Exploration of the mechanism of action of Xiayuxue Tang against hepatic fibrosis based on GEO data mining, network pharmacology and molecular docking technology

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Author contributions

Rui-Zhu Jiang wrote the article; Yu-Hong Ling was responsible for data collection; Xin-Rui Xian was responsible for key target screening; Xian-Ling Yuan was responsible for data visualization; Yang Zheng was responsible for revising the article; Jia-Hui Wang was responsible for molecular docking; and Tie-Jian Zhao was responsible for the design of the article idea and the financial support.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

ECM, extracellular matrix; NCBI, National Center for Biotechnology Information; GEO, Gene Expression Omnibus; TCMSP, Traditional Chinese Medicine Systematic Pharmacology; OB, Oral Bioavailability; DI, Drug-Likeness; BP, Biological Processes; CC, Cellular Components; MF, Molecular Functions; NAFLD, Non-alcoholic fatty hepatic disease; TNF, tumor necrosis factor; PI3K, phosphatidylinositol-3-kinase; TLR, Toll-like receptor; Nrf2, nuclear factor-related factor 2; LOXL2, Lysyl Oxidase Like 2.

Citation


Abstract

Objective: To study the mechanism of action of Xiayuxue Tang in treating hepatic fibrosis by combining GEO data mining, network pharmacology, and molecular docking technology, and provide new research directions for the treatment of hepatic fibrosis. Method: Utilizing multiple databases, we aim to identify the relevant targets of various components in Xiayuxue Tang and their associations with hepatic fibrosis. After pinpointing the key targets through interaction analysis, we will construct both the compound-target network and the protein interaction network for Xiayuxue Tang. Conclusively, we will conduct GO and KEGG enrichment analyses on these key targets, followed by molecular docking verification. Result: Through mining the GEO database, 171 related targets were identified. When combined with other databases, a total of 2,343 hepatic fibrosis-related targets were obtained. Xiayuxue Tang comprises 82 related components, which include 26 active components from rhubarb, 1 from ground beetle worm, 46 from peach kernels, with a total of 314 predicted targets. The GO enrichment analysis revealed 748 biological processes, 32 cellular components, and 73 molecular functions, while the KEGG enrichment analysis identified 222 pathways. Molecular docking verification confirmed that effective compounds can bind stably to key proteins, exhibiting strong binding activity. This underscores the potential efficacy of Xiayuxue Tang in addressing hepatic fibrosis. Conclusion: Xiayuxue Tang exerts regulatory effects on hepatic fibrosis through different targets and pathways, suggesting that the herbal compound has the characteristics of multiple pathways and targets.

Keywords: Xiayuxue Tang; hepatic fibrosis; network pharmacology; molecular docking technology
Hepatic fibrosis is a pathological process characterized by the diffuse deposition of extracellular matrix (ECM), particularly an excessive accumulation of type I collagen α1, within the liver. This process signifies the body’s reparative response to liver injury and is a common pathological alteration observed in various chronic liver diseases [1]. Furthermore, hepatic fibrosis represents a critical stage in the progression of diseases such as hepatitis, cirrhosis, and potentially, carcinoma [2]. While early-stage hepatic fibrosis tends to be reversible, there are currently no targeted therapeutic drugs specifically designed to treat it at this stage. In the context of Traditional Chinese Medicine (TCM), the liver is considered the primary reservoir of blood. The characteristic stagnation of blood within the liver, when in a pathological state, is easily induced [3]. However, there is no direct reference to ‘hepatic fibrosis’ within ancient Chinese medicinal texts. Instead, based on the clinical symptoms, conditions akin to hepatic fibrosis can be classified under categories such as “jaundice”, “hypochondriac pain”, “dropsy”, and “hepatic accumulation” [4].

This formula is from Zhang Zhongjing’s “The Essentials of the Golden Chamber”, which is composed of 9 grams of rhubarb, 20 pieces of peach kernels and 20 pieces of earthworms. Rhubarb swings away blood stasis, peach kernel moistens and invigorates blood stasis, and earthworm expels blood stasis and breaks up knots. The combination of the three herbs is effective in invigorating blood and removing blood stasis, breaking up blood stasis and dispersing knots, and pushing out the old to bring in the new [5]. This formula, which was established 1,800 years ago, has been extensively utilized in clinical settings, demonstrating substantial therapeutic efficacy. Furthermore, a decade ago, it was reported that Xiaxyuxue Tang could significantly inhibit the progression of hepatic fibrosis under continuous stimulation from carbon tetrachloride [6, 7].

