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Exploring the effects and mechanism of peony pollen in treating benign prostatic hyperplasia

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A R T I C L E I N F O

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A B S T R A C T

Paeonia suffruticosa is widely cultivated globally due to its medicinal and ornamental value. Peony pollen (PP) is commonly used in Chinese folk medicine to make tea to treat benign prostatic hyperplasia (BPH), but its molecular mechanism against BPH is yet to be comprehended. The objective of this research was to experimentally verify the effect of PP in the treatment of BPH and to preliminarily reveal its mechanism of action on BPH using network pharmacology methods. The results revealed that PP could decrease prostate volume and prostate index, serum testosterone (T), dihydrotestosterone (DHT), and estradiol (E2) levels. Moreover, it could improve prostate tissue structure in BPH model animals as well. Additionally, database searches and disease target matching revealed 81 compounds in PP. Of these, 3, 7, 8, 2′-tetrahydroxyflavone, Chrysin, Wogonin, Limocitrin, and Sexangularetin were the top five compounds associated with the therapeutic effects of BPH. Furthermore, 177 therapeutic targets for BPH were retrieved from databases of Swiss Target, DisGeNET, Drugbank, Genecards, OMIM, TTD, and Uniprot. In contrast, core targets AKT1, EGFR, IL6, TNF, and VEGFA were obtained by PPI network diagram. Molecular docking also showed that the main efficacy components and potential core targets in PP had good binding capacity. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomics (KEGG) analysis established that the effect of PP in BPH therapy was mainly through regulating the expression levels of protein kinase B on phosphatidylinositol 3-kinase and phosphatidylinositol 3-kinase-protein kinase B pathways. Additionally, Western blot experiments also exhibited a significant elevation in the activated PI3K and AKT proteins in the model (Mod) group relative to the control (Con) group, and the expression of these activated proteins was significantly reduced after PP administration. In summary, this research provides a scientific basis for employing PP to treat BPH, preliminarily reveals its mechanism of action and potential targets, and lays the foundation for further research and development.

Abbreviations: BPH, benign prostatic hyperplasia; PP, peony pollen; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PI3K, phosphatidylinositol 3-kinases; Akt, protein kinase B; PPI, protein–protein interaction; T, testosterone; DHT, dihydrotestosterone; E2, estradiol; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E_2; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; BAD, Bcl-Xl/Bcl-2asociated death promoter; PPH, peony pollen high dose; PPM, peony pollen medium dose; PPL, peony pollen low dose; QLK, Qianliekang; FIN, Finasteride.

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1. Introduction

Benign prostatic hyperplasia (BPH) is among the most prevalent benign diseases leading to urinary disorders in middle-aged and elderly males. Moreover, it is especially common in elderly men [1]. Age is considered one of the most significant risk factors for developing BPH lesions, with autopsy-proven histological prevalence reaching 60 % at 60 and 80 % at 80 years [2]. According to a recent meta-analysis, the lifetime prevalence of BPH is estimated to be 26.2 %, irrespective of ethnic background [3]. Additionally, if left untreated, patients will experience worsening voiding and urinary storage symptoms with increasing age. Risk factors that have been found to predispose to the further development of prostate enlargement include metabolic disorders, diabetes, obesity, hypertension, and genetic variables [4]. The presently employed treatments for prostatic disease are surgical and pharmacological methods. However, these treatments still face significant challenges, including multidrug resistance, severe side effects, such as impotence, decreased libido, and retrograde ejaculation, as well as post-surgery adverse effects [5–7]. Hence, it is especially important to find more efficient and safer drugs to treat BPH.

The Chinese folk drink peony pollen (PP) tea to relieve BPH in men and dysmenorrhea in women. By studying the composition and content of fatty acids and flavonoids in peony, Shuai Shao [8] screened out a peony species with high content of anti-BPH active ingredients. Additionally, earlier studies have shown that many natural products, including pollen, have anti-BPH potential [9,10]. Moreover, Qianliekang (QLK), a Chinese medicine employed to treat BPH, has been marketed in China, and its main raw material is rapeseed pollen. However, its main efficacy components and mechanism of action are unclear. More importantly, rapeseed is a economic crop and is the main source of edible oil, so excessive harvesting of its pollen can affect the yield of rapeseed crops. Peony is a deciduous shrub native to China cultivated for medicinal value. China has planted more than 0.2 million hectare of peony, and the resources of the pollen of *P. suffruticosa* are abundant but little exploited in practice; a lot of resources are even wasted. Therefore, it is believed that confirming the effect of PP in the treatment of BPH and studying its main efficacy components and mechanisms of action holds considerable importance for the development of drugs to treat BPH. Moreover, the reuse of resources from abandoned PP holds significant medical and social value.

Network pharmacology offers an emerging approach as an integrated multidisciplinary concept based on systems biology and pharmacology, providing a novel network model of "multi-target-multi-effect-complex disease." Due to its benefits in drug development, such as improved efficacy and success in clinical trials, it has been extensively used to investigate the therapeutic mechanisms of drugs, thereby obviating the need for drug discovery and speeding up clinical translation [11]. In this study, animal experiments demonstrated that PP does have a good therapeutic effect on BPH. Subsequently, the research preliminarily explored its possible efficacy components, mechanism of action, and potential targets of action through network pharmacological methods. It lays the foundation for subsequent studies.

