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ORIGINAL RESEARCH

Mechanism of Action and Efficacy of Wu-Hua-Yan-Xiao in the Treatment of Pediatric Acute Pharyngitis Based on Network Pharmacology and Experimental Validation

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Ethnopharmacological Relevance: Wu-Hua-Yan-Xiao (WHYX) is an innovative volatile oil formulation derived from the traditional Yinqiao-Mabo decoction, developed for the treatment of pediatric acute pharyngitis.

Materials and Methods: Network pharmacology was utilized to identify active components and potential therapeutic targets of WHYX in acute pharyngitis. Compounds in WHYX were characterized using UHPLC-QE-MS. A pediatric acute pharyngitis rat model was established by administering 25% ammonia to the pharyngeal mucosa of young rats. WHYX was delivered via aerosol inhalation at gradient concentrations. Histopathological changes in pharyngeal tissues were evaluated by H&E staining. Serum levels of IL-6, IL-1β, TNF-α, and PGE2 were quantified by ELISA. Expression levels of TNF-α, TP53, IL17A, IL6, and Bcl-2 were assessed by qRT-PCR and Western blotting. Apoptosis was analyzed through immunofluorescence staining for Caspase-3 and TUNEL.

Results: Network pharmacology identified 130 active compounds and 600 gene targets, with 194 overlapping drug-disease targets. TP53 signaling emerged as a central regulatory pathway. Compared with the model group, the high-dose WHYX volatile oil group showed marked improvements in pharyngeal pathology, significant reductions in inflammatory cytokines, downregulation of TNF-α, TP53, IL17A, IL6, and Bcl-2 expression, and suppressed apoptosis ($P < 0.05$). Therapeutic effects were comparable to or exceeded those observed in the positive control group. ($P < 0.05$).

Conclusion: The WHYX formula alleviates inflammation, reduces apoptosis, and protects pharyngeal tissue in young rats with acute pharyngitis. Aerosol inhalation of WHYX presents a direct, effective, and non-invasive strategy for pediatric acute pharyngitis management.

Keywords: pediatric acute pharyngitis, traditional Chinese medicine volatile oil, atomization inhalation, network pharmacology, cell apoptosis, anti-inflammatory effects

Introduction

Acute pharyngitis is among the most common upper respiratory tract infections in children.[1] In the United States, approximately 7.3 million children experience pharyngeal pain annually, with acute pharyngitis accounting for 34% of these cases.[2] The annual economic burden for diagnosis, treatment, and management of pediatric pharyngitis is estimated at $224 to $539 million.[3] Clinically, pediatric acute pharyngitis presents as acute, non-specific inflammation of the pharyngeal mucosa and submucosal tissues. Its etiology includes infectious agents, such as viruses, bacteria, mycoplasma, and chlamydia, and non-infectious stimuli, including dust, smoke, irritant gases, and stimulation.[4] Notably, viral infections account for up to 70% to

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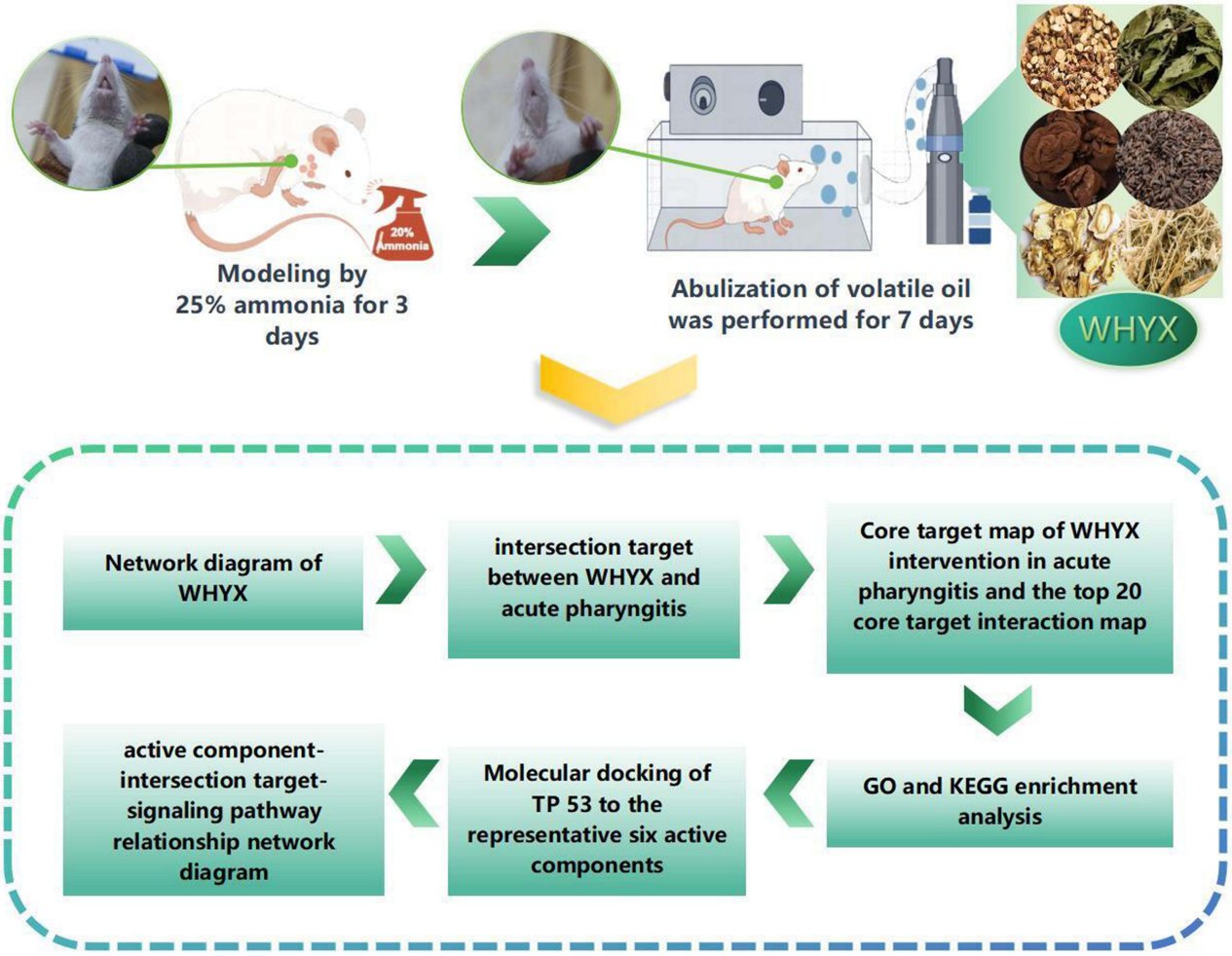
Graphical Abstract



95% of acute pharyngitis cases, including those caused by respiratory viruses and enteric viruses.[2,5,6] The disease manifests with hyperplasia, congestion of lymphoid follicles on the posterior pharyngeal wall and inflammation of the palatine tonsils.[7] Clinically, patients may present with redness, swelling, and pharyngeal pain.[8] In severe cases, symptoms may include fever, chills, hoarseness, and dysphagia.[9] Current treatment strategies primarily involve antibiotics, antivirals, and glucocorticoids.[7] However, over 90% of pediatric cases are viral,[10] and effective antiviral therapies remain limited. Misuse of antibiotics remains a concern, contributing to drug resistance, adverse reactions and dysbiosis of microflora.[11] In addition, high medication costs and potential toxicity pose further challenges.[12]

Traditional Chinese medicine (TCM), rooted in millennia of empirical practice, is distinguished by its multi-herbal, multi-component, and multi-target therapeutic approach. In the context of pediatric acute pharyngitis, TCM has demonstrated efficacy in alleviating clinical symptoms and preventing complications. Wu Jutong's Detailed Analysis of Epidemic Warm Diseases, a seminal text from the Qing Dynasty, recommends the use of Yinqiao-Mabo decoction for treating sore throats associated with damp-warm syndromes. Modern clinical studies confirm that both the original and modified forms of this decoction yield favorable therapeutic outcomes in conditions such as acute tonsillitis, pediatric acute pharyngitis, and asthma,[13–15] highlighting the potential of these classical formulations for novel therapeutic development. Despite these advantages, the administration of traditional decoctions in pediatric populations remains

problematic. Young children often struggle with treatment compliance and are particularly sensitive to the bitter taste of herbal decoctions, frequently leading to refusal or vomiting. These factors compromise therapeutic outcomes and place an additional burden on caregivers and clinicians. In this context, atomized delivery of TCM represents an innovative and targeted strategy for pediatric acute pharyngitis, allowing direct deposition of therapeutic agents onto inflamed pharyngeal tissues. This non-invasive method improves patient compliance and ensures rapid onset of action while minimizing discomfort during administration. Clinical reports indicate that atomization therapy is well tolerated and associated with low adverse effects in children.[16,17] However, limitations persist with conventional atomization approaches, which typically utilize water decoctions. During the decoction process, volatile oils containing key bioactive components, such as cinnamaldehyde, limonene, citral, and zedoary oil, may undergo substantial loss.[18]

In response to the limitations associated with conventional water decoction-based atomization, an innovative aerosol formulation, Wu-Hua-Yan-Xiao (WHYX), was developed based on the Yinqiao-Mabo prescription. Volatile oils were extracted from Niubangzi (*Arctium Lappa L)*., Mabo (*Lasiosphaera seu Calvatia)*., Yuxingcao (*Houttuynia cordata Thunb)*., Jiegeng (*Platycodon Grandiflorus (Jacq)*.), Bohe (*Mentha haplocalyx Briq)* and Gancao (*Glycyrrhiza uralensis Fisch)*., and incorporated into the compound. The formulation was further optimized in accordance with the classical compatibility principles of the original recipe. *Arctium Lappa L., Platycodon Grandiflorus (Jacq)* and *Lasiosphaera seu Calvatia*. serve as the core components of the Yinqiao-Mabo formula, traditionally recognized for their effectiveness in relieving pharyngeal inflammation. Based on historical usage, *Glycyrrhiza uralensis Fisch*. was added due to its compatibility with *Arctium Lappa L*. in treating pharyngeal disorders. Modern research demonstrates that *Glycyrrhiza uralensis Fisch*. exhibits anti-inflammatory properties and synergistic effects, significantly enhancing the therapeutic efficacy.[19] *Mentha haplocalyx Briq*., commonly used for respiratory disorders, contains volatile oils with expectorant activity and the ability to inhibit airway hyperresponsiveness in asthmatic mice.[20,21] Moreover, the volatile oil of *Mentha haplocalyx Briq*. exhibits strong anti-inflammatory activity, effectively alleviating airway inflammation.[22] Clinical studies have confirmed its anti-inflammatory effects, demonstrating a reduction in systemic inflammatory responses and notable efficacy in treating respiratory conditions such as pneumonia, chronic bronchitis, respiratory tract infections, pharyngitis, and pulmonary tuberculosis.[23] By integrating these herbs, the study aims to exploit the multi-target and multi-pathway advantages of traditional Chinese medicine, offering a safe and effective therapeutic strategy for pediatric acute pharyngitis. To elucidate the underlying mechanisms of WHYX in treating this condition, network pharmacology analysis and molecular docking were conducted. Furthermore, an acute pharyngitis model in young rats was established to evaluate the therapeutic effects of WHYX aerosol at histopathological, molecular, and genetic levels.

