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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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

Introduction

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 Xiayuxue 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 Xiayuxue 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 Xiayuxue 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 ≥ 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 Xiayuxue 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 Xiayuxue 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 Xiayuxue 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 Xiayuxue Tang.

Molecular docking
The compounds with the top 6 degree values in Xiayuxue Tang were selected in the compound-target network map of traditional Chinese medicine, and the files of the corresponding components were obtained by using TCMSP 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 SailVina final software.
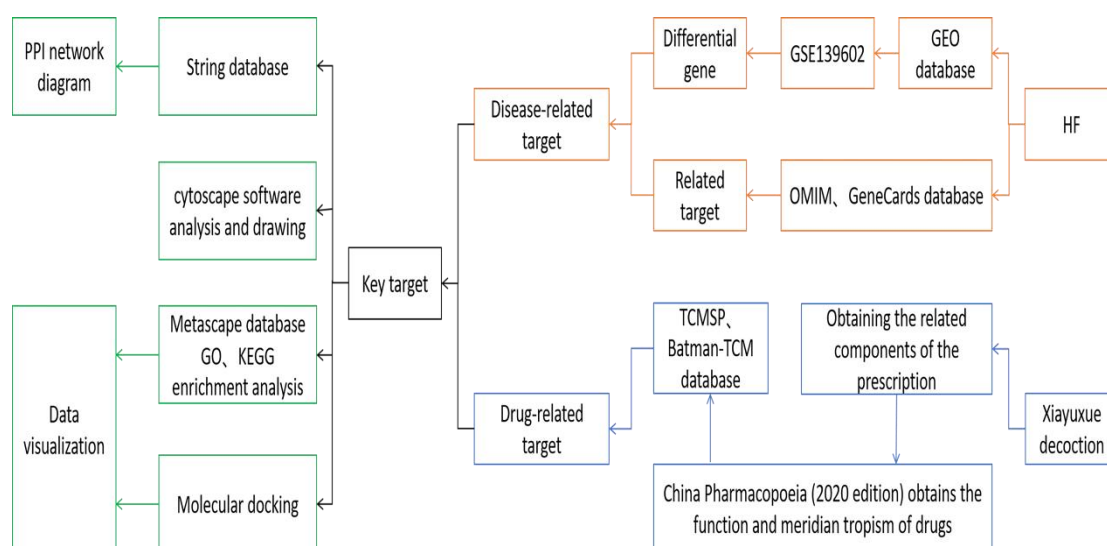
Figure 1 Exploring the research process of the treatment of hepatic fibrosis

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 |logFC| > 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 Table1.

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
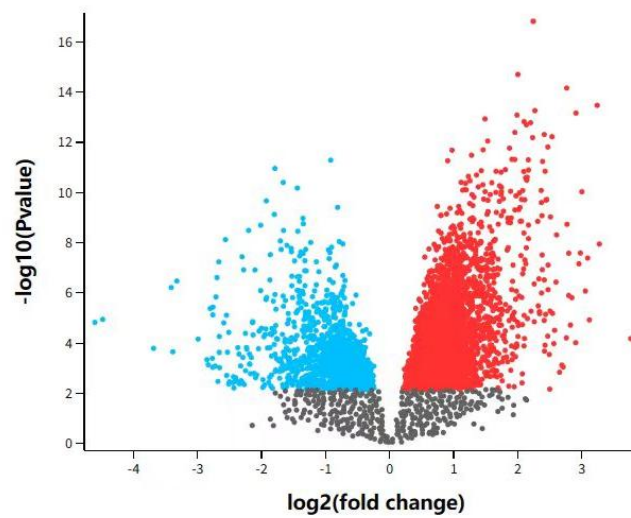
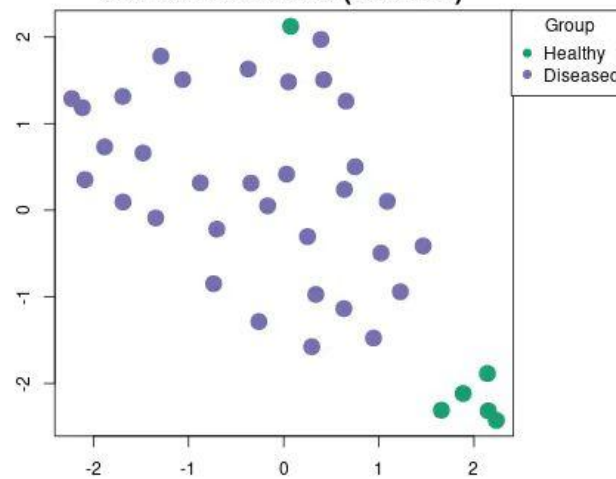## Healthy vs Diseased, Padj<0.05

Figure 2 Volcano plot of differential genes

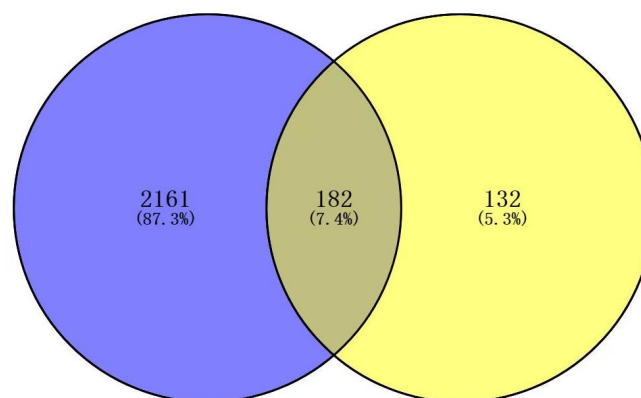**Figure 3 Scatter plots of differential genes**

