Effects of Gynostemma pentaphyllum on hypolipidemic and the mechanism of network pharmacology and molecular docking in treating hyperlipidaemia

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Article

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Abstract

Objective: To explore the material basis and potential molecular mechanism of Gynostemma pentaphyllum's hypolipidaemic effects.

Methods: A hyperlipidaemic rat model was established to evaluate the hypolipidaemic effect of Gynostemma pentaphyllum. Next, we verified the bioactive components (BCI) and potential targets of Gynostemma pentaphyllum in the treatment of hyperlipidaemia using network pharmacology and molecular docking, and constructed the pharmacological network and PPI network. Then the core genes were enriched by GO and KEGG. Finally, the bioactive components were docked with the key targets to verify the binding ability between them.

Results: The results showed that the serum TC, LDL-C and HDL-C levels were significantly decreased ($P<0.05$), the number of liver vacuoles was also significantly reduced and the relative expression of PPARγ and SOD mRNA increased ($P>0.05$) after gynostemma administration. What's more, a total of 24 active ingredients and 125 common targets of the drug and the disease. PPI network and functional analysis showed that AKT1, TNF, IL6, VEGFA, EGFR, PPARG are very important which were mainly involved in the cancer pathway, the chemo-carcinogenesis-receptor activation pathway, the lipid and atherosclerosis pathway, proteoglycans in cancer, the PI3K-Akt signalling pathway. Molecular docking results showed that the core active components of gibberellin, gibberellin saponin XXVIII, 3'-methylrhodopsin and gibberellin showed good affinity with the Hub gene.

Conclusion: Gynostemma has a significant hypolipidaemic effect and may affect lipid metabolism by acting on multiple targets through multiple components.

Introduction

Hyperlipidaemia is a disease caused by abnormal blood lipid levels that is also known as abnormal lipid metabolism and is characterized by abnormally high serum levels of total cholesterol (TC), triacylglycerol (TG) and low-density lipoprotein cholesterol (LDL-C) or abnormally low levels of high-density lipoprotein cholesterol (HDL-C)[1]. With the improvement in people's living standards, a high-energy and high-fat diet has led to a significant increase in the incidence of hyperlipidaemia, and studies have shown that hyperlipidaemia significantly increases the risk of other serious cardiovascular diseases (CVDs), such as fatty liver and atherosclerosis[2-4], and hyperlipidaemia has thus become an important health problem. Therefore, lowering lipid levels is considered an important way to prevent or slow down the progression of atherosclerosis[5], and it is important to focus on strategies for preventing and treating dyslipidaemia.

The chemical drugs commonly used in clinical practice to treat hyperlipidaemia are statins, cholesterol absorption inhibitors, fibrate derivatives, bile acid chelators and PCSK9 inhibitors. Although these chemical drugs are widely used in clinical practice, they are associated with many adverse effects, such as elevated blood glucose, muscle pain, nausea, and even rhabdomyolysis and hepatotoxicity with long-

term administration[6]. In addition, lipid synthesis, transport, uptake and catabolism are regulated by multiple gene expression and signalling pathways that involve pathological mechanisms such as abnormal glucolipid metabolism, inflammation, and endoplasmic reticulum stress[7-8]; therefore, it is necessary to explore multitarget therapeutic drugs for hyperlipidaemia characterized by disorders of lipid metabolism. Traditional Chinese medicine (TCM) has received increasing attention because of its “multicomponent and multitarget” features, minimal adverse effects and high safety characteristics. Moreover, lipid-lowering herbal medicines such as Zelenia and the Chinese compound Danshen[9-10] have shown lipid-lowering effects in the treatment of hyperlipidaemia in recent years, indicating that Chinese medicine has unique efficacy in the treatment of hyperlipidaemia and shows great advantages and prospects for development and application.

Materials And Methods

This study was conducted in accordance with ARRIVE guidelines (PLoS Bio 8(6), e1000412,2010) (https://arriveguidelines.org) and approved by the Medical Ethics Committee of Zunyi Medical University (Zunyi Medical Ethics (2022) No.1-303). All methods were performed in accordance with the relevant guidelines and regulations and all experimental protocols were approved by Experimental Management Center of Zunyi Medical University.

Database

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

The platforms and databases used in this study are shown in Table 1.

Table 1 Database and analysis platform websites

Database and software name	Website
TCMSP	https://old.tcmsp-e.com/tcmsp.php
PubChem	https://pubchem.ncbi.nlm.nih.gov/
Swiss Targe Prediction	www.swisstargetprediction.ch/
DisGeNET	https://www.disgenet.org/
Gene Card	www.genecards.org/
STRING	https://string-db.org/
Cytoscape 3.7.0	https://cytoscape.org/
Metascape	https://metascape.org/
Venny 2.1.0	https://bioinfogp.cnb.csic.es/tools/venny/index.html
bioinformatics	http://www.bioinformatics.com.cn/

Animals and reagents

24 Male Sprague-Dawley (SD) rats were used, weighing 150–180g, production licence No. SCXK (Xiang) 2019-0004, were purchased from Hunan Sleek Jingda Laboratory Animal Co. Gynostemma (mass fraction > 98%, lot number: 200703) was purchased from Shaanxi Zhongxin Biotechnology Co., Ltd. Simvastatin was acquired from State Drug Administration (H20010053). Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) kits were purchased from Nanjing Jiancheng Biotechnology Co.

Establishment and administration of the hyperlipidaemia model

Male SD rats were randomly divided into 4 groups of 6 rats each. The rats in the control group (normal diet, ND) were fed a basal diet, and the rest were fed a high-fat diet (HFD; 60% fat, purchased from Jiangsu Synergy Biotechnology Co., Ltd.). The body weights of the rats were recorded weekly, and the serum lipid indexes (TC, TG, HDL-C and LDL-C) of the rats in the blank group and the model group (HFD) were measured after 17 weeks to determine the success of the model. The rats in the gibberellin administration group (GP+HFD) were given gibberellin 125 mg/kg body weight per day by gavage, and the rats in the positive drug simvastatin (SIM+HFD) group were given simvastatin 5 mg/kg body weight per day by gavage. Rats in the control (ND) and model (HFD) groups were administered saline in equal volumes by gavage daily. The rats in the control group (ND) and the model group (HFD) were fed the same volume of saline daily by gavage. After 16 weeks of administration, animals were food-deprived for 18 hours before surgery, but allowed free access to water. Cervical dislocation was killed after pentobarbital anesthesia (We consulting the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020)), the serum and liver of the rats were stored at -80°C for further analysis.

Measurement of biochemical parameters and haematoxylin and eosin (HE) staining of the liver

Serum TC, TGs, LDL-C, and HDL-C were determined using commercial kits, and the results were measured by enzyme markers. Liver tissues were collected and fixed for HE staining. The liver lesions of rats were observed under the microscope after blocking the slices.

Q-PCR detection of the expression of SOD, FXR and PPARγ mRNA in rat liver

The expression of liver peroxisome proliferator-activated receptor γ (PPARγ), superoxide dismutase (SOD), and farnesyl receptor (FXR) mRNA was detected by fluorescence quantitative Q-PCR, and the primer sequences are shown in Table 2. Rat liver was ground and homogenized, and 1 mL of TRIzol was used to extract the RNA from the tissue. The purity and concentration of the RNA were determined. Following the instructions of the reverse transcription reagent, the RNA was reverse transcribed to cDNA. The cDNA was mixed and spiked with the target gene to be measured, and PCR amplification was performed under the instructions of the SYBR kit, with predenaturing at 95°C for 10 min, denaturing at 95°C for 10 s and annealing at 58°C for 30 s for 40 cycles. The Ct value of each sample was obtained by using GAPDH as the internal reference control, and the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative mRNA expression of each gene for statistical analysis.

