Molecular mechanism underlying the hypolipidemic effect of *Shanmei* Capsule based on network pharmacology and molecular docking

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Abstract.

**BACKGROUND:** *Shanmei* Capsule is a famous preparation in China. However, the related mechanism of *Shanmei* Capsule against hyperlipidemia has yet to be revealed.

**OBJECTIVE:** To elucidate underlying mechanism of *Shanmei* Capsule against hyperlipidemia through network pharmacology approach and molecular docking.

**METHODS:** Active ingredients, targets of *Shanmei* Capsule as well as targets for hyperlipidemia were screened based on database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed via Database for Annotation, Visualization, and Integrated Discovery (DAVID) 6.8 database. Ingredient-target-disease-pathway network was visualized utilizing Cytoscape software and molecular docking was performed by Autodock Vina.

**RESULTS:** Seventeen active ingredients in *Shanmei* Capsule were screened out with a closely connection with 34 hyperlipidemia-related targets. GO analysis revealed 40 biological processes, 5 cellular components and 29 molecular functions. A total of 15 signal pathways were enriched by KEGG pathway enrichment analysis. The docking results indicated that the binding activities of key ingredients for PPAR-\(\alpha\) are equivalent to that of the positive drug lifibrate.

**CONCLUSIONS:** The possible molecular mechanism mainly involved PPAR signaling pathway, Bile secretion and TNF signaling pathway via acting on MAPK8, PPAR\(\gamma\), MMP9, PPAR\(\alpha\), FABP4 and NOS2 targets.

Keywords: *Shanmei* Capsule, hyperlipidemia, network pharmacology, molecular docking

1. Introduction

Hyperlipidemia is one of the most prevalent global chronic metabolic diseases, which is closely related
to many high-incidence cardiovascular diseases, such as hypertension, diabetes, coronary heart disease and atherosclerosis [1–3]. It is characterized as elevated contents of total cholesterol (TC), triglyceride (TG), low-density-lipoprotein cholesterol (LDL-C), as well as decreased high-density-lipoprotein cholesterol (HDL-C) levels [4]. With the elevating prevalence and morbidity, hyperlipidemia has become a critical global issue for public health. Regulating lipid metabolism disorders may be a potential approach to decelerating or preventing the progression of hyperlipidemia. At present, statins, such as atorvastatin, lovastatin and simvastatin, are among the most commonly used lipid-lowering drugs to reduce plasma lipids. Statins are generally regarded as safe and tolerable drugs, however, certain controversies remain. A survey analyzed all case reports from the FDA AE Reporting System database linking muscle-related adverse events to statin use. The results show that dose-dependent side effects occur across the statin class [5–8]. Thus, it is necessary to develop and identify an alternative drug that may be valuable in regulating lipid metabolism. Compound formulae of traditional Chinese medicine (TCM) emphasizes the synergism among active ingredients, with proper herbs and relevant dosage to synergize the desirable effects and minimize side effects integrally. Compared with Western medicine, TCM are considered relatively safe and produces fewer adverse reactions [9,10].

TCM has been used for thousands of years for the treatment of hyperlipidemia in China, with advantages of multitarget mechanisms, remarkable curative effect and safety. They are featured by global regulation of glucose and lipid metabolism, as well as energy homeostasis via active ingredients in a prescription [11]. Shanmei Capsule, a famous preparation in China, is made of a combination of Crataegi Folium (called Shanzhaye in Chinese, the leaves of Grataegus pinnatifida Bge. var. major N.E. Br. or Grataegus pinnatifida Bge.) and Dahurian rose fruit (called Cimeiguo in Chinese, the fruit of Rosa davurica Pall. var. davurica) ethanol extract in proportion [12]. Crataegi Folium and Dahurian rose fruit both are well-known traditional medicinal plants, which have been confirmed to possess various biological and pharmacological activities, such as anti-inflammatory, antioxidant, hypocholesterolaemic and hypolipidaemic effects [13–15]. In clinical application, they are included in remedies on their own or in combination with other herbs to regulate lipid metabolism disorders induced by cardiovascular diseases. The above evidence suggests that Shanmei Capsule may be an alternative approach for treating hyperlipidemia. However, the hypolipidemic effect and related activity mechanisms of Shanmei Capsule against hyperlipidemia have yet to be revealed. Due to the complexity of active ingredients and the unknown synergistic effects of multiple components, it remains a laborious task to clarify the molecular mechanism of the Shanmei Capsule.

