# Qiangli Wuhu Mixture Alleviates LPS-Induced Pneumonia by Inhibiting the TLR4/NF-κB/NLRP3 Pathway: A Study Based on Network Pharmacology

**Jie Tian**
Ningxia Chinese Medicine Research Center
**Xiao-Long Wang**
Ningxia Medical University
**Long-Cheng Wang**
Ningxia Chinese Medicine Research Center
**Fei Chen**
Ningxia Medical University
**Yun Tian**
Monash University
**Li Ma**
Ningxia Chinese Medicine Research Center
**Chao-Yun Pan**
Ningxia Chinese Medicine Research Center
**Yan-Pinig Wang**
138950138060163.com

ningxia chinese medicine research center https://orcid.org/0000-0002-1585-2746

**Research**

**Keywords:** Qiangli Wuhu Mixture, network pharmacology, LPS, pneumonia, TLR signal pathway

**Posted Date:** October 28th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-965011/v1

**Version of Record:** A version of this preprint was published at Pharmaceutical Biology on July 12th, 2022. See the published version at https://doi.org/10.1080/13880209.2022.2093384.

# Abstract

**Background:** This study aimed to clarify the mechanism of action of Qiangli Wuhu Mixture used in the treatment of pneumonia.

**Methods:** The potential targets of Qiangli Wuhu Mixture in the treatment of pneumonia were predicted by network pharmacology. ICR male mice were randomly divided into 5 groups: normal control (Control), pneumonia model (Vehicle), low and high dose groups of Qiangli Wuhu Mixture, and dexamethasone positive control group with 12 mice in each. The model was established by tracheal instillation of lipopolysaccharide for 3 consecutive days. After the establishment of the animal model, Qiangli Wuhu Mixture was intragastrically administered for 5 days; the expression of inflammatory factors tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and anti-inflammatory factor interleukin-10 (IL-10) in the serum of mice in each group was detected by ELISA kit; the protective effect of Qiangli Wuhu Mixture on the lung tissue of pneumonia mice was observed by staining; Western Blot was used to detect the expression of Toll-like receptor 4 (TLR4), nuclear factor kappa B (NF-κB) and NLRP3 signal pathway related proteins in lung tissue.

**Results:** Network pharmacology predicted that the target of Qiangli Wuhu Mixture in treating pneumonia might be related to lipopolysaccharide reaction and Toll-like receptor signaling pathway. the in vivo results indicated that Qiangli Wuhu Mixture had a strong protective effect on acute pneumonia in mice induced by lipopolysaccharide, decreased the levels of inflammatory factors TNF-α, IL-1β and IL-6 in serum of model mice, and decreased the activation of TLR4, NF- κ B, and the expression of NLRP3 protein in lung tissue.

**Conclusion:** Qiangli Wuhu Mixture has a strong protective effect on lipopolysaccharide-induced pneumonia in mice, and its mechanism of action may be related to reducing the pathological changes of lung tissue, reducing the level of inflammatory cytokines in blood, and inhibiting the inflammatory signal pathway of TLR4/NF-κB/NLRP3.


# Background

Respiratory diseases are common and frequently occurring. They can seriously endanger health, and in recent years, they have caused a great burden on global health and economy[1]. It is reported that respiratory diseases rank third in the global mortality rate of non-communicable diseases. Pneumonia is among the commonest forms of respiratory disease, with about 120 million new cases every year, and 1.3 million deaths. The number of deaths caused by severe pneumonia accounts for 8.7% of all deaths each year. Therefore, pneumonia has become a major health problem that requires urgent attention. Pneumonia refers to the inflammation between the terminal airway, alveoli, and lung parenchyma. It can be caused by infection, physical and chemical factors, immune injury, allergy and through the use of drugs. Infection is the most common cause of pneumonia. Almost all pathogenic microorganisms and parasites can cause pneumonia[2]. The main clinical manifestations are fever, cough, dyspnea, and moist

rales in the lung. Common pathogens include *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Klebsiella pneumoniae*[3]. In recent years, the excessive prophylactic use of antibiotics, their combined use, increased use of high-level antibiotics, empirical use, and frequent replacement of antibiotics have all contributed to a significant increase in the rate of drug resistance[4–6]. As a result, the efficacy of antibiotics is greatly affected. The long-term use of antibiotics also has certain side effects on the health of patients[7]. For these reasons, researchers continue to explore the efficacy of traditional Chinese medicine (TCM) in the treatment of pneumonia, aiming to reduce the dependence on antibiotics.

Qiangli Wuhu Mixture is a medical concoction approved and registered by Ningxia Medical Products Administration. This mixture has been prepared by modifying the classic traditional prescriptions of Chinese medicine, "Ma Xing Shi Gan Decoction" and "Sangbaipi Decoction". The following constituents of the original prescriptions were retained in the Qiangli Wuhu mixture: ephedra, bitter almond, Ephedrae herba, Semen armeniacae amarum, Mori cortex, Radix Scutellariae and Pinelliae Rhizoma Praeparatum Cum Alumine. In addition, the following constituents were added to the mixture: *Bombyx batryticatus*, Pheretima, Stemonae Radix, Folium Eriobotryae, Descurainiae Semen LepidII Seneb, Uncariae Ramulus Cum Uncis and FritillariaeThunbergII Bulbus[8, 9]. The mixture has the following effects: relieving spasm, cough and asthma, clearing heat and resolving phlegm. In the past 20 years of clinical application, Qiangli Wuhu Mixture has had a significant effect on cough, acute and chronic bronchitis, pneumonia and other diseases caused by infections of the respiratory tract. The mixture can be used in many types of pneumonia, especially in children with phlegm-heat sealed lung (a type of symptom recognised in TCM) pneumonia[10, 11]. However, its mechanism of action has not been clarified yet. With the collaboration between computer network technology and life science, and the rapid development of interdisciplinary technology, network pharmacology integrates the academic theories of systems biology and pharmacology[12]. Computer network technology is used to summarise and analyse the results from previous research into drug molecular structure, target characteristics and the interaction rules of genes, proteins and metabolites. This is done to establish a variety of openly accessible scientific databases. It provides an opportunity for the study of multi-target mechanisms of action in TCM. Additionally, it provides a sense of direction for further exploring the materials and potential mechanisms of action of TCM compound mixtures in the treatment of pneumonia[13]. Therefore, this study is based on network pharmacology. In this study, we first retrieve the effective components and action targets of the Qiangli Wuhu Mixture, interactively analyze the correlation between the mixture and the therapeutic target of pneumonia. We then predict the therapeutic target of Qiangli Wuhu Mixture in the treatment of pneumonia and verify the results by conducting experiments. The purpose of this study is to lay a foundation for further exploring the mechanism of action of Qiangli Wuhu Mixture in the treatment of pneumonia, and to provide a basis for its extensive clinical application.

# Materials And Methods

2.1 Network pharmacology

2.1.1 Database and analysis software

(1)Drug target screening: TCMSP database (http://tcmspw.com/tcmsp.php), TCMID database (http://119.3.41.228:8000/tcmid/), Pubchem database (https://pubchem.ncbi.nlm.nih.gov/), Swiss Target Prediction database (http://www.swisstargetprediction.ch/) ;

(2)Disease target screening: Disgenet database (https://www.disgenet.org/), Genecards database (https://www.genecards.org/),TTD database (http://db.idrblab.net/ttd/), STRING database (https://string-db.org/); Drawing and analysis software: Venny2.1 online software mapping tools (https://bioinfogp.cnb.csic.es/ tools/ venny/), Cytoscape3.7.2 software, R3.6.1 software.

2.2 Experimental materials

ICR mice weighing 20±2g were provided by the Animal Experimental Center of Ningxia Medical University. The mice were adapted to a moderate environment of 21-25℃, humidity of 50±20% for 3 days, 12 hours of light per day, and adequate food and water were guaranteed. Qiangli Wuhu Mixture was produced by the Chinese Medicine Research Center of Ningxia (batch number: 20201217, crude drug content: 1.05g/mL). Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone was purchased from Meilunbio (Dalian, Liaoning, China). ELISA kits (TNF- α, IL-6, IL-1β, IL-4 and IL-10) were purchased from Servicebio (Wuhan, China). Antibodies: TLR4, 1:500; MyD88, 1:1000; NLRP3, 1:1000 were purchased from Abcam, (Cambridge, MA, USA); IKK-beta, 1:500; P-IKK-beta, 1:500; IκB-α, 1:1000; p-IκB-α, 1:1000, NF-κB, 1:1000; p-NF-κB, 1:1000; GAPDH, 1:2000; α-tubulin, 1:1000 and HRP-Anti-Rabbit Secondary Antibody, 1:5000 were purchased from ProteinTech, (Wuhan, China). SUPER ECL Assay Kit and BCA Protein Assay Kit were purchased from Nanjing KeyGen Biotech Co., Ltd., (Nanjing, China).

