

To Explore the Mechanism of Hawthorn in the Intervention of Hyperlipidemia Based on the Gene Expression Omnibus and Network Pharmacology

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Song *et al.*: Mechanism of Hawthorn on Hyperlipidemia Based on the Gene Expression Omnibus and Network Pharmacology

A common clinical treatment for hyperlipidemia in China is hawthorn, a traditional Chinese herb. However, few researches have been done on its active ingredients and their possible mechanisms for treating hyperlipidemia. The active chemical constituents of hawthorn were obtained from the encyclopedia of traditional Chinese medicine, the bioavailability and druglikeness properties of each active ingredient of hawthorn were analyzed using SwissADME and their possible targets of action were predicted using SwissTargetPrediction and PharmMapper. Then, we used the Gene Expression Omnibus-related gene microarray data to mine the differentially expressed genes for hyperlipidemia. Meanwhile, we collected relevant targets of hyperlipidemia through the DrugBank, Online Mendelian Inheritance in Man, GeneCards, DisGeNET and Therapeutic Target Database. Based on this data, a component-target-disease pathway and a protein-protein interaction networks were constructed using search tool for the retrieval of interacting genes/proteins. Finally, Metascape was systematically used to analyze the targets of hawthorn intervention in hyperlipidemia. A total of 8 active components and 312 hawthorn targets were gathered and 343 prospective targets associated with hyperlipidemia were predicted, 25 of which shared targets with hawthorn-related targets. Gene ontology enrichment analysis revealed that the mechanism of action of hawthorn in intervention of hyperlipidemia lies in the regulation of lipid metabolism, nutrition level, hormone level and cholesterol transport process. The Kyoto encyclopedia of genes and genomes enrichment analysis revealed that the peroxisome proliferator activated receptor signaling pathway contained the majority of the core targets. Molecular docking verified that hawthorn could bind closely to the core targets. According to the findings of network pharmacology and enrichment analysis, we predicted that the main active components of hawthorn to intervene in hyperlipidemia are epicatechin, officinalic acid and quercetin, which mainly intervene in hyperlipidemia through the peroxisome proliferator activated receptor signaling pathway.

Key words: Network pharmacology, hawthorn, hyperlipidemia, gene expression omnibus database

Cardiovascular Disease (CVD) is the major cause of morbidity and death in the world today. In a global survey of CVD, it is reported that the number of people with CVD worldwide increased rapidly from 1.271 billion to 523 million between 1990 and 2019, and the number of deaths increased from 12.1 million to 18.6 million, with 9.6 million men and 8.9 million women^[1]. Metabolic CVD is a class of diseases in which disorders of multiple nutrient metabolites impair the heart and blood vessel function. It is closely related to CVD development and mainly includes abnormalities in blood lipids, blood glucose,

uric acid and obesity^[2]. A prospective cohort study of 6255 subjects found that patients with metabolic syndrome had a prominently increased risk of death from CVD than healthy subjects and patients with diabetes combined with CVD were at higher risk^[3]. Hyperlipidemia is a common metabolic syndrome characterized by elevated levels of Total Cholesterol (TC), Triglycerides (TGs), Low-Density Lipoprotein Cholesterol (LDL-C) and decreased High-Density Lipoprotein Cholesterol (HDL-C)^[4]. It is a significant risk factor for coronary artery disease^[5]. Furthermore, lowering TC, TGs and LDL-C levels in blood can

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considerably lower the incidence of coronary artery disease and the risk of death^[6]. Therefore, effective lipid control is essential for the treatment of CVD and for improving the quality of life of patients. At present, hyperlipidemia cannot be cured temporarily and requires long-term oral drug treatment. Atorvastatin calcium, rosuvastatin calcium, simvastatin and other Hydroxymethylglutaryl-Coenzyme A (HMG-CoA) reductase inhibitors and some other lipid-lowering drugs are commonly used in the first line of clinical practice, and long-term clinical observation has found that they have good lipid-lowering effects^[7]. However, there are certain adverse effects such as muscle damage, liver function impairment, allergy, etc.^[8]. Therefore, the development of effective drugs with less toxic side effects is an inevitable clinical requirement.

Most interventions in Chinese medicine for hyperlipidemia use methods to strengthen the spleen and resolve phlegm, and hawthorn is one of the most used single herbs with a long history^[9]. Metabolomic analysis shows that hawthorn exerts its activity by regulating lipid metabolism, energy metabolism, oxidative stress and amino acid metabolism^[10]. In addition, hawthorn extract can effectively lower serum cholesterol, TGs, LDL-C and increase HDL-C levels, reducing a series of inflammatory factors, inhibits progression and protects vascular endothelial cells^[11]. Meanwhile, Shator *et al.* found that hawthorn can effectively reduce blood lipids and reverse atherosclerosis in hyperlipidemic rats through an antioxidant mechanism and regulation of oxidized LDL-C (ox-LDL-C) levels^[12]. It has potential value in the treatment of hyperlipidemia. However, the specific pharmacological mechanism of hawthorn intervention in hyperlipidemia is still unclear. This study comprehensively elucidated the potential therapeutic targets of hawthorn intervention in hyperlipidemia using network pharmacology, Gene Expression Omnibus (GEO) and molecular docking to provide a strong theoretical basis for clinical research and drug use.

