from Gibico (California, USA). Trypticase Soy Broth was supplied by Qingdao Hope Bio-Technology (Qingdao, China). ELISA kits were purchased from Cloud-clone Crop (Wuhan, China). Antibodies for interleukin-17A (IL-17A), phospho-nuclear factor-kappa B p65 (NF-κB pp65), NF-κB p65 and TNF receptor associated factor 6 (TRAF6) were obtained from Abcam (Shanghai, China). Antibodies for Interleukin-1β (IL-1β), β-Actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Proteintech (Wuhan, China). Antibodies for phospho-protein kinase B (p-AKT) and AKT were obtained from Santa Cruz Biotechnology (Shanghai, China). Antibodies for phospho-signal transducer and activator of transcription 3 (p-STAT3), STAT3, phospho-signal transducer and activator of transcription 1 (p-STAT1), and STAT1 were obtained from Cell Signaling Technology (Boston, America). Bicinchoninic acid (BCA) protein assay kits were from Beyotime Biotech Inc Co., Ltd. (Shanghai, China).

2.2. Collection of effective chemical compounds of SFJD

Chemical compounds of eight Chinese medicines contained in SFJD were collected from the traditional Chinese medicine systems pharmacology analysis (TCMSP) database and high performance liquid chromatography-mass spectrometry (HPLC-MS) (Zhang et al., 2020). TCMSP is a system pharmacology platform that provides Chinese herbal medicine chemical compounds and related targets (Ru et al., 2014). As recommended by TCMSP, two preset standard parameters of OB $\geq$ 30% and DL $\geq$ 0.18 were adopted to obtain the potential composition (Chen et al., 2021). In addition, by referring to *Chinese Pharmacopoeia* (2020 edition), HPLC-MS analysis and relevant literature, the effective compounds that do not meet the above screening criteria but have high recognition were supplemented.

2.3. Screening SFJD targets and BP-related genes

Targets of each potential component were searched from TCMSP. "Bacterial pneumonia" was used as the keyword to identify BP-related targets in the following databases: GeneCards database (https://www.genecards.org/), CTD database (https://ctdbase.org/), OMIM database (https://www.omim.org/), PharmGKB database (https://www.pharmgkb.org/) and DrugBank database (Huang et al., 2020).

2.4. Interaction network

Potential components and intersection targets of SFJD-BP were input into the Ctoyscape 3.7.0 software and constructed and analyzed the SFJD-components-targets-BP interaction network and screened compounds-target core nodes. The potential targets of SFJD and "bacterial pneumonia" were input into the STRING database (Szklarczyk et al., 2021) to construct the protein-protein interaction (PPI) network. Cytoscape 3.7.0 was employed to visualize the PPI network.

2.5. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

To further explore the potential associated with potential targets, the Matescape platform (https://metascape.org/gp/index.html) was used for GO and KEGG pathway enrichment. The heatmap was plotted by https://www.bioinformatics.com.cn, an online platform for data analysis and visualization (Tang et al., 2023).

2.6. Molecular docking technology

Autodock Vina 1.1.2 was employed to dock potential components and targets (Wu, Liu, & Li, 2022), and the docking results were visualized by Pymol.

2.7. Animals experiment

2.7.1. Animals

ICR mice, SPF grade, weight (14 ± 1) g, were maintained in IVC cages in an ABLS-2 barrier animal facility and fed standard food and distilled water (SYXK2019-003). All laboratory animal experiments were conducted abiding by the provisions of the People's Republic of China on protocols of experimental animals (Yao et al., 2016). All the animal protocols were approved by the Ethics Committee of the China Academy of Chinese Medical Sciences (Certificate number: D2021059).

2.7.2. Bacteria and medicine

PA (Schroeter) Migula (BAA-2110, ATCC, Manassas, USA) was grew aerobically in Trypticase Soy Broth at 37 °C for 24 h and quantified by turbidimeter.

Levofloxacin Tab (Lot Number: BY098G1) was supplied by Daiichi Sankyo Pharmaceutical Co., Ltd. (Beijing, China). The dosage of levofloxacin group was equivalent to clinical dosage. The dosage of levofloxacin = 500 mg × 11 / 60 kg = 0.09 g/kg. SFJD-M group (Lot

Number: 3201010) was equivalent to clinical dosage. The dosage of SFJD-M = 0.52 g × 12 capsules (daily dose) × 11 / 60 kg = 1.14 g/kg.

2.7.3. Establishment of lethal mice model

A total of 100 ICR mice were randomly separated into five equal groups (with 20 mice per group): model group, levofloxacin group (levofloxacin treatment, 0.09 g/kg), SFJD-H group (2.28 g/kg), SFJD-M group (1.14 g/kg), and SFJD-L group (0.57 g/kg). Mice were infected by intranasal 35 μL 1×10^8 CFU/mL PA inoculation. Since the first day of infection, SFJD and levofloxacin groups were gavaged for 4 d. The model group mice were given corresponding volumes of distilled water. Survival and behavior were recorded daily for 14 d.

2.7.4. Establishment of mouse BP model

Sixty ICR mice were randomly separated into six equal groups: normal control group, model group, levofloxacin group (levofloxacin treatment, 0.09 g/kg), SFJD-H group (2.28 g/kg), SFJD-M group (1.14 g/kg), and SFJD-L group (0.57 g/kg), with 10 mice per group. Mice were infected by intranasal 35 μL 1×10^7 CFU/mL PA inoculation. Mice in the normal control group were treated by nasal inhalation of PBS. Mice were weighed and sacrificed 4 d after infection, and lungs were weighed and stored for histopathological study, Western blot, and cytokine assay.

2.7.5. Micro-computed tomographic analysis

Mouse lung tissues were performed using standard resolution CT protocol (Boudewijns et al., 2020). Technical specifications: 70 kV, 114 μA, 2 min, and 36 mm. Mice were monitored through scanning. Visualization and quantification were performed by CT viewer software. Images were reconstructed and visualized (Kim et al., 2022).

