DOI: 10.53469/jcmp.2024.06(08).10

Mechanism of Action of Taohong Huazhuo Decoction the Empirical Prescription Made by Professor Yang Zhen of Master of Traditional Chinese Medicine in Treating Hepatic Fibrosis based on Network Pharmacology and Molecular Docking Techniques

Li Zhang[1], Yuan Ren[2], Jianmei Hao[2,*]

[1]Shaanxi University of Traditional Chinese Medicine, 2023 Master's Degree, Xianyang 712046, Shaanxi, China
[2]Xi'an Traditional Chinese Medicine Hospital, Xi'an 710021, Shaanxi, China
Correspondence Author

Abstract: ***Objective:*** *To explore the mechanism of Taohong Huazhuo Decoction, the empirical prescription made by professor Yang Zhen of master of traditional Chinese medicine(TCM) in the treatment of hepatic fibrosis based on network pharmacology and molecular docking technology.* ***Methods:*** *The active ingredients and their corresponding effective targets of Taohong Huazhuo Decoction were screened using the TCM pharmacology database and analysis platform; the potential targets of hepatic fibrosis were obtained from GeneCards database and OMIM database, and then were processed to obtain common potential targets of hepatic fibrosis, thereby obtaining common targets of Taohong Huazhuo Decoction and hepatic fibrosis; Cytoscape 3.8.0 software was used to plot the network map of "traditional Chinese medicine-active substance-disease potential targets"; the intersection targets of drugs and diseases were imported into the String database, and the protein-protein interaction network (PPI) map was drawn by Cytoscape 3.8.0 software, and the intersection targets were analyzed by GO enrichment analysis and KEGG pathway enrichment analysis; small molecule ligand file 2D structures were searched in the PubChem database, the UniProt database was used to find the ID of the key gene of the disease, and the corresponding three-dimensional spatial structure of the disease protein was screened from the PDB database for download. Subsequently, molecular docking of small molecule ligand files and disease protein ligands was performed.* ***Results:*** *A total of 182 active ingredients of Taohong Huazhuo Decoction, 240 component targets, 1,749 hepatic fibrosis-related targets, and 28 disease intersection targets were obtained through each platform. In Taohong Huazhuo Decoction, it was found that there were 144 targets for anti-hepatic fibrosis, and the top 10 key active ingredients were quercetin, luteolin, kaempferol, naringenin, nephrin irisin, baicalein, nobiletin, acacetin, β-carotene and isorhamnetin. The top 10 genes screened by PPI were AKT1, TNF, TP53, IL6, IL1B, MMP9, CASP3, HIF1A, PTGS2 and EGFR. GO functional enrichment analysis showed that the targets were enriched in the response to oxidative stress in biological process (BP); in terms of cellular component (CC), the targets were enriched in membrane rafts and membrane microdomains; In terms of molecular function (MF), targets were enriched in DNA-binding transcription factor binding and so on. The enriched KEGG pathways included TNF signaling pathway, hepatitis B, small cell lung cancer, etc. Molecular docking results showed that there was a strong interaction between the core active components of Taohong Huazhuo Decoction and the core targets of hepatic fibrosis.* ***Conclusion:*** *The study preliminarily uncovers the potential mechanisms of action of Taohong Huazhuo Decoction in the treatment of liver fibrosis based on network pharmacology and molecular docking technique in combination. Besides, it has identified the key bioactive ingredients of the prescription, potential protein targets associated with liver fibrosis, and elucidates the cellular pathways that they may be involved. These findings provide a theoretical basis for the clinical application of Taohong Huazhuo Decoction in the prevention and treatment of hepatic fibrosis.*

Keywords: Taohong Huazhuo Decoction, Zhen Yang, Hepatic Fibrosis, Network Pharmacology, Molecular Docking.

1. Introduction

Hepatic fibrosis (HF), a complex pathological process, arises from various causes of liver disease. During the occurrence of multiple chronic liver diseases and its progression, an imbalance in the synthesis and degradation of liver fiber leads to excessive collagen deposition, which triggers inflammatory reactions in the liver and ultimately progresses to liver cirrhosis. In this process, multiple types of cells are involved [1]. Traditional Chinese Medicine (TCM) holds unique advantages in the treatment of hepatic fibrosis. Its distinctiveness lies in the principle of syndrome differentiation and treatment, as well as its comprehensive therapeutic effects that operate through multiple pathways, at multiple levels, and target multiple biological mechanisms. TCM can individually tailor herbal prescriptions and formulas based on the patient's specific constitutional characteristics, ensuring a personalized approach to treatment [2]. Taohong Huazhuo Decoction (THHZD), an empirical formula developed by master physician Zhen Yang for treating dampness-heat and excess fire syndromes in liver diseases, possesses the functions of aromatic dampness-resolving and promoting qi circulation to remove blood stasis. Professor Yang believes that the treatment of dampness-heat and excess fire of liver and kidney syndrome in liver diseases should focus on the treatment principles of removing dampness without damaging yin, clearing heat without exacerbating dampness, eliminating dampness with aromatics and utilizing pungent herbs with pungent for opening and bitter drugs for descending, thereby achieving better curative efficacy [3].