Materials and Methods

Reagent Materials

The following reagents were used in the study: 25% ammonia water (500 mL; lot no. A801005) was acquired from Shanghai MacLean Biochemical Technology Co., Ltd. (Shanghai, China). 1,2-propylene glycol and vegetable glycerol were provided by Shanghai Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and Procter & Gamble Company (Shanghai, China), respectively. ELISA kits for rat interleukin-6 (IL-6; lot no. YD-30219), interleukin-1β (IL-1β; lot no. YD-30206), tumor necrosis factor-α (TNF-α; lot no. YD-31063), and prostaglandin E2 (PGE2; lot no. LCSJZF30697) were obtained from Xiamen Lun Changshuo Biotechnology Co., Ltd. (Xiamen, China). Primary antibodies, including caspase-3 (lot no. AF6311), β-actin (lot no. AF7018), TNF-α (lot no. AF7018 and AF7014), p53 (lot no. AF0879), IL-17a (lot no. DF6127), IL-6 (lot no. DF6087), and Bcl-2 (lot no. AF6139), were purchased from Jiangsu Pro Biological Research Center Co., Ltd. (Jiangsu, China). Protein extraction reagents (lot no. MDL91201), protease inhibitors (lot no. MD912893), BCA protein quantification kits (lot no. MD913053), Western blot secondary antibodies (lot no. MD912565), and SDS-PAGE precast gel kits (lot no. MD911919) were supplied by MDL Beijing Biomedical Technology Co., Ltd. (Beijing, China). The protein molecular weight marker (lot no. BP06193S) was acquired from Suzhou Boolong Technology Co., Ltd. (Suzhou, China). UltraPure agarose (lot no. SH441-01) was purchased from Beijing Bomide Biotechnology Co., Ltd. (Beijing, China), and the SuperScript III RT reverse transcription kit (lot no. A502) was obtained from EXONGEN Rong Wei Gene Biotechnology Co., Ltd. (Chengdu, China). CY3-conjugated goat

anti-rabbit secondary antibody (1:500; lot no. PN0051) was purchased from Pinofi Biotechnology Co., Ltd. (Wuhan, China). Essential oils used in the preparation of WHYX aerosol included those of *Arctium Lappa L*. (lot number: 230508), *Houttuynia cordata Thunb*. (lot number: 230803), *Mentha haplocalyx Briq*. (lot number: 230709), *Lasiosphaera seu Calvatia* (lot number: 230902), *Platycodon Grandiflorus (Jacq)*.(lot number: 230731), *Glycyrrhiza uralensis Fisch*. (lot number: 230620), all sourced from Asus Spice Oil Co., Ltd. (Jian, China).

WHYX Preparation of Decoction and Volatile Oil

The WHYX decoction was prepared using the following crude herbal components: 15g of *Arctium Lappa L*., 10g of *Mentha haplocalyx Briq*., 10g of *Houttuynia cordata Thunb*., 10g of *Lasiosphaera seu Calvatia*, 10g of *Platycodon Grandiflorus (Jacq)*., and 5g of *Glycyrrhiza uralensis Fisch*. Six batches were purchased from the First Affiliated Hospital of Hunan University of Chinese Medicine and authenticated by Professor Xie Mengzhou, in accordance with the 2020 edition of the Pharmacopoeia of the People's Republic of China. All materials were stored at the Key Laboratory of Diagnostics of Traditional Chinese Medicine of Hunan Province at Hunan University of Chinese Medicine. To prepare the decoction, the herbs were boiled in a ceramic container with ultrapure water, followed by extraction under reduced pressure. The extract was concentrated to 250 mL to yield a solution with a 1.2 g/mL density.

The volatile oil aerosol formulation of WHYX was prepared by mixing the essential oils in the following proportions: 15 parts of *Fructus Arctii*, 10 parts of *Lasiosphaera seu Calvatia*, 10 parts of *Houttuyniae Herba*, 10 parts of *Platycodon Grandiforus*, 10 parts of *Menthae Herba* and 5 parts of *licorice*. All medicinal materials were stored at 4°C until use.

UPLC-MS Quantification

The compounds in WHYX aerosol were identified with ultra-performance liquid chromatography (Vanquish, UPLC, Thermo, USA) and a high-resolution mass spectrometer (Q Exactive HFX, Thermo, USA). Samples were injected into a reversed-phase column (100 × 2.1 mm, 1.8 μm particle size). The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with a constant flow rate of 0.3 mL/min. The column temperature was maintained at 40°C throughout the analysis. Mass spectrometric parameters were configured as follows: ion transport tube temperature was set to 320°C; sheath gas flow rate was 40 arbitrary units; auxiliary gas flow rate was 10 arbitrary units. Electrospray ionization was performed in both positive and negative ion modes, with a spray voltage of +3.0 kV in positive mode and –2.8 kV in negative mode.

Construction and Analysis of Network Pharmacology

Screening of Active Ingredients and Target Prediction for WHYX

The bioactive constituents of *Arctium Lappa L., Lasiosphaera seu Calvatia., Houttuynia cordata Thunb., Platycodon Grandiflorus (Jacq)., Mentha haplocalyx Briq*. and *Glycyrrhiza uralensis Fisch*. were identified using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/index.php). Screening criteria included oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18, ensuring the selection of compounds with favorable pharmacokinetic profiles and drug-like properties. The corresponding target proteins for these selected compounds were retrieved and standardized using the UniProt database (https://beta.uniprot.org/), with filtering parameters set to "Reviewed" status and species limited to "Homo sapiens." Protein names were then converted into standardized gene names for subsequent analysis. To complement the target dataset, the Similarity Ensemble Approach (SEA) database (https://sea.bkslab.org/) was utilized to obtain target information not available within TCMSP.

Construction for a Compound Ingredient-Target Network Diagram

A compound–target interaction network was constructed using network pharmacology approaches to elucidate the relationships between active ingredients in the formulation and their corresponding target proteins. The network illustrates the connections between multiple bioactive components and their predicted targets. To generate the Key Chemical Component–Common Target Network, all target genes and their associated compounds were organized into a tabular dataset, which was subsequently imported into Cytoscape software (version 3.7.2) to visualize the interactions between key constituents and their related target genes.

Acquisition of Compound-Disease Common Targets and PPI Networks

Target genes related to acute pharyngitis were retrieved from the GeneCards database (https://www.genecards.org/) and the DisGeNET database (https://www.disgenet.org/). The predicted target proteins of the active compounds were mapped against these disease-related genes to identify overlapping targets, thereby revealing shared targets between the compound formulation and acute pharyngitis, as well as the corresponding key chemical constituents. The common targets were subsequently submitted to the STRING database (https://cn.string-db.org/) to construct a protein-protein interaction (PPI) network. A confidence score threshold of 0.9 was applied to enhance the reliability of the interaction network. To further analyze the network, the CytoNCA plug-in in Cytoscape was utilized to calculate key topological parameters, including Degree Centrality (DC), Betweenness Centrality (BC), Closeness Centrality (CC), and Neighborhood Connectivity (NC). Core targets were identified by filtering nodes based on the median values of each parameter, using the following thresholds: DC $\geq$ 5.000, BC $\geq$ 81.308, CC $\geq$ 0.122, and NC $\geq$ 19.477. These criteria facilitated the identification and visualization of hub nodes within the PPI network.

GO and KEGG Enrichment Analysis

Common target genes were submitted to the Metascape database (http://metascape.org/gp/index.html) for enrichment analysis. Parameters were set as follows: P value $\leq$ 0.01, minimum overlap $\geq$ 3, and enrichment score $\geq$ 1.5. The analysis included Gene Ontology (GO) categories—biological process (BP), cellular component (CC), molecular function (MF) and KEGG pathway enrichment. Bubble plots were generated online using the ImageGP platform to visualize the top enrichment results. To further explore the molecular mechanisms of acute pharyngitis, the target–pathway interaction network was constructed using Cytoscape software. Core targets were also analyzed through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (www.kegg.jp/kegg/pathway. html). The KEGG Mapper "Color Tool" function was applied by selecting the "hsa" (Homo sapiens) model to identify relevant signaling pathways and visualize the distribution of target proteins within these pathways.

Construction of the Relationship Network of "WHYX - Active Ingredients - Intersection Targets - Signaling Pathways - Acute Pharyngitis"

Network topology analysis was performed using the CytoNCA plug-in in Cytoscape to construct an integrated relationship network connecting the WHYX formulation, its active ingredients, common targets, signaling pathways, and acute pharyngitis. Median values of DC, BC, and CC were used as thresholds for core node identification. Screening criteria for core active compounds were set as follows: DC $\geq$ 10.000, BC $\geq$ 0.391, and CC $\geq$ 127.796. Core targets were filtered using the following parameters: DC $\geq$ 4.500, BC $\geq$ 0.346, and CC $\geq$ 37.511.

Molecular Docking Simulation

Three-dimensional structures of active compounds and their corresponding target proteins were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and the Protein Data Bank (https://www.rcsb.org/), respectively. Hydrogen atoms were added, and docking parameters for receptor proteins were set using AutoDock Tools version 1.5.6. Molecular docking was performed using AutoDock software, and binding affinities were calculated to evaluate ligand-receptor interactions. Docking results were visualized utilizing PyMOL software. Binding energies were interpreted as follows: values below −4.25 kcal/mol indicated a certain degree of binding activity, below −5.0 kcal/mol indicated good binding activity, and below −7.0 kcal/mol suggested strong binding affinity between ligand and receptor. Finally, the results were visualized by PyMol software (docking score < −5.0 kcal/mol).

Animal Experiment

Model Construction and Grouping

A total of 56 specific pathogen-free (SPF) Sprague-Dawley (SD) rats, including 28 males and 28 females aged 3–4 weeks and weighing (50 ± 20) g, were obtained from Hunan Slake Jingda Experimental Animal Co., Ltd. (License No. SCXK (Xiang) 2019–0004). All animals were housed at the Experimental Animal Center of Hunan University of Traditional Chinese Medicine under standard laboratory conditions: three rats per cage, with ad libitum access to food and water, maintained at a temperature of (21.0 ± 2.0) °C and humidity of (50.0 ± 10.0)%, with a 12-hour light/dark cycle. Animal

care and experimental procedures were performed according to the Guidelines for Animal Experimentation of Hunan University of Chinese Medicine, with approval from the Hunan University of Chinese Medicine Experimental Animal Ethics Committee. The ethical number is HNUCM21-2310-03.

Rats were randomly assigned into seven groups (n = 8 per group) using a random number table: normal group, model group, WHYX volatile oil low-dose group (WHYX-VO (L)), medium-dose group (WHYX-VO (M)), high-dose group (WHYX-VO (H)), decoction group (WHYX-D) and positive control group treated with HouYanQing granules (HYQ). An acute pharyngitis model was established by administering 25% ammonia solution (0.2–0.4 mL/rat) twice daily for three consecutive days. The solution was delivered using a type A laryngeal sprayer (purchased from Jiangsu Ping An Medical Device Co., Ltd)., targeting a 150 mm^2 area of the posterior pharyngeal wall. Congestion and edema of the pharyngeal mucosa were observed, consistent with successful model induction, which could be sustained for up to 10 days.[24] Dosages of WHYX were calculated by converting human clinical doses to equivalent animal doses based on body surface area normalization, further adjusted for the bioavailability of aerosol administration. Low-, medium-, and high-dose concentrations were defined as 40 mg/kg, 80 mg/kg, and 160 mg/kg, respectively. The volatile oil formulation was diluted with 1,2-propylene glycol and administered once daily via aerosol inhalation using a volatile oil atomizer (Hangzhou NetEase Yanxuan Trading Co., Ltd., Hangzhou, China) for 30 minutes over 7 consecutive days. The WHYX-D group received ultrasonic nebulization of 250 mL of the herbal decoction daily for 30 minutes over the same 7-day period, along with oral administration of normal saline. The HYQ group was treated with HouYanQing granules (1.2 g/mL) at a dose of 2.25 mL/rat via oral gavage once daily for 7 days, combined with aerosolized normal saline. Both the normal and model groups received equal volumes of normal saline by oral gavage and inhalation. Body weight and food intake were recorded daily.

At the conclusion of the treatment period, all rats were fasted for 12 hours. On the following morning, animals were weighed and anesthetized with 50 mg/kg of 3% sodium pentobarbital. Blood samples were collected from the abdominal aorta under open abdominal surgery. Sections of pharyngeal mucosa and submucosal tissues were harvested and fixed in 4% paraformaldehyde for histological analysis while the remaining tissue samples were snap-frozen in liquid nitrogen for molecular studies.