Table 2 qPCR primer sequences

Gene	Upstream Sequence	Downstream Sequence
PPARγ	GGACATCCAAGACAACCTGC	TGCTCTGTGACAATCTGCCT
FXR	AAAGGATAGAGAGGCAGTGGA	CGTGGTGATGGTTGAATGTC
SOD	GATGAAGAGAGGCATGTGGGA	AAGTCATCTTGTTTCTCGTGGA
GAPDH	GTCGGTGTGAACGGATGTGG	GTGCCGTGGAACTGGCCG

Acquisition of the active components and targets of action of gynostemma

In the search box of the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), the Chinese medicine name “Gynostemma” was entered. The active ingredients of the drug were obtained by clicking on the Latin name. Two pharmacokinetic properties, namely, oral bioavailability (OB) and drug likeness (DL), were used to screen the active ingredients in the drugs, which had to meet both OB ≥ 30% and DL ≥ 0.18. The chemical structure formula of the active ingredients was obtained by using the PubChem database. The Swiss Target Prediction database was used to obtain the targets of active ingredients and their gene symbols.

Construction of the “drug-component-target” network

Cytoscape 3.7.0 mapping software was used to create the “drug-component-target” interaction network diagram. The size of the degree determines the size of the node for identifying the important components

of the drug and the strength of the interaction.

Acquisition of targets for hyperlipidaemia and screening of the related target proteins

The key word “hyperlipidaemia” was used to search the Gene Card database and DisGeNET database, the targets were collected, and duplicate values were removed.

Screening of common targets between the active ingredients of gynostemma and hyperlipidaemia and construction of a PPI protein interaction network map

We used the online mapping website Venny 2.1.0 for screening the common targets of gibberellic acid active ingredients and hyperlipidaemia to create a Venn diagram to obtain the potential targets of gibberellic acid for the treatment of hyperlipidaemia. The screened potential targets were imported into the STRING protein interaction analysis platform, and the drug-disease intersection genes were entered by selecting “Multiple proteins” and selecting “Homo sapiens” as the species. The protein–protein interaction (PPI) networks were constructed with a confidence level of 0.4. After removing invalid proteins without interactions with other proteins, the PNG and TSV format files were exported. The TSV files were imported into Cytoscape 3.7.0, and the “network analyser” function was used to obtain the degree of the participating proteins. Based on the results of multiple interceptions of the median degree values, the core targets were obtained, and the degree value size was used to determine the node size and the size of the bound score to determine the thickness of the edges for constructing the PPI network graph.

“Drug-component-disease target” network construction

Cytoscape 3.7.0 mapping software was used to create the “drug-component-disease target” interaction network map, and the “network analyser” function was used to obtain the degree of drug-component-disease target interaction to identify the more important components and the strength of interaction of the drug for the treatment of hyperlipidaemia.

GO bioenrichment analysis and KEGG pathway enrichment analysis

Using the Metascape database, we limited the species to “Homo sapiens” (human source) and set the p value to less than 0.01 to perform gene ontology (GO) biological analysis of drug-disease genes, including GO Biological Process, GO Molecular Function, and GO Cellular Component. GO Molecular Function and GO Cellular Component were used to study the main biological processes of gibberellins in the treatment of hyperlipidaemia, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted. The analysis was performed to study the signalling pathways associated with the treatment effects of the drug on the disease.

Molecular docking verification

Molecular docking verification of the six most core active ingredients of gynostemma with the core targets was performed using AutoDock software. The structure format files of the main active ingredients

and target proteins of gynostemma were downloaded from the TCMSP database as well as the Pubchem database and PDB database and were exported after converting the SDF format to PDB format by Open Babel software. The core targets were modified by PyMOL software, and their PDB files were saved. The composition and target files were then imported into AutoDock software for molecular docking, and the docking results were visualized using PyMOL software. The flaw chart of this study as displayed in Figure 1.

Results

Gynostemma significantly ameliorates HFD-induced disorders of lipid metabolism

Analysis of lipid levels in rats showed that serum TC, LDL-C and HDL-C levels were significantly higher in the model group (HFD) than in the blank group (ND) ($P<0.05$); compared with levels in the model group, serum TC, LDL-C and HDL-C levels were significantly lower in the gibberellin group (GP+HFD) and the positive drug group (SIM+HFD) ($P<0.05$). In addition, compared with levels in the blank group (ND), serum triglyceride levels were reduced in the model group (HFD) rats, but the difference was not significant (see Figure 2 and supplementary file 1).

Gynostemma significantly improves liver lipid deposition in rats

Figure 3 shows the representative microscopic morphology of liver tissue sections stained by HE. In the HFD group, the HFD induced diffuse steatosis in the liver, which was manifested by the presence of a large number of fat vacuoles in hepatocytes (shown by black arrows). After gibberellin and simvastatin drug treatment, attenuated lipid accumulation and a significant reduction in fat vacuoles in the cells could be observed in the rat liver.

Expression of related genes

As shown in Figure 4, compared with expression in the control group, the relative expression of FXR mRNA was increased in the liver of rats in the model group, but the difference was not statistically significant; the relative expression of SOD was significantly lower ($P<0.05$), and PPARγ was not significantly different ($P>0.05$). Compared with the model group, the relative expression of PPARγ and SOD mRNA increased in the gibberellin-administered group, but the difference was not significant. The relative expression of FXR mRNA was decreased supplementary file 2).

Gynostemma candidate pharmacophores and their target proteins

Through the TCMSP database, all active ingredients of gibberellins were collected, based on the settings of OB ≥ 30% and DL ≥ 0.18, and a total of 24 effective active ingredients, including 3'-methylrhodopsin, rhamnetin, and glutathione, were screened. The Chinese names of the effective active ingredients were obtained by combining with relevant literature[11] (see Table 3). The core active ingredients for the treatment of hyperlipidaemia were gibberellin saponins, quercetin, rhamnetin plumbagin, etc.The Swiss

Target Prediction database was searched for target proteins corresponding to the active ingredients of gynostemma, and 1167 target genes were obtained, with a total of 338 after deduplication. The target genes were imported into Cytoscape 3.7.0 mapping software to construct a gibberellin active ingredient-target interaction network diagram, as shown in Figure 5 supplementary file 3 , which had 361 nodes and 1191 edges. The active ingredient-target network diagram revealed the interaction relationship between the active ingredient of gynostemma and potential targets. A certain active ingredient of the drug could interact with multiple potential targets, and different active ingredients could also act on the same target, reflecting the multicomponent and multitarget action characteristics of the Chinese medicine gynostemma.

Table 3 Information on the effective active ingredients of Gynostemma pentaphyllum

NO.	Mol ID	Molecule Name	OB (%)	DL
JGL1	MOL000338	3'-Methyleriodictyol	51.61	0.27
JGL2	MOL000351	Rhamnazin	47.14	0.34
JGL3	MOL000359	Sitosterol	36.91	0.75
JGL4	MOL004350	Ruvoside	36.12	0.76
JGL5	MOL004355	Spinasterol	42.98	0.76
JGL6	MOL005438	Campesterol	37.58	0.71
JGL7	MOL005440	Isofucosterol	43.78	0.76
JGL8	MOL007475	Ginsenoside f2	36.43	0.25
JGL9	MOL000953	CLR	37.87	0.68
JGL10	MOL000098	Quercetin	46.43	0.28
JGL11	MOL009855	(24S)-Ethylcholesta-5,22,25-trans-3beta-ol	46.91	0.76
JGL12	MOL009867	4α,14α-Dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol	46.29	0.76
JGL13	MOL009877	Cucurbita-5,24-dienol	44.02	0.74
JGL14	MOL009878	Cyclobuxine	84.48	0.7
JGL15	MOL009888	Gypenoside XXXVI	37.85	0.78
JGL16	MOL009928	Gypenoside LXXIV	34.21	0.24
JGL17	MOL009929	Gypenoside LXXIX	37.75	0.25
JGL18	MOL009938	Gypenoside XII	36.43	0.25
JGL19	MOL009943	Gypenoside XL	30.89	0.21
JGL20	MOL009969	Gypenoside XXXV	37.73	0.78
JGL21	MOL009971	Gypenoside XXVII	30.21	0.74
JGL22	MOL009973	Gypenoside XXVIII	32.08	0.74
JGL23	MOL009976	Gypenoside XXXII	34.24	0.25
JGL24	MOL009986	Gypentonoside A	36.13	0.8

Intersection of the targets of action of gibberellic acid active ingredients and hyperlipidaemia

The key word “hyperlipidaemia” was used to search the Gene Card database and DisGeNET database to collect disease targets, the obtained targets were summarized, and duplicate values were removed to obtain a total of 1663 hyperlipidaemia disease targets.