2. Material and methods

2.1. Drugs and reagents

PP (NO. 20191009) was purchased from Shanxi Fengdan Zhengyuan Biotechnology Co., Ltd. (Shanxi), Latin scientific name *Paeonia ostii* T.Hong & J.X.Zhang (the appraisal report is in Supplementary Materials Figs. S1 and S2). Its plant name can be found at http://www.theplantlist.org. Ethyl carbamate (urethane) was purchased from Shanghai Caoyang Second Reagent Factory. Tissue fixative was purchased from Wuhan Saiwei Biotechnology Co., Ltd. Rat testosterone (T), dihydrotestosterone (DHT) and estradiol (E2) kits were obtained from Shanghai Xitang Biotechnology Co., Ltd. Moreover, QLK tablets were acquired from Zhejiang Conba Pharmaceutical Co., Ltd. (Zhejiang, China), Finasteride (FIN) was purchased from Merck (Kenneworth, USA), and testosterone propionate was purchased from Tianjin Jinyao Pharmaceutical Co., Ltd.

2.2. HPLC-MS analysis of PP

HPLC-MS analysis was performed using HPLC on AB SCIEX ExionLC system (SCIEX, Framingham, MA). The chromatographic column is a Kinetex C18 column (100 × 2.1 mm, 2.6 μm), the mobile phase consisted of acetonitrile (B) and water containing 0.1 % formic acid (A) following a gradient elution program: 0–2 min: 5 % (B); 2–14min: 5%–55 % (B). The flow rate was 0.3 mL/min and the injection volume was 2 μL, which was injected into the detector for HPLC analysis. Mass spectrometry data were recorded on a SCIEX X500R mass spectrometer (SCIEX, Framingham, MA) and data acquisition was controlled by SCIEX OS software (SCIEX, Framingham, MA). Specific results are shown in Fig. S3 and Fig. S4 in the Supplementary Material.

2.3. Animal experiment validation

Seventy male Sprague-Dawley rats were acquired from the Chengdu Docetaxel Laboratory Animal Co., Ltd. (Approved No.: SCXY (Chuan) 2020-030): the rats were segregated randomly into seven groups: control group (Con), model group (Mod), PP high (PPH), PP medium (PPM), PP low (PPL) dose groups, and two positive control QLK group and 1 g/kg (hormones currently used clinically to treat BPH) group. Following five days of habituation, rats in all groups except the normal group received orchiectomy and subcutaneous injected testosterone propionate. Rats in the normal group received an injection of the corresponding dose of olive oil according to body weight. Rats in each group were also administered the corresponding drugs for 28 days. The administration method and dosage are as follow: PPH group oral administrate PP 2 g/kg, PPM group oral administrate PP 1 g/kg, PPL group oral administrate PP 0.5 g/kg,

QLK group oral administrate QLK 1 g/kg, FIN group oral administrate FIN 1 mg/kg, Con and Mod groups were given the same dose of distilled water.

2.3.1. Sample collection

Serum T, DHT, and E2 levels were measured using an automatic microplate reader produced by Hangzhou Aosheng Instrument Co., Ltd. In addition, the effect of the drug on the prostate gland was examined, and the prostate index was computed according to equation (1).

$$\text{Visceralindex} = \frac{\text{Visceralweight(g)}}{\text{Visceralweight(g)}} \times 100\% \quad (1)$$

2.3.2. Histopathology

Prostate tissue was fixed in tissue fixative for more than 24 h. Following this, the tissue was removed, trimmed with a scalpel, labeled, and stored in the processing box. For dewatering wax extraction, the dehydration box was placed in the dehydrator with 75 % ethanol for 4 h, 85 %, and 90 % ethanol for 2 h, 95 % ethanol for 1 h, absolute ethanol for 30min twice, alcohol-benzene for 10min, xylene for 10min twice in turn for dehydration. The paraffin was then melted thrice at 65 °C for 1 h for wax extraction operation. To embed the tissue, the wax-soaked tissue was placed in the embedding machine for embedding. Then, the four embedded wax blocks were fixed on a microtome and cut into 4 μm slices, baked in an oven at 60 °C, bake in the water dry and melt the wax, removed, and finally, stored at room temperature until needed. After HE staining, histopathological changes were observed under a microscope, and the thickness of the prostate epithelium was statistically analyzed with Image J software.

2.3.3. Western blot analysis

WB experiments [12] were performed in the following experimental order: gel preparation-loading – electrophoresis-transfer – milk powder blocking – primary antibody incubation – overnight at 4 °C – secondary antibody incubation – color development.

Each sample was mixed with a lysis buffer containing protease and phosphatase inhibitors followed by trituration and centrifugation. The protein concentration was measured using BCA protein assay, and 30 μg of protein samples were separated by 9 % SDS-PAGE gel and blotted onto PVDF membranes (Millipore). Membranes were blocked with 5 % skim milk dissolved in PBST for 2 h at room temperature and were then incubated with the primary antibody diluted in PBST at 4 °C overnight. The primary antibodies used included polyclonal antirabbit GAPDH (Affinity, AF7021, 1:1000), PI3K (Affinity, AF6241, 1:1000), p-PI3K (Affinity, AF3241, 1:1000), AKT (Affinity, AF6261, 1:1000), *p*-AKT (Affinity, AF0016, 1:1000). The membranes were washed in PBST and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (Affinity, 1:10000), and immunoreactive bands were visualized by ECL (Elabscience). Band intensities were measured with a Tanon 5200 imager and quantified by Image J (National Institutes of Health). The proteins were normalized against GAPDH.

