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Investigating the mechanism of action of Yanghe Pingchuan Granule in the treatment of bronchial asthma based on bioinformatics and experimental validation

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ABSTRACT

Background: Yanghe Pingchuan Granule (YPG) is a patented Chinese medicine developed independently by the Anhui Provincial Hospital of Traditional Chinese Medicine. For many years, it has been used for the treatment of asthma with remarkable clinical effects. However, the composition of YPG is complex, and its potential active ingredients and mechanism of action for the treatment of asthma are unknown.

Materials and methods: In this study, we investigated the potential mechanism of action of YPG in the treatment of asthma through a combination of bioinformatics and in vivo experimental validation. We searched for active compounds in YPG and asthma targets from multiple databases and obtained common targets. Subsequently, a protein-protein interaction (PPI) network for compound disease was constructed using the protein interaction database for Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Finally, hematoxylin and eosin (H&E) staining, Masson staining, enzyme-linked immunosorbent assay (ELISA) analysis, immunofluorescence (IF) experiments, and Western blot (WB) experiments were performed to verify the possible mechanism of action of YPG for asthma treatment.

Results: We obtained 72 active ingredients and 318 drug target genes that overlap with asthma. Serine/threonine-protein kinase (AKT1), tumor protein p53 (TP53), tumor necrosis factor (TNF), interleukin (IL)-6, IL-1β, vascular endothelial growth factor-A (VEGFA), prostaglandin-endoperoxide synthase 2 (PTGS2), caspase-3 (CASP3), mitogen-activated protein kinase 3 (MAPK3) and epidermal growth factor receptor (EGFR) were the most relevant genes in the PPI network. KEGG analysis showed a high number of genes enriched for the nuclear factor kappa-B (NF-κB) signaling pathway. Animal experiments confirmed that YPG reduced inflammatory cell infiltration and down-regulated the expression of ovalbumin-induced inflammatory factors. Furthermore, YPG treatment decreased the protein expression of NFκB1, nuclear factor kappa B kinase subunit beta (IKBKB), vascular endothelial growth factor (VEGF), and vascular endothelial growth factor receptor 2 (VEGFR2) in lung tissue.

Conclusion: YPG has a positive effect on asthma by interfering with multiple targets. Furthermore, YPG may significantly inhibit the follicle-induced inflammatory response through the NF-κB signaling pathway.

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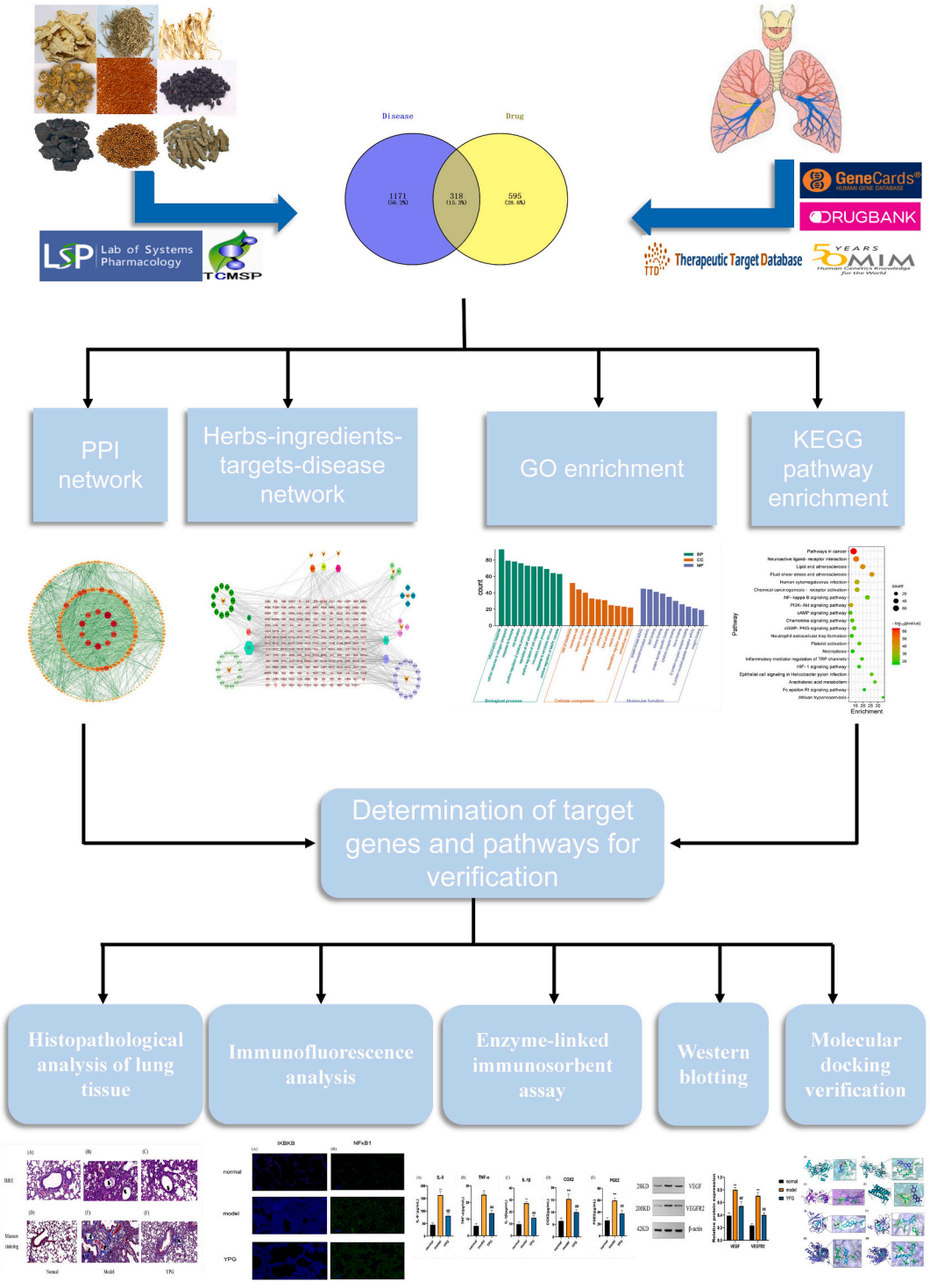

Fig. 1. Flow chart of anti-asthma design and analysis of Yanghe Pingchuan Granule.

1. Introduction

Asthma is a severe inflammatory disease of the airways that affects all age groups, especially children [1,2]. International Study on Asthma and Allergy in Childhood (ISAAC) reported a global prevalence of 13.7 % in children [3]. A variety of inflammatory cells, inflammatory mediators, and cytokines are involved in the pathogenesis of asthma, which is characterized by recurrent episodes of wheezing, chest tightness, shortness of breath, or cough [4]. Currently, available treatments include inhaled glucocorticoids, β2-adrenergic agonists, anticholinergics, and theophyllines as primary treatments [5]. However, due to the complexity of the causes of asthma, knowledge and research on herbal medicine have grown in recent years, and herbal medicine has become an alternative treatment option for asthma [6].