2.3. Establishment of pneumonia model, grouping and administration of mice

(1) Dosage: The daily dose of Qiangli Wuhu Mixture for 60 kg adults is 100 mL, and the crude drug concentration is 1.01g /mL. According to the formula, the daily equivalent dose of mice is about 20 g/kg[14]. This was set as the high dose. The low dose was set at 10 g/kg. Dexamethasone was used as a positive control drug at a dose of 10 mg/kg.

$$HED = 1.68\mathrm{g/kg}, MouseKm = 3, HumanKm = 37$$

$$MED = HED/(MouseKm/HumanKm) \approx 20\mathrm{g/kg}$$

(2) ICR male mice were randomly divided into 5 groups, including normal Control group (Control), pneumonia model group (Vehicle), Qiangli Wuhu Mixture low-dose (10 g/kg), high-dose (20 g/kg) group, and dexamethasone positive Control group (DXM), with 12 mice in each group. Modeling was performed by endotracheal infusion of LPS 10 mg/kg, 5 μL at a time, for 3 consecutive days. After the animal model was established, the drug was administered by intragastric administration twice a day for 5 days. Vehicle mice were given equal volume of 0.9% normal saline

2.4.1 Hematoxylin and eosin staining

Mice were anaesthetised by i.p. 10% chloral hydrate, thoracic cavity was exposed. phosphate buffer saline was the first perfusion through the right ventricle, followed by perfusion fixation with 4% paraformaldehyde solution. The right lung was extracted and fixed in 10% formaldehyde solution, paraffin section was prepared, hematoxylin-eosin (hematoxylin, eosin, eosin, eosin, eosin, eosin, eosin, eosin, eosin, HE). Histopathological changes were observed in the lung by staining to observe the degree of damage of alveolar epithelial cells, inflammatory cell infiltration and alveolar wall thickening.

# 2.4.2 ELISA

The eyeballs of the experimental mice were clipped with tweezers and removed quickly, and the blood flowed into the anticoagulant tube from the orbit. The plasma supernatant was then extracted by centrifugation and stored at -80 ℃. According to the steps of ELISA kit, the concentrations of TNF- α, IL-1 β, IL-6 and IL-10 in serum were detected in turn. After the termination of the reaction, the enzyme plate was put into the enzyme meter, 450 nm wavelength detection was selected. The standard and blank control areas were determined, and the corresponding optical density (OD) values were detected. The standard curve was then drawn, and the concentration of the sample was calculated through the standard curve.

2.5 Western blot.

Lung tissues of mice were collected and stored at -80 ℃ for later use. Radio-Immune Precipitation Assay lysate was added to the lung tissue to split homogenate, and protein was extracted and determined by BCA method. The protein was concentrated, and then transferred to Polyvinylidene fluoride membrane by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, then sealed with 5% skim milk at room temperature for 2 h, incubated at 4 ℃ overnight with primary antibody, rinsed 4 times with TBST for 8 min, incubated at room temperature with secondary antibody for 90 min, rinsed 3 times with TBST. Developing reaction was performed by SUPER ECL Assay Kit and images were collected then. Image J software was used to analyse gray values to count protein expression.

2.6 Statistical analysis

Data analyses and graph generation were performed using SPSS version 23.0 and GraphPad Prism 7 software. Data are shown as the mean ± standard error of the mean (SEM). One-way ANOVA was used to perform statistical analysis followed by Tukey’s post-hoc pairwise test, and significant difference was considered at the $P<0.05$ level.

# Results

3.1 Network pharmacology

3.1.1 Screening of active ingredients and their targets

Drug composition and target screening: The compound drugs were searched in TCMSP database, and the ingredients not included in TCMSP were searched by TCMID as potential active ingredients. The structures of the above components were obtained by using Pubchem database and imported into Swiss Target Prediction database. Targets with predicted scores greater than 0 were selected as drug targets.

Results: OB ≥ 30% and DL ≥ 0.18 were set in TCMSP database, and the active components of Epherdrae herba, Armeniacae semen amarum, Mori cortex, Scutellariae radix, Bombyx Batryticatus, Pheretima, Stemonae Radix, Eriobotryae Folium, Descurainiae Semen LepidII Seneb, Uncariae Ramulus Cum Uncis, Fritillariae ThunbergII Bulbus and Pinelliae Rhizoma Praeparatum Cum Alumine were retrieved. Then 203 potential active compounds and 1129 drug targets were screened out using Swiss Target Prediction database (Table 1).

Table 1
Active components and targets of Qiangli Wuhu Mixture

| Medicinal herbs | Active compounds | Predicted targets |
|---|---|---|
| Epherdrae herba | 23 | 348 |
| Armeniacae semen amarum | 19 | 398 |
| Mori cortex | 31 | 229 |
| Scutellariae radix | 36 | 442 |
| Bombyx Batryticatus | 4 | 23 |
| Pheretima | 10 | 186 |
| Stemonae Radix | 32 | 653 |
| Eriobotryae Folium | 18 | 367 |
| Descurainiae Semen LepidII Seneb | 12 | 262 |
| Uncariae Ramulus Cum Uncis | 33 | 675 |
| Fritillariae ThunbergII Bulbus | 7 | 328 |
| Pinelliae Rhizoma Praeparatum Cum Alumine | 13 | 464 |

3.1.2 Screening of pathogenic targets for pneumonia

The disease targets were retrieved with "pneumonia" as a keyword by using Disgenet, Genecards and TTD databases. Results: A total of 854 disease targets were obtained by using "pneumonia" as a keyword in Disgenet, Genecards and TTD databases.

3.1.3 Screening of drug-disease common targets

Drug targets numbering 1129, and 854 disease targets were input on Venny2.1 online software mapping tool platform to draw Venn diagram, and 135 drug-disease common targets were obtained after

intersection of the two parts (Figure 1).

3.1.4 Network Construction

Cytoscape 3.7.2 software was used to construct a Network diagram of "drug-component-target-disease", and Network Analyzer function was used to analyse the main active ingredients of TCM compound. Results: 203 potential active ingredients and 135 drug-disease common targets in TCM compound were inputted into Cytoscape software. Isolated components without intersection with targets were deleted, and the network diagram of "drug-component-target-disease" interaction was drawn (Figure 2).

3.1.5 Another method to construct the relationship between proteins

The above 135 common targets were input into STRING database for retrieval. The protein type was set as "*Homo sapiens*" and the minimum interaction threshold was 0.4. The network relationship data of target interaction was obtained and imported into Cytoscape software to draw the protein interaction network diagram (Figure 3). The color and its shades represent the size of the degree value.

3.1.5 Core target screening based on topology analysis

PPI network was imported into Cystoscape 3.7.2, and topology analysis was conducted by Network Analyzer tool. Genes with degree values greater than average scores were selected as core targets, and bar charts were drawn for the first 30 targets using R 3.6.1 (Figure 4).

3.1.6 GO enrichment analysis

Bioconductor bioinformatics software was used to conduct functional enrichment analysis of key target genes GO and KEGG, based on R software (P value <0.05, Q value <0.05), and output the results in the form of a bubble graph (Figure 5).

Results: The biological process, cell component and molecular function of 135 common targets were selected by GO analysis after R language operation. GO results showed that the intersection gene set was enriched in 2387 biological process pathways, including bacterial infection, LPS response, inflammatory response and T cell activation; the intersection gene set was enriched to 62 cell components; the set of intersected genes was enriched to 130 in the process related to molecular function, mainly including cytokine receptor and primary lysosomal sac.

