Network Pharmacology and Molecular Docking Analysis to Explore the Mechanism of Huaiqihuahuang-Mediated Alleviation of Henoch–Schönlein Purpura Nephritis

Qingqing Liu,1 Jiahua Liu,2 Yaya Du,1 Weiyan Guo,3 Jie Mi,4 and Yanyan Guo1

1Precision Pharmacy & Drug Development Center, Department of Pharmacy, Tangdu Hospital, Air Force Medical University, Xi’an, Shaanxi 710038, China
2Department of Clinical Laboratory, Xi’an Children’s Hospital, Xi’an, Shaanxi 710003, China
3Department of Pharmacy, The First Hospital of Xi’an, Xi’an, Shaanxi 710002, China
4Department of Pharmacy, Xi’an Children’s Hospital, Xi’an, Shaanxi 710003, China

Correspondence should be addressed to Jie Mi; mijie666@163.com and Yanyan Guo; gyy712@163.com

Received 13 August 2022; Accepted 8 October 2022; Published 4 November 2022

Objective. Henoch–Schönlein purpura nephritis (HSPN) is considered a major cause of chronic renal failure and is the most common secondary glomerular disease in children. Huaiqihuahuang (HQH), a traditional Chinese herbal formula, exhibits therapeutic effects against HSPN in clinical practice. However, the potential molecular targets and mechanisms underlying HSPN treatment remain unclear.

Methods. By constructing a protein-protein interaction (PPI) network, core targets related to HQH and HSPN were identified. Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes pathways were analyzed to identify the main pathways related to HSPN based on the core targets. To screen the main active ingredients of HQH against HSPN, an ingredient-target-pathway network was constructed using the top 10 main pathways associated with HSPN. Then, molecular docking was performed to explore the interactions and binding patterns between molecules and proteins.

Results. Clinical data showed that HQH combined with conventional medicine significantly reduced 24-hour urine protein excretion, urine microalbumin levels, and erythrocyte counts in the urine sediment of HSPN patients. By constructing PPI models, 15 potential core targets were identified. The top 10 main pathways showed higher enrichment ratios, including the cytokine–cytokine receptor interaction and signaling pathways related to NOD-like receptor, IL-17, etc. Through the ingredient-target-pathway network and molecular docking, we revealed that five active ingredients of HQH had good affinities with three core targets, AKT1, MMP9, and SERPINE1, which may be vital in treating HSPN.

Conclusions. The study preliminarily explored the active ingredients, targets, and pathways involved in HQH therapy for HSPN. The mechanism of HQH therapy may be attributed to the modulation of inflammatory response, immune response, and oxidative stress. Combined with clinical data, our results indicate that HQH is highly effective in treating HSPN.

1. Introduction

In childhood, Henoch–Schönlein purpura nephritis (HSPN) is a common vasculitis disease [1]. HSP can affect glomerular capillaries and interstitial blood vessels and cause kidney damage. Henoch–Schönlein purpura nephritis (HSPN) is the most severe complication of HSP. Approximately 30–50% children with HSP develop HSPN [2]. If HSPN is left untreated, it eventually leads to an unexpected chronic kidney disease in up to 20% of affected children [3], which can be further life-threatening.

Currently, glucocorticoid steroids combined with immunosuppressive therapy are recommended to treat HSPN [4]. However, clinical data suggest that this treatment has evident
side effects such as gastrointestinal diseases, oncogenesis, and myelosuppression [5]. Therefore, it is necessary to identify new, effective, and safe strategies for the treatment of HSPN. In recent years, traditional Chinese medicine (TCM), including Tripterygium glycosides [6] and Huaqiuhuang (HQH) [7], has shown prominent effects in preventing and treating HSPN.

HQH is a traditional Chinese herbal formula, which comprises Trametes robianiphila Murr (Huaier), Polypodium sibiricum (HJ), and Lycium barbarum (GQZ). HQH has been used as an adjuvant for primary nephropathy, mycoplasma pneumonia, and bronchial asthma in children [5, 8]. HQH exhibits a significant therapeutic effect against kidney diseases by enhancing immune function and reducing proteinuria and hematuria [9, 10]. Moreover, HQH compensates for the defects of cyclophosphamide (CYP) by reducing nephrotoxicity [11]. However, our understanding of HQH in HSPN treatment remains unclear.

It has been widely known that network pharmacology can explain the complex mechanisms and multiple effects of TCM [12]. Molecular docking is performed to predict the target proteins and active molecules. In the present study, we used network pharmacology and molecular docking to explore potential targets and molecules to understand the mechanism of action of HQH in HSPN treatment. Figure 1 depicts the process flowchart in detail.

2. Methods

2.1. Analysis of HQH Clinical Efficacy on HSPN. This study included 30 children diagnosed with HSPN who underwent drug treatment in the Xi’an Children’s Hospital from June 2021 to December 2021. All data were sourced retrospectively from the hospital information system. The control group consisted of 15 children who received conventional treatment with an oral prednisone tablet and an intravenous infusion of CYP. The remaining 15 children in the experimental group were administered HQH granules combined with the conventional treatment. Renal function indicators, including 24-hour urine protein excretion, urinary microalbumin, and erythrocyte count in urine sediment, were assessed before and after 3-month treatment. SPSS 20.0 software was used for data processing and analysis.

