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RESEARCH ARTICLE

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Integrated approach of network pharmacology, molecular docking, and clinical observations in evaluating the efficacy and safety of Bufei Huoxue capsules for pulmonary hypertension associated with chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is a persistent and progressive disorder characterized by airway or alveolar abnormalities, commonly leading to pulmonary hypertension (PH). This clinical observational study investigates the therapeutic mechanisms of Bufei Huoxue capsules (BHC) in treating PH in patients with COPD-linked PH (COPD-PH) using network pharmacology and molecular docking methods, and assesses the therapeutic efficacy and safety of BHCs. The active compounds and their target proteins in BHCs were sourced from the Traditional Chinese Medicine Systems Pharmacology database, with additional target proteins derived from the GeneCards and OMIM databases. An active network was constructed using Cytoscape 3.7.1, and interaction networks were established. Intersecting targets underwent Gene Ontology

Wenjun He, Chunli Liu, and Xuanyi Li contributed equally to this work, and they share first authorship.

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(GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the Metascape database. Network pharmacology and molecular docking studies demonstrated favorable binding affinities of BHC active ingredients, such as quercetin, bavachalcone, and isobavachin, for key targets including PTGS1, ESR1, and PTGS2. Gene Ontology enrichment analysis highlighted the involvement of these targets in processes such as the positive regulation of locomotion, the transmembrane receptor protein tyrosine kinase signaling pathway, and peptidyl-tyrosine phosphorylation. KEGG pathway analysis indicated their roles in pathways related to cancer, AGE-RAGE signaling in diabetic complications, and prostate cancer. BHCs exhibit therapeutic effects on COPD-PH through multi-component, multi-target, and multi-pathway interactions. This clinical observational study confirms the efficacy and safety of BHCs in improving cardiac and pulmonary functions, enhancing exercise tolerance, and elevating the quality of life in patients with COPD-PH.

KEYWORDS

Bufei Huoxue capsule, COPD-PH, molecular docking, network pharmacology, therapeutic efficacy and safety

INTRODUCTION

Pulmonary hypertension (PH) is a clinical and pathophysiological syndrome characterized by structural and functional alterations in the pulmonary vasculature. These changes culminate in elevated pulmonary arterial pressure and pulmonary vascular resistance, which escalate the risks of right-sided heart failure and mortality.[1] In particular, chronic obstructive pulmonary disease (COPD), a progressive ailment featuring airflow restriction and airway or alveolar anomalies, has emerged as a prominent cause of PH that significantly exacerbates disease progression and patient prognosis.[2,3]

The prevalence of COPD-linked PH (COPD-PH) ranks second only to PH associated with left-sided heart disease, underscoring the imperative for dedicated research on COPD-PH.[4–7]

Current therapeutic strategies for this variant of PH primarily concentrate on addressing the underlying lung ailment. The therapeutic arsenal encompasses bronchodilators for COPD, immunosuppressants, and antifibrotic agents for interstitial lung disease and guideline-recommended long-term oxygen therapy. Notably, targeted therapies specifically tailored for COPD-PH lack endorsement by the European Respiratory Society guidelines, underscoring the urgent demand for comprehensive research in this domain.

Traditional Chinese medicines, renowned for their historical use and minimal side effects, present a promising avenue for pioneering COPD-PH treatments.[8–11] Noteworthy among them is the Bufei Huoxue capsules (BHC), a traditional Chinese medicine formulated from *Astragali radix*, *Paeoniae radix rubra*, and *Psoraleae fructus* with proven potential.[12–14] BHC has demonstrated efficacy in enhancing lung ventilation function, reducing pulmonary artery systolic pressure and blood viscosity, improving cardiopulmonary function, and significantly boosting the immune system in patients with PH.[15–17]

Accordingly, the current study assessed the clinical efficacy and safety of BHC in COPD-PH treatment. We endeavor to unravel the intricate mechanisms underlying the actions of BHC and their alignment with the pathological and physiological aspects of COPD-PH, furnishing robust scientific validation for clinical use. This objective was pursued through a comprehensive amalgamation of network pharmacology, molecular docking, and clinical observational approaches, thereby illuminating the multi-target and multi-pathway interactions of this traditional Chinese medicine with COPD-PH.[18]

METHODS

Screening of effective ingredients and target prediction for BHC

BHC encompasses three traditional Chinese medicines: *Astragali radix*, *Paeoniae radix rubra*, and *Psoraleae*

fructus. Initially, the effective ingredients and relevant targets of *Astragalus radix* and *Paeonia fructus* in BHCs were retrieved from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php). The screening criteria necessitated oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. For *Achyranthes bidentata*, ingredients and target information were sourced from the Traditional Chinese Medicine Integrated Database (TCMID) and Swiss Target Prediction databases (http://www.swisstargetprediction.ch/). The corresponding gene names of the target proteins were acquired from the UniProt database (https://www.uniprot.org/).

Target screening for COPD-PH

Employing "pulmonary hypertension in chronic obstructive pulmonary disease" as the keyword, disease target genes were identified by querying the GeneCards (https://www.genecards.org/) and OMIM databases (https://www.omim.org/). The screening criterion for the GeneCards database was a relevance score exceeding 20, and COPD-PH targets were compiled after removing duplicate targets.

Construction of active ingredient-target and protein–protein interaction (PPI) networks

Intersection targets for COPD-PH treatment with BHC were deduced from the online Venn diagram tool Venny 2.1 to map drug and disease targets. Cytoscape 3.7.1 was used to construct the active ingredient–target network by analyzing their topological properties. Subsequently, the PPI network was established by submitting the intersection targets to STRING version 11.0 with a species designation of "human" and a confidence score threshold of 0.4 while eliminating isolated nodes.

Gene Ontology (GO) function analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway enrichment analysis

The Metascape database (https://metascape.org/gp/index.html#/main/step1) facilitated GO enrichment and KEGG pathway analysis of the COPD-PH treatment targets of BHC. Visualization and analysis were performed using an advanced bubble chart in the bioinformatics platform. Targets significant at $p < 0.01$ were selected to unravel the biological functions and signaling pathways.