3.1.7 KEGG enrichment analysis

A total of 158 KEGG pathways were obtained by running 135 common targets through R Programming Language. The first 20 results formed a bar graph with KEGG functional enrichment (Figure 6). P adjust represented the significance of enrichment, and the redder the color, the higher the significance. The results showed that the involved pathways were highly correlated with TNF signaling pathway, Toll-like

receptor signaling pathway, PI3K signaling pathway and MAPK signaling pathway. Considering that the biological processes involved in the treatment of pneumonia by Qiangli Wuhu Mixture are highly correlated with inflammatory response, LPS reaction and bacterial infection, TNF-α, Toll-like receptor signaling pathway and NF-κB signaling pathway were highly related to the involved signaling pathways. In the next experiment, LPS tracheal drip was used to stimulate mice to establish a pneumonia model to evaluate the therapeutic effect of Qiangli Wuhu Mixture on pneumonia and to verify the related predicted signaling pathways.

3.2 Effect of Qiangli Wuhu Mixture on serum inflammatory factors of mice with LPS induced pneumonia

Mice were injected with LPS tracheal drops for 3 consecutive days and intragastric administration of strong Wuhu Mixture (10 and 20 g/kg) was conducted to detect the effects of strong Wuhu Mixture (10 and 20 g/kg) on serum inflammatory factors TNF-α, IL-1β, IL-6 and anti-inflammatory factor IL-10. The results showed that the levels of TNF-α, IL-1β and IL-6 in serum of mice in model group were significantly increased, and the levels of TNF-α, IL-1β and IL-6 were significantly decreased by intragastric treatment with different dosages of Qiangli Wuhu Mixture (Fig. 7. A-C). At the same time, Qiangli Wuhu Mixture effectively increased the serum level of anti-inflammatory factor IL-10 (Fig. 7. D).

3.3 Hematoxylin and eosin staining

The lung tissues of the control group were stained clearly, and the cells were uniform in size without inflammatory cell infiltration. The model group had a few intact alveolar structures with uneven alveolar cell size and narrow airway. Inflammatory infiltration, vascular hyperplasia, and fibrosis with small blood vessels, lymphocytes, and a few macrophages can be seen. The lung tissue lesions of mice in the high-dose and low-dose Qiangli Wuhu Mixture groups were slight, with lung interstitial thickening and the presence of individual inflammatory cells, which had been significantly improved compared with the pathological model group.

3.4 Effect of Qiangli Wuhu Mixture on inflammatory factors and NLRP3/TLR4/NF-κB signaling pathway in mice with LPS-induced pneumonia

According to the results of network pharmacology analysis, NLRP3 is an important protein in the process. In addition, TLR4 is the LPS recognition receptor, while MyD88 is the connector protein of this inflammatory process. The levels of NLRP3, TLR4 and MyD88 were detected by Western blot. As shown in Figure 9 (A), the expressions of NLRP3, TLR4 and MyD88 were significantly upregulated in the model group compared with the control group, but the levels of NLRP3, TLR4 and MyD88 were effectively inhibited by administration of Qiangli Wuhu Mixture. Considering that MyD88 signal transduction mediates NF-κB pathway, we evaluated the effect of Qiangli Wuhu Mixture on NF-κB pathway by Western blot analysis[15]. As shown in the results (Figure 9. B-D), LPS stimulation significantly increased the level of p-IκB-α/IκB-α p-IκB-α/IκB-α and p-NF-κB/NF-κB. However, the levels of p-IκB-α/IκB-α p-IκB-α/IκB-α and p-NF-κB/NF-κB in mice could be effectively reduced by intragastric administration of different doses of Qiangli Wuhu Mixture.

# Discussion

The British Thoracic Society guidelines recommend that the clinical diagnosis of pneumonia should be explicitly treated with antibiotics because bacterial and viral pneumonia cannot be reliably distinguished[16]. Some reports showed that viral and bacterial co-infection of pneumonia accounted for 30% of the cases. In addition, in the clinical treatment of pneumonia, due to the lag in identification and diagnosis of pathogenic bacteria, targeted antibacterial treatment is difficult[17]. Therefore, the use of antibiotics is mostly based on the age of patients, the most likely pathogens, and the experience of clinicians[18]. Penicillin, cephalosporin and macrolides are the most used antibiotics[19]. The increase in bacterial resistance makes the treatment of pneumonia difficult, and the abuse of antibiotics also has a variety of side effects, such as liver and kidney damage, gastrointestinal symptoms, double infection, and allergy.

In the treatment of pneumonia, TCM has many functions, such as inhibiting virus proliferation, promoting the expression of interferons, inhibiting inflammation, and enhancing immunity. Viruses with RNA as genetic material, such as influenza and coronaviruses, are more prone to mismatches and mutations during replication than DNA viruses. Their high variability makes it harder to develop vaccines and easier to develop resistance to single chemicals[20]. Chinese herbal medicine and compound medicine have the characteristics of multi-component, multi-pathway, and multi-pathway complex network. Therefore, drug resistance is relatively rare in TCM clinical practice. Moreover, increasingly, evidence suggests that Chinese medicine has definite therapeutic activity and few adverse reactions, and Chinese medicine has good curative effects and significant safety in treating pneumonia. In nearly 20 years of clinical use, Qiangli Wuhu Mixture has a significant curative effect on children with bronchopneumonia with an effective rate of 97.30%, but its mechanism of action is still unclear. The systematic view of network pharmacology resembles the holistic view of TCM, which embodies the viewpoint of system theory. Information about drug targets serves as the bridge to the effective treatment of a disease using drugs[21]. In this study, 994 active ingredients were found through network pharmacology, corresponding to 135 targets for the treatment of pneumonia, which reflected the characteristics of TCM compound mixture with multiple targets. PPI topological analysis revealed that the core target genes were mainly TNF-α, IL-6, IL-1β, NF-κB, NLRP3 and other inflammatory factors. KEGG enrichment analysis showed that Toll-like receptor signaling pathway, NF-kappa B signaling pathway, T cell receptor signaling Pathway and NOD-like receptor signaling pathway are highly correlated in the treatment of pneumonia by Qiangli Wuhu Mixture.

NF-κB is mainly involved in immune and inflammatory responses and can induce the expression of downstream inflammatory cytokines such as TNF-α and IL-6 to regulate the inflammatory response of disease[22]. TNF-α and TNF receptor 2 can be expressed on immune cells and regulate T-cell survival, activation, and proliferation. One of the main causes of lung infection caused by Gram-negative bacteria, LPS of its cell wall outer membrane will effectively stimulate immune and structural cells. Introduction of LPS into the systemic circulation may lead to harmful inflammatory systemic immune responses[23]. Therefore, LPS is often used to establish animal inflammatory models[24–26]. Reliable evidence

indicates that LPS tracheal drip can establish a pneumonia model in mice[27–29]. NLRP3 inflammasome is mainly expressed in neutrophils, macrophages, monocytes, and dendritic cells and induces a primary immune response against pathogen invasion. When TLR and NLR are overactivated, it can lead to excessive release of IL-β and IL-18, which can cause a continuous and massive inflammatory response, leading to further aggravation of tissue damage[30, 31]. TLR4 recognizes and binds to Gram-negative bacterial lipopolysaccharides, leading to the activation of nuclear transcription factor-κB and expression of inflammatory factors such as IL-6 and TNF-α through myeloid differentiation factor 88 (MyD88) dependent and independent signaling pathways[15]. The study found that the expression levels of TLR2 and TLR4 in patients with pneumonia were significantly higher than those in normal people, suggesting that the increase in expression levels of is related to pneumonia[32]. TLR4, as an indicator for evaluating pneumonia can be studied[33]. Therefore, TLR4/NF-κB/NLRP3 pathway can be used as an important pathway to study pneumonia.

Based on the prediction and analysis of network pharmacology, we used LPS tracheal drip to establish a pneumonia model in mice and conducted confirmatory experiments on the predicted results. The results showed that Qiangli Wuhu Mixture significantly improved the lung tissue of LPS-induced pneumonia mice and reduced the expression of serum inflammatory factors. Meanwhile, Western Blot results suggested that the therapeutic effect of Qiangli Wuhu Mixture might be realized by inhibiting TLR4/NF-κB/NLRP3 signaling pathway. In addition, network pharmacology results predicted and analysed that the effects of Qiangli Wuhu Mixture on the treatment of pneumonia may also involve the likes of MAPK signaling pathway, apoptosis, intestinal immune network for IgA production, and inflammatory bowel disease. This suggests that the effect of Qiangli Wuhu Mixture on the treatment of pneumonia may also involve intestinal mucosal immunity, energy regulation and apoptosis. In the follow-up study, we will further explore other related targets and pathways. Although network pharmacology reveals the drug-gene-disease relationship through the analysis of network regulatory effects and protein interaction networks, its principle is similar to the overall concept of TCM. But network pharmacology also has some limitations. At present, there are not enough effective data of TCM clinical and basic research, and there are also few dedicated pharmacological databases. Secondly, the weights of each component of the mixture are not clear, and the results are often redundant and not specific, so there is scope for improving the analysis algorithm.