Network pharmacology is a systematic analysis method based on the interaction of disease, drugs, active components, target gene and target protein^[13]. It emphasizes the analysis of potential targets of drug interventions on diseases from a systemic level and the holistic perspective of network data, which coincides with the holistic concept of Chinese medicine and is important for studying the role of Traditional

Chinese Medicine at the micro level^[14]. The GEO database is a publicly accessible archive of data from a wide range of high-throughput genetic studies, including single and dual-channel microarray-based assays that detect messenger Ribonucleic Acid (mRNA), genomic Deoxyribonucleic Acid (DNA), and protein abundance, as well as non-array technologies^[15,16]. Combining network pharmacology and GEO could reveal the disease mechanisms and molecular targets for drug therapy^[17]. Therefore, this study will systematically use network pharmacology combined with GEO to analyze the mechanism of hawthorn intervention on hyperlipidemia (fig. 1). First, we obtained its composition and potential targets to intervene in hyperlipidemia. Protein-Protein Interaction (PPI) models of potential targets for intervention in hyperlipidemia were constructed using the potential targets. Following that, a comprehensive examination of possible targets and biofunctional modules was carried out. Meanwhile, molecular docking was used to validate the interactions between active components and key targets.

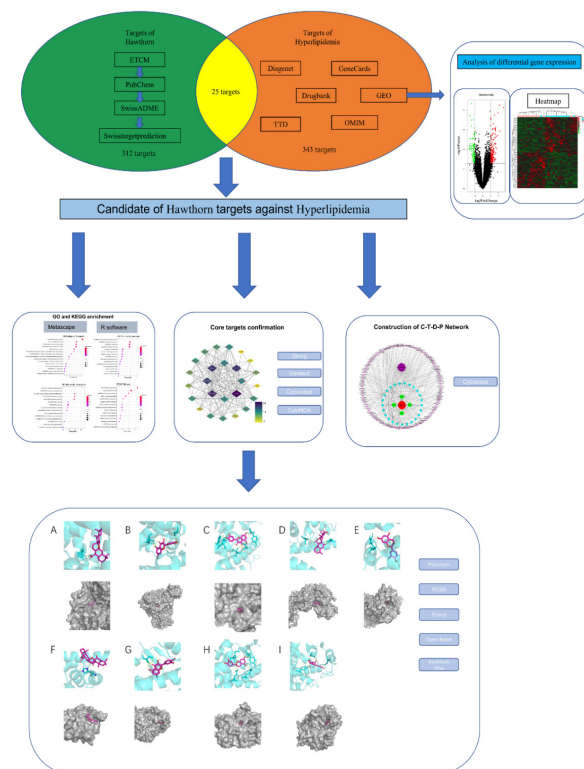


Fig. 1: The flow chart of the study

MATERIALS AND METHODS

Hawthorn target acquisition:

Firstly, using the Encyclopedia of Traditional Chinese

Medicine (ETCM)^[18] and published literature the individual active components of hawthorn are collected. Then, all Structure Data Format (SDF) files were obtained from PubChem^[19]. Next, the obtained SDF files were imported into SwissADME^[20] for absorption prediction. The criteria for setting the potential core compounds were, Pharmacokinetics-Gastro Intestinal (GI) absorption was set to “High”, indicating good oral bioavailability of the ingredient; Druglikeness was set to 5 categories of pharmacophore prediction (Lipinski, Ghose, Veber, Egan, Muegge) with two or more “Yes” results^[21,22]. Finally, the screened core chemical components were uploaded to SwissTargetPrediction^[23] and PharmMapper^[24] to predict potential targets and the screened targets were combined and de-duplicated.

Therapy targets of hyperlipidemia:

The datasets were retrieved using the keyword “hyperlipidemia” in the GEO database and downloaded the retrieved gene chip related files. Converting probe IDs to gene names using the R software limma package^[25], obtaining the expression matrix and ultimately screening for differential genes with Fold Change (FC)>2 and p value<0.05 as the threshold, using R software pheatmap package^[26] to draw heat map. Utilizing ggplot2^[27], we created a volcano plot and differentially expressed gene sets were normalized to the UniProt protein database^[28].

Online Mendelian Inheritance in Man (OMIM)^[29], DrugBank^[30], GeneCards^[31], DisGeNET^[32] and Therapeutic Target Database (TTD)^[33] can provide the latest data on human genes and free information on currently known disease-related targets in which hyperlipidemia was used as a keyword with the condition “human” or “*Homo sapiens*.” The relevant hyperlipidemia targets were gathered in the aforementioned database, merged and de-duplicated with the differentially expressed gene set obtained from GEO analysis and all targets were standardized using UniProt.

Component-Target-Disease-Pathway (C-T-D-P) network construction:

Based on the data gathered, we constructed the C-T-D-P network using Cytoscape 3.8.0^[34] to demonstrate the correlation between bioactive compounds, potential targets and pathways. Potential targets were used for subsequent protein interaction and functional enrichment analysis.

PPI analysis:

We extracted intersect of targets in hyperlipidemia and hawthorn and defined them as potential targets for hawthorn intervention in hyperlipidemia. PPI^[35] refers to studying correlations between compounds and disease-related proteins from the perspective of biochemistry, signal transduction pathways and genetic networks. PPI analysis of disease-associated proteins allows better presentation of their relevance to drug compounds. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a web-based online tool^[36] that provides online analysis of PPIs by uploading potential intervention targets to STRING to obtain PPI network relationships. The results are imported into Cytoscape for further visualization and analysis. CytoNCA plugin^[37] of cytoscape was used to analyze and evaluate PPI networks and Molecular Complex Detection (MCODE)^[38] was used to obtain potential protein functional modules and analyze related functions. In addition, in order to identify the core of the potential targets more accurately, we imported the potential targets of hawthorn intervention in hyperlipidemia into the VarElect dataset^[39] for correlation analysis of potential targets with hyperlipidemia.