The collected information on the potential action targets of gibberellin and hyperlipidaemic disease targets was imported into the Venny 2.1.0 online website, and a Venn diagram was drawn (Figure 6 and supplementary file 4), which resulted in a total of 125 drug-disease intersection target genes, mainly acting on the core targets AKT1, TNF, IL6, VEGFA, EGFR, PPARG, PTGS2, etc.

Construction of the PPI network diagram and screening of the core networks

The STRING database was used. The drug-disease intersection target was entered, the species was set to Homo sapiens, the linkage colour was set to evidence (evidence type), the confidence was set to 0.4, hidden free node was selected, and the rest of the settings were left unchanged to build the original PPI network diagram between the disease and the drug (Figure 7A and supplementary file 5) and to analyse the drug-disease intersection target protein interaction relationship. Online analysis using the database showed that a total of 1279 edges were contained. A total of 1279 protein interactions existed with an average degree value of 20.5, an average clustering coefficient of 0.555 and a P value < 1.0e-16, representing a strong protein interaction between herbal medicine and hyperlipidaemia. The TSV format of the protein interactions in the library was exported to Cytoscape 3.7.0 software, where the “network analyser” function was used to obtain the degree of the proteins involved in the interactions. The top degree sizes belong to AKT1, TNF, IL6, VEGFA, EGFR, PPARG, PTGS2, etc. The larger the degree value is, the larger the protein displayed in the graph and the darker the colour. The thickness of the line is set according to the interprotein binding score, and the higher the score is, the thicker the line is (see Figure 7B and supplementary file 5 for details).

The Excel file after network analysis was exported, and 18 core targets were obtained based on the results of multiple interceptions of the median degree values. It is hypothesized that these 18 targets are the key targets of gibberellin action in hyperlipidaemia, among which AKT1, TNF, IL6, VEGFA, EGFR, and PPARG are ranked in the top six nodal degree values, and it is hypothesized that these targets may be the core targets for the treatment of hyperlipidaemia.

Construction of an active ingredient-target-hyperlipidaemia network map

The 18 core targets were imported into Cytoscape 3.7.0 mapping software to create a “drug-component-disease target” interaction network (Figure 8 and supplementary file 6). With the help of the “network analyser” function, we obtained the degree of drug-component-disease target interactions, among which the top interactions were ESR1, PPARG, PPARA, NR3C1, PTGS2, etc. The larger the degree value is, the larger the corresponding node displayed in the graph.

Pathway enrichment analysis of key targets

With the help of the Metascape database, KEGG enrichment analysis was performed on 125 potential targets of gynostemma for the treatment of metabolism-related hyperlipidaemia, and $p<0.01$ was set. A total of 178 pathways were obtained, and the top 30 pathways were screened according to the smallest to largest Log(p) values. The larger the bubble, the more genes there are that are enriched on that

entry (see Table 4). The main pathways are the cancer pathway, chemo-oncogenic-receptor activation pathway, lipid and atherosclerosis pathway, proteoglycan in cancer, PI3K-Akt signalling pathway, etc. The results suggest that the active ingredients of gynostemma may act through modulation of these signalling pathways in metabolism-related hyperlipidaemia (Figure 9 and supplementary file 7).

Table 4 Top 10 ranked KEGG enrichment pathways of Gynostemma pentaphyllum active ingredients in the treatment of hyperlipidaemia

NO.	Items	KEGG	Gene No.	P value
1	hsa05200	Pathways in cancer	35	3.483E-32
2	hsa05207	Chemical carcinogenesis - receptor activation	22	1.22142E-24
3	hsa05417	Lipid and atherosclerosis	22	1.67466E-24
4	hsa05205	Proteoglycans in cancer	21	1.97569E-23
5	hsa04151	PI3K-Akt signalling pathway	21	1.82478E-18
6	hsa05010	Alzheimer disease	20	1.568E-16
7	hsa01522	Endocrine resistance	19	8.274E-27
8	hsa05167	Kaposi sarcoma-associated herpesvirus infection	19	6.72872E-21
9	hsa04931	Insulin resistance	18	3.61056E-24
10	hsa04932	Non-alcoholic fatty liver disease	17	1.19662E-19

GO analysis consists of three parts, namely, biological process (BP), cellular component (CC), and molecular function (MF), which together describe the function of the gene product. GO functional analysis of potential targets of gibberellin for the treatment of hyperlipidaemia was performed with the help of the Metascape database. With the setting of $P<0.01$, a total of 1580 entries were obtained, including 1344 BP entries, 84 CC entries and 152 MF entries. The top 30 GO entries were selected according to Log(p) sorting from smallest to largest, and the enrichment bubble plot was drawn using the microbiological letter website. The larger the bubble is, the more genes are enriched in that GO entry (see Figure 10 and supplementary file 8). GO analysis showed that biological processes mainly included responses to hormones, cellular responses to lipids, intracellular receptor signalling pathways, and cellular responses to organic cyclic compounds. The cellular components mainly included membrane rafts, membrane microregions, receptor complexes, etc. The molecular functions mainly included nuclear receptor activity, ligand-activated transcription factor activity, steroid binding, etc. The biological processes, cellular components and molecular functions of gibberellins are closely related to the development of hyperlipidaemia.

Molecular docking analysis

To further investigate the relationship between the effects of gypenoside on hyperlipidaemia, molecular docking was performed with the aid of AutoDock software, and the six active ingredients with the highest degree values, namely, gypentonoside A, quercetin, gypenoside XXXV, rhamnazin, gypenoside XXVIII, and 3'-methyleriodictyol, were associated with the six most closely related proteins AKT1 (1H10), TNF (1A8M), IL6 (1ALU), VEGFA (1BJ1), EGFR (1IVO), and PPARG (1FM6) for molecular docking. The results showed that the interaction energies of the small molecules with the target proteins were all <0, indicating that each active ingredient had a high affinity for each protein. Among them, rhamnetin had the highest affinity for AKT1 (-6.06), and gibberellin XXVIII had the highest affinity for TNF, IL6 and VEGFA (-6.04, -8.09 and -4.64, respectively). The affinity of 3'-methylurodiol for EGFR and PPARG was -3.26 and -4.05, respectively, indicating that these six active ingredients bind well to the corresponding core target proteins, suggesting that the data and results of this experiment are reliable and have high reference value (see Figure 11 and supplementary file 9).

Discussion

Hyperlipidaemia is a disease in which blood lipids are higher than normal due to abnormal fat metabolism or transport in the body and is one of the main causative factors of many cardiovascular diseases. Studies have shown that the Chinese herb gynostemma can treat hyperlipidaemia with excellent lipid-lowering effects and few toxic side effects. The results of this study found that gynostemma total saponin significantly reduced serum TC and LDL-C levels and improved hepatic lipid accumulation in rats. To investigate the mechanism of the hypolipidaemic effect of gynostemma, the mRNA expression of genes related to lipid metabolism and oxidative stress in the liver was determined in this study. Among them, PPARγ can inhibit the accumulation of triglycerides, neutral lipids and total fatty acids in macrophages and enhance cholesterol efflux through the regulation of the expression of fatty acid transporter protein 1[12]. It has been shown that inhibition of macrophage lipid accumulation through inhibition of PPARγ activation is beneficial in reducing the risk of diabetic atherosclerosis[13-14]. The results of this study revealed that the level of PPARγ mRNA expression in rat liver was reduced compared with that in the normal control group, indicating that the high-fat diet state decreased the expression of PPARγ, which in turn affected TG biosynthesis and the activities of fatty acid oxidase and lipoprotein lipase to aggravate the level of lipid deposition in rat liver, thereby further aggravating the development of hyperlipidaemia. The expression level of PPARγ mRNA in rat liver was increased after gynostemma intervention, suggesting that gynostemma total saponins may play a role in the prevention and treatment of cardiovascular and cerebrovascular diseases such as hyperlipidaemia by increasing PPARγ expression and thus accelerating the process of lipid metabolism and reducing hepatic lipid deposition. FXR is a member of the orphan nuclear receptor superfamily that plays an important role in bile acid and triglyceride metabolism and glucose metabolism[15]. The activation of hepatic FXR regulates the expression of many hepatic genes involved in lipoprotein metabolism, including scavenger receptor class B type 1 (SR-B1), apolipoprotein ApoC-II, ApoC-III and ApoA-I[16-19]. Activation of FXR in rodents has also been shown to regulate adipogenic pathways and to prevent atherosclerosis and non-alcoholic fatty liver disease (NAFLD)[20]. The results of the present study showed that hepatic FXR expression was elevated