# Conclusion

In conclusion, the results of this study suggest that Qiangli Wuhu Mixture has a strong protective effect on LPS-induced pneumonia in mice, and that the mechanism may be related to reducing lung tissue lesions and the level of inflammatory factors in blood and inhibiting TLR4/NF-κB/NLRP3 inflammatory signaling pathway.

# Abbreviations

LPS

Lipopolysaccharide
QLWH
Qiangli Wuhu Mixture
TCMSP
Traditional Chinese Medicine System Pharmacology Database and Analysis Platform
TTD
Therapeutic Target Database
PPI
Protein-Protein Interaction Network
GO
Gene ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
OB
Oral bioavailability
DL
Drug-like-ness
TCM
Traditional Chinese Medicine.

# Declarations


### Acknowledgements

Not applicable.


### Author Contributions

Jie Tian and Long-Cheng Wang developed hypotheses and conceived the project; Xiao-Long Wang and Fei Chen designed the experiments and performed animal experiments; Yun Tian and Xiao-Long Wang wrote the manuscript; Li Ma and Chao-Yun Pan analysed the data and validated it; Yan-Ping Wang was involved in the administration of the project and in acquiring funding. All authors read and approved the final manuscript.


### Funding

This work was supported by Ningxia Natural Science Foundation (No. 2020AAC03432)


### Availability of data and materials

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

### Ethics approval and consent to participate

Animal experiments were carried out in accordance with the recommendations of the institutional and national guidelines for animal care and use. The protocol was approved by the Committee on the Ethics

of Animal Experiments of Ningxia Medical University, Yinchuan, China (January 10, 2021).

### Consent for publication

Not applicable.

### Competing interests

The authors declare no conflicts of interest.

### Author details

a Ningxia Chinese Medicine Research Center, Yinchuan 750011, China. b Ningxia Medical University, Yinchuan 750004, China. c Monash University, Melbourne 3800, Australia

# References

1. Bhutta ZA, Das JK. Global burden of childhood diarrhea and pneumonia: what can and should be done? Pediatrics. 2013;131(4):634–6.
2. Mulholland K. Problems with the WHO guidelines for management of childhood pneumonia. Lancet Glob Health. 2018;6(1):e8–9.
3. Ottosen J, Evans H. Pneumonia: challenges in the definition, diagnosis, and management of disease. Surg Clin North Am. 2014;94(6):1305–17.
4. Ho J, Ip M. Antibiotic-Resistant Community-Acquired Bacterial Pneumonia. Infect Dis Clin North Am. 2019;33(4):1087–103.
5. Wunderink RG, Waterer G. Advances in the causes and management of community acquired pneumonia in adults. BMJ. 2017;358:j2471.
6. Rodrigues CMC. Challenges of Empirical Antibiotic Therapy for Community-Acquired Pneumonia in Children. Curr Ther Res Clin Exp. 2017;84:e7–11.
7. Mathur S, Fuchs A, Bielicki J, Van Den Anker J, Sharland M. Antibiotic use for community-acquired pneumonia in neonates and children: WHO evidence review. Paediatr Int Child Health. 2018;38(sup1):66–75.
8. Tian J, Wang Y-P, Ning Y, Wang Z-Y, Xu J. **Optimization of Water Extraction Process of Qiangli Wuhu Mixtures by Orthogonal Test**. *Mod Chin Med* 2016, 18(6). [in Chinese].
9. Tian J, Ning Y, Xu J, Wang Z-Y, Wang Y-P, Zhang Z. **Study on Process of Qiangli Wuhu Mixture purification**. *Ningxia Med J* 2018, 40. [in Chinese].

10. Wang H-M, Li Q-R. Clinical study of Qiangli Wuhu Mixture in treating children bronchopneumonia with phlegm-heat sealed lung. Ningxia Med J. 2020;42(2):41–10. [in Chinese].
11. Bryce J, Boschi-Pinto C, Shibuya K, Black RE, Group WHOCHER. WHO estimates of the causes of death in children. Lancet. 2005;365(9465):1147–52.
12. Wu X-M, Wu C-F. Network pharmacology: A new approach to unveiling Traditional Chinese Medicine. Chin J Nat Med. 2015;13(1):1–2.
13. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin J Nat Med. 2014;11(2):110–20.
14. Janhavi P, Divyashree S, Sanjailal KP, Muthukumar SP. **DoseCal: a virtual calculator for dosage conversion between human and different animal species**. *Arch Physiol Biochem* 2019:1–5.
15. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009;1(4):a000034.
16. Cardinale F, Cappiello AR, Mastrototaro MF, Pignatelli M, Esposito S. Community-acquired pneumonia in children. Early Hum Dev. 2013;89(Suppl 3):49–52.
17. Wallihan R, Ramilo O. Community-acquired pneumonia in children: current challenges and future directions. J Infect. 2014;69(Suppl 1):87–90.
18. Das RR, Singh M. Treatment of severe community-acquired pneumonia with oral amoxicillin in under-five children in developing country: a systematic review. PLoS One. 2013;8(6):e66232.
19. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM Jr, Musher DM, Niederman MS, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44(Suppl 2):27–72.
20. Woolhouse ME, Brierley L, McCaffery C, Lycett S. Assessing the Epidemic Potential of RNA and DNA Viruses. Emerg Infect Dis. 2016;22(12):2037–44.
21. Liu C-x, Liu R, Fan H-r, Xiao X-f, Chen X-p, Xu H-y. Lin Y-p: **Network Pharmacology Bridges Traditional Application and Modern Development of Traditional Chinese Medicine**. Chinese Herbal Medicines. 2015;7(1):3–17.
22. Koch L, Frommhold D, Buschmann K, Kuss N, Poeschl J, Ruef P. LPS- and LTA-induced expression of IL-6 and TNF-alpha in neonatal and adult blood: role of MAPKs and NF-kappaB. Mediators Inflamm. 2014;2014:283126.
23. Coimbra R, Melbostad H, Loomis W, Porcides RD, Wolf P, Tobar M, Hoyt DB. LPS-induced acute lung injury is attenuated by phosphodiesterase inhibition: effects on proinflammatory mediators, metalloproteinases, NF-kappaB, and ICAM-1 expression. J Trauma. 2006;60(1):115–25.
24. Gnauck A, Lentle RG, Kruger MC. The Characteristics and Function of Bacterial Lipopolysaccharides and Their Endotoxic Potential in Humans. Int Rev Immunol. 2015;35(3):189–218.
25. Whitfield C, Trent MS. Biosynthesis and export of bacterial lipopolysaccharides. Annu Rev Biochem. 2014;83:99–128.

26. Sender V, Stamme C. Lung cell-specific modulation of LPS-induced TLR4 receptor and adaptor localization. Commun Integr Biol. 2014;7:e29053.
27. Tao H, Li N, Zhang Z, Mu H, Meng C, Xia H, Fu L, Xu Y, Zhang S. Erlotinib Protects LPS-Induced Acute Lung Injury in Mice by Inhibiting EGFR/TLR4 Signaling Pathway. Shock. 2019;51(1):131–8.
28. Chang JS, Lin HJ, Deng JS, Wu WT, Huang SS, Huang GJ. Preventive Effects of Velvet Antler (Cervus elaphus) against Lipopolysaccharide-Induced Acute Lung Injury in Mice by Inhibiting MAPK/NF-kappaB Activation and Inducing AMPK/Nrf2 Pathways. Evid Based Complement Alternat Med. 2018;2018:2870503.
29. Niu X, Liu F, Li W, Zhi W, Zhang H, Wang X, He Z. Cavidine Ameliorates Lipopolysaccharide-Induced Acute Lung Injury via NF-kappaB Signaling Pathway in vivo and in vitro. Inflammation. 2017;40(4):1111–22.
30. Abrahams VM. The role of the Nod-like receptor family in trophoblast innate immune responses. J Reprod Immunol. 2011;88(2):112–7.
31. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J Biol Chem. 1999;274(16):10689–92.
32. Hauber HP, Tulic MK, Tsicopoulos A, Wallaert B, Olivenstein R, Daigneault P, Hamid Q. Toll-like receptors 4 and 2 expression in the bronchial mucosa of patients with cystic fibrosis. Can Respir J. 2005;12(1):13–8.
33. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature. 2000;406(6797):782–7.

