

Network Pharmacology and Experimental Verification Reveal the Regulatory Mechanism of Chuanbeimu in Treating Chronic Obstructive Pulmonary Disease

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Background: Chronic obstructive pulmonary disease (COPD) is a common respiratory disorder in pulmonology. Chuanbeimu (CBM) is a traditional Chinese medicinal herb for treating COPD and has been widely utilized in clinical practice. However, the mechanism of CBM in the treatment of COPD remains incompletely understood. This study aims to investigate the underlying therapeutic mechanism of CBM for COPD using network pharmacology and experimental approaches.

Methods: Active ingredients and their targets were obtained from the Traditional Chinese Medicine Systems Pharmacology database. COPD-associated targets were retrieved from the GeneCards database. The common targets for CBM and COPD were identified through Venn diagram analysis. Protein-protein interaction (PPI) networks and disease-herb-ingredient-target networks were constructed. Subsequently, the results of the network pharmacology were validated by molecular docking and in vitro experiments.

Results: Seven active ingredients and 32 potential targets for CBM were identified as closely associated with COPD. The results of the disease-herb-ingredient-target network and PPI network showed that peimisine emerged as the core ingredient, and *SRC*, *ADRB2*, *MMP2*, and *NOS3* were the potential targets for CBM in treating COPD. Molecular docking analysis confirmed that peimisine exhibited high binding affinity with *SRC*, *ADRB2*, *MMP2*, and *NOS3*. In vitro experiments demonstrated that peimisine significantly upregulated the expression of *ADRB2* and *NOS3* and downregulated the expression of *SRC* and *MMP2*.

Conclusion: These findings indicate that CBM may modulate the expression of *SRC*, *ADRB2*, *MMP2*, and *NOS3*, thereby exerting a protective effect against COPD.

Keywords: chuanbeimu, molecular docking, network pharmacology, experiment verification, chronic obstructive pulmonary disease

Introduction

Chronic obstructive pulmonary disease (COPD) is a prevalent respiratory condition within pulmonary disease. In clinical practice, it is essential to consider respiratory difficulties, long-term chronic cough or expectoration, and recurrent lower respiratory tract infections when accompanied by long-term exposure to risk factors such as smoking, exposure to fumes, as well as air pollution. According to the 2022 revision of Global Initiative for Chronic Obstructive Lung Disease (GOLD), the initial assessment and diagnosis categorizes COPD into two categories: stable COPD and Acute exacerbation of COPD (AECOPD), based on evaluation results and characteristics.¹ The prevalence and mortality of COPD in

China are increasing annually due to an aging population, an increasing number of smokers, and rising levels environmental pollution. According to World Health Organization (WHO) statistics, it is estimated that approximately 5.4 million individuals will succumb to COPD and associated diseases by 2060.² The pathogenesis of COPD remains elusive, however, studies reveal that lung parenchymal damage, inflammatory responses, and structural alterations are the most characteristic pathological changes in the occurrence and development of COPD.³ Pulmonary function tests are currently objective indicator for the diagnosis and evaluation of COPD. When combined with the COPD Assessment Test (CAT), it can markedly improve the sensitivity of COPD diagnosis.⁴

Currently, some drugs such as glucocorticoids, bronchodilators, antibiotics, anti-inflammatory drugs, and immunosuppressants, are commonly used in the treatment of COPD.^{5,6} While these medications have demonstrated certain efficacy in treating COPD, they also trigger various adverse reactions such as increased drug resistance and hormone-related side effects.⁷ Thus, alternative therapies such as traditional Chinese medicine (TCM) may be a treatment option for COPD with fewer side effects and lower cost. TCM, a system with a long history, has demonstrated unique therapeutic advantages and significant research potential in treating COPD. TCM emphasizes holistic conditioning, individualized treatment, and herbal medicine, offering significant advantages in alleviating symptoms, reducing the frequency of acute exacerbation, and improving the quality of life in COPD.^{8,9} Unlike western medicine, which often targets specific symptoms, TCM aims to harmonize the balance of Yin and Yang in the human body, thereby improving the quality of life of COPD patients through a comprehensive treatment approach.¹⁰ Recent studies have provided further evidence supporting the effectiveness and elucidating the mechanisms of TCM in treating COPD,^{11,12} suggesting that TCM offers a broader range of treatment options and substantial potential for ongoing research in this field.

Chuanbeimu (CBM) is a valued and time-honored Chinese medicinal material with a rich medicinal history. Over the past few decades, several chemical components of CBM have been identified, which primarily include steroid alkaloids, saponins, and terpenoids.¹³ In recent years, numerous studies have shown that CBM exhibits a multiple biological activities, including expectorant, antitussive, antiasthmatic, anti-inflammatory, anti-tumor, and anti-oxidant effects.^{14–16} Historically, CBM has been used both in China and abroad for the treatment of lung diseases.¹⁷ Its therapeutic effects are primarily mediated by biological processes, notably inflammation, to confer therapeutic benefits in lung diseases.¹⁸ Currently, over 200 CBM-related products are available on the market. Examples include Nin Jiom Pei Pa Koa, Chuanbei Zhike Lu, and Chuanbei Pipa Capsules. These products are widely used in clinical practice for treating respiratory difficulties, bronchitis, COPD, and other pulmonary conditions.^{19–22} Wang et al found that imperiline, a steroidal alkaloid derived from CBM, may alleviate both lung function and structural integrity in a rat model of COPD, potentially delaying disease progression.²² Investigating the mechanism of CBM in the treatment of COPD holds significant importance. COPD, a debilitating respiratory disease, profoundly affects patients' quality of life. Among the pharmacologically active components of CBM, total alkaloids have demonstrated potential therapeutic effects on COPD symptoms in animal studies. These components may exert their mechanism of action through the inhibition of TGF- β and NF- κ B signaling pathways, both of which play crucial roles in the inflammatory response and pulmonary fibrosis associated with COPD.²³ However, the research on CBM for COPD treatment encounters challenges primarily stemming from the intricate nature of its chemical composition. Therefore, further investigation is necessary to identify effective components and elucidate their mechanisms of action.