Biology enrichment analysis:

Functional enrichment analysis (Gene Ontology (GO)^[40] and Kyoto Encyclopedia of Genes and Genomes (KEGG)^[41]) was performed on potential targets to facilitate mechanistic understanding of hawthorn intervention in hyperlipidemia. Metascape is a web-based tool that enables researchers thorough gene list annotation and functional enrichment^[42]. Potential targets were uploaded to Metascape for GO Biological Process (BP), Cell Component (CC), Molecular Function (MF) and KEGG pathway enrichment analysis, and entries with p value<0.01 are considered significantly enriched. Finally, the top 15 eligible GO enrichment analysis results and KEGG pathways were selected for further research.

Molecular docking:

Import the core compound “SDF” file of Hawthorn into Open Babel 3.1.1^[43] to convert the file format to “mol2”. Protein Data Bank (PDB)^[44] collects the structures of the core targets and saves them as “mol2” file format, using PyMOL software^[45] to separate modified ligands and remove water. AutoDockTools^[46] was used to add polar hydrogens

and assign Gasteiger charges for proteins and ligands, and the resulting structure was saved in Protein Data Bank, Partial Charge (Q), and Atom Type (T) (PDBQT) format. Finally, in order to determine whether the ligand and proteins selected after this procedure would bind well, dockings were performed with AutoDock Vina^[47], where a docking score affinity < -4.25 kcal/mol can suggest binding activity between ligand and proteins, score < -5.0 kcal/mol indicates improved binding activity and score < -7.0 kcal/mol indicates substantial docking activity between ligand and proteins^[48].

RESULTS AND DISCUSSION

Hawthorn targets were shown here. Active components of hawthorn were obtained from ETCM and their structures were downloaded through PubChem after uploading the "SDF" files to SwissADME to assess the GI absorption and druglikeness. A total of 8 potential core compounds were obtained and shown in Table 1. 209 potential hawthorn targets were collected after importing them into SwissTargetPrediction and 369 potential targets of hawthorn were collected after prediction by PharmMapper. After removing duplicates, a total of 312 potential targets of hawthorn were obtained.

Targets of hyperlipidemia were shown here. After searching from the GEO datasets, the original dataset of the GSE1010 chip and its annotation file GPL96 were downloaded and analyzed using R software. There were 253 differentially expressed genes which were obtained, of which 164 had their increased expression and 89 were downregulated. Following this, a heat map for hyperlipidemia target was created using the pheatmap R package (fig. 2). Finally, a volcano diagram was developed using the R package ggplot2 (fig. 3). We searched OMIM, DrugBank, GeneCards, DisGeNET and TTD. A total of 141 hyperlipidemia-associated targets were obtained. After pooling and de-duplication with GEO

differential expressed genes, 343 hyperlipidemia-associated genes were collected.

Using Cytoscape, we created a C-T-D-P network based on the data presented above. The network consisted of 142 nodes, including four active components of hawthorn, hyperlipidemia and 25 common targets, with 321 signaling pathways connected between the nodes (fig. 4). This network has initially revealed a complex mechanism of multiple targets and signaling pathways for compounds of hawthorn to intervene in hyperlipidemia.

PPI network analysis was explained here. The further analysis used, STRING to analyze PPI for all 25 common targets. The data was loaded into Cytoscape and degree values of each gene were determined with the CytoNCA plugin. Meanwhile, PPI networks were constructed based on the STRING database (fig. 5). The targets with top 8 degree values were defined as hub targets. The names and functions of these hub genes are shown in Table 2. Using the MCODE plugin, a working module was also identified (fig. 6). In order to determine the target of the hub genes more accurately, we used VarElect to analyze the correlation. According to the findings, 17 targets were directly associated to hyperlipidemia, whereas 8 were indirectly related to hyperlipidemia (Table 3). After comparing with the hub target, it was found that Albumin (ALB), Peroxisome Proliferator Activated Receptor Alpha (PPARA) and Peroxisome Proliferator Activated Receptor Gamma (PPARG) had the highest score of correlation. Each active component of hawthorn will be molecularly docked to 3 hub targets.

Functional enrichment analysis and its results were explained here. 25 potential targets were uploaded to Metascape for GO enrichment analysis: BP, CC, MF and KEGG pathways. The results of $p < 0.01$ were derived and the bubble diagram of enrichment analysis was drawn using the ggplot package in R language (fig. 7).

TABLE 1: POTENTIAL CORE COMPOUNDS OF HAWTHORN

Compound	PubChem ID	Molecular formula
Acetic acid	176	C ₂ H ₄ O ₂
Epicatechin	72 276	C ₁₅ H ₁₄ O ₆
Malic acid	525	C ₄ H ₆ O ₅
Officinalic acid	10 577 334	C ₃₀ H ₄₄ O ₆
Oxalic acid	971	C ₂ H ₂ O ₄
Propionic acid	1032	C ₃ H ₆ O ₂
Quercetin	5 280 343	C ₁₅ H ₁₀ O ₇
Succinic acid	1110	C ₄ H ₆ O ₄