With the booming development of systems biology and bioinformatics, network pharmacology approach has been used successfully for the discovery of the key targets and their potential molecular mechanisms [16,17]. Contrary to “one drug, one target, one disease” principle of traditional drug design, network pharmacology based on the “drug-disease-target” interaction network, which comprehensively and systematically evaluates the intervention or effect of drugs on diseases. Network pharmacology combines the multi-level information of “ingredient-disease-target-pathway”, which corresponded with the characteristics of TCM. Molecular docking is an important computer-aided drug discovery method, which can be used to predict the interactions between drug ingredients and target proteins, and the possible binding sites of drugs [18,19]. Interactions between drug molecules and target proteins provide important information on drug discovery, which will help towards the development of drugs to treat hyperlipidemia.

In this study, network pharmacology approach was applied to investigate the relationships between active ingredients, core targets and pathways. Based on the results of network analysis and molecular docking, multi-ingredient, multi-target, and multi-pathway potential mechanisms related to Shanmei Capsule against hyperlipidemia were predicted.
2. Methods

2.1. Collection of active ingredients for Shanmei Capsule

*Crataegi Folium* and Dahurian rose fruit were used as keywords to screen the candidate ingredients of Shanmei Capsule. All the chemical ingredients were queried based on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com/tcmsp.php) and literatures. The structural information regarding biological activities of small molecules, including CAS numbers and Canonical SMILES strings, were obtained from the Scifinder database (https://scifinder.cas.org/scifinder/) and PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [20].

2.2. Target acquisition for drug and disease-related

Target prediction of Shanmei Capsule was performed using Swiss Target Prediction (http://www.swisstargetprediction.ch/) [21]. The Canonical SMILES strings obtained in 2.1 was introduced into the Swiss Target Prediction to obtain human-related targets for active ingredients. Using hyperlipidemia as a keyword, the candidate target of hyperlipidemia was searched by the GeneCard database (version 4.14, https://www.genecards.org/). After removing the duplicate targets, the overlapping targets related to active ingredients and hyperlipidemia were selected as the candidate targets, and then imported into the STRING database (http://string-db.org/) for gene interaction analysis.

2.3. GO and KEGG pathway enrichment analysis

Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis were carried out based on the Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.8, http://www.david.niaid.nih.gov). Results with values of $P < 0.01$ were selected, and Human was defined as the species. DAVID is a powerful gene functional database, which can provide comprehensive functional annotations of pathways, including biological processes, cellular component and molecular functions. After getting the results, these pathways were visualized as a bubble chart by imageGP (http://www.ehbio.com/ImageGP/), a free online tool for data analysis.

2.4. Network construction and analysis

Network construction and analysis were performed using Cytoscape 3.3.0 (download from https://cytoscape.org/). The nodes in different shapes and colors of the network represented active ingredients, targets, pathways, and the edges indicated the interaction of them. In this study, degree refers to the number of directly connected nodes with one another. It is considered that the greater the number of degree is, the greater the influence is. The topological parameters of network, such as degree, were calculated by Network Analyzer (Cytoscape plugin).

2.5. Molecular docking

The candidate ingredients of Shanmei Capsule and lifibrate (positive control drug) were chosen for molecular docking, and the results were visualized using PyMOL software (https://www.pymol.org/). The detailed steps were as follows. Firstly, molecular structure of active ingredients and the protein crystal structure of the hyperlipidemia-related target were downloaded from the ZINC 15 (http://zinc.docking.
org/) and RCSB PDB database (https://www.rcsb.org/), respectively. Prior to performing the docking process, the target protein structure was pretreated by AutoTools, including removal of water molecules, addition with hydrogens and so on. Secondly, the active pocket on protein structure was predicted by GetBox Plugin (PyMOL plugin) [22,23]. The values of the utilized parameters in autodock software were presented in Table S1. Finally, molecular docking simulation was performed using the Autodock Vina software. According to the scoring method, the docking models with the lowest score and binding-energy value were selected and visualized for subsequent analysis.

2.6. ADME and toxicology prediction

The 2D structures (in .sdf format) and simplified molecular input line entry specification (SMILES) codes of 17 key active ingredients were downloaded from the PubChem database (http://pubchem.ncbi.nlm.nih.gov). Then, a SwissADME web tool (http://www.swissadme.ch/index.php) was employed to predict ADME (Absorption, Distribution, Metabolism and Excretion) parameters, including bioavailability score, gastrointestinal (GIT) absorption and blood-brain barrier (BBB). Toxicity analysis was done by Toxtree (version 3.1.0.1851), a generic open source application, which is able to estimate toxic hazard by making predictions for a number of toxicological endpoints by different modules, such as carcinogenicity prediction (genotoxic and non-genotoxic) and Ames carcinogenicity tests.