Therefore, this study will explore the mechanism of THHZD, a formula developed by Master Physician Zhen Yang, in treating HF based on network pharmacology.

2. Materials and Methods

2.1 Screening of Active Components of THHZD and Prediction of Targets

There were 12 herbs in THHZD, including Persicae Semen, Carthami Flos, Herba Moslae, Fortune Eupatorium Herb, Herba Agastachis Rugosae, Herba Artemisiae Scopariae, Poria cocos, Fried Semen Coicis, Citri Reticulatae Pericarpium, Curcumae Radix, Imperatae Rhizoma, and Isatidis Radix via searching TCMSP database. The chemical compounds with a bioavailability ≥30% and drug-likeness ≥0.18 were selected as criteria for screening the chemical components of THHZD. We searched the corresponding protein targets of these active ingredients in the TCMSP database, and validated gene names were obtained from the uniprot database and subsequently utilized as probes to convert the target information of TCM components into standardized gene information.

2.2 Acquisition of Disease Targets

We searched the GeneCards and OMIM databases with the keyword "hepatic fibrosis" to obtain potential targets related to liver fibrosis. After obtaining disease targets, we processed the information obtained from the two databases to identify common targets of the disease.

2.3 Obtaining Intersection Targets of THHZD and HF

The acquired drug and disease targets were imported into the R software (version 4.3.1) for comprehensive Venn analysis. This process enabled the precise identification of overlapping targets between drugs and diseases, subsequently a Venn diagram that visually represents their shared targets was plotted.

2.4 Construction of Interaction Network "Chinese Medicine-Active Ingredients-Potential Disease Targets"

Using Cytoscape 3.8.0 software, we utilized “the Analyze Network” function under Tools to analyze the intersection targets of THHZD for treating liver fibrosis, along with corresponding information and active ingredients. Degree values were utilized to determine node size, with larger nodes signifying greater importance. A network map of "Chinese Medicine-Pharmacologically Active Compounds-Potential Disease Targets" was constructed.

2.5 Construction of Protein-Protein Interaction (PPI) Network

We imported information on intersection targets of THHZD and liver fibrosis from the String database into Cytoscape 3.8.0 software. Selecting "Homo sapiens" under Organisms, we analyzed interactions between drugs and disease intersection targets at the cellular expression and protein functional levels, constructing a PPI network diagram and exporting analysis data. We used the CytoNCA plugin in Cytoscape 3.8.0 software to analyze the network's topological parameters, sorting them based on Degree values to draw a PPI network diagram.

2.6 Functional Enrichment Analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways for Intersection Targets of THHZD in Treating HF

Using R 4.3.1 software and Bioconductor packages such as "org.Hs.eg.db," we analyzed information by importing intersection targets of drugs and diseases for entrezID conversion. Modules including "colorspace" "stringi" "ggplot2" and bioconductor packages like "DOSE" "clusterProfiler" "enrichplot" and "pathview" were used for GO functional enrichment analysis and KEGG pathway enrichment analysis of potential therapeutic targets of THHZD for treating liver fibrosis, setting parameters for enrichment analysis at $P < 0.05$, and generating corresponding bar and bubble charts.

2.7 Molecular Docking

In the PubChem database, we searched for small molecule ligand files based on key disease targets and downloaded their 2D structures. Using the UniProt database, we identified key disease gene IDs and screened for corresponding disease protein 3D spatial structures in the PDB database for download. We introduced the small molecule ligand files of effective active ingredients and disease protein ligands into the CB-DOCK2 online molecular docking platform (https://cadd.labshare.cn/cb-dock2/php/index.php). The optimal design scheme and binding energy with minimum binding energy were obtained by molecular docking method.

3. Results

3.1 Acquisition of Active Ingredients and Targets of THHZD

Through screening on the TCMSP database platform, a total of 182 effective active ingredients were identified in THHZD, including 23 types of Persicae Semen, 22 types of Carthami Flos, 15 types of Herba Moslae, 11 types of Fortune Eupatorium HerbHerba Agastachis RugosaeFortune Eupatorium herb, 13 types of Herba Artemisiae Scopariae, 15 types of Poria cocos, 9 types of Fried Semen Coicis, 5 types of Citri Reticulatae Pericarpium, 15 types of Curcumae Radix, 4 types of Imperatae Rhizoma, and 39 types of Isatidis Radix. By querying the drug-target information of these effective active ingredients, a total of 2,077 target proteins were identified, including 101 proteins in Persicae Semen, 382 in Carthami Flos, 414 in Herba Moslae, 101 in Fortune Eupatorium Herb, 213 in Herba Agastachis Rugosae, 306 in Herba Artemisiae Scopariae, 23 in Poria cocos, 42 in Fried Semen Coicis, 94 in Citri Reticulatae Pericarpium, 65 in Curcumae Radix, 64 in Imperatae Rhizoma, and 272 in Isatidis Radix. The identified active ingredients and target information of THHZD were proofread and validated using Perl software in combination with the UniProt KB platform. Finally, the target information of 240 active ingredients was obtained.