Molecular docking of ingredients and targets

Molecular docking was performed to identify interactions between the active ingredients of BHC and relevant COPD-PH targets. Compound structures in the SDF format were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and Chem 3D software was used to convert them to the mol2 format. The PDB format structures of PTGS1 (PDB ID:6Y3C), ESR1 (PDB ID:1UOM), and PTGS2 (PDB ID:5F19) were retrieved from the RCSB database (https://www.rcsb.org/), and Pymol software was employed to remove solvent molecules and ligands. AutoDock Tools 1.5.6 software facilitated hydrogen addition, charge calculation, and atom type assignment, and the structures were saved in the pdbqt format. Molecular docking was performed using AutoDock Vina 1.1.2, and the docking conformations were analyzed using Discovery Studio 2020 software.

Clinical study design

In this single-center, randomized, controlled, single-blind study, patients were allocated to the BHC ($n = 12$) and control groups ($n = 6$). Eligible patients received BHC capsules or placebo capsules at an oral dose of 1.4 g (four capsules) thrice daily for 12 months concomitantly with rehabilitation therapy. All patients underwent monthly follow-up for 3 months. Basic information, clinical symptoms, vital signs, medication status, and adverse events were recorded to evaluate the participants' exercise tolerance and degree of symptom improvement. The primary indicators included improvements in 6-min walking distance (6MWD) and the pulmonary function index. The secondary efficacy measurements were as follows: World Health Organization (WHO) pulmonary arterial hypertension classification, Borg dyspnea score, Minnesota Living with Heart Failure (MLHF) quality of life score, BNP levels, pulmonary arterial systolic pressure (PASP), and coagulation parameters. Additionally, any side effects or adverse events during the 12-month follow-up period were recorded. The cohort for this study was sourced from the First Affiliated Hospital of Guangzhou Medical University. Patients with COPD-PH patients admitted between September 2018 and October 2019 were eligible, and their participation was contingent upon the provision of informed consent and the approval of the ethical review committee and their

provision of informed consent. The ethical approval reference number is "2018 NO. K-29," and the trial registration number is ChiCTR1800016955.

The inclusion criteria were as follows: 48–80 years, diagnosis of pulmonary hypertension (mean pulmonary arterial pressure ≥20 mmHg and pulmonary capillary wedge pressure ≤15 mmHg at rest via right heart catheterization), stable group 3 PH in accordance with COPD per the 2015 ESC/ERS classification, WHO functional class II/III, baseline 6MWD of 150–550 m, forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio <70% following the inhalation of 400 μg of salbutamol, no targeted therapy within the past 3 months, ability to communicate and complete trial-required tests, and provision of written informed consent.

Exclusion criteria were as follows: (1) Low systemic blood pressure (< 90/50 mmHg) or uncontrolled hypertension (>170/110 mmHg). (2) Active infectious or connective tissue diseases. (3) Severe infections, particularly pulmonary. (4) Shock or other hemodynamic instability. (5) Liver cirrhosis or resultant portal hypertension. (6) Severe bleeding or bleeding tendencies. (7) Hemolytic anemia or glucose-6-phosphate dehydrogenase deficiency. (8) Requirement or use of drugs affecting the experiment. (9) Acute/chronic organic diseases impeding study adherence (excluding respiratory distress). (10) AST and ALT levels >3× upper limit or Ccr ≤50 mL/min. (11) Known drug component allergy. (12) Pregnancy or lactation.

Statistical methods

R version 3.6.1 statistical software (R Foundation for Statistical Computing) was employed for statistical analyses. Quantitative data were presented as the mean ± standard deviation, and non-parametric statistics such as *t*-tests, analysis of variance, or Wilcoxon's rank-sum test were selected according to the variable distribution. Qualitative data were expressed as *n* (%) and analyzed using the chi-squared test or Fisher's exact probability test. All statistical inferences were two-sided tests with a significance level of 0.05. Parameters' confidence intervals were calculated using a two-sided 95% confidence interval.

RESULTS

Identification of active ingredients, BHC targets, and COPD-PH targets

The active ingredients of BHC and their corresponding targets were ascertained by querying the TCMSP and TCMID databases. This search revealed 20, 29, and 30 active ingredients in *Astragali radix*, *Paeoniae radix rubra*, and *Psoraleae fructus*, respectively. Upon associating gene names with each ingredient's targets, 2011 target genes were identified. Specifically, *Astragali radix* targeted 462 genes, *Paeoniae radix rubra* targeted 158 genes, and *Psoraleae fructus* targeted 1391 genes.

Subsequently, the keyword "pulmonary hypertension in chronic obstructive pulmonary disease" was employed to search the GeneCards database, yielding 1188 targets. An additional search of the OMIM database resulted in the identification of 176 targets. After merging and removing duplicates, 1324 disease-related targets were obtained. Intersection analysis using Venny 2.1 uncovered 203 shared targets between the two datasets. These shared targets could potentially serve as therapeutic targets for COPD-PH treatment with BHC. The identified active ingredients, targets of BHC, and COPD-PH targets are presented in Figure 1.

Active ingredient–target network construction

The active ingredient–target network, consisting of 51 compound nodes, 203 target nodes, and 758 edges, is depicted in Figure 2. This network illustrates BHC's multi-component and multi-target characteristics in COPD-PH treatment, indicating that a single active ingredient can interact with various targets and vice versa. Topological analysis identified quercetin, bavachalcone, and isobavachin as key components because of their high "degree" values.

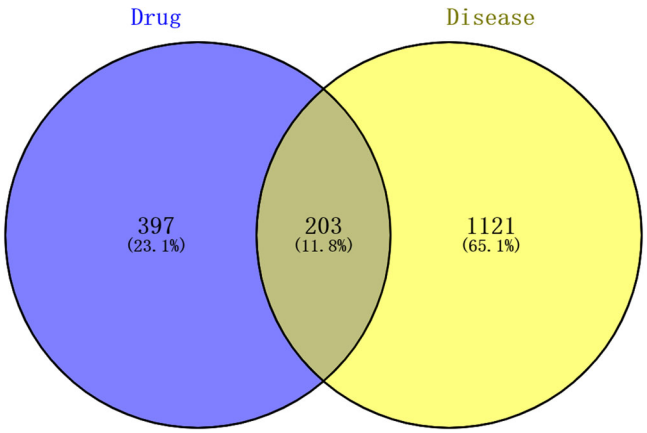

FIGURE 1 Targets of Bufei Huoxue capsules (BHC) in the treatment of chronic obstructive pulmonary disease-linked pulmonary hypertension.