2.7.6. Histopathological study

The lungs were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5 μm sections. Then, the sections were stained by hematoxylin & eosin (H&E) reagent. The mice lung histopathology changes were visualized under a NIKON microscope (DS-U3, Tokyo, Japan), and the pathological scores of alveolar septal widening were evaluated as follows criteria: "−": normal; "+": alveolar septa minor widened; "++": alveolar septa mild widened; "+++": alveolar septa moderately widened; "++++": lung consolidation and massive inflammatory cell immersion. Pathological scores of perivascular inflammatory infiltration were evaluated as follows criteria: "−": normal; "+": slight, 1–2 perivascular inflammatory cell infiltration; "++": mild, 3–7 perivascular inflammatory cell infiltration; "+++": moderate, 7–12 perivascular inflammatory cell infiltration; "++++": severe, > 12 perivascular inflammatory cell infiltration, diffuse inflammatory cell infiltration.

2.7.7. ELISAS

Lung samples were homogenized, and levels of IL-6, TNF-α, and IL-1β were determined by ELISA as per the manufacturer's instructions.

2.7.8. Western blot

Lung samples with radioimmunoprecipitation assay (RIPA) buffer containing 0.5% protease inhibitor cocktail and 0.5 mmol/L phenylmethanesulfonyl fluoride (PMSF) were homogenized by sample disruption instrument (FastPrep 5G, California, USA). Samples were laid in ice for 30 min and then centrifuged. Nuclear protein was extracted by the nuclear and cytoplasmic protein extraction kit. The BCA kit determined the supernatant protein contents. Equal proteins (30 μg) were loaded per well, separated in 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto PVDF membranes. Blocking for 10 min and then washing by tris-buffered saline and Tween 20 (TBST) for 3 min. After washing four times, membranes were incubated at 4 °C with primary antibodies for 12 h (Dong et al., 2018), which were washed four times again and incubated with the appropriate secondary antibody. ECL reagents (Beyotime, Shanghai, China) were used to visualize.

2.8. Statistical analysis

ANOVA followed by Bonferroni's multiple comparison test (GraphPad Prism) was used for analysis. *P*-values below 0.05 were considered significant. Data were analyzed by GraphPad Prism.

3. Results

3.1. Prediction and identification of potential components and targets of SFJD

The potential components were obtained from the TCMSP based on ADME and supplemented with HPLC-MS. Potential SFJD targets were predicted, and 425 targets of 172 potential compounds were identified.

3.2. BP-related targets screening

As shown in Fig. 1A, 2 028 targets were collected, including 726 targets from the CTD, 1 216 targets from the GeneCards, 188 targets from the OMIM, and 207 targets from the PharmGKB, which intersected as the BP-related targets.

3.3. Construction of a SFJD-compound-targets-BP network

A total of 200 common genes were screened out, based on the SFJD target database and the BP-related target database from the five databases (SFJD, OMIM, Gene Cards, Drug Bank, PharmGKB), as potential targets for SFJD treatment of BP (Fig. 1A). A network was built to analyze the complex interactions of SFJD drugs, compounds, and targets (Fig. S2). In this network of 172 active compounds interacting with 200 BP targets, there were 1 862 edges connecting 382 nodes. In addition, connections were counted using NetworkAnalyzer, an analysis tool in Cytoscape (Xu et al., 2021). The top 10 potential compounds and information were shown in Table 1.

3.4. Screening key targets

PPI networks were predicted by STRING. PPI showed that targets had complexly functional associations (Szklarczyk et al., 2021). Cytoscape was used for further analysis to explore the key targets (Li, Lei, Zhang, Kong, & Qin, 2017). We used a series of parameters such as BetweennessCentrality, ClosenessCentrality, ClusteringCoefficient, and Degree to analyze the key targets. The top four key targets were confirmed: IL-6, TNF, IL-1β, and AKT (Fig. 1B). Furthermore, the top 2 targets were molecularly docked with potential compounds.

3.5. Enrichment analysis

Furtherly, the correlation between these potential targets and SFJD resistance to BP, GO and KEGG enrichment analyses was performed by metascape (Zhao et al., 2021; Zhou et al., 2019). GO biological process (BP), GO cellular component (CC) and GO molecular function (MF) terms were analyzed, top 10 for each term were shown in Fig. 2A–C. Results of GO BP mainly included "response

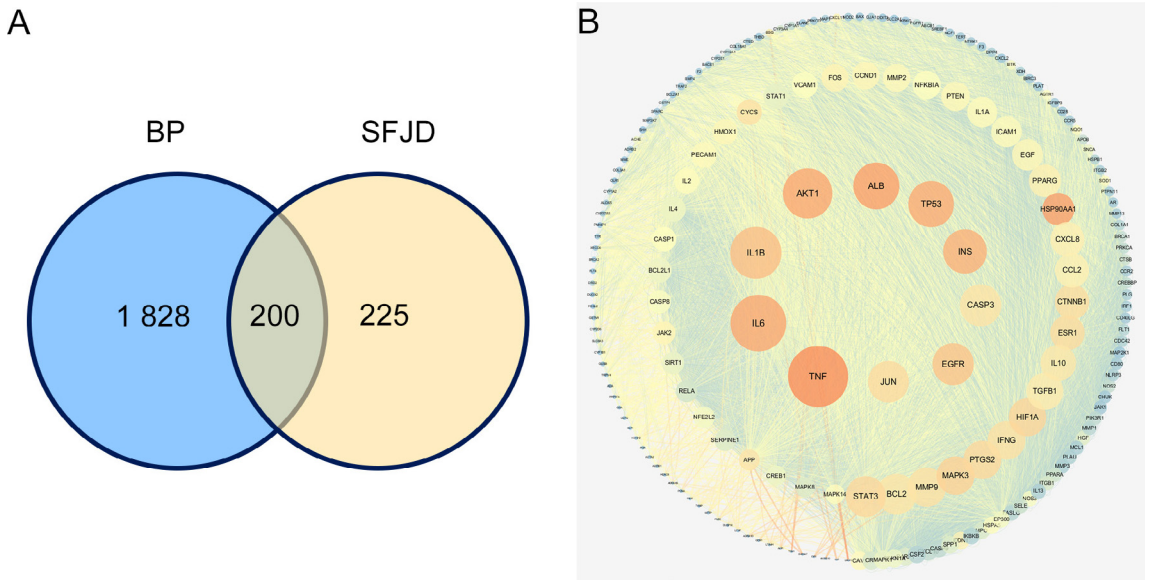

Fig. 1. Screening key targets of SFJD for treating BP. (A) Venn figure of SFJD and BP-related targets. (B) Protein-protein interaction (PPI) network.