Network pharmacology is a widely employed approach for investigating the biological mechanisms of TCM in complex diseases at the molecular level. This method utilizes the principles of multi-directional pharmacology, systems biology, network analysis, and computational biology to systematically elucidate the potential targets and mechanisms of TCM.²⁴ Moreover, it facilitates the establishment of a relationship network encompassing the disease-herb-compound-target pathway, thus uncovering the molecular basis and predicting the pharmacological mechanism.²⁵ Network pharmacology is gaining popularity as a method for investigating the mechanisms through which TCM treats a wide range of diseases.

Molecular docking plays a crucial role in contemporary drug design and discovery. It facilitates the comprehension of drug mechanisms, prediction of efficacy, and assessment of side effects by simulating the interactions between drugs and their target receptors.²⁶ Molecular docking enables precise demonstration of the interactions between drugs and their receptors. It facilitates the determination of the drug's position within the binding pocket and the forces involved with amino acids, both of which are essential for understanding the drug's affinity and specificity. For instance, Hamza et al

determined the binding mode of Pyrazolo-pyridazinone derivatives as FGFR1 inhibitors through docking analysis.²⁷ Molecular docking analysis assists in optimizing drug structures, thereby enhancing their activity and selectivity. Through simulating interactions between various structures and the receptor, this approach enables the design of more effective drugs. For instance, previous studies have guided the design of pyrrolo-pyrimidine analogs as FGFR1 inhibitors through docking analysis.²⁸ In summary, molecular docking plays a pivotal role in drug development, offering efficient computational tools for design and the elucidation of mechanisms.

In this study, we screened the key active ingredients and targets for CBM against COPD and investigated the potential mechanism of CBM in the treatment of COPD through network pharmacology, molecular docking, and in vitro experiments. The flowchart of our research is illustrated in Figure 1.

Materials and Methods

Screening of Active Ingredients and Targets of CBM

The chemical components of CBM were screened using the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (<https://old.tcmsp-e.com/index.php>).²⁹ From these, we selected active components based on the criteria of oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 .³⁰ Subsequently, TCMSP was employed to

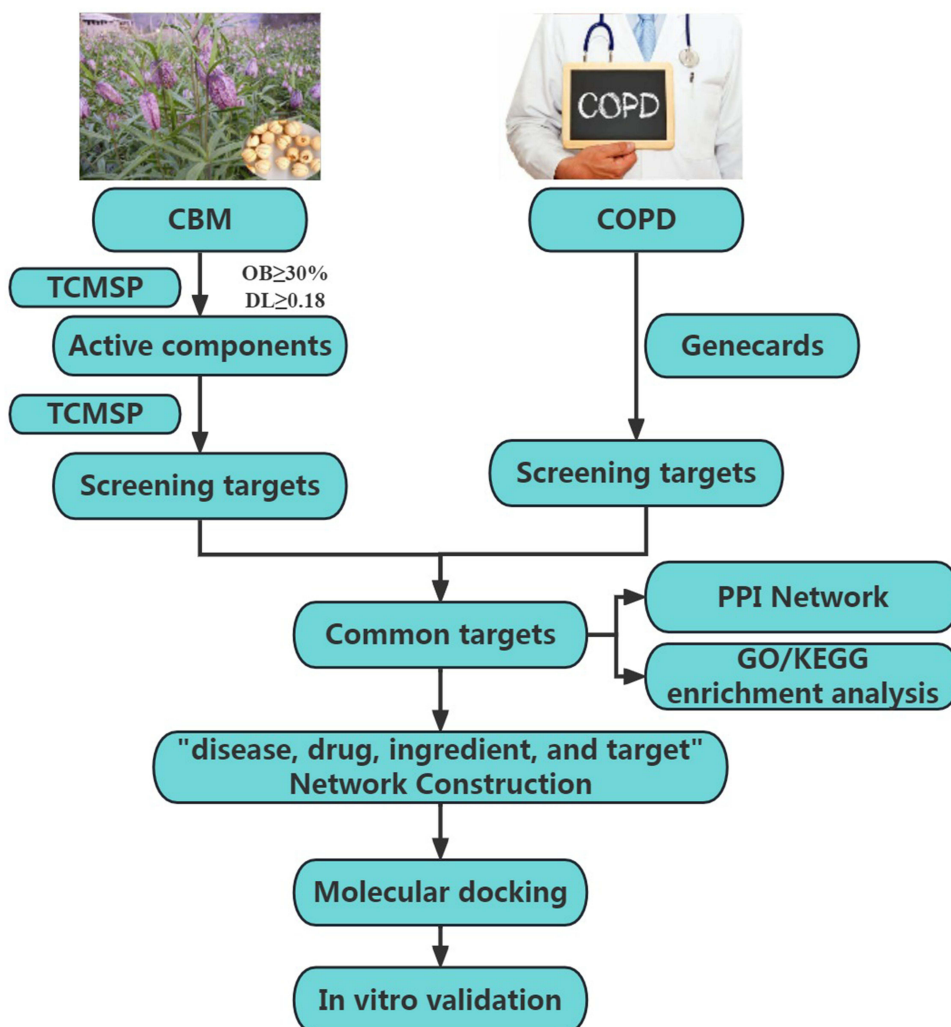


Figure 1 The overall flow chart of this work.

Abbreviations: CMB, chuanbeimu; COPD, chronic obstructive pulmonary disease; TCMSP, Traditional Chinese Medicine Systems Pharmacology; OB, oral bioavailability; DL, drug-likeness; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

predict targets of the active components. Furthermore, the UniProt database (<https://www.uniprot.org/>) was utilized to standardize the targets.

Prediction of COPD Targets

The search for targets associated with COPD was conducted in the GeneCards database (<https://www.genecards.org/>).³¹ The keyword used for the search was “chronic obstructive pulmonary disease”. The targets associated with COPD were identified based on a relevance score greater than 1.

Screening for CBM-COPD Common Targets and Construction of a Protein-Protein Interaction (PPI) Network

The overlapping targets between CBM and COPD were identified using the Venn diagram tool 2.1.0 (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>). The potential targets of CBM for the treatment of COPD were then entered into the STRING database (<http://string-db.org/>)³² for PPI analysis. The biological species “Homo sapiens” was selected as the study organism, the isolated protein was masked, and the TSV file was exported. Cytoscape 3.10.0 was utilized to generate the PPI network diagram and conduct topological analysis to identify the central protein.