General Condition and Throat Endoscopy Observation

The general physical conditions of the rats were monitored throughout the experimental period, including changes in body weight, activity levels, fur condition, respiratory symptoms (cough and wheezing), mental state, and urinary and fecal output. Prior to sample collection, throat observations were conducted using the T01 pharyngeal endoscope (Shenzhen HengTechnology Co., Ltd., Shenzhen, China). The semi-quantitative evaluation was performed to assess the therapeutic efficacy of treatments based on visible alterations in the pharyngeal region. The criteria for the rat conditions are as follows: Grade "–" was assigned for slight discoloration in the pharyngeal region, mild redness and swelling over a small area, slight hair loss around the mouth, reduced saliva secretion, absence of cough or wheezing, shiny fur, stable body weight, and normal activity; Grade "+" indicated moderate discoloration in the pharyngeal region, redness and swelling over a medium area, moderate hair loss around the mouth, moderate saliva secretion, mild cough and wheezing, dry and dull fur, slight weight loss, and mildly reduced activity; Grade "++" reflected pronounced discoloration in the pharyngeal region, extensive redness and swelling, significant hair loss around the mouth, excessive saliva secretion, severe cough and wheezing, markedly dull fur, substantial weight loss, and severely impaired activity.[24]

Routine Blood Test

Whole blood (100 μL) was collected from each rat into tubes containing EDTA as an anticoagulant. Routine hematological parameters were analyzed immediately utilizing a TEK-VET3 automated hematology analyzer (Tekang Science and Technology, Jiangxi, China).

HE Staining for Pathological Changes Observation

All rats were harvested from pharyngeal mucosa and submucosal tissues, fixed in 4% paraformaldehyde solution, embedded in paraffin, sectioned, and stained by hematoxylin-eosin (H&E) at 100 times magnification under an upright light microscope (Model Nikon Eclipse E100, Nikon, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood samples were centrifuged at 3,000 rpm for 15 minutes at 4°C to isolate serum. Serum levels of IL-6, IL-1β, TNF-α, and PGE2 were quantified utilizing commercial ELISA kits. Absorbance was measured using a microplate reader (Rayto RT-6100, Shenzhen Ledu Life Sciences Co., China).

qRT-PCR

Key genes were selected based on network pharmacology analysis. Total RNA was extracted from pharyngeal mucosal tissues using TRIzol reagent, followed by cDNA synthesis using a high-capacity cDNA reverse transcription kit. TNF-α, TP53, IL17A, IL6, and Bcl-2 expression levels were analyzed. Primers were designed via PrimerBank and synthesized by GenScript (Wuhan Jinkui Biotechnology Co., Ltd., China), with sequences listed in Table 1. The melting curve analysis was obtained on the Q2000B fluorescence quantitative PCR instrument (Hangzhou Langji Scientific Instrument Co., Ltd., China), and relative expression levels were calculated utilizing the ΔΔCt method with base factors.

Western Blotting

Pharyngeal mucosal tissues were lysed using protein lysis buffer, and total protein concentrations were determined utilizing a BCA protein assay kit. Equal amounts of protein were separated via SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked and incubated overnight at 4°C with primary antibodies against TNF-α(1:1000), p53 (1:1000), IL17A (1:1000), IL6 (1:1000), and Bcl-2 (1:1000). On the following day, membranes were washed with TBST and incubated with secondary antibodies at a dilution of 1:300. After additional washes, enhanced ECL reagent was applied for signal development. Membranes were subsequently imaged, and band intensities were analyzed utilizing ImageJ software (2.14.0).

Double Immunofluorescence Staining for Caspase-3 and TUNEL

Paraffin-embedded sections of pharyngeal mucosa were dewaxed in distilled water, followed by antigen retrieval and membrane permeabilization. Reagents from the TUNEL assay kit were prepared by mixing TdT (Reagent 1) and dUTP (Reagent 2) at a 2:29 ratio. The mixture was applied to the tissue sections, which were placed flat in a humidified chamber and incubated at 37°C for 2 hours. To maintain humidity, a small volume of water was added to the chamber. PBS was added dropwise to the sections, which were then incubated overnight at 4°C. The tissues were subsequently incubated with a primary antibody corresponding to the appropriate species for 50 minutes at room temperature. Nuclear staining was performed using DAPI at 37°C, and the sections were sealed with an anti-fluorescence quenching mounting medium. Fluorescence imaging was conducted using a Nikon Eclipse Ti-SR microscope (Nikon, Japan). Red fluorescence indicated Caspase-3, green fluorescence marked TUNEL-positive cells, and blue fluorescence represented nuclei. Cells exhibiting overlap of all three signals were considered TUNEL/Caspase-3 double-positive and were quantified utilizing Image-Pro Plus software (version 6.0).

Table 1 The Primer Sequence Information

Gene Name	Primer Sequences (5′–3′)		Length
TNF-a	TNF-a-F	GTTCCATGGCCCAGACCCT	100bp
	TNF-a-R	AGCTGCTCCTCCGCTTGGTG	
TP53	TP53-F	TGCCCACCAGCACAAGCTC	110bp
	TP53-R	CAGCTCTCGGAACATCTCGAAG	
IL-17A	IL-17A-F	CCCTGCGTTTCTCTGCAAAC	110bp
	IL-17A-R	CAGGGTGGAAGGCAGACAAT	
IL-6	IL-6-F	TGAGAAAAGAGTTGTGCAAT	67bp
	IL-6-R	TTGTTTTCTGACAGTGCAT	
BCL2	BCL2-F	CCTGAATGACCACCTAGAGCCTT	87bp
	BCL2-R	TCGGCTGCTGCATTGTTCCC	

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Statistical Analysis

Statistical analysis was performed utilizing SPSS 24.0 software. Data following a normal distribution were presented as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance (ANOVA) was applied for comparisons among multiple groups when variances were homogeneous, followed by LSD tests for pairwise comparisons. In cases of unequal variances, Tamhane's T2 test was used. A P value of <0.05 was deemed statistically significant.

Results

UPLC-MS Analysis

To ensure the quality, consistency, and stability of the WHYX-VO, UPLC-MS analysis was performed. Given the complex matrix of the WHYX extract, which comprises multiple herbal components with diverse chemical compositions and concentrations, both positive and negative ion modes were employed for comprehensive compound profiling. Representative base peak intensity (BPI) chromatograms of three independent batches of WHYX are presented in Figure 1A. All major peaks appeared within 10 minutes, with uniformly distributed chromatographic signals across all samples, indicating consistent retention and separation performance. No significant differences were observed in the chemical composition among the three batches, demonstrating the compositional stability and reproducibility of the formulation. In Figure 1B and C, a total of 14 major components were identified: 4-hydroxy cassia bark acid, ferulic acid, protocatechaldehyde, 1-heptanol, 3′-O-Demethylarctigenin, vanillic acid, 7-Demethylsuberosin, Velutin, Lobetyol, methyleugenol, Aurantiamide acetate, (R) -camphor, Isobavachin, and Ganoderma acid H. Detailed compound information is provided in Table S1.

Network Pharmacology-Based Analysis

A total of 130 active compounds and 600 corresponding gene targets were identified from the TCMSP database across six herbal components (Figure 2A). From disease databases, 2,089 gene targets associated with acute pharyngitis were retrieved. By mapping compound targets to disease-related genes, 194 overlapping targets were identified, along with their associated key chemical constituents (Figure 2B). PPI networks were conducted to explore the relationships between the shared targets. Fifty core targets relevant to WHYX treatment of acute pharyngitis were identified and visualized in the PPI network (Figure 2C), with the top 20 core gene interaction pairs highlighted in Figure 2D and Table S2.

GO functional enrichment analysis revealed key BP, CC, and MF associated with the overlapping targets. KEGG pathway enrichment indicated that signaling pathways such as cellular Pathways in cancer, Lipid and atherosclerosis, Prostate cancer, Kaposi sarcoma-associated herpesvirus infection, and TNF signaling may contribute to the therapeutic effects of WHYX (Figure 2E and F). The top 20 enriched pathways are listed in Table S3. Integration of KEGG and GO analyses suggested that the TP53 signaling may serve as a primary regulatory mechanism in the treatment of acute pharyngitis.

A compound–target network was constructed to further elucidate the core interactions underlying the therapeutic effects of WHYX. The network included 332 nodes and 23,651 edges, reflecting its multi-component, multi-target nature. Cluster analysis identified a subset of 45 core components and 79 hub targets (Figure 2G), with the top 10 key compounds listed in Table S4.

Molecular Docking

Based on network pharmacology results and literature review, six key target genes (TP53, TNF, IL-6, IL-1β, BCL2, CASP3) and six representative active compounds with moderate PPI values (quercetin, kaempferol, luteolin, 7-methoxy-2-methyl-3-phenyl-4-H-acacetin-4, and beta-sitosterol) were selected for molecular docking. The 3D structures of the active components and related proteins were downloaded through the PubChem CID database and the PDB database, respectively. Hydrogen atoms were added, and receptor docking parameters were configured using AutoDock Tools 1.5.6. Molecular docking was performed with AutoDock software, and the binding energies were calculated. Docking conformations were visualized using PyMOL, with detailed results provided in Tables S5 and S6.

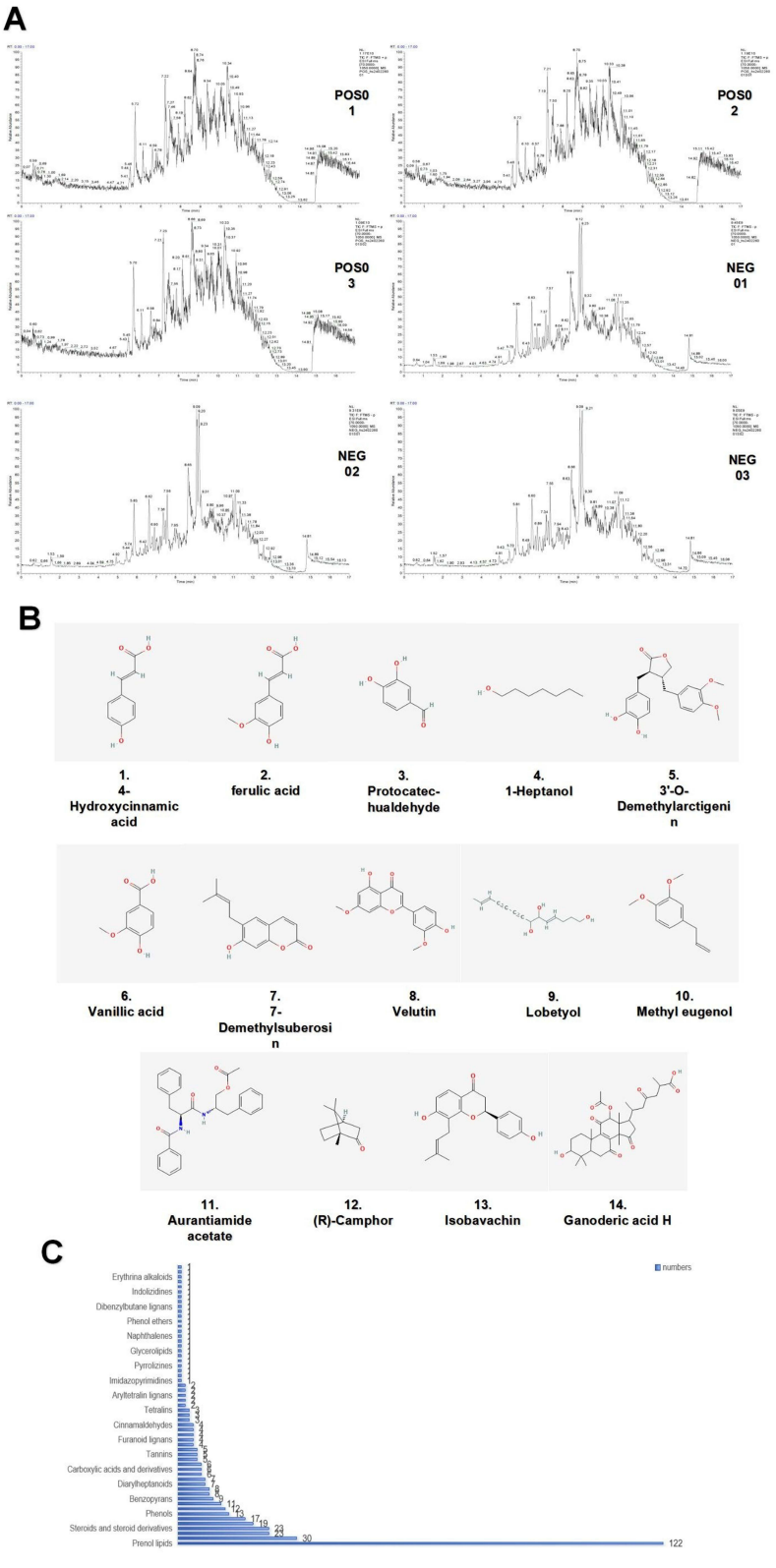

Figure 1 UPLC-MS analysis of WHYX. (**A**) Chromatogram of base peak intensity; (**B**) Structure of main active components; (**C**) Histogram representing the distribution of all types of active ingredients.