For a long time, traditional Chinese medicine has provided excellent treatment for asthma in clinical practice [7]. Yanghe Pingchuan Granule (YPG) is a hospital preparation of Anhui Provincial Hospital of Traditional Chinese Medicine, which was gradually optimized by Dr. Qiaowu Hu of our hospital on the based of Yanghe decoction and Pingchuan decoction, combined with years of clinical application experience [8]. YPG is composed of nine medicinal herbs, including *Radix Morindae Officinalis* (Common English: Ephedra Stem; Chinese pinyin: Ba Ji Tian), *Semen Lepidii* (Common English: Tingli Seed; Chinese pinyin: Ting Li Zi), *Radix Platycodi* (Common English: Balloon Flower Root; Chinese pinyin: Jie Geng), *Radix Rehmanniae Preparata* (Common English: Prepared Chinese Foxglove Root; Chinese pinyin: Shu Di Huang), *Flos Inulae* (Common English: Inula Flower; Chinese pinyin: Xuan Fu Hua), *Semen Sinapis* (Common English: White Mustard Seed; Chinese pinyin: Bai Jie Zi), *Herba Ephedrae* (Common English: Ephedra Stem; Chinese pinyin: Ma Huang), *Fructus Schisandrae* (Common English: Schisandra Fruit; Chinese pinyin: Wu Wei Zi), and *Radix Angelicae Sinensis* (Common English: Chinese Angelica Root; Chinese pinyin: Dang Gui) at a weight ratio of 10: 10: 10: 15: 9: 6: 6: 6: 10 [9]. Plant names have been checked through https://www.americandragon.com/index.htm. YPG has been clinically used in our hospital for more than 10 years and has excellent therapeutic effects in clinical practice [10]. In our previous study, we conducted quality control experiments on YPG, and the results showed that sinapine thiocyanate, ferulic acid, acteoside, quercetin, and schizandra were considered the main active ingredients for the pharmacological activity of YPG [11]. In addition, the content of YPG was determined and it was found that each bag of YPG (10 g/bag) contained 0.762 mg of ephedrine hydrochloride, 0.403 mg of schizandra and 2.181 mg of sinapine thiocyanate [12]. Clinical studies have shown that YPG can improve the clinical symptoms of acute exacerbation of the chronic obstructive pulmonary disease (AECOPD) in patients with kidney deficiency and phlegm turbidity syndrome and improve lung ventilation function [13]. Guangchuan Dai et al. found that YPG combined with conventional western medicine for asthma-chronic obstructive pulmonary disease-overlap (ACO) with kidney deficiency and phlegm could reduce the degree of inflammation, decrease airway hyperresponsiveness, and improve lung function of patients [14]. Meanwhile, the therapeutic effect of YPG on asthma could reduce airway inflammation by upregulating miR-139-5p and downregulating Notch1/Hes1 pathway to inhibit T helper (Th) 2 inflammatory response [15]. However, the potential pharmacological mechanism of action of YPG remains largely unknown and requires further study.

Bioinformatics is an interdisciplinary study exploring the correlation between drugs and diseases. Its completeness, systemic nature, and focus on drug-target interactions are consistent with the fundamental characteristics of traditional Chinese medicine (TCM), which can systematically reflect the network mechanisms of drug intervention in diseases. In recent years, it has been widely used in Chinese medicine research and advanced drug discovery [16,17]. In this study, we focus on the main active components of YPG against asthma and their mechanism of action. We investigated the action and mechanism of YPG anti-asthma by bioinformatics and in vivo experiments. A flowchart of the study design is illustrated in Fig. 1.

2. Materials and methods

2.1. Bioinformatics

2.1.1. Screening of active compounds in YPG

The chemical components of nine herbs in YPG were identified using the Traditional Chinese Medicine System Pharmacological Analysis Platform [18] (https://tcmspw.com/tcmsp.php, TCMSP). Screening of ingredients based on absorption, distribution, metabolism, and excretion (ADME) of YPG. The screening was carried out according to the following conditions: oral bioavailability (OB) $\geq$ 30 %, drug similarity (DL) $\geq$ 0.18 [19].

2.1.2. Asthma-related target collection

GeneCards database (https://www.genecards.org/) [20], Online Mendelian Inherited Man (OMIM) database (https://www.omim.org/) [21], Therapeutic Target Database (TTD) (http://db.idrblab.net/ttd) [22], the DrugBank database (https://www.drugbank.ca) [23] and PharmGbk database (https://www.pharmgkb.org/) [24] were searched using the keyword "asthma" to collect targets related to asthma treatment. UniProt (http://www.uniprot.org/) [25] was used to remove duplicate and normalized target genes to obtain YPG and asthma-related targets.

2.1.3. Herbs-ingredients-targets-disease network construction

Common potential targets of YPG in asthma treatment were obtained using Venn 2.1.0 (http://bioinfo.cnb.csic.es/tools/venny/index.html) [26]. These common target proteins were correlated using Cytoscape 3.9.1 to construct herbs-components-targets correlation networks [27].

2.1.4. Protein-protein interaction (PPI) network analysis

Import the common targets of YPG and diseases into the string database (https://string-db.org/cgi/input.pl) [28], and build a PPI network. Download the PPI network file and import Cytoscape to further filter the core targets with degree, betweenness, and closeness as the reference values and greater than the median as the filter condition [29].

2.1.5. GO annotation and KEGG pathway analysis

Drug and asthma overlap targets were imported into Metascape [30] (https://metascape.org/) for Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The biological species was selected as Homo sapiens. The threshold criteria are the p value ≤ 0.01, the minimum overlap value ≥ 3, and the minimum enrichment value ≥ 1.5. GO analysis can be divided into biological process (BP), cellular component (CC), and molecular function (MF) [31].

2.1.6. Molecular docking simulation

Autodock vina 1.2.0 was used to dock the main compunds of YPG with the core targets relevant to the treatment of asthma [32]. Small molecule compounds were downloaded from the PubChem website and converted from 2D to mol2 structures using OpenBable 3.1.1 software. The secondary structures of the target protein and ligand were downloaded from the PDB database (https://www.rcsb.org/) [33] and the water molecules and small molecule ligands were removed using PyMOL software. Finally, LigPlot software was used to demonstrate the interaction between each chemical bond, and binding energies were obtained from AutoDock vina to show the binding strength between the ligand and target protein. The binding energy results are used to generate hot maps using the Microbiology website (https://www.bioinformatics.com.cn/), an online platform for data analysis and visualization.

2.2. Validation of experiment

2.2.1. Chemicals and reagents

YPG is composed of 10 g of *Radix Morindae Officinalis*, 10 g of *Semen Lepidii*, 10 g of *Radix Platycodi*, 15 g of Radix Rehmanniae Preparata., 9 g of *Flos Inulae*, 6 g of *Semen Sinapis*, 6 g of *Herba Ephedrae*, 6 g of *Fructus Schisandrae* and 10 g of *Radix Angelicae Sinensis*. The preparation was supplied by the Chinese Pharmacy of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (10 g/bag, Lot No. 202111208). Aluminum hydroxide (Lot No. C11882499) was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Ovalbumin (OVA, Lot No. CLCB9757) was provided by Sigma-Aldrich (St. Louis, MO, USA). Interleukin(IL)-8 (RX303109R), tumor necrosis factor (TNF)-α (RX30301010R), IL-1β (RX303111R), cyclooxygenase (COX)2 (RX303080R) and prostaglandin (PG)E2 (RX302407R) were purchased from Quanzhou Rexin Biotechnology Co Ltd (Fujian, China).

2.2.2. Animals

Thirty healthy specific pathogen-free (SPF) Sprague Dawley (SD) male rats weighing 250 ± 20 g were provided by the Laboratory Animal Management Center of Anhui Medical University and housed for one week on a 12 h cycle with light and darkness, at 22–25 °C and 50 %–70 % relative humidity, with daily food and water. All animals are approved by the Animal Experiment Ethics Committee of Anhui University of Traditional Chinese Medicine (license number: LLCS20160336).