2.3.4. Statistical analysis

Experimental data were expressed as mean ± standard deviation (mean ± S.D.). Protein bands were assessed utilizing Image J software, and data were statistically analyzed utilizing SPSS software. One-way ANOVA statistical test method was used.

2.4. Screening of drug targets

All ingredients in PP were summarized by literature review. Bioactivity was assessed by the Organic Small Molecule Bioactivity Database (PubChem, https://pubchem.ncbi.nlm.nih.gov/), and the two-dimensional structures of all drug ingredients were downloaded. Furthermore, the SwissADME database (http://www.swissadme.ch/) components were screened to select those that met Lipinski's rules and literature validation as final active components.

2.5. Collection of drug and disease targets

Swiss Target Prediction Database (http://www.swisstargetprediction.ch/) was used to predict the target of the active ingredient, but the species was restricted to *Homo sapiens*. "Probability >0" was employed as a screening criterion for the active ingredient target of PP. Moreover, the targets associated with BPH were identified from DisGeNET (https://www.disgenet.org/), Drugbank (https://go.drugbank.com/), Genecards (https://www.genecards.org/), OMIM (https://www.omim.org/), and Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/). Following this, the resulting data were pooled and de-duplicated to obtain disease targets. Furthermore, venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) helped to obtain a drug-disease cross-target, which is a potential target for PP in treating BPH.

2.6. Protein interaction

Using a database called STRING (https://cn.string-db.org/), a PPI network of overlapping targets between PP and BPH was developed. An interaction score ≥0.4 was used as a threshold, and software called Cytoscape 3.7.2 was applied to visualize the network.

2.7. Enrichment analysis

Metascape (https://metascape.org/gp/index.html#/main/step1) was utilized to perform gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The Microbiology Information Cloud Platform (http://www.bioinformatics.com.cn/) was used to present the results of the GO and KEGG analysis. In addition to this, a "drug-compound-target-pathway-disease" network was built to obtain core components and targets.

2.8. Molecular docking

The two-dimensional structures of 3,7,8,2′-Tetrahydroxyflavone, chrysin, wogonin, limocitrin, and sexangularetin were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and were then converted into three-dimensional structures using chem3D software. Moreover, the structure of the core target was obtained from the PDB database (https://www.rcsb.org/). Water and residual molecules were removed from the target using Pymol software. Finally, molecular docking of ligands and receptors was accomplished using Autodock Vina software.

3. Results

3.1. PP significantly inhibited prostate index in BPH rats

On 29th day, the rats were weighed and dissected, and the prostate tissues were also removed and weighed. The results of rat body weight showed that the body weight of rats in each group was considerably lower relative to the Con group after castration. The body weight of rats in the PPH and FIN groups was considerably lower relative to the Mod group ($P < 0.05$), as depicted in Fig. 1A. The