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Binding energies less than 4.25, 5.0, and 7.0 kcal/mol were considered to reflect certain, good, and strong binding activity, respectively (1 kcal = 4.184 kJ). Binding energy values are listed in Table S7 and S8. Visualization revealed that most interactions occurred via hydrogen bonding. Among the targets, TP53 showed the strongest binding affinity with the selected compounds. Docking conformations and heatmap visualizations are shown in Figure 3.

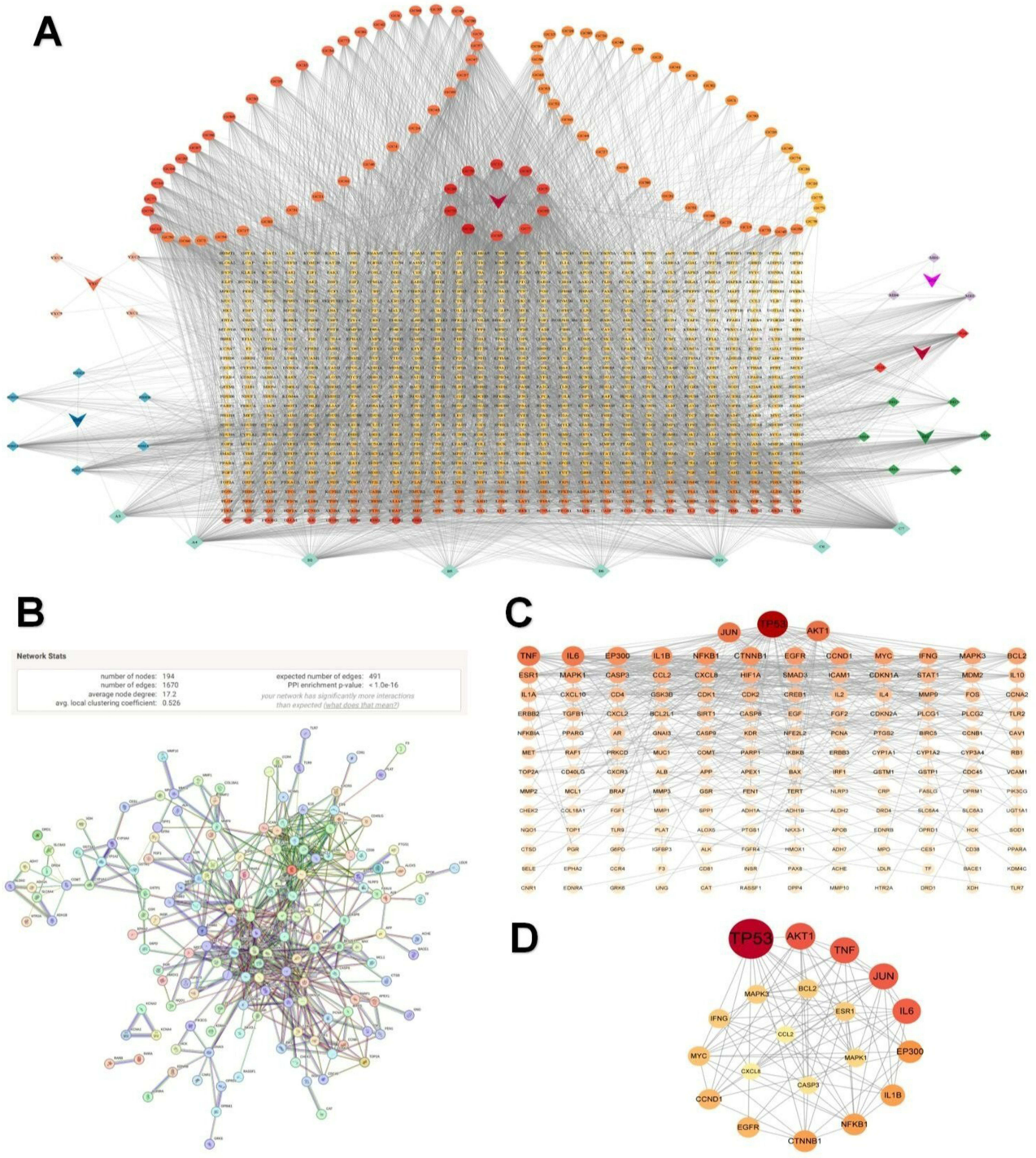

Figure 2 Continued.

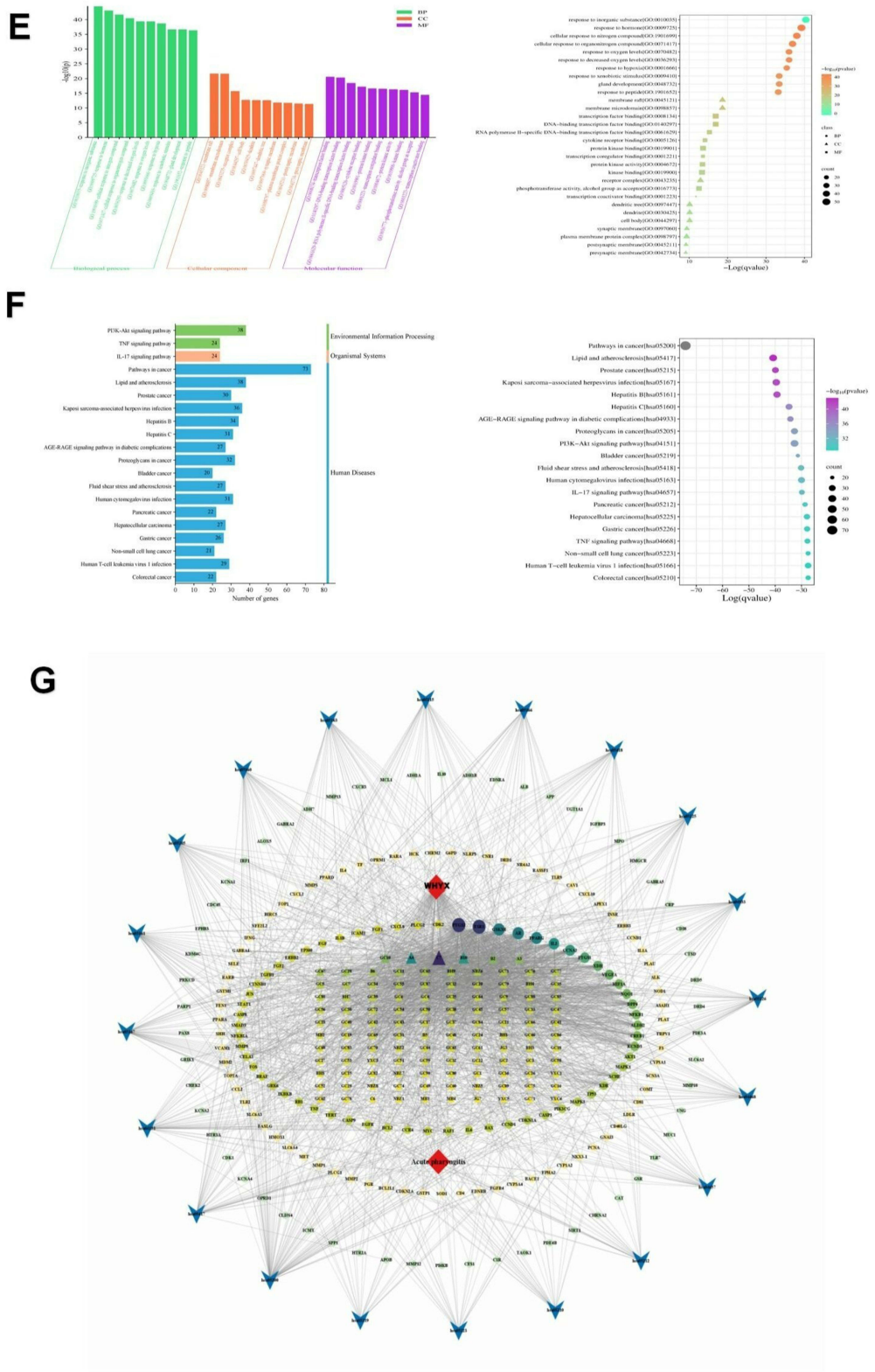

Figure 2 Network pharmacology analysis of WHYX. (**A**) Network diagram of WHYX; (**B**) A PPI network diagram of WHYX and acute pharyngitis; (**C**) Core target map of WHYX intervention in acute pharyngitis; (**D**) The top 20 core target interaction map; (**E**) GO enrichment analysis; (**F**) KEGG enrichment analysis; (**G**) Active component-intersection target-signaling pathway relationship network diagram.

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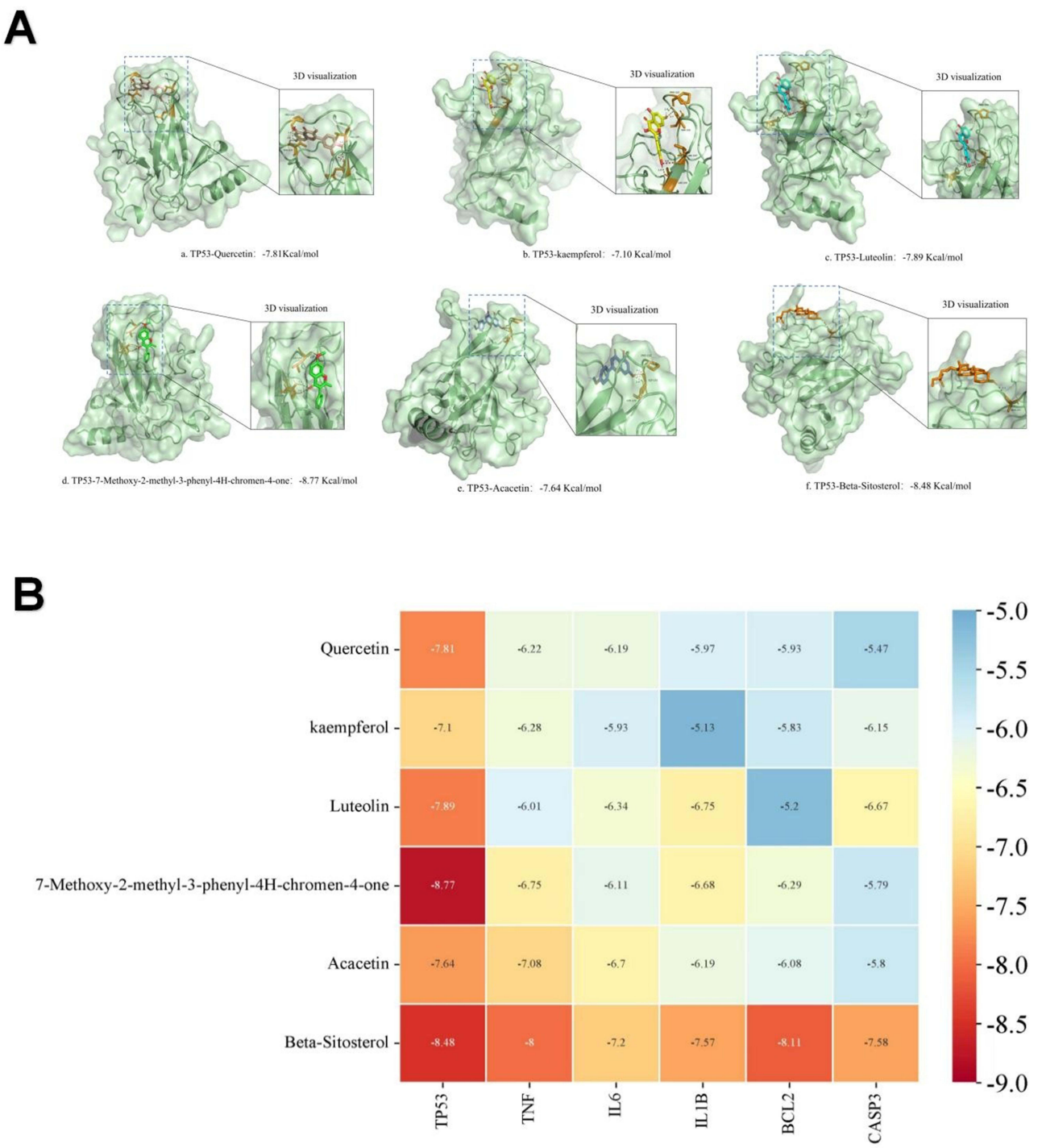

Figure 3 Molecular docking of WHYX active components with TP53. (**A**) Docking conformations between TP53 and six representative active compounds; (**B**) Heatmap of the molecular docking.