2.2.3. Grouping and modeling

All male SD rats (n = 30) were randomly divided into three groups: normal group, model group, and YPG group (14.76 g/kg). Ten rats were placed in each group. Replication of the asthma model as reported in the relevant literature [34]. Rats in the model group and YPG group were sensitized by intraperitoneal injection of 10 % OVA (containing 100 mg ovalbumin, 100 mg aluminum hydroxide, and 1 ml saline) on days 1 and 8, respectively. Starting on day 15, rats in the other two groups inhaled a 1 % OVA solution for 30 min every other day for a total of 14 days, except for the normal group, which used normal saline instead. On day 15, gavage was started simultaneously in all rats. After half an hour of daily nebulized stimulation, 14.76 g/kg of YPG was given to the administered group and an equal amount of saline was given to the normal and model groups. All drugs were used for two weeks. Twenty-four hours after the last dose, 10 rats were selected from each group, anesthetized with 2 % pentobarbital, and blood and lung samples were collected.

2.2.4. Histopathological analysis of lung tissue

The left lung tissue was fixed with 4 % paraformaldehyde for 24 h, dehydrated, embedded in paraffin, cut into 50 μm sections, stained with hematoxylin and eosin (H&E) and Masson, sealed with neutral glue, and visualized under a light microscope.

2.2.5. Enzyme-linked immunosorbent assay (ELISA)

Serum from rats stored at −80 °C was taken and the levels of IL-8, TNF-α, IL-1β, COX2, and PGE2 in serum of three rats in each group were measured using commercially available ELISA kits.

2.2.6. Immunofluorescence (IF) analysis

Fixed lung tissue was incubated with the inhibitor of IKBKB antibody (Affinity, 1:200) and NFκB1 antibody (Affinity, 1:1000) in a refrigerator at 4 °C overnight and then washed three times with phosphate buffered saline (PBS). The fluorescent secondary antibody was added dropwise, incubated for 1 h at room temperature and protected from light, and washed three times with PBS. 4′-6-diamidino-2-phenylindole (DAPI) was incubated in the dark for 10 min, then turned off and photographed under a fluorescence microscope at 400 × magnification.

2.2.7. Western blotting (WB)

Lung tissue was collected and 1:100 of RIPA buffer (containing 1 mM phenylmethanesulfonyl fluoride (PMSF)) was added to obtain total protein. After separating equal amounts of proteins by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the bands were transferred to polyvinylidene fluoride (PVDF) membranes and blocked with 5 % skimmed milk at room temperature. Primary antibodies: β-actin (1:1,000, Abcam, Cambridge, UK), VEGF (1:1,000, Abcam, Cambridge, UK), and VEGFR2 (1:1,000, Protein Technology, Chicago, USA) were added and incubated overnight at 4 °C. Wash 3 times with Tris-buffered saline Tween-20 (TBST) and then incubated with horseradish peroxidase (HRP)-labeled secondary antibody (1:10,000) for 2 h at room temperature with repeated washes. Protein strips were developed with a cartridge of allergenic chemiluminescent color development reagent and analyzed with Image J software.

2.3. Statistical analysis

The study was statistically analyzed using GraphPad Prism 9.0 software, with mean ± standard deviation (x ± s) for independent samples, *t*-test for group comparisons, and one-factor ANOVA for multiple group comparisons $^{**}p < 0.01$, $p < 0.05$ indicating statistical difference. Differential analysis of GO and KEGG pathway enrichment was analyzed using Metascape software, and results were considered statistically significant at $p < 0.01$.

3. Results

3.1. Results of bioinformatics

3.1.1. Active ingredients of YPG

The chemical components of nine herbs contained in YPG were searched in the TCMSP database. We obtained a total of 72 potentially active ingredients, including 16 compounds from Inula japonica Thunb, 16 compounds from *Radix Morindae Officinalis* (Ba Ji Tian), 2 compounds from *Radix Angelicae Sinensis* (Dang Gui), 1 compound from *Semen Sinapis* (Bai Jie Zi), 4 compounds from *Radix Platycodi* (Jie Geng), 20 compounds from *Herba Ephedrae* (Ma Huang), 2 compounds from *Radix Rehmanniae Preparata* (Shu Di Huang), 7 compounds from *Semen Lepidii* (Ting Li Zi), and 4 compounds from *Fructus Schisandrae* (Wu Wei Zi).

3.1.2. Potential targets identification

The TCMSP database was utilized to search for the targets corresponding to the 72 active ingredients, and 913 targets related to the active ingredients of YPG were obtained (Supplementary Table 1). By searching Genecards database, OMIM database, TTD database, DrugBank database, and PharmGkb database, summarizing and removing duplicates, 1489 asthma-related targets were obtained (Supplementary Table 2). The Venn diagram results showed a total of 318 overlapping targets between YPG and asthma targets (Fig. 2 and Supplementary Table 3).

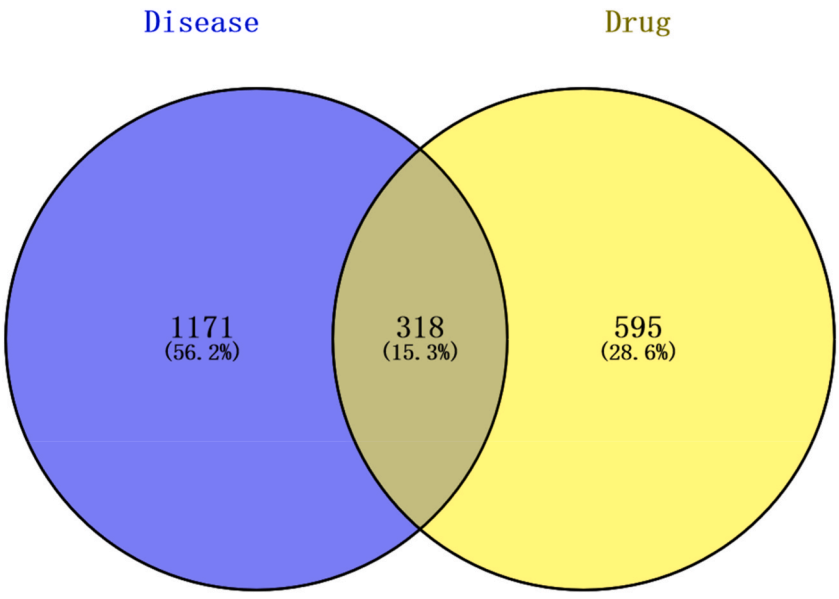

Fig. 2. The overlap targets of YPG and asthma with Venny. The blue circle indicates the targets of asthma, the yellow circle indicates the targets of YPG, and the intersecting part of the two circles indicates the common targets of both.

3.1.3. Herbs-components-targets network construction

To analyze the relationship between herbs, components, and related targets, we constructed herbs-components-targets networks using Cytoscape 3.9.1 software. As shown in Fig. 3, the herbs-components-targets graph of YPG has 261 nodes and 646 edges. The six compounds with the highest number of corresponding targets are quercetin, kaempferol, luteolin, beta-sitosterol, stigmasterol, and isorhamnetin, which may be the key compounds for YPG in the treatment of asthma (Table 1). In addition, we constructed a PPI network containing 318 potential targets and visualized it using Cytoscape 3.9.1 software. According to the distribution of the network diagram (Fig. 4), the core targets were Serine/threonine-protein kinase (AKT1), tumor protein p53 (TP53), tumor necrosis factor (TNF), interleukin (IL)-6, IL-1β, vascular endothelial growth factor-A (VEGFA), prostaglandin-endoperoxide synthase 2 (PTGS2), caspase-3 (CASP3), mitogen-activated protein kinase 3 (MAPK3) and epidermal growth factor receptor (EGFR). Therefore, we hypothesize that these targets may be key targets for the treatment of asthma in YPG.