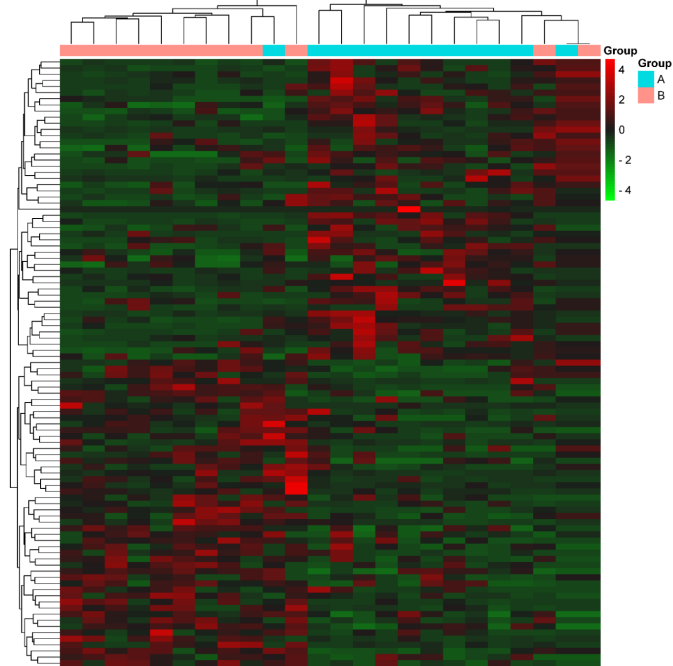


Fig. 2: The heat map of differentially expressed genes.

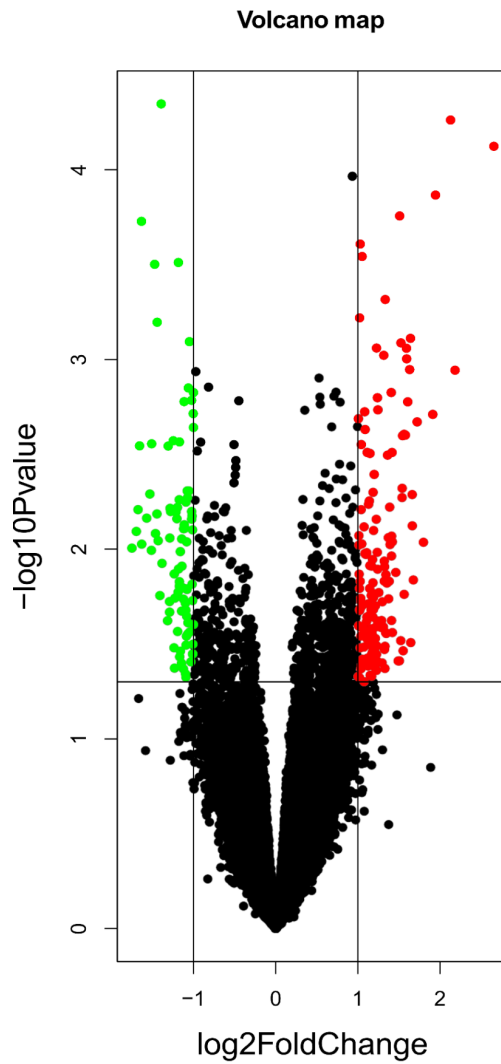


Fig. 3: The volcano map, (●) Upregulated; (●) Downregulated and (●) Neutral genes