WHYX Alleviates Pathological Damage in the Throat of Young Rats with Acute Pharyngitis

General observations showed that rats in the normal group maintained good health, exhibited no abnormal behaviors, and presented with clear pharyngeal mucosa and lustrous fur. In contrast, rats in the model group displayed symptoms including persistent coughing, weight loss, decreased food intake, pharyngeal swelling, excessive mucus secretion, and hair loss around the mouth. Some animals also showed signs of laryngeal edema. Treatment groups exhibited a notable reduction in cough and wheezing. The pharyngeal mucosa gradually returned to normal coloration, and laryngeal edema was reduced (see Figure 4A).

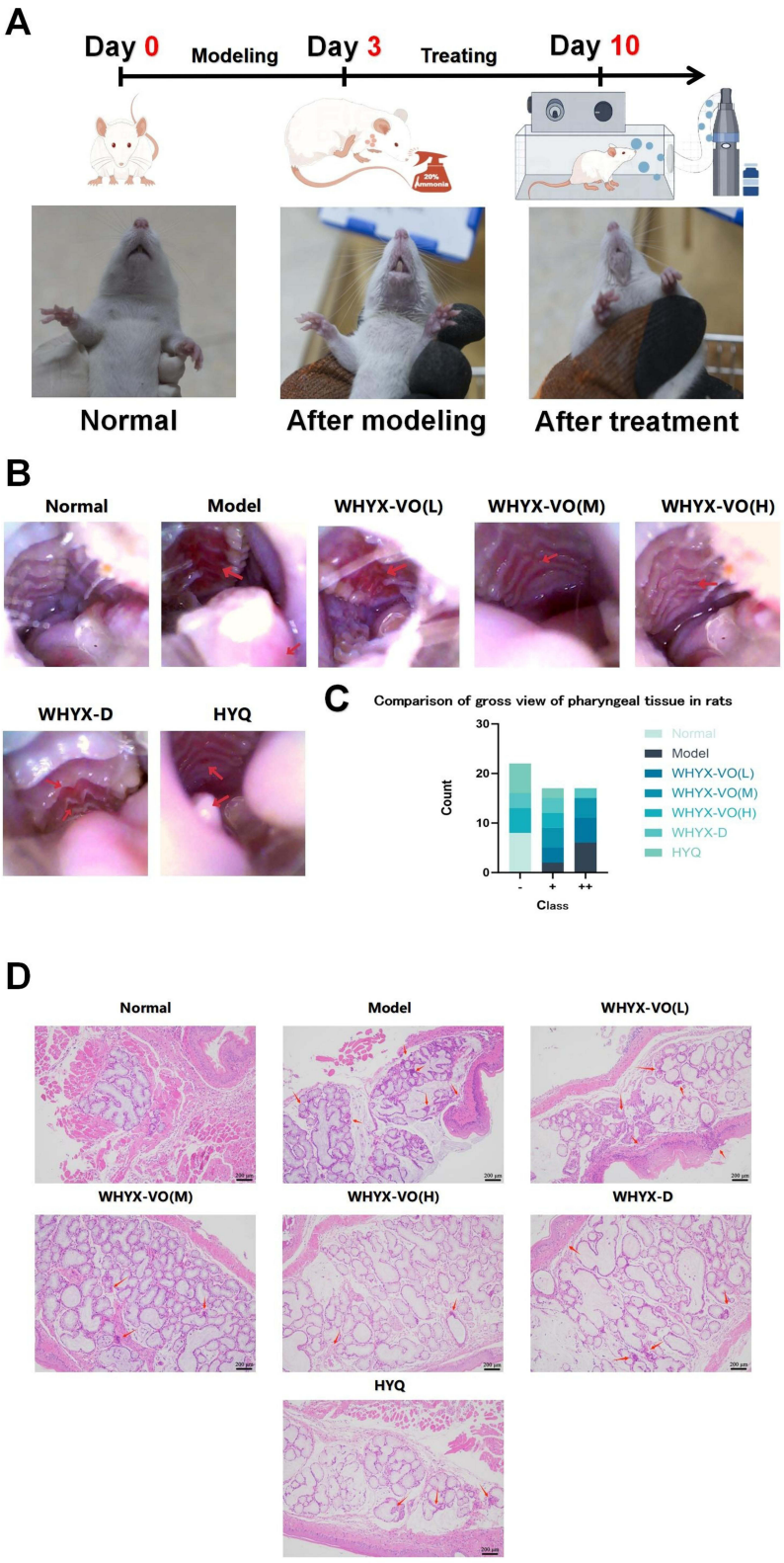

Figure 4 WHYX reduces pathological injury in young rats with acute pharyngitis. (**A**) Overall flow chart of the experiment; (**B**) Throyngeal endoscopy in each group; (**C**) Semiquantitative analysis of throat pathology; (**D**) Histological sections of pharyngeal tissue stained with H&E (×100). Red arrows indicate reddened and ulcerated regions of the oral cavity and pharynx in young rats.

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Endoscopic imaging revealed pronounced mucosal congestion and redness in the model group, which were visibly ameliorated following treatment. The semi-quantitative analysis confirmed that tissue damage scores improved across all treatment groups, with the most substantial improvement observed in the WHYX-VO (H) group, which showed a marked reduction in redness and fewer bleeding points (Figure 4B and C). Histopathological analysis by H&E staining revealed that the normal group displayed intact squamous epithelium, connective tissue, vasculature, muscle fibers, and glands, with no signs of inflammation. In contrast, the model group exhibited epithelial hyperplasia, vascular congestion, and substantial inflammatory cell infiltration. Inflammatory responses remained apparent in the WHYX-VO (M) and HYQ groups but were notably attenuated in the WHYX-D group. Representative histological features are shown in Figure 4D.

WHYX Attenuates Inflammation in Young Rats with Acute Pharyngitis

Hematological analysis revealed a significant elevation in white blood cell (WBC) counts in the model group compared to the normal group ($P < 0.05$). Treatment with WHYX-VO (M) and WHYX-VO (H) reduced WBC levels ($P < 0.05$). Lymphocyte counts were also decreased in the WHYX-VO (M), WHYX-VO (H), and WHYX-D groups compared to the model cohort ($P < 0.05$), as illustrated in Figure 5A and B. ELISA results revealed that IL-6, IL-1β, TNF-α, and PGE2 levels in the model group were elevated compared to those in the normal group ($P < 0.01$). All treatment groups

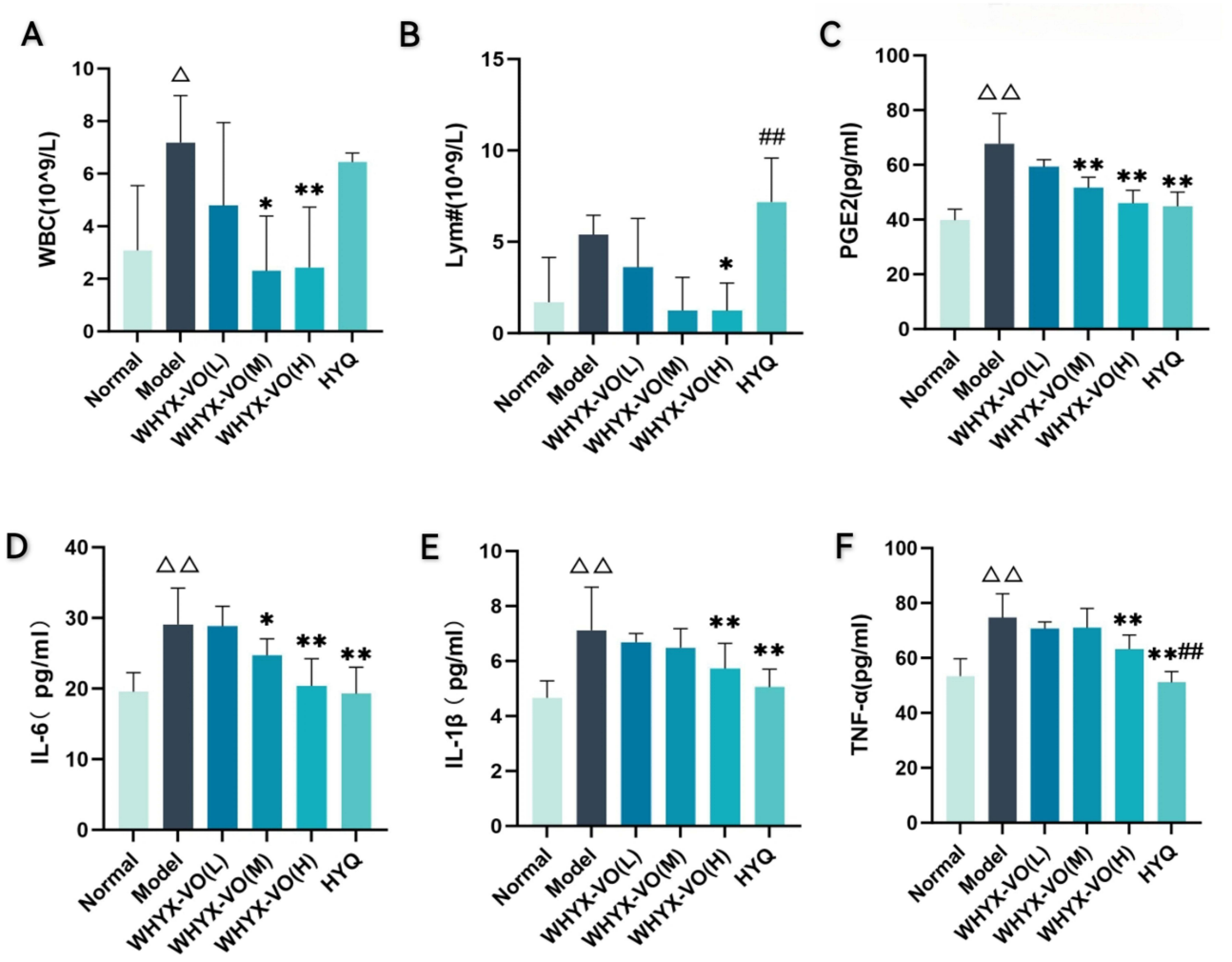


Figure 5 WHYX attenuates inflammation in young rats with acute pharyngitis (**A** and **B**) Routine blood tests of young rats with acute pharyngitis, (**A**) white blood cell; (**B**) lymphocyte. (**C–F**) ELISA analysis of PGE2, IL-6, IL-1β and TNF-α in serum, respectively. Data are expressed as mean±standard deviation (n=6). $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, compared with normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with nodel group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with WHYX-VO(H) group. Data are mean ± SD from two independent technical replicates for (**A–F**) and represent two independent biological experiments for (**A–F**).

exhibited significant reductions in these cytokines, with a dose-dependent trend. The WHYX-VO (H) group demonstrated anti-inflammatory effects comparable to those of the WHYX-D and HYQ groups. These findings are depicted in Figure 5C–F.