3.1.4. Results of enrichment analysis

We analyzed 318 overlapping genes for for enrichment with GO and KEGG. The GO enrichment results showed that a total of 2545 items were enriched for BP, 138 items for CC, and 333 items for MF (Supplementary Table 4). The top 10 enrichment results were selected for analysis, $p < 0.01$ was used as the screening criterion, and each item was displayed as a bar graph. The results showed that the target of action of YPG in the treatment of asthma in terms of BP mainly involves inflammatory response, cell activation. CC mainly occurs on the side of the membrane, receptor complex. MF is mainly associated with involved protein kinase activity, lipid binding, and protein homodimerization activity (Fig. 5). KEGG enrichment analysis yielded 219 signaling pathways (Supplementary Table 5), and the results showed that many target genes were associated with inflammatory and immune-related pathways, such as cGMP-PKG signaling pathway, NF- κB signal pathway, Fc epsilon RI signal pathway, and PI3K- Akt signal pathway. The top 20 pathways were selected and the enrichment pathways were visualized by enrichment bubble plots (Fig. 6).

3.1.5. Molecular docking verification

The main method to evaluate the binding ability of small and large molecules is the energy value through binding. The smaller the value, the stronger the binding ability and the easier the active ingredient is to bind to the receptor. A docking score < -5.0 indicates good binding activity between the active substance and the protein, and a docking score < -7.0 indicates strong binding activity. The results of molecular docking are shown in Fig. 7, and the structures of partial docking are shown in Fig. 8A–H. The top eight simulators are TP53-luteolin (−8.7 kcal/mol), TP53-quercetin (−8.4 kcal/mol), TP53-kaempferol (−7.3 kcal/mol), IL-6-quercetin (−7.2 kcal/mol), IL-1β-β-sitosterol (−7.2 kcal/mol), IL-1β-isorhamnetin (−7.2 kcal/mol), PGE2-quercetin (−8.5 kcal/mol), and PGE2-isorhamnetin (−7.3 kcal/mol). This indicates that kaempferol, quercetin, luteolin, β-sitosterol, stigmasterol, and isorhamnetin are sufficiently bound to the TNF, IL-6, TP5, VEGFA, AKT1, IL-1β, MAPK3, CASP3, PTGS2, EGFR, IL-8, COX2, and PGE2.

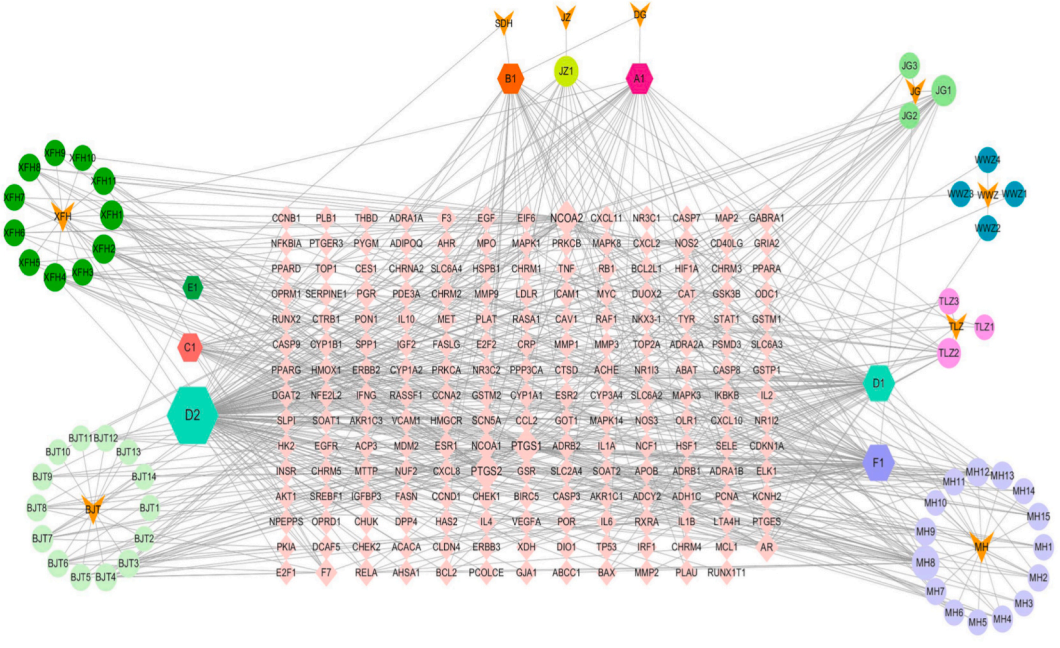

Fig. 3. Herbs-compounds-targets diagram of YPG. The pink diamond indicates the intersection target of disease and compounds, the orange square indicates the names of the herbs, and the circles indicate compounds.

Table 1
Basic information on main compounds of Yanghe Pingchuan Granule.

MOLID	chemical component	Number of targets	Herbs containing the compound
MOL000098	quercetin	126	*Herba Ephedrae, Flos Inulae, Semen Lepidii*
MOL000422	kaempferol	51	*Herba Ephedrae, Flos Inulae, Semen Lepidii*
MOL000006	luteolin	51	*Herba Ephedrae, Flos Inulae, Radix Platycodi*
MOL000358	beta-sitosterol	31	*Herba Ephedrae, Flos Inulae, Radix Morindae Officinalis, Radix Angelicae Sinensis*
MOL000449	stigmasterol	29	*Herba Ephedrae, Radix Rehmanniae Preparata, Radix Angelicae Sinensis*
MOL000354	isorhamnetin	23	*Flos Inulae, Semen Lepidii*

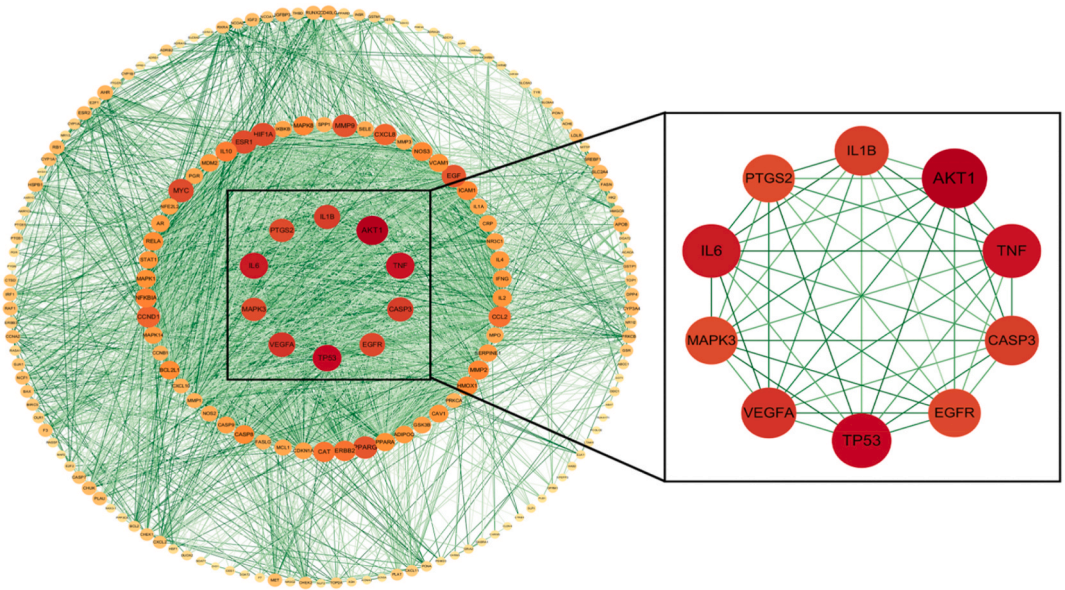

Fig. 4. PPI network of potential targets of YPG for asthma treatment.