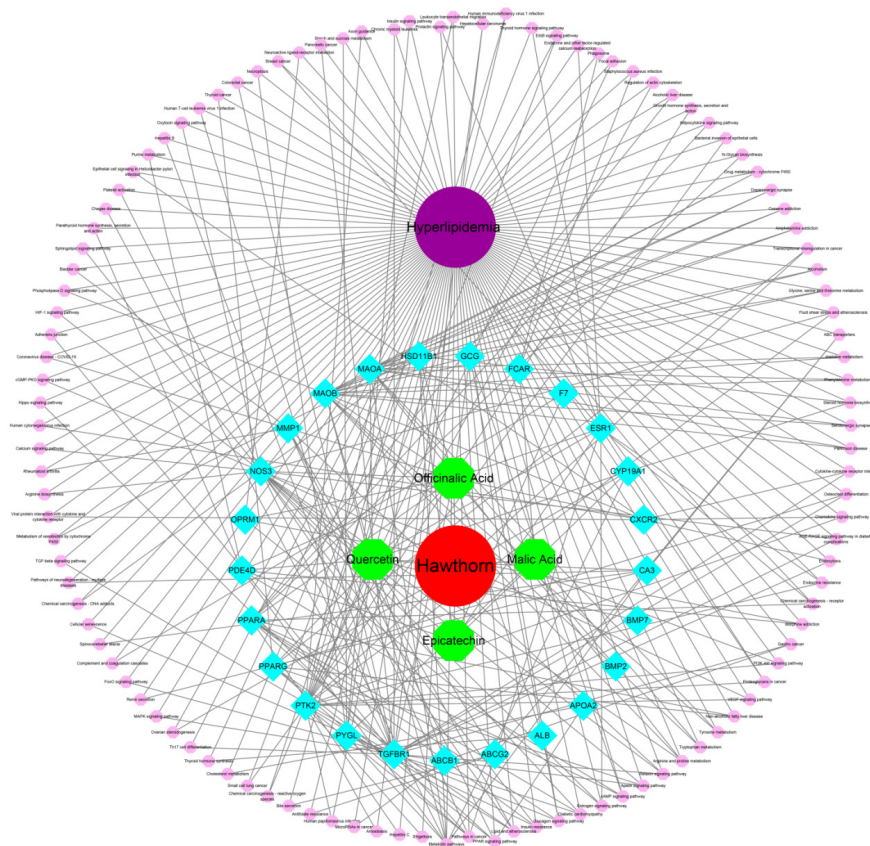


Fig. 4: The C-T-D-P network

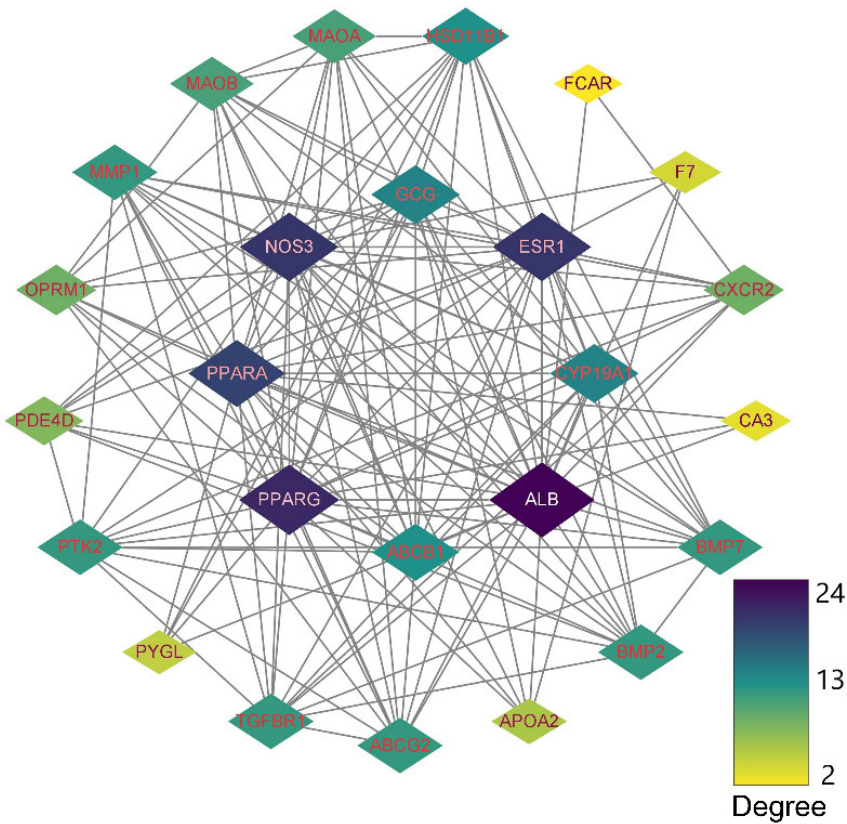
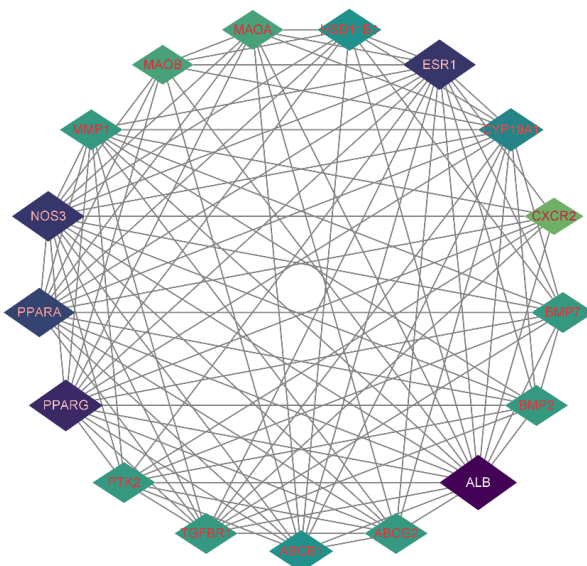


Fig. 5: The PPI network
Note: All nodes are coloured according to the degree value

TABLE 2: NAMES AND TOPOLOGICAL PARAMETERS OF THE HUB TARGETS

Gene symbol	Degree	Betweenness centrality	Closeness centrality
ALB	24	99.3894	1
PPARG	21	46.5841	0.88889
NOS3	20	31.1672	0.85714
ESR1	20	29.3894	0.85714
PPARA	19	36.4794	0.82759
CYP19A1	14	5.18066	0.70588
ABCB1	13	4.07669	0.68571
HSD11B1	13	6.72114	0.68571

**Fig. 6: The function modules identified using MCODE plugin****TABLE 3: THE INFORMATION OF TARGETS GENES DIRECTLY ASSOCIATED TO HYPERLIPIDEMIA**

Symbol	Description	Scores
APOA2	Apolipoprotein A2	16.74
ALB	Albumin	11.15
PPARA	Peroxisome Proliferator Activated Receptor Alpha	11.06
PPARG	Peroxisome Proliferator Activated Receptor Gamma	8.9
NOS3	Nitric Oxide Synthase 3	3.81
PYGL	Glycogen Phosphorylase L	3.59
CYP19A1	Cytochrome P450 Family 19 Subfamily A Member 1	3.05
ESR1	Estrogen Receptor 1	2.97
F7	Coagulation Factor VII	2.63
GCG	Glucagon	2.2
ABCB1	ATP Binding Cassette Subfamily B Member 1	0.9
HSD11B1	Hydroxysteroid 11-Beta Dehydrogenase 1	0.5
PTK2	Protein Tyrosine Kinase 2	0.49
MMP1	Matrix Metalloproteinase 1	0.38
BMP2	Bone Morphogenetic Protein 2	0.22
PDE4D	Phosphodiesterase 4D	0.12
ABCG2	ATP Binding Cassette Subfamily G Member 2 (Junior Blood Group)	0.12

Note: The score is an indication of the strength of the connection between the gene and the disease