WHYX Inhibits Cell Apoptosis by Down-Regulating Inflammatory Factors

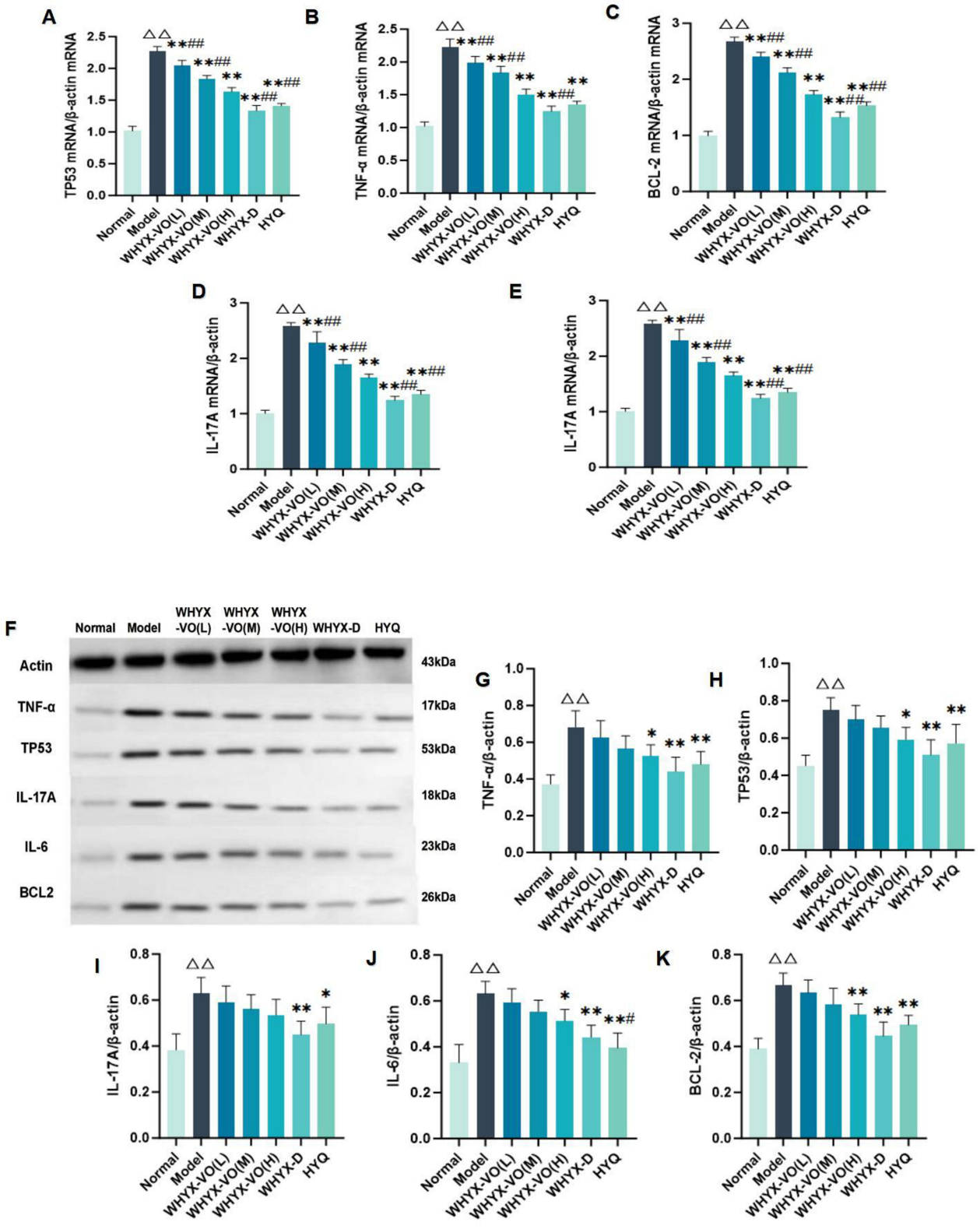
Network pharmacology analysis identified TP53 and multiple inflammatory mediators, including TNF-α, IL-6, IL-17A, and BCL2, as key targets involved in the therapeutic mechanism of WHYX against acute pharyngitis. To validate these findings, qRT-PCR, Western blotting, and double immunofluorescence staining were performed to assess gene and protein expression levels and to evaluate apoptotic activity in vivo.

As shown in Figure 6A–E, mRNA expression levels of TNF-α, TP53, IL-17A, IL-6, and BCL2 were elevated in the model group compared to the normal group ($P < 0.01$)Treatment with WHYX led to a dose-dependent reduction in these gene expression levels, with all treatment groups showing significant decreases relative to the model group ($P < 0.01$). Protein expression analysis revealed a similar trend. As shown in Figure 6F–K, protein levels of the same targets were markedly increased in pharyngeal tissues of model rats ($P < 0.01$) and decreased in the WHYX-VO (H), WHYX-D, and HYQ groups ($P < 0.05$ or $P < 0.01$). As shown in Figure 6L–N, the TUNEL (green) and caspase-3 (red) double staining was performed under fluorescence microscopy. Minimal fluorescence was observed in the normal group, while strong signals for both markers were widely distributed in the model group ($P < 0.05$), indicating extensive apoptosis. Treatment reduced the number of TUNEL- and caspase-3-positive cells in a dose-dependent manner, with the WHYX-VO (H) group exhibiting the most pronounced reduction ($P < 0.05$). The results above verified the protective effects of WHYX in suppressing TP53-mediated apoptosis in acute pharyngitis.

Discussion

Upper respiratory tract infection (URTI) is the most common infectious condition in children, with preschool-aged children experiencing an average of six to eight episodes annually.[25] Pediatric acute pharyngitis, a frequent manifestation of URTI, is characterized by inflammation of the pharyngeal mucosa, submucosal tissue, and associated lymphoid structures.[26] Pediatric acute pharyngitis is a leading cause of outpatient referrals and antibiotic prescriptions for children.[27–29] Although some scholars consider pediatric acute pharyngitis to be a self-limiting condition, antibiotics are still widely prescribed by clinicians to prevent suppurative inflammation and potential complications. LeviE et al[30] reported that group A β-hemolytic streptococcus (GABHS) accounts for approximately 15% to 30% of pediatric cases. However, pharyngeal cultures are performed in only 8% of cases, while antibiotics are prescribed in 55%. Data from pediatric practices in the United States indicate that antibiotics are administered in 60% of acute pharyngitis cases, despite the predominantly viral etiology.[31] These findings highlight a pattern of antibiotic overuse, which may not improve clinical outcomes and raises concerns about antimicrobial resistance.

The therapeutic efficacy of TCM compounds has been widely acknowledged in clinical practice due to their multifaceted mechanisms, targets, and pathways. In the treatment of acute pharyngitis, the TCM principle of syndrome differentiation offers unique advantages, exerting analgesic, antiviral, and anti-inflammatory effects. This approach can promptly alleviate pharyngeal pain symptoms, enhance overall condition, reduce the incidence of complications, and improve the quality of life for pediatric patients.[32,33] Moreover, TCM formulations contribute to the restoration of microecological balance, modulation of immune responses, infection prevention, and mitigation of drug resistance development. These properties position TCM as a promising alternative to address the widespread overuse of antibiotics in pediatric acute pharyngitis.[34] Building on this foundation, the present study focuses on volatile oil-based atomization agents derived from TCM, which offer greater acceptability in pediatric applications. Volatile oil atomization provides a key pharmacokinetic advantage by minimizing the loss of active ingredients and bypassing the hepatic first-pass effect associated with oral administration. Unlike intravenous, oral, or rectal routes, aerosolized delivery allows fine particles of TCM solutions to be uniformly distributed across the respiratory mucosa. This method enhances local drug absorption, enables rapid onset of action, and achieves high concentrations at the target site. Approximately 70% of the inhaled dose can directly reach respiratory mucosa and secretions. Furthermore, this approach hydrates mucosal surfaces, protects epithelial cells and cilia, reduces inflammation, promotes mucus clearance, and improves pulmonary circulation, thereby



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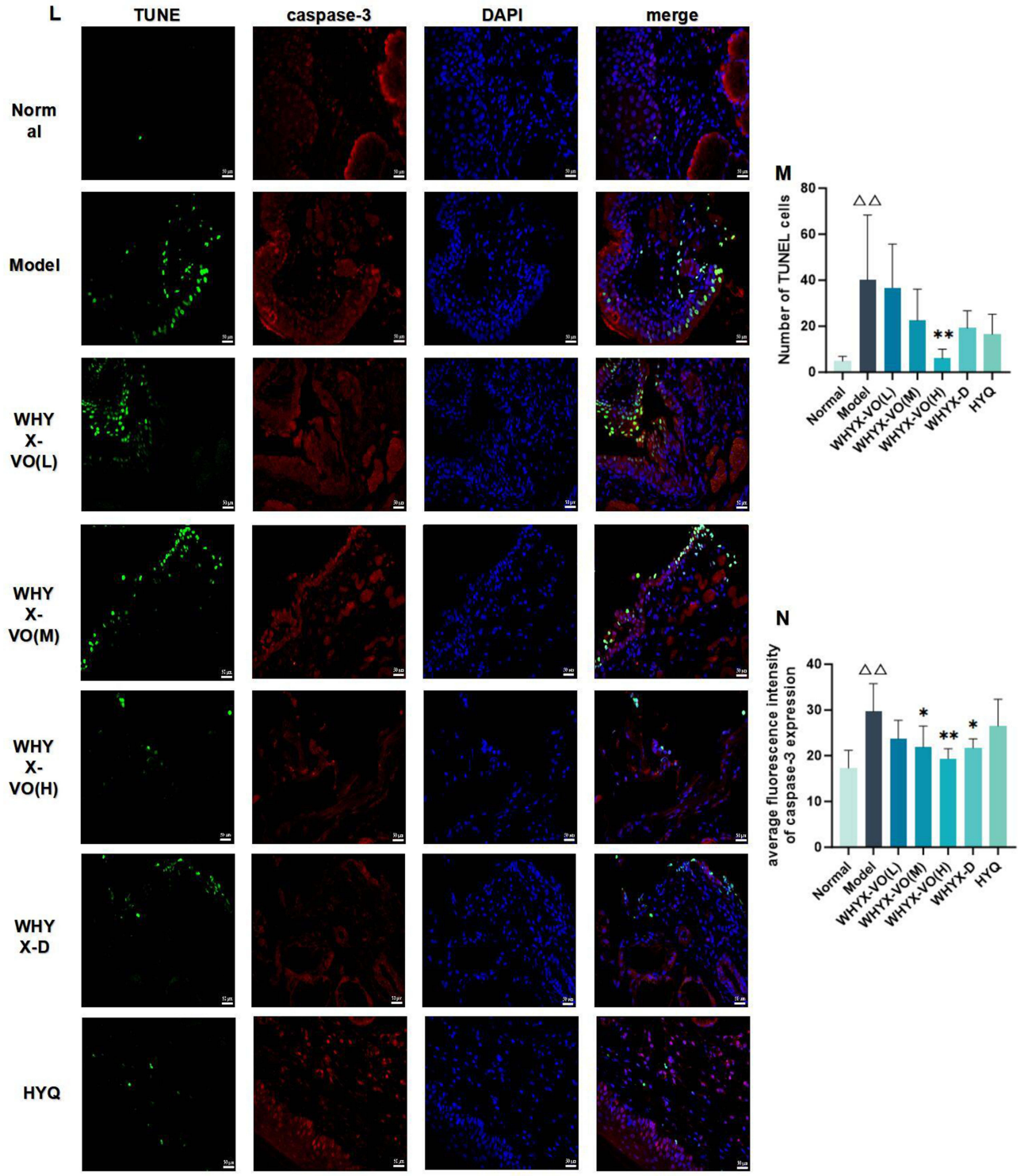

Figure 6 WHYX inhibits TP53-mediated apoptosis. (**A–E**) The relative mRNA levels of TP53, TNF- α, BCL 2, IL-17A, and IL-6 in the pharyngeal mucosal tissue. (**F–K**) Representative protein bands and quantification of protein levels in pharyngeal mucosa. (**L–N**) Immunofluorescence analysis of TUNEL and caspase-3 double staining (Immunofluorescence staining, ×400). Data are expressed as mean±standard deviation (n=6). $^{\Delta\Delta}P < 0.01$, compared with normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with nodel group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with WHYX-VO(H) group. Data are mean ± SD from three independent technical replicates for (**A–N**) and represent three independent biological experiments for (**A–N**).