# Figures

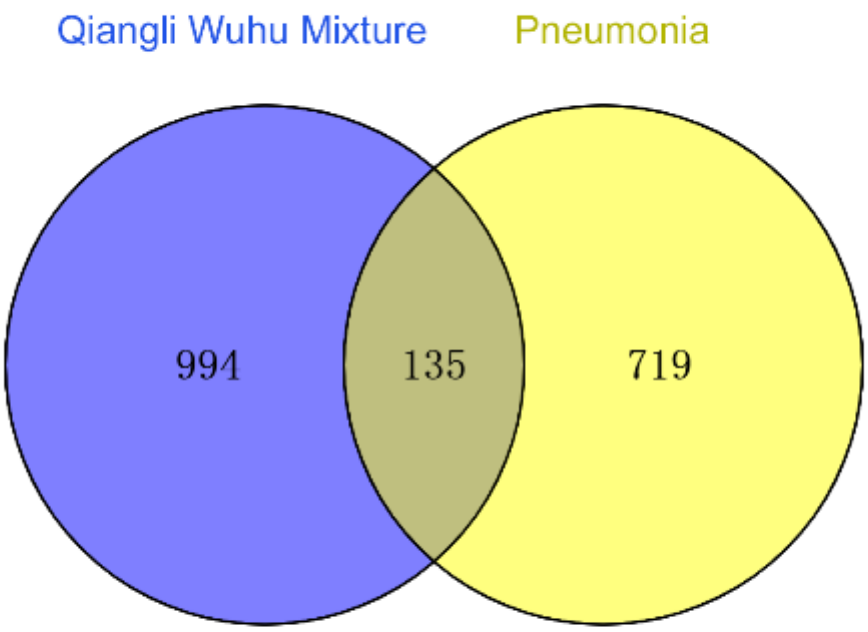
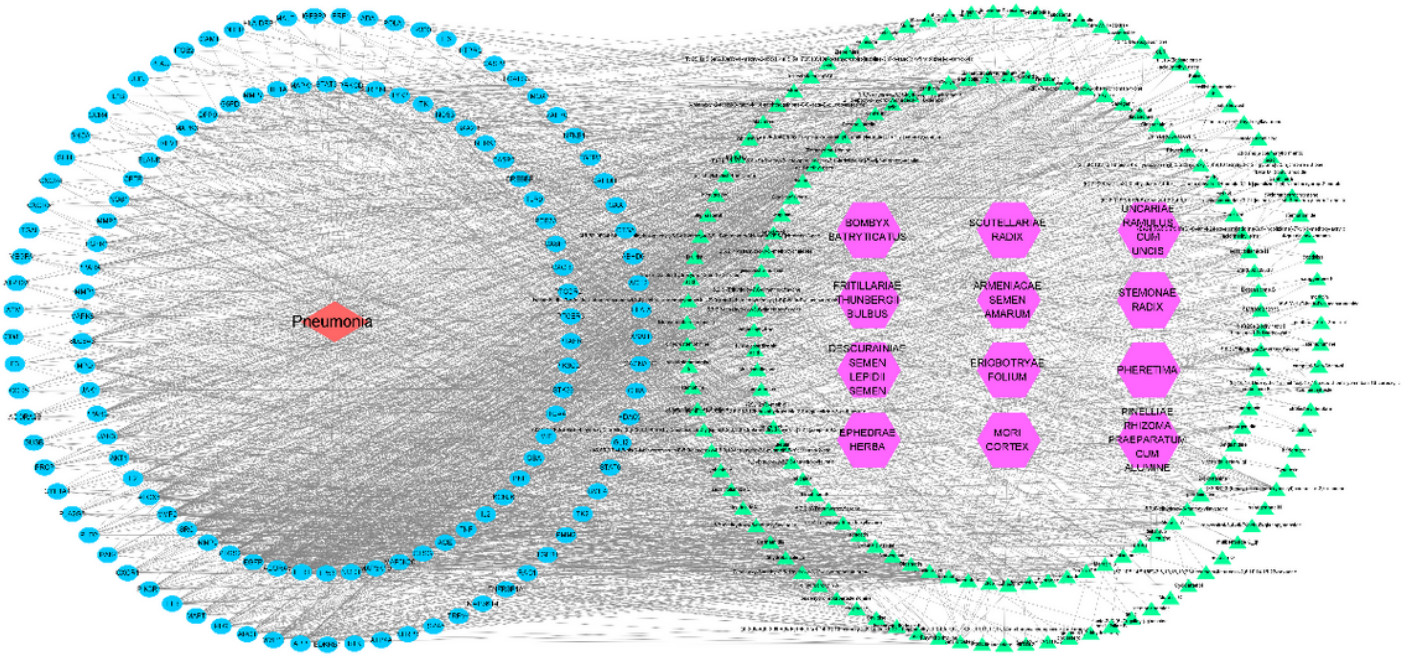


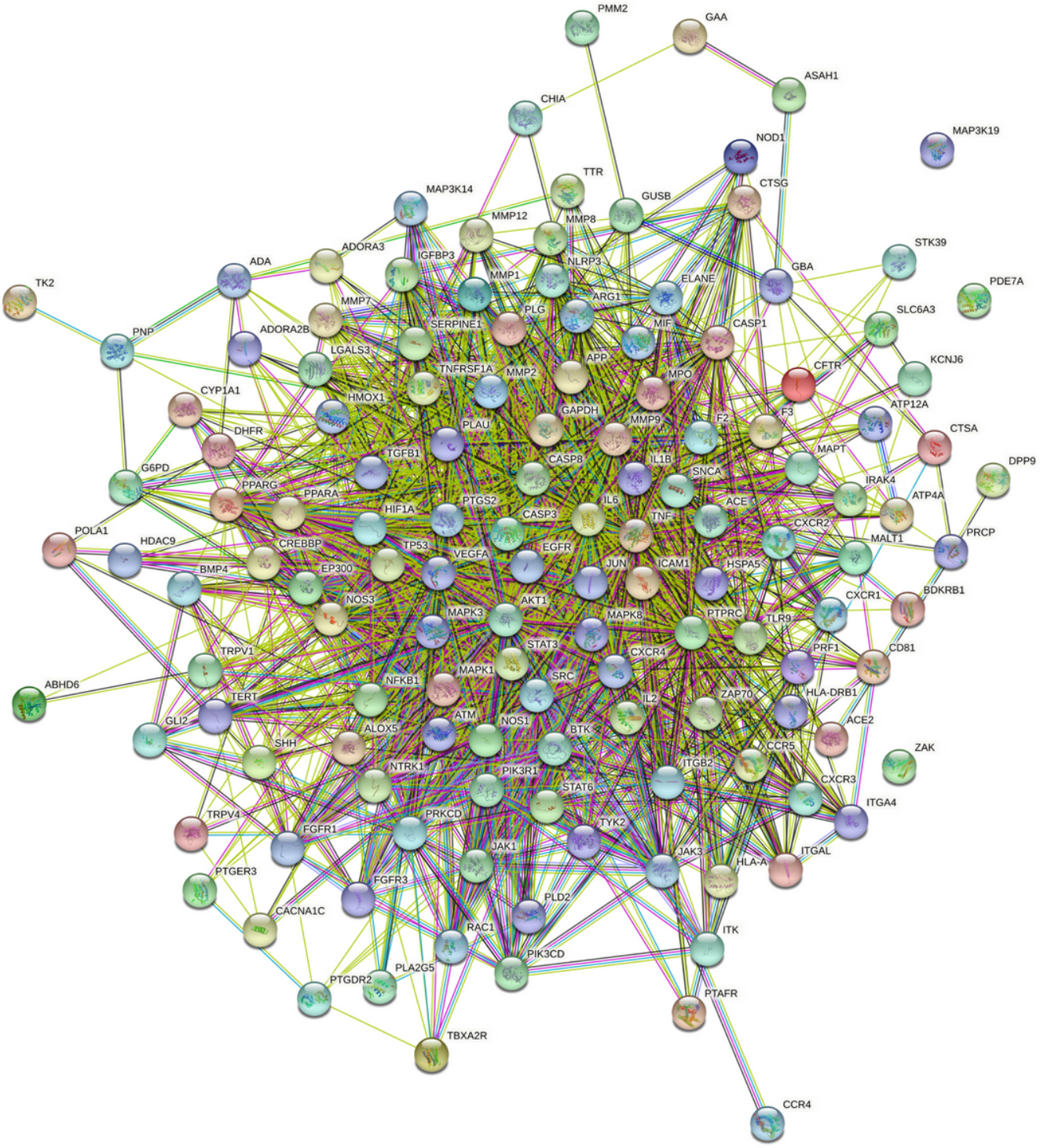

**Figure 1**

Venn diagrams of therapeutic and pathogenic targets




## Figure 2

Drug-component-target-disease interaction network diagram (In the figure, purple represents drugs, green represents 171 active components in Qiangli Wuhu Mixture, blue represents 135 common targets, and red represents diseases.)