Table 1
Top 10 components based on value of degree.

Compounds	MOL ID	BetweennessCentrality	ClosenessCentrality	Degree
Quercetin	MOL000098	0.070 372 95	0.480 552 07	91
Resveratrol	MOL012744	0.051 044 62	0.459 232 61	82
Apigenin	MOL000008	0.014 914 13	0.424 141 75	39
Kaempferol	MOL000422	0.016 675 7	0.422 737 31	38
Ursolic acid	MOL000511	0.009 320 26	0.414 054 05	36
Luteolin	MOL000006	0.011 824 42	0.422 271 22	35
Naringenin	MOL004328	0.007 785 6	0.399 374 35	21
Emodin	MOL000472	0.005 753 24	0.391 615 54	20
Sinensetin	MOL001803	0.004 823 8	0.401 888 77	19
Beta-carotene	MOL002773	0.003 900 68	0.383 767 54	19

to lipopolysaccharide" and "positive regulation of cytokine production". The results of GO MF mainly included "cytokine activity".

KEGG results showed that 200 potential targets associated with SFJD treatment of BP were significantly correlated with multiple key pathways, for example the "IL-17 signaling pathway" and "JAK/STAT signaling pathway". The top 20 signaling pathways of KEGG enrichment were shown in Fig. 2D. These results suggested that SFJD regulated host inflammation and immunity may be the main mechanism for treating BP.

3.6. Molecular docking

The top two key targets (IL-6 and TNF-α) were docked with the top six active compounds (Table 2) (PDB-ID: TNF-α:1ext, IL-6:1ALU; Ligand: TNF-α:SO4, IL-6:TLA). The smaller the docking binding energy is, the better the stability of the compound and target is. The value < −5.0 kJ/mol indicated stable binding activity. The docking affinity of the top six compounds lower than −5.0 kJ/mol indicated well binding ability with these targets. Visualisations of the top two key targets with better docking binding performance were shown in Fig. 3. And these compounds may be potential chemical substances basis of SFJD against BP.

3.7. Effect of SFJD on lethal BP model

In order to determine the protective efficacy of SFJD against the lethal PA infection model, mortality and survival time for ICR mice were determined (Fig. 4). Seven days after infection, 17 of 20 mice in the model group died, while the mortality of the SFJD-H, SFJD-M, and SFJD-L treatment groups was significantly decreased to 50%, 65%, and 70%. In addition, SFJD treatment groups significantly increased the survival time of the mice.

3.8. Micro-computed tomography

Computed tomography (CT) is a widespread application for diagnosing pneumonia (Bachmann et al., 2018; Hani et al., 2020). To determine whether SFJD could alleviate pneumonia caused by PA, we determined the histological and structural changes by micro-CT. As shown in Fig. 5A, 96 h after infection, pulmonary consolidation was discovered in the model group. While less consolidation was found in SFJD-treated groups. Quantification of lung non-aerated lung volume reflects the consolidations in the lungs. As shown in Fig. 5B micro-CT images, model group consolidation was similar, while the treated group alleviated.

3.9. Effect of SFJD on BP

The lung index was calculated to assess lung edema. Compared with the normal group, lung index in the model group increased significantly. Compared with model group lung index, the SFJD treatment groups showed a significant reduction (Fig. 5C).

In concordance with the result of the lung index, milder thickened alveoli septum and inflammatory cells infiltrating around