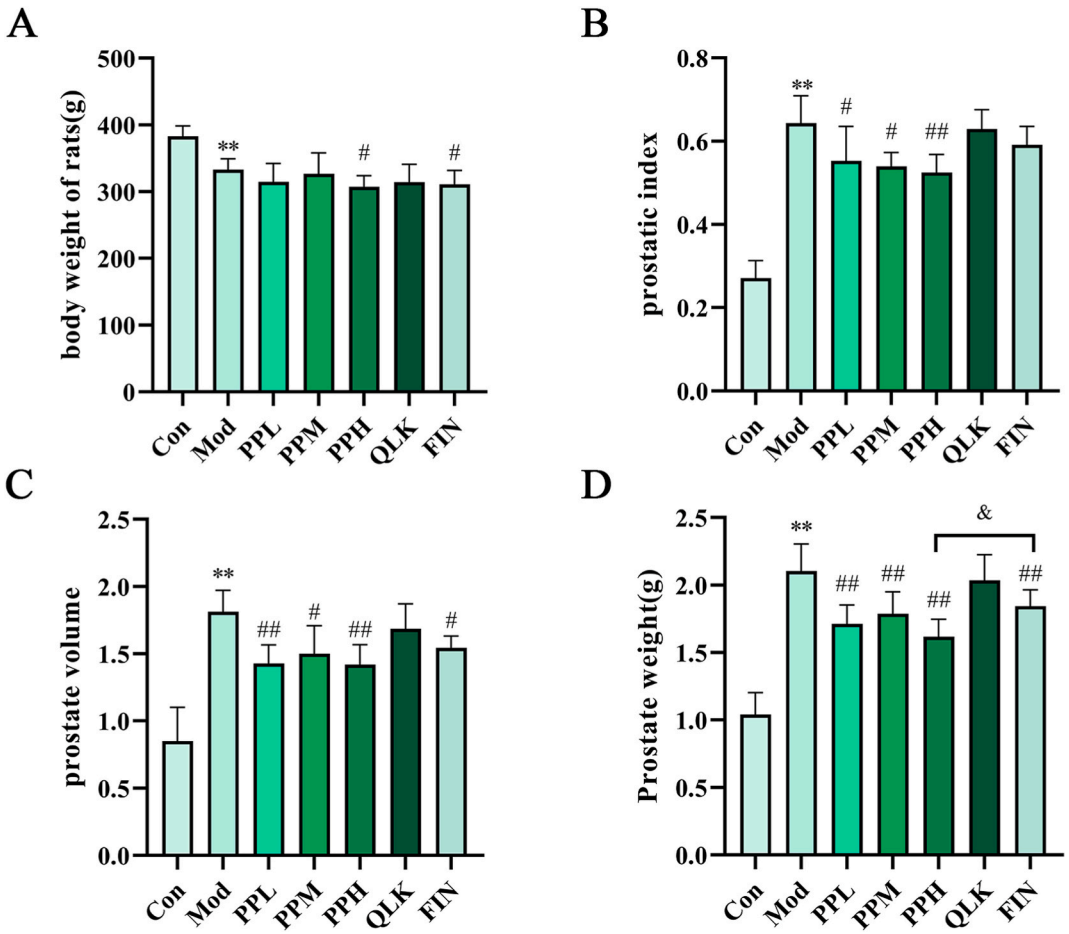

Fig. 1. PP significantly inhibited prostate index in BPH rats. A: Body weight of rats, B: Prostate index, C: Prostate volume and D: Prostate weight. Data were expressed as mean ± S.D., (n = 10). Compared with Con, $^{**}P < 0.01$; compared with Mod, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$; compared between dosing groups, $^{\&}P < 0.05$.

prostate index was the largest difference, and PPL, PPM, and PPH significantly improved the prostate index (x) of rats than the Mod group ($P < 0.05$), as depicted in Fig. 1B. Prostate weight was measured by drainage method, and the findings established that the PPL and PPH groups had the most significant inhibitory effect on prostate volume ($P < 0.01$), as shown in Fig. 1C. Among each dose group, prostate weight in the QLK group was not different from that in the Mod group, whereas the other dose groups significantly decreased prostate weight ($P < 0.01$), as shown in Fig. 1D.

3.2. PP significantly improved hormone levels in BPH rats

The findings established that the contents of serum T, DHT, and E2 in the Mod group were considerably elevated as opposed to the Con group after subcutaneous injection of T for 28 consecutive days ($P < 0.01$). The serum T content of rats in each dose group was considerably lower than Mod group ($P < 0.05$). However, the decrease in PPL was not significant, as shown in Fig. 2A. Additionally, in the serum DHT assay, the content of DHT in each treatment group was significantly lower than the Mod group, and the decrease in the PPH, PPM, and PPL groups was dose-dependent, as shown in Fig. 2B. As for the E2 assay, only the PPM and PPH groups could significantly reduce the content of E2 in the serum than the Mod group ($P < 0.05$) (Fig. 2C).

3.3. PP significantly improves the histomorphology of the prostate in BPH rats

Histomorphological observation of prostate tissues was performed by tissue embedding and HE staining (Fig. 2D). In the Con group,