and blood lipid levels were significantly reduced in hyperlipidaemic rats after gibberellic acid administration, suggesting a good therapeutic effect of gibberellic acid in hyperlipidaemic rats. In addition, studies have shown that oxidative stress is closely related to the development of obesity, hyperlipidaemia and atherosclerosis and that inhibition of oxidative stress may be beneficial in controlling obesity and hyperlipidaemia and protecting liver tissues; additionally, the antioxidant defence system in the body plays a crucial role in inhibiting oxidative stress, while SOD is an important antioxidant enzyme in liver tissues[21]. The results of this experiment showed that gynostemma could increase the expression of SOD mRNA in the liver of hyperlipidaemic rats, indicating that gynostemma could regulate the expression of mRNA related to liver lipid metabolism and inhibit oxidative stress to improve the disordered lipid metabolism in hyperlipidaemic rats to some extent. All of the above results indicated that gynostemma had good hypolipidaemic effects in hyperlipidaemic rats.

Through network pharmacological analysis of the active ingredients of gynostemma for the treatment of hyperlipidaemia, a total of 24 effective active ingredients, such as 3'-methylrhodopsin, rhamnolipid and glutathione, and 125 effective targets acting on hyperlipidaemia were screened. The active ingredient-target network analysis showed that gypentonoside A, quercetin, 4α, 14α-dimethyl-5α-ergosta-7, 9(11), 24(28)-trien-3β-ol, gypenoside XXXV, and gypenoside XXVIII had relatively more associated targets and were closely linked to hyperlipidaemia. Gynostemma saponin can significantly reduce blood lipids and repair liver damage in rats with experimental hyperlipidaemia and can significantly improve the disordered lipid metabolism caused by experimental hyperlipidaemia, with preventive and therapeutic effects on hyperlipidaemia and atherosclerosis[22-23], and its lipid-regulating effects are related to the inhibition of free fatty acid production and the synthesis of neutral fat in adipocytes. Wang Wei[24] found through experiments that the parent nucleus structure of gibberellin total saponin is very similar to the parent nucleus structure of endogenous bile acids in vivo, which can activate the bile acid receptor FXR on the liver, decrease the expression of nuclear receptor SHP, reduce the binding to hepatic nuclear receptors Lrh1 and Hnf4a, upregulate the key enzymes CYP8B1 and CYP7A1 involved in bile acid synthesis, promote the synthesis and secretion of bile acids, promote lipid metabolism, and finally play a role in lowering blood lipids. Quercetin is a flavonol compound with various biological activities and has good effects on lowering blood lipids and blood pressure and dilating coronary arteries[25]. Quercetin effectively reverses hyperlipidaemia by lowering lipids mainly through lipid catabolism-related genes that reduce hepatic oxidative stress and inflammatory damage, improve hepatic lipid metabolism disorders, and reduce triacylglycerol accumulation and insulin resistance[26]. Some studies have shown that quercetin reduces lipid levels and prevents the formation of atherosclerosis in hyperlipidaemia model mice[27]. The prediction of drug targets reveals important information about the drug and molecular mechanisms of action, which is important for promoting drug development. In the screening of gibberellin targets for the treatment of hyperlipidaemia, the targets with the strongest nodal correlation based on the PPI network diagrams were AKT1, TNF, IL6, VEGFA, EGFR, and PPARG. Among them, AKT kinase is an insulin effector with key in vivo functions in adipocytes, such as stimulating glucose uptake and glycogen synthesis, as well as protein synthesis[28]. AKT1, one of the major isoforms of AKT, mediates many of the metabolic, mitogenic and anti-apoptotic effects of insulin, IGF-1, IL-3, and other

growth factors and is an important pathogenic factor involved in adipogenesis[29]. Related experiments have demonstrated that AKT1 increases the activity of CDK2 (AKT phosphorylated cyclin-dependent kinase), which speeds up the growth cycle of 3T3-L1 cells and affects their proliferation and growth[30]. Interleukin 6 (IL6) acts directly or indirectly as an important factor in bone homeostasis and vascularity by inducing VEGF, leading to increased angiogenic activity and vascular permeability. VEGF is involved in metabolic control, as it is discharged into the bloodstream after muscle contraction to increase lipolysis and improve insulin resistance and mediate crosstalk between insulin-sensitive tissues, intestinal L cells and islets via GLP-1 to adapt to changes in insulin requirements. Vascular endothelial growth factor A (VEGFA) plays an important regulatory role in adipogenesis and energy metabolism. VEGFA belongs to the VEGF family and is involved in processes such as angiogenesis, the regulation of vascular permeability, and maintenance of vascular physiological function. VEGFA is also directly related to the regulation of body obesity, as adipose tissue stimulates the expansion of endothelial cells to produce VEGFA, which in turn promotes the formation of new capillaries, and subsequently, the body controls angiogenesis by increasing serum leptin levels to counteract the accumulation of lipids in adipocytes[31]. Epidermal growth factor receptor (EGFR), a member of the EGF family of receptor tyrosine kinases, binds ligands and activates several signalling cascades to translate extracellular signals into appropriate cellular responses. Ligand binding triggers receptor homo- and/or heterodimerization and autophosphorylation on key cytoplasmic residues. Phosphorylated receptors recruit bridging proteins such as GRB2, thereby activating a complex downstream signalling cascade. This complex activates at least 4 major downstream signalling cascades, including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC, and STAT modules. Peroxisome proliferator-activated receptor gamma (PPARG) binds nuclear receptors for peroxisome proliferators such as those utilized by lipid-lowering drugs and fatty acids. Once activated by the ligand, the nuclear receptor binds to DNA-specific PPAR response elements (PPREs) and regulates the transcription of its target genes, such as acyl coenzyme A oxidase. Thus, PPARG controls the peroxisomal β-oxidation pathway of fatty acids, a key regulator of adipocyte differentiation and glucose homeostasis.

Among the molecular mechanisms of gibberellin for the treatment of hyperlipidaemia, the main pathways with more enriched targets based on KEGG enrichment analysis were the cancer pathway, chemo-oncogenic-receptor activation pathway, lipid and atherosclerosis pathway, proteoglycans in cancer, and PI3K-Akt signalling pathway. GO was used to analyse biological processes, including the hormone response, lipid response, intracellular receptor signalling pathway, and organic cyclic compound response. The cellular components included membrane rafts, membrane microregions, and receptor complexes. The molecular functions included nuclear receptor activity, ligand-activated transcription factor activity, and steroid binding. The biological processes, cellular components and molecular functions of gibberellins are closely related to the development of hyperlipidaemia.

Conclusion

The molecular docking results showed that the core active components of gibberellin, gibberellin saponin XXVIII, 3'-methylrhodopsin and gibberellin act on the key targets of hyperlipidaemia. AKT1, TNF, IL6, VEGFA, EGFR, and PPARG all exhibited good affinity, further validating the network pharmacology prediction of this study that gibberellin may affect the development of metabolism-related fatty liver disease through multicomponent actions on multiple targets. In summary, this study used the methods and techniques of network pharmacology to make preliminary predictions; to analyse the active components, potential targets and mechanism of action of gynostemma in the treatment of hyperlipidaemia; and to elaborate the synergistic effects of gynostemma through multiple components, targets and pathways, which provided new ideas and directions for subsequent deeper exploration of the mechanism of gynostemma in the treatment of hyperlipidaemia and the development and utilization of gynostemma drugs.