3.2. Experimental results

3.2.1. Effects of YPG on the histological changes in OVA-induced asthmatic rats' lungs

Two different staining methods are used to examine OVA-induced airway inflammation: H&E staining showed (Fig. 9(A-C)) that the alveoli of rats in the normal group were structurally intact with clear crystal structures and no obvious infiltration of inflammatory cells. Compared with the normal group, the rats in the ova-induced asthma model group had thickened tracheal walls and thickened peribronchial and perivascular connective tissue airway walls with severe inflammatory cell infiltration, which was ameliorated in the YPG group. Masson staining showed that the lung tissue of normal group rats had only a small amount of collagen deposition and thin edges (Fig. 9D), while the lung tissue of model group rats had significantly increased collagen deposition (Fig. 9E). Rats treated with YPG showed decreased collagen deposition in the peribronchial area (Fig. 9F).

3.2.2. The expression of inflammatory factors

According to the expected results of bioinformatics, YPG treatment of asthma is closely related to inflammation. Therefore, we investigated the expression levels of various inflammatory factors in a serum model of asthma rats. The expression levels of IL-8, TNF-α, IL-1β, COX2, and PGE2 were significantly higher in the lung tissue of the model group compared with the normal group ($P < 0.01$). Compared with the model group, YPG effectively reduced the expression levels of IL-8, TNF-α, IL-1β, COX2, and PGE2 in serum ($P < 0.05$), decreased the levels of inflammatory factors, and relieved the symptoms of asthma (Fig. 10A–E).

3.2.3. The expression of NFκB1, IKBKB, VEGF and VEGFR2 in rat lung tissue

IF results showed that the model group had the strongest expression of NFκB1 and IKBKB fluorescent signals, which were significantly higher than the normal group. After treatment, the fluorescence expression of NFκB1 and IKBKB was significantly attenuated (Fig. 11A and B). Based on the results of bioinformatics, VEGF, and VEGFR2 were selected for WB analysis to further verify their gene expression at the protein level. The results of WB assay showed that the levels of VEGF and VEGFR2 proteins were significantly higher in the model group compared to the normal group ($p < 0.01$). Compared with the model group, the VEGF and VEGFR2 protein levels were significantly lower in the administered group ($p < 0.05$) (Fig. 12 and Supplementary Fig. 1). The results showed that YPG significantly inhibited the expression of VEGF and VEGFR2 proteins in asthmatic lung tissues.

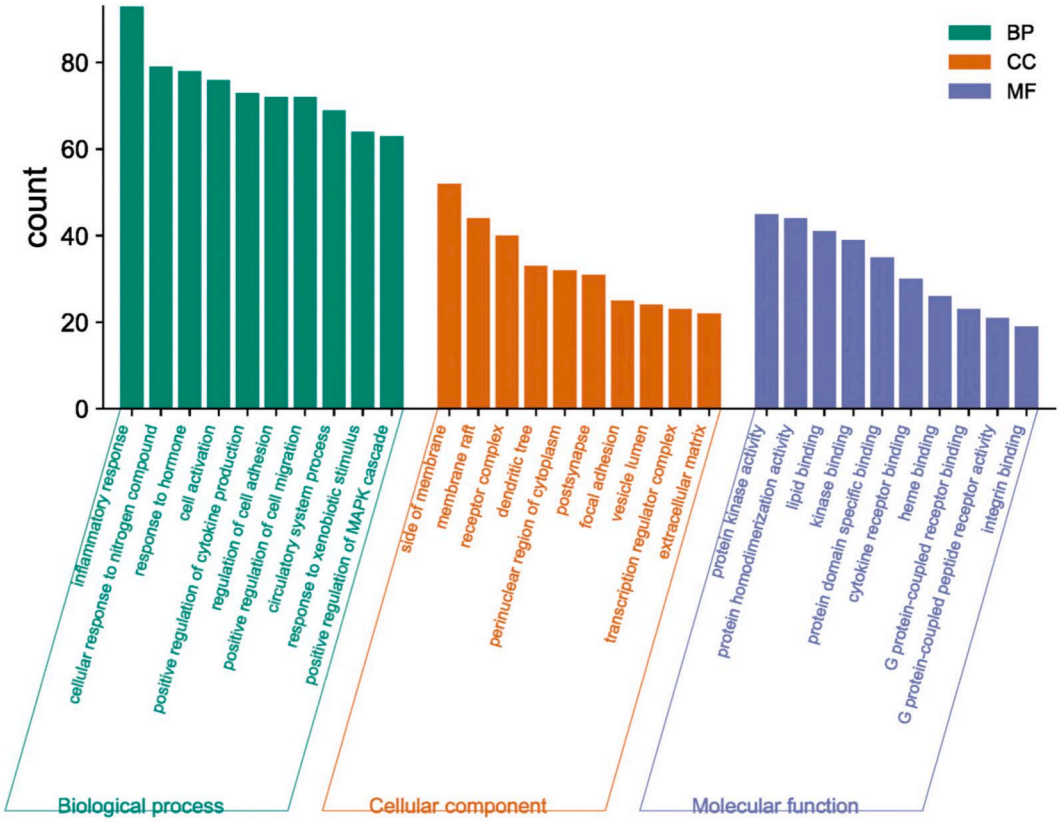

Fig. 5. Histogram of Gene Ontology (GO) analysis. GO enrichment analysis of 318 overlapping targets by Metoscape database. Green represents the biological process, orange represents the cellular component, and blue represents the molecular function, $p < 0.01$.

4. Discussion

YPG is a granule produced by the respiratory medicine department of our hospital after many years of clinical experience, which has been used clinically in the treatment of bronchial asthma with remarkable efficacy [35]. YPG has been shown to improve airway mucus hypersecretion in asthmatic rats and to reduce chronic airway inflammation in asthmatic patients [36]. However, due to the complexity and diversity of the components involved, the exact mechanism of action of YPG in the treatment of asthma remains unclear. Rapid advances in bioinformatics have allowed us to screen the active components of YPG and the core gene targets associated with YPG for asthma therapy.

Using bioinformatics techniques, a network of herbal compound targets of YPG components was established to predict the possible anti-inflammatory mechanisms of YPG. It was shown that quercetin, kaempferol, luteolin, β-sitosterol, stigmasterol, and isorhamnetin might be the main active compounds of YPG for asthma treatment. TNF, IL-6, TP5, VEGFA, AKT1, IL-1β, MAPK3, CASP3, PTGS2, EGFR, IL-8, COX2, and PGE2 might be the main anti-asthma targets of YPG. In molecular docking experiments, we selected TNF, IL-6, TP5, VEGFA, AKT1, IL-1β, MAPK3, CASP3, PTGS2, EGFR, IL-8, COX2, and PGE2 and six core compounds (quercetin, kaempferol, lignocerotoxin, β-sitosterol, stigmasterol, and isorhodopsin) with a high recognition rate and related to asthma for molecular docking validation. To investigate the interaction between protein receptors and active compounds, we selected binding modes with optimal docking fractions. All docking fractions were <-5 kcal/mol, indicating a good binding affinity between the six compounds and the core target. These results suggest that the six active components of YPG may reduce airway inflammation by binding to TNF, IL-6, TP53, VEGFA, AKT1, IL-1β, MAPK3, CASP3, PTGS2, EGFR, IL-8, COX2, and PGE2.