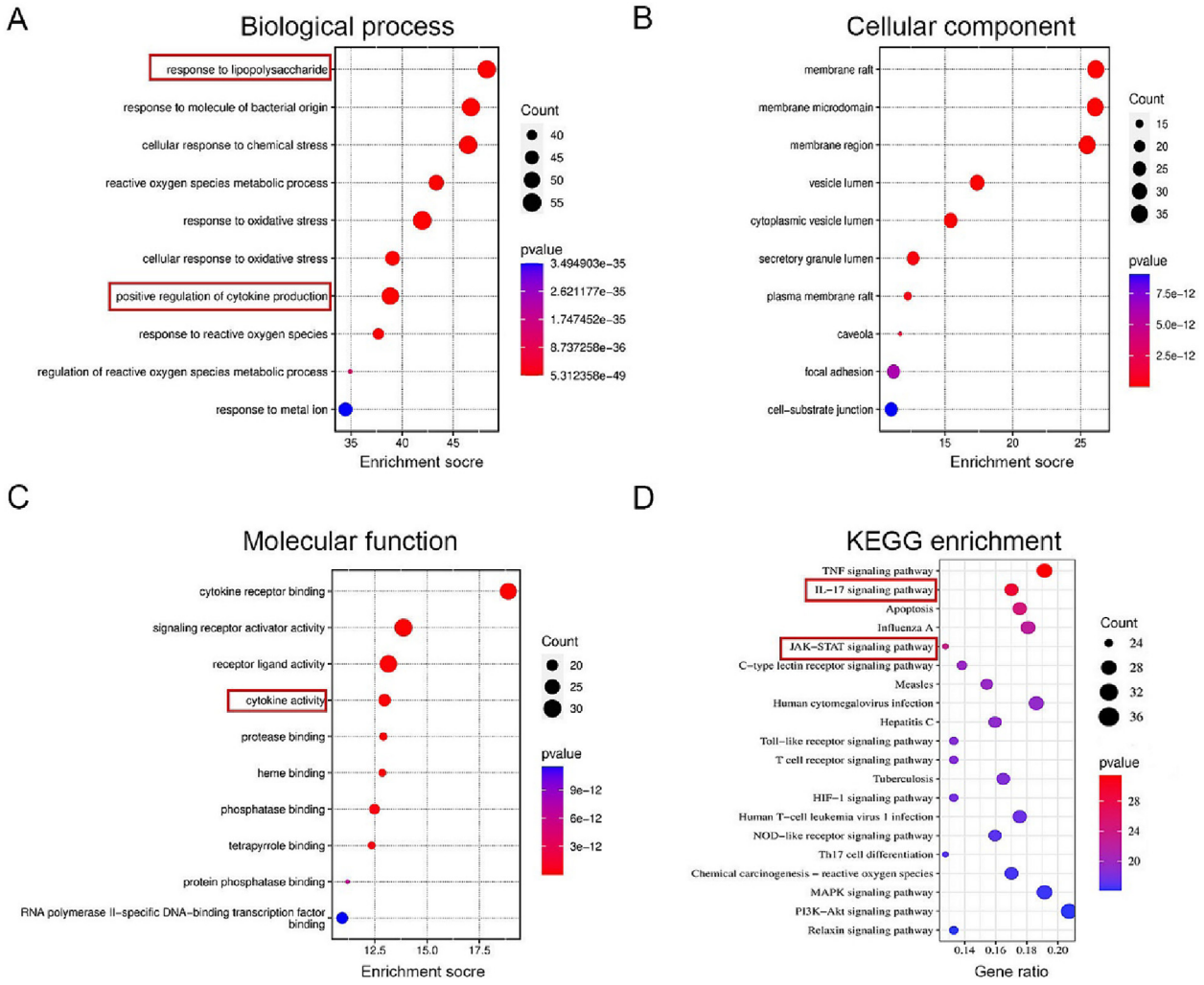

Fig. 2. GO Enrichment analyses of biological process (A), cell composition (B), molecular function (C), and KEGG enrichment result (D).

Table 2
Molecular docking score of top six potential compounds and core targets.

Compounds	Docking binding energy with TNF-α (kJ/mol)	Docking binding energy with IL-6 (kJ/mol)
Quercetin	−5.9	−6.3
Resveratrol	−5.3	−5.6
Apigenin	−7.7	−6.8
Kaempferol	−6.1	−6.8
Ursolic acid	−6.7	−7.3
Luteolin	−6.8	−6.6
Ligand	−8.1	−7.2

the vessels pathology were observed. In the model group, PA strain infection caused diffuse parenchymal lung disease, massive inflammatory cell infiltration, and capillary congestion. Treatment with SFJD reduced histological injury, with fewer alveolar septa and recruitment of inflammatory infiltrates (Fig. 6).

3.10. Effect of SFJD on levels of inflammatory factors in lung

The expression levels of IL-6, IL-1β, and TNF-α in the model group were significantly increased compared with those in the normal group. Meanwhile, the expression levels of IL-6, IL-1β, and TNF-α in the SFJD treatment groups were significantly decreased (Fig. 7). This result showed that SFJD could inhibit excessive inflammatory responses of lung tissue.

3.11. SFJD treated BP through IL-17 signaling pathway

The expression of IL-17 signaling pathway proteins in the lung tissues of mice in each group was shown in Fig. 8. The expression of IL-17A, TRAF6, nuclear NF-κB p65 and IL-1β was significantly increased in the lung tissues of mice in the model group compared with the control group ($P < 0.05$, $P < 0.01$). The expression of IL-17A, TRAF6, p-IκBα/IκBα, nuclear NF-κB p65, p-NF-κB p65 and IL-1β was significantly decreased in lung tissues of mice in SFJD treatment groups compared with the model group ($P < 0.05$, $P < 0.01$). These results indicated that SFJD significantly suppressed the excessive inflammatory expression of IL-17 signaling pathway caused by PA infection.

3.12. SFJD treated BP through JAK/STAT signaling pathway

The expression of JAK/STAT signaling pathway and AKT proteins in the lung tissues of mice in each group were shown in Fig. 9. The expressions of p-STAT3/STAT3, p-STAT1/STAT1 and p-AKT/AKT were significantly increased in the lung tissues of mice in the model group compared with the control group ($P < 0.05$, $P < 0.01$). The expressions of p-STAT3/STAT3, p-STAT1/STAT1 and

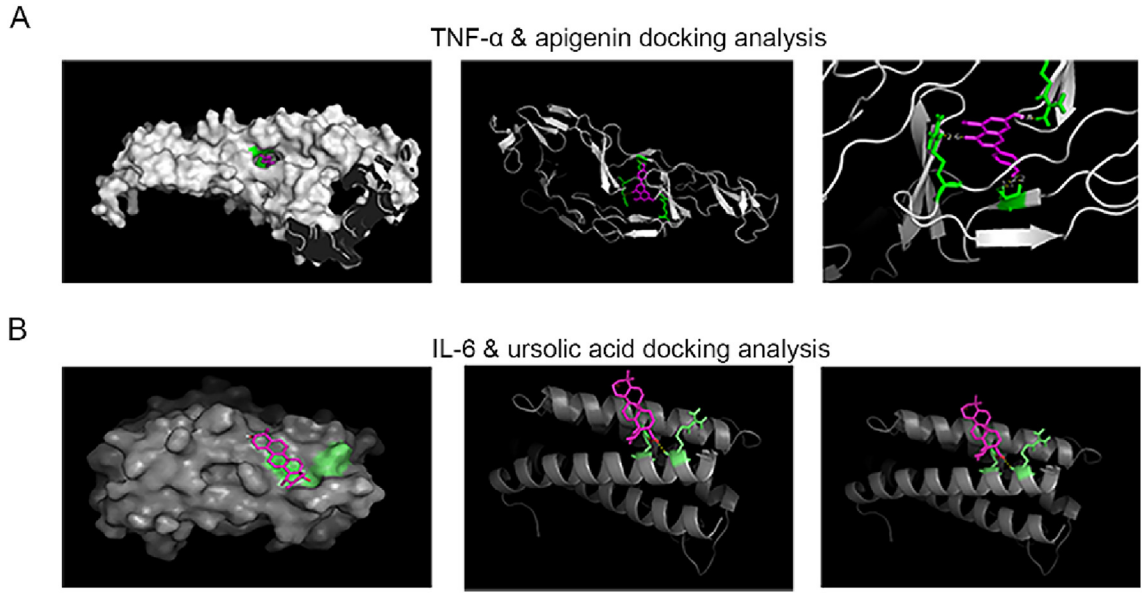

Fig. 3. Molecular docking analysis of TNF-α & apigenin (A) and IL-6 & ursolic acid (B).