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Figures

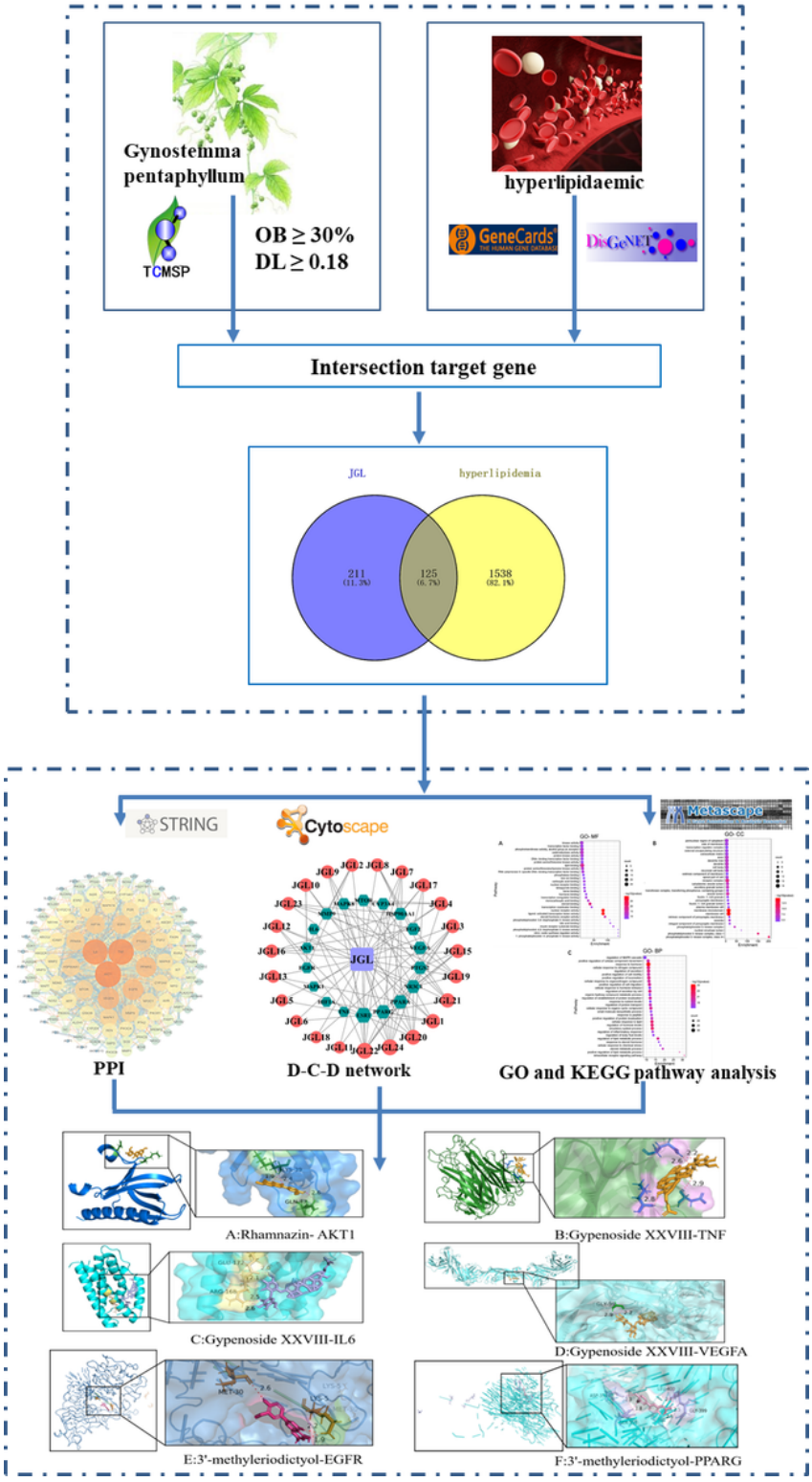

Figure 1

Flow chart of network pharmacology to study the potential molecular mechanism of gynostemma in the treatment of hyperlipidaemia

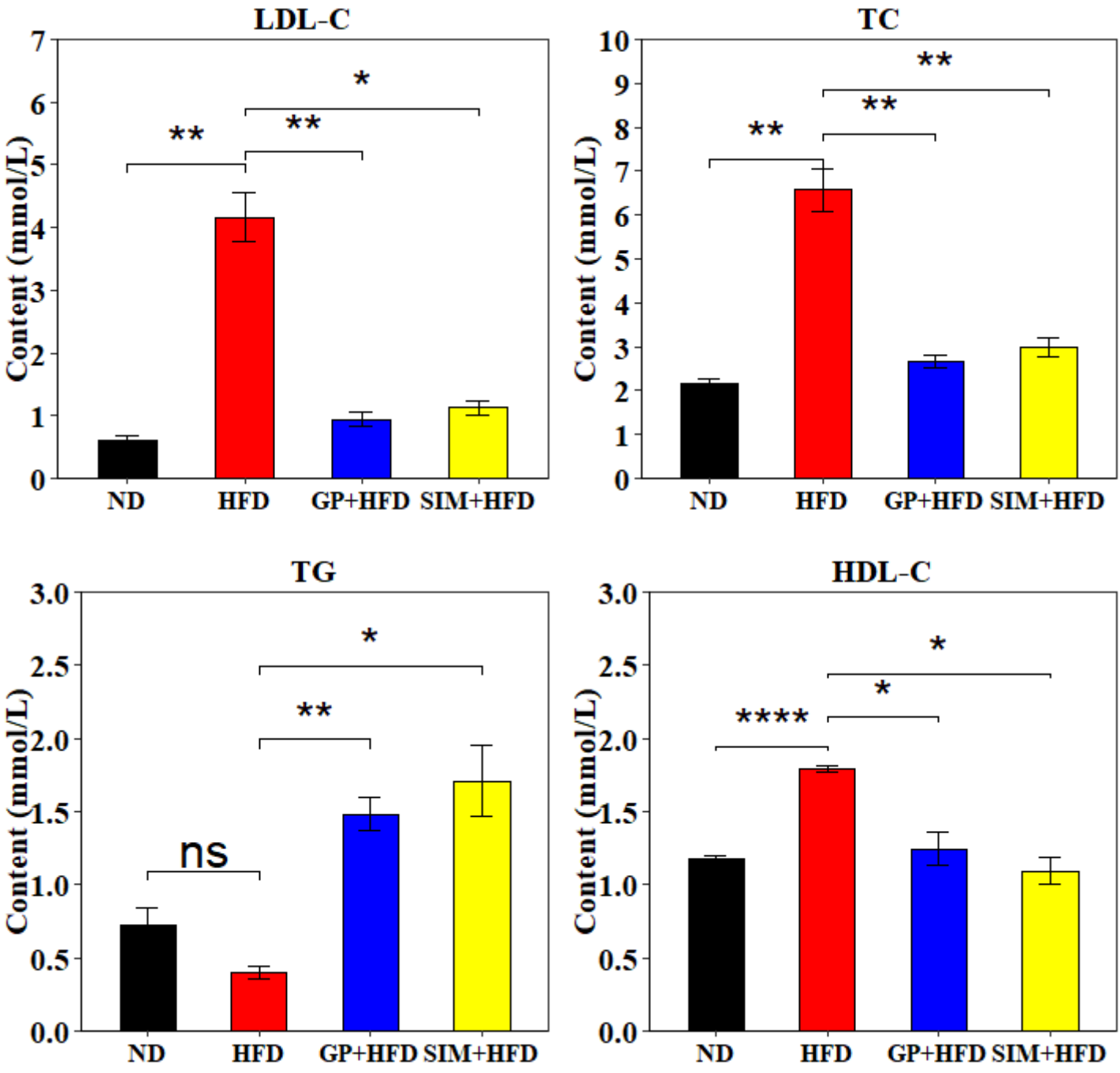

Figure 2

Gynostemma pentaphyllum can significantly improve disordered blood lipid metabolism in hyperlipidaemic rats