Inflammation is considered an important molecular mechanism in asthma and can be classified into type 1 (TNF-α and IL-1β) and type 2 responses (IL-4, IL-6, and IL-8) [37]. TNF-α and IL-1β are common inflammatory mediators associated with lung disease and these markers regulate the maturation of dendritic cells and promote neutrophil PGE2 is the most secreted prostaglandin in the body and plays a significant role in the regulation of inflammatory processes [38]. Prostaglandins are arachidonic acid (AA) metabolites produced by enzymes with cyclooxygenase (COX) activity. COX2 can be induced by the cytokines IL-β and TNF-α, which play an important role in the pathogenesis of asthma [39]. To further explore and verify the pharmacological mechanism of action of YPG, we observed that YPG could improve the bronchial inflammation phenomenon in asthma model rats in H&E staining and Masson staining. Our data suggested that YPG improved lung function and inhibited the development of airway inflammation by reducing serum expression of several cytokines associated with inflammation, mainly IL-8, TNF-α, IL-1β, COX2, and PGE2. VEGF is a mitogen of

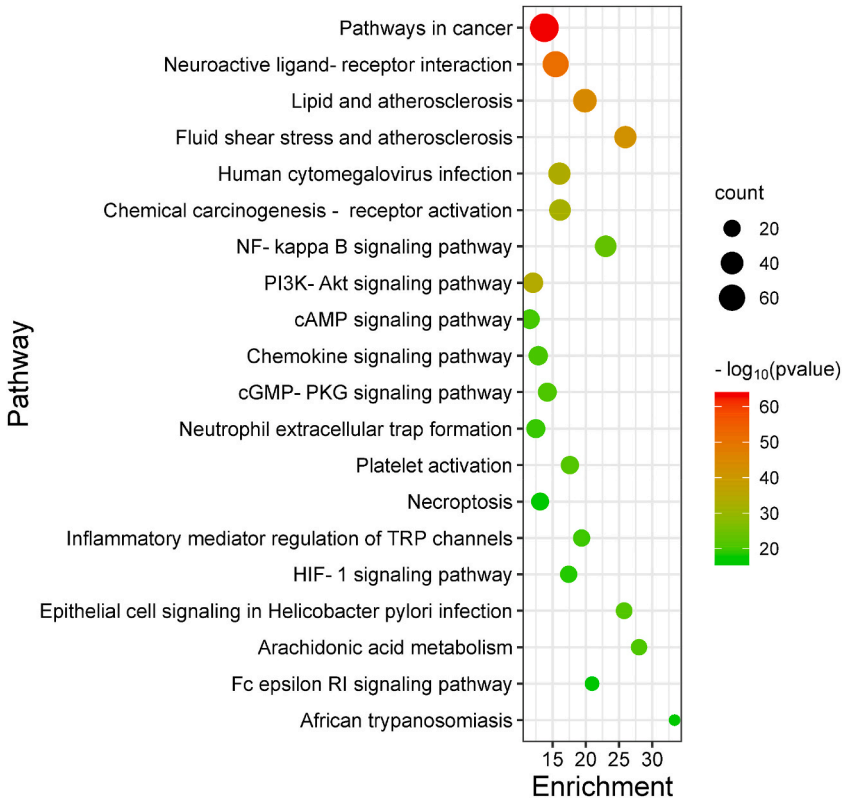

Fig. 6. The top 20 significantly enriched pathways were selected. The Y-axis represents the main pathway, and the X-axis represents the enrichment score, $p < 0.01$.

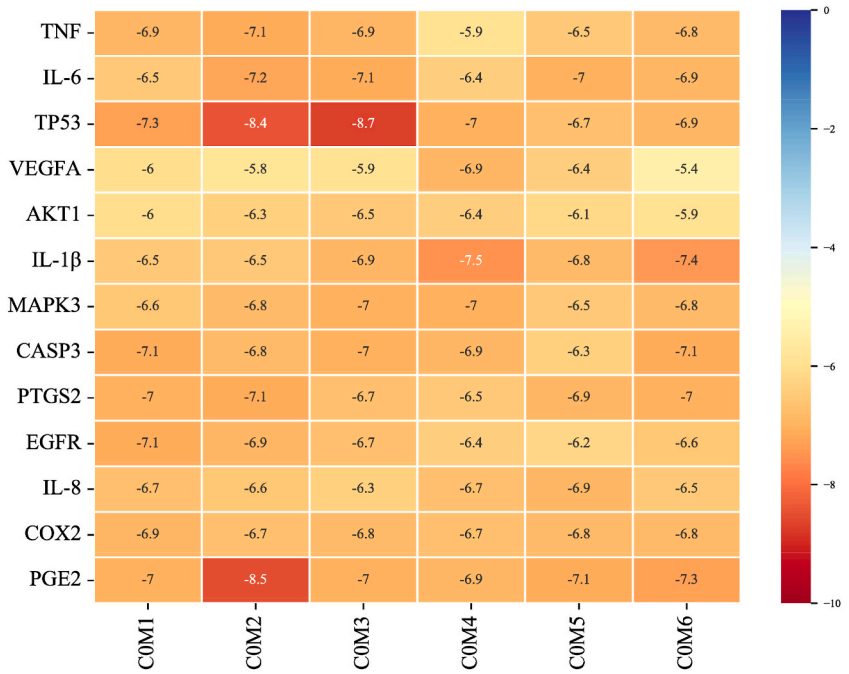

Fig. 7. Heatmap of molecular docking results. COM1: kaempferol, COM2: quercetin, COM3: luteolin, COM4: β-sitosterol, COM5: stigmasterol, COM6: isorhamnetin.