Network pharmacology is an emerging discipline integrating genomics, high-throughput screening, network visualization and pharmacological analysis of drug networks, which can verify, analyze and evaluate the actual efficacy of drugs, adverse effects and pharmacological effects and transformation mechanisms produced by drugs on the human body, and provide important help to reduce the toxicity and side-effects of drugs on the human body, improve the comprehensive utilization of drugs by the human body, and the development of new drugs and other research. Molecular docking, on the other hand, allows the study of the binding capacity between protein receptors and drug small molecules. In this study, network pharmacology was used to predict the potential targets of action and signaling pathways of Xiaxyuxue Tang against hepatic fibrosis, explaining the synergistic effects of multi-component and multi-target interventions in TCM and its relationship with various diseases in terms of the TCM system and holistic diagnosis and treatment perspectives. It helps to understand the potential mechanism of multi-component, multi-target, and multi-pathway combined therapy in TCM, and at the same time validate its core targets by combining with molecular docking technology, in order to systematically and comprehensively reveal the relationship between drugs and diseases, and to provide new evidences and ideas for related clinical applications and mechanism studies.

**Material**

The database, software and related procedures used in this study are detailed in Figure 1.

**Method**

**Acquisition of Disease-Related Targets**

We accessed the National Center for Biotechnology Information (NCBI) and utilized the Gene Expression Omnibus (GEO) database, searching for pertinent microarrays using the keyword “hepatic fibrosis” to identify related targets. Subsequently, we employed the OMIM and GeneCards platforms to search for hepatic fibrosis-associated targets. By cross-referencing these targets with those from the GEO database, we successfully pinpointed the relevant targets for hepatic fibrosis.

**Acquisition of drug-related targets**

We employed the Traditional Chinese Medicine Systematic Pharmacology Platform (TCMSP) to identify the active ingredients of each constituent of Xiaxyuxue Tang. By selecting “Herb name”, we conducted searches for rhubarb, peach kernel, and turkey berry, respectively, setting the Oral Bioavailability (OB) to ≥ 30% and Drug-Likeness (DL) to ≥ 0.18 to obtain the corresponding active ingredients, components, and target predictions of their active components.

Additionally, we used the bioinformatics analysis tool for molecular mechanism of TCM (BATMAN-TCM) to identify relevant active ingredients and corresponding protein targets, setting the compound relevance score (score cutoff) to ≥ 0.80 and adjusted P value to ≥ 0.05. The results sourced from both databases were filtered to eliminate duplicates, enabling us to compile a comprehensive list of all the active ingredients and protein targets of the components in Xiaxyuxue Tang.

**Acquisition of the common targets**

The targets involved in drugs and diseases were interactively processed using Venn diagrams to obtain the common targets between drugs and diseases, as a predictive target for the action of Xiaxyuxue Tang on hepatic fibrosis.

**Construction of compound-potential target map**

Common targets were matched to their respective compounds, and a herbal medicine-component-network diagram was constructed using Cytoscape 3.8.2 software. Subsequently, compounds with elevated betweenness centrality, closeness centrality, and degree value were selected for molecular docking.

**Construct the protein interaction network diagram**

The common targets were uploaded to the String database, and the preliminary protein interaction linkage network diagram between Xiaxyuxue Tang on hepatic fibrosis was obtained, and its TSV file was downloaded and imported into Cytoscape 3.8.2 software to perform topological analysis, modular analysis, and screening of key targets for the protein interaction network.

**Gene enrichment analysis and pathway analysis of targets**

The common targets were imported into the Metascape database for KEGG pathway enrichment analysis and GO biological process enrichment analysis, and the results were imported into RStudio software for visualization.

**Construction of traditional Chinese medicine-meridian network diagram**

By searching the 2020 edition of the *Chinese Pharmacopoeia*, we obtained the meridians of rhubarb, peach kernel, and earthworm, and thus constructed the network diagram of Chinese medicine-meridian attribution for Xiaxyuxue Tang.