3.2 Acquisition of Hepatic Fibrosis Target Information

Using the GeneCards database (https://www.genecards.org/), we searched the keyword "hepatic fibrosis" and obtained 7,032 disease target information entries. Due to the large number of targets, a filtering criterion of relevance score > 5 was applied, resulting in 1,775 potential disease targets. Additionally, searching on the OMIM database (https://omim.org/) yielded 69 disease target entries. By consolidating and deduplicating the data from GeneCards and OMIM databases, a total of 1,749 disease-related target entries and 28 intersection targets were obtained, as shown in Figure 1.

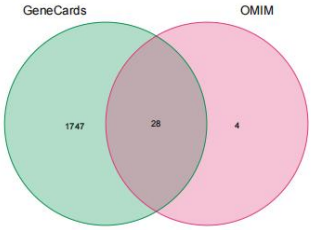


Figure 1: VEEN diagram of hepatic fibrosis protein target in GeneCards and OMIM databases

3.3 Acquisition of Target Sites for the Action of THHZD on HF

The effective active ingredients of THHZD for treating HF and disease-related target sites were imported into the R 4.3.1 software to draw a Venn diagram. This process identified 144 target sites where THHZD components act against HF, as shown in Figure 2.

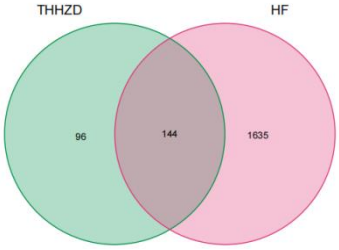


Figure 2: VEEN diagram of effective active ingredient targets of THHZD and disease targets of liver fibrosis

3.4 Construction of Interaction Network "Chinese Medicine-Active Ingredients-Target Genes"

The action target sites for treating hepatic fibrosis with THHZD and its effective active ingredients were imported into Cytoscape 3.8.0 software to create a network diagram of the Chinese herbal formula regulatory network. The network diagram "Chinese Medicine-Active Ingredients-Target Genes" was generated and analyzed, revealing a total of 255 nodes and 660 edges in the diagram. Nodes with larger degree values indicate greater importancOn the left side, circular nodes representthe 12 traditional Chinese herbs and effective components in THHZD. On the right side, square nodes represent the 144 potential target sites of the treatment of hepatic fibrosis by THHZD. Based on degree values, the top 10 key active ingredients in THHZD were identified as Quercetin, Luteolin, Kaempferol, Naringenin, Irisolidone, Baicalein, nobiletin, acacetin, β-Carotene, and Isovitexin, as shown in Figure 3 and Table 1.

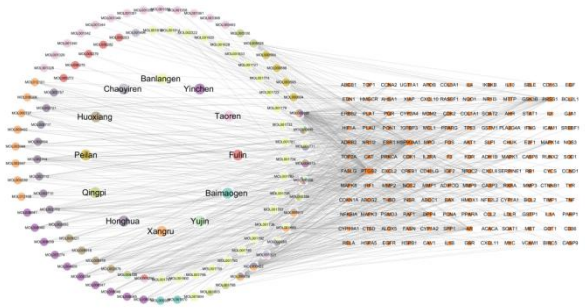


Figure 3: "TCM - active ingredients - potential targets" network diagram

Notes: Taoren refers to Persicae Semen, Honghua refers to Carthami Flos, Xiangru refers to Herba Moslae, Peilan refers to Herba Agastachis Rugosae, Huoxiang refers to Fortune Eupatorium Herb, Yinchen refers to Artemisia scoparia, Fuling refers to Poria cocos, Chaoyiren refers to Fried Semen Coicis, Qingpi refers to Citri Reticulatae Pericarpium, Yujin refers to Curcumae Radix, Baimaogen refers to Imperatae Rhizoma, and Banlangen refers to Isatidis Radix.