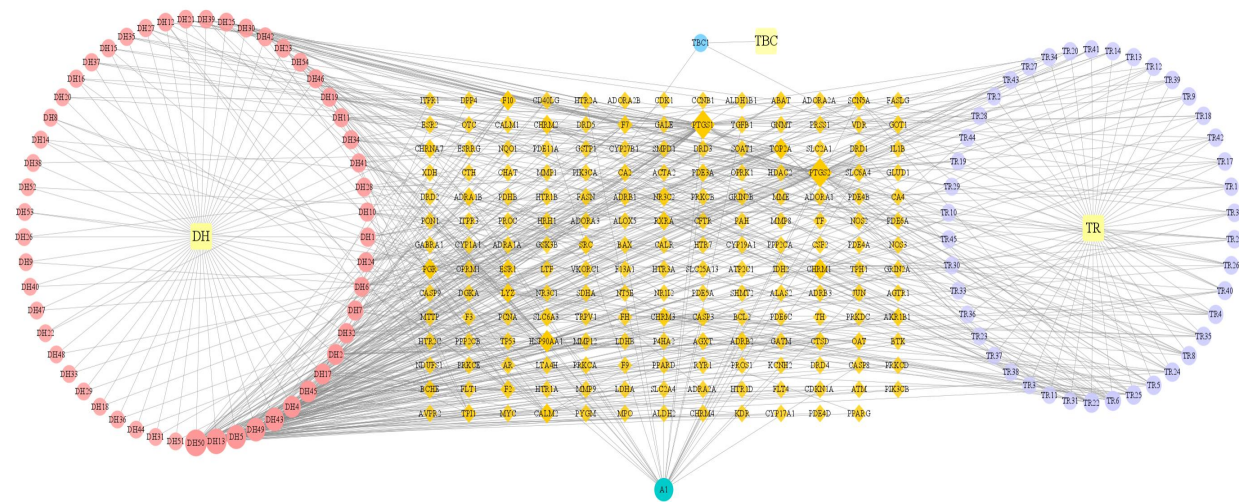**Figure 4 Venn diagram of lower stasis soup and hepatic fibrosis**

Figure 5 Network diagram of TCM-components-potential targets

Table 1 The top 10 compounds in their degree values

Betweenness Centrality	Closeness Centrality	Degree	Number	Name
0.190706	0.39485	42	DH50	Serotonine
0.127365	0.39485	36	DH13	OXL
0.108711	0.389281	30	DH5	emodin
0.153036	0.369478	28	DH49	Progesterone
0.082644	0.384937	27	DH43	DLA
0.074889	0.37247	27	A1	beta-sitosterol
0.054234998	0.375510204	20	DH4	aloe-emodin
0.075118115	0.376534789	19	DH45	(E)-4-Phenyl-3-Buten-2-One
0.023568272	0.348045397	16	DH2	succinic acid
0.034510693	0.375510204	16	DH17	EUPATIN

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
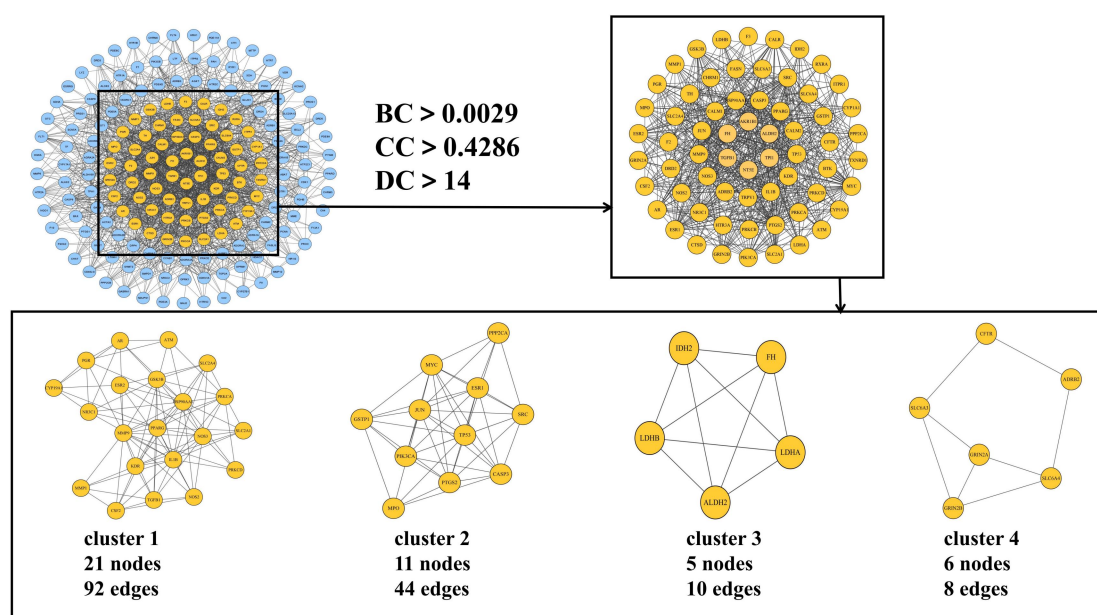
Figure 6 PPI network diagram and module analysis