The inclusion criteria were as follows: (1) Clinical Guidelines for Nephrology [13] was used for diagnosis of HSPN; (2) all cases and laboratory data were available; and (3) the patients showed good compliance.

The exclusion criteria were as follows: (1) glomerulonephritis, IgA nephropathy, and other kidney diseases; (2) allergic to the medicine during treatment; and (3) patients with other autoimmune diseases.

The outcome was measured based on (1) 24-hour urine protein excretion, (2) urinary microalbumin content, and (3) erythrocyte count in urine sediment.

2.2. Search and Collection of Active Ingredients of HJ and GQZ. The Chinese State Food and Drug Administration has authorized the application of Huaier granules to treat various cancers. Evidence showed that proteoglycans were active ingredients of Huaier [14]. The active ingredients of HJ and GQZ were searched in the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (http://tcmsp.com/tcmsp.php) conforming to oral bioavailability ≥ 30% and drug-likeness ≥ 0.18 [15]. The structure-data file (SDF) format of all active ingredients was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for subsequent prediction of potential targets and molecular docking.

2.3. Identification of the Active Ingredient Targets and Disease-Related Genes. The SDF files of active ingredients were imported into the Swiss Target Prediction database (http://www.swisstargetprediction.ch) to obtain active ingredient targets. Since the targets for Huaier could not be obtained here, we searched and collected them from PubMed and China National Knowledge Infrastructure (CNKI) databases.

Disease-related genes were obtained through GeneCards (https://www.genecards.org/), OMIM (https://www.omim.org/), and DisGeNET (http://www.disgenet.org) with the keywords “Henoch–Schönlein purpura nephritis.” The genes were limited to “Homo sapiens.” All genes were downloaded and integrated using Microsoft Excel.

2.4. Protein-Protein Interaction (PPI) Network. Venn diagrams (https://bioinfogp.cnb.csic.es/tools/venny_old/) were generated to determine the common targets between HQH and disease. Then, the PPI network of common targets was constructed by the STRING database (https://string-db.org/). “Homo sapiens” was chosen, and a medium confidence score > 0.4 was selected. The above results were imported into Cytoscape (v3.7.2) (https://cytoscape.org/) for visualization. These targets were defined as core targets if their degree value exceeded the average.

2.5. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis. The Metascape database (http://metascape.org/) was used to perform GO and KEGG analysis. The core targets were imported, and “Homo sapiens” was selected. An online bioinformatics web (http://www.bioinformaticss.com.cn) was used to visualize the results. The cutoff chosen for the p value was <0.01.

2.6. Molecular Docking. Molecular docking was carried out using Discovery Studio 2021 to explore the interactions and binding patterns between molecules and proteins. The active ingredients (ligands) of HQH were stored as SDF files. The main protein targets were obtained from the Protein Data Bank (http://rcsb.org/). Additionally, the best protein crystal structure was characterized based on the images with a low resolution with observable ligands and a relatively intact structure. The ligands were prepared using the Ligand Preparation module and minimized using the CHARMM force field to generate 10 conformers. Using the Receptor-Ligand Pharmacophore Generation module to define the ligand-binding site, original ligands were extracted from the target proteins. The docking results were evaluated based on -CDocker energy, hydrogen bond interaction, and the pattern of binding mode.
3. Results