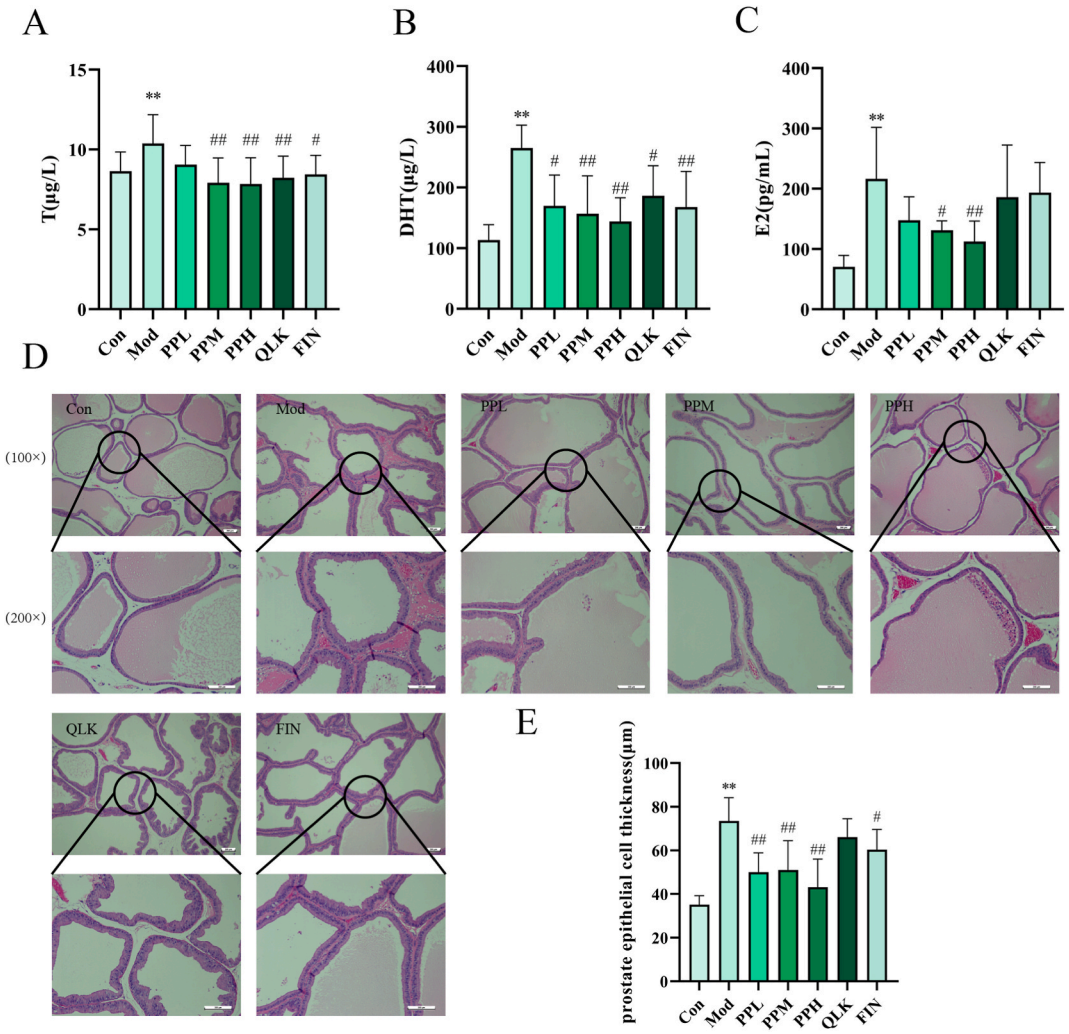

Fig. 2. *PP significantly improved hormone levels and histomorphology structure in BPH rats.* A: the results of the serum T levels in each group; B: the results of the serum DHT levels in each group; C: the results of serum E2 levels in each group; D: histomorphology structure of HE staining in rats of each group (× 100 and × 200); E: Statistical results of prostate epithelial cell thickness in each group. Data were expressed as mean ± S.D., (n = 10). Compared with Con group, $^{**}P < 0.01$; compared with Mod group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

the prostate tissues were clearly arranged, the epithelial cells were closely arranged in a single columnar pattern, the surface was smooth, and the acinar epithelial folds were less. However, compare to the Con group, the prostate glands in the Mod group had significant hyperplasia, the epithelial cells showed multi-layer arrangement, the rough folds were more, the glandular lumen area was increased, and the epithelial cell thickness was also increased ($P < 0.01$). Additionally, relative to the Mod group, the PPL group had moderate hyperplasia of prostatic glands, increased glandular lumen area, and significantly decreased epithelial cell thickness ($P < 0.01$); the PPM group had mild hyperplasia of prostatic glands, slightly increased glandular lumen area, and significantly decreased epithelial cell thickness ($P < 0.01$); the PPH group had mild hyperplasia of prostatic glands, slightly increased glandular lumen area,