Table 2 The top 10 key targets

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

Table 3 Docking and binding energies of small molecules and targets

Active ingredients	binding energy (kcal $\times$ mol^{-1})					
	TP53 (3DCY)	SRC (4U5J)	CASP3 (5JFT)	MYC (6UOZ)	JUN (2GMX)	HSP90AA1 (5H22)
Serotonine	-1.5	-6.1	-4.7	-6.1	-3.2	-7.0
OXL	-3.0	-2.8	-2.6	-2.5	-2.5	-3.1
Emodin	-12.7	-8.8	-5.3	-7.4		-8.7
Progesterone	-16.9	-7.8	-5.4	-6.4		-8.2
DLA	-3.8	-3.2	-3.0	-3.1	-2.7	-3.6
Beta-sitosterol	-19.2	-8.3	-3.1	-7.1		-9.4

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 that that β-sitosterol is a potential therapeutic agent for hepatic fibrosis [22]. Serotonine serotonin, also known as 5-hydroxytryptamine, is a component that is closely associated with tumors, including tumor cell proliferation, invasion, and induction of
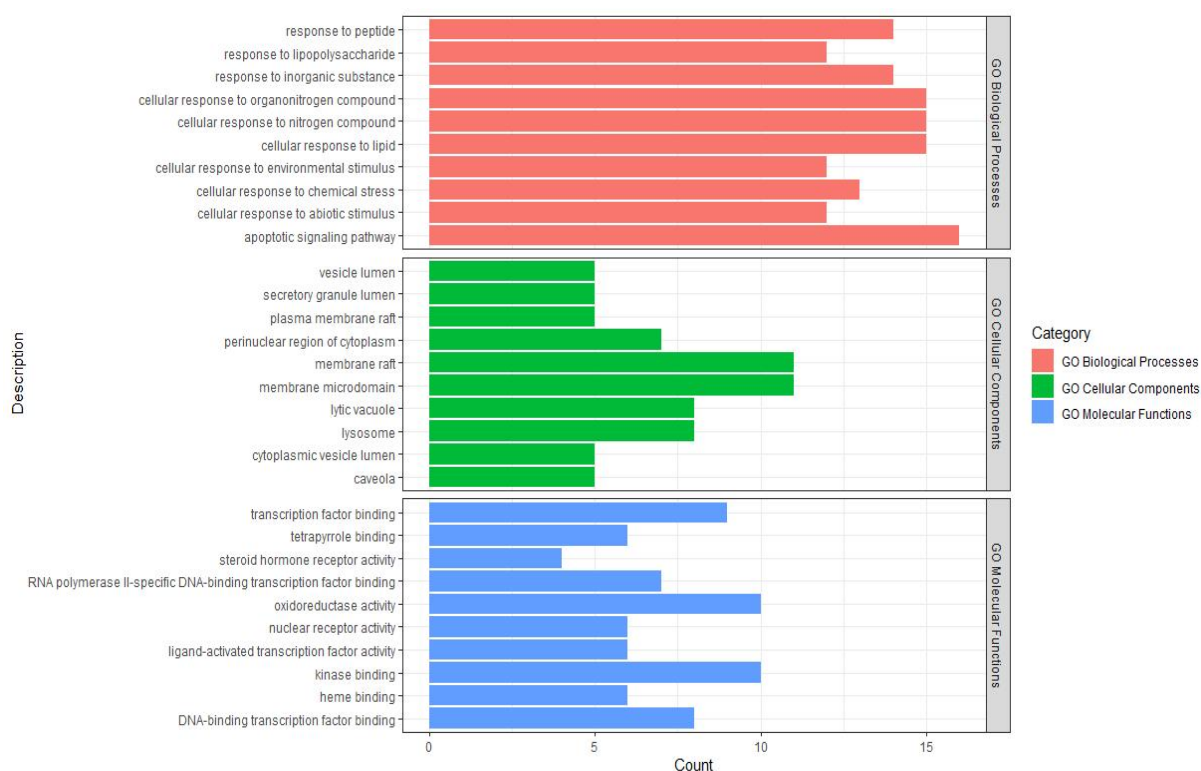
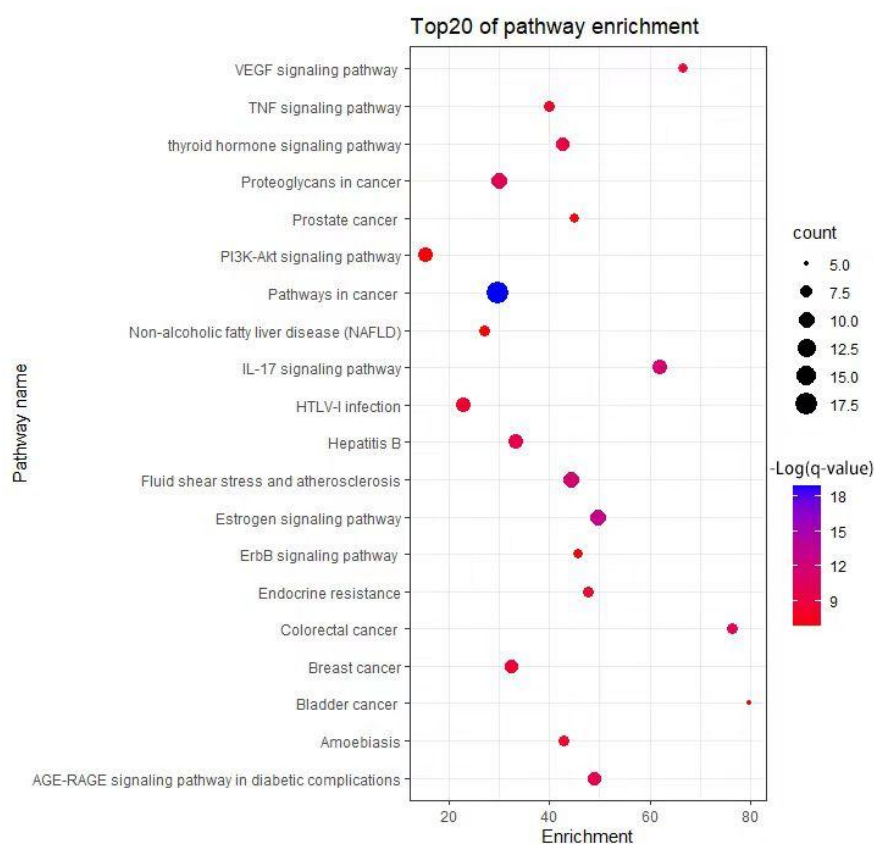**Figure 7 GO enrichment analysis**