## Figure 3

Protein interaction network

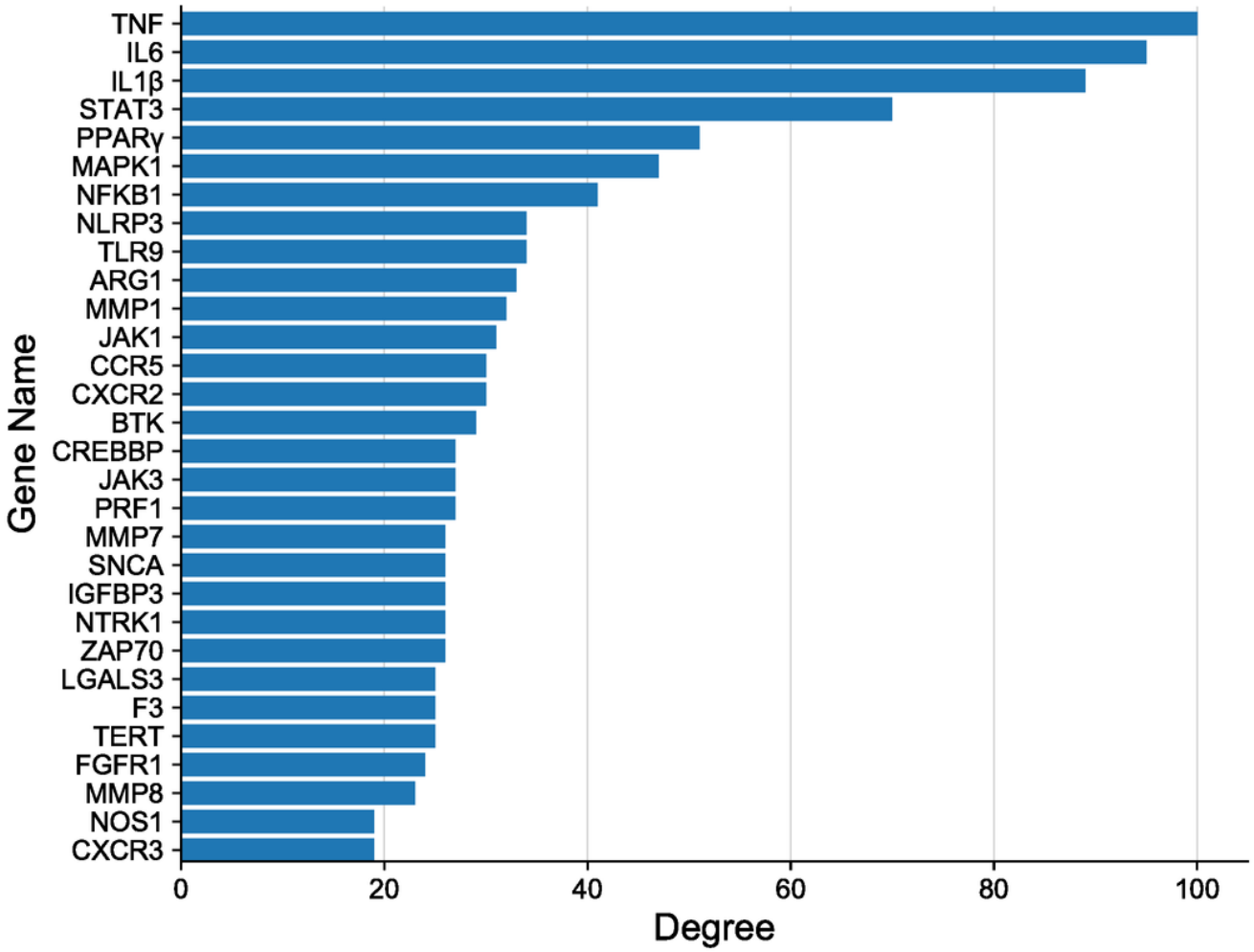
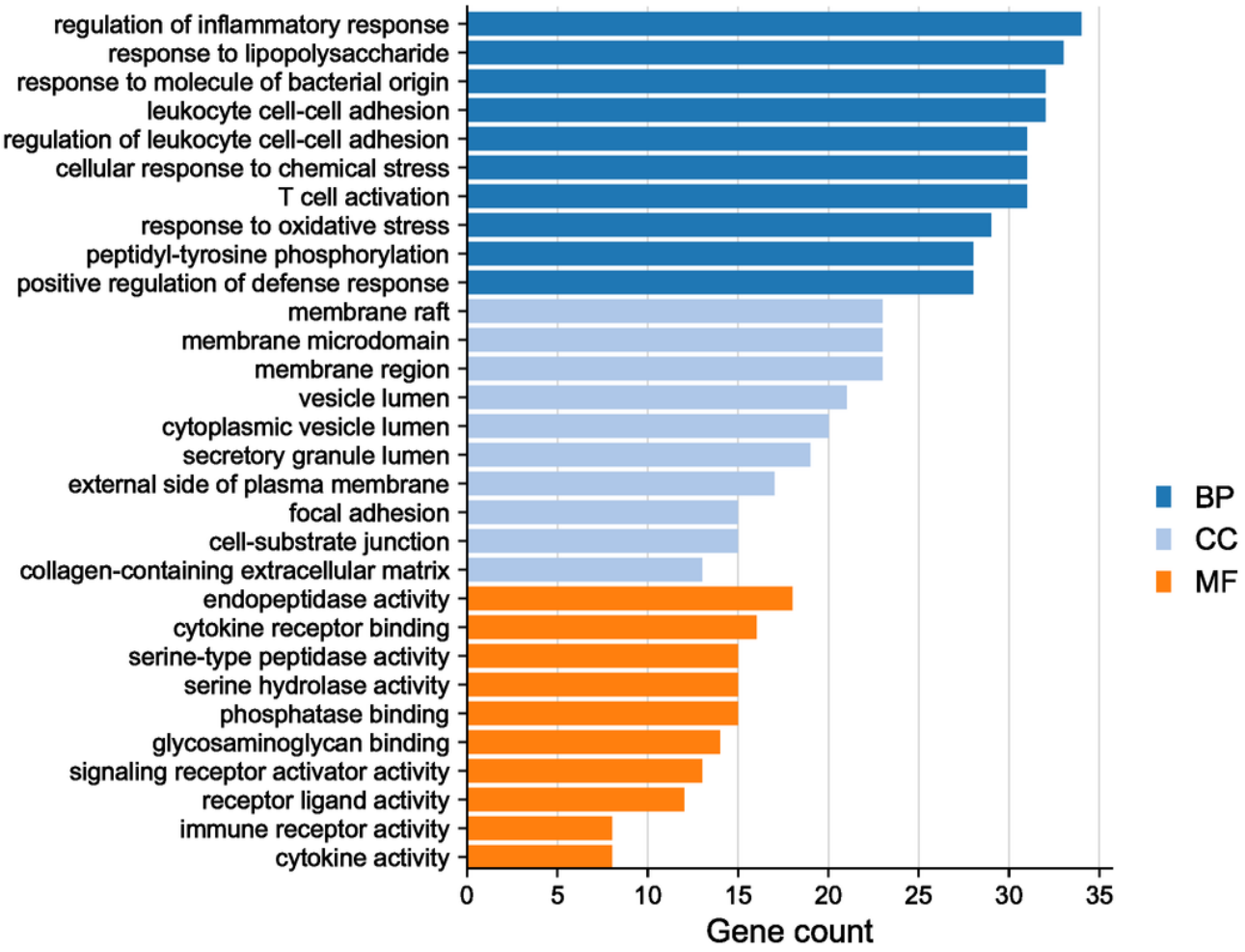