**Molecular docking**

The compounds with the top 6 degree values in Xiaxyuxue Tang were selected in the compound-target network map of traditional Chinese medicine, and the files of the corresponding components were obtained by using TCMP and pubchem databases; the target genes, which were selected as the top 6 degree values in the key targets, were then queried by using Uniprot and RCSB PDB databases, and then the files were downloaded in their PDB format, and the results were analyzed and the relevant images were drawn after molecular docking of the compounds and the protein targets through the SaliVina final software.
Results

Disease-related targets
The chip data numbered GSE139602 was selected from the GEO database. This chip data contains 39 samples, of which 33 are test group and 6 are control group. Differential gene volcano plots and scatter plots were drawn after organizing the experimental data provided by the chip, see Figure 2 and Figure 3, after which 171 genes were screened with \( \log_{2}F \) > 1, \( P < 0.05 \) as the differential gene condition. Among the 171 genes, 102 were up-regulated and 69 were down-regulated. 100 hepatic fibrosis targets were obtained from OMIM database, and 2175 hepatic fibrosis targets were obtained from Genecards database. A total of 2,343 relevant targets were obtained.

Drug-related targets
A total of 82 active compounds were obtained from the screening, including 26 active components of Rhubarb, 1 of tupelo and 46 of peach kernel. A total of 314 predicted targets were obtained.

Common targets
The 314 drug-related targets and the 2,343 disease-related targets were processed interactively to yield a total of 182 common targets between the drugs and diseases. These represent potential targets for the treatment of hepatic fibrosis. The results were visualized using Venn diagrams, as shown in Figure 4.

Construction of traditional Chinese medicine-component-potential target network
The predicted targets were matched to their respective compounds, which were then numbered. A network graph was plotted using Cytoscape 3.8.2, resulting in a total of 281 nodes and 689 edges, as depicted in Figure 5. Compounds with a higher degree value were selected for molecular docking, with details of the top 10 compounds presented in Table 1.

PPI network of targets of blood stasis decoction on hepatic fibrosis
After importing the common targets into the string database, the free targets were removed and the PPI network diagram was initially obtained. After downloading the TSV file and importing it into Cytoscape 3.8.2, the PPI network map was further plotted (see Figure 6). The closer the target point is to the center of the circle and the denser the connecting lines are, the more important the target point is and the more likely it is to be used as a key target point. After the key targets were screened according to the conditions, the MCODE plug-in was then used to perform modular analysis of these targets, and then these targets were summarized for subsequent GO and KEGG enrichment analysis. The top 10 targets were selected and the information is detailed in Table 2.

GO and KEGG, and the enrichment analysis
Using a \( P \) Value Cutoff of 0.01 and a Min Enrichment value of 1.5 as qualifying conditions, we queried the Metascape database and obtained 748 GO Biological Processes (BPs), 32 Cellular Components (CCs), and 73 Molecular Functions (MFs). We plotted bubble diagrams for the top 10 entries in each category, with detailed information provided in Table 3. From the 222 KEGG entries, we selected the top 20 to create bubble charts. All selected entries were visualized and analyzed using Rstudio software, with the GO image shown in Figure 7 and the KEGG image in Figure 8.

Chinese medicine-meridian network
The drug-attribution network was constructed using Cytoscape 3.8.2 software, resulting in a herbal-attribution network as shown in Figure 9. This reveals that the drug exhibits the highest number of connections in the hepatic meridian.

Molecular docking
After docking the small molecules and target proteins, a total of 33 results were obtained, and the target JUN was only successfully docked with DLA, OXL, and SEROTONINE, so JUN only showed three results. During the docking process, the more stable the conformation is, the lower the binding energy is, and usually a binding energy < 0 kcal/mol suggests binding activity between molecules [8]. According to the magnitude of the binding energies, the nine docking results with the smallest values were selected and plotted as 3D and 2D images in Figure 10 and Figure 11, and the related binding energy information is shown in Table 3.

Discussion
Xiayuxue Tang is composed of three ingredients: rhubarb, peach kernel, and earthworm. According to Traditional Chinese Medicine (TCM), hepatic fibrosis’s fundamental patterns are a deficiency of positive qi and blood stasis in the hepatic meridians and collaterals. The primary role of Haoyuishuetang is to drain heat and eliminate blood stasis. In the formula, peach kernel belongs to heart, hepatic and large intestine meridians, and enters heart and hepatic blood meridians, with strong power to dispel blood stasis and has the function of breaking blood; rhubarb belongs to spleen, stomach, large intestine, hepatic and pericardium meridians, which can invigorate...
Figure 2 Volcano plot of differential genes