3.1. HQH Enhanced Conventional Medicine Efficacy for Treating HSPN Children. In our study, the experimental group consisted of eight boys and seven girls aged between 3.75 and 13.33 (8.15 ± 2.7) years. The control group consisted of ten boys and five girls, and their ages ranged from 3.75 to 12.25 (9.03 ± 2.28) years. The degree of renal function damage is a prognostic factor for HSPN, as HSPN manifestation is primarily marked by abnormal urine, including a decrease in proteinuria and hematuria. In clinics, 24-hour urine protein excretion, urine microalbumin, and erythrocyte counts in urine sediment are used as renal function indices. Therefore, we used these three indices to evaluate the
| Drug source | Molecule ID | Active ingredients | OB (%) | DL |
|-------------|-------------|---------------------|--------|----|
| GQZ         | MOL001323   | Sitosterol alpha 1  | 43.28  | 0.78 |
|             | MOL03578    | Cycloartenol        | 38.69  | 0.78 |
|             | MOL001494   | Mandenol            | 42     | 0.19 |
|             | MOL001495   | Ethyl linolenate    | 46.1   | 0.2  |
|             | MOL001979   | LAN                 | 42.12  | 0.75 |
|             | MOL000449   | Stigmasterol        | 43.83  | 0.76 |
|             | MOL000358   | Beta-sitosterol     | 36.91  | 0.75 |
|             | MOL005406   | Atropine            | 45.97  | 0.19 |
|             | MOL005438   | Campesterol         | 37.58  | 0.71 |
|             | MOL006209   | Cyanin              | 47.42  | 0.76 |
|             | MOL007449   | 24-Methylidenelophranol |   | 44.19 | 0.75 |
|             | MOL008173   | Daucoosterol_qt     | 36.91  | 0.75 |
|             | MOL008400   | Glycitein           | 50.48  | 0.24 |
|             | MOL010234   | Delta-carotene      | 31.8   | 0.55 |
|             | MOL009653   | CLR                 | 37.87  | 0.68 |
|             | MOL009640   | 14b-Pregnane        | 34.78  | 0.34 |
|             | MOL009612   | (24R)-4Alpha-methyl-24-ethylcholesta-7,25-dien-3beta-ylacetate | 46.36 | 0.84 |
|             | MOL009615   | 24-Methylencycloartan-3beta,21-diol | 37.32 | 0.8 |
|             | MOL009617   | 24-Ethylcholest-22-enol | 37.09 | 0.75 |
|             | MOL009618   | 24-Ethylcholesta-5,22-dienol | 43.83 | 0.76 |
|             | MOL009620   | 24-Methyl-31-norlanost-9(11)-enol | 38 | 0.75 |
|             | MOL009621   | 24-Methylenealanes-8-enol | 42.37 | 0.77 |
|             | MOL009622   | Fucosterol          | 43.78  | 0.76 |
|             | MOL009631   | 31-Norcyclolaudenol | 38.68  | 0.81 |
|             | MOL009633   | 31-Norlanost-9(11)-enol | 38.35 | 0.72 |
|             | MOL009634   | 31-Norlanosterol    | 42.2   | 0.73 |
|             | MOL009635   | 4,24-Methylphenol   | 37.83  | 0.75 |
|             | MOL009639   | Lophenol            | 38.13  | 0.71 |
|             | MOL009640   | 4Alpha,14alpha,24-trimethylcholesta-8,24-dienol | 38.91 | 0.76 |
|             | MOL009641   | 4Alpha,24-dimethylcholesta-7,24-dienol | 42.65 | 0.75 |
|             | MOL009642   | 4Alpha-methyl-24-ethylcholesta-7,24-dienol | 42.3 | 0.78 |
|             | MOL009644   | 6-Fluoroinodo-7-dehydrocholesterol | 43.73 | 0.72 |
|             | MOL009646   | 7-O-Methylulutein-6-C-beta-glucoside_qt | 40.77 | 0.3 |
|             | MOL009650   | Atropine            | 42.16  | 0.19 |
|             | MOL009651   | Cryptoxanthin monoeoxide | 46.95 | 0.56 |
|             | MOL009653   | Cycloecacalolol     | 39.73  | 0.79 |
|             | MOL009656   | (E,E)-1-Ethyl octadeca-3,13-dienoate | 42 | 0.19 |
|             | MOL009660   | Methyl (1R,4aS,7R,7aS)-4a,7-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,5,6,7a-tetrahydrocyclopenta[d]pyran-4-carboxylate | 39.43 | 0.47 |
|             | MOL009662   | Lantadene A         | 38.68  | 0.57 |
|             | MOL009664   | Physalin A          | 91.71  | 0.27 |
|             | MOL009665   | Physcion-8-O-beta-D-gentiobioside | 43.9 | 0.62 |
|             | MOL009677   | Lanost-8-en-3beta-ol | 34.23 | 0.74 |
|             | MOL009678   | Lanost-8-enol       | 34.23  | 0.74 |
|             | MOL009681   | Obtusifoliol        | 42.55  | 0.76 |
|             | MOL00098    | Quercetin           | 46.43  | 0.28 |
therapeutic effect of HQH in this study. Before treatment, the groups showed no significant differences in 24-hour urine protein excretion, urine microalbumin levels, and erythrocyte counts in urine sediment ($p > 0.05$). However, all indices significantly decreased after 3-month treatment in both groups ($p < 0.05$). Moreover, the experimental group showed indices lower than the control group ($p < 0.05$) (Table 1). Therefore, HQH was effective as an adjuvant for treating HSPN.

3.2. Identification of Active Ingredients of HQH and the Corresponding Targets. The total number of active ingredients selected from GQZ and HJ was 45 and 12, respectively (Table 2). The Swiss Target Prediction database suggested more than 524 targets of GQZ and HJ, and 482 targets related to GQZ-HJ were selected after removing duplicates for further studies. Another component of HQH is Huaier, which is a fungus. The PubMed and CNKI databases suggested 73 valid targets of Huaier. Taken together, we identified 540 HQH targets for further study.

3.3. Identification of Target Genes of the Diseases. We searched for HSPN-related genes using GeneCards, DisGeNET, and OMIM and identified 84, 29, and 63 HSPN-related genes, respectively. After removing the duplicate genes and integrating the results, 157 candidate genes were identified.