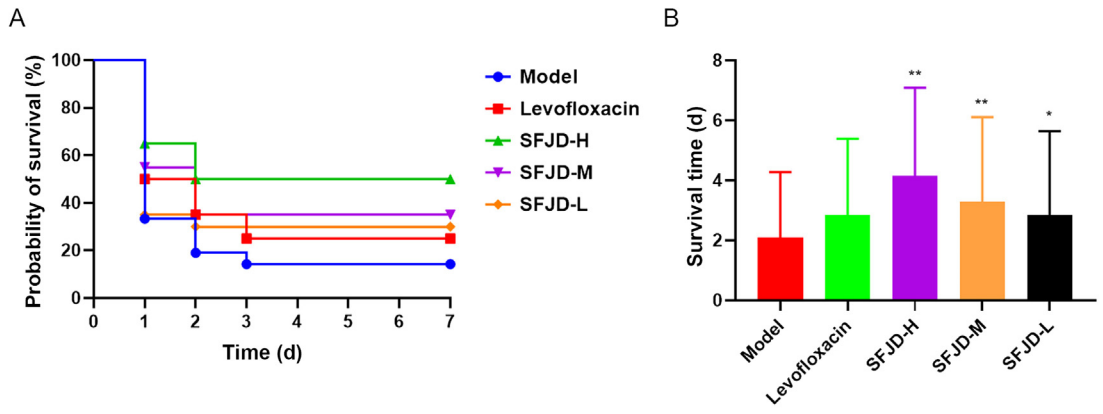

Fig. 4. Effect of SFJD on lethal BP model. (A) Survival curve (n = 20). (B) Survival time in lethal model of each group (means ± SD, n = 20). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* model group.

p-AKT/AKT were significantly decreased in lung tissues of mice in SFJD treatment groups compared with the model group ($P < 0.05$, $P < 0.01$). These results indicated that SFJD significantly suppressed the expression of p-STAT3, p-STAT1 and p-AKT caused by PA infection.

4. Discussion

SFJD showed broad-spectrum antibacterial action, reduced mortality, and increased survival time in mice *Staphylococcus aureus* or *Streptococcus* infection model (Bao, Gao, & Cui, 2016). SFJD has shown activity in acute lung injury (ALI) based on its anti-inflammatory and immunomodulatory effects (Chen, Lin; Niu, & Xiao, 2021). Therefore, this paper aims to reveal the potential mechanism from the point of the antibacterial link to BP of SFJD.

Network pharmacology methods are efficient in discovering the potential components of TCM and predicting the potential mechanisms (Lyu et al., 2021; Nogales et al., 2022; Wu et al., 2022; Zhou, Qian, Li, Gao, & Li, 2022). Through the research of the SFJD-compounds-target-BP network, the potential components of SFJD were identified, including quercetin, resveratrol, apigenin, kaempferol, ursolic acid, luteolin. BP causes severe lung injury and cytokine storms. Quercetin can significantly reduce PA bacteria copies *in vivo* (Wang et al., 2018) and regulate IL-1β production (Chanjitwiriya, Roytrakul, & Kunthalert, 2020). Kaempferol pretreatment reduced IL-6, TNF-α, and IL-1β in ALI and alleviated oxidative stress (Yang et al., 2021). Luteolin can inhibit macrophage polarization and downregulate the expression of inflammatory factors (Wang et al., 2020). Rutin and isorhamnetin can downregulate levels of IL-6, TNF-α and reduce oxidative stress