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effectively relieving clinical symptoms.[18] As a result, volatile oil atomization has emerged as one of the safest and most effective delivery methods for respiratory diseases. In this study, WHYX is an optimized formulation based on the classical "Yinqiao-Mabo decoction". Its primary components include volatile oils from *Arctium Lappa L., Lasiosphaera seu Calvatia., Houttuynia cordata Thunb., Platycodon Grandiflorus (Jacq)., Mentha haplocalyx Briq*. and *Glycyrrhiza uralensis Fisch*. In line with TCM theory, these herbs are traditionally used for heat-clearing, detoxification, and alleviation of pharyngeal discomfort and cough. Numerous studies have confirmed the anti-inflammatory and immunomodulatory effects of each component. For instance, *Fructus Arctii* has been shown to suppress the production and release of pro-inflammatory cytokines such as IL-6,[35] while volatile oil from *Houttuyniae Herba* exhibits potent anti-inflammatory activity.[36]

To investigate the efficacy and mechanism of action of WHYX, we conducted network pharmacology analysis alongside animal experiments. In this study, fourteen major active constituents of WHYX were identified, and 194 shared target genes associated with pediatric acute pharyngitis were screened. A PPI network was constructed based on these targets. GO and KEGG pathway enrichment analyses revealed the pivotal role of TP53 and its associated signaling pathways within the network. Molecular docking analysis demonstrated strong binding affinities between TP53 and several key active compounds in WHYX, including quercetin, kaempferol, luteolin, 7-methoxy-2-methyl-3-phenyl-4H-chromen-4-one, acacetin, and beta-sitosterol. All six compounds were detected through UPLC-MS analysis, supporting the relevance of these interactions. These results provide important mechanistic insights into the pharmacological action of WHYX. Network pharmacology also predicted that WHYX may exert anti-inflammatory effects by modulating cytokines such as TNF-α, IL-17A, and IL-6. Consistent with these predictions, subsequent animal experiments revealed that expression levels of p53 and related inflammatory mediators were significantly altered following WHYX treatment. These findings suggest that WHYX may exert therapeutic effects in pediatric acute pharyngitis by modulating the p53 pathway.

TP53 was the first tumor suppressor gene to be identified and plays a central role in maintaining genomic stability and suppressing tumorigenesis. It regulates critical cellular processes, including cell cycle progression, senescence, and apoptosis. Inflammatory microenvironments, often characterized by elevated reactive oxygen species (ROS) and pro-inflammatory cytokines from activated immune cells, promote the accumulation of TP53 mutations.[37,38] Sustained exposure to these factors can lead to TP53 activation, resulting in cell cycle arrest or apoptosis. Under conditions of severe cellular damage, TP53 favors apoptosis or induces replicative senescence to prevent the propagation of dysfunctional cells.[39] TP53-mediated apoptosis is regulated through several pathways, including death receptor signaling via TNF receptors and Fas.[40] The Bcl-2 family protein Bax, a pro-apoptotic factor, is a well-known downstream effector directly transcriptionally activated by p53-binding sites in its promoter region. In acute pharyngitis, infection, irritation, or injury to the pharyngeal mucosa initiates a local inflammatory response. Activation of immune cells results in the excessive secretion of pro-inflammatory cytokines, including TNF-α, IL-17A, and IL-6, which contribute to the exacerbation of symptoms and accelerate disease progression. These inflammatory signals trigger intracellular stress pathways that activate p53, a key regulator of cellular damage responses. Once activated, p53 initiates the apoptotic program to eliminate damaged epithelial cells, thereby limiting further tissue injury and maintaining mucosal integrity.

Based on the preceding discussion, severe damage to pharyngeal epithelial cells activates TP53, which upregulates pro-apoptotic genes such as BAX and PUMA to eliminate impaired cells and prevent the further spread of inflammation. In pediatric patients, the immaturity of the immune system renders the regulation of TP53 particularly important. Dysfunctional TP53 signaling may lead to uncontrolled inflammation or delayed tissue repair, thereby exacerbating mucosal injury. Bcl-2, a key anti-apoptotic protein, inhibits cytochrome C release by preserving mitochondrial membrane integrity and suppresses the downstream activation of the caspase cascade. Elevated Bcl-2 expression protects mucosal epithelial cells from excessive apoptosis and helps maintain epithelial barrier function during acute inflammation.[41] However, in chronic inflammatory conditions, prolonged Bcl-2 expression may promote abnormal cell survival and persistent immune cell infiltration, contributing to sustained tissue damage.[42] Maintaining a dynamic balance in Bcl-2 expression is essential to prevent the progression from acute to chronic pharyngitis. Caspase-3 functions as a terminal effector of apoptosis, inducing programmed cell death through the cleavage of substrate proteins such as PARP and nuclear lamins. In pathogen infection or chemical irritation models, Caspase-3 activation plays a critical role in eliminating infected cells and limiting pathogen dissemination.[43]

However, excessive activation may lead to extensive detachment of the mucosal epithelium, aggravating symptoms such as sore throat and dysphagia.

Given the thinner and more fragile pharyngeal mucosa in children, moderate regulation of Caspase-3 activity is crucial to achieving a balance between inflammation resolution and tissue repair. The pediatric immune system is highly active but lacks mature regulatory control, making the TP53/Bcl-2/Caspase-3 axis particularly sensitive to infection and pharmacological intervention. Overactivation of apoptosis may delay mucosal healing and impair essential functions such as swallowing and breathing, underscoring the need for precise dosing in therapeutic strategies.

To validate the findings from network pharmacology and further investigate the efficacy and mechanisms of WHYX, an acute pharyngitis model was established in young rats through ammonia spray-induced pharyngeal injury. This method produced acute mucosal irritation, congestion, and swelling, leading to inflammation and activation of the immune response, thus establishing a model of acute pharyngitis relevant to pediatric cases.[24] Serological analyses revealed that, compared to the normal group, the model group exhibited significantly elevated levels of inflammatory cytokines, including IL-6, IL-1β, TNF-α, and PGE2, as well as increased infiltration of innate immune cells. TP53 expression was also upregulated in the model group. Immunofluorescence staining further indicated a significant increase in apoptotic cells, confirming heightened cell death in pharyngeal tissues. Clinically, the model group showed symptoms such as increased mucus secretion, bright red pharyngeal mucosa, persistent coughing and wheezing, dull fur, and reduced vitality. These overall observations suggest a successful model establishment.

Following a 7-day intervention, both high and low-dose groups of WHYX-VO exhibited notable symptom relief, including a significant reduction in pharyngeal mucosal congestion, epithelial hyperplasia, and inflammatory cell infiltration. Serum concentrations of pro-inflammatory cytokines IL-6, IL-1β, TNF-α, and PGE2 were markedly decreased compared to the model group, reflecting a substantial reduction in systemic inflammation. These changes suggest both protective and reparative effects of WHYX on pharyngeal tissue and modulation of immune responses. Besides, significant downregulation of TNF-α, TP53, IL-17A, and IL-6 was observed at both the mRNA and protein levels in pharyngeal tissues. TUNEL and caspase-3 double staining confirmed reduced apoptosis in mucosal epithelial cells, indicating that WHYX mitigates inflammation-induced cell death. All observed effects were dose-dependent, with the high-dose group showing the most pronounced efficacy, further highlighting the therapeutic potential of WHYX in protecting pharyngeal mucosa and regulating inflammatory injury.

Houyanqing granule (HYQ), composed of *Achyranthes bidentata* Bl., *Kalimeris indica* (L). Sch.-Bip., *Plantago asiatica* L. and *Carpesium abrotanoides* L., are strongly recommended by expert consensus for relieving sore throat, fever, and cough in patients with acute pharyngitis.[44] Accordingly, HYQ was selected as the positive control in this study. Given the traditional use of decoction in Chinese medicine, a WHYX decoction group (WHYX-D) was included to assess the comparative efficacy of the volatile oil formulation. Surprisingly, the group of WHYX-VO (H) exhibited greater efficacy than the WHYX-D and HYQ groups in regulating TP53, inhibiting apoptosis, and reducing levels of TNF-α, IL-6, and IL-17A while mitigating the inflammatory response ($P < 0.05$). Notably, the WHYX-VO (H) group exhibited superior efficacy compared to the HYQ positive control group and WHYX-D group, indicating the potential of WHYX as an alternative or adjunct therapy to conventional treatments. The enhanced efficacy of WHYX-VO (H) appears closely linked to its unique mode of administration. Traditional decoction preparation involves prolonged heating and boiling, which often leads to substantial loss of volatile, oil-based active compounds. In contrast, aerosolized delivery of herbal volatile oils allows direct deposition onto inflamed pharyngeal mucosa, ensuring rapid local absorption and avoiding hepatic first-pass metabolism associated with oral delivery. This targeted approach enables a faster onset of action, more concentrated drug exposure at the site of inflammation, and improved mucosal protection. Moreover, aerosol administration is better tolerated by pediatric patients, supporting improved compliance. Experimental data further revealed that WHYX volatile oil may exert therapeutic effects by downregulating the TP53/p53 signaling pathway, thereby suppressing the production of TNF-α and IL-6. Concurrently, modulation of the Bcl-2/Caspase-3 axis contributes to the inhibition of excessive apoptosis. This dual anti-inflammatory and anti-apoptotic mechanism is particularly well-suited for pediatric applications, given the heightened sensitivity and rapid regenerative capacity of children's pharyngeal mucosa. Collectively, these findings support the use of WHYX volatile oil atomization as a safe, effective, and pediatric-friendly therapeutic strategy for managing acute pharyngitis, with the potential to enhance clinical outcomes while reducing reliance on systemic medications.

However, several aspects require further investigation in future studies. Although this research highlights the regulatory effects of WHYX-VO on inflammatory mediators and introduces an innovative therapeutic strategy, the exploration of underlying molecular mechanisms and signaling pathways remains incomplete. Current findings suggest that p53 plays a central role in mediating the immunomodulatory and anti-apoptotic effects of WHYX. For example, TREM-1 has been reported to mediate immunosuppression via cytokines such as IL-8 and IL-1β, while SRMS inhibits apoptosis through the MKK4–JNK–MCL1 pathway.[45,46] Given the central role of TP53 identified through network pharmacology and its consistent regulatory effects observed in animal experiments, future studies will focus on elucidating the detailed mechanisms underlying its involvement in the therapeutic action of WHYX.

Conclusion

The WHYX formula significantly alleviates pharyngeal inflammation by downregulating pro-inflammatory markers such as TNF-α and TP53, suppresses apoptosis, and reduces pathological damage in a rat model of pediatric acute pharyngitis. Through its active ingredients, WHYX volatile oil atomization provides a targeted, non-invasive, and effective treatment strategy. Its rapid action, high local bioavailability, and pediatric-friendly administration make it a promising candidate for the clinical management of pediatric acute pharyngitis.