3.4. Acquiring the Core Targets by PPI Network. To obtain the potential key genes targeted by HQH during HSPN treatment, we considered the overlap of HQH targets and disease-related genes and identified 24 common targets correlated with HSPN (Figure 2), including AKT1, CCL2, CXCL8, ESR1, IL10, IL18, IL6, STAT3, RELA, IL1β, TLR4, TNF, TGFβ1, CFD, F2, IGF1, ILK, MIF, MMP9, MPO, NR3C1, PRSS1, SERPINE1, and TTR. Importantly, AKT1, MMP9, ESR1, and STAT3 were correlated with Huaier, GQZ-HJ, and HSPN.

The degree parameter was calculated to analyze the importance of common targets in the PPI network using
Cytoscape software. A total of 24 nodes and 179 edges were visualized (Figure 3). Larger size and darker color indicate a higher degree of nodes, indicating a stronger interaction with other targets. All targets with degree values greater than the average were considered core targets (average degree = 14.90). The core targets related to HSPN are shown in Table 3, which include IL6, TNF, AKT1, CCL2, MMP9, SERPINE1, IL1B, CXCL8, IL10, STAT3, IGF1, TLR4, TGFβ1, IL18, and ESR1.

3.5. KEGG Pathway and GO Analysis. In total, 79 KEGG pathways were significantly enriched. These pathways were mainly related to bacterial and viral diseases and signal transduction pathways (Figure 4(a)). Because we aimed to explore the potential biological mechanism of HSPN, pathway terms directly related to other diseases and different functional categories were removed. The HSPN-related signal transduction pathways were further analyzed and are illustrated in Figure 4(b). Compared with other pathways, the top 10 main pathways are presented in Table 4.

GO analysis was performed to explore the molecular functions, biological processes, and cellular components of the core targets (Figures 4(c)–4(e)). By setting the filter as a p value cutoff < 0.01, the molecular function of 15 core targets primarily focused on cytokine activity, receptor-ligand interaction, activity of growth factors, and growth factor-receptor binding. The biological processes of HSPN mainly involve the inflammatory response, regulation of cell migration, regulation of cell proliferation and apoptosis by modulating the cellular response to lipopolysaccharide, leukocyte migration, and regulation of interferon-6 production. Cellular components were related to the side of the membrane, extracellular matrix, vesicle lumen, and platelet alpha granules. In summary, we found that the 15 core targets were enriched in signaling pathways and biological mechanisms associated with inflammation and immune response.

3.6. Construction of the Ingredient-Target-Pathway Network. To understand which ingredient regulates these core targets for treating HSPN through the top 10 main pathways, we constructed the ingredient-target-pathway interaction network using Cytoscape. ESR1 was excluded from the network because it was not enriched in these pathways. The remaining 14 core targets were identified by Huaier and different active ingredients from HJ and GQZ, including MOL003889, MOL009760, MOL000098, MOL001792, MOL004941, MOL009646, MOL009644, and MOL006331 (Figure 5(a)). Finally, we screened eight active ingredients from this network for further analysis.

Furthermore, we found that MOL003889, MOL009760, MOL006331, MOL001792, and MOL004941 were correlated to HJ, and MOL009646, MOL009644, and MOL000098 were associated with GQZ (Figure 5(b)). Our results showed that Huaier mainly acted on targets associated with immunity and inflammation, such as IL6, IL10, IL1β, TLR4, and CCL2. The three common targets, AKT1, MMP9, and STAT3, were modulated by Huaier, MOL000098, MOL006331, MOL003889, and MOL009760. In addition, MOL001792, MOL004941, MOL009646, and MOL009644 interacted with SERPINE1 and IGF1. Therefore, Huaier and active ingredients from HJ and GQZ may be utilized to treat HSPN by regulating their respective targets.

3.7. Molecular Docking. To verify whether the eight active ingredients could interact with targets, we used molecular docking to analyze their binding mode and affinity. We set the top hit to 10 and pose cluster radius to 0.5 to ensure as diverse docking conformations as possible. A high -CDOCKER energy indicates a high affinity between the molecules and protein receptors. We selected ingredients with lower -CDOCKER energy than that of the original ligand and the target protein as potentially active molecules.

Table 3: Information of core targets from HQH for HSPN treatment.