GO and KEGG Enrichment Analysis

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://david.abcc.ncifcrf.gov>)³³ and KEGG Orthology Based Annotation System (KOBAS) (<https://www.biostars.org/p/200126/>) databases were utilized to search for the relevant gene functions. Gene ontology (GO) functional enrichment analysis and KEGG pathway enrichment analysis were performed using R package version 4.1.1. The variables for plotting were selected based on the *p*-values.

Construction of Disease-Herb- Ingredient-Target Network

Cytoscape 3.10.0 (<https://cytoscape.org/>)³⁴ was used to generate a network diagram illustrating the interactions among disease, herb, active ingredients, and targets in CBM for the treatment of COPD. Nodes represent diseases, drugs, active ingredients, and targets. Edges represent the connections between nodes.

Molecular Docking

To gain a more comprehensive understanding of the correlation between key active ingredients and their targets, we assessed the strength and pattern of their interactions through molecular docking. The 3D molecular structure of the active ingredient was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The crystal structure of the target proteins were obtained from the RCSB Protein Data Bank (PDB, <http://www.rcsb.org/>).³⁵ Subsequently, molecular docking between the ligands and receptors was performed using Autodock 4.2 software.³⁶ The results of molecular docking were analyzed using Discovery Studio Visualizer software and PyMOL.

Experimental Verification

Preparation of Cigarette Smoke Extract (CSE)

Septwolves cigarettes (tobacco type of tar: 10 mg, nicotine content: 0.8 mg, and carbon monoxide fumes: 12 mg) were purchased from China Tobacco Fujian Industry Limited Liability Company (Xiamen, China) and used to prepare CSE through a puffing mechanism mimicking a standardized human smoking pattern (volume 30 mL/puff, duration 2 s/puff, and frequency 1 puff/min) as described elsewhere with minor modifications.³⁷ A total of 300 mL cigarette smoke was collected by a mechanical vacuum pump and bubbled in 10 mL DMEM medium without serum. The crude CSE was adjusted pH to 7 and filtered through a 0.22 µm filter, and this solution was defined as 100% CSE. Working concentration was prepared by dilution the 100% CSE with culture medium.

Establishing a COPD Cell Model

The human bronchial epithelial cell line (BEAS-2B) was purchased from the Procell Life Science and Technology Co., Ltd. (Wuhan, China) and cultured in DMEM medium (Procell, Wuhan, China) in a humidified incubator under 5% CO₂ at 37 °C. The cells were exposed to varying concentrations of CSE (0%, 1%, 2.5%, 5%, 7.5%, 10%, 25%) for different durations (24 h and 48 h). Following the replacement of the CCK-8 mixture (Beyotime, Hangzhou, China), the cells were incubated for 4 hours. Subsequently, the absorbance values of each well were measured using a full-spectrum microplate reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 450 nm. Specific concentrations (low, medium, and high) were then selected, and a singular treatment time was chosen for subsequent model validation experiments. For the cell apoptosis assessment, these cells were stained with Muse Annexin V & Dead Cell kit (Muse, Beijing, China), and then was detected and analyzed by the flow cytometry. RT-qPCR was conducted to evaluate the levels of inflammatory factors IL-1 β and TNF- α . Integrating the results from the above experiments allows for a comprehensive evaluation of the successful construction of the model and aids in identifying the most suitable modeling concentration.

Drug Treatment of BEAS-2B

Peimisine (purity \geq 98%) was purchased from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). Cell viability was assessed using the CCK-8 proliferation assay (Beyotime, Hangzhou, China). BEAS-2B cells were seeded in a 96-well plate at a density of 3×10^5 , and after culturing for 24 h, different concentrations of peimisine (0 μ M, 2.5 μ M, 5 μ M, 10 μ M, 20 μ M, 40 μ M, 80 μ M, 160 μ M, and 320 μ M) were added, respectively. Following 24 h and 48 h of incubation, CCK8 was added to each well and the cells were cultured at 37 °C for 4 h. The OD value (450 nm) of each well was measured using a full-spectrum microplate reader (Molecular Devices, Sunnyvale, CA, USA) to determine the safe concentration range of peimisine.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Analysis

Total RNA was extracted from cells using Trizol reagent (AgiBio, Hunan, China) following the manufacturer's protocol. A NanoDrop 2000 (Thermo Fisher Scientific, USA) was used to determine the RNA purity and concentration. A reverse transcription kit (TaKaRa, Clontech) was employed to transcribe RNA into cDNA. After reverse transcription, qRT-PCR was conducted using the ABI 7500 Real-Time PCR System (Applied Biosystems, USA). The primer sequences are provided in [Table S1](#), and the expression of IL-1 β , TNF- α , *SRC*, *ADRB2*, and *MMP2* were assessed. The qRT-PCR reaction system had a total volume of 20 μ L, comprising 10 μ L $2 \times$ TB Green Premix Ex Taq II, 0.8 μ L of 10 μ M forward and reverse primers each, 0.4 μ L $50 \times$ ROX Reference Dye II, 2 μ L cDNA, supplemented with double-distilled water. The qRT-PCR conditions were as follows: 40 cycles of denaturation at 95 °C for 30s, followed by denaturation at 95 °C for 5s, and annealing at 60 °C for 34s. GAPDH served as the internal control, and data analysis utilized the $2^{-\Delta\Delta Ct}$ method. The experiment was repeated three times.

Statistical Analysis

The data were analyzed using GraphPad Prism 8 software for statistical analysis. All experimental data obtained from the study were collected from three biological replicates. Measurement data were presented as mean \pm standard deviation (SD). Inter-group comparisons were conducted using one-way analysis of variance (ANOVA) or Student's *t*-test. The results were statistically significant, with a *p*-value of less than 0.05. * *p* < 0.05, ** *p* < 0.01, and *** *p* < 0.001.