3. Results

3.1. Screening of Shanmei Capsule active ingredients

A total of 59 candidate active ingredients were screened from the TCMSP, including 25 from *Crataegi Folium*, 29 from Dahurian rose fruit, and 5 shared ingredients. Details are shown in Table 1. The obtained candidate ingredients and all of the potential targets were applied to construct a network of ingredient-target interactions, including 330 nodes (59 ingredients and 271 targets) and 928 edges, as shown in Fig. 1. The degree of targets was adopted as a characteristic topological parameter to define the significance of candidate ingredients. The interactions indicated that one ingredient could regulate numerous targets, such as quercetin, kaempferol, β-sitosterol, isorhamnetin, ursolic acid, chlorogenic acid and so on, suggesting that Shanmei Capsule has multi-ingredients and multi-target characteristics for the treatment of hyperlipidemia.

3.2. Prediction of candidate targets of hyperlipidemia

A total of 1018 potential targets to have correlations with hyperlipidemia were found via the GeneCards database. After merging hyperlipidemia-related targets and active ingredient targets, 34 overlapping targets were considered as candidate targets (Table 2, Fig. 2). Further linking of target ingredients with candidate targets based on Cytoscap 3.3.0 resulted in a network of disease-ingredient-target, with 51 nodes and 301 edges (Fig. 3). Among them, 17 red dovetail nodes represent active ingredients of Shanmei Capsule, and 34 purple ellipse nodes represent candidate targets of hyperlipidemia. After analyzing the topological characteristics of each node in the interaction network, it shows that the node degrees of eight targets, HMGCR, MMP9, MAPK8, PPARα, PPARγ, FABP4, ALOX5 and NOS2 are more than 13, which implied that these targets might be key targets for hyperlipidemia. Previous study found that these targets were primarily involved in inflammatory reactions, lipid metabolism, etc.
A list of the selected compounds in Shanmei Capsule for network analysis:

| Source                  | Compound            | Molecular formula | CAS number      |
|-------------------------|---------------------|-------------------|-----------------|
| Dahurian rose fruit     | 2-furan-2-yl-pentanal | C                   | 10              |
| Dahurian rose fruit     | n-hexyl furan       | C                  | 27              |
| Dahurian rose fruit     | Betulinic acid      | C                  | 18              |
| Dahurian rose fruit     | Cerotic acid        | C                  | 30              |
| Dahurian rose fruit     | Linolenic acid      | C                  | 27              |
| Dahurian rose fruit     | Linoleic acid       | C                  | 18              |
| Dahurian rose fruit     | Oleic acid          | C                  | 18              |
| Dahurian rose fruit     | Oleanolic acid      | C                  | 30              |
| Dahurian rose fruit     | Hyperoside          | C                  | 21              |
| Dahurian rose fruit     | luteolin-7-O-rutinoside | C              | 29              |
| Crataegi Folium         | Diethylamine        | C                  | 165             |
| Crataegi Folium         | Vitexin-2-O-rhamnoside | C             | 251             |
| Crataegi Folium         | Thiamine            | C                  | 251             |
| Crataegi Folium         | Maslinic acid       | C                  | 251             |
| Crataegi Folium         | Stigmasterol        | C                  | 251             |
| Crataegi Folium         | Nicotinic acid      | C                  | 251             |
| Crataegi Folium         | Vitexin             | C                  | 251             |
| Crataegi Folium         | Caffeic acid        | C                  | 251             |
| Crataegi Folium         | Heriguard           | C                  | 251             |
| Crataegi Folium         | Cyanidol            | C                  | 251             |
| Crataegi Folium         | Tyramine            | C                  | 251             |
| Q. Wang et al. / Molecular mechanism underlying the hypolipidemic effect of Shanmei Capsule | | | |
Table 1, continued

| Source            | Compound                              | Molecular formula | CAS number |
|-------------------|---------------------------------------|-------------------|------------|
| Dahurian rose fruit | 2-amino-propionic acid ethyl ester | C_5H_11NO_2       | 17344-99-9 |
| Dahurian rose fruit | Isobutyl formate                      | C_5H_10O_2        | 542-55-2   |
| Dahurian rose fruit | Isoamyl acetate                       | C_7H_14O_2        | 123-92-2   |
| Dahurian rose fruit | Ethyl hexanoate                       | C_6H_{12}O_2      | 123-66-0   |
| Dahurian rose fruit | Ethyl 1-acetylcyclopropane-1-carboxylate | C_6H_{13}O_2    | 32933-03-2 |
| Dahurian rose fruit | Ethyl palmitoleate                    | C_{16}H_{32}O_2   | 56219-10-4 |