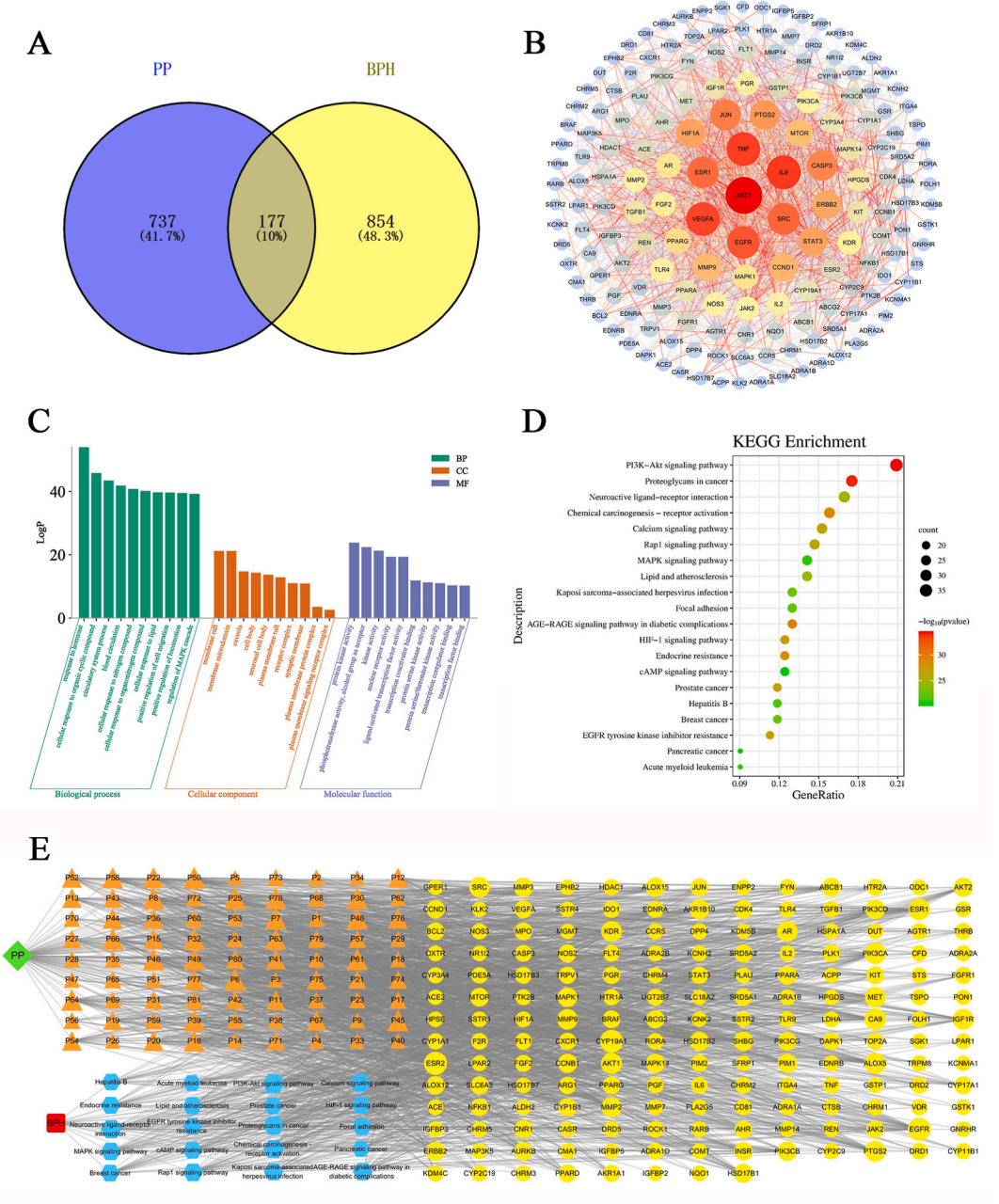

Fig. 3. Network Pharmacology Results. A: Venn diagram for common targets of PP and BPH; B: PPI network diagram of protein interactions. The size and color of the circles are linked to the degree value size, from small to large, from blue to red, indicating that the degree value from small to large; the thickness and color of the line are linked to the total score, from fine to thick, from blue to red, indicating that the total score from low to high. C: Histograms of GO enrichment analysis for common targets; D: Bubble plots enriched for common targets of the KEGG signaling pathways. Larger bubbles represent more targets, and more green bubbles represent smaller *P*-values; E: "Drug-compound-target-pathway-disease" network diagram.

and significantly decreased epithelial cell thickness ($P < 0.01$), with the structure tending toward normality.

3.4. Preliminary study on the substance composition and mechanism of PP in BPH therapy

From the literature review, 196 chemical constituents were identified in PP. Among these chemical constituents, 81 active ingredients had good bioavailability and gastrointestinal absorption. Moreover, 914 and 1031 active ingredients and targets for BPH were collected from the above drug databases, respectively. Finally, 177 overlapping targets were obtained, and protein interaction analysis was performed, followed by GO and KEGG analysis (Fig. 3A–D) to build the "drug-compound-target-pathway-disease" network (Fig. 3E). Additionally, the therapeutic effects of PP were found to be associated with multiple biological functions, including responses to hormones, circulatory processes, and cellular responses to nitrogen compounds. KEGG analysis showed that these effects could be achieved through epidermal growth factor receptor tyrosine kinase inhibitor resistance, the PI3K/Akt signaling cascade, and the HIF-1 signaling cascade. In the enrichment analysis part, the KEGG pathway enrichment analysis diagram shows that the PI3K/Akt pathway has the highest number of enriched differential genes. Hence, it is believed that the mechanism of PP in the treatment of BPH is related to the PI3K/Akt pathway, and thus this pathway was selected for this study.

3.5. High binding of efficacy components in PP to key targets of BPH

In the PPI network, the top five core targets of degree values, namely AKT1, EGFR, IL6, TNF, and VEGFA, were selected for molecular docking with the top five compounds of degree values. Notably, all five core targets associated with PP were enriched in the PI3K/Akt pathway. Molecular docking results are shown in Table 1; heat maps are shown in Fig. 4A, and docking mode maps are shown in Fig. 4B–G. The remaining molecular docking mode maps are shown in Supplementary Material Fig. S5.

3.6. PP inhibits key proteins expression in PI3K/Akt pathway of BPH

As shown in Fig. 5A–C, in terms of p-PI3K/PI3K protein expression, the phosphorylated PI3K protein expression in the PPH group decreased most significantly in each treatment group ($P < 0.01$). As for *p*-AKT/AKT protein expression, except for the absence of a decreasing trend in *p*-AKT protein expression in the QLK group, *p*-AKT protein expression decreased in all other treatment groups. Moreover, relative to the PPL group, *p*-AKT protein expression decreased more significantly in the PPM, PPH, and FIN groups ($P < 0.01$).

4. Discussion

BPH is among the most prevalent benign diseases causing urinary disorders. Current theories on the pathogenesis of BPH include the hormonal-endocrine theory, the growth factor theory, the epithelial-mesenchymal interaction theory, the apoptosis and gene regulation theory, and the inflammation theory [13]. The pathological features of BPH mainly include inflammation, prostate enlargement, intraprostatic fluid accumulation, and epithelial tissue hyperplasia [14]. To confirm the therapeutic effect of PP on BPH, a traditional folk usage, this study employed animal experiments and demonstrated that PP has a good treatment effect on BPH. This was accomplished by reducing hormone levels such as T, DHT, and E2, as well as lowering gold indicators such as prostate index and prostate volume. The effect was particularly significant, surpassing that of the positive control drugs QLK and FIN. The former is a Chinese patent medicines for treating BPH in China, and its main raw material is rapeseed pollen, whereas the latter is a frequently used steroid for the clinical treatment of BPH. Nevertheless, the efficacy components, mechanism of action, and targets of PP in treating BPH are still unclear.