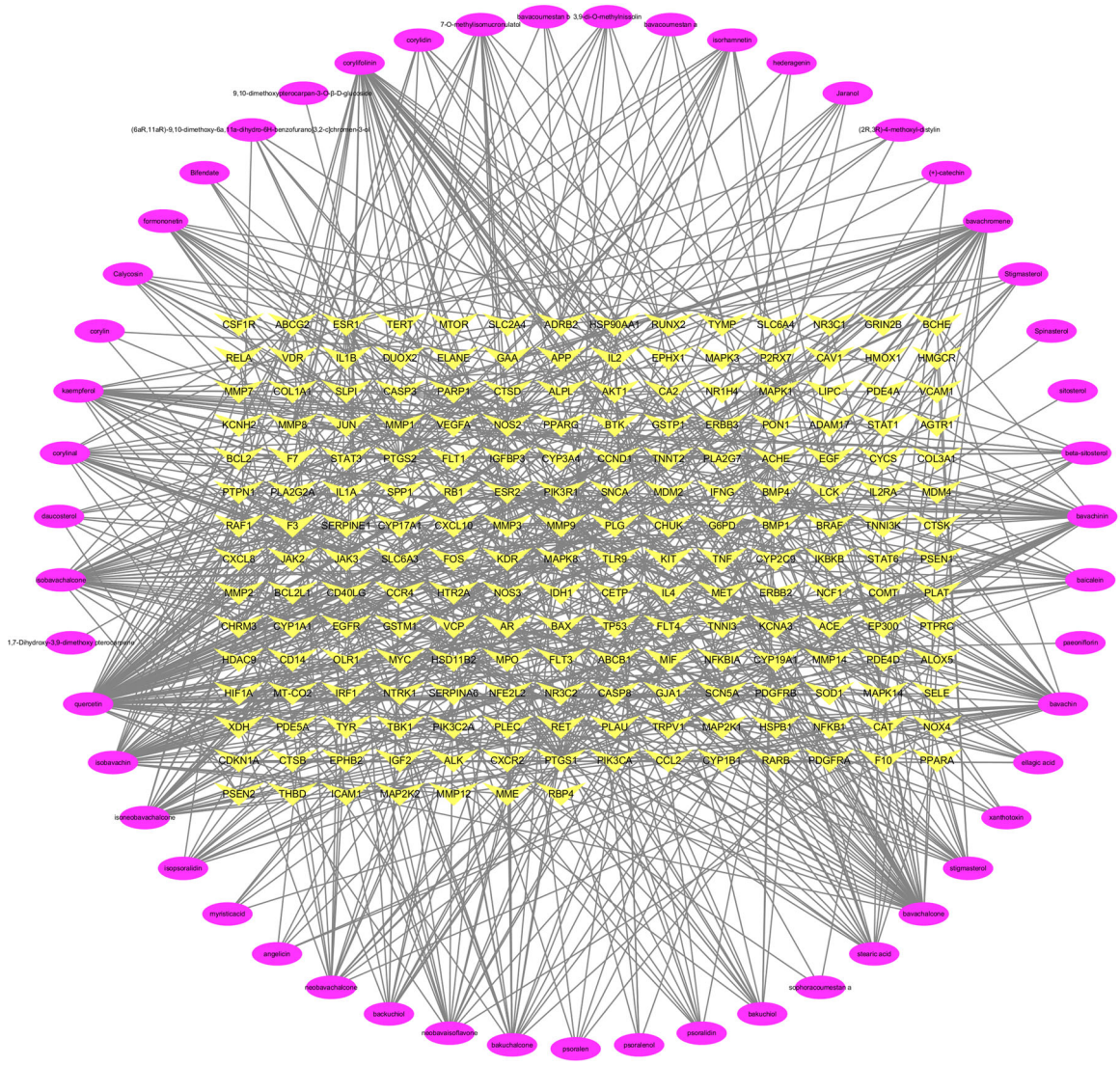

FIGURE 2 Active ingredient target network.

PPI network construction

As illustrated in Figure 3, the PPI network of the target proteins was established using STRING version 11.0 with "*Homo sapiens*" as the selected species and a minimum interaction confidence threshold of "Medium confidence" (0.4). The key target proteins, including AKT1, VEGFA, and TNF, identified in this network could play pivotal roles in the therapeutic effects of BHC on COPD-PH.

GO functional and KEGG pathway enrichment analyses

The 203 proteins in the BHC component–target network were subjected to GO functional enrichment and KEGG pathway enrichment analyses. Enriched biological processes and metabolic pathways were filtered using a criterion of $p < 0.01$, favoring pathways with higher gene enrichment. GO enrichment analysis yielded 7974 GO terms, including 6466 biological process-related terms such as positive regulation of locomotion, transmembrane receptor protein tyrosine kinase signaling pathway, and peptidyl-tyrosine phosphorylation. Meanwhile, 971 molecular function-related terms were identified, including protein kinase activity and phosphatase binding. Finally, 537 terms were associated with cellular components, including membrane rafts and receptor complexes. The top 10 terms ranked by count are presented in Figure 4a.

KEGG pathway enrichment analysis identified 487 significant pathways, including pathways in cancer,