Fig. 1. Establishment of component-target network. Components are shown as red dovetails nodes and target are marked as purple circular nodes.
Table 2

| No. | Uniprot ID | Target gene | Target protein |
|-----|------------|-------------|----------------|
| 1   | P15121     | AKR1B1      | Aldo-keto reductase family 1 member B1 |
| 2   | O60218     | AKR1B10     | Aldo-keto reductase family 1 member B10 |
| 3   | P08253     | MMP2        | 72 kDa type IV collagenase |
| 4   | P14780     | MMP9        | Matrix metalloproteinase-9 |
| 5   | P03956     | MMP1        | Interstitial collagenase |
| 6   | P08254     | MMP3        | Stromelysin-1 |
| 7   | P10636     | MAPT        | Microtubule-associated protein tau |
| 8   | P10275     | AR          | Androgen receptor |
| 9   | P04035     | HMGCR       | 3-hydroxy-3-methylglutaryl-coenzyme A reductase |
| 10  | P11511     | CYP19A1     | Aromatase |
| 11  | P01130     | LDLR        | Low-density lipoprotein receptor |
| 12  | P03372     | ESR1        | Estrogen receptor |
| 13  | Q92731     | ESR2        | Estrogen receptor beta |
| 14  | P09917     | ALOX5       | Arachidonate 5-lipoxygenase |
| 15  | P08183     | ABCB1       | ATP-dependent translocase ABCB1 |
| 16  | P35869     | AHR         | Aryl hydrocarbon receptor |
| 17  | P04626     | ERBB2       | Receptor tyrosine-protein kinase erbB-2 |
| 18  | P09053     | EGFR        | Epidermal growth factor receptor |
| 19  | P05164     | MPO         | Myeloperoxidase |
| 20  | Q9UNQ0     | ABCG2       | ATP-binding cassette sub-family G member 2 |
| 21  | P15090     | FABP4       | Fatty acid-binding protein |
| 22  | P07148     | FABP1       | Fatty acid-binding protein |
| 23  | P37231     | PPARγ       | Peroxisome proliferator-activated receptor gamma |
| 24  | Q07669     | PPARα       | Peroxisome proliferator-activated receptor alpha |
| 25  | P21554     | CNR1        | Cannabinoid receptor 1 |
| 26  | P35554     | PTGS2       | Prostaglandin G/H synthase 2 |
| 27  | P00734     | F2          | Prothrombin |
| 28  | Q96R11     | NR1H4       | Bile acid receptor |
| 29  | P08246     | ELANE       | Neutrophil elastase |
| 30  | P07900     | HSP90AA1    | Heat shock protein HSP 90-alpha |
| 31  | P45983     | MAPK8       | Mitogen-activated protein kinase 8 |
| 32  | Q16539     | MAPK14      | Mitogen-activated protein kinase 14 |
| 33  | P29474     | NOS3        | Nitric oxide synthase |
| 34  | P35228     | NOS2        | Nitric oxide synthase |

3.3. GO and KEGG pathway enrichment analysis

To further clarify relevant functions and pathways, GO functional analyses and KEGG pathway enrichment were performed for above 34 core targets using DAVID database. GO enrichment analysis was annotated in the following three categories, including biological process, cellular component and molecular function, with $P < 0.01$ serving as the threshold. There were 40 biological processes involved, including oxidation-reduction processes, intracellular receptor signaling pathway, regulation of inflammatory response, positive regulation of MAP kinase activity, etc. (Fig. 4A). In the cellular component group, GO terms were mainly included nucleus, nucleoplasm, extracellular space, perinuclear region of cytoplasm and basolateral plasma membrane (Fig. 4B). Figure 4C shows that the enriched molecular functions of targets are mainly associated with RNA polymerase II transcription factor activity, enzyme binding, steroid hormone receptor activity, etc. These results indicated that the potential mechanism of Shanmei Capsule against hyperlipidemia was related to oxidation-reduction processes, intracellular receptor signaling, and inflammatory response. A total of 15 signal pathways were enriched by KEGG pathway enrichment analysis (Table 3), including PPAR signaling pathway, Bile secretion and TNF signaling pathway, et al. Among them, Pathway in cancer is a cancer-related signaling pathway. Recent
Fig. 2. Target-target interaction network between active ingredients of Shanmei Capsule against hyperlipidemia.

Fig. 3. Component-target-disease interactive network of Shanmei Capsule. Red dovetails nodes are the main active ingredients of Shanmei Capsule, and purple circular nodes are the potential targets for treating hyperlipidemia.
Table 3
Pathway enrichment of potential targets based on KEGG pathway analysis