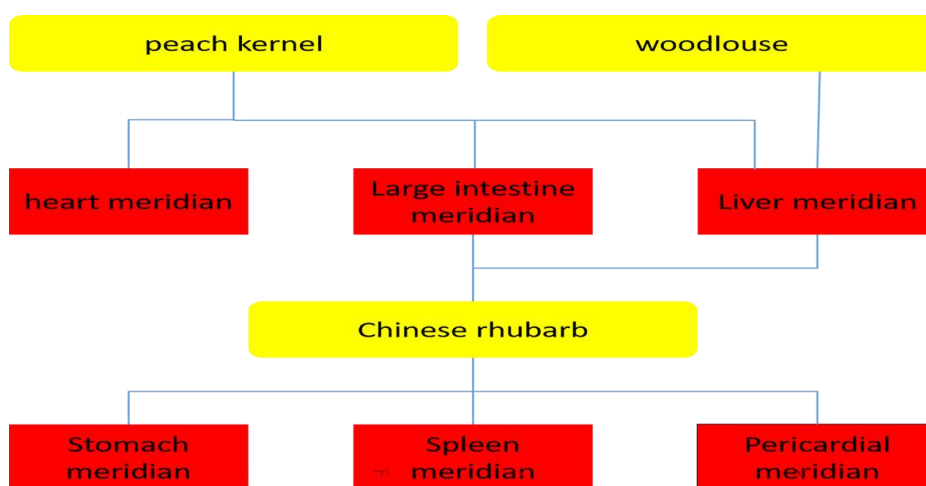**Figure 8 KEGG enrichment analysis**

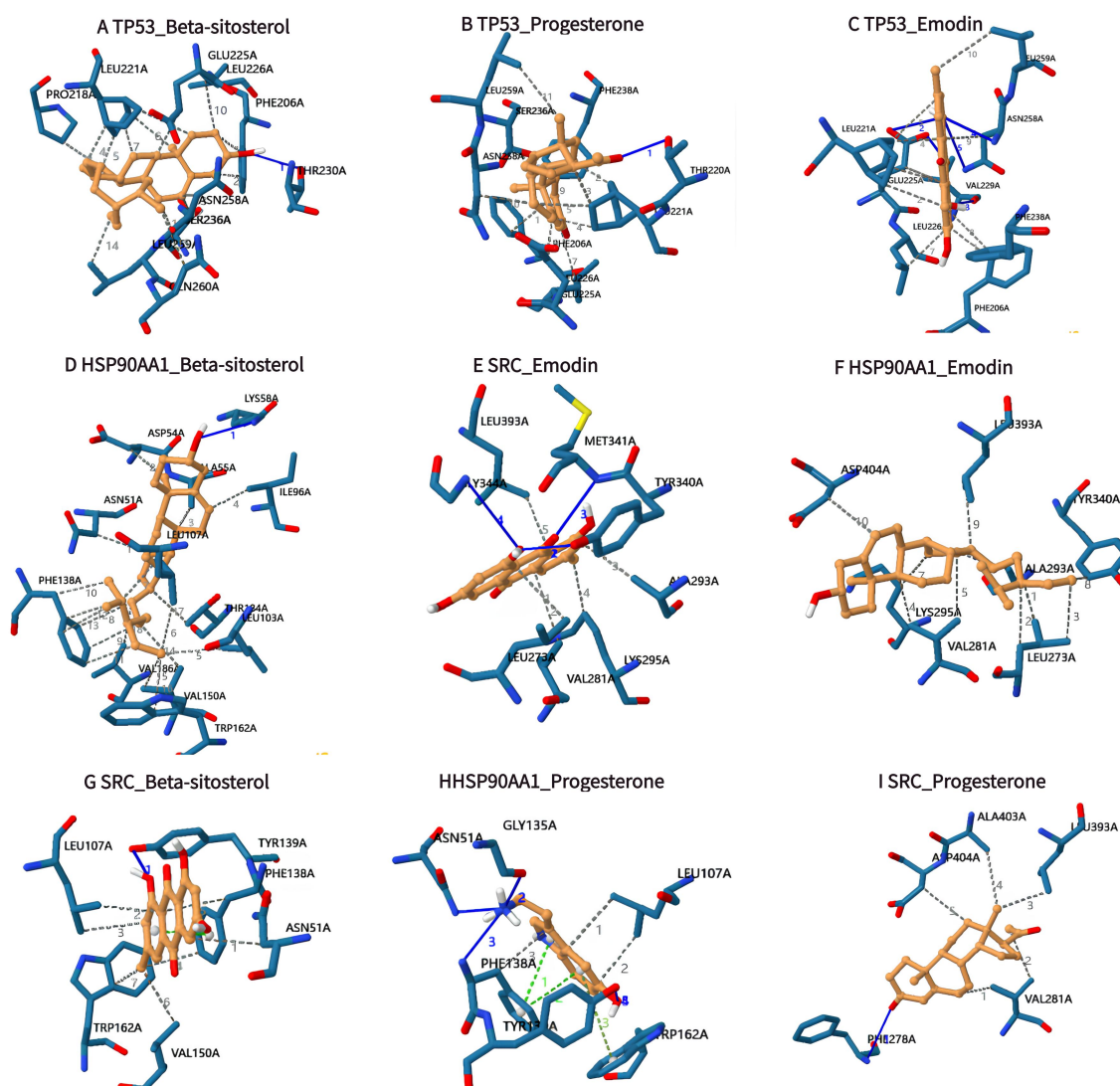Figure 9 Chinese medicines in Xiayuxue Tang-Categorization network