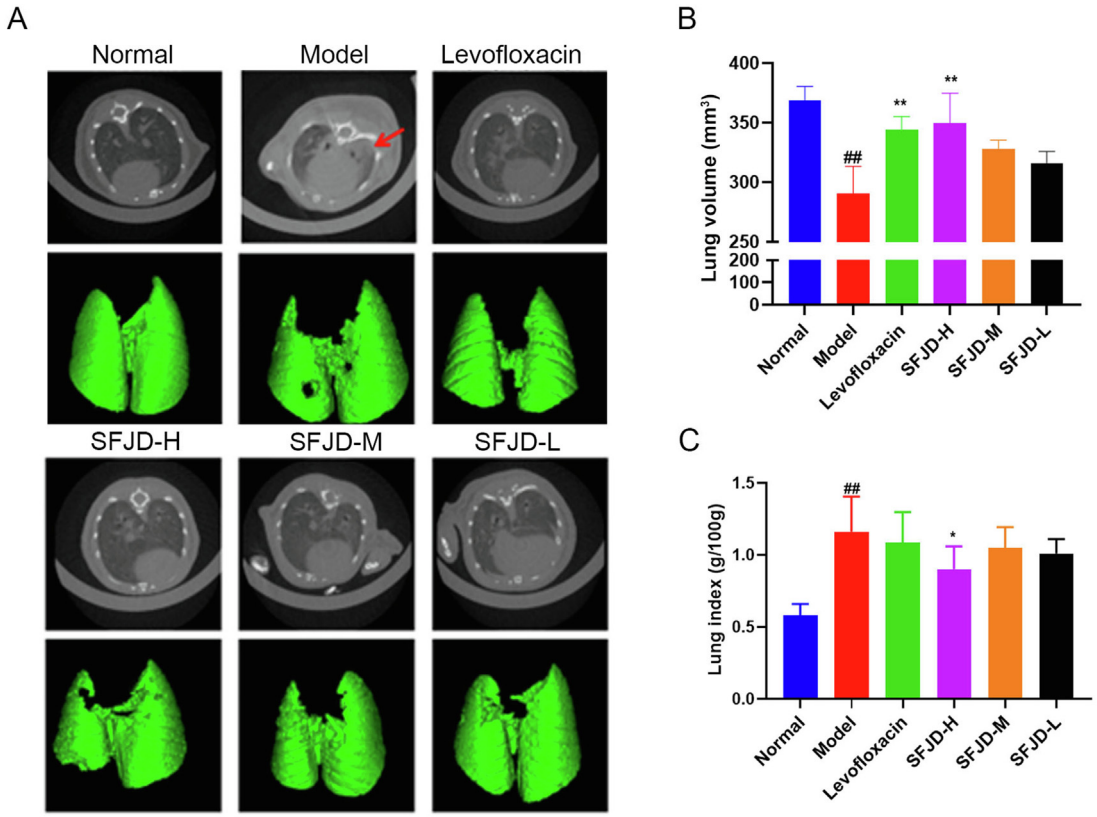

Fig. 5. SFJD treatment improved pulmonary consolidations. (A) Representative transversal micro-CT revealed visualization of pneumonia disease process and treatment of SFJD. (B) Quantification of lung non-aerated lung volume (mean ± SD, n = 3). (C) Lung index (mean ± SD, n = 10). $^{\#\#}P < 0.01$ *vs* normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* model group.

(Dong, Li, Ma, Liu, & Liu, 2022; Tosun et al., 2021; Yang et al., 2016; Yeh, Yang, Yang, Li, & Kuan, 2014).

Following KEGG pathway analysis, we found that the IL-17 signaling pathway, Jak-STAT signaling pathway and P13K-AKT signaling pathway were mainly relative to SFJD treatment on BP. SFJD can alleviate pneumonia by regulating the host immune inflammation response. SFJD significantly reduced the mortality and prolonged survival days in lethal models. The pneumonia model experiments showed that SFJD could decrease lung index and alleviate pathological changes through attenuating cytokines release. SFJD could significantly decrease the expression of IL-17A, TRAF6, p-IκB/IκB, NF-κB pp65, p-AKT/AKT, p-STAT3/STAT3, p-STAT1/STAT1, and the protein level of IL-6, TNF-α, and IL-1β in lung tissue.

IL-17 signaling pathway plays a considerable role in host defense against BP (Chen et al., 2016). IL-17A is essential to the defense against bacteria invasion of the lung and is significantly correlated with the development of BP (Chen & Kolls, 2013). Bacterial infection and inflammation increase IL-17A accumulation in the host lung (Lorè et al., 2016). During acute bacterial infections with lung injury, IL-17A and IL-17F are produced by $CD4^+$ and $CD8^+$ T cells, γδ T cells, and various innate immune cell populations (Mills, 2023). IL-17A activates the NF-κB signaling pathway via NF-κB-p65, and NF-κB stimulates the production of downstream inflammatory factors (Li et al., 2017). In clinical practice, TNF-α, IL-1β, and IL-6 are critical potential biomarkers among cytokines. The downstream pro-inflammatory factor levels are known as the critical factors of BP and pneumonia-induced sepsis (Hu et al., 2021). Although IL-17A plays an important role in bacterial clearance, IL-17A can lead to a cascading response of neutrophil recruitment, inflammation, and host defense mechanisms that produces excessive inflammation and significant tissue damage. Excessive expression of host inflammation often leads to poorer clinical outcomes (Li et al., 2023). Therefore, suppression of inflammation and modulation of immunity have also become necessary therapeutic approaches.

BP remains a major burden worldwide. Although an inflammatory response in the lungs is required to fight pathogens, neutrophils that persistently reside in the tissues in non-resolving pneumonia can induce collateral tissue damage and precipitate acute lung injury. JAK-STAT is an important signaling pathway regulated by cytokines that is essential for initiating innate immunity, orchestrating adaptive immune mechanisms, and ultimately suppressing inflammatory and immune responses. lL-17A plays an important role in the prevention and control of bacterial infections through enhancing IL-6 secretion and indirectly or directly stimulating STAT3 and STAT1 phosphorylation. IL-6 induces phosphorylation of lung signaling and transcriptional activators STAT1 and STAT3 (Jones et al., 2006). LPS produced by Gram-negative bacteria also induces phosphorylation of STAT1 and STAT3 (He, Li, Zhou, Gu, & Jiang, 2021). In this study, the SFJD treatment group significantly inhibited STAT3 and STAT1 phosphorylation and ameliorated the excessive inflammatory response in BP infection.