Figure 3 Scatter plots of differential genes

Figure 4 Venn diagram of lower stasis soup and hepatic fibrosis
hepatic blood, expel hepatic blood stasis, and pass hepatic meridians, and Toupouche worms enter hepatic meridians, with a good disposition to run away, and breaks blood and expels blood stasis in hepatic meridians, which, matched with rhubarb and peach kernel, can strengthen the effect of activating blood and passing through meridians of the formula to achieve the aim of treating hepatic fibrosis. Modern studies have demonstrated that rhubarb possesses several medicinal properties, including the ability to cool the blood, stop bleeding, activate blood circulation, remove stagnation, and act as an antimicrobial, anti-inflammatory, antifibrotic, and anticancer drug [9]. Peach kernel has been found to have immunomodulatory, antitumor, anti-inflammatory, and antioxidant effects [10]. Turkey berry is known to enhance the body’s immunity and is a preferred drug for regulating blood rheology [11]. The Treatise on Medicinal Properties notes that turkey berry is effective in “breaking the accumulation of retained blood,” while the Classic of the Materia Medica describes it as “the mainstay of evidence of blood closure in the obstruction of the abdomen and blood accumulation in the heart and abdomen.” The Divine Husbandman’s Classic of the Materia Medica recorded that it is “the mainstay of cold and heat in the heart and abdomen, for washing and washing, for the accumulation of blood in the masses, for breaking up hardness, and for lowering the closure of blood” [12].

Hepatic fibrosis serves as a pivotal stage in the progression towards cirrhosis and potentially primary hepatic cancer. While it’s a reversible condition, managing or reversing its course is essential for preventing and treating both cirrhosis and hepatic cancer. There is a large body of literature showing that epithelial-mesenchymal transition occurs when hepatocytes grow malignantly to hepatocellular carcinoma, and when cancer cells detach from hepatocytes to hepatocellular carcinoma and acquire the ability to migrate and invade [13, 14], and epithelial-mesenchymal transition of hepatocytes is an important mechanism of hepatic fibrosis occurring in vivo. In primary biliary cirrhosis and non-alcoholic fatty cirrhosis models, hepatocytes and biliary epithelial cells can be transformed into myofibroblasts through the process of epithelial-mesenchymal transition, which participates in the development of hepatic fibrosis, and at the same time, epithelial-mesenchymal transition is also the initial stage in which the cancerous cells of the primary tumors leave the ordered tissue structure, which is an important sign of the initiation of cancer metastatic program [15, 16]. Inhibition of intrahepatic bile duct epithelial cell activation and proliferation, antagonism of pathological angiogenesis and remodeling, promotion of functional vascular neovascularization, inhibition of hepatic inflammatory response, and inhibition of hepatic oxidative stress all play an inhibitory role in hepatic fibrosis [2, 17].

Beta-sitosterol (β-sitosterol), a common constituent of rhubarb and peach kernel, is a naturally occurring phytosterol with water-resistant

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Table 1 The top 10 compounds in their degree values

<table>
<thead>
<tr>
<th>Betweenness Centrality</th>
<th>Closeness Centrality</th>
<th>Degree</th>
<th>Number</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.190706</td>
<td>0.39485</td>
<td>42</td>
<td>DH50</td>
<td>Serotonine</td>
</tr>
<tr>
<td>0.127365</td>
<td>0.39485</td>
<td>36</td>
<td>DH13</td>
<td>OXL</td>
</tr>
<tr>
<td>0.108711</td>
<td>0.389281</td>
<td>30</td>
<td>DH5</td>
<td>emodin</td>
</tr>
<tr>
<td>0.153036</td>
<td>0.369478</td>
<td>28</td>
<td>DH49</td>
<td>Progesterone</td>
</tr>
<tr>
<td>0.082644</td>
<td>0.384937</td>
<td>27</td>
<td>DH4</td>
<td>DLA</td>
</tr>
<tr>
<td>0.074889</td>
<td>0.37247</td>
<td>27</td>
<td>A1</td>
<td>beta-sitosterol</td>
</tr>
<tr>
<td>0.054234998</td>
<td>0.375510204</td>
<td>20</td>
<td>DH4</td>
<td>aloe-emodin</td>
</tr>
<tr>
<td>0.075118115</td>
<td>0.376534789</td>
<td>19</td>
<td>DH45</td>
<td>(E)-4-Phenyl-3-Buten-2-One</td>
</tr>
<tr>
<td>0.023568272</td>
<td>0.348045397</td>
<td>16</td>
<td>DH2</td>
<td>succinic acid</td>
</tr>
<tr>
<td>0.034510693</td>
<td>0.375510204</td>
<td>16</td>
<td>DH17</td>
<td>EUPATIN</td>
</tr>
</tbody>
</table>