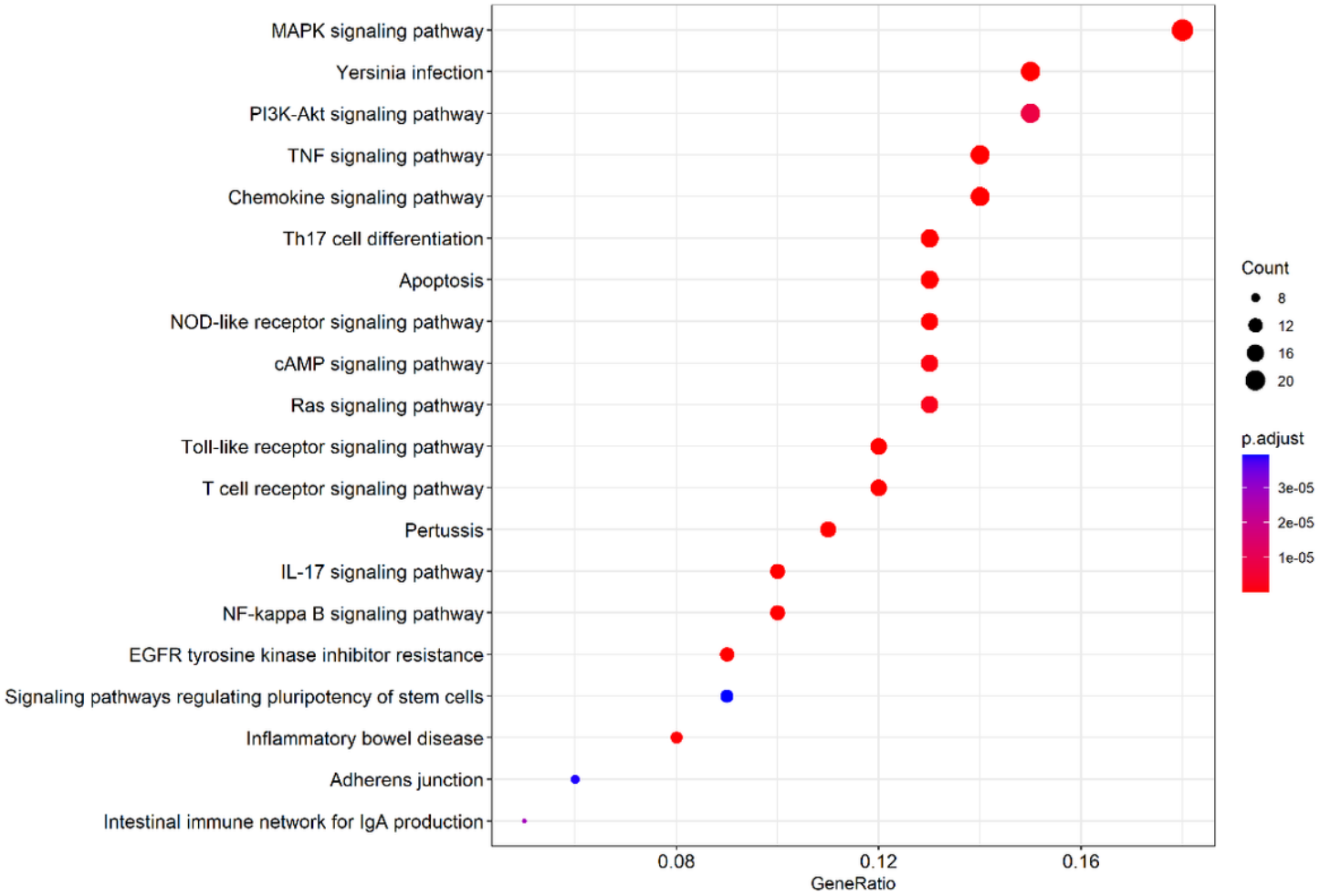

**Figure 4**

Ranking of core targets based on PPI topology analysis



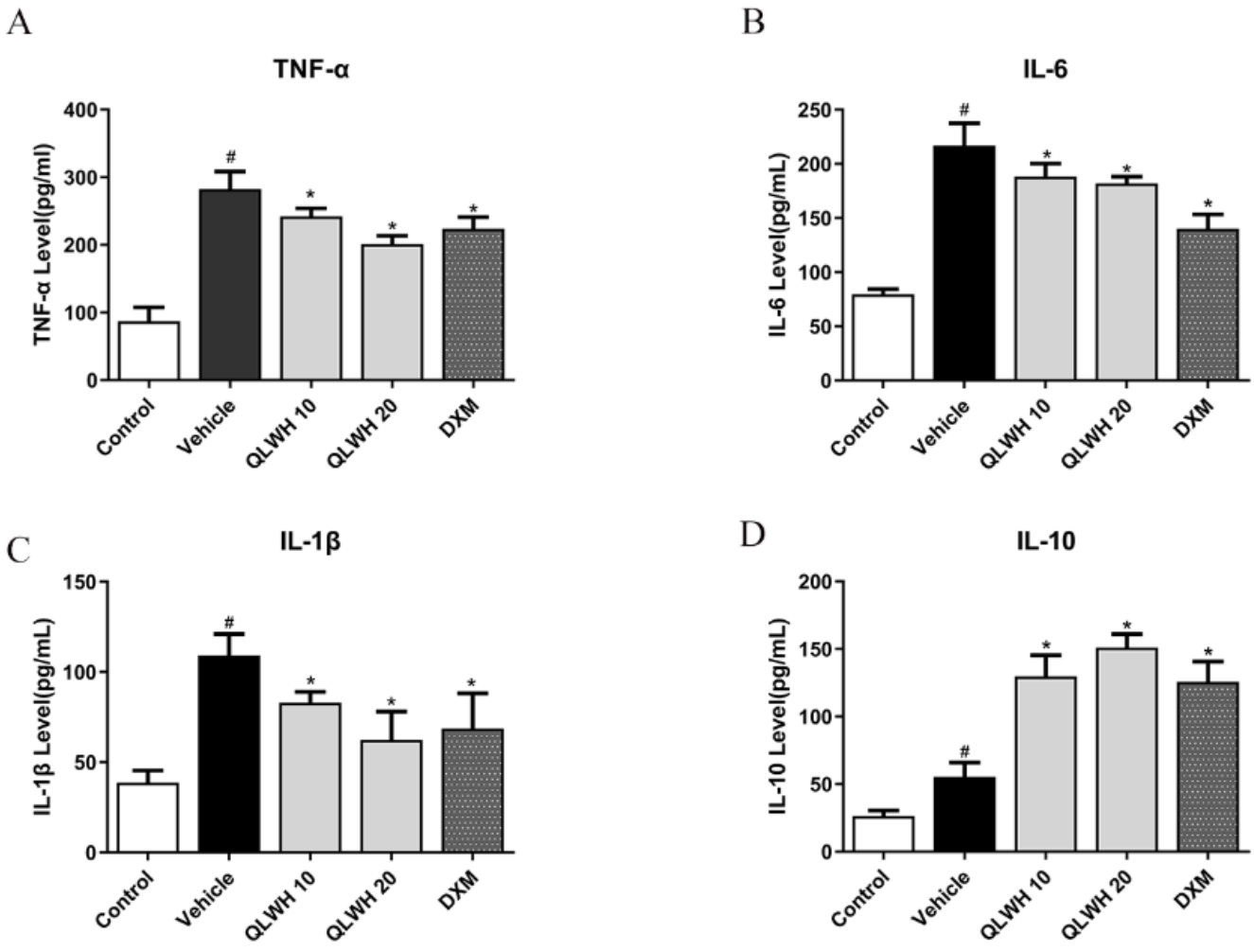

## Figure 5

GO enrichment analysis of Qiangli Wuhu Mixture used in the treatment of pneumonia (Biological processes, BP; Cell component, CC; Molecular function, MF)