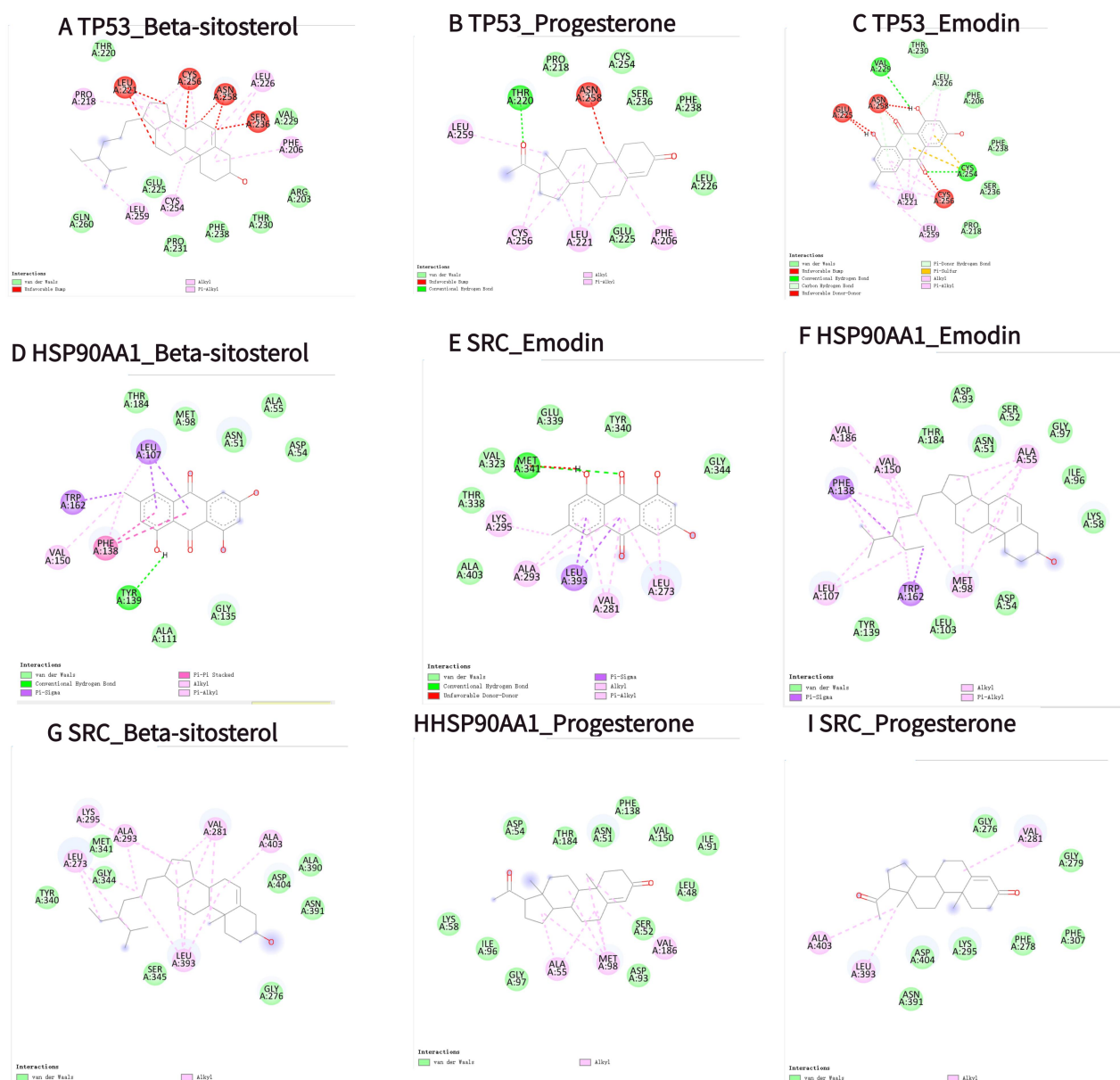Figure 10 Top 9 protein-small molecule 3D docking map

Figure 11 Top 9 protein-small molecule 2D 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 antihepatic fibrosis [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 oncogenes [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)

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 collagenI, 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 hepatocyte 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, coagulation, 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 choledochal 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 cell differentiation, inhibiting Th17 cell production, 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 angiopoietin-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, thus initiating 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 isoniazid-induced hepatic In isoniazid-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 hepatics, 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 Xiayuxue 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 Xiayuxue 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