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Figure 5 Network diagram of TCM-components-potential targets

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resin and hepatoprotective properties [18], and the substance also possesses anti-inflammatory, immunomodulation, antitumor, and central nervous system modulatory effects [19, 20]. Hepatic fibrosis is the major pathological change in chronic hepatic disease, and some pathways [20]. After the activation of hepatic stellate cells due to altered growth factor beta (TGF-beta), β-sitosterol was able to down-regulate the mRNA and protein expression levels of collagen-1 and α-SMA, preventing the accumulation of collagen, which proved studies suggest that β-sitosterol is hepatoprotective not only against CCl4-induced oxidative stress-mediated chronic hepatic disease [21], but also against CCL4-induced hepatic fibrosis in mice, and the mechanism may be related to the TGFβ/Smad2/3 and TNF-α, NF-κB pathways that β-sitosterol is a potential therapeutic agent for hepatic fibrosis [22]. Serotonin, serotonin, also known as 5-hydroxytryptamine, is a component that is closely associated with tumors, including tumor cell proliferation, invasion, and induction of

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### Table 2 The top 10 key targets

<table>
<thead>
<tr>
<th>Betweenness Centrality</th>
<th>Closeness Centrality</th>
<th>Degree</th>
<th>gene symbol</th>
<th>name</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.044539</td>
<td>0.571429</td>
<td>67</td>
<td>TP53</td>
<td>Cellular tumor antigen p53</td>
</tr>
<tr>
<td>0.063159</td>
<td>0.582524</td>
<td>65</td>
<td>SRC</td>
<td>Proto-oncogene tyrosine-protein kinase Src</td>
</tr>
<tr>
<td>0.066023</td>
<td>0.582524</td>
<td>63</td>
<td>IL1B</td>
<td>Interleukin-1 beta</td>
</tr>
<tr>
<td>0.043405</td>
<td>0.567823</td>
<td>61</td>
<td>CASP3</td>
<td>Caspase-3</td>
</tr>
<tr>
<td>0.060842</td>
<td>0.564263</td>
<td>60</td>
<td>MYC</td>
<td>Myc proto-oncogene protein</td>
</tr>
<tr>
<td>0.037343</td>
<td>0.560748</td>
<td>59</td>
<td>JUN</td>
<td>Transcription factor AP-1</td>
</tr>
<tr>
<td>0.031789</td>
<td>0.552147</td>
<td>54</td>
<td>HSP90AA1</td>
<td>Heat shock protein HSP 90-alpha</td>
</tr>
<tr>
<td>0.071766</td>
<td>0.564263</td>
<td>52</td>
<td>NOS3</td>
<td>Nitric oxide synthase, endothelial</td>
</tr>
<tr>
<td>0.029838</td>
<td>0.542169</td>
<td>51</td>
<td>ESR1</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>0.018225</td>
<td>0.535714</td>
<td>50</td>
<td>PTGS2</td>
<td>Prostaglandin G/H synthase 2</td>
</tr>
</tbody>
</table>

### Table 3 Docking and binding energies of small molecules and targets

<table>
<thead>
<tr>
<th>Active ingredients</th>
<th>binding energy (kcal × mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP53 (3DCY)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>−1.5</td>
</tr>
<tr>
<td>OXL</td>
<td>−3.0</td>
</tr>
<tr>
<td>Emolin</td>
<td>−12.7</td>
</tr>
<tr>
<td>Progesterone</td>
<td>−16.9</td>
</tr>
<tr>
<td>DLA</td>
<td>−3.8</td>
</tr>
<tr>
<td>Beta-sitosterol</td>
<td>−19.2</td>
</tr>
</tbody>
</table>
**Figure 7 GO enrichment analysis**