Through the previous HPLC-QTOF-MS study, we found that PP contains multiple chemical components, including flavonoids, terpenoids, phenolic acids and spermidines. However, the effective component of BPH treatment is not known. In this research, through document retrieval and the Swiss target prediction platform, 81 active ingredients in PP, represented by the top five compounds 3,7,8,2′-tetrahydroxyflavone, chrysin, wogonin, limocitrin, and sexangularetin, were screened out to treat BPH. Although the effectiveness of these compounds on BPH has not been verified yet, numerous literature reports demonstrate their strong association with improving the level of inflammation in the body. Chrysin is a naturally occurring flavonoid that contributes to the regulation of pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and prostaglandins [15]. It has a range of biological activities, including anti-inflammatory, neuroprotective, anti-cancer, and antioxidant effects [16–18]. Moreover, *in vitro* results have shown that Wogonin

Table 1
Binding energy of compound to target (kJ•mol^{-1}).

Receptor	Ligand				
	3,7,8,2′-Tetrahydroxyflavone	Chrysin	Wogonin	Limocitrin	Sexangularetin
AKT1	−6.6	−6.9	−6	−6.4	−6.3
EGFR	−8.4	−9.1	−9.1	−9.0	−9.0
IL6	−6.7	−6.7	−6.4	−6.2	−6.2
TNF	−7.1	−8.8	−6.6	−7.7	−6.2
VEGFA	−7.2	−8	−7.5	−7.4	−7.5

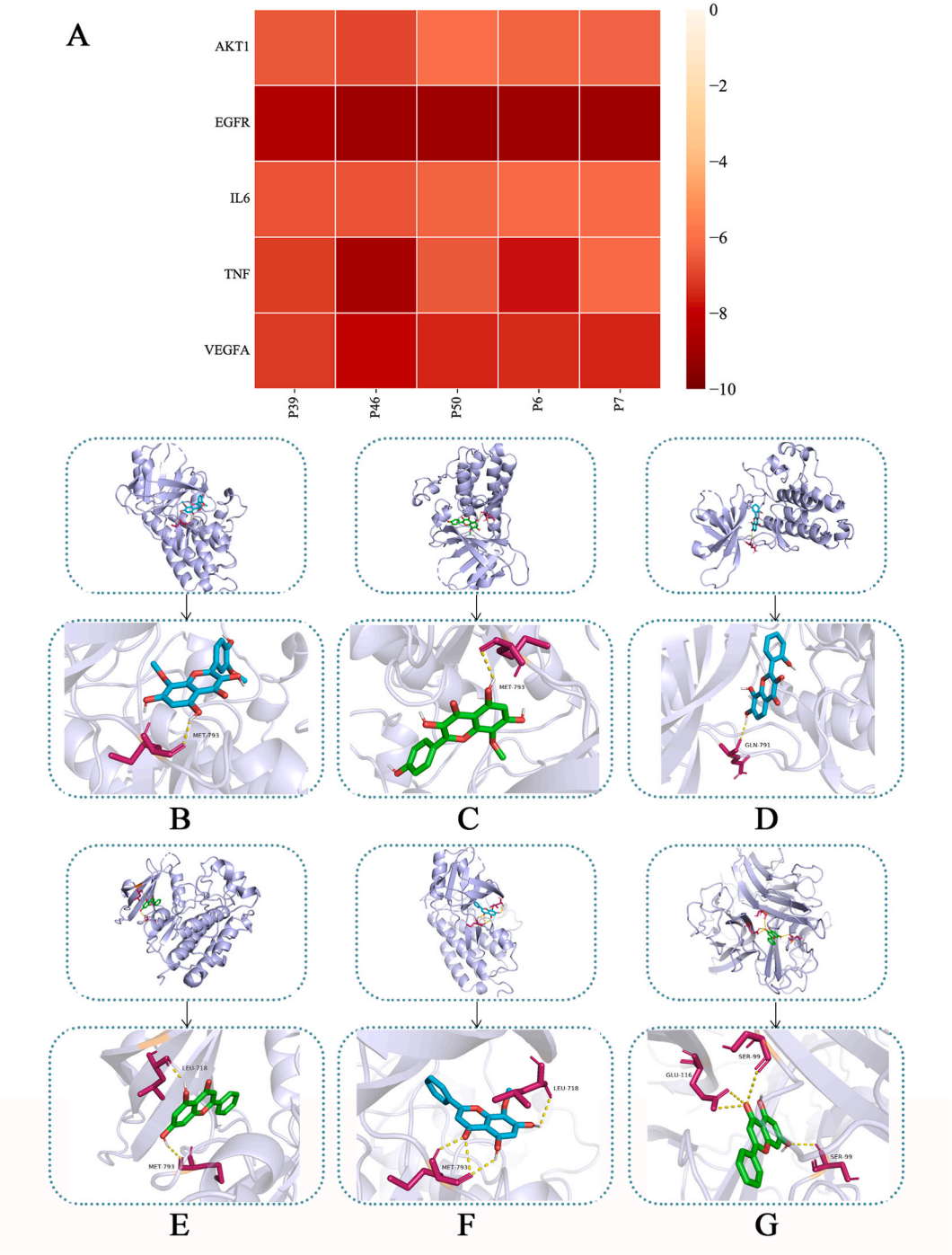

Fig. 4. Molecular docking. A: Molecular docking heatmap. P39: 3, 7, 8, 2′-tetrahydroxyflavone; P46: Chrysin; P50: Wogonin; P6: Limocitrin; P7: Sexangularetin. B–G: Molecular docking pattern diagram; B: EGFR with 3, 7, 8, 2′-Tetrahydroxyflavone; C: EGFR with Chrysin; D: EGFR with Wogonin; E: EGFR with Limocitrin; F: EGFR with Sexangularetin; G: TNF with Chrysin.

inhibits the expression of 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-stimulated mouse skin fibroblast-induced COX-2 and prostaglandin E2 (PGE2) production [19]. Other studies consistently confirmed that Wogonin inhibited COX-2 and inducible nitric oxide synthase (iNOS) expression in macrophages from LPS-treated mice [20,21]. Next, the anti-BPH activity of these compounds must be validated through *in vivo* and *in vitro* experiments.