1. Zhao SS, Shao RG, He HW. Potential targets of action for anti-hepatic fibrosis. *J Pharm* 2014;49(10):1365–1371. (Chinese) Available at:
   http://doi.org/10.16438/j.0513-4870.2014.10.011
2. Roehlen N, Crouchet E, Baumert TF. Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives. *Cells* 2020;9(4):875. Available:
   http://doi.org/10.3390/cells9040875
3. Berumen J, Baglieri J, Kisseleva T, Mekeel K. Liver fibrosis: Pathophysiology and clinical implications. *WIREs Mech Dis* 2021;13(1):e1499. Available at:
   http://doi.org/10.1002/wsbm.1499
4. Wei LX, Ding MP, Wang ZW, Yao N, Pang YR. Exploring the mechanism of anti-hepatic fibrosis effects of Cornus officinalis-Mudan Pi medication based on network pharmacology. *Chin J New Drugs Clin* 2021;40(1):64–70. (Chinese) Available at:
   http://doi.org/10.14109/j.cnki.xyylc.2021.01.13
5. Zhang DQ, Xu Y, Mu YP, Liu W, Liu P. Progress in the study of anti-hepatic fibrosis effect and its mechanism of Xiayuyuetang. *Chin J Exp Formu* 2018;24(21):219–224. (Chinese) Available at:
   http://doi.org/10.13422/j.cnki.syfjx.20181908
6. Mu YP, Liu P, Liu Y, et al. Discussion on the inhibitory effect of Xiayuyuxi Tang on liver fibrosis and its formula in rats with progressive stage. *J Tradi Chin Med* 2006;(3):215–218. (Chinese) Available at:
   http://doi.org/10.13288/j.11-2166/r.2006.03.035
7. Wang Y, Yuan Y, Wang W, et al. Mechanisms underlying the therapeutic effects of Qingfeiyin in treating acute lung injury based on GEO datasets, network pharmacology and molecular docking. *Comput Biol Med* 2022;145:105454. Available at:
   http://doi.org/10.1016/j.compbiomed.2022.105454
8. Li S, Wang Y, Li C, et al. Study on Hepatotoxicity of Rhubarb Based on Metabolomics and Network Pharmacology. *Drug Des Devel Ther* 2021;15:1883–1902. Available at:
   http://doi.org/10.2147/DDDT.S301417
9. Xiang H, Zuo J, Guo F, Dong D. What we already know about rhubarb: a comprehensive review. *Chin Med* 2020;15:88. Available at:
   http://doi.org/10.1186/s13020-020-00370-6
10. Xin J. New progress in the study of the pharmacological action mechanism of peach kernel for clinical application. *Health Road* 2016;15(01):20. (Chinese) Available at:
    https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44Y
    LTlOAiTRKibYlV5Vjs7iLvwzaWL4OdVinibbbzj3pP5Kq-f5EykRL
    PGcqf7kr3rp3tBfNioDuFUhx6F4Tukm&uniplatform=NZKPT
11. Lee KH, Morris-Natschke S, Qian K, et al. Recent Progress of Research on Herbal Products Used in Traditional Chinese Medicine: the Herbs belonging to The Divine Husbandman's Herbal Foundation Canon (Shén Nóng Běn Cǎo Jīng). *J Tradit Complement Med* 2012;2(1):6–26. Available at:
    http://doi.org/10.1016/s2225-4110(16)30066-9
12. Wang M, Ye Q, Mao D, Li H. Research Progress in Liver-Regenerating Microenvironment and DNA Methylation in Hepatocellular Carcinoma: The Role of Traditional Chinese Medicine. *Med Sci Monit* 2020;26:e920310. Available at:
    http://doi.org/10.12659/MSM.920310
13. Pelletier L, Rebouissou S, Vignjevic D, Bioulac-Sage P, Zucman-Rossi J. HNF1α inhibition triggers epithelial-mesenchymal transition in human liver cancer cell lines. *BMC Cancer* 2011;11:427. Available at:
    http://doi.org/10.1186/1471-2407-11-427
14. Zhao X, Sun T, Che N, et al. Promotion of hepatocellular carcinoma metastasis through matrix metalloproteinase activation by epithelial-mesenchymal transition regulator Twist1. *J Cell Mol Med* 2010;15(3):691–700. Available at:
    http://doi.org/10.1111/j.1582-4934.2010.01052.x
15. Wei C, Yang C, Wang S, et al. Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. *Mol Cancer* 2019;18(1):64. Available at:
    http://doi.org/10.1186/s12943-019-0976-4
16. Kong D, Zhang Z, Chen L, et al. Curcumin blunts epithelial-mesenchymal transition of hepatocytes to alleviate hepatic fibrosis through regulating oxidative stress and autophagy. *Redox Biol* 2020;36:101600. Available at:
    http://doi.org/10.1016/j.redox.2020.101600
17. Wu XM, He Q, You SJ, Li YN, Zhang YJ, Yan HM. Overview of the study on the mechanism of anti-hepatic fibrosis effect of Chinese medicine compound. *Beijing Chin Med* 2021,40(06):675–680. Chinese Available at:
    http://doi.org/10.16025/j.1674-1307.2021.06.031
18. Gumede NM, Lembede BW, Nkomozepi P, Brooksbank RL, Erlwanger KH, Chivandi E. β-Sitosterol mitigates the development of high-fructose diet-induced nonalcoholic fatty liver disease in growing male Sprague–Dawley rats. *Can J Physiol Pharmacol* 2020;98(1):44–50. Available at:
    http://doi.org/10.1139/cjpp-2019-0295
19. Raj D, Sharma V, Upadhyaya A, et al. Swertia purpurascens Wall ethanolic extract mitigates hepatic fibrosis and restores hepatic hepcidin levels via inhibition of TGFβ/SMAD/NF κ B signaling in rats. *J Ethnopharmacol* 2022;284:114741. Available at:
    http://doi.org/10.1016/j.jep.2021.114741
20. Kim KS, Yang HJ, Lee JY, et al. Effects of β-sitosterol derived from Artemisia capillaris on the activated human hepatic stellate cells and dimethylnitrosamine-induced mouse liver fibrosis. *BMC Complement Altern Med* 2014;14:363. Available at:
    http://doi.org/10.1186/1472-6882-14-363
21. Devaraj E, Roy A, Royapuram Veeraragavan G, et al. β-Sitosterol attenuates carbon tetrachloride–induced oxidative stress and chronic liver injury in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2020;393(6):1067–1075. Available at:
    http://doi.org/10.1007/s00210-020-01810-8
22. Kim KS, Yang HJ, Lee JY, et al. Effects of β-sitosterol derived from Artemisia capillaris on the activated human hepatic stellate cells and dimethylnitrosamine-induced mouse liver fibrosis. *BMC Complement Altern Med* 2014;14:363. Available at:
    http://doi.org/10.1186/1472-6882-14-363
23. Hu N, Liu J, Xue X, Li Y. The effect of emodin on liver disease --