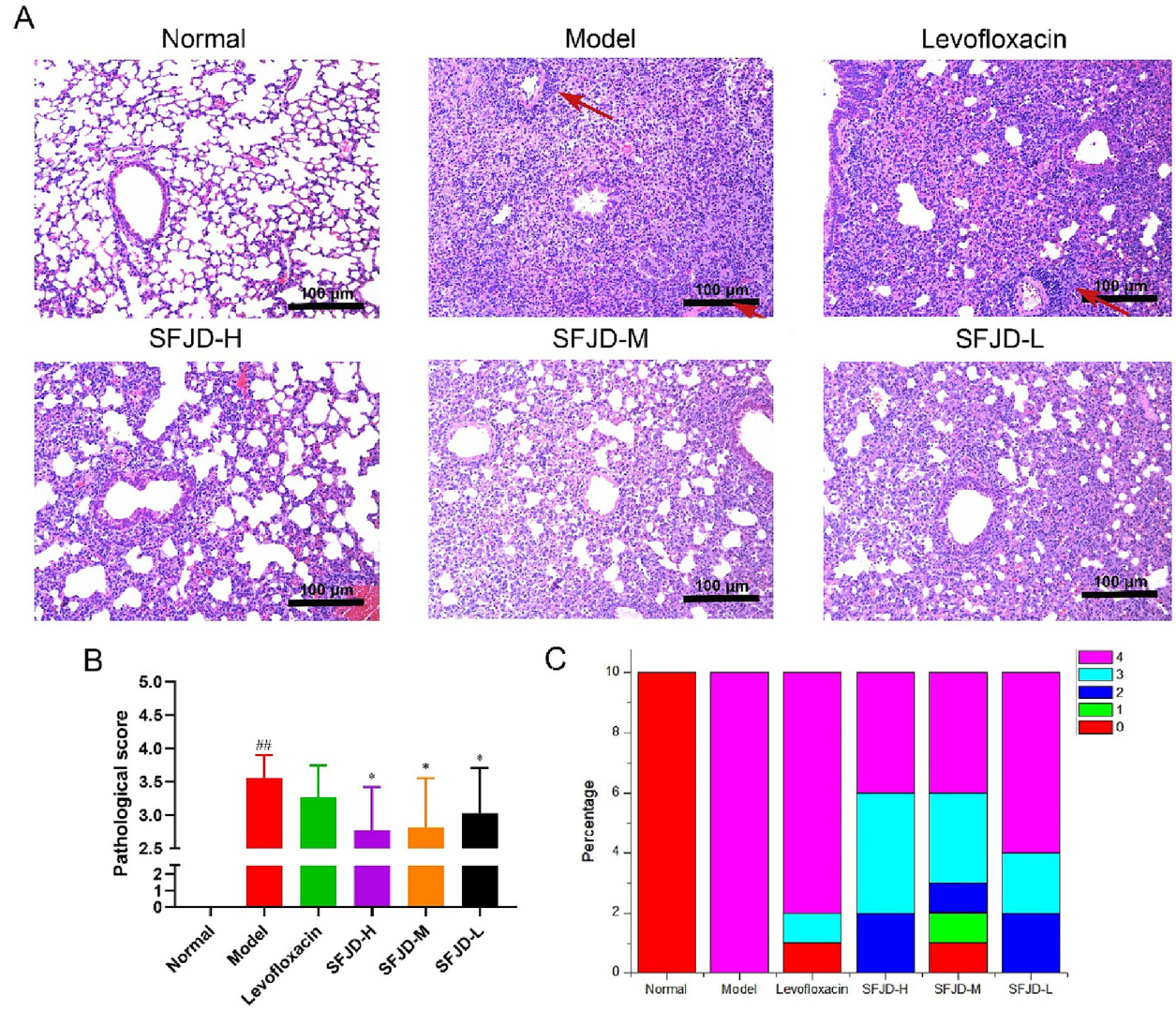

Fig. 6. SFJD treatment improved pulmonary infiltrates. (A) Typical HE staining of lung tissues in different groups (× 100). (B) Pathological scores of alveolar septal widening (mean ± SD, n = 10). (C) Pathological scores of perivascular inflammatory infiltration (mean ± SD, n = 10). $^{\#\#}P < 0.01$ *vs* normal group; $^{*}P < 0.05$ *vs* model group.

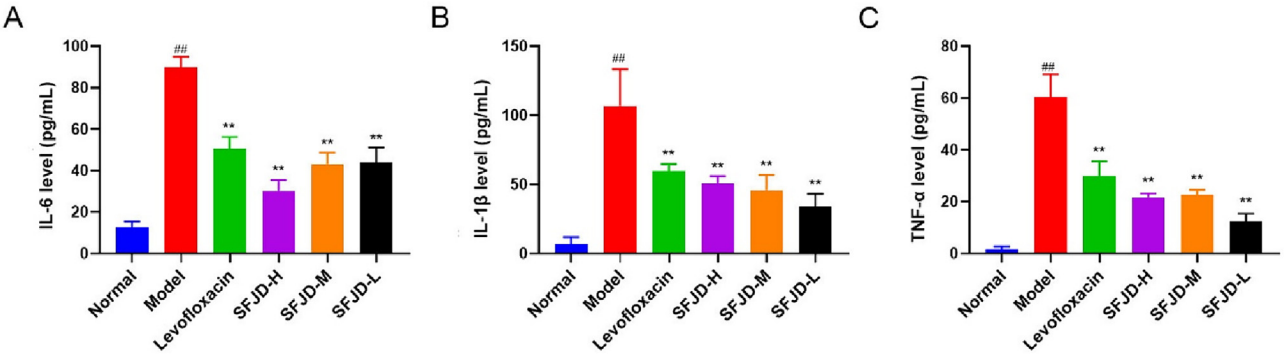

Fig. 7. SFJD inhibited overproduction of inflammatory factors. (A–C) Influence of SFJD on levels of IL-6, IL-1β, and TNF-α in lung (mean ± SD, n = 5). $^{\#\#}P < 0.01$ *vs* normal group; $^{**}P < 0.01$ *vs* model group.