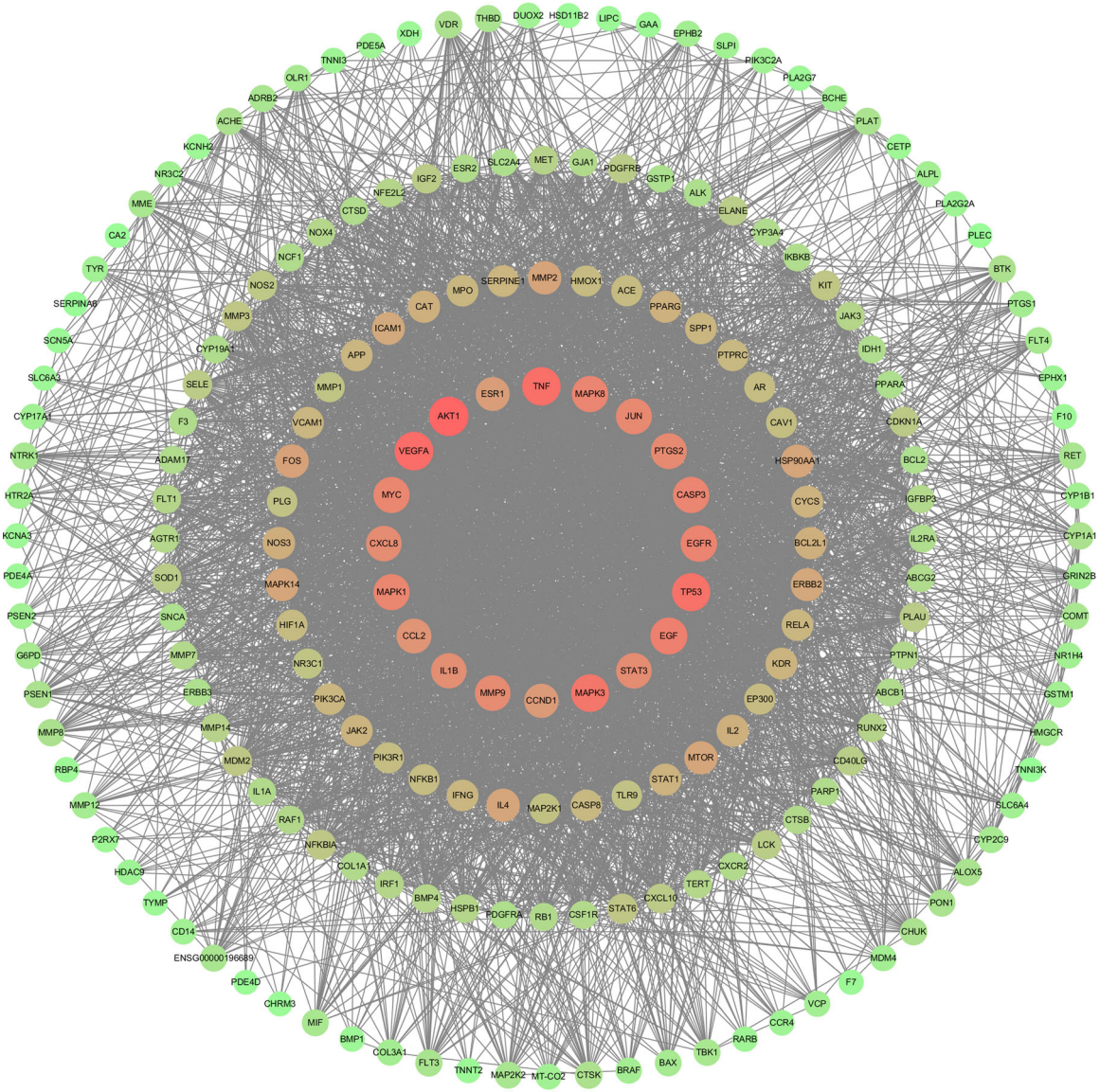

FIGURE 3 Protein–protein interaction network of Bufei Huoxue capsules in the treatment of COPD-PH.

AGE-RAGE signaling in diabetic complications, and prostate cancer. The 20 most significant pathways are depicted in Figure 4b.

Molecular docking validation of key BHC components

As displayed in Table 1, molecular docking was performed on the top three high-degree compounds in the active ingredient–target network (quercetin, bavachalcone, and isobavachin) and three key targets (PTGS1, ESR1, and PTGS2). Each compound was individually docked to its corresponding target. Conventionally, lower binding energy implies stable ligand-receptor binding. Binding energies of −5.0 kcal/mol or lower suggest feasible binding, and those of −7.0 kcal/mol or lower indicate strong binding. The docking results demonstrated favorable binding between PTGS1, ESR1, and PTGS2 and quercetin, bavachalcone, and isobavachin, respectively.

Analysis of molecular docking binding modes

The binding modes of selected compounds with their respective target proteins were analyzed, as illustrated in Figure 5a–d.

Figure 5a displays that isobavachin, when docked with PTGS1, formed hydrophobic interactions with