Results

Identification of Bioactive Ingredients and Targets in CBM

The OB and DL indices are critical tools for the screening of active ingredients in TCM. OB indicates the degree of absorption of compounds within TCM formulations, where substances with an OB \geq 30% are regarded as having enhanced pharmaceutical potential. DL reflects the potential for pharmaceutical development of compounds, with those exhibiting a DL \geq 0.18 chosen as markers of substantial drug delivery potential. Integrating these two indices can improve the precision of predicting TCM efficacy and optimize the in vivo absorption and utilization. In this study,

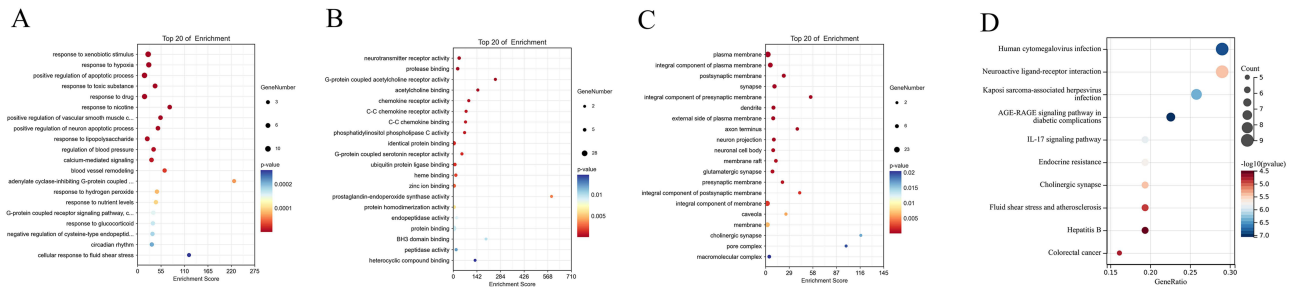


Figure 3 Functional enrichment analysis of the common targets. **(A)** Bubble chart of the biological process category terms; **(B)** Bubble chart of the molecular function category terms; **(C)** Bubble chart of the cellular component category terms; **(D)** Bubble chart showing the top 10 enriched KEGG terms. **Abbreviation:** KEGG, Kyoto Encyclopedia of Genes and Genomes.

terms are illustrated using bubble charts in **Figures 3A–C**. Among these terms, the BP category showed predominant enrichment in processes such as response to xenobiotic stimulus, hypoxia, and positive regulation of the apoptotic process. In the MF category, enrichment was primarily observed in functions including neurotransmitter receptor activity, protease binding, and G-protein-coupled acetylcholine receptor activity. The CC category was primarily enriched in components such as the plasma membrane, integral components of the plasma membrane, and postsynaptic membrane. The top 10 enriched KEGG terms are displayed in the bubble chart depicted in **Figure 3D**.

Construction and Analysis of the Disease-Herb-Ingredient-Target Network

We constructed a disease-herb-ingredient-target network using Cytoscape 3.10.0 (**Figure 4A**), comprising 41 nodes: 7 representing active ingredients, 32 for targets, one for disease, and one for herb). **Table 2** illustrated that certain ingredients, such as peimisine, sincipemine, and beta-sitosterol, exhibited higher degree values. Notably, peimisine, with the highest degree value of 16, may play a pivotal role in COPD treatment. Additionally, we constructed an ingredient-target network centered on peimisine and its associated targets (**Figure 4B**). The target with the highest node degree were SRC, ADRB2, NOS3, GSK3B, and MMP2, as depicted in **Figure 4C**. Integrating this with the PPI network of the 32 common targets, where SRC, ADRB2, NOS3, and MMP2 exhibited the highest degree values, we hypothesize that peimisine may regulate the expression of SRC, ADRB2, NOS3, and MMP2, contributing to its therapeutic potential in COPD treatment.

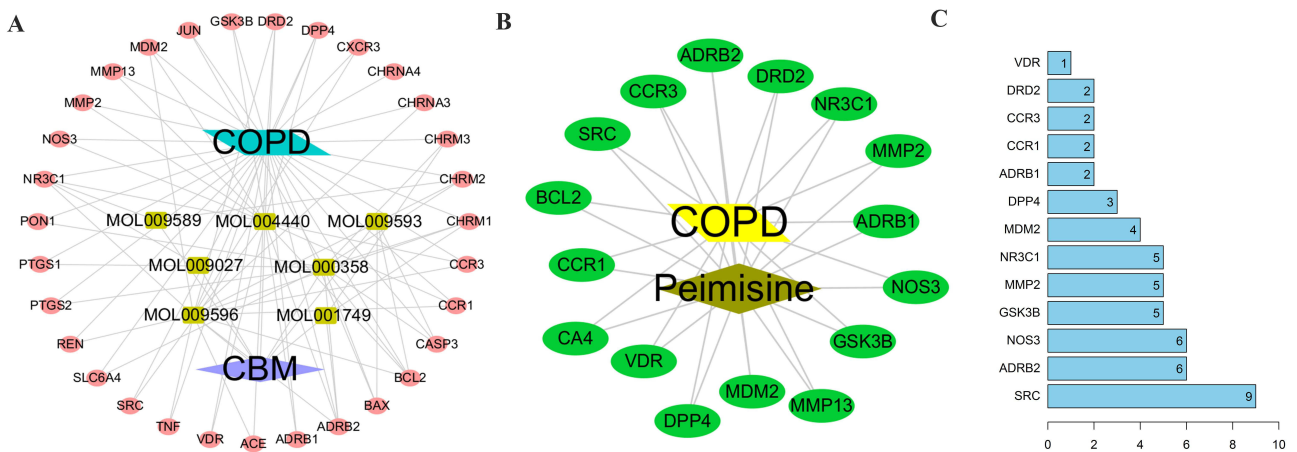


Figure 4 Network construction of the herb, ingredient, and targets. **(A)** Network of the disease-herb-ingredient-target for the treatment of COPD using the CBM; **(B)** Peimisine and disease-target network; **(C)** Barplot of the 16 targets of peimisine. **Abbreviations:** CBM, chuanbeimu; COPD, chronic obstructive pulmonary disease.