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO Biological Processes</td>
<td>response to peptide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>response to lipopolysaccharide</td>
<td></td>
</tr>
<tr>
<td></td>
<td>response to inorganic substances</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cellular response to organohalogen compound</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cellular response to nitrogen compound</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cellular response to lipid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cellular response to environmental stimulus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cellular response to chemical stimulus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cellular response to abiotic stimulus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>apoptotic signaling pathway</td>
<td></td>
</tr>
<tr>
<td>GO Cellular Components</td>
<td>vesicle lumen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>secretory granule lumen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>alpha membrane Brf</td>
<td></td>
</tr>
<tr>
<td></td>
<td>perinuclear region of cytoplasmic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>membrane associated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lysosomes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cytoplasmic vesicle lumen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cavela</td>
<td></td>
</tr>
<tr>
<td>GO Molecular Functions</td>
<td>transcription factor binding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tRNAylation binding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>steroid hormone receptor activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA polymerase II-specific DNA binding transcription factor binding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>securin transduction activity</td>
<td></td>
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<tr>
<td></td>
<td>nuclear receptor activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ligand-activated transcription factor activity</td>
<td></td>
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<tr>
<td></td>
<td>kinase binding</td>
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<tr>
<td></td>
<td>heme binding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA-binding transcription factor binding</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 8 KEGG enrichment analysis**

- VEGF signaling pathway
- TNF signaling pathway
- Thyroid hormone signaling pathway
- Progestins in cancer
- Prostate cancer
- PGF-AH signaling pathway
- Pathways in cancer
- Non-alcoholic fatty liver disease (NAFLD)
- IL-17 signaling pathway
- HTLV infection
- Hepatitis B
- Fluid shear stress and atherosclerosis
- Estrogen signaling pathway
- EMT signaling pathway
- Endocrine resistance
- Colon cancer
- Breast cancer
- Bladder cancer
- Amoeboas
- AGE-RAGE signaling pathway in diabetic complications

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Figure 9 Chinese medicines in Xiayuxue Tang-Categorization network

Figure 10 Top 9 protein-small molecule 3D docking map
angiogenesis. Angiogenesis includes physiological and pathological, pathological angiogenesis and remodeling is an important pathological mechanism of hepatic fibrosis, and anti-angiogenesis can effectively inhibit the development of hepatic fibrosis [17]. Emodin can reduce collagen synthesis and antiangiogenesis [23]. There is increasing evidence that rhodopsin can induce apoptosis in human hepatocellular carcinoma cells by activating p53 [24, 25], and some studies have shown that inhibition of hematopoietic stem cell activation is an effective strategy for reversing hepatic fibrosis [25], and p53 is an important target molecule for the activated hematopoietic stem cells against hepatic fibrosis [24].

In predicting relevant targets, several key targets such as TP53, SRC, IL1B, among others, were identified. Notably, Cellular tumor antigen TP53, a tumor suppressor, was found to have the highest degree value in this study, as determined by Protein-Protein Interaction (PPI) network mapping and analysis. TP53 not only regulates genotoxic stress by inducing cell cycle blockage and apoptosis, but also regulates other cellular processes, including autophagy, stem cell self-renewal and reprogramming of differentiated cells into stem cells, the immune

rhodopsin has a variety of biological functions such as anti-inflammatory, antimicrobial, antioxidant, and anticancer, which can inhibit bile production, promote bile secretion, and protect against intrahepatic cholestasis, and at the same time, rhodopsin also system and metastasis. More than 50% of human tumors have mutations in the TP53 gene, most of which are missense and mutant, and thus regulation of TP53 presumably suppresses tumor activity [26]. Additionally, in the aforementioned discussion, we illustrated the mechanism by which TP53 counters hepatic fibrosis. The protein Proto-oncogene tyrosine-protein kinase Src is also a key protein in this study, and Src and Src family protein kinases play key roles in cell morphology, viability, proliferation and survival as primary oncopgenes [27]. It has been extensively studied as part of a large family of non-receptor tyrosine kinases over the past decades. This has led to the recognition that Src is able to regulate a number of signaling pathways that affect tumor cell behavior, including proliferation, survival, migration, invasion, and angiogenesis [28]. During the development of hepatic fibrosis, activated hepatic stellate cells are augmented to produce collagen-based extracellular matrix (ECM)

Figure 11 Top 9 protein-small molecule 2D docking map
components, which accumulate in large quantities in the hepatic, disrupting its structure and function [24], while fibrotic ECM stimulates cell proliferation and alters cellular polarity, thereby promoting tumor development and growth. Regarding the protein Interleukin-1 beta, IL1B is expressed in a variety of tissues and cells, especially in macrophages of lymphoid organs. Activated IL1B, when recognized by cell surface receptors, can play an important role in acute and chronic inflammation in a variety of diseases, leading to the initiation of adaptive anti-tumor responses, yet chronic inflammation increases the risk of cancer. It has been shown that common variants in the IL6 and IL1B genes may increase susceptibility to nonalcoholic steatohepatitis and increase the risk of hepatic parenchymal injury [29], whereas a decrease in parenchymal cell necrosis leads to increasing connective fibrous tissue in the stroma leading to fibrosis [30]. The protein Caspase-3, a cysteine-aspartate protease, plays a central role in the execution phase of apoptosis, with which the development of hepatic fibrosis happens to be inextricably linked, and the protein is also involved in signaling pathways of apoptosis, necrosis and inflammation.