| No. | Name | Degree | No. | Name | Degree |
|-----|------|--------|-----|------|--------|
| 1   | IL6  | 21     | 13  | TGFβ1| 16     |
| 2   | TNF  | 21     | 14  | IL18 | 16     |
| 3   | AKT1 | 20     | 15  | ESR1 | 15     |
| 4   | CCL2 | 20     | 16  | F2   | 14     |
| 5   | MMP9 | 20     | 17  | RELA | 14     |
| 6   | SERPINE1| 19   | 18  | MPO  | 13     |
| 7   | IL1B | 19     | 19  | NR3C1| 12     |
| 8   | CXCL8| 19     | 20  | MIF  | 11     |
| 9   | IL10 | 19     | 21  | ILK  | 5      |
| 10  | STAT3| 18     | 22  | CFD  | 4      |
| 11  | IGF1 | 18     | 23  | TTR  | 4      |
| 12  | TLR4 | 18     | 24  | PRSS1| 2      |
Figure 4: Continued.
Except for STAT31 and IGF1, other active ingredients showed good affinity to the targets. Five bioactive molecules were screened based on their energy, as shown in Table 5. Among these, MOL000098 had the highest affinity for AKT1. MOL000098 interacted with Lys179, Asp292, Asn279, and Glu278 via hydrogen bonding and entered the active pocket of AKT1 (Figure 6). MOL000098 and MOL006331 fit well within the active pocket of MMP9 and interacted with Ala189, Gln227, and Asp249 to form hydrogen bonds. MOL001792, MOL004941, and MOL009646 occupied the active pocket of SERPINE1 and formed stable hydrogen bonds with Asp95, Arg76, and Tyr37. The names and structures of the potentially active ingredients are listed in Table 6.

4. Discussion

HQH is effective in treating HSPN. However, its active ingredients and underlying biological mechanisms are unclear. Our study prospectively predicts the bioactive ingredients and potential targets of HQH for HSPN treatment from the perspective of network pharmacology. We found that HQH improved kidney function indices to enhance the efficacy of conventional medicine in treating children with HSPN. Second, network pharmacology demonstrated that HQH affected 15 core targets related to HSPN. Subsequently, GO and KEGG enrichment analyses showed that these core targets may participate in the immune and inflammatory responses and oxidative stress.
Finally, molecular docking defined suitable active ingredients and potential target proteins. Together, our results suggest that the five ingredients from HJ and GQZ could treat HSPN by regulating AKT1, MMP9, and SERPINE1 with the exception of Huaier.

HSPN is a self-limiting immune disease, which can cure spontaneously or lead to chronic kidney disease. Our results showed that HQH combined with conventional medicine significantly reduced 24-hour urine protein excretion, urine microalbumin levels, and erythrocyte counts in urine sediment. Therefore, HQH can alleviate kidney damage and enhance the effect of conventional medicines on HSPN. In addition, conventional medicines, such as CYP, increase nephrotoxicity while treating HSPN [16]. Importantly, HQH has a protective effect against CYP-induced nephrotoxicity.

Table 4: Top 10 KEGG pathway analyses for the treatment of HSPN.

| Rank | Pathways                                      | p value | Enrichment | Targets                      |
|------|-----------------------------------------------|---------|------------|------------------------------|
| 1    | Cytokine-cytokine receptor interaction        | $4.57 \times 10^{-13}$ | 0.027119   | IL1B, IL6, CXCL8, IL10, IL18, CCL2, TGFB1, TNF |
| 2    | NOD-like receptor signaling pathway           | $1.72 \times 10^{-12}$ | 0.038043   | IL1B, IL6, CXCL8, IL18, CCL2, TLR4, TNF |
| 3    | IL-17 signaling pathway                       | $3.78 \times 10^{-12}$ | 0.06383    | IL1B, IL6, CXCL8, MMP9, CCL2, TNF |
| 4    | Viral protein interaction with cytokine and cytokine receptor | $5.53 \times 10^{-12}$ | 0.06       | IL6, CXCL8, IL10, IL18, CCL2, TNF |
| 5    | Toll-like receptor signaling pathway          | $7.04 \times 10^{-12}$ | 0.057692   | AKT1, IL1B, IL6, CXCL8, TLR4, TNF |
| 6    | HIF-1 signaling pathway                       | $9.38 \times 10^{-12}$ | 0.055046   | AKT1, IGF1, IL6, SERPINE1, STAT3, TLR4 |
| 7    | TNF signaling pathway                         | $1.11 \times 10^{-11}$ | 0.053571   | AKT1, IL1B, IL6, MMP9, CCL2, TNF |
| 8    | FoxO signaling pathway                        | $2.88 \times 10^{-11}$ | 0.045802   | AKT1, IGF1, IL6, IL10, STAT3, TGFB1 |
| 9    | C-type lectin receptor signaling pathway      | $1.28 \times 10^{-9}$  | 0.048077   | AKT1, IL1B, IL6, IL10, TNF |
| 10   | NF-kB signaling pathway                       | $1.76 \times 10^{-7}$  | 0.038462   | IL1B, CXCL8, TLR4, TNF |

Table 5: Molecular docking score.

| Gene | PDB | Molecular | -CDOCKER energy |
|------|-----|-----------|-----------------|
| STAT3| 6NJS | MOL009760 | $-64.40$       |
| AKT1 | 3OCB | MOL000098 | $49.06$        |
| MMP9 | 6ESM | MOL006331 | $34.22$        |
| SERPINE1 | 4AQH | MOL009646 | $41.10$        |
| IGF1 | 5HZN | MOL009644 | $-40.60$       |

Figure 5: (a) Ingredient-target-pathway network; (b) ingredient-target network.
Therefore, HQH may be a special adjunctive drug in treating HSPN.