Table 2 CBM Active Ingredients Correspond to Targets

Mol ID	Molecule Name	Targets Number	Targets
MOL004440	Peimisine	16	NR3C1
MOL004440	Peimisine		NOS3
MOL004440	Peimisine		DPP4
MOL004440	Peimisine		CCR3
MOL004440	Peimisine		DRD2
MOL004440	Peimisine		CCR1
MOL004440	Peimisine		VDR
MOL004440	Peimisine		MDM2
MOL004440	Peimisine		SRC
MOL004440	Peimisine		MMP13
MOL004440	Peimisine		MMP2
MOL004440	Peimisine		GSK3B
MOL004440	Peimisine		BCL2
MOL004440	Peimisine		ADRB2
MOL004440	Peimisine		ADRB1
MOL004440	Peimisine		CA4
MOL009596	Sinpemine	15	CHRM2
MOL009596	Sinpemine		CHRM1
MOL009596	Sinpemine		REN
MOL009596	Sinpemine		MDM2
MOL009596	Sinpemine		DPP4
MOL009596	Sinpemine		ADRB2
MOL009596	Sinpemine		CCR1
MOL009596	Sinpemine		CCR3
MOL009596	Sinpemine		NR3C1
MOL009596	Sinpemine		CHRNA3
MOL009596	Sinpemine		CXCR3
MOL009596	Sinpemine		DRD2
MOL009596	Sinpemine		BCL2
MOL009596	Sinpemine		TNF
MOL009596	Sinpemine		ACE
MOL000358	Beta-sitosterol	12	PTGS1
MOL000358	Beta-sitosterol		PTGS2
MOL000358	Beta-sitosterol		CHRM3
MOL000358	Beta-sitosterol		CHRM1
MOL000358	Beta-sitosterol		CHRM2
MOL000358	Beta-sitosterol		ADRB2
MOL000358	Beta-sitosterol		SLC6A4
MOL000358	Beta-sitosterol		BCL2
MOL000358	Beta-sitosterol		BAX
MOL000358	Beta-sitosterol		JUN
MOL000358	Beta-sitosterol		CASP3
MOL000358	Beta-sitosterol		PONI
MOL001749	ZINC03860434	3	CHRM3
MOL001749	ZINC03860434		ADRB2
MOL001749	ZINC03860434		CHRM1
MOL009593	Verticinone	3	BCL2
MOL009593	Verticinone		BAX
MOL009593	Verticinone		CASP3
MOL009589	Korseverinine	2	CHRM2
MOL009589	Korseverinine		NR3C1
MOL009027	Cycloamine	1	NR3C1

Abbreviations: CBM, Chuanbeimu; ID, Identity document.

Molecular Docking results

To assess the validity of molecule-target interactions and ascertain the exact binding patterns, we identified peimisine as the principal molecule, with *SRC*, *ADRB2*, *MMP2*, and *NOS3* according to the preceding results. Lower binding energy correlates with increased stability of the ligand-receptor binding conformation. A binding energy of -5 kcal/mol or lower signifies robust binding affinity between molecules and proteins.

As depicted in Figure 5 and Table 3, our findings indicate that peimisine exhibits strong interactions with *SRC*, *ADRB2*, *MMP2*, and *NOS3*, characterized by binding energies of -9.1 kcal/mol, -9.2 kcal/mol, -8.0 kcal/mol, and -10 kcal/mol, respectively. This affirms the stability of the binding activity between the core active ingredient and the key target proteins.

Establishment and Evaluation of a Cell Model for COPD

To assess the successful establishment of the COPD model, we evaluated cell proliferation with CCK-8 and quantified inflammatory factor levels via PCR. CCK-8 assays (Figure 6A) demonstrated cytotoxicity to the cells at a 25% CSE concentration. Consequently, we selected CSE concentrations of 5%, 7.5%, and 10% for the subsequent 24-hour experiments. PCR analyses showed that various CSE concentrations significantly upregulated the expression of inflammatory markers *IL-1 β* and *TNF- α* , as depicted in Figure 6B and C. Furthermore, apoptosis assays indicated that disparate CSE concentrations induced apoptosis, evidenced by Figure 6D. Taken together with the apoptosis data, these findings suggest a successful construction of our COPD cell model. In light of these findings, we selected a 10% CSE concentration as optimal for further experimentation.

Effect of Peimisine on Cell Viability in a Cell Model of COPD

In this study, we determined the safe concentration range of peimisine for BEAS-2B cells to accurately assess its therapeutic potential for COPD. The results indicated that peimisine addition led to a reduction in cell proliferation (Figure 7A). Following the experimental results, we selected a concentration of 160 μ M peimisine for a 24-hour exposure as the optimal concentration for subsequent experiments.

The Influence of CBM on the Expression Levels of *SRC*, *ADRB2*, *MMP2*, and *NOS3*

To examine the effects of peimisine on the expression of target genes in COPD, we quantified the mRNA expression levels of *SRC*, *ADRB2*, *MMP2*, and *NOS3* using qRT-qPCR. The results demonstrated that peimisine significantly increased *ADRB2* and *NOS3* mRNA expression levels ($p < 0.001$), while notably reducing *SRC* and *MMP2* mRNA expression levels of ($p < 0.001$), as shown in Figure 7B–E.

Discussion

TCM exerts a beneficial therapeutic effect on COPD. CBM represents a TCM therapeutic strategy for COPD, with prior research illustrating the significant role of CBM and its components in the treatment of COPD.^{22,38} However, the complex composition of TCM poses challenges to exploring the potential therapeutic mechanisms underlying CBM. Consequently, this study validated the mechanism of action of CBM for treating COPD through network pharmacology and in vitro experimentation.