Table 1: Information table of the main active ingredients of THHZD

English name	Chinese name	Constitute ID	Degreevalue	Type of chemical compound	Source of Chinese herbs
quercetin	槲皮素	MOL000098	98	flavonoids	Carthami Flos, Herba Moslae,Herba Agastachis Rugosae,Herba Artemisiae Scopariae
luteolin	木犀草素	MOL000006	42	flavonoids	Carthami Flos, Herba Moslae,Fortune Eupatorium Herb
kaempferol	山柰酚	MOL000422	36	flavonoids	Carthami Flos,Herba Moslae
naringenin	柚皮素	MOL004328	28	flavonoids	Citri Reticulatae Pericarpium, Curcumae Radix
irisolidone	尼泊尔鸢尾素	MOL005916	20	flavonoids	Herba Agastachis Rugosae
baicalein	黄芩素	MOL002714	19	flavonoids	Carthami Flos
nobiletin	川陈皮素	MOL005828	19	flavonoids	Citri Reticulatae Pericarpium
acacetin	金合欢素	MOL001689	18	flavonoids	Herba Moslae, Isatidis Radix
beta-carotene	β-胡萝卜素	MOL002773	16	terpene	Carthami Flos
isorhamnetin	异鼠李素	MOL000354	13	flavonoids	Herba Artemisiae Scopariae

3.5 Construction of PPINetwork

The obtained 144 drug-disease intersection targets were uploaded to the String database for constructing a protein interaction network. Using Cytoscape 3.8.0 software, the data was analyzed and a PPI network diagram was created. The diagram showed a total of 144 nodes and 3,743 edges. Node size and color in the diagram represent their degree values: larger node sizes and darker colors indicate greater importance, as shown in Figure 4.After analysis, the top 10 genes were identified: serine/threonine-protein kinase 1 (AKT1), tumor necrosis factor (TNF), tumor protein p53

(TP53), interleukin-6 (IL6), interleukin-1 beta (IL1B), matrix metalloproteinase-9 (MMP9), caspase-3 (CASP3), hypoxia-inducible factor 1-alpha (HIF1A), prostaglandin-endoperoxide synthase 2 (PTGS2), and epidermal growth factor receptor (EGFR). It is hypothesized that THHZD may exert its anti-hepatic fibrosis effects by regulating these 10 target genes.

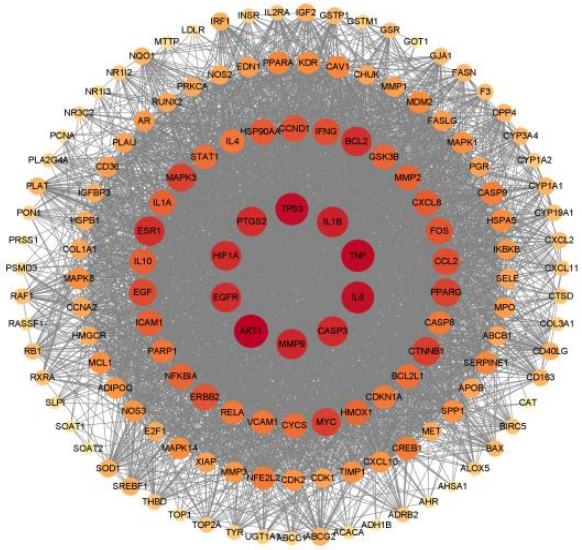
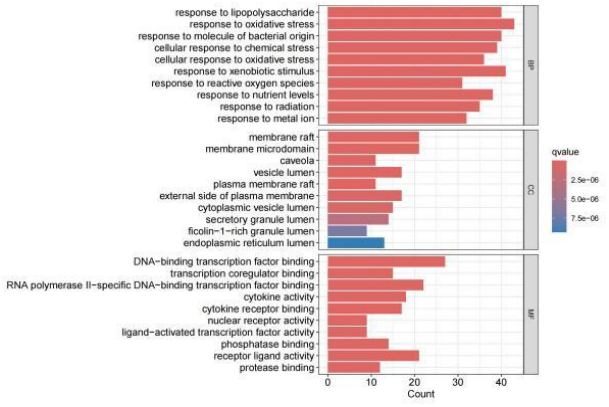


Figure 4: PPI of target protein of THHZD for anti-liver fibrosis

3.6 Potential Target Enrichment Analysis of THHZD in Treating HF

GOenrichment analysis was conducted on the data from three dimensions: biological process (BP), cellular component (CC), and molecular function (MF). The top 10 most credible entries were selected for annotation, as shown in Figure 6. In BP, potential target enrichment was primarily observed in responses to oxidative stress and responses to xenobiotic stimulus. In CC, target proteins were concentrated in membrane rafts and membrane microdomains. In MF, potential target enrichment focused on DNA-binding transcription factor binding and RNA polymerase II-specific DNA-binding transcription factor binding. This analysis indicated that the potential targets of THHZD in treating hepatic fibrosis are involved in these specific BP, CC, and MF.



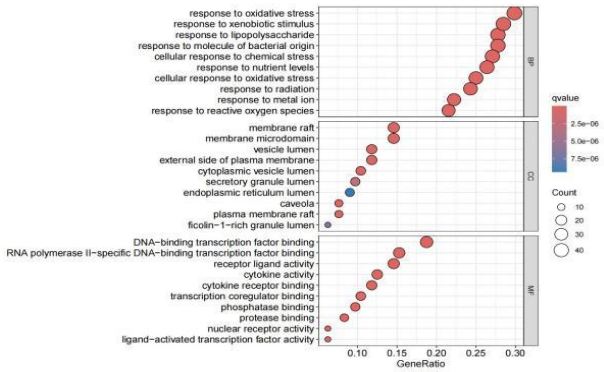


Figure 6: GO functional enrichment analysis diagram

Notes: The size of the dots in the bubble map indicates the number of enriched genes, and the degree of red in the dots indicates the degree of confidence in enrichment.