By KEGG enrichment analysis, 222 relevant pathways were obtained, including IL-17 signaling pathway, TNF signaling pathway, VEGF signaling pathway, PI3K-Akt signaling pathway, Non-alcoholic fatty hepatic disease (NAFLD) and 222 other related pathways. The study of molecular pathways is extremely important, and molecular signaling pathways are one of the keys to the treatment of diseases with drugs. Different active ingredients of TCM can affect the same molecular signaling pathway or participate in multiple signaling pathways, and the active ingredients of TCM are the basis of the anti-hepatic fibrosis effect of TCM [2]. CD4+ T-lymphocytes are the main cells regulating the immune response in the hepatic, and the Th17 cells are the most important cells participating in the development and progression of hepatic fibrosis. CD4+ T lymphocytes are the main cells regulating the immune response in the hepatic, of which Th17 cells are the most important cells involved in the occurrence and development of hepatic fibrosis [31], and the pro-inflammatory cytokine interleukin IL-17 is the most important bioactive factor secreted by Th17, which has strong pro-fibrotic effects, inducing hepatic stellate cells to secrete a large amount of collagen, which then leads to an imbalance in the production and degradation of the extracellular matrix. Inflammation is important for the initiation of hepatic fibrosis, and pro-inflammatory cytokines may promote hepatic stellate cell pro-fibrotic activity [32], meanwhile, inflammatory mechanism is a common process in the development of chronic hepatic disease from various etiological factors, and is the core mechanism for the progression of hepatic fibrosis [33], and this remedy involves a lot of related inflammatory factors and pathways in the process of combating hepatic fibrosis, which suggests that it may play an important therapeutic role in combating hepatic fibrosis process, suggesting that the remedy may play an important therapeutic role in the fight against hepatic fibrosis. Meanwhile, some studies have shown that regulatory T lymphocytes isolated from the peripheral blood of patients with chronic hepatitis B cirrhosis can inhibit the activation and proliferation of hepatic stellate cells, while IL-17 acts in the opposite way [34, 35]. According to the relevant experiments of Yin Yan et al, it was confirmed that IL-17 and its related proteins are closely related to hepatic fibrosis formation [35]. TNF (tumor necrosis factor), also known as TNF-α, is a participant in CCL4-induced hepatic fibrosis [20], can induce hepatic apoptosis, up-regulates TIMP-1 and down-regulates BAMBI in hepatic stellate cells, promotes hepatic stellate cell survival and proliferation, and activates hepatic macrophages [36], and in the early stages of hepatic injury, TNF-α-mediated stromal breakdown may be essential [32], and TNF-α can stimulate increased Kupffer cell activity and enhance the stellate cell pro-fibrotic effect [37]. In addition, TNF-α is capable of influencing lipid metabolism, cosgulation, insulin resistance and endothelial function, a cytokine that can directly kill tumor cells, without significant cytotoxicity to normal cells, and a cell signaling protein involved in systemic inflammation, one of the cytokines constituting the acute-phase response, which is exacerbated in hepatic fibrosis with choleodochal ligation [38]. According to studies, it is suggested that TNF-α can act synergistically with IL-17 on various cells including hepatocytes or skin and synovial fibroblasts, and IL-17 enhances the function of TNF-α by acting on some pro-inflammatory genes [32]. It has also been demonstrated that inhibition of TNF-α can attenuate hepatic fibrosis during hepatic injury [1, 39]. Clinical studies have also shown that patients with alcoholic hepatitis treated with TNF-α monoclonal antibodies have improved their hepatitis and fibrosis symptoms [1, 40]. It has been suggested that the phosphatidylinositol-3-kinase (PI3K) pathway is one of the most important signaling pathways in a variety of malignant tumors. In cancer cells, PI3K-AKT activity is increased, which in turn activates cell proliferation, cell growth, and migration [41]. The PI3K-Akt pathway contains major signaling pathways such as mTOR, MAPK, and VEGF, among which the mTOR signaling pathway is involved in hepatocarcinogenesis, including cell growth, metabolism, proliferation, and apoptosis inhibition. In addition, inhibition of PI3K signaling during Toll-like receptor (TLR)-mediated inflammation inhibits the secretion of pro-inflammatory factors from macrophages and dendritic cells as well as increases the secretion of the anti-inflammatory factor IL-10 [42, 43]. Relevant studies showed that intraperitoneal injection of CCL4 in mice significantly induced phosphorylation of the PI3K-Akt-mTOR signaling pathway, and at the same time, the PI3K-Akt-mTOR pathway had the effect of promoting Treg inhibiting Th17 cell proliferation and regulating immune responses in hepatic tissues. The VEGF signaling pathway is an important component of neurodevelopmental and vascular regulatory epithelial branching morphogenesis [44], and with fibroblast growth factor-induced activation of hepatic stellate cells and hepatic endothelial cells accelerated hepatic fibrosis and angiogenesis. VEGF and angiopeptin-1 induce migration of hepatic stellate cells, neointimal formation with fibrinolytic imbalance, and collagen production, leading to accelerated progression of hepatic disease. The binding of VEGF and hepatocyte growth factor to the extracellular matrix not only induced proliferation of hepatic endothelial cells, but also influences the interaction of hepatic endothelial cells with the extracellular matrix through the production and activation of proteases and cellular receptors in angiogenesis and tumor progression, a process that has been shown to be closely associated with hepatic fibrosis [45]. The involvement of the non-alcoholic fatty hepatic disease (NAFLD) pathway suggests a close relationship between NAFLD and hepatic fibrosis. The occurrence of hepatic fibrosis is considered to be an important pathological change in the disease progression of NAFLD [46], and related studies have reported that the severity of hepatic fibrosis is not only an important predictor of hepatic-related diseases in patients with NAFLD, but also a strong predictor of extrahepatic diseases, including cardiovascular diseases and extrahepatic malignancies [46, 47]. Meanwhile, NAFLD mainly includes: simple fatty hepatic, nonalcoholic steatohepatitis and its associated cirrhosis and hepatocellular carcinoma [48]. Some data show that patients with NAFLD develop hepatic fibrosis within 3 to 4 years [49].