| No. | Pathway description               | P value | Nr. Genes | Ratio |
|-----|-----------------------------------|---------|-----------|-------|
| 1   | Pathways in cancer                | 0.00001 | 11        | 32.4  |
| 2   | Estrogen signaling pathway        | 0.00001 | 7         | 20.6  |
| 3   | Bladder cancer                    | 0.00004 | 5         | 14.7  |
| 4   | PPAR signaling pathway            | 0.00024 | 5         | 14.7  |
| 5   | Bile secretion                    | 0.00027 | 5         | 14.7  |
| 6   | TNF signaling pathway             | 0.00140 | 5         | 14.7  |
| 7   | Ovarian steroidogenesis           | 0.00150 | 4         | 11.8  |
| 8   | Toxoplasmosis                     | 0.00160 | 5         | 14.7  |
| 9   | Proteoglycans in cancer           | 0.00210 | 6         | 17.6  |
| 10  | Hepatitis C                       | 0.00320 | 5         | 14.7  |
| 11  | Prolactin signaling pathway       | 0.00420 | 4         | 11.8  |
| 12  | Transcriptional misregulation in cancer | 0.00710 | 5     | 14.7  |
| 13  | Prostate cancer                   | 0.00770 | 4         | 11.8  |
| 14  | GnRH signaling pathway            | 0.00840 | 4         | 11.8  |
| 15  | HIF-1 signaling pathway           | 0.00970 | 4         | 11.8  |

Fig. 4. Go and KEGG pathway enrichment bubble diagrams for candidate targets \((P \leq 0.01)\). (A) GO analysis of Biological processes, (B) GO analysis of cellular components, (C) GO analysis of molecular functions, (D) KEGG analysis.

studies have reported that a carcinogenic role of the metabolic syndrome in variety of cancers [24]. Based on these results, Shanmei Capsule may attenuate hyperlipidemia partially by regulating inflammation or apoptosis, lipid metabolism and decrease oxidative stress during hyperlipidemia progression.

At last, in order to elucidate the connection between the core targets and corresponding pathways, a
component-target-pathway interaction network map was generated by Cytoscape 3.3.0 (Fig. 5). The 17 red dovetails nodes represent the active pharmaceutical ingredient, the 34 purple circular nodes denote the potential targets, and the 15 rose red parallelogram nodes represent the pathways. And the node sizes depend on degree value. The larger the node is, the larger the degree value is. There were 66 nodes and 340 edges were involved in the association between these targets, which reflected the biological process of the key targets. These results were revealed that Shanmei Capsule take effect in hyperlipidemia treatment by multi-components, multi-targets and multi-pathways.

3.4. Docking results analysis

Molecular docking was performed among 17 ingredients and 34 potential targets using the Autodock
Fig. 6. The heat map of molecule docking scores.

Vina software, and detailed results are presented in Fig. 6. Affinity was the binding score for the molecular docking, and when the docking score value was smaller, the binding affinity to target protein was stronger. The docking score \(< -7\) indicated a high binding activity [25]. The molecular docking results revealed that the docking scores of 47% active ingredients were lower than \(-7\), and the ingredients with the lowest scores among these targets were quercetin and chlorogenic acid (heriguard) (Fig. 6). PPAR-\(\alpha\) is a key target of lifibrate (positive drug), which belongs to the nuclear hormone receptor family, which is linked to dyslipidemia and diabetes. The docking results showed that the binding activity of quercetin, kaempferol, isorhamnisn and chlorogenic acid for PPAR-\(\alpha\) are equivalent to, and even better than that of the positive drug lifibrate. The molecular docking models are illustrated in Fig. 7. These findings indirectly verified that Shanmei Capsule had a regulatory effect on hyperlipidemia targets. At the same time, the above molecular docking results were consistent with those of previous network screening, which verified the reliability of network pharmacology.

3.5. Results of drug ADME and toxicity estimations

The ADME properties of 17 active ingredients were calculated by SwissADME web tool. As is can be observed in Table 4, all the ingredients, besides heriguard, have bioavailability score above the threshold of 0.5, which might suggest good oral bioavailability. The majority of the tested ingredients have no ability to penetrate the blood-brain barrier, thus presenting a low risk for central nervous system side effects. Based on toxicity tests, it appears that all the ingredients have no nongenetic carcinogen properties, with only vitaminG was predicted to be genetic carcinogen. The results of SwissADME calculations and toxicity tests provided useful information about the selected ingredients and found to be free from high risks of undesired effects.
Table 4
ADME and toxicity prediction results of 17 key ingredients in Shanmei Capsule