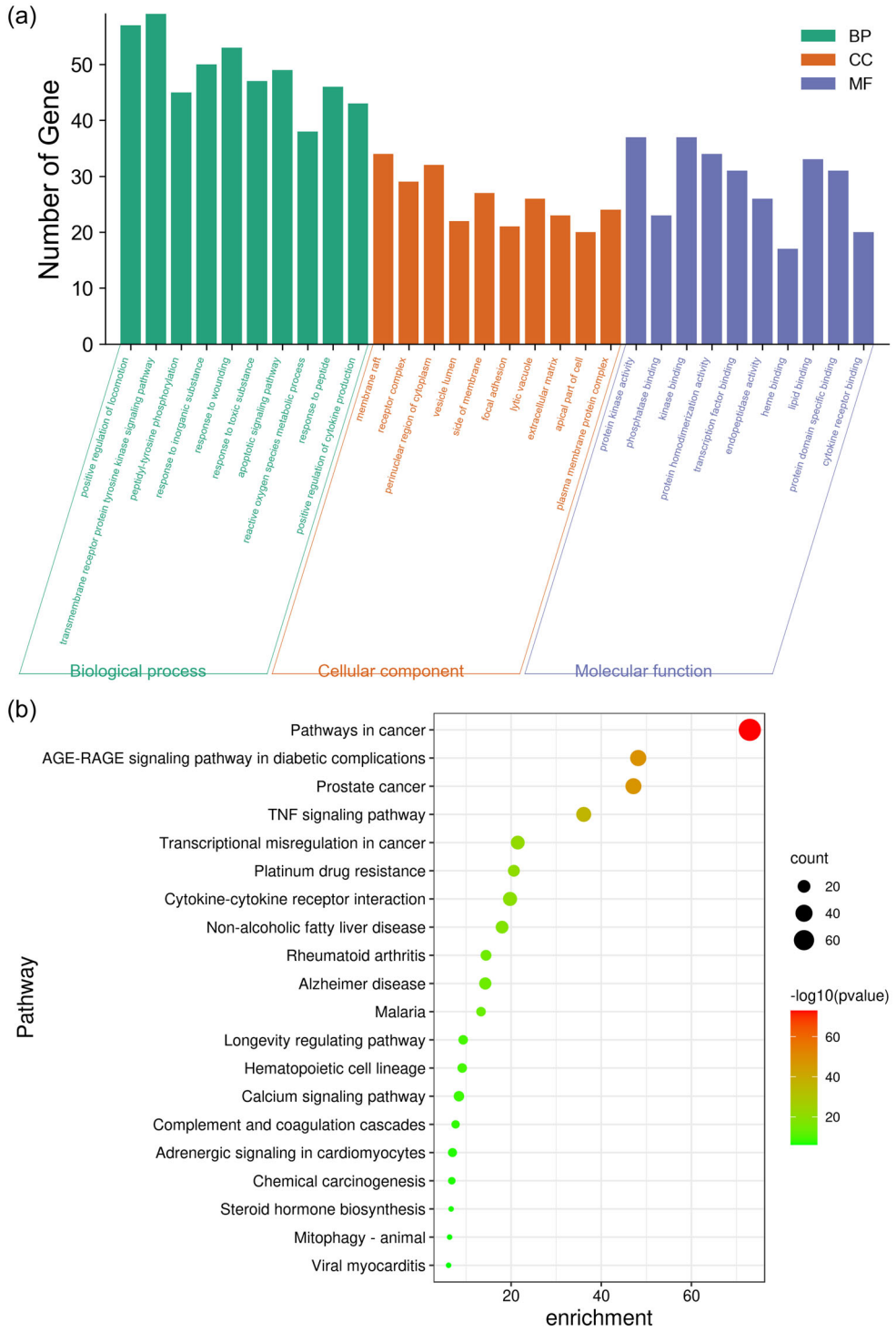

FIGURE 4 Analysis of the key and core targets of BHC in COPD-PH: (a) Gene Ontology analysis of the key targets of BHC against COPD-PH and (b) Kyoto Encyclopedia of Genes and Genomes analysis of core targets of BHC against COPD-PH.

Leu123 and Val119 in the a-ring and with Pro86, Leu115, and Val119 in the b-ring.

The interactions of bavachalcone with ESR1 are presented in Figure 5b. The a-ring formed a π-sigma interaction with Ile424, a π–π stacking interaction with His524, and a hydrogen bond with Leu525. Additionally, the a-ring exhibited hydrophobic interactions with Leu384, Leu387, Leu525, Trp383, and Ala350. The b-ring displayed hydrophobic interactions with Ala350, Leu391, and Leu387 and a π–π stacking interaction with

TABLE 1 Docking results of core components and core targets.

Protein	PDB number	Ligand molecule	Actual binding free energy (kcal/mol)
PTGS1	6Y3C	Bavachalcone	−7
		Isobavachin	−7.2
		Quercetin	−7
ESR1	1UOM	Bavachalcone	−9
		Isobavachin	−9
		Quercetin	−8.8
PTGS2	5F19	Bavachalcone	−9.1
		Isobavachin	−8.6
		Quercetin	−9.2

Phe404, and the phenolic hydroxyl group formed a hydrogen bond with Leu387.

Figure 5c presents the docking of isobavachin with ESR1. The a-ring formed hydrophobic interactions with Leu387, Leu391, and Ala350 and a π–π stacking interaction with Phe404, and the phenolic hydroxyl group formed a hydrogen bond with Glu353. The b-ring displayed a π–π stacking interaction with His524 and hydrophobic interactions with Ile424, Ala350, Trp383, Leu384, Leu387, and Leu525. The phenolic hydroxyl group formed a hydrogen bond with Gly521.

The docking of quercetin with PTGS2 is illustrated in Figure 5d. The figure illustrates that the a-ring formed a π–π stacking interaction with His388 and the phenolic hydroxyl group formed a hydrogen bond with Ala199. The b-ring exhibited a π–cation interaction with His207 and a π–π stacking interaction with His386. The c-ring formed π–cation interactions with His207 and His386, and the phenolic hydroxyl group formed a hydrogen bond with Asn382.

Participant demographics and baseline characteristics

The mean ages were 67.83 ± 8.53 and 69.00 ± 6.20 years in the BHC and control groups, respectively. No significant differences were observed between the two groups regarding age, heart rate, blood pressure, or percutaneous oxygen saturation, indicating comparable baseline characteristics. Detailed data are presented in Table 2.

Primary outcome measures

Changes in 6MWD

As demonstrated in Table 3, after 3 months of treatment, no statistically significant differences were observed in the change of 6MWD between the BHC group (-9.30 ± 65.77, $n = 12$) and the control groups (0.83 ± 53.42, $n = 6$). However, at 6 months, the BHC group exhibited a notable increase in 6MWD, in stark contrast to the decline observed in the control group. By the 9th and 12th months, 6MWD was significantly higher in the BHC group than in the control group, and notably greater than baseline ($p < 0.01$). These findings suggest that BHC have the potential to enhance 6MWD, improve cardiac functional status, and enhance exercise tolerance in patients with COPD-PH.

Pulmonary function changes

At baseline, no significant differences in percent predicted FEV1 were observed between the two groups. Following 6 months of treatment, percent predicted FEV1 in the BHC group exhibited a significant increase compared with baseline ($p < 0.05$), whereas the control group experienced a minor decline. At 12 months, the percent predicted FEV1 was significantly lower in the control group than that in the BHC group ($p < 0.05$, Table 3). No significant changes were noted in the FEV1/FVC ratio in either group (Table 3), implying that BHC can enhance pulmonary function in patients with COPD-PH without affecting airflow limitation.

Patient wellness and biochemical assessments

WHO functional classification of pulmonary hypertension comparison

Both groups exhibited a WHO functional classification of 2 or 3 at baseline with no significant difference between them. After 3 months of BHC treatment, the precapillary PH classification reduced, and this reduction became significant after 12 months ($p < 0.05$). Conversely, the control group displayed no significant changes after treatment. The functional classification of the BHC group was lower than that of the control group after treatment, with the difference becoming significant at 9 months ($p < 0.05$, Table 4). This suggests the potential of BHC to delay disease progression and enhance the survival of COPD-PH patients.