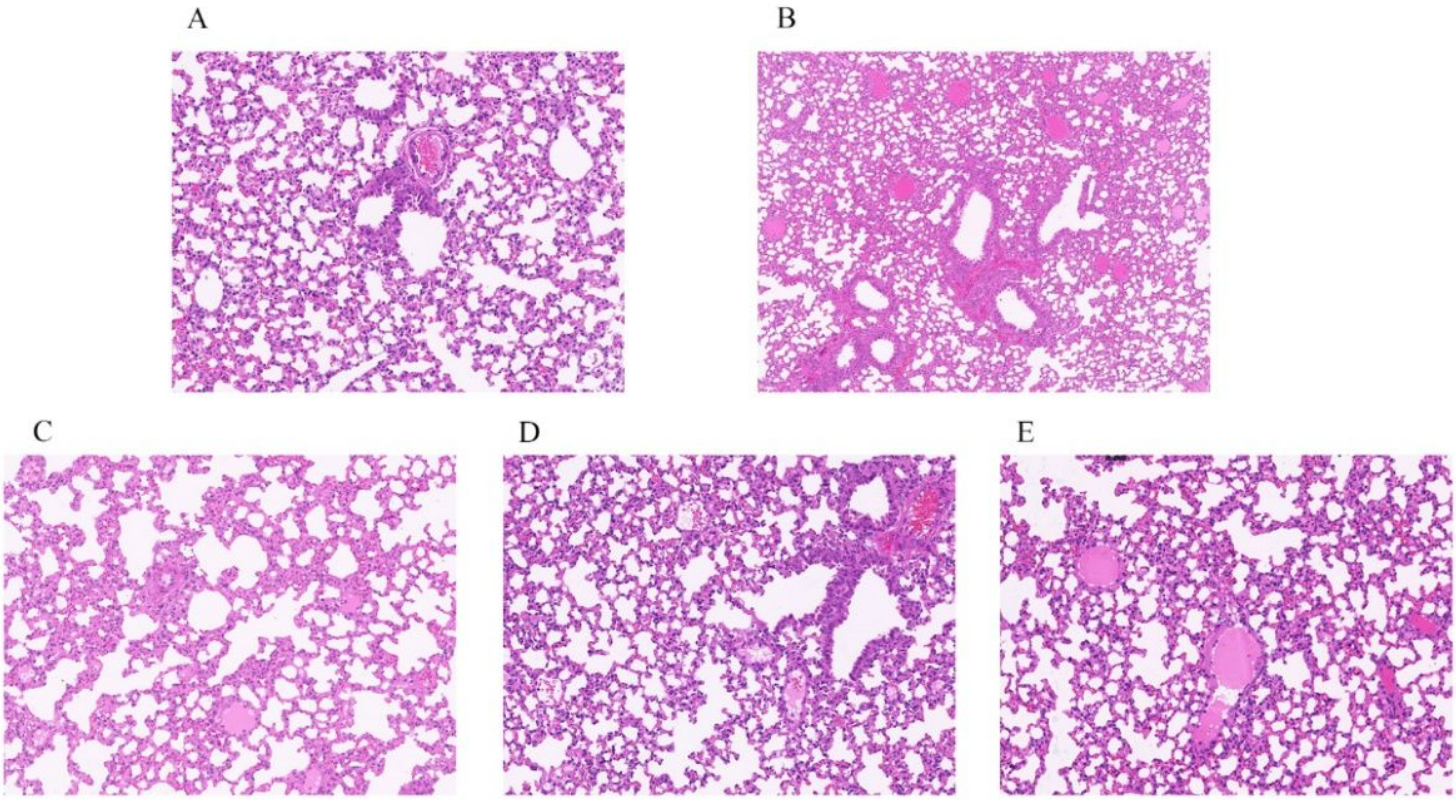

## Figure 6

KEGG enrichment analysis of Qiangli Wuhu Mixture in the treatment of pneumonia



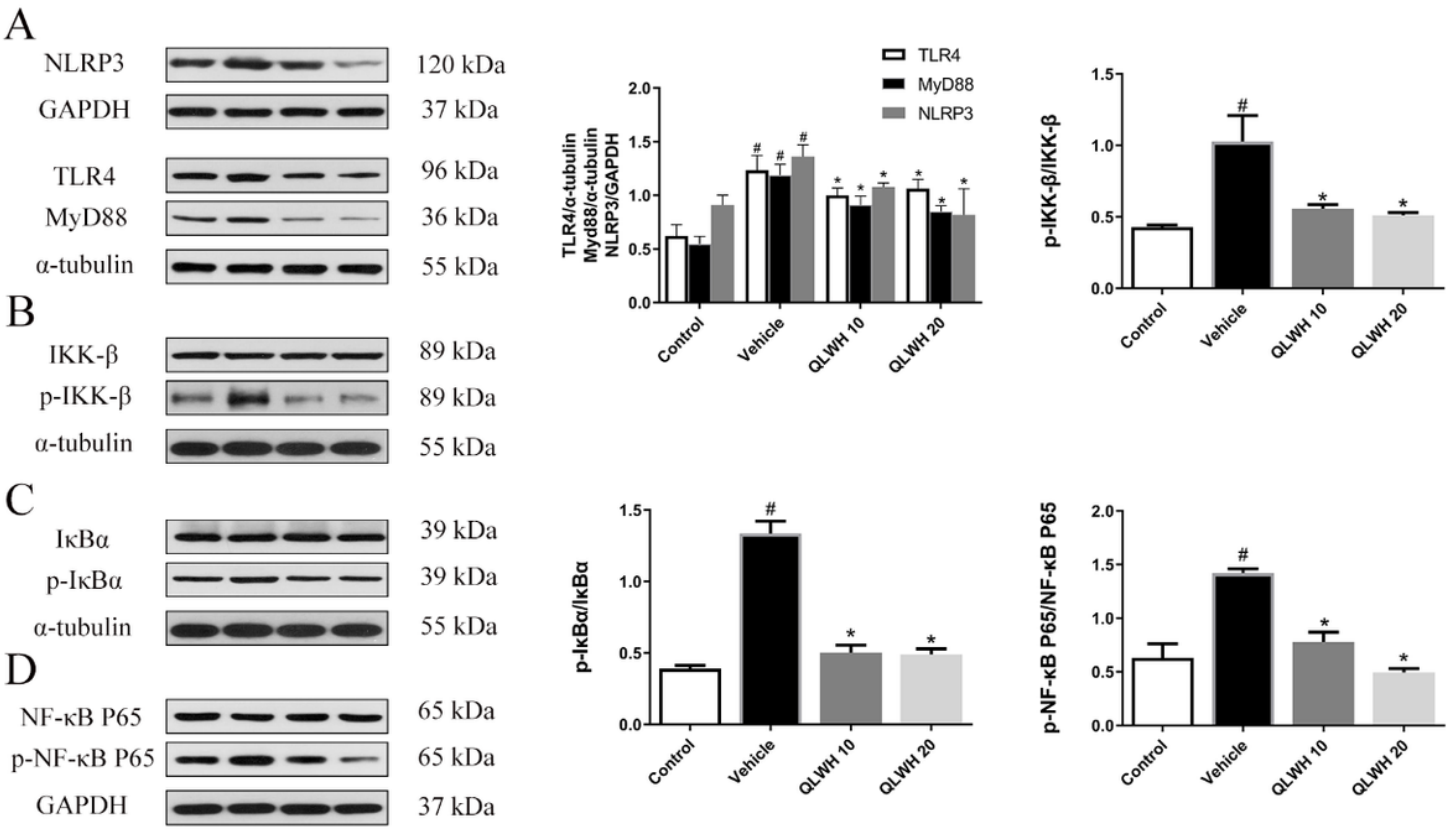

## Figure 7

Effects of Qiangli Wuhu Mixture on serum levels of inflammatory cytokines TNF-α, IL-1β, IL-6 and anti-inflammatory cytokines IL-10 in mice with LPS-induced pneumonia #p < 0.05 compared with the Control group ∗p < 0.05 compared with the Vehicle group




## Figure 8

Effect of Qiangli Wuhu Mixture on lung tissue injury of mice with LPS-induced pneumonia A: Control group B: Vehicle group C: Qiangli Wuhu 10 g/kg D: Qiangli Wuhu 20 g/kg E: DXM 10 mg/kg




## Figure 9

Effect of Qiangli Wuhu Mixture on NLRP3/TLR4/MyD88 pathway in mice with LPS-induced pneumonia. A. the protein levels of NLRP3, MyD88, TLR4 was detected by Western blot, B-D. the protein levels of NF-kappa B, p-NF-kappa B, IκB-α, p-IκB-α, IKK-β and p-IKK-β were detected by Western blot. #$p < 0.05$ compared with the Control group ∗$p < 0.05$ compared with the Vehicle group