Biological processes including apoptotic signaling pathway were obtained in GO enrichment analysis, suggesting that the pathogenesis of hepatic fibrosis is inextricably linked to apoptosis, and in the early stage of apoptosis, the pro-apoptotic gene Bax is expressed, which induces the release of cytochrome C from the mitochondrial membrane, triggering the apoptotic process [50]. Sustained apoptosis of hepatocytes activates hepatic stellate cells, and the reduction of activated hepatic stellate cells upon reversal of hepatic fibrosis is also mainly through apoptotic mechanisms rather than phenotypic transformation [17]; related biological processes also include response to peptide, and according to Liao, Peng-Ying [51] et al. small molecule peptides were able to ameliorate the structural lesions of hepatic histopathology in mice, and significantly reduce CCL4 induced hepatic fibrosis mice serum AST and ALT levels and hepatic tissue MDA and Hyp contents, and hepatic tissue GSH-Px and SOD contents were significantly increased.

In cellular components including lysosomal lysosome and other
components, in ionization-induced hepatic In ionization-induced hepatic fibrosis, α-tocotrienol was able to selectively induce apoptosis of activated hepatic stellate cells through the lysosomal-mitochondrial axis [52]. The molecular functions include heme binding, nuclear receptor activity, oxidoreductase activity, etc. Oxidative stress is one of the key factors in the development of hepatic fibrosis, and nuclear factor-related factor 2 (NRF2) is a central part of the hepatic anti-oxidative stress system [53]. system [17]; Lysyl Oxidase Like 2 (LOXL2). LOXL2 is virtually absent in healthy but strongly induced in fibrotic hepaties, and in fibrosis established by thioacetamide, LOXL2 not only inhibits and promotes fibrosis reversal, but also enhanced the splitting and thinning of fibrotic septa [53].

Conclusion

The molecular docking validation indicated that the effective compounds in the Xiayuuxue Tang had strong activities with the key targets, so all the targets predicted on this basis had a high degree of confidence, highlighting the authenticity of this study. The ability of Xiayuuxue Tang to exert regulatory effects on hepatic fibrosis at different targets and pathways can suggest the rationality of this prescription against hepatic fibrosis, which can work by affecting the relevant cells and cytokines, and it also indicates that the Chinese herbal compound has the characteristics of multi-pathway and multi-targets.

References

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