The top 20 pathways with the highest confidence were selected from the enrichment analysis results of KEGG pathway, as shown in Figure 7. Signal transduction pathways with high expression levels of target genes include lipid and atherosclerosis, PI3K-Akt signaling pathway, and apoptosis. Additionally, this process is closely related to viral infections such as Epstein-Barr virus infection, hepatitis B, toxoplasmosis, hepatitis C, and human cytomegalovirus infection. It also involves pathways related to tumors such as small cell lung cancer, bladder cancer, prostate cancer, and pancreatic cancer, which indicates that THHZD enables to treat hepatic fibrosis via multiple targets and pathways.

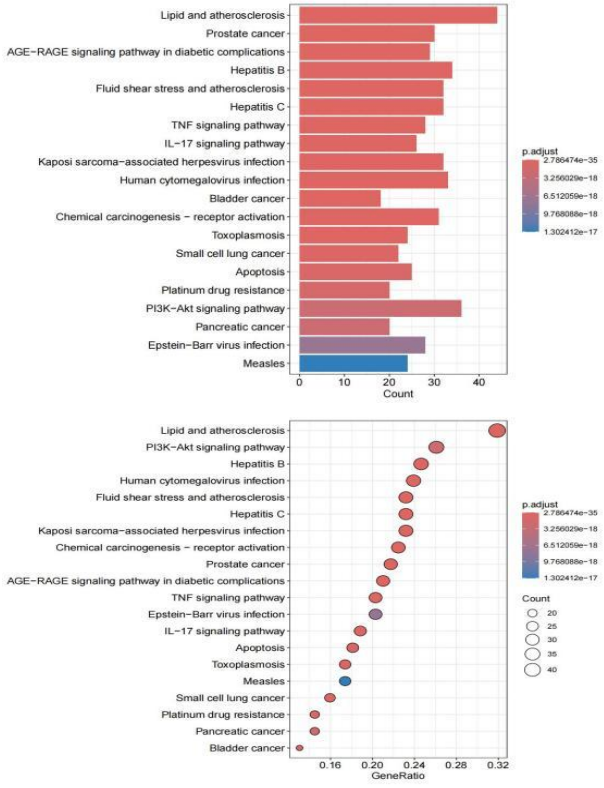


Figure 7: Diagram of enrichment analysis of KEGG pathway

3.7 Molecular Docking

The top 10 active ingredients with the highest degree values from the "Chinese Medicine-Active Ingredients-Potential Target" network diagram were selected for molecular docking

with the 10 most important key targets from the PPI diagram of THHZD in treating hepatic fibrosis. The docking results are shown in Table 2.

Typically, it is believed that the lower the binding energy between ligands and receptors was, the greater the stability and binding strength of molecular docking conformations will be, indicating a higher likelihood of interaction between molecules. According to the results of this molecular docking, we found that AKT1 interacted with 6 types of active ingredients, TNF with 4 types, TP53 with 5 types, IL6 with 2 types, IL1B with 2 types, MMP9 with 5 types, CASP3 with 7 types, HIF1A with 2 types, PTGS2 with 10 types, and EGFR with 2 types of active ingredients. Among these compounds, PTGS2 showed the strongest affinity with beta-carotene, while the binding strength between AKT1 and kaempferol was relatively low. TNF, MMP9, CASP3, and PTGS2 demonstrated strong binding with various active ingredients. Therefore, these four protein targets are considered the main targets of THHZD in treating hepatic fibrosis. The selection of the four with the lowest binding energy, indicating the strongest binding activity, is presented in Figure 8.

Table 2: Molecular docking results of the key active ingredient and core target of THHZD in treating liver fibrosis