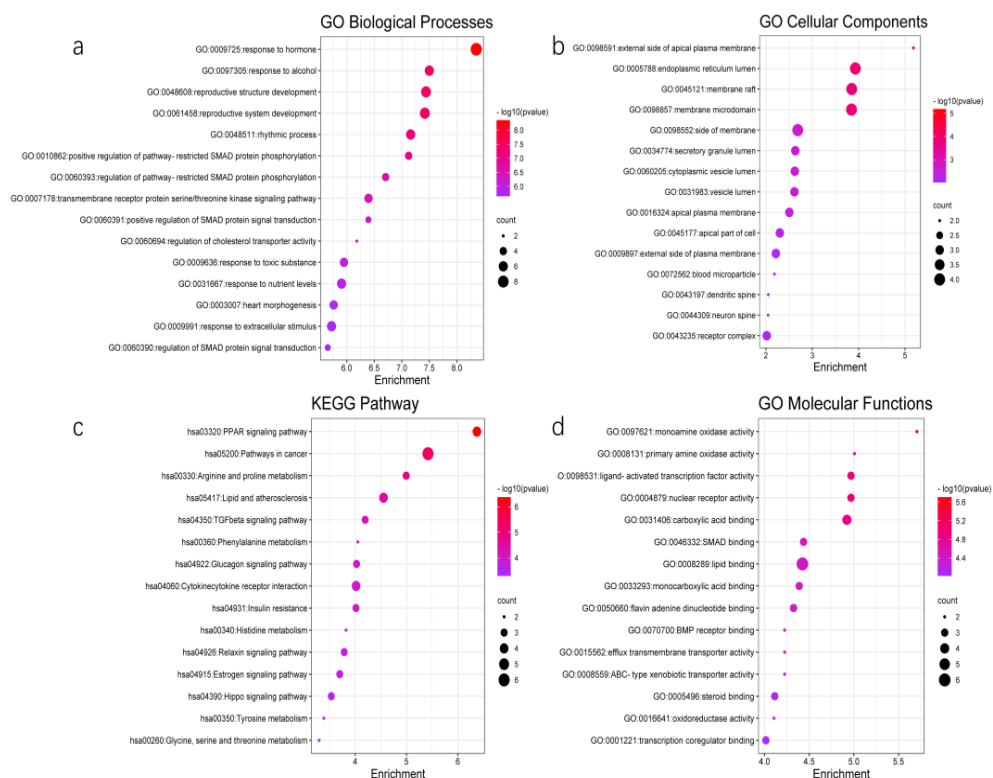


Fig. 7: The functional enrichment analysis of (a) GO-BPs; (b) GO-CCs; (c) KEGG pathway and (d) GO-MFs

Potential targets in GO BPs were mainly involved in nutrient level regulation, hormone levels, growth, heart rate, cholesterol transport and other processes (fig. 7a). GO CCs are involved in the apical plasma membrane, endoplasmic reticulum, plasma membrane rafts, vesicle lumen and secretory granule lumen (fig. 7b). The KEGG pathway focuses on PPAR signaling pathway, lipid and atherosclerosis, Transforming Growth Factor beta (TGF- β) signaling pathway, glucagon signaling pathway, insulin resistance and cytokine receptor interactions (fig. 7c). GO MFs are mainly involved in monoamine oxidase activity, nuclear receptor activity, lipid binding, transcription factor activity, steroid binding, carboxylic acid-binding, etc. (fig. 7d).

The functional modules were subjected to GO and KEGG enrichment analyses and their findings were shown in R using the ggplot package (fig. 8). The enrichment analysis showed that the modules could be involved in cancer pathways, lipid binding, transport, steroid hormones bioregulation, TGF- β -Suppressor of Mothers against Decapentaplegic (TGF- β -SMAD) signaling pathway regulation, redox, etc.

Molecular docking results were explained here.

The protein PDB files (ALB: 4L8U, PPARA: 1I7G and PPARG: 1FM6) were obtained from the PDB database. In further analysis, they were preprocessed with ligands for molecular docking using AutoDock Vina and results were exported (fig. 9). The results were visualized using PyMOL (fig. 10). As a result, 24 receptor-ligand docking results were obtained, which depicted that, the docking model becomes more stable if the binding energy is less. Finally, we included 9 groups with the least binding energy. In the binding model of officinalic acid to ALB, the officinalic acid core can form hydrogen bonds with residues H242 (fig. 10a). In the binding model of quercetin to ALB, the quercetin core can form hydrogen bonds with residues K199, A210 (fig. 10b). In the binding model of quercetin to PPARA, the quercetin core can form hydrogen bonds with residues Y214, M320, T283, T279 and Y334 (fig. 10c). In the binding model of officinalic acid to PPARG, the officinalic acid core can form hydrogen bonds with residues L476, K457 (fig. 10d). In the binding model of epicatechin to ALB, the epicatechin core can form hydrogen bonds with residues R10 (fig. 10e). In the binding model of officinalic acid to ALB, the officinalic acid core can form hydrogen bonds with residues H411 (fig. 10f).

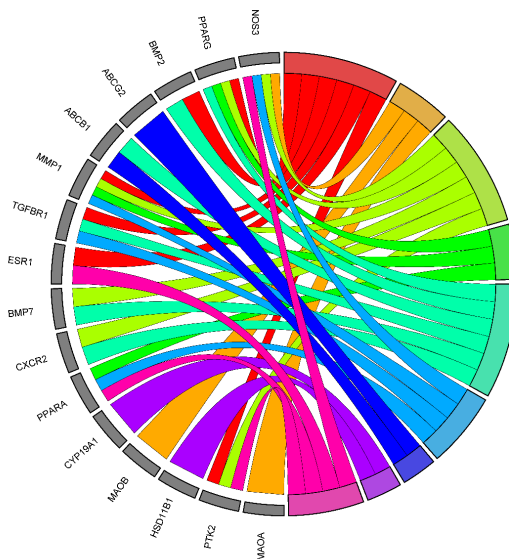


Fig. 8: The enrichment analysis of function modules

Note: (■) Pathways in cancer; (■) Arginine and proline metabolites; (■) Lipid and atherosclerosis; (■) PPAR signaling pathway; (■) Cytokine-cytokine receptor interactions; (■) Relaxin signaling pathway; (■) ABC transporters; (■) Steroid hormone biosynthesis and (■) Endocrine resistance