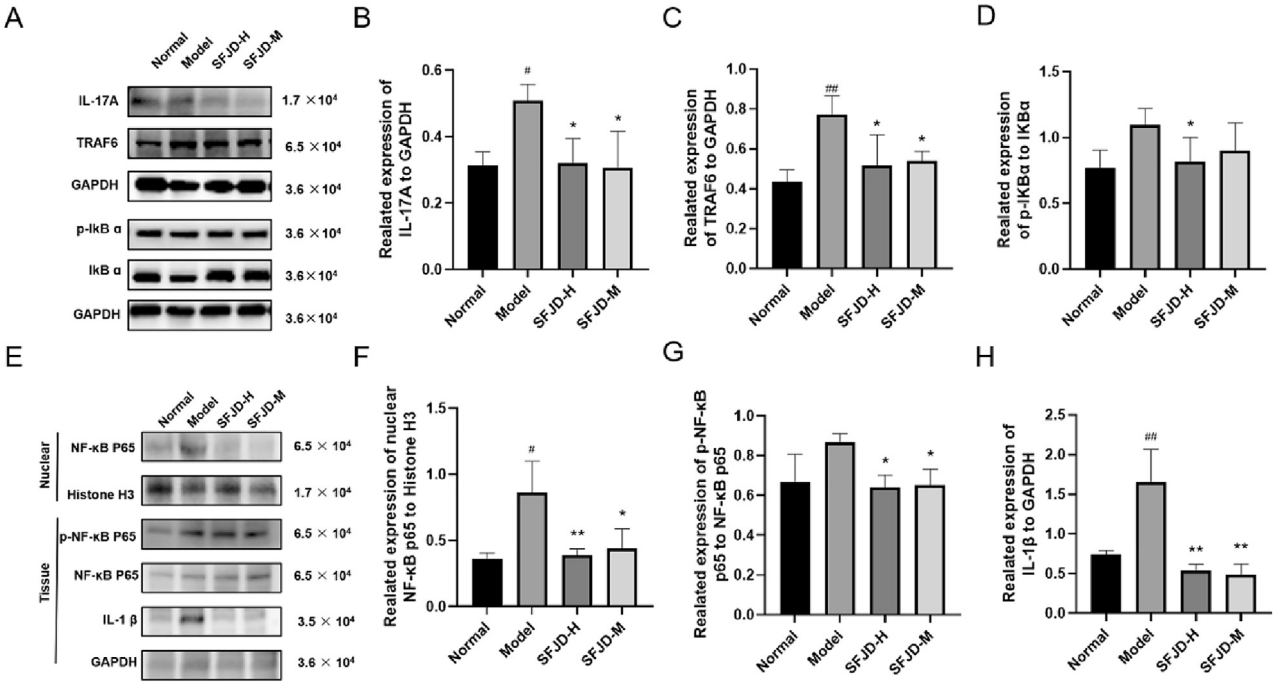

Fig. 8. SFJD inhibited overexpression of IL-17 signaling pathway in BP mice (mean ± SD, n = 3). (A and E) Western blot analysis of related proteins expression in lung. (B−D and F−H) Expression of IL-17A, TRAF6, p-IκB/IκB, nuclear NF-κB p65, p-NF-κB p65 and IL-1β. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* model group.

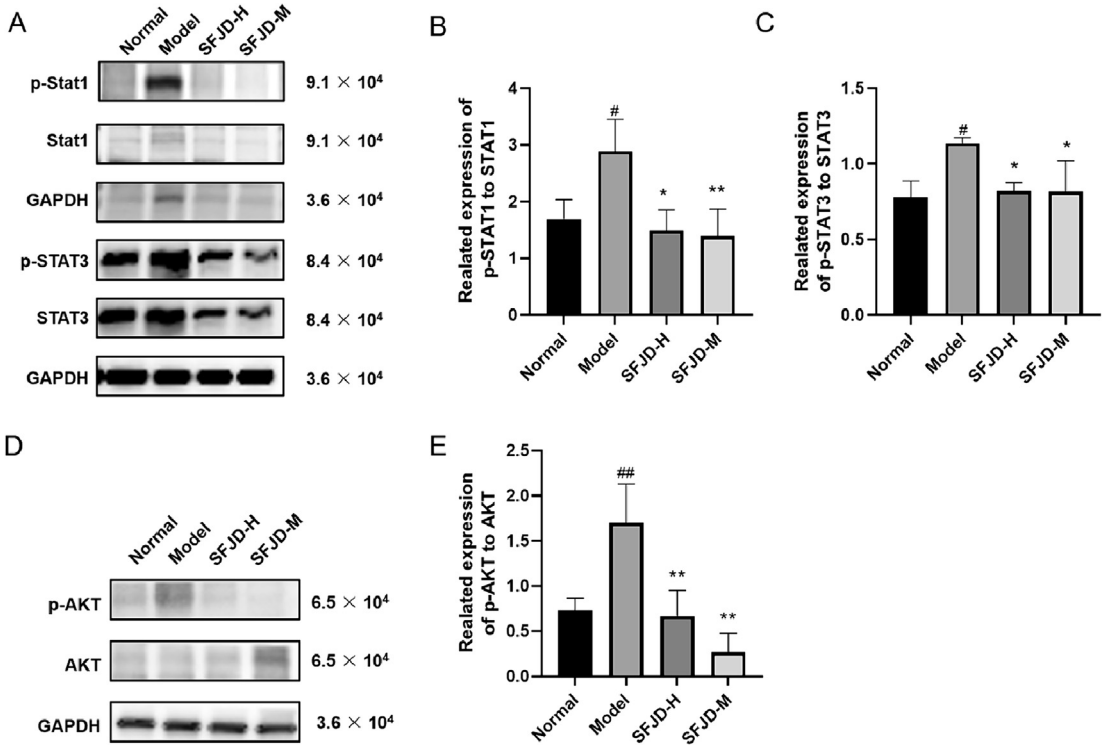

Fig. 9. SFJD inhibited overexpression of JAK/STAT signaling pathway in BP mice (mean ± SD, n = 3). (A and D) Western blot analysis of related proteins expression in lung. (B −C) Expression of p-STAT3/STAT3, p-STAT1/STAT1 and (E) p-AKT/AKT. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* model group.

5. Conclusion

SFJD is reported to play an essential role in anti-BP. Combined with network pharmacology and *in vivo* study, it provides new evidence for treatments of BP through regulating host immune inflammation response, suppressing the IL-17 signaling pathway and JAK/STAT signaling pathway.

CRediT authorship contribution statement

Yingli Xu: Data curation, Writing – review & editing. **Lei Bao:** Methodology. **Ronghua Zhao:** Methodology. **Zihan Geng:** Methodology. **Shuran Li:** Methodology. **Bo Pang:** Methodology. **Qiyue Sun:** Methodology. **Shanshan Guo:** Methodology. **Xiaolan Cui:** Project administration, Methodology. **Jing Sun:** Project administration, Methodology, Writing – review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2024.01.002.

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