| No. | Compound                                | GI absorption | BBB permeant | Bioavailability score | Genetic carcinogen | Nongenetic carcinogen | Ames |
|-----|-----------------------------------------|---------------|--------------|-----------------------|--------------------|-----------------------|------|
| 1   | β-Sitosterolacetate                      | Low           | No           | 0.55                  | −                  | −                     | −    |
| 2   | Eicosadienoicacid                       | High          | No           | 0.56                  | −                  | −                     | −    |
| 3   | Ergosterol                               | Low           | No           | 0.55                  | −                  | −                     | −    |
| 4   | 2-amino-propionic acid ethyl ester      | High          | No           | 0.55                  | −                  | −                     | −    |
| 5   | Ethylpalmitoleate                        | High          | Yes          | 0.55                  | −                  | −                     | −    |
| 6   | Heriguard                                | Low           | No           | 0.11                  | −                  | −                     | −    |
| 7   | Isoliquiritigenin                       | High          | Yes          | 0.55                  | −                  | +                     | −    |
| 8   | Isorhamnetin                             | High          | No           | 0.55                  | −                  | −                     | +    |
| 9   | Kaempferol                               | High          | No           | 0.55                  | −                  | −                     | −    |
| 10  | Linoleic acid                            | High          | Yes          | 0.56                  | −                  | −                     | −    |
| 11  | Linolenic acid                           | High          | Yes          | 0.56                  | −                  | −                     | −    |
| 12  | Oleic acid                               | High          | No           | 0.56                  | −                  | −                     | −    |
| 13  | Quercetin                                | High          | No           | 0.55                  | −                  | −                     | +    |
| 14  | Sitosterol                               | Low           | No           | 0.55                  | −                  | −                     | −    |
| 15  | Stigmasterol                             | Low           | No           | 0.55                  | −                  | −                     | −    |
| 16  | Ursolic acid                             | Low           | No           | 0.56                  | −                  | −                     | −    |
| 17  | VitaminG                                 | Low           | No           | 0.55                  | +                  | −                     | +    |

Notes: (−) negative; (+) positive.

4. Discussion

Hyperlipidemia is one of the most prevalent global chronic metabolic diseases, which usually involved in a disorder of lipid metabolism and accompanied by elevated contents of TC, TG, LDL-C, as well as decreased HDL-C levels. During this process, blood lipid level, oxidative stress, inflammation, and other factors might be the key link of the development of dyslipidemia-induced hyperlipidemia. Shanmei Capsule, a famous preparation in China, is made of a combination of Crataegi Folium and Dahurian rose fruit ethanol extract in proportion. Crataegi Folium and Dahurian rose fruit both are well-known traditional medicinal plants, which have been confirmed to possess various biological and pharmacological activities, such as anti-inflammatory, antioxidant and hypolipidaemic effects. Therefore, in this study, network pharmacology approach and molecular docking were applied to investigate the
potential mechanism of multi-ingredient, multi-targets, and multi-pathway on Shanmei Capsule against hyperlipidemia (Fig. 8) [26–30].

Via network pharmacology analysis, a total of 17 key active ingredients were regarded to be effective on hyperlipidemia, including quercetin, kaempferol, isorhamnetin, chlorogenic acid, ursolic acid and \( \beta \)-sitosterol, etc. Quercetin, one of antioxidant substances, has been reported that it could upregulate the expression of PPAR\( \alpha \). Quercetin and its metabolites induced eNOS activity by AMPK phosphorylation, which resulted in an improvement in vessel function. They were also inhibited the formation of foam cell through activating PPAR\( \gamma \)-ABCA1 pathway, and thereby improving lipid levels [31,32]. Foam cell accumulation in atherosclerotic lesions is a critical early stage process of atherosclerosis. Treatment with kaempferol markedly suppresses oxidized low-density lipoprotein-induced macrophage foam cell formation, which promotes an increase in cholesterol efflux and a decrease in lipid accumulation in foam cells [33]. Zhang et al. [34] indicated that isorhamnetin is a dietary source of PPAR\( \gamma \) antagonist. Isorhamnetin treatment inhibited the adipocyte differentiation induced by the PPAR\( \gamma \) agonist rosiglitazone, and ameliorated insulin resistance, which may be beneficial to prevent obesity development and hepatic steatosis. Several researchers reported that anti-lipidemic and Anti-diabetic effects of chlorogenic acid are mediated through the activation of AMPK (AMP-activated protein kinase). Chronic administration of chlorogenic acid inhibited hepatic glucose-6-phosphatase expression and activity, improved lipid homeostasis and skeletal muscle glucose uptake, attenuated hepatic steatosis, which in turn improved glucose tolerance, fasting glucose level, dyslipidemia and insulin sensitivity in Leprdb/db mice [35,36]. Ursolic acid is a triterpenoid compound commonly present in fruits, vegetables and TCM, and that have a wide range of biological functions, such as anti-inflammatory, antihyperlipidemic, hypoglycemic
and antioxidant properties [37]. He et al. [38] showed that ursolic acid inhibited 3T3-L1 preadipocyte differentiation and adipogenesis via the LKB1/AMPK pathway, so as to suppress 3T3-L1 preadipocyte differentiation and lipid accumulation by regulating the transcriptional factors and their downstream lipogenic targets. β-sitosterol is a plant sterol. Experimental and clinical studies have shown that β-sitosterol has hypolipidemic, anti-diabetic, hepatoprotective, anti-cancer, and anti-arthritic role [39]. In summary, the main active ingredients (e.g., quercetin, kaempferol, isorhamnetin, chlorogenic acid, ursolic acid and β-sitosterol) of Shanmei Capsule in the treatment of hyperlipidemia could regulate multiple cellular signaling pathways, thereby involving in the regulation of lipid synthesis and metabolism, lowering cholesterol levels, and preventing or delaying the development of dyslipidemia.