Within the framework of TCM, compounds that lack appropriate pharmacokinetic profiles are unable to effectively reach the target organs to manifest their biological activity. Consequently, we screened active compounds of CBM based on $OB \geq 30\%$ and $DL \geq 0.18$, revealing that the selected compounds exhibited pharmacokinetic properties, which suggests their potential for effective absorption and metabolism in humans.²⁹ Significantly, research has indicated that ingredient with a high degree value may exert therapeutic benefits for COPD. In our study, peimisine might be the primary active ingredient of CBM in the treatment of COPD with a high degree value. Peimisine, an alkaloid found in CBM, has expectorant and cough-relieving effects.³⁹ A study has shown that peimisine, a steroid alkaloid from CBM, reduces the expression of p-AKT and phosphorylated glycogen synthase kinase 3β (p-GSK3 β), while increasing the expression of phosphorylated myosin light chain 2 (p-MLC2) to prevent the progression of COPD.³⁸ Meanwhile, peimisine can alleviate inflammation, acute lung injury, and oxidative stress.⁴⁰

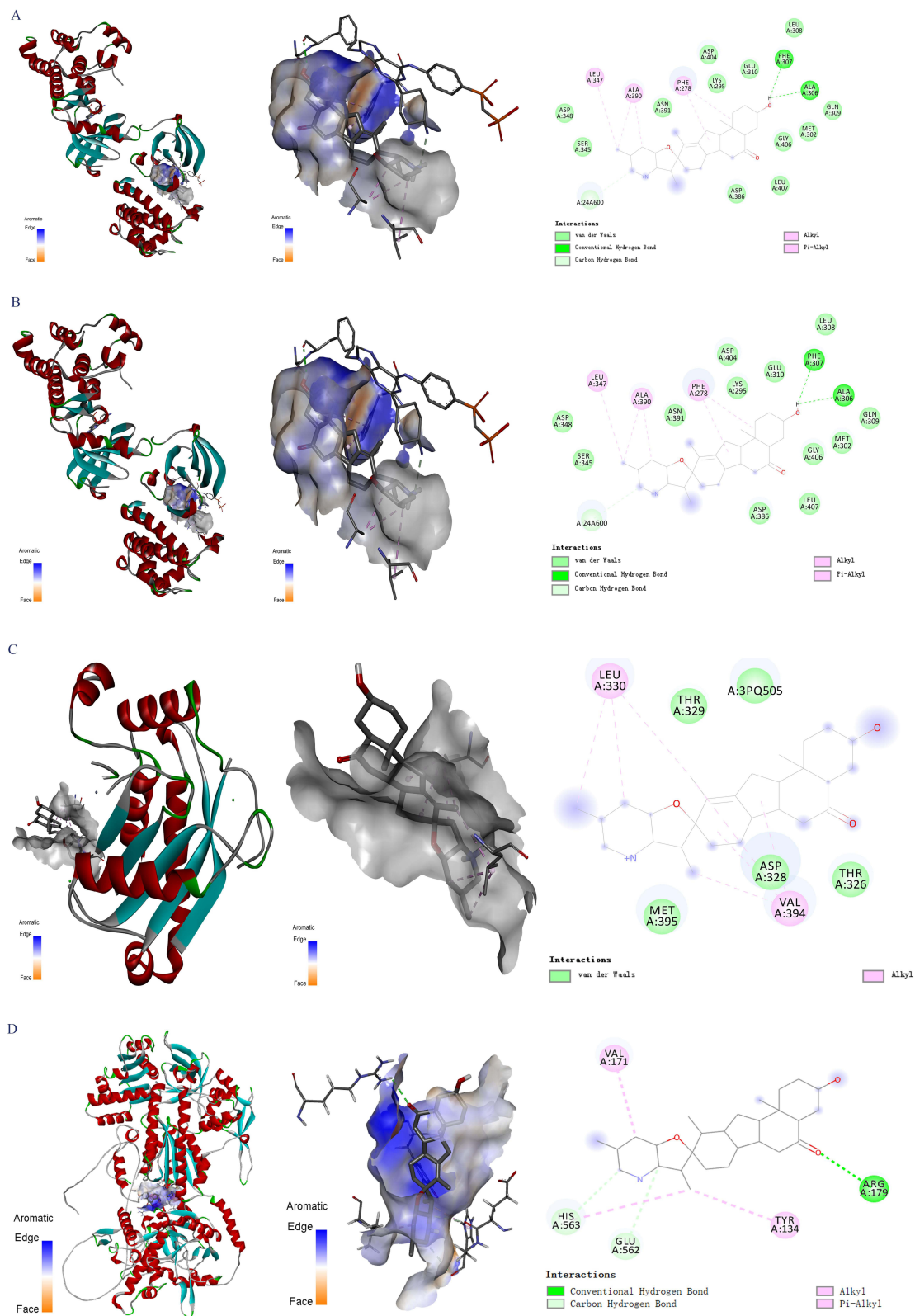


Figure 5 Molecular docking analysis between peimisine and the targets. **(A)** SRC, **(B)** ADRB2, **(C)** MMP2, and **(D)** NOS3.

Table 3 Molecular Docking Information of Peimisine and Targets

Compound	Target	PDB ID	Affinity (kcal/mol)
Peimisine	NOS3	P29474	-10.0
Peimisine	SRC	2BDF	-9.1
Peimisine	ADRB2	6PS2	-9.2
Peimisine	MMP2	4WK7	-8.0

Abbreviations: PDB, Protein data bank; ID, Identity document.

Chen et al discovered that peimisine was mainly distributed in tissues such as the lungs, spleen, and kidneys in rats.⁴¹ Jin et al discovered that peimisine (10 mg/kg) significantly decreased the levels of *TNF- α* and *IL-1 β* , and mitigated the extent of lung tissue damage.⁴² *TNF- α* and *IL-1 β* are the primary inflammatory factors in human COPD.⁴³ Therefore, peimisine may have certain anti-inflammatory effects by reducing the levels of inflammatory factors. As a result, it has potential in the treatment of COPD. Furthermore, the PPI analysis and disease-herb-ingredient-target network revealed that *SRC*, *ADRB2*, *MMP2*, and *NOS3* were the potential targets for CBM in treating COPD. The study has shown that *SRC* is activated in the lungs of patients with COPD.⁴⁴ The Beta 2-adrenergic receptor (*ADRB2*) plays a crucial role in regulating airway smooth muscle tension in COPD.⁴⁵ In COPD, mutations that impair *ADRB2* function may increase the risk of the disease or reduce the response to naturally occurring and inhaled adrenergic agonists.⁴⁵ Chi et al found elevated *MMP2* expression in the lung tissue of rats with COPD.⁴⁶ Smoking can increase *MMP2* activity in human lung fibroblasts and mouse bronchoalveolar lavage fluid.⁴⁷ *NOS3* has a substantial role in the development of COPD is substantial. For example, Ben Nasr et al demonstrated that *NOS3* polymorphism is associated with an increased susceptibility to COPD, which strongly associated with NO levels and the degree of airflow limitation.⁴⁸ In addition, Barreiro et al observed that in the quadriceps femoris of patients with COPD, *NOS3* activity is elevated and protein oxidation levels are higher.⁴⁹ Ito et al further highlighted the pivotal role of *NOS3* in the pathophysiology of COPD, particularly in maintaining and repairing alveolar structures.⁵⁰ Furthermore, Csoma et al reported that dysregulation of the