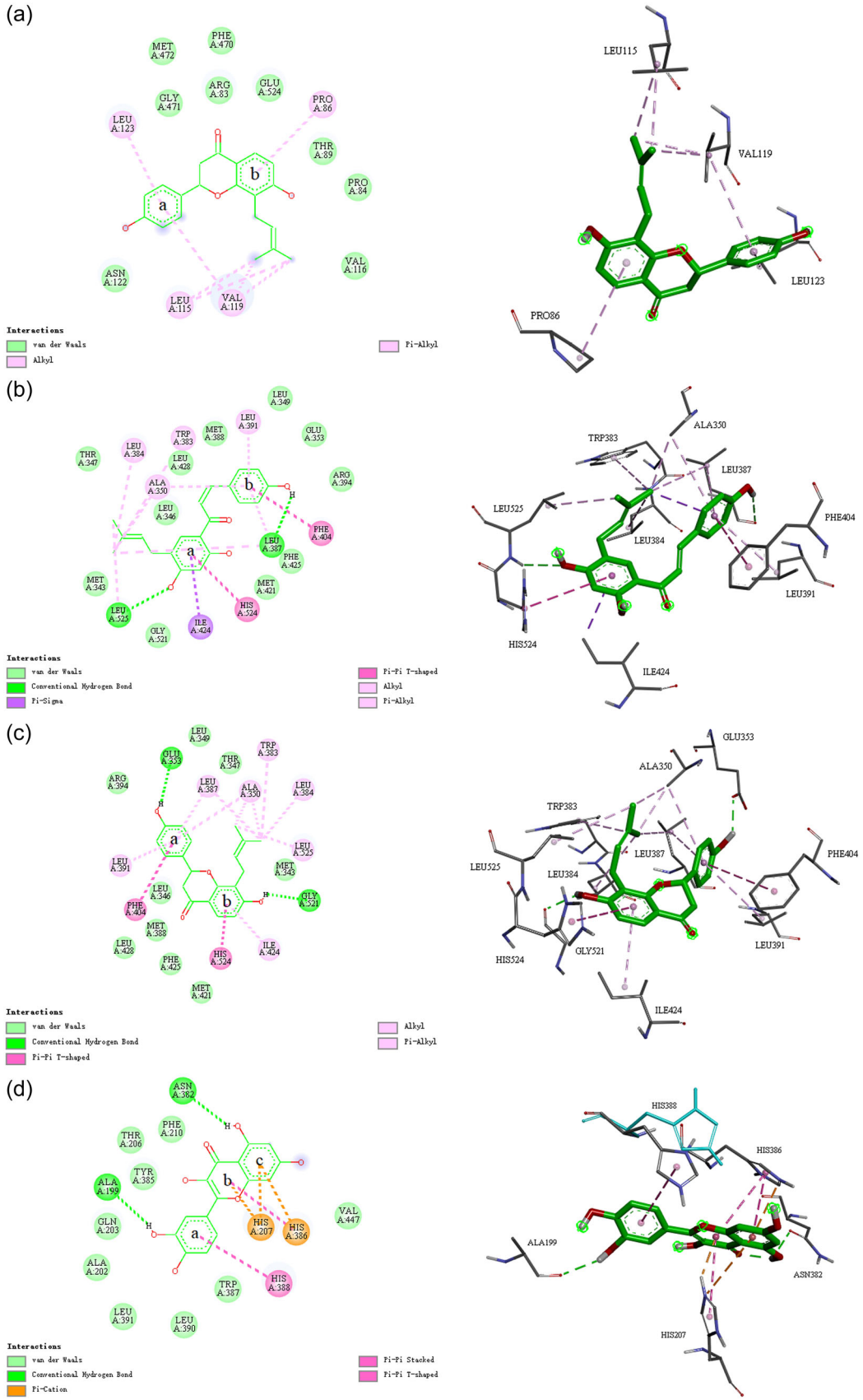

FIGURE 5 Interaction diagrams between select compounds and their molecular targets. (a) Interaction diagram between isobavachin and PTGS1. (b) Interaction diagram between bavachalcone and ESR1. (c) Interaction diagram between isobavachin and ESR1. (d) Interaction diagram between quercetin and PTGS2.

TABLE 2 Baseline information for both groups.

Group	Sample size	Age (*years* $\bar{x} \pm s$)	Weight (kg)	Vital signs				
				Respiration rate (breaths/min)	Heart rate (beats/min)	Diastolic blood pressure (mmHg)	Systolic blood pressure (mmHg)	SpO2 (%)
BHC group	12	67.83 ± 8.53	67.33 ± 29.47	19.83 ± 3.43	81.09 ± 17.21	77.25 ± 11.00	129.83 ± 15.39	93.40 ± 4.79
Control group	6	69.00 ± 6.20	53.50 ± 5.89	18.17 ± 2.04	81.67 ± 19.98	85.50 ± 12.71	136.83 ± 26.17	94.17 ± 1.60
p Value	/	0.7461	0.1423	0.2172	0.9538	0.2085	0.5649	0.6505

Abbreviation: BHC, Bufei Huoxue capsule.

Borg dyspnea score comparison

The baseline Borg dyspnea score was comparable between the BHC (1.46 ± 1.47) and control groups (0.92 ± 1.56). After 3 months of treatment, the BHC group displayed a decline in the Borg dyspnea score, which was lower than that in the control group. After 12 months, a significant difference was observed in the Borg dyspnea score between the two groups ($p < 0.05$, Table 4), indicating the potential of BHCs to alleviate respiratory difficulties in COPD-PH patients.

MLHF quality of life score comparison

The baseline MLHF quality of life score did not significantly differ between the BHC and control groups. Throughout the treatment period, the MLHF score gradually declined in the BHC group, reaching statistical significance after 6 months ($p < 0.05$). Meanwhile, the score also decreased in the control group (Table 4). These results indicate that BHC can enhance the quality of life among COPD-PH patients.

BNP level comparison

BNP levels tended to decrease over time in the BHC group, albeit without significance, whereas a minor decrease was observed at 6 months in the control group (Table 4). These results indicate the potential of BHCs to moderately reduce BNP levels in patients with COPD-PH.

PASP comparison

PASP exhibited no significant differences between the BHC and control groups either before or after treatment (Table 4), indicating a lack of effects of the treatment on PASP in patients with COPD-PH.

Coagulation parameters comparison

Pre- and post-treatment coagulation parameters did not differ between the BHC and control groups (Table 4). However, a slight prolongation of activated partial thromboplastin time (APTT) was observed in the BHC group compared with that in the control group. Conversely, prothrombin time (PT) decreased post-treatment in the control group, indicating that BHCs mildly improved the thrombotic state of patients with

TABLE 3 Changes in primary indicators after 3, 6, 9, and 12 months of treatment.