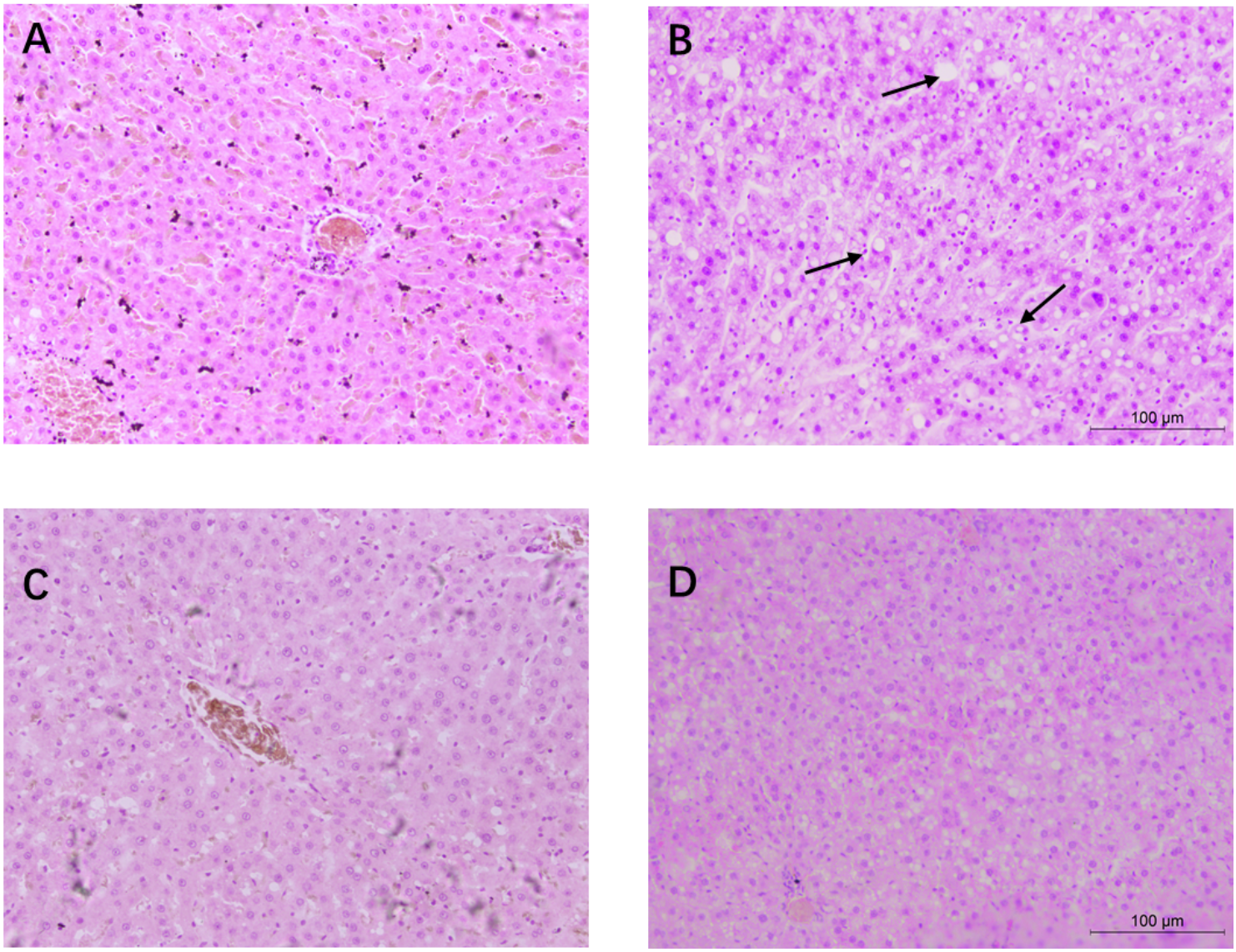

Figure 3

HE staining of rat liver (X200)

A: Control group; B: model group; C: Gynostemma pentaphyllum group; D: positive drug group

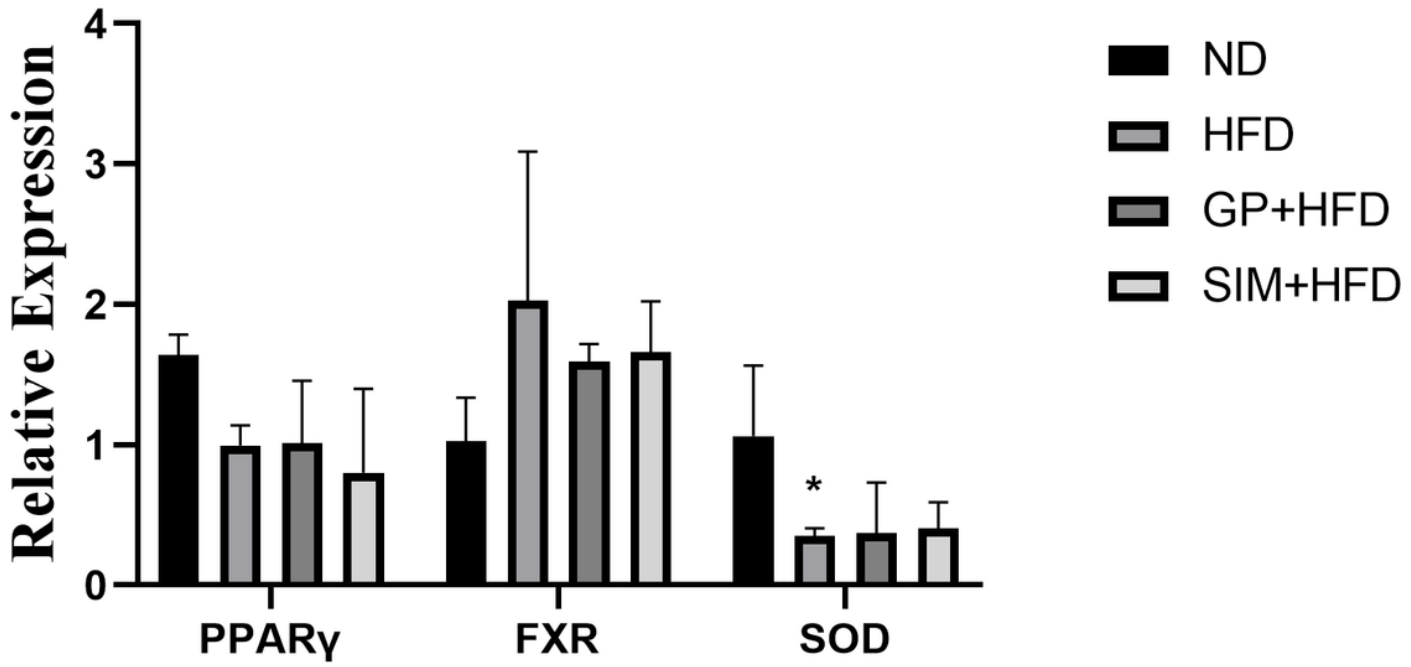

Figure 4

Effect of Gynostemma pentaphyllum on the expression levels of *PPARγ*, *FXR*, and *SOD* mRNA in rat liver

ND: control group; HFD: model group; GP + HFD: Gynostemma pentaphyllum group; Sim + HFD: positive drug group

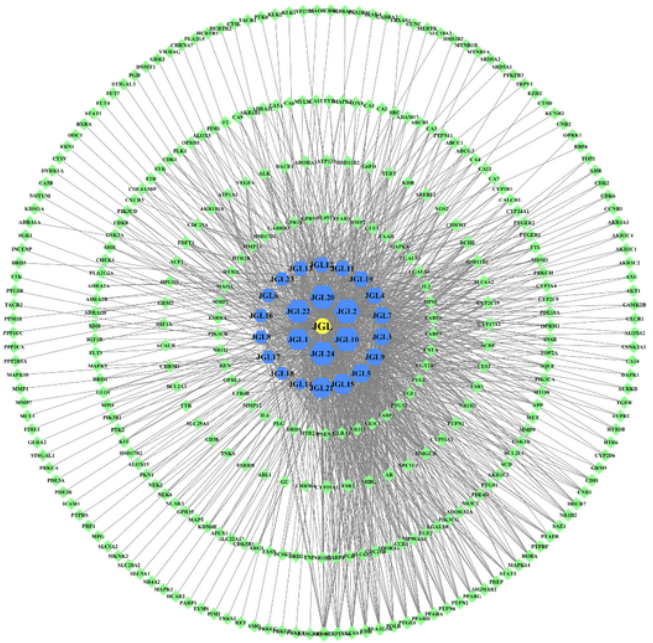

Figure 5

Network diagram of effective active component targets of Gynostemma pentaphyllum

Note: JGL is the acronym for Gynostemma pentaphyllum, yellow is the drug Gynostemma pentaphyllum, blue is the active component of Gynostemma pentaphyllum, and green is the key target.