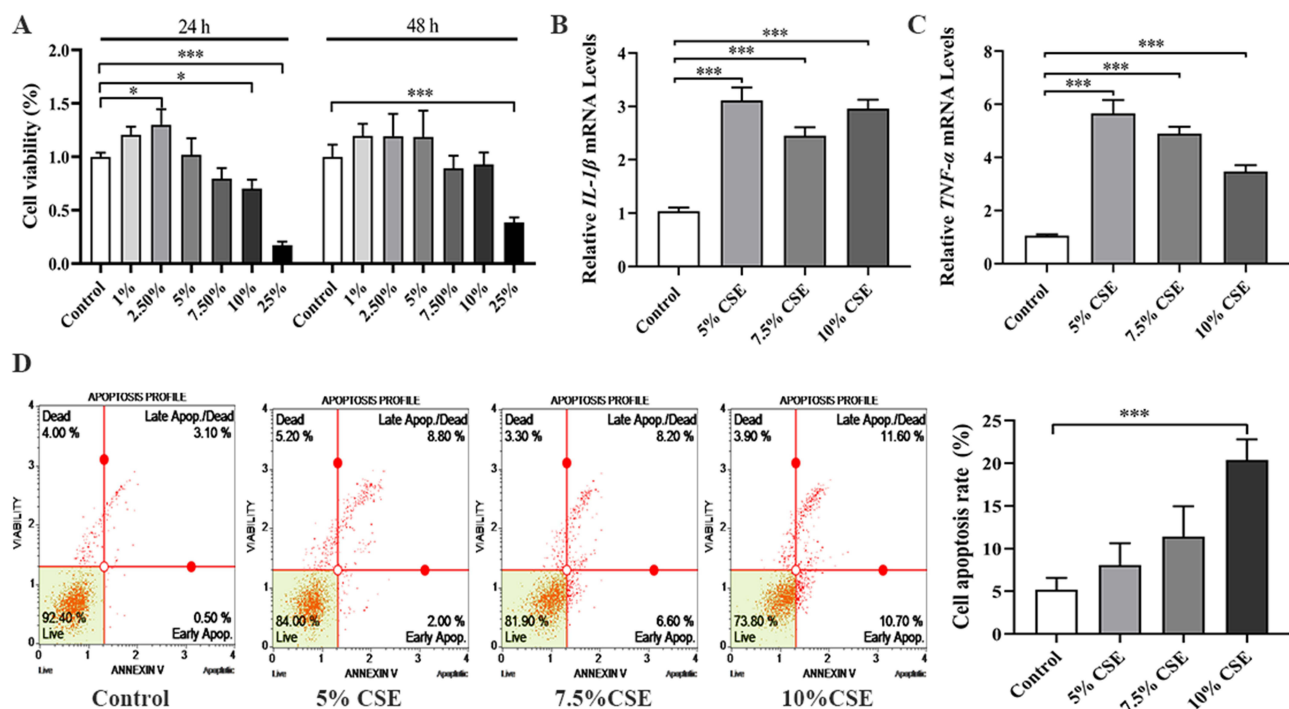


Figure 6 Establishment and evaluation of COPD cell model. (A) Cell proliferation was detected by CCK8 assay at 24 h and 48 h; (B and C) Detection of inflammatory factors; (D) Detection of cell apoptosis. All in vitro experiments were repeated three times independently. * $p < 0.05$ and *** $p < 0.001$.

Abbreviations: COPD, chronic obstructive pulmonary disease; CCK-8, Cell Counting Kit-8.