Thirty-four potential targets consist of HMGCR, MMP9, MAPK, PPARα, PPARγ, FABP4, NOS2, etc., were screened as effective targets of Shanmei Capsule against hyperlipidemia. HMGCR (HMG-CoA reductase), is a rate-limiting enzyme of cholesterol biosynthesis. HMGCR inhibitors (e.g., statins) are currently the mainstay of treatment for dyslipidemia, which can reduce the levels of cholesterol via blocking mevalonate synthesis [40]. MMP9, belongs to matrix metalloproteinase (MMP) molecular family, has been considered as a key target for treating metabolic diseases. Studies have shown that MMP inhibitor was closely associated with calcific aortic valve disease (CAVD) among high fat induced mice, and that these enzymes might be important targets for preventing CAVD development [41]. Intracellular mitogen-activated protein kinases (MAPK) are an important class of proline-dependent protein kinases, which involved in the regulation of various biological functions, such as inflammation, cell proliferation and differentiation. Aouadi et al. [42] demonstrated that p38MAPK increased adipogenesis via inhibition of C/EBPβ and PPARγ activations. PPAR, a member of the nuclear receptor transcription factor family, has 3 isoforms: PPARα, PPARβ and PPARγ. It is known that lipid metabolism could be regulated by PPAR, which were shown to decrease lipoprotein secretion, accelerate triglyceride catabolism, and influences serum total- and LDL-cholesterol levels. On the other hand, PPARγ stimulates the fatty acids storage in adipocytes and regulates glucose metabolism, thereby improving insulin sensitivity [43]. FABP4, also known as Adipocyte fatty acid–binding protein (A-FABP), is a major cytoplasmic protein in adipocytes. It is shown that FABP4 is expressed at high levels in adipose tissues, especially in adipocyte differentiation [44–46]. NO is generated from L-arginine catalyzed by a class of nitric oxide synthases (NOS), including neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). All three NOS isoforms were expressed in atherosclerotic plaque. Once the enzyme is expressed, iNOS is regulated by the transcription fact and catalyzes the high expression of NO. On the contrary, eNOS and nNOS produce a small number of NO in a highly regulated way [47]. The above results indicated that Shanmei Capsule treatment may play its role of hypolipidemic via multi-targets.

The KEGG enrichment analysis indicated that the main pathways involved in the hyperlipidemia treatment process are PPAR signaling pathway, Bile secretion and TNF signaling pathway. PPAR signaling pathway participate in many physiological processes implicated in cardiovascular diseases associated with hyperlipidemia and diabetes, including lipid metabolisms, energy metabolisms, and inflammatory reactions, etc. PPARα is an essential transcription factor involved in lipid metabolism and inflammation, and there is predominantly expressed in β-oxidation activities and tissues with high mitochondrial (e.g., liver) [48]. While PPARγ is mainly expressed in immune system and adipose tissue, where it modulates genes involved in cell differentiation, cell cycle regulation, insulin sensitivity and glucose uptake [49]. Bile acids are famous for their effects on lipid digestion and cholesterol homeostasis. The conversion of cholesterol to bile acids (i.e., bile acid synthesis) in the liver is the major pathway of cholesterol removal from the body [50], which itself is endogenous ligands of the farnesoid X receptor (FXR). FXR, together with small heterodimer partner (SHP), plays an important role in linking bile acids
regulation with lipid, lipoprotein, and glucose metabolism [51,52]. As for TNF signaling pathway, it has direct effect on inflammation, whereas many cytokines such as TNF-α are able to increase lipolysis, which in turn contributes to a chronic state of insulin resistance and increases circulating free fatty acids. It has been reported that expanding adipose tissue results in chronic low-grade inflammation that leads to the development of metabolic disorders, including insulin resistance, dyslipidemia, and type 2 diabetes [53,54].

The potential binding sites and intermolecular interaction of active ingredients for target proteins were further elaborated by docking analysis. PPARα is commonly considered as a key target of lifibrate, and at the same time it is also an important target screened from the network pharmacology of Shanmei Capsule. The melocule docking results confirmed the potential hypolipidemic mechanism of Shanmei Capsule, showing excellent affinity towards PPARα target protein. The above findings also support the network pharmacology analysis results of Shanmei Capsule. Therefore, such compounds could provide a molecular foundation for the subsequent development of novel pharmacological inhibitors against hyperlipidemia.