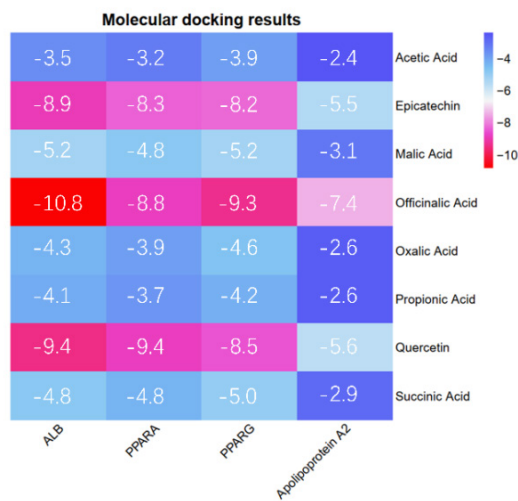


Fig. 9: The results of molecular docking

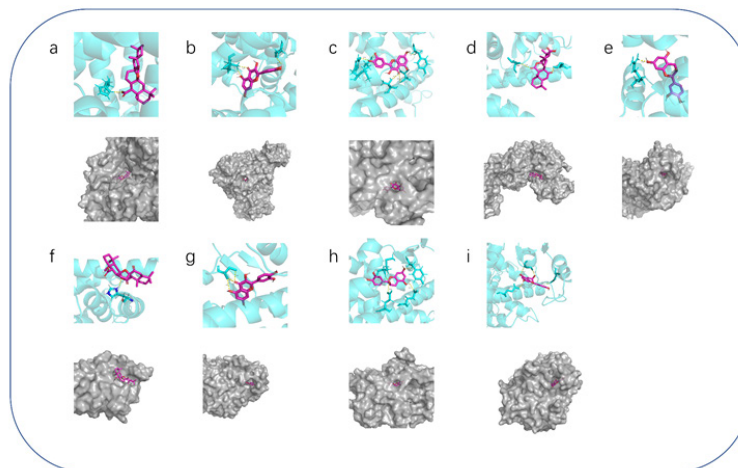


Fig. 10: The top 10 binding energy molecular docking models

Note: Binding models of (a) Officinalic acid and ALB; (b) Quercetin and ALB; (c) Quercetin and PPARA; (d) Officinalic acid and PPARG; (e) Epicatechin and ALB; (f) Officinalic acid and PPARA; (g) Quercetin and PPARG; (h) Epicatechin and PPARA and (i) Epicatechin and PPARG

In the binding model of quercetin to PPARG, the quercetin core can form hydrogen bonds with residues S342 (fig. 10g). In the binding model of epicatechin to PPARA, the epicatechin core can form hydrogen bonds with residues M220, E282, E286 and Y334 (fig. 10h). In the binding model of epicatechin to PPARG, epicatechin core can form hydrogen bonds with residues E259, E291, S342 (fig. 10i).

Hyperlipidemia belongs to “lipid turbidity” in traditional Chinese medicine. Most medical practitioners believe that the method of removing stagnation and strengthening the spleen is to resolve phlegm which is crucial in intervening in hyperlipidemia^[9]. Modern clinical studies have shown that almost patients with hyperlipidemia have disorders of lipid metabolism due to decreased activity of lipoprotein degrading enzymes and increased rate of lipoprotein synthesis, or abnormal lipoprotein structure and lipoprotein receptor structure, ultimately leading to excessive accumulation of lipids in the body^[49]. Several studies have shown that hawthorn can regulate lipid metabolism, effectively reducing cholesterol and TG content in hyperlipidemic rats^[10-12]. Based on the network pharmacological screening, 3 active compounds (quercetin, epicatechin and officinalic acid) of hawthorn had a potential role in the intervention in hyperlipidemia. Quercetin is a flavonol compound with various biological activities. In the pharmacological effect study of quercetin, it was found that quercetin could significantly improve lipid metabolism in rats, which includes inhibiting the activity of HMG-CoA reductase, down-regulating the HMG-COA expression, reduction in TC, TGs and LDL levels^[50]. In addition, quercetin also induces TC and HDL binding through activation of the PPARG/Liver X Receptor- α (LXR α) pathway to increase HDL-C selective lipid uptake and promotes cholesterol transportation to the liver, thereby reducing lipid accumulation^[51,52]. Meanwhile, Cheng *et al.* showed that epicatechin could effectively reduce TC, TGs and LDL by regulating the Insulin-Induced Gene 1 protein (INSIG1)-Sterol Regulatory Element-Binding Protein (SREBP)-SREBP Cleavage-Activating Protein (SCAP) pathway and other genes related to lipid metabolism (including LXR α , Fas Cell Surface death receptor (FAS) and Sirtuin 1 (SIRT1)), which could increase the amount of HDL in serum, significantly improving lipid metabolism^[53]. Currently, clinical experiments have confirmed that epicatechin can reduce the level of membrane lipid peroxidation, which can improve

the membrane fluidity and lipid transport in patients with hypercholesterolemia and healthy donors^[54]. It is expected that these active components will be used in potential directions for hawthorn intervention in hyperlipidemia.