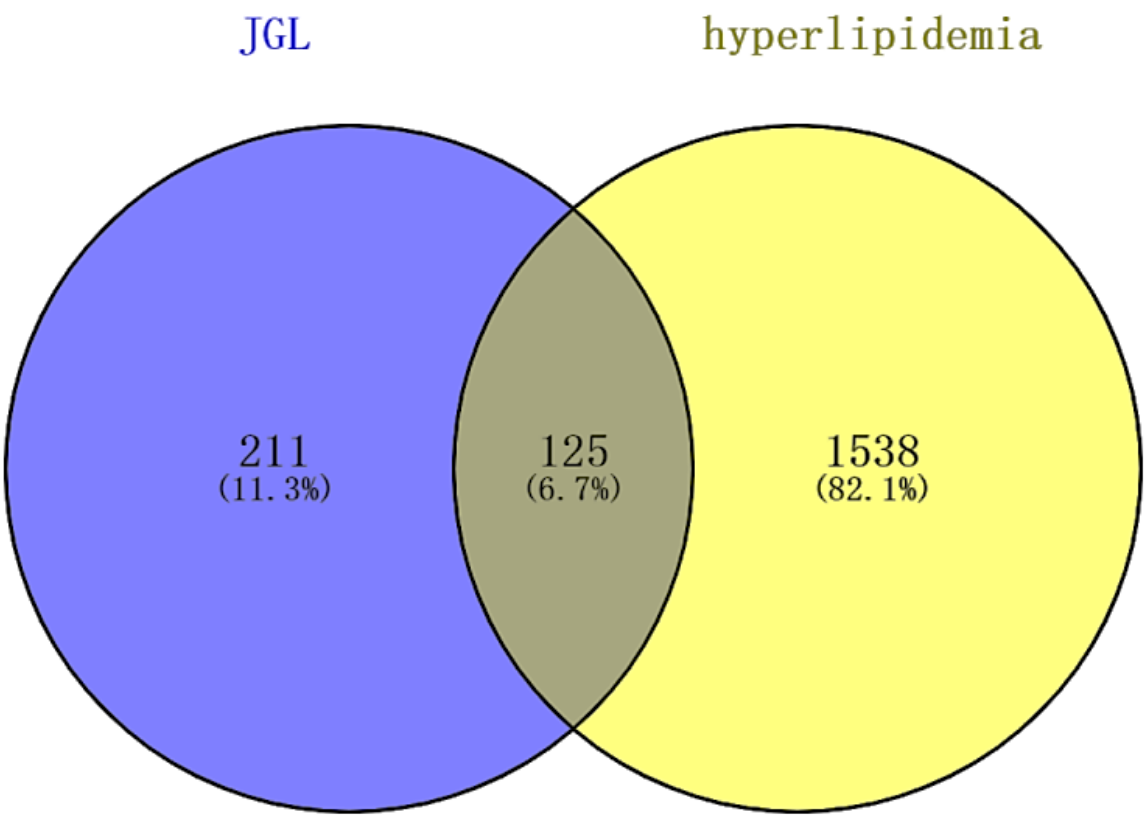

Figure 6

Wayne diagram of the intersection of the targets of Gynostemma pentaphyllum active components and hyperlipidaemia

Note: the purple circle represents the targets of the effective active components of Gynostemma pentaphyllum, the yellow circle represents the targets of hyperlipidaemia, and the overlapping part represents the targets of Gynostemma pentaphyllum on hyperlipidaemia.

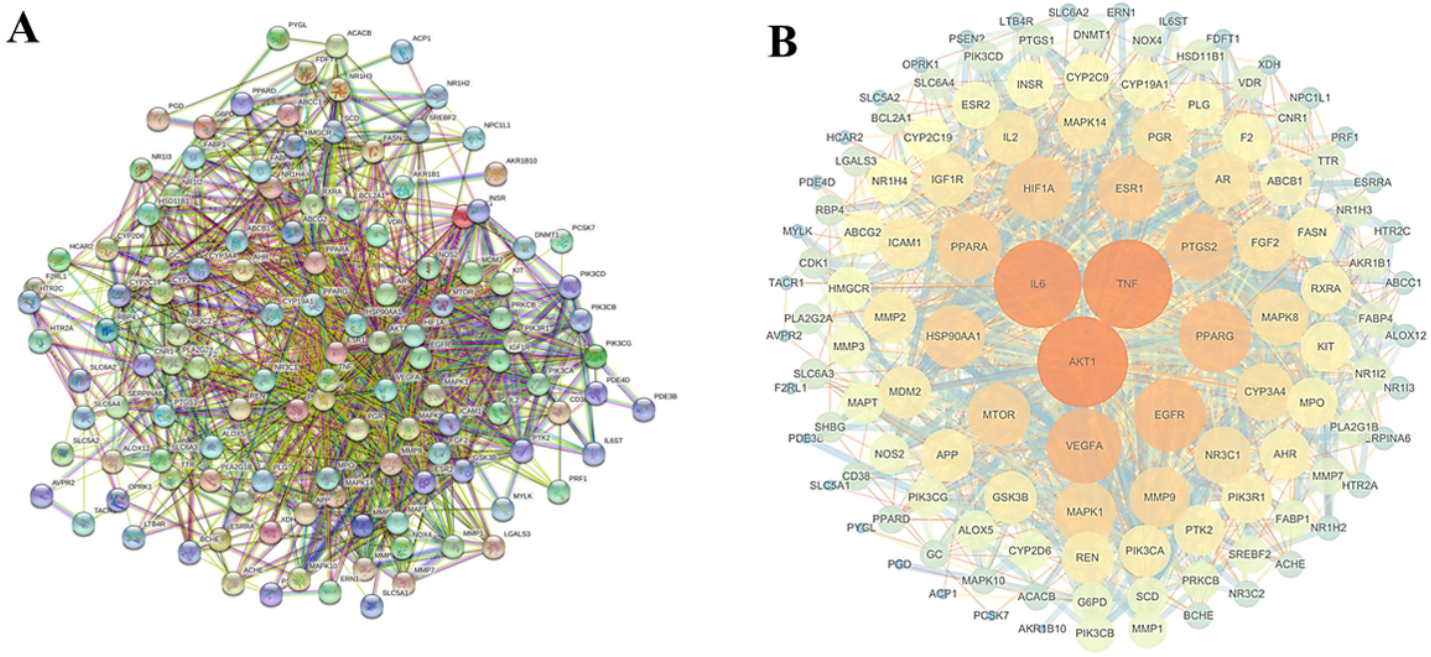

Figure 7

PPI network diagram of Gynostemma pentaphyllum in the treatment of hyperlipidaemia

Note: the circular node represents the target, and the node size represents the interaction degree between targets (the interaction degree of the warm colour system is high, and the interaction degree of the cold colour system is low).