HQH is a Chinese herbal compound comprising Huaier, GQZ, and HJ. Huaier has demonstrated promising curative effects in clinical treatment for various cancers [17]. The anticancer activity of Huaier results from the action of its polysaccharides [18] that show antitumor and immunomodulatory effects [14]. Huaier protects the kidney and relieves nephrotoxicity by reducing oxidative stress and inflammation, promoting the recovery of mitochondrial function and inhibiting the NF-κB signaling pathway [19]. GQZ and HJ, the main ingredients of HQH, have been extensively used to treat various chronic kidney diseases. However, the specific roles of GQZ and HJ in treating HSPN are yet unknown.

Consistent with previous results, our study showed that Huaier mainly regulated immune-related targets (IL6, TNF, CCL2, IL1B, CXCL8, IL10, TLR4, TGFβ1, and IL18). Huaier protects the kidney and relieves nephrotoxicity by reducing oxidative stress and inflammation, promoting the recovery of mitochondrial function and inhibiting the NF-κB signaling pathway [19]. GQZ and HJ, the main ingredients of HQH, have been extensively used to treat various chronic kidney diseases. However, the specific roles of GQZ and HJ in treating HSPN are yet unknown.

Consistent with previous results, our study showed that Huaier mainly regulated immune-related targets (IL6, TNF, CCL2, IL1B, CXCL8, IL10, TLR4, TGFβ1, and IL18). Huaier protects the kidney and relieves nephrotoxicity by reducing oxidative stress and inflammation, promoting the recovery of mitochondrial function and inhibiting the NF-κB signaling pathway [19]. GQZ and HJ, the main ingredients of HQH, have been extensively used to treat various chronic kidney diseases. However, the specific roles of GQZ and HJ in treating HSPN are yet unknown.

Immune abnormalities and inflammatory injuries are involved in the pathogenesis of HSPN [20]. Children with HSPN show an imbalance in Th17/Treg cells [21, 22]. IL-6 is an important cytokine in Th17 cells. Treg cells exert their effects via TGF-β and IL-10. Patients with HSPN present high levels of IL-8, CCL2, TNF-α, IL-10, and IL-6 [23, 24]. HQH administration elevates Treg and lowers Th17 levels [5, 25]. Consistent with previous results, our study showed that Huaier acts on IL6, CCL2, IL10, and TNF. These targets are covered by signaling pathway related to NOD-like receptor and IL-17, viral protein interaction with the cytokine and cytokine receptor, and cytokine-cytokine receptor interaction. An important role for STAT3 is to regulate anti-tumor immunity response. STAT3 activation can produce various immunosuppressive factors such as IL-6, IL-10, TGFβ, and CCL2 [26]. Previous studies have shown that Huaier can interact with STAT3 [27]. Unexpectedly, we observed a poor binding between HJ (MOL003889 and MOL009760) and STAT3.

AKT1 (also known as mitochondrial protein kinase B) indirectly prevents the development of glomerulosclerosis and subsequent chronic kidney disease [28]. We observed that MOL000098 and Huaier interacted to stabilize AKT1. MMP9 controls developmental processes, tissue remodeling, and inflammatory responses [29]. MMP9 has been recognized in chronic kidney diseases and is an important indicator for the early diagnosis of HSPN [30]. In our study, Huaier, MOL000098 (GQZ), and MOL006331 (HJ) regulated MMP9 expression. In comparison with the control groups, the level of IGF-1 in HSPN children was significantly increased [31]. Unfortunately, MOL009644 had poor binding energy with IGF-1. SERPINE1, also known as plasminogen activator inhibitor type 1 (PAI-1), is produced in small amounts in healthy kidneys but is highly expressed in chronic kidney diseases [32]. SERPINE1 might be used as a potential biomarker for the pathology and progression of kidney diseases [33]. We found that SERPINE1 expression was regulated by HJ and GQZ. Altogether, our study indicates that HQH can be effective in treating HSPN through multiple important targets.

Oxidative stress pathologically aggravates kidney diseases. HQH can alleviate oxidative damage [11]. Moreover, HQH relieves CYP-induced kidney damage by suppressing the MAPK/NF-κB pathway, which is associated with oxidative stress regulation. In the present study, the NF-κB pathway was regulated by IL1B, CXCL8, TLR4, and TNF. In addition, HIF-1 activation is closely related to inflammatory responses, while FOXO can regulate oxidative stress.

Figure 6: Docking results of active ingredients with target proteins.
autophagy, and apoptosis. Both HIF-1 and FOXO were covered by core genes from HQH. Taken together, HQH exhibits significant antioxidant effects, reduces inflammatory damage, and improves humoral immunity.

5. Conclusions

HQH plays an important role in improving HSPN and shows promising therapeutic efficacy by modulating inflammatory response, immune response, and oxidative stress. In this study, the clinical application evidence of HQH in treating HSPN is provided. Furthermore, some shortcomings remain in the study. The prediction of the ingredients, targets, and pathways still needs further pharmacological experimental verification.