Through the Swiss Target Prediction database, DisGeNET, Drugbank, Genecards, OMIM, and Therapeutic Target Database, 177 intersecting targets were identified. AKT1, EGFR, IL-6, TNF, and VEGFA were selected as the top five potential targets for PP treatment

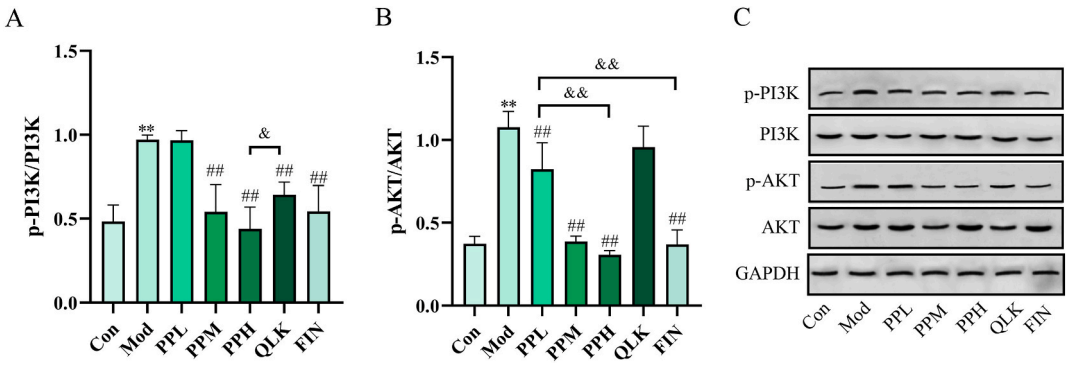

Fig. 5. PP inhibits key proteins expression in PI3K/Akt pathway of BPH. Data were expressed as mean ± S.D., (n = 10). Compared with Con, $^{**}P < 0.01$; compared with Mod, $^{\#\#}P < 0.01$, compared between dosing groups, $^{\&}P < 0.05$, $^{\&\&}P < 0.01$.

of BPH. PI3K is normally activated by extracellular signals, including growth factors, cytokines, and hormones [22]. The kinases of the protein kinase B family are an integral and important component of the growth factor signaling pathway, activated downstream of membrane-bound PI3K. Akt gets phosphorylated and activated, further phosphorylating many other proteins that regulate a range of cellular processes. These processes include protein synthesis, cell growth and survival, proliferation, metabolism, and migration [23, 24]. Epidermal growth factor receptor EGFR, a receptor for epithelial growth factor (EGF) cell proliferation and signaling, is a glycoprotein, a tyrosine kinase-type receptor. Evidence suggests that EGF and androgens synergistically stimulate EGFR synthesis and prostate cell proliferation *in vitro* [25]. Furthermore, growth factor activation of the PI3K/Akt signaling pathway leads to the phosphorylation of pro-apoptotic proteins (BAD), which results in the attachment of BAD and anti-apoptotic molecules such as Bcl-xL and Bcl-2. It can also inhibit apoptosis [26] and lead to prostate tumorigenesis [27]. Therefore, blocking the PI3K/Akt signaling pathway has an inhibitory effect on BPH [28].

The molecular docking outcomes showed that they have good binding energy with the top five ligands. This also suggests that PP may exert therapeutic effects on BPH through multiple components and targets via multiple pathways. Since the KEGG enrichment analysis revealed that these effects could be achieved by the PI3K/Akt signaling pathway, the expression of PI3K and AKT proteins was detected in animal prostate tissue. The findings established a significant increase in the activated PI3K and AKT proteins in the Mod group relative to the Con group, thereby indicating that the PI3K/AKT signaling pathway was activated. Following PP gavage, the expression of activated PI3K and AKT was significantly reduced, thus indicating that PP has an inhibitory effect on the PI3K/AKT signaling pathway. Next, systematic research on the selected compounds and targets must be conducted, employing PI3K/AKT inhibitors to verify the potential drug targets and pathways of action.

In conclusion, the therapeutic effect of PP on BPH was verified through animal experiments and network pharmacology in this research. The effective components for treating BPH in PP were screened, and its mechanism of action and potential targets were preliminarily elucidated. This laid the foundation for subsequent research and the development of drugs for treating BPH.

Data availability statement

The original contributions presented in the study are included in the article or supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Laboratory Animal Welfare and Ethics Committee of Air Force Medical University.

CRediT authorship contribution statement

Jun Mu: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Junsheng Wu:** Validation, Methodology, Investigation. **Linrui Duan:** Resources, Project administration, Formal analysis. **Qian Yang:** Resources, Project administration, Formal analysis. **Xiaoting Liu:** Writing – review & editing, Visualization. **Huixin Bai:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Yanhua Xie:** Supervision, Software, Resources. **Jie Li:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis. **Siwang Wang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis.

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.e22212.

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