After screening the related datasets, a total of 25 potential intervention targets were collected. Based on this target, a PPI network was constructed to evaluate its association and a C-T-D-P diagram was drawn to visually express the relevant targets and pathways of hawthorn components intervening in hyperlipidemia. Meanwhile, potential functional modules were identified through the MCODE plugin. The potential targets in GO BPs are mainly involved in regulating nutrient levels, hormone levels, growth, heart rate, cholesterol transport and other processes. The KEGG pathway focuses on PPAR signaling pathway, lipid and atherosclerosis, TGF- β signaling pathway, glucagon signaling pathway, insulin resistance, and cytokine receptor interactions. The enrichment analysis showed that the modules could be involved in cancer pathways, lipid binding, transport, steroid hormone bioregulation, TGF- β -SMAD signaling pathway regulation and redox regulation.

Impaired lipid metabolism is a significant factor in the development of hyperlipidemia and PPAR signaling was the most enriched KEGG pathway in the analysis of the potential target. Furthermore, the PPAR signaling is closely related to the lipid metabolism pathway. Therefore, we hypothesize that hawthorn intervenes in hyperlipidemia by modulating the PPAR pathway to improve lipid metabolism. The PPAR signaling pathway can be involved in regulating a variety of cellular functions. Nan *et al.* found that up-regulating the expression of Lipoprotein Lipase (LPL) through the PPAR signaling pathway and inhibiting the expression of Fatty Acid Translocase (FAT)/Cluster of Differentiation 36 (CD36) signaling pathway could effectively improve lipid metabolism in hyperlipidemic rats and correct the lipid metabolism and lipid deposition imbalances in the liver^[55]. LPL, a critical enzyme involved in lipid metabolism, plays an essential role in lipoprotein metabolism and lipid partitioning^[56]. Mutations in the human LPL gene cause various abnormalities in lipid metabolism and lead to familial hyperlipidemia^[57]. CD36 is a membrane receptor known to play a role in lipid metabolism by promoting the uptake of long-chain fatty acids^[58]. Meanwhile, TGF- β -SMAD is an

important signaling pathway related to the regulation of cardiovascular function. It plays an important role in BPs such as atherosclerosis, myocardial fibrosis, regulation of cardiomyocyte apoptosis and vascular remodeling^[59]. TGF- β -SMAD is also closely related to lipid metabolism. Lu *et al.* found that calcitriol could inhibit the activation of Interleukin-1 beta (IL-1 β) and Tumor Necrosis Factor alpha (TNF- α), effectively reducing TC, TGs and LDL-C through the TGF- β -SMAD signaling pathway, which can effectively improve lipid metabolism and atherosclerosis in hyperlipidemic rats^[60].

Finally, PPI and disease association analysis was performed and the core targets (ALB, PPARA and PPARG) were identified. ALB has the highest degree of value, association and centrality, making it a primary target not just for PPI networks but also for possible functional modules. In addition, disease association analysis using VarElect showed that ALB was strongly associated with hyperlipidemia. It can be concluded that ALB is the main target of hawthorn to intervene in hyperlipidemia. ALB, the most abundant protein in human blood, plays a key role in lipid and fatty acid metabolism, binding and transport^[61]. In addition, studies on congenital hypoalbuminemia have found that congenital hypoalbuminemia significantly affects lipid metabolism, leading to severe hypoalbuminemia and elevated levels of LDL-C, leading to the occurrence of hyperlipidemia^[62]. It has also been noted that people suffering from severe anaplasmosis are often accompanied by severe hypercholesterolemia and elevated LDL. These aberrant metabolisms might be the product of a compensation mechanism for severe ALB deficit and they give evidence for a putative function of serum ALB in the regulation of lipoprotein metabolism^[63]. Faingold *et al.* found that the ALB-bound nitrosyl iron complex can inhibit oxidative stress-induced lipid peroxidation^[64]. PPARA is a key regulator of lipid metabolism, which can regulate the β -oxidation of fatty acids and accelerates the bioavailability of lipids. A further study by Eurlings *et al.* verified that PPARA was identified as a gene that regulates intra and extracellular lipid metabolism, such as lipolysis and mitochondrial β -oxidation. A change in these factors, in turn, leads to decreased expression of Fatty Acid Binding Protein (FABP) and Fatty Acid Transport Protein (FATP). Interestingly, the absorption of Free Fatty Acids (FFA) requires the mediation of FABP and FATP, leading to blocked FFA entry into hepatocytes for β -oxidation. These changes

could increase the TG synthesis from FFA and Very-Low-Density Lipoprotein (VLDL) production^[65-67]. Meanwhile, experiments conducted by Stec *et al.* confirmed that PPARA mutant mice acquire obesity in a sexually dimorphic way, showing that PPARA plays an important role in the prevention of hyperlipidemia and fatty liver disease^[68]. PPARG, a key pathway of the PPAR signaling pathway, has a positive role in regulating lipids^[69]. Ruotolo *et al.* found that PPARG can significantly improve dyslipidemia by modulating insulin resistance^[70]. Recently, an animal study suggests that PPARG can regulate adipocyte differentiation to suppress hyperlipidemia^[71]. Further mechanism investigations have revealed that PPARG mutation and lipid metabolism related to PPARG are important in the progression of hyperlipidemia, which is one of the cores of the development of hyperlipidemia^[72,73]. In conclusion, based on the results of network pharmacology and enrichment analysis, we predicted that the main active components of hawthorn to intervene in hyperlipidemia are epicatechin, officinalic acid and quercetin, which mainly intervene in hyperlipidemia through the PPAR signaling pathway.

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Author's contributions:

Xuejing Song, Hong Li and Chao Tang conceived, designed and planned the study. Hong Li, Yi Liang and Xuejing Song acquired and analysed the data. Hong Li and Jun Chen interpreted the results. Hong Li and Chao Tang drafted the manuscript. Yi Liang and Xuejing Song contributed to the critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

Conflicts of interest:

The authors declare that there is no conflict of interest.

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