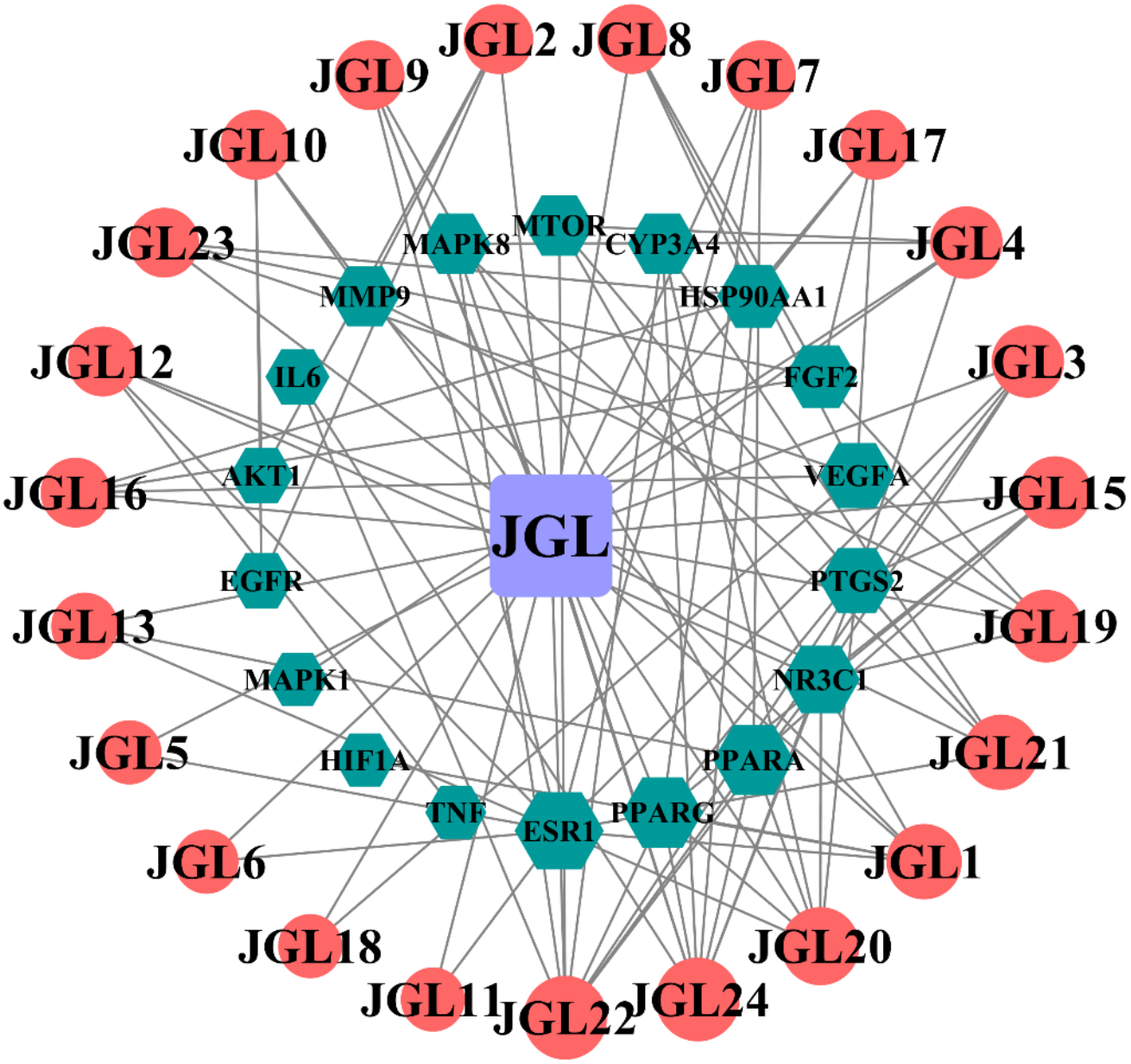

Figure 8

Active ingredient target hyperlipidaemia network

Note: purple squares represent Gynostemma pentaphyllum, red circles represent active components, and dark green hexagons represent hyperlipidaemia targets.

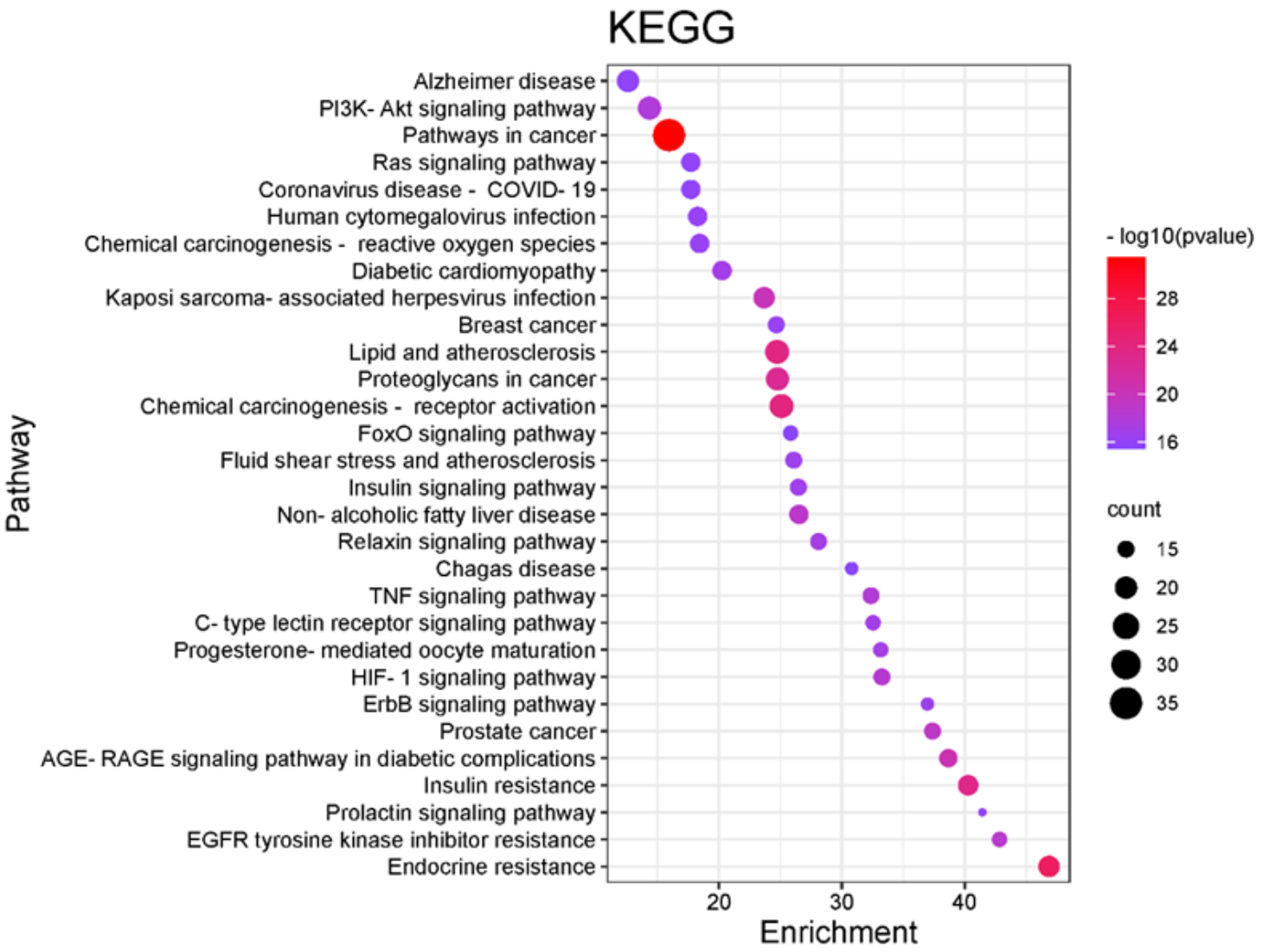

Figure 9

Enrichment bubble diagram of the KEGG pathway of the Gynostemma pentaphyllum active ingredient in the treatment of hyperlipidaemia