comprehensive advances in molecular mechanisms. *Eur J Pharmacol* 2020;882:173269. Available at:
http://doi.org/10.1016/j.ejphar.2020.173269

24. Liang B, Gao L, Wang F, et al. The mechanism research on the anti-liver fibrosis of emodin based on network pharmacology. *IUBMB Life* 2021;73(9):1166–1179. Available at:
http://doi.org/10.1002/iub.2523

25. Zhang F, Lu S, He J, et al. Ligand Activation of PPARγ by Ligustrazine Suppresses Pericyte Functions of Hepatic Stellate Cells via SMRT-Mediated Transrepression of HIF-1α. *Theranostics* 2018;8(3):610–626. Available at:
http://doi.org/10.7150/thno.22237

26. Kamada R, Toguchi Y, Nomura T, Imagawa T, Sakaguchi K. Tetramer formation of tumor suppressor protein p53: Structure, function, and applications. *Biopolymers* 2016;106(4):598–612. Available at:
http://doi.org/10.1002/bip.22772

27. Roskoski R Jr. Src protein–tyrosine kinase structure and regulation. *Biochem Biophys Res Commun* 2004;324(4):1155–1164. Available at:
http://doi.org/10.1016/j.bbrc.2004.09.171

28. Creedon H, Brunton VG. Src Kinase Inhibitors: Promising Cancer Therapeutics? *Crit Rev Oncog* 2012;17(2):145–159. Available at:
http://doi.org/10.1615/CritRevOncog.v17.i2.20

29. Huang T (Dazhong), Behary J, Zekry A. Non-alcoholic fatty liver disease: a review of epidemiology, risk factors, diagnosis and management. *Intern Med J* 2020;50(9):1038–1047. Available at:
http://doi.org/10.1111/imj.14709

30. Wu M, Sun J, Wang L, et al. The lncRNA HOTAIR via miR-17-5p is involved in arsenite-induced hepatic fibrosis through regulation of Th17 cell differentiation. *J Hazard Mater* 2023;443(Pt B):130276. Available at:
http://doi.org/10.1016/j.jhazmat.2022.130276

31. Murphy FR, Issa R, Zhou X, et al. Inhibition of Apoptosis of Activated Hepatic Stellate Cells by Tissue Inhibitor of Metalloproteinase-1 Is Mediated via Effects on Matrix Metalloproteinase Inhibition: implications for reversibility of liver fibrosis. *J Biol Chem* 2002;277(13):11069–11076. Available at:
http://doi.org/10.1074/jbc.M111490200

32. Beringer A, Miossec P. IL-17 and TNF-α co-operation contributes to the proinflammatory response of hepatic stellate cells. *Clin Exp Immunol* 2019;198(1):111–120. Available at:
http://doi.org/10.1111/cei.13316

33. Wree A, McGeough MD, Inzaugarat ME, et al. NLRP3 inflammasome driven liver injury and fibrosis: Roles of IL-17 and TNF in mice. *Hepatology* 2018;67(2):736–749. Available at:
http://doi.org/10.1002/hep.29523

34. Kong X, Feng D, Wang H, et al. Interleukin-22 induces hepatic stellate cell senescence and restricts liver fibrosis in mice. *Hepatology* 2012;56(3):1150–1159. Available at:
http://doi.org/10.1002/hep.25744

35. Yin Y, Li XT, Guo YG, Zhou LY, Wang L. Mechanism of the inhibitory effect of $1,25(OH)_2D_3$ on rat liver fibrosis by interfering with IL-17 pathway. *J Gastr Hepat* 2017;26(09):1011–1015. (Chinese) Available at:
https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44Y LTlOAiTRKibYlV5Vjs7iAEhECQAQ9aTiC5BjCgn0RpGKgIsEsTB c63M2cMWQxuEa0MpEph-Zp4Zc_-4TLQQ6&uniplatform=NZ KPT

36. Seki E, Schwabe RF. Hepatic inflammation and fibrosis: Functional links and key pathways. *Hepatology* 2015;61(3):1066–1079. Available at:
http://doi.org/10.1002/hep.27332

37. Hu JC, Hu GT. Clinical efficacy of soft liver anti-fibrotic soup combined with entecavir dispersible tablets in the treatment of cirrhosis and liver fibrosis. *J Clin Ratio Drug* 2021;14(33):93–95. (Chinese) Available at:

http://doi.org/10.15887/j.cnki.13-1389/r.2021.33.031

38. Roh YS, Cho A, Cha YS, Oh SH, Lim CW, Kim B. Lactobacillus Aggravate Bile Duct Ligation-Induced Liver Inflammation and Fibrosis in Mice. *Toxicol Res* 2018;34(3):241–247. Available at:
http://doi.org/10.5487/TR.2018.34.3.241