Indicator	Group	Pretreatment	Treatment after 3 months	Treatment after 6 months	Treatment after 9 months	Treatment after 12 months
6-min walking distance with baseline difference (m)	BHC group	315.58 ± 53.31 ($N = 12$)	−9.30 ± 65.77 ($N = 10$)	2.50 ± 74.52 ($N = 10$)	78.89 ± 60.85,*# ($N = 9$)	82.3 ± 66.16,*# ($N = 10$)
	Control group	394.50 ± 66.54 ($N = 6$)	0.83 ± 53.42 ($N = 6$)	−24.00 ± 11.92 ($N = 4$)	−43.4 ± 13.22 ($N = 5$)	−71.33 ± 41.86 ($N = 6$)
Percentage of the predicted value of FEV1(%)	BHC group	30.68 ± 10.81 ($N = 11$)		38.13 ± 13.69 ($N = 11$)		37.85 ± 13.48*# ($N = 8$)
	Control group	30.17 ± 4.01 ($N = 6$)		27.77 ± 6.34 ($N = 6$)		27.17 ± 8.94 ($N = 8$)
FEV1/FVC ratio (%)	BHC group	14.34 ± 20.87 ($N = 11$)		6.67 ± 14.11 ($N = 11$)		26.07 ± 28.86 ($N = 8$)
	Control group	32.03 ± 18.15 ($N = 5$)		25.74 ± 20.06 ($N = 6$)		19.11 ± 22.13 ($N = 4$)

Abbreviation: BHC, Bufei Huoxue capsule.

*$p < 0.01$ versus control group.

#$p < 0.01$ versus prior treatment.

COPD-PH. Both groups displayed increased fibrinogen (FIB) levels post-treatment. However, after 12 months, the FIB level was lower in the BHC group than in the control group (Table 4), suggesting that BHC might somewhat delay blood coagulation progression in patients with COPD-PH.

Assessment of treatment safety

No adverse or serious adverse events were observed in either group as assessed by the incidence rates of adverse and serious adverse events.

DISCUSSION

This study comprehensively explored the potential therapeutic mechanisms, clinical effectiveness, and safety of BHC in managing COPD-PH. Through the integration of network pharmacology analysis, molecular docking, and clinical observation, our findings underscore the promising utility of BHC as a treatment option for COPD-PH.

COPD-PH is categorized as group 3 PH. Left unchecked, COPD-PH can lead to serious complications such as cor pulmonale, right heart failure, and even mortality.[19,20] Despite extensive research, the intricate pathogenesis of COPD-PH remains enigmatic, involving a multitude of mechanisms. Unfortunately, current clinical interventions, unfortunately, often fail to provide definitive efficacy. TCM classifies group 3 PH as "lung swelling" based on its clinical presentation, and it displays efficacy in ameliorating symptoms, slowing disease progression, and enhancing prognosis in stable patients.[21–23]

BHC, representing a novel Chinese medicine formulation, amalgamates three distinct herbal medicines: *Astragali radix*, *Paeoniae radix rubra*, and *Psoraleae fructus*.[24] The composite herbal concoction targets the lungs, spleen, and kidneys, addressing both the root cause and manifestations.[24] Contemporary pharmacological investigations revealed the active ingredients in these herbs that potentially mitigate COPD-PH symptoms. The core disease mechanism primarily involves deficiencies in lung, spleen, and kidney qi, with BHC's formulation aiming to replenish lung function, stimulate blood circulation, and fortify kidney health. This multifaceted approach imparts several advantageous effects such as antioxidant, antimicrobial, and antithrombotic effects, culminating in improved pulmonary circulation and notable relief of clinical symptoms in patients with COPD-PH, ultimately enhancing exercise tolerance and cardiopulmonary function.[12–14,24]

Our investigation employed the 6MWD test and WHO functional classification to gauge exercise tolerance among patients with PH. Notably, the BHC group exhibited significant augmentation in 6MWD and improvement in the WHO functional classification following treatment, suggesting the potential of BHCs to enhance exercise tolerance and slow disease progression. Moreover, BHC improved lung function and

TABLE 4 Changes in secondary indicators after 3, 6, 9, and 12 months of treatment.

Indicators	Group	Pretreatment	Treatment after 3 months	Treatment after 6 months	Treatment after 9 months	Treatment after 12 months
WHO PH grades with baseline difference	BHC group	2.56 + 0.53 ($N = 9$)	−0.56 ± 0.53# ($N = 9$)	−0.38 ± 0.52 ($N = 8$)	−0.56 ± 0.73* ($N = 9$)	−0.63 ± 0.74# ($N = 8$)
	Control group	2.67 + 0.52 ($N = 6$)	−0.17 ± 0.75 ($N = 6$)	0 ± 0.63 ($N = 6$)	0.4 ± 0.55 ($N = 5$)	0.17 ± 0.75 ($N = 6$)
Borg dyspnea score	BHC group	1.46 ± 1.47 ($N = 9$)	1.41 ± 0.94 ($N = 11$)	1.00 ± 1.33 ($N = 10$)	1.00 ± 1.27 ($N = 10$)	1.40 ± 1.07* ($N = 10$)
	Control group	0.92 ± 1.56 ($N = 6$)	1.67 ± 1.40 ($N = 6$)	1.90 ± 1.56 ($N = 5$)	2.40 ± 2.30 ($N = 5$)	2.58 ± 1.20 ($N = 10$)
Minnesota heart failure (MLHF) quality of life scores	BHC group	33.92 ± 26.04 ($N = 12$)	22.82 ± 16.31 ($N = 11$)	16.10 ± 6.94# ($N = 10$)	15.64 ± 9.07 ($N = 11$)	10.40 ± 10.00# ($N = 10$)
	Control group	28.17 ± 16.51 ($N = 6$)	17.17 ± 7.44 ($N = 6$)	26.33 ± 17.66 ($N = 6$)	21.6 ± 6.50 ($N = 5$)	23.67 ± 12.61 ($N = 6$)
Blood-BNP (ng/L)	BHC group	759.55 ± 1467.42 ($N = 12$)		547.23 ± 1277.13 ($N = 11$)		490.85 ± 995.65 ($N = 9$)
	Control group	90.80 ± 64.28 ($N = 6$)		88.53 ± 84.75 ($N = 6$)		116.33 ± 107.84 ($N = 6$)
Pulmonary artery systolic blood pressure (mmHg)	BHC group	47.83 ± 22.07 ($N = 12$)		48.44 ± 14.10 ($N = 9$)		51.25 ± 15.85 ($N = 8$)
	Control group	43.33 ± 7.20 ($N = 6$)		40.50 ± 8.36 ($N = 6$)		47.75 ± 16.36 ($N = 4$)
Prothrombin time (PT)(s)	BHC group	15.18 ± 1.82 ($N = 12$)		15.70 ± 1.77 ($N = 11$)		16.27 ± 1.37 ($N = 9$)
	Control group	16.53 ± 0.54 ($N = 6$)		16.43 ± 0.57 ($N = 6$)		16.62 ± 1.02 ($N = 6$)
Activated partial thromboplastin time (APTT) (s)	BHC group	37.64 ± 5.44 ($N = 12$)		36.02 ± 6.60 ($N = 11$)		38.41 ± 6.73 ($N = 9$)
	Control group	38.00 ± 3.12 ($N = 6$)		37.60 ± 3.16 ($N = 6$)		36.38 ± 4.46 ($N = 6$)
Plasma fibrinogen (FIB) (g/L)	BHC group	3.35 ± 0.76 ($N = 12$)		3.63 ± 1.02 ($N = 11$)		3.59 ± 0.67 ($N = 9$)