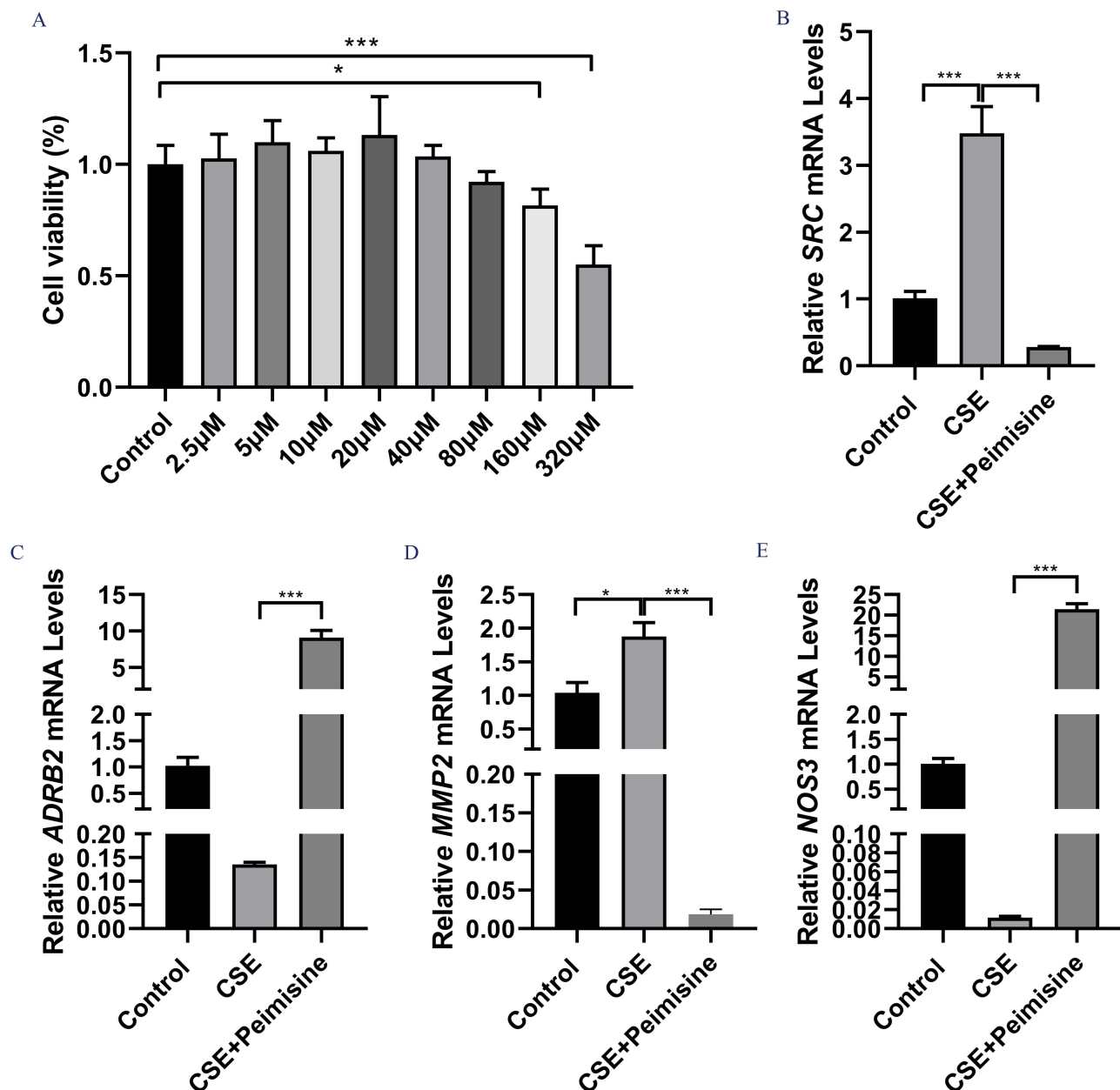


Figure 7 To clarify the effect of Peimisine on target gene expression in COPD. Effect of Peimisine on cell viability at 24h (A). RT-qPCR was used to detect the mRNA expression levels of SRC (B), ADRB2 (C), MMP2 (D), and NOS3 (E). All experiments were repeated three times independently. * $p < 0.05$ and *** $p < 0.001$. **Abbreviation:** COPD, chronic obstructive pulmonary disease.

endothelial nitric oxide pathway is linked to airway inflammation in patients with COPD.⁵¹ These findings suggest that *SRC*, *ADRB2*, *MMP2*, and *NOS3* have an important role in COPD for treatment with CBM.

Molecular docking represents a pivotal computational strategy employed to simulate the interactions between pharmaceutical agents and protein targets, aiding researchers in predicting the binding modes and affinity of pharmaceutical compounds to proteins. In the field of traditional Chinese medicine research, this technology is indispensable for elucidating the interactions of complex herbal constituents with biomolecules and their pharmacological impacts.⁵² For instance, the research conducted by Liu et al identified the principal constituents of Huanglian-Jiedu decoction and their potential targets using molecular docking, which furnished novel insights into the treatment of sepsis.⁸ Furthermore, Jiao et al utilized molecular docking and network pharmacology approaches to comprehensively predict the biological activities of TCM constituents and elucidate their mechanisms of action.⁵³ In our research, we performed molecular

docking to assess the validity of molecule-target interactions, peimisine demonstrates a strong binding affinity with *SRC*, *ADRB2*, *MMP2*, and *NOS3*.

Furthermore, we substantiated our findings with additional validation through in vitro experiments. First, we established a cell model for COPD by preparing CSE. CS is a primary causes of COPD,⁵⁴ as it contains high concentrations of reactive oxygen species that can induce chemokines and lead to neutrophil accumulation in the lungs.⁵⁵ CSE comprises numerous harmful substances, including tar, nitric oxide, nicotine, and oxides. Upon inhaling these harmful substances through smoke, ciliary motility of tracheal mucosal epithelial cells is inhibited, secretion is stimulated, and macrophage phagocytosis is impaired, ultimately resulting in inflammation.⁵⁶ Consequently, we validated the COPD cell model by identifying cellular inflammatory factors. Yuan et al demonstrated that the severity of lung injury can be evaluated based on the release of various inflammatory mediators, including macrophages, neutrophils, and cytokines.⁵⁷ Inflammation plays a crucial role in COPD. In the early stages of COPD development, macrophages and neutrophils infiltrate the airways and alveoli. These inflammatory cells subsequently elevate the levels of a complex cascade of inflammatory mediators, such as *TNF- α* , *IL-1 β* , and *MMPs*,⁵⁸ which aligns with our findings. Finally, the results indicated that peimisine significantly elevated the mRNA expression levels of *ADRB2* and *NOS3* and notably decreased the mRNA expression levels of *SRC* and *MMP2*. Collectively, our findings suggest that CBM may treat COPD by modulating the expression of *SRC*, *ADRB2*, *MMP2*, and *NOS3*.

It is noteworthy that network pharmacology and in vitro experiments may offer preliminary insights into the mechanisms underlying CBM in treating COPD. However, further investigation, encompassing both in vivo studies and clinical trials, is essential to confirm the therapeutic efficacy of CBM for COPD management.

Conclusion

In conclusion, our study is the pioneering investigation into the core ingredient, key targets, and underlying mechanisms of CBM for treating COPD. The findings indicate that peimisine, the core ingredient of CBM, may exert an anti-COPD effect by modulating the expression of *SRC*, *ADRB2*, *MMP2*, and *NOS3*. This study provides a novel outlook on the development of drugs for COPD by elucidating the underlying mechanisms responsible for the therapeutic effects of CBM. Subsequent research will entail animal experiments to further validate the safety and efficacy of CBM in the treatment of COPD.

Data Sharing Statement

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

This study was approved by the Ethics Committee of Hainan Medical College, and the data used in this study obtained the informed consent of the Ethics Committee of Hainan Medical College.

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Disclosure

The authors declare that they have no competing interests in this work.

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