Data Availability

The data of this research is obtained through authoritative online databases and software analysis and can be acquired from this study and supplementary material (available here).

Conflicts of Interest

All authors report no competing interests regarding the publication of this manuscript.

Authors’ Contributions

Qingqing Liu, Jiahua Liu, and Yaya Du contributed equally to this work.
Acknowledgments

This research was supported by the hospital-level project of Xi’an Children’s Hospital (No. 2021H09).

Supplementary Materials

Table 1: references of Huaier

Table 2: targets of Huaier, GQZ-HJ, HQH, and HSPN. (Supplementary Materials)

References

[1] A. K. C. Leung, A. H. C. Wong, and S. S. N. Barg, “Proteinuria in children: evaluation and differential diagnosis,” American Family Physician, vol. 95, no. 4, pp. 248–254, 2017.

[2] J. C. Davin and R. Coppo, “Henocho–Schönlein purpura nephritis in children,” Nature Reviews Nephrology, vol. 10, no. 10, pp. 563–573, 2014.

[3] M. Jelusic, M. Sestan, R. Cimaz, and S. Ozen, “Different histological classifications for Henoch–Schönlein purpura nephritis: which one should be used?,” Pediatric Rheumatology Online Journal, vol. 17, no. 1, p. 10, 2019.

[4] J. T. Flynn, W. E. Smoyer, T. E. Bunchman, D. B. Kershaw, and A. B. Sedman, “Treatment of Henoch-Schönlein Purpura glomerulonephritis in children with high-dose corticosteroids plus oral cyclophosphamide,” American Journal of Nephrology, vol. 21, no. 2, pp. 128–133, 2001.

[5] P. Zhou, Q. Xiao, L. Chen et al., “Effects of Huaqihuang granules adjuvant therapy in children with primary nephrotic syndrome,” Open Life Sciences, vol. 14, no. 1, pp. 519–527, 2019.

[6] Y. Jin, Y. Wang, S. Wang, Q. Zhao, D. Zhang, and X. Feng, “The Efficacy of Tripterygium Glycosides Combined with LMWH in Treatment of HSPN in Children,” Evidence-Based Complementary and Alternative Medicine, vol. 2021, Article ID 7223613, 6 pages, 2021.

[7] X. Xue, X. H. Liu, C. L. Lu et al., “Chinese patent herbal medicine Huaqiuhuang for Henoch-Schönlein purpura nephritis in children: a systematic review of randomized controlled trials,” BMC Complementary Medicine and Therapies, vol. 21, no. 1, p. 278, 2021.

[8] Y. Dai, M. Zhao, F. Qiu, X. Yan, Y. Fan, and C. Sun, “Investigation of the effect of Huaqiuhuang granules via adjuvant treatment in children with relapsed systemic lupus erythematosus,” American Journal of Translational Research, vol. 13, no. 4, pp. 3222–3229, 2021.

[9] L. T. Li, M. Y. Shi, S. Y. Wei, T. Li, and B. Li, “Huai Qi Huang ameliorates proteinuria and hematuria in mild IgA nephropathy patients: a prospective randomized controlled study,” Journal of the Formosan Medical Association, vol. 112, no. 12, pp. 766–772, 2013.

[10] T. Li, J. Mao, L. Huang et al., “Huaqiuhuang may protect from proteinuria by resisting MCP5 podocyte damage via targeting p-ERK/CHOP pathway,” Bosnian Journal of Basic Medical Sciences, vol. 16, no. 3, pp. 193–200, 2016.

[11] Y. Zhang, J. Chang, H. Gao et al., “Huaqihuang (HQH) granule alleviates cyclophosphamide-induced nephrotoxicity via suppressing the MAPK/NF-kB pathway and NLRP3 inflammasome activation,” Pharmaceutical Biology, vol. 59, no. 1, pp. 1425–1431, 2021.

[12] A. L. Hopkins, “Network pharmacology: the next paradigm in drug discovery,” Nature Chemical Biology, vol. 4, no. 11, pp. 682–690, 2008.

[13] Subspeciality Group of Nephrology, Society of Pediatrics, and Chinese Medical Association, “Evidence-based guidelines on diagnosis and treatment of childhood common renal diseases (II): evidence-based guideline on diagnosis and treatment of Henoch-Schönlein purpura nephritis,” Zhonghua Er Ke Za Zhi = Chinese Journal of Pediatrics, vol. 47, no. 12, pp. 911–913, 2009.

[14] Y. Sun, T. Sun, F. Wang et al., “A polysaccharide from the funghi of Huaier exhibits anti-tumor potential and immunomodulatory effects,” Carbohydrate Polymers, vol. 92, no. 1, pp. 577–582, 2013.

[15] J. Ru, P. Li, J. Wang et al., “TCMSP: a database of systems pharmacology for drug discovery from herbal medicines,” Journal of Cheminformatics, vol. 6, no. 1, p. 13, 2014.