Targets	Protein ID	Constitute ID	Name of constitutes	Vinascore (kcal/mol)
AKT1	1H10	MOL000098	quercetin	-6.1
AKT1		MOL000006	luteolin	-6.1
AKT1		MOL000422	kaempferol	-6.0
AKT1		MOL004328	naringenin	-6.2
AKT1		MOL002714	baicalein	-6.7
AKT1		MOL002773	beta-carotene	-7.0
TNF	1A8M	MOL000098	quercetin	-9.3
TNF		MOL000006	luteolin	-9.0
TNF		MOL000422	kaempferol	-9.2
TNF		MOL005916	irisolidone	-9.5
TP53	1GZH	MOL000098	quercetin	-8.3
TP53		MOL000006	luteolin	-8.2
TP53		MOL002714	baicalein	-7.6
TP53		MOL005828	nobiletin	-8.0
TP53		MOL001689	acacetin	-7.9
IL6	1IL6	MOL000098	quercetin	-9.3
IL6		MOL000006	luteolin	-8.9
IL1B	1L2H	MOL000098	quercetin	-7.1
IL1B		MOL005916	irisolidone	-6.7
MMP9	1GKC	MOL000098	quercetin	-9.9
MMP9		MOL000006	luteolin	-9.7
MMP9		MOL005916	irisolidone	-10.4
MMP9		MOL002714	baicalein	-9.6
MMP9		MOL005828	nobiletin	-10.4
CASP3	1RE1	MOL000098	quercetin	-9.3
CASP3		MOL000006	luteolin	-9.0
CASP3		MOL000422	kaempferol	-9.2
CASP3		MOL004328	naringenin	-9.0
CASP3		MOL002714	baicalein	-9.2
CASP3		MOL001689	acacetin	-9.1
CASP3		MOL002773	beta-carotene	-7.9
HIF1A	1H2K	MOL000098	quercetin	-7.8
HIF1A		MOL002714	baicalein	-7.6
PTGS2	5F19	MOL000098	quercetin	-10.2
PTGS2		MOL000006	luteolin	-9.7
PTGS2		MOL000422	kaempferol	-9.5
PTGS2		MOL004328	naringenin	-9.4
PTGS2		MOL005916	irisolidone	-9.7
PTGS2		MOL002714	baicalein	-9.5
PTGS2		MOL005828	nobiletin	-8.4
PTGS2		MOL001689	acacetin	-9.8
PTGS2		MOL002773	beta-carotene	-10.6
PTGS2		MOL000354	isorhamnetin	-9.2
EGFR	2GS2	MOL000098	quercetin	-7.9
EGFR		MOL000006	luteolin	-7.2

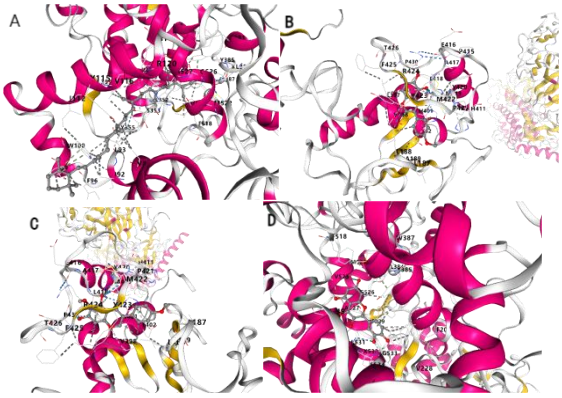


Figure 8: The molecular docking between the active ingredients and the targets

Notes: PTGS2 and beta-carotene (A); MMP9 and irisolidone (B); MMP9 and nobiletin (C); PTGS2 and quercetin (D)

4. Discussion

Liver fibrosis is a pathological condition resulting from various factors such as viral infections (e.g., hepatitis B and C viruses), non-alcoholic fatty liver disease, cholestasis, metabolic disorders, and excessive alcohol consumption [4]. In recent years, TCM research on anti-liver fibrosis has witnessed a surge of interest, highlighting its advantages and distinctive characteristics. Notably, we fail to find a explicit name of "liver fibrosis" in TCM, but it is recognized as the broader categories such as "accumulation" and related syndromes ". Professor Zhen Yang, a highly experienced practitioner in the treatment of liver diseases, has made an innovative contribution by introducing the novel terminology Ganbi(liver fibrosis)..His self-formulated THHZD has demonstrated definitive therapeutic efficacy in combating liver fibrosis[5]. However, the intricate nature of the active components within THHZD poses formidable challenges in unraveling the underlying molecular mechanisms responsible for its anti-fibrotic effects. The current lack of clarity in these mechanisms precludes the provision of a robust scientific basis for the clinical application of THHZD. Thus, further exploration is imperative to elucidate the intricate interplay of its constituents and their roles in adjusting the pathophysiology of liver fibrosis.

This study found that the formula THHZD contains 182 effective active components, with 240 corresponding effective targets. There are 1,749 potential targets related to liver fibrosis, and after processing, 28 intersecting targets were identified. The main compounds in THHZD for treating liver fibrosis include quercetin, luteolin, kaempferol, naringenin, irigenin, baicalin, hesperidin, acacetin, β-carotene, and isorhamnetin, indicating that flavonoids are the primary effective active ingredients. Related studies have found that flavonoids have antioxidant, anti-inflammatory, lipid-regulating, glucose metabolism-regulating, and anti-apoptotic effects [6]. Quercetin has various pharmacological activities, such as free radical scavenging, anti-liver fibrosis, and hepatoprotective effects [7]. Research by Diao Ying et al. [8] showed that luteolin can antagonize liver fibrosis by regulating hepatic stellate cell activation, extracellular matrix release, inhibiting migration, and transdifferentiation, and regulating liver fibrosis through multiple pathways affecting metabolism. Furthermore, studies