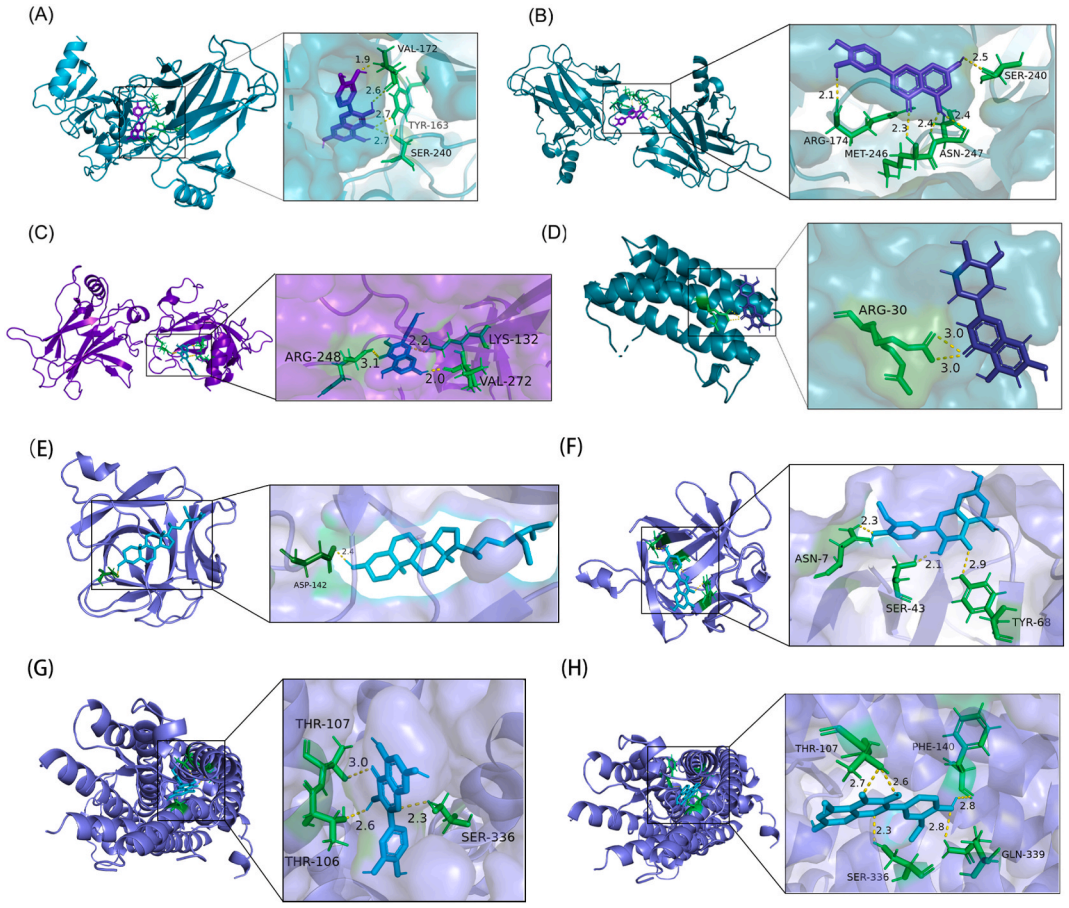

Fig. 8. Molecular docking models of main chemical compounds binding to potential targets. (A) TP53-quercetin, (B) TP53-luteolin, (C) TP53-kaempferol, (D) IL6-quercetin, (E) IL-1β-β-sitosterol, (F) IL-1β-isorhamnetin, (G) PGE2-quercetin, (H) PGE2-isorhamnetin.

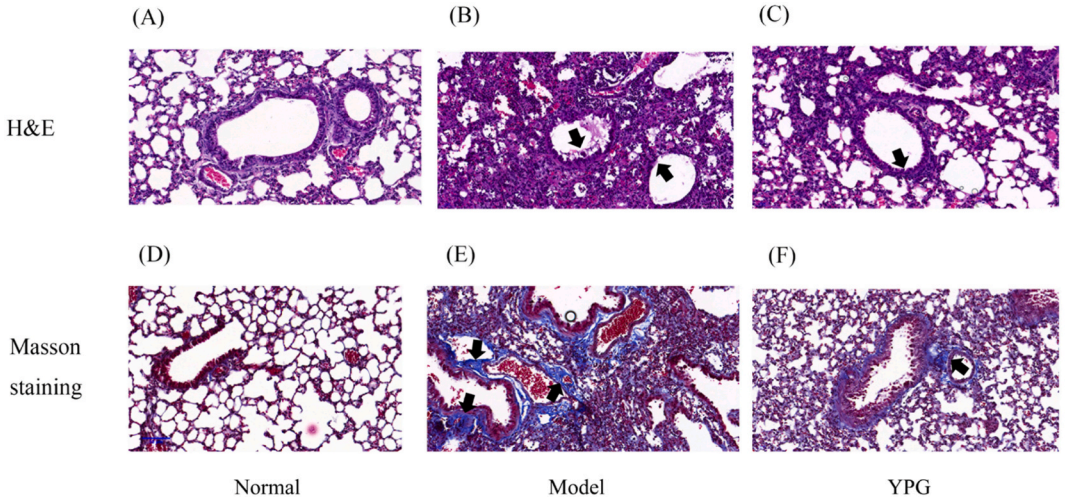

Fig. 9. Pathological features of YPG in attenuating airway inflammation in OVA-induced asthmatic rats. N = 10; magnification, × 400; scale bars, 50 μm. (A–F) H&E staining and Masson staining images of normal, model, and YPG-treated (14.76 g/kg/d) lung tissue of rats.

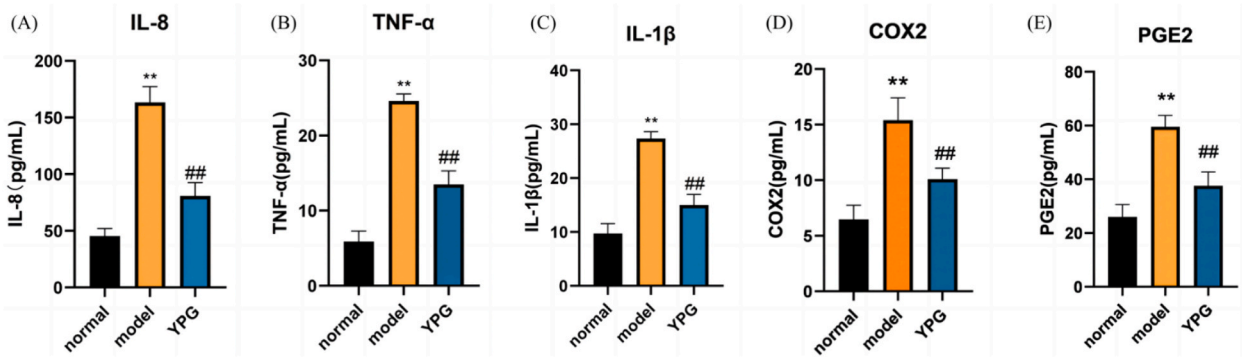

Fig. 10. Effect of YPG on the levels of inflammatory cytokines (A) IL-8, (B) TNF-α, (C) IL-1β, (D) COX2, and (E) PGE2 in lung tissue of rats with OVA-induced asthma. N = 10. $^{**}p < 0.01$ indicate significant difference from normal group, $^{\#\#}p < 0.05$ indicate significant difference from model group.