[16] M. Ahlmann and G. Hempel, “The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy,” Cancer Chemotherapy and Pharmacology, vol. 78, no. 4, pp. 661–671, 2016.

[17] W. Wang, X. Wang, C. Li et al., “Huai suppresses breast cancer progression via linc00339/miR-4566/CSN2K2 signaling pathway,” Frontiers in Oncology, vol. 9, p. 1195, 2019.

[18] A. Yang, H. Fan, Y. Zhao et al., “An immune-stimulating proteoglycan from the medicinal mushroom Huaier up-regulates NF-κB and MAPK signaling via Toll-like receptor 4,” The Journal of Biological Chemistry, vol. 294, no. 8, pp. 628–5268, 2019.

[19] X. Zhang, Y. Cheng, Q. Zhou et al., “The effect of Chinese traditional medicine Huaqiuhuang (HQH) on the protection of nephropathy,” Oxidative Medicine and Cellular Longevity, vol. 2020, Article ID 2153912, 10 pages, 2020.

[20] W. Liu, Z. Gao, and M. Zhang, “Clinical effect of combined western medicine and traditional Chinese medicine on children with Henoch-Schönlein purpura nephritis,” American Journal of Translational Research, vol. 13, no. 4, pp. 3323–3329, 2021.

[21] X. Shao, C. Jiang, Y. Li et al., “Function of CD4(+) CD25(+) regulatory T cells in Henoch-Schönlein purpura nephritis in children,” Zhonghua Er Ke Za Zhi = Chinese Journal of Pediatrics, vol. 52, no. 7, pp. 516–520, 2014.

[22] B. Li, Q. Ren, J. Ling, Z. Tao, X. Yang, and Y. Li, “The change of Th17/Treg cells and IL-10/IL-17 in Chinese children with Henoch-Schönlein purpura: a PRISMA-compliant meta-analysis,” Medicine, vol. 98, no. 3, article e13991, 2019.

[23] M. Sugiyama, Y. Wada, N. Kanazawa et al., “A cross-sectional analysis of clinicopathologic similarities and differences between Henoch-Schönlein purpura nephritis and IgA nephropathy,” PLoS One, vol. 15, no. 4, article e0232194, 2020.

[24] L. Yuan, Q. Wang, S. Zhang, and L. Zhang, “Correlation between serum inflammatory factors TNF-α, IL-8, IL-10 and Henoch-Schönlein purpura with renal function impairment,” Experimental and Therapeutic Medicine, vol. 15, no. 4, pp. 3924–3928, 2018.

[25] P. Liang, S. Peng, M. Zhang, Y. Ma, X. Zhen, and H. Li, “Huai Qi Huang corrects the balance of Th1/Th2 and Treg/Th17 in an ovalbumin-induced asthma mouse model,” Bioscience Reports, vol. 37, no. 6, 2017.
[26] S. Zou, Q. Tong, B. Liu, W. Huang, Y. Tian, and X. Fu, “Targeting STAT3 in cancer immunotherapy,” Molecular Cancer, vol. 19, no. 1, p. 145, 2020.

[27] Y. F. Zou, Y. M. Rong, Z. X. Chen et al., “Effects of Huaier extract on ameliorating colitis-associated colorectal tumorigenesis in mice,” OncoTargets and Therapy, vol. 13, pp. 8691–8704, 2020.

[28] H. Y. Lin, Y. Chen, Y. H. Chen et al., “Tubular mitochondrial AKT1 is activated during ischemia reperfusion injury and has a critical role in predisposition to chronic kidney disease,” Kidney International, vol. 99, no. 4, pp. 870–884, 2021.

[29] J. Wozniak, J. Floege, T. Ostendorf, and A. Ludwig, “Key metalloproteinase-mediated pathways in the kidney,” Nature Reviews Nephrology, vol. 17, no. 8, pp. 513–527, 2021.

[30] Y. H. Qin, T. B. Zhou, F. Y. Lei et al., “Cut-off values for serum matrix metalloproteinase-9: is there a threshold to predict renal involvement for Henoch-Schonlein purpura in children?,” Nephrology, vol. 16, no. 1, pp. 93–99, 2011.

[31] L. Ru, A. Abudouhaer, and Y. F. Guo, “Clinical significance of serum levels of IGF-1 and IGFBP-3 in children with Henoch-Schonlein purpura or Henoch-Schonlein purpura nephritis,” Zhongguo Dang Dai Er Ke Za Zhi = Chinese Journal of Contemporary Pediatrics, vol. 15, no. 11, pp. 1009–1013, 2013.

[32] A. A. Eddy and A. B. Fogo, “Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action,” Journal of the American Society of Nephrology, vol. 17, no. 11, pp. 2999–3012, 2006.

[33] J. Chen, Y. Chen, A. Olivero, and X. Chen, “Identification and validation of potential biomarkers and their functions in acute kidney injury,” Frontiers in Genetics, vol. 11, p. 411, 2020.