have identified that kaempferol has a pronounced inhibitory effect on the growth of HSC-T6 cells, which is attributed to its ability to promote apoptosis in these cells.. This finding indicates that the anti-liver fibrosis mechanism of kaempferol is related to inducing cell apoptosis [9]. Naringenin exhibits the ability to hinder the activation of HSCs by activating the apoptotic signal in LX2 cells, subsequently ameliorating liver fibrosis [10]. Irigenin, on the other hand, possesses multifaceted properties including anti-inflammatory, antioxidant, anticancer effects, and provides protection against alcoholic liver disease. Baicalin exerts its anti-fibrotic effect through a comprehensive mechanism that encompasses inhibiting the expression of pro-fibrotic factors, modulating fibrosis signaling pathways, and eliciting anti-inflammatory and antioxidant effects [11]. Hesperidin has multiple pharmacological effects, primarily notable for its capacity to suppress the secretion of inflammatory factors, thereby exhibiting both anti-inflammatory and anti-fibrotic properties [12]. Acacetin not only demonstrates antioxidant, anti-inflammatory, antibacterial, and anti-fibrotic effects but also exhibits broad anticancer effects [13]. Additionally, β-carotene may alleviate liver damage by inhibiting the release of inflammatory cytokines, providing a valuable approach to mitigate hepatic injury [14]. Isorhamnetin enables to improve liver cell necrosis and apoptosis caused by various factors, which is related to its antioxidant effect [15].

The main core genes involved in the treatment of liver fibrosis with THHZD include AKT1, TNF, TP53, IL6, IL1B, MMP9, CASP3, HIF1A, PTGS2, and EGFR. This indicates that THHZD has significant anti-liver fibrosis effects closely related to pathways involving viral infection, tumors, and signal transduction. AKT1 primarily serves as a pivotal regulator in cell growth, metabolism, and proliferation. Studies have shown that AKT1 and TP53 exert a significant inhibitory effect on the proliferation, migration, invasion, and epithelial-mesenchymal transition of human liver cancer cells and concurrently significantly promote apoptosis in these cells [16]. TNF-α, IL-6, and IL-1β, being pivotal inflammatory mediators, have the capacity to activate immune cells and liver cells, thereby fostering liver inflammation and fibrosis. Research conducted by Xian Guanxiu et al. [17] elucidated a positive correlation between serum TNF-α levels and the severity of liver fibrosis, implicating its role in the progression towards cirrhosis. Furthermore, IL-6 has been shown to accelerate liver fibrosis by activating HSCs via MAPK, JAK/STAT3, and other signaling pathways [18]. Wu Yi [19] found that neutrophils are the main source of MMP9. Notably, the accumulation of neutrophils is directly proportional to the enhanced expression of MMP9, which exerts anti-fibrotic effects by accelerating fiber degradation. Additionally, literature suggests that the liberation of cytochrome c activates caspase 9, which ultimately activates CASP3, initiating apoptosis. This process is crucial in maintaining cellular homeostasis and achieving anti-fibrotic outcomes [20], thereby illustrating the intricate interplay between inflammation, fibrosis, and cell death in liver pathology.[20]. In the pathological process of fibrosis, hypoxia induces the activation of the HIF-1 transcription system, thereby upregulating fibrosis-related cytokines such as TGF-β to induce the expression of pro-fibrosis and anti-fibrosis factors, playing a core regulatory role in the fibrosis process [21]. Under the stimulation of inflammatory mediators and cytokines, the expression of PTGS2 becomes upregulated, which subsequently fosters the proliferation of HSCs and contributes to the development of liver fibrosis [22]. Studies have revealed a link between the activation of HSCs during liver fibrosis and EGFR, and EGFR is highly expressed in activated HSCs. Consequently, inhibiting the phosphorylation of EGFR has been shown to slow down the progression of liver fibrosis [23]. In summary, this study utilized network pharmacology and molecular docking techniques to preliminarily explore the mechanisms of THHZD in treating liver fibrosis. The study revealed the effective active ingredients, potential disease targets, and possible cellular pathways involved in THHZD 's treatment of liver fibrosis. Previous research indicates that THHZD operates through multiple targets, multiple pathways, and synergistic therapeutic effects. This provides theoretical support for the clinical application of THHZD in the treatment of liver fibrosis. Therefore, further animal experiments will be designed to verify the results of network pharmacology studies, allowing for a more in-depth exploration of the mechanisms and specific targets of this formula.