39. Bahcecioglu IH, Koca SS, Poyrazoglu OK, et al. Hepatoprotective Effect of Infliximab, an Anti-TNF-α Agent, on Carbon Tetrachloride-Induced Hepatic Fibrosis. *Inflammation* 2008;31(4):215–221. Available at:
http://doi.org/10.1007/s10753-008-9067-1

40. Tilg H, Jalan R, Kaser A, et al. Anti-tumor necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis. *J Hepatol* 2003;38(4):419–425. Available at:
http://doi.org/10.1016/S0168-8278(02)00442-7

41. Wang Y, Chu F, Lin J, et al. Erianin, the main active ingredient of Dendrobium chrysotoxum Lindl, inhibits precancerous lesions of gastric cancer (PLGC) through suppression of the HRAS-PI3K-AKT signaling pathway as revealed by network pharmacology and in vitro experimental verification. *J Ethnopharmacol* 2021;279:114399. Available at:
http://doi.org/10.1016/j.jep.2021.114399

42. Liang Z, Mao Q, Gao Q, et al. Establishing China's national standards of antigen content and neutralizing antibody responses for evaluation of enterovirus 71 (EV71) vaccines. *Vaccine* 2011;29(52):9668–9674. Available at:
http://doi.org/10.1016/j.vaccine.2011.10.018

43. Niu YB, Wang XL, Chen C, Ren YF, Zhang HL, Dong SG. Effect of metformin inhibition of PI3K-AKT-mTOR signaling pathway on carbon tetrachloride-induced acute liver injury in mice. *Chin J Biolo Prod* 2021;34(09):1062–1068＋1075. (Chinese) Available at:
http://doi.org/10.13200/j.cnki.cjb.003425

44. Eichmann A, Simons M. VEGF signaling inside vascular endothelial cells and beyond. *Curr Opin Cell Biol* 2012;24(2):188–193. Available at:
http://doi.org/10.1016/j.ceb.2012.02.002

45. Wang J, Zhang Q, Cheng J, et al. Angiogenesis and Hepatic Fibrosis: Western and Chinese Medicine Therapies on the Road. *Chin J Integr Med* 2018;24(9):713–720. Available at:
http://doi.org/10.1007/s11655-018-3007-1

46. Li PH, Liu XD, Huang L, Zhu HM, Wu TX, Pang HZ. Relationship between liver fibrosis progression and endothelin-1/nitric oxide in nonalcoholic fatty liver disease. *J Clin Hepat Dise* 2021;37(11):2558–2562. (Chinese) Available at:
https://kns.cnki.net/kcms/article/abstract?v=3uoqIhG8C44Y LTlOAiTRKibYlV5Vjs7iy_Rpms2pqwbFRRUtoUImHcZ3J_9RdIH KYyiD266qZoquapzLXjoqzPjpv0MUej_g&uniplatform=NZKPT

47. Mikolasevic I, Orlic L, Franjic N, Hauser G, Stimac D, Milic S. Transient elastography (FibroScan(®)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand? *World J Gastroenterol* 2016;22(32):7236–7251. Available at:
http://doi.org/10.3748/wjg.v22.i32.7236

48. Qi SY, Huang H, Li YK, Pei LG, Zhang WJ. Effects and mechanisms of curcumol on liver function and liver fibrosis in rats with nonalcoholic fatty liver. *Chin J Appli Physi* 2021;37(6):611–615. (Chinese) Available at:
http://doi.org/10.12047/j.cjap.6058.2021.082

49. Shen YH, Chi HX, Xia D, Liu XJ, An JK, Liu JK. Clinical study of telmisartan in the treatment of nonalcoholic fatty liver fibrosis. *Liver* 2016;21(04):323–325. (Chinese) Available at:
http://doi.org/10.14000/j.cnki.issn.1008-1704.2016.04.026

50. Pan GT, Liu YH, Zhou FY, Shang LR,Yang SG. Protective mechanism of rhodopsin against lipopolysaccharide-induced acute liver injury by attenuating oxidative stress and hepatocyte apoptosis. *World Sci Tech-Moder Tradi Chin Med* 2021;23(7):2294–2301. (Chinese) Available at:
https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44Y

LTlOAiTRKibYlV5Vjs7iy_Rpms2pqwbFRRUtoUImHTnfdy5DC0t
TlPrQvsH-mpOWBJAJ9S0OID8-FBpW7Ez5&uniplatform＝NZK
PT

51. Liao PY, Yang YR, Wei YJ, et al. Preliminary study on the effect of small molecular peptide LK-5 on the prevention and treatment of liver fibrosis in mice. *Chin Ethn Folk Med* 2021;30(17):38–41＋57. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v＝3uoqIhG8C44Y LTlOAiTRKibYlV5Vjs7iy_Rpms2pqwbFRRUtoUImHS9o9JVS1y XHGvlLnUMgeTD8K2ZejfDkoC_00oi8AtDj&uniplatform＝NZKP T

52. Santra S, Chowdhury A, Bishnu D, Dhali GK, Santra A. Alpha Toocotrienol causes apoptosis of activated Hepatic Stelate cells through lysosome mitochondrial axis in INH induced liver fibrosis: an in-vitro study. *J Hepatol* 2017;66(1):S395–S396. Available at: http://doi.org/10.1016/S0168-8278(17)31145-5

53. Ikenaga N, Peng ZW, Vaid KA, et al. Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. *Gut* 2017;66(9):1697–1708. Available at: http://doi.org/10.1136/gutjnl-2016-312473