TABLE 4 (Continued)

Indicators	Group	Pretreatment	Treatment after 3 months	Treatment after 6 months	Treatment after 9 months	Treatment after 12 months
	Control group	3.46 ± 0.94 (N = 6)		3.82 ± 0.81 (N = 6)		4.06 ± 1.13 (N = 6)
Plasma prothrombin time (PT) (s)	BHC group	12.38 ± 1.27 (N = 12)		13.17 ± 2.00 (N = 11)		12.59 ± 1.49 (N = 9)
	Control group	29.77 ± 40.78 (N = 6)		12.80 ± 0.51 (N = 6)		12.83 ± 0.99 (N = 6)

Abbreviations: BHC, Bufei Huoxue capsules; PH, pulmonary hypertension.

*$p < 0.01$ versus control group.

#$p < 0.01$ versus prior treatment.

immune responses in patients. In our study, the BHC group displayed a substantial increase in percent predicted FEV1, indicating the potential of BHC to bolster lung function in individuals with COPD-PH. Furthermore, we observed that BNP levels, which were initially high in patients with COPD-PH in the BHC group, decreased after BHC treatment. Our study also highlighted a mild exacerbation of the hypercoagulable state in the control group posttreatment. Additionally, BHC moderately extended APTT and improved the thrombotic status of patients with COPD-PH.

Moreover, our network pharmacology analysis provided insights into the potential mechanisms of action of BHC. The effects of BHC were potentially linked to the positive regulation of locomotion, transmembrane receptor protein tyrosine kinase signaling pathways, peptidyl-tyrosine phosphorylation, and other processes. These findings suggest that BHC regulate specific pathological processes in COPD-PH, including inflammation, cell proliferation, and remodeling of the pulmonary vasculature.

Molecular docking studies confirmed that BHC's active ingredients, namely quercetin, isobavachin, and bavachalcone, bind effectively to the key proteins PTGS1, ESR1, and PTGS2. PTGS1 and PTGS2 are pivotal in prostaglandin synthesis, implicating them in the inflammatory and vascular remodeling processes observed in PH. By contrast, ESR1 plays a protective role in PH. Consequently, these interactions might underlie BHC's pharmacological actions in treating COPD-PH, contributing to their anti-inflammatory, vasodilatory, and remodeling-inhibitory effects. This identification of several BHC active ingredients, including quercetin, isobavachin, and bavachalcone, highlights their potential influence on biological targets PTGS1, ESR1, and PTGS2.

Moreover, BHC's active ingredients display pharmacological properties potentially relevant to treating PH. Research indicates that quercetin can reverse pulmonary arterial hypertension through TrkA pathway modulation, emphasizing its therapeutic promise.[25] Evidence supports quercetin's ability to lower blood pressure in a dose-dependent manner, which is beneficial for managing pulmonary arterial hypertension.[26] Reviews suggest that quercetin's antihypertensive action reduces oxidative stress and enhances endothelial function, offering cardiovascular benefits.[27] The attenuation of HIF-1α activity by bavachalcone, which is critical to PH-associated hypoxia, demonstrates its anti-inflammatory potential.[28] Its ability to delay endothelial cell senescence further suggests vascular protective properties.[29] Isobavachin's activation of Nrf2, which modulates oxidative stress, is implicated in PH pathophysiology.[28] The antioxidant and anti-inflammatory activities of these

compounds are essential in the treatment of cardiovascular diseases that share etiological factors with PH.[30]

Analytical techniques have substantiated the presence and stability of these compounds, reinforcing their therapeutic validity. The absence of direct PH model studies notwithstanding, current evidence reasonably indicates the potential effectiveness of bavachalcone and isobavachin in PH treatment, justifying further investigation.

Although our study provides initial insights into the clinical efficacy and safety of BHC in COPD-PH treatment, network pharmacology and molecular docking results offer foundational knowledge of the involved mechanisms. Identifying the specific pathways and biological processes affected by these active compounds, as well as their roles in the observed clinical outcomes, requires further study. Additionally, our study did not reveal a significant decrease in PASP in patients with COPD-PH treated with BHC. Moreover, the limited sample size of this study necessitates the validation of these findings in larger multi-center studies.

CONCLUSION

In summary, our investigation furnished theoretical and clinical substantiation for the use of BHCs in COPD-PH treatment. Furthermore, it unearthed novel avenues for in-depth research and exploration in the future.

AUTHOR CONTRIBUTIONS

Wenjun He, Bihua Zhong, Xuanyi Li, Qian Jiang, Ning Lai, Yuanhui Xiong, Weici Feng, Yilin Chen, Dansha Zhou, and Wenju Lu conducted experiments and data analysis; Jian Wang and Yuqin Chen provided guidance, and supervision throughout the study; consultations were made with Defu Li, Jurjan Aman, and Harm Jan Bogaard, among others, and discussions were held. Wenjun He and Yuqin Chen wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

We confirm that all data presented in this manuscript are accurate and reliable, and they have been appropriately analyzed and interpreted. The raw data and any additional materials required to reproduce the findings presented in the manuscript will be made available upon request.

ETHICS STATEMENT

This Ethics Committee is independent, and its composition and procedures comply with the ICH-GCP principles and relevant national laws and regulations.

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