References

[1] Chinese Society of Hepatology Liver Fibrosis Chemistry Group. Consensus on Diagnosis and Efficacy Evaluation of Liver Fibrosis [J]. Chinese Journal of Hepatology, 2002, (05): 8-9

[2] Yang Jing, Li Ying, Zeng Li et al. Research progress on traditional Chinese medicine treatment of fibrotic diseases [J/OL]. Chinese Journal of Experimental Formulary, 1-9

[3] Yang Zhen; Hao Jianmei The application of "Xianghuo theory" in the diagnosis and treatment of liver disease [J]. Journal of Integrated Traditional Chinese and Western Medicine for Liver Disease, 2018, 28 (05): 257-260

[4] MOON A M, SINGAL A G, TAPPER E B. Contemporary epidemiolo- gy of chronic liver disease and cirrhosis[J]. Clin Gastroenterol Hepa- tol, 2020,18(12):2650-2666.

[5] Chen Xiangni; Hao Jianmei; Yang Zhen. Clinical Observation of Self made Taohong Huazhuo Decoction in the Treatment of Liver Fibrosis with Damp Heat Intrinsic Type [J]. Yunnan Journal of Traditional Chinese Medicine, 2013, 34 (07)

[6] Yao LH, Jiang YM, Shi J, et al. Flavonoids in food and their health benefits [J]. Plant Foods Hum Nutr,2004,59(3):113-122.

[7] Xu Huolong; Nie Lei; Li Chunyu; Zhao Dongmei; Wang Limin. Research progress on the anti fibrotic mechanism of quercetin [J]. Trace Elements and Health Research, 2021, 38 (03): 70-73

[8] Diao Ying; Wang Zhongjuan; Li Min. Study on the inhibitory effect and mechanism of magnolol on hepatic stellate cell activation and CCl4 induced liver fibrosis in mice [J]. Journal of Henan Normal University (Natural Science Edition)

[9] Kong Wen. Investigating the preventive and therapeutic effects of kaempferol-3-O-rutinoside on liver fibrosis based on zebrafish juvenile model [D]. Southern Medical University, 2023

[10] Wu Lixia; Wang Yuwei; Wu Hongyan. Naringin inhibits hepatic stellate cell activation by activating apoptotic signals [J]. Chinese Pharmacy, 2023, 34 (10): 1187-1192
[11] Zhu Qingjun. Research progress on the treatment of fibrotic diseases with baicalin and baicalein [J]. Chinese Journal of Traditional Chinese Medicine, 2017, 42 (07)
[12] Yang Jingfan; Li Minyan; Qin Yanqin; Li Jiansheng. Research progress on pharmacological activity of Chenpi extract [J]. Chinese Journal of Traditional Chinese Medicine, 2023, 38 (04)
[13] Mana; Li Yajing; Fan Jiping. Pharmacological research progress of acacetin [J]. Chinese Journal of Modern Applied Pharmacy, 2018, 35 (10)
[14] Song Yang. Effects of β - carotene on ulcerative colitis and liver injury in mice [D]. Jilin Agricultural University, 2021
[15] Liu N, Feng J, Lu X, et al. Isorhamnetin inhibits liver fibrosis by reducing autophagy and inhibiting extracellular matrix formation via the TGF- β 1/Smad3 and TGF- β 1/p38 MAPK pathways [J]. Mediators Inflamm,2019,2019:6175091.
[16] Chen Xiaolong. Synergistic inhibition of proliferation, migration, and invasion of hepatocellular carcinoma cells by co expression of Akt1 specific siRNA and P53 based on plasmids [D]. Chongqing Medical University, 2021
[17] Xian Guanxiu; Chen Cong; Ma Yanni; Bu Danxia; Huang Shutong. Correlation analysis between the content of TNF - α , IL-1 β in the serum of patients with hepatitis B cirrhosis and the degree of liver fibrosis [J]. Heilongjiang Medical Journal, 2020,44 (05): 642-644
[18] Kagan P, Sultan M, Tachlytski I, et al. Both MAPK and STAT3 signal transduction pathways are necessary for IL-6-dependent hepatic stellate cells activation[J]. PLoS One,2017,12(5):e0176173.
[19] Wu Yi. Targeted apoptosis inhibitor protein alleviates CCl4 induced liver fibrosis by increasing the expression of MMP9 derived from neutrophils [D]. Southern Medical University, 2022
[20] Gao Z, Kang X, Hu J, et al. Induction of apoptosis with mitochondrial membrane depolarization by a glycyrrhetinic acid derivative in human leukemia K562 cells [J]. Cytotechnology, 2012, 64(4): 421-428.
[21] Lin Lin; Ling Tianqian. The effect of hypoxia inducible factor-1 on fibrosis [J]. Journal of Clinical Stomatology, 2014, 30 (07): 440-442
[22] Yin Wei; Yang Xuefeng. Effects of phospholipase C γ and cyclooxygenase-2 on proliferation of hepatic stellate cells [J]. Modern Medicine and Health, 2014, 30 (03): 389-391
[23] Fuchs B C, Hoshida Y, Fujii T, et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma[J]. Hepatology, 2014, 59(4): 1577-1590.