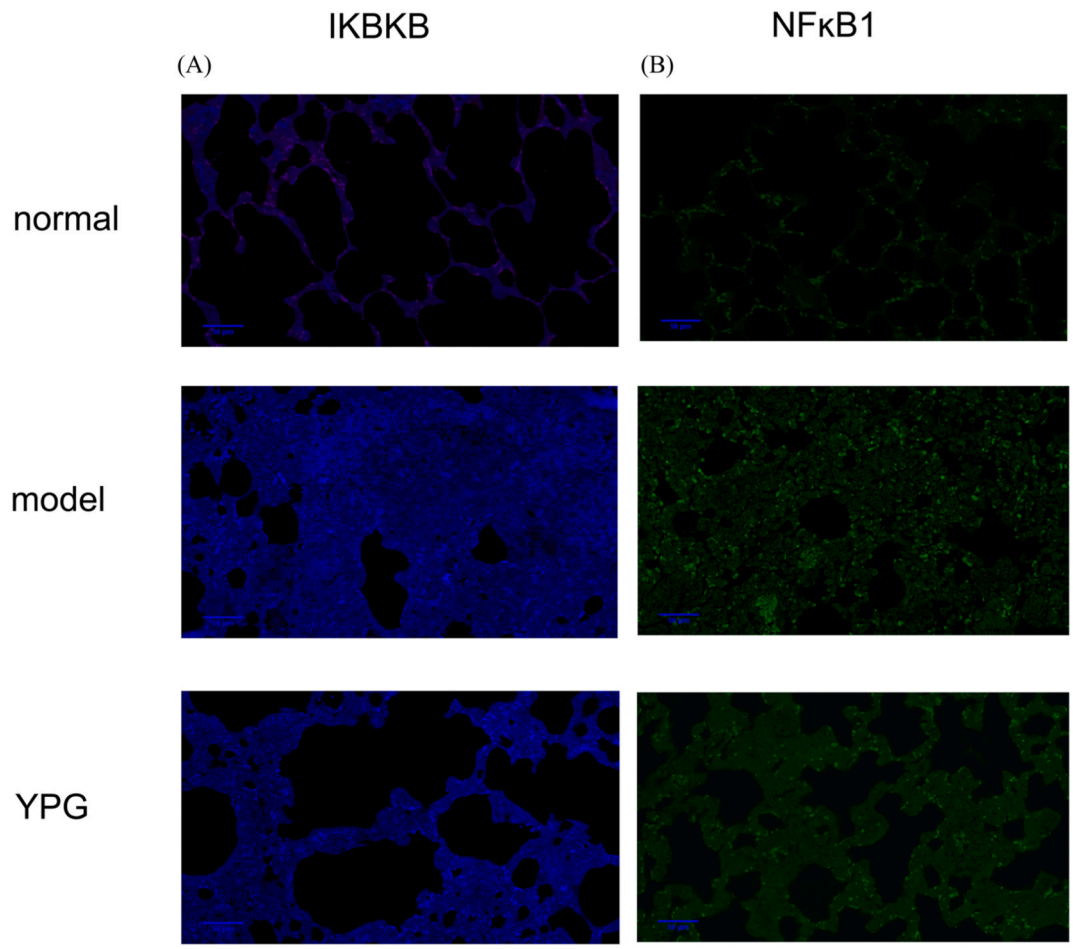

Fig. 11. Immunofluorescence staining to determine the expression of (A) IKBKB and (B) NFκB1. N = 10; magnification, × 400.

endothelial cells and promotes extracellular matrix degradation, migration, proliferation, lumen formation, and vascular stabilization through the tyrosine kinase receptor VEGFR2. VEGFA is widely distributed in many tissues in the body and is also highly expressed in the lung [40,41], where they are thought to be the main factors regulating airway vascular growth in asthmatics, and increased vascular permeability and release of inflammatory mediators are important pathological processes in asthmatics. Therefore, we hypothesized that YPG may improve asthma symptoms by regulating the release of inflammatory factors and improving vascular permeability.

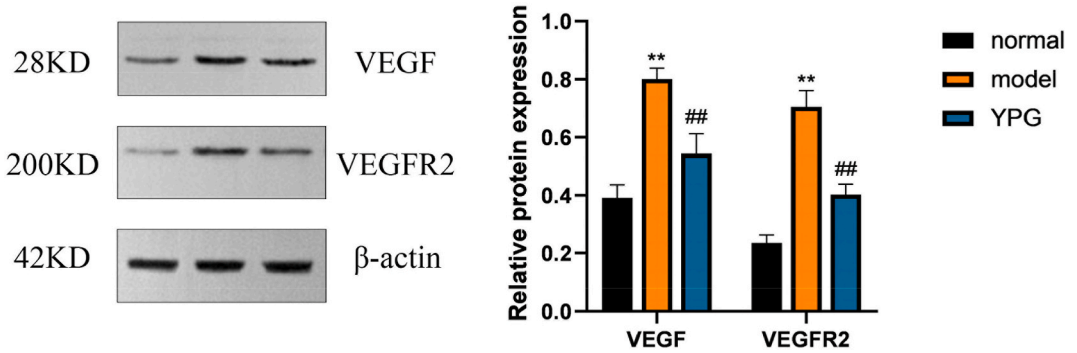

Fig. 12. Effects of YPG on the expression of VEGF and VEGFR2 proteins in the lung tissue. The original WB images were available in the supplementary file. One-way ANOVA was used to compare data between all groups. $^{**}p < 0.01$, compared with the normal group; $^{\#\#}p < 0.05$, compared with the model group.

According to the results of KEGG enrichment analysis, there were multiple pathways associated with asthma, and the NF-κB signaling pathway had the highest expression among the 20 signaling pathways, indicating that the largest number of targets were involved. Therefore, the NF-κB signaling pathway may be a potential pathway of action for YPG in the treatment of asthma. NF-κB is a multifunctional proteinaceous nuclear transcription factor and is associated with inflammatory changes in many diseases such as rheumatoid arthritis, bronchial asthma, and brain diseases [42,43]. NF-κB binds to nuclear factor-κB (IκB) inhibitors in the cytoplasm in an inactive state [44]. When the upstream signal (NF-κB signaling pathway) activates IKK (IκB kinase), the activated IKK ubiquitinates, phosphorylates, and degrades IκB, allowing NF-κB to be activated and transferred from the cytoplasm to the nucleus [45]. IKBKB is an important catalytic subunit that constitutes the IKK complex and is used to recognize and phosphorylate the downstream substrate IKB family proteins, namely IkBα and NFκB1 [46]. During inflammation, phosphorylation of NFκB1 (p105) is highly activated. We used immunofluorescence to detect the expression of NF–B1 and IKBKB in lung tissue. The results showed that YPG inhibited the upregulated NF-κB signaling pathway with effects similar to those of dexamethasone, while exposure to OVA accelerated activation of NF-KB in asthmatic rats.

In the present experiments, YPG may control asthma by regulating the anti-inflammatory and immunomodulatory targets of NF-κB. The results of immunofluorescence and ELISA experiments showed that the expression levels of NFκB1, IKBKB protein, and inflammatory factors (TNF-α, IL-1β, and IL-8) were higher in the asthma model group than in the control group, leading to increased inflammatory response. Therefore, the multi-targeted action of YPG may exert anti-asthma effects by regulating the imbalance of NF-κB signaling pathway and inhibiting the inflammatory response of lung tissue and airway structural remodeling. However, based on the search of bioinformatics, the target predictions were based on existing databases. Therefore, the accuracy depends heavily on the web server and computer algorithms. Furthermore, the experimental validation method adopted in this study is relatively single and has not yet been combined with multidimensional methods for the same index. It is necessary to conduct more in-depth clinical experiments to provide a more accurate experimental basis for the prediction results of this study.

5. Conclusion

In this study, the pharmacological mechanisms of YPG in asthma were investigated using bioinformatics and experimental methods. YPG has been shown to have an inhibitory effect on the inflammatory response, reducing the expression of IL-8, TNF-α, IL-1β, COX2 and PGE2 in serum, and exerting its therapeutic effects by downregulating NFκB1 and IKBKB. YPG reduced the expression of VEGF and VEGFR2 protein expression. This study provides a certain experimental basis for the pharmacological mechanism of action of asthma and also provides new research ideas to further explore the possible intervention pathways of TCM on asthma and new drugs for asthma prevention and treatment.

Ethics statement

All procedures were conducted following the Guide for Animal Welfare Ethics Review of Laboratory Animals (GB/T 35892-2018, China) and were approved by the Animal Experiment Ethics Committee of Anhui University of Traditional Chinese Medicine (license number: LLCS20160336).

Data availability statement

Data included in article/supp. material/referenced in article.

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CRediT authorship contribution statement

Chunxia Gong: Conceptualization, Writing – original draft. **Lingyu Pan:** Data curation, Methodology. **Yeke Jiang:** Validation, Writing – original draft. **Yehong Sun:** Data curation, Software. **Yanquan Han:** Validation, Writing – review & editing. **Dianlei Wang:** Writing – review & editing. **Yongzhong Wang:** Funding acquisition, Supervision, 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.heliyon.2023.e21936.

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