Abbreviations

BC, Betweenness Centrality; OB, bioavailability; CC, Closeness Centrality; DC, Degree Centrality; DL, drug-likeness; ELISA, Enzyme-linked immunosorbent assay; GABHS, group A β-hemolytic streptococcus; HPLC, high-performance liquid chromatography; HYQ, HouYanQing granule group; NC, Neighborhood Connectivity; qRT-PCR, real-time quantitative polymerase chain reaction; ROS, reactive oxygen species; SEA, Similarity ensemble approach; TCM, Traditional Chinese medicine; URTI, Upper respiratory tract infection; WBC, white blood cell; WHYX, Wu-Hua-Yan-Xiao; WHYX-VO(H), WuHuaYanXiao-volatile high-dose group; WHYX-VO(L), WuHuaYanXiao-volatile low-dose group; WHYX-VO(M), WuHuaYanXiao-volatile medium-dose group.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare that there is no conflict of interest for this work.

References

1. Sun H, Zhang L. *Otolaryngology, Head and Neck Surgery*. Beijing: People's Medical Publishing House; 2018:275.
2. Linder JA, Bates DW, Lee GM, Finkelstein JA. Antibiotic treatment of children with sore throat. *JAMA*. 2005;294(18):2315–2322. doi:10.1001/jama.294.18.2315

3. Zahid M, Masoumeh G. Diagnostic Methods, Clinical Guidelines, and Antibiotic Treatment for Group A Streptococcal Pharyngitis: a Narrative Review. *Front Cell Infect Mi*. 2020;10:563627. doi:10.3389/fcimb.2020.563627
4. Tian YQ. *Otolaryngology, Head and Neck Surgery*. 7th edition. Beijing: People's Medical Publishing House; 2008. Vol. 2008: 136–138.
5. Gilbey P, Livshits L, Sharabi-Nov A, Avraham Y, Miron D. Probiotics in addition to antibiotics for the treatment of acute tonsillitis: a randomized, placebo-controlled study. *Eur J Clin Microbiol Infect Dis*. 2015;34(5):1011–1015. doi:10.1007/s10096-015-2315-z
6. Roggen I, Berlaer VG, Gordts F, Pierard D, Hubloue I. Acute sore throat in children at the emergency department: best medical practice? *Eur J Emerg Med*. 2015;22(5):343–347. doi:10.1097/MEJ.0000000000000175
7. PUMCH. The Eleventh Revised International Classification of Diseases (ICD-11) Global Information Standard for Diagnostic Health. 2021. Available from: https://icd11.pumch.cn/. Accessed Oct 25, 2024.
8. Dao VA, Overhagen S, Bilstein A, Kolot C, Sonnemann U, Mösges R. Ectoine lozenges in the treatment of acute viral pharyngitis: a prospective, active-controlled clinical study. *Eur Arch Otorhinolaryngol*. 2019;276(3):775–783. doi:10.1007/s00405-019-05324-9
9. Zhang YM, Zhang TY. *Practical Pediatric Otolaryngology Science*. Vol. 2011. Beijing: People's Medical Publishing House; 2011.
10. Department of Pediatric Pharmacy, Hospital Pharmacy Committee of Chinese Pharmaceutical Association. Guiding Opinions on Clinical Rational Use of Antibacterials in Pediatrics. *J. Pediatr. Pharm*. 2006;04:46–51. doi:10.13407/j.cnki.jpp.1672-108x.2006.04.022
11. Harris AM, Hicks LA, Qaseem A. and High Value Care Task Force of the American College of Physicians and for the Centers for Disease Control and Prevention. *Ann Intern Med*. 2016;164(6):425–434. doi:10.7326/M15-1840
12. Tadesse M, Hailu Y, Biset S, Ferede G, Gelaw B. Prevalence, Antibiotic Susceptibility Profile and Associated Factors of Group A Streptococcal pharyngitis Among Pediatric Patients with Acute Pharyngitis in Gondar, Northwest Ethiopia. *Infect Drug Resist*. 2023;16:1637–1648. doi:10.2147/IDR.S402292
13. Chen HF, Sun XJ, Wu LY. Overview of Yinqiao-Mabo decoction's clinical application in pediatrics. *Chin Pediat Integr Trad West Med*. 2020;12(6):496–498.
14. Ke S, Xu L, Li SF, Zhang YB, Lan ZH, Liu LJ. Experience of Hong Guangxiang in Treating Cough Variant Asthma with Wenluo Decoction Combined with Yinqiao Maboge San. *J Tradit Chin Med*. 2022;63(01):8–11. doi:10.13288/j.11-2166/r.2022.01.003
15. Xiong JX, Zhao XM, Zhang QZ. Fifty cases of treating children's upper airway cough syndrome with Yinqiao Maboge San combined with Shengxian San and acupoint patch application. *Jiangxi Zhongyiyao*. 2022;53(03):53–55.
16. Shen QJ. Effect of aerosolized inhalation therapy for acute pharyngitis in children. *Health Frontier*. 2016;23(6):30.
17. Lu M, Tan MJ, Jiang L. Clinical efficacy of open-throat sword spray for pediatric acute pyogenic tonsillitis. *Chin. J. Biochem. Pharm*. 2017;37(1):113–115.
18. Li SC, Tao YK. Clinical application of TCM for aerosol inhalation. *West China J. Pharm. Sci*. 2019;34(5):532–537. doi:10.13375/j.cnki.wcjps.2019.05.020
19. He H, Zhang XR, Zhao SS, Zhu XY, Cui ZJ, Shao J. Network pharmacology study on Radix Taraxaci-Margareti and Glycyrrhiza Decoction in the treatment of pharyngitis and establishment of a HPLC method for simultaneous determination of 13 active components. *Yaowu Fenxi Zazhi*. 2022;42(09):1522–1536. doi:10.16155/j.0254-1793.2022.09.05
20. Ha MA, Smith GJ, Cichocki JA, et al. Menthol attenuates respiratory irritation and elevates blood cotinine in cigarette smoke exposed mice. *PLoS One*. 2015;10(2):e0117128. doi:10.1371/journal.pone.0117128
21. Yang R, Chen XH, Li J, He J, Chang YX. Research Progress on Chemical Components and Pharmacological Activities of Mentha. *Tianjin Zhongyiyao Daxue Xuebao*. 2022;41(01):4–13.
22. Chen X, Zhang S, Xuan Z, et al. The Phenolic Fraction of Mentha haplocalyx and Its Constituent Linarin Ameliorate Inflammatory Response through Inactivation of NF-κB and MAPKs in Lipopolysaccharide-Induced RAW264.7 Cells. *Molecules*. 2017;22(5):811. doi:10.3390/molecules22050811
23. Cai HX, Liu JN, Chen SJ, Cao G, Chen HD. Research progress on chemical constituents, biological activities and clinical applications of Houttuynia cordata. *Zhongchengyao*. 2019;41(11):2719–2728.
24. Miao MS, Chang BJ, Bai M, Bai L. Specification for animal model preparation of acute pharyngitis (draft). *Pharmacol. Clin. Chin. Mater. Med*. 2018;34(01):175–178. doi:10.13412/j.cnki.zyyl.2018.01.044
25. Korppi M, Heikkilä P, Palmu S, Huhtala H, Csonka P. Antibiotic prescribing for children with upper respiratory tract infection: a Finnish nationwide 7-year observational study. *Eur J Pediatr*. 2022;181(8):2981–2990. doi:10.1007/s00431-022-04512-w
26. Zhang F, Shi XY, Zhao D, et al. Virus spectrum and epidemic characteristics of acute upper respiratory tract infection in Qingdao during 2017–2018. *Chin J Dis Control Prev*. 2019;23(6):694–699. doi:10.16462/j.cnki.zhjbkz.2019.06.015
27. Barbieri E, Donà D, Cantarutti A, et al. Antibiotic prescriptions in acute otitis media and pharyngitis in Italian pediatric outpatients. *Ital J Pediatr*. 2019;45(1):103. doi:10.1186/s13052-019-0696-9
28. Dona D, Baraldi M, Brigadoi G, et al. The impact of clinical pathways on antibiotic pre-scribing for acute otitis media and pharyngitis in the emergency department. *Pediatr Infect Dis J*. 2018;37(9):901–907. doi:10.1097/INF.0000000000001976
29. Sykes EA, Wu V, Beyea MM, Simpson MTW, Beyea JA. Pharyngitis: approach to diagnosis and treatment. *Can Fam Physician*. 2020;66(4):251–257.
30. Levi E, Ronen O. Community clinic and emergency department physicians' adherence to acute pharyngitis antibiotic treatment guidelines. *Eur J Clin Invest*. 2021;51(1):e13355. doi:10.1111/eci.13355
31. Dooling KL, Shapiro DJ, Van BC, Hersh AL, Hicks LA. Overprescribing and inappropriate antibiotic selection for children with pharyngitis in the United States, 1997-2010. *JAMA Pediatr*. 2014;168(11):1073–1074. doi:10.1001/jamapediatrics.2014.1582
32. Chen Y, Su P, Zhang HJ, Zhang GP, Ye ZG. Exploring the mechanism of anti-diarrhea effect of Mongolian Sulongga-4 particles based on network pharmacology. *Drug Eval. Res*. 2020;43(7):1259–1266.
33. Zhang X, Xie YM, Li G, et al. Advantages and problems of TCM in treating acute pharyngitis. *China J Chin Mater*. 2017;42(19):3819–3825.
34. Wu W, Sun YZ, Liu DX, et al. Treatment of clinical dominant diseases in the field of otolaryngology head and neck surgery. *Chin J Exp Tradit Med. Formulae*. 2021;27(14):208–214. doi:10.13422/j.cnki.syfjx.20211497
35. Han YH, Mun JG, Jeon HD, et al. The Extract of Arctium lappa L. Fruit (Arctii Fructus) Improves Cancer-Induced Cachexia by Inhibiting Weight Loss of Skeletal Muscle and Adipose Tissue. *Nutrients*. 2020;12(10):3195. doi:10.3390/nu12103195
36. Nan HY, Zhang WJ, Zhang JM, Xu BL. Pharmacological effects of volatile oil from supercritical CO2. *Strait Pharmaceutical Journal*. 2006;18(3):46–48.

37. Hofseth LJ, Hussain SW, Wogan GN, Harris CC. Nitric oxide in cancer and chemoprevention. *Free Radic Biol Med*. 2003;34(8):955–968. doi:10.1016/s0891-5849(02)01363-1
38. Vendramini-Costa DB, Carvalho JE. Molecular link mechanisms between inflammation and cancer. *Curr. Pharm. Des*. 2012;18(26):3831–3852. doi:10.2174/138161212802083707
39. Carvajal LA, Manfredi JJ. Another fork in the road—life or death decisions by the tumour suppressor p53. *EMBO Rep*. 2013;14(5):414–421. doi:10.1038/embor.2013.25
40. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*. 2000;408(6810):307–310. doi:10.1038/35042675
41. Radha G, Raghavan SC. BCL2: a promising cancer therapeutic target. *Biochimica Et Biophysica Acta. Reviews on Cancer*. 2017;1868(1):309–314. doi:10.1016/j.bbcan.2017.06.004
42. Kaloni D, Diepstraten ST, Strasser A, Kelly GL. BCL-2 protein family: attractive targets for cancer therapy. *Apoptosis*. 2022;28(1–2):20–38. doi:10.1007/s10495-022-01780-7
43. Li F, He Z, Shen J, et al. Apoptotic caspases regulate induction of iPSCs from human fibroblasts. *Cell Stem Cell*. 2010;7(4):508–520. doi:10.1016/j.stem.2010.09.003
44. Yan R, Daxin L, Weiping H, Yu G. Expert consensus on the clinical application of oral liquid (granules). *Chin J Otorhinol Integ Med*. 2022;30(4):241–245. doi:10.16542/j.cnki.issn.1007-4856.2022.04.001
45. Jiang Y, Song L, Lin Y, et al. ROS-mediated SRMS activation confers platinum resistance in ovarian cancer. *Oncogene*. 2023;42(20):1672–1684. doi:10.1038/s41388-023-02679-6
46. Pullikuth AK, Routh ED, Zimmerman KD, et al. Bulk and Single-Cell Profiling of Breast Tumors Identifies TREM-1 as a Dominant Immune Suppressive Marker Associated With Poor Outcomes. *Frontiers in Oncology*. 2021;11:734959. doi:10.3389/fonc.2021.734959