5. Conclusion

In the present study, a network pharmacology approach in combination with molecular docking was proposed to explore the underlying mechanism of action of Shanmei Capsule against hyperlipidemia. The active ingredients of Shanmei Capsule in hyperlipidemia treatment are composed of 59 ingredients. Among them, quercetin, kaempferol, isorhamnins and chlorogenic acid are main active ingredients. The possible molecular mechanism mainly involved PPAR signaling pathway, Bile secretion and TNF signaling pathway via acting on MAPK8, PPARγ, MMP9, PPARα, FABP4 and NOS2 targets. In conclusion, Shanmei Capsule, as a traditional Chinese medicine preparation, has multiple constituents, multiple targets and multiple pathways properties, which could serve as a promising therapeutic drug for hyperlipidemia.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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**Supplementary material**

**Table S1**

| Protein | PDB ID | Grid parameters of the protein-ligand interaction | Center x | Center y | Center z | Size x | Size y | Size z |
|---------|--------|---------------------------------------------------|----------|----------|----------|--------|--------|--------|
| AKR1B10| 1ZUA   | -24.9 23.9 15.0 60.0 60.0 60.0                  |          |          |          |        |        |        |
| AR      | 2AX6   | 26.9 3.5 4.4 17.8 18.5 12.5                      |          |          |          |        |        |        |
| CNR1    | 5TGZ   | 32.3 19.0 292.2 39.8 73.1 70.4                   |          |          |          |        |        |        |
| CYP19A1 | 3EQM   | 85.2 51.3 42.8 21.9 24.7 21.4                    |          |          |          |        |        |        |
| EGFR    | 5U8L   | 13.4 -4.7 -15.9 21.7 29.1 47.6                    |          |          |          |        |        |        |
| ELANE   | 1H1B   | 10.5 8.9 15.9 39.2 52.8 31.0                      |          |          |          |        |        |        |
| ERBB2   | 3PP0   | 16.4 17.4 26.2 17.4 23.9 17.2                      |          |          |          |        |        |        |
| ESR1    | 3UUC   | -10.1 -16.2 -3.9 18.1 26.0 25.5                    |          |          |          |        |        |        |
| ESR2    | 3OLL   | -10.6 45.0 10.2 20.4 32.2 16.6                      |          |          |          |        |        |        |
| F2      | 3K6S   | 22.9 0.9 2.4 16.9 22.7 14.6                      |          |          |          |        |        |        |
| FABP4   | 5Y0F   | 5.6 -7.5 -19.2 17.4 16.1 18.6                      |          |          |          |        |        |        |
| LDLR    | 1JQ    | -3.1 39.3 47.6 50.0 48.0 52.0                      |          |          |          |        |        |        |
| MAPK8   | 4L7F   | -5.1 53.8 4.3 19.1 17.3 27.5                      |          |          |          |        |        |        |
| MAPK14  | 6SF1   | 53.4 69.4 17.4 19.7 23.8 21.7                      |          |          |          |        |        |        |
| MMP1    | 966C   | 8.8 -10.9 38.9 20.4 18.4 18.8                      |          |          |          |        |        |        |
| MMP3    | 1D7X   | -7.4 19.8 29.6 40.0 40.0 40.0                      |          |          |          |        |        |        |
| MMP9    | 4H1Q   | 29.2 6.0 18.9 37.5 22.8 25.2                      |          |          |          |        |        |        |
| MPO     | 5MFA   | -27.5 4.9 0.3 64.4 65.2 84.7                      |          |          |          |        |        |        |
| NOS2    | 4NOS   | 3.1 95.2 17.2 26.8 19.1 30.0                      |          |          |          |        |        |        |
| NR1H4   | 6HL1   | 10.9 14.7 12.3 17.9 23.7 14.5                      |          |          |          |        |        |        |
| PPARA   | 5HYK   | 8.7 33.9 19.3 14.5 18.0 19.8                      |          |          |          |        |        |        |
| PPARG   | 6T9C   | 14.8 17.3 17.7 21.9 17.8 18.8                      |          |          |          |        |        |        |
| PTGS2   | 5F19   | 28.1 41.0 56.6 54.1 75.2 45.6                      |          |          |          |        |        |        |
| AKR1B1  | 5OUK   | 4.5 2.7 4.8 27.0 24.7 20.5                      |          |          |          |        |        |        |
| FABP1   | 3STK   | -0.9 -8.8 19.4 24.2 18.4 28.0                      |          |          |          |        |        |        |
| HAP90AA1| 4LWE   | -0.7 -36.0 -25.3 17.1 17.9 17.3                  |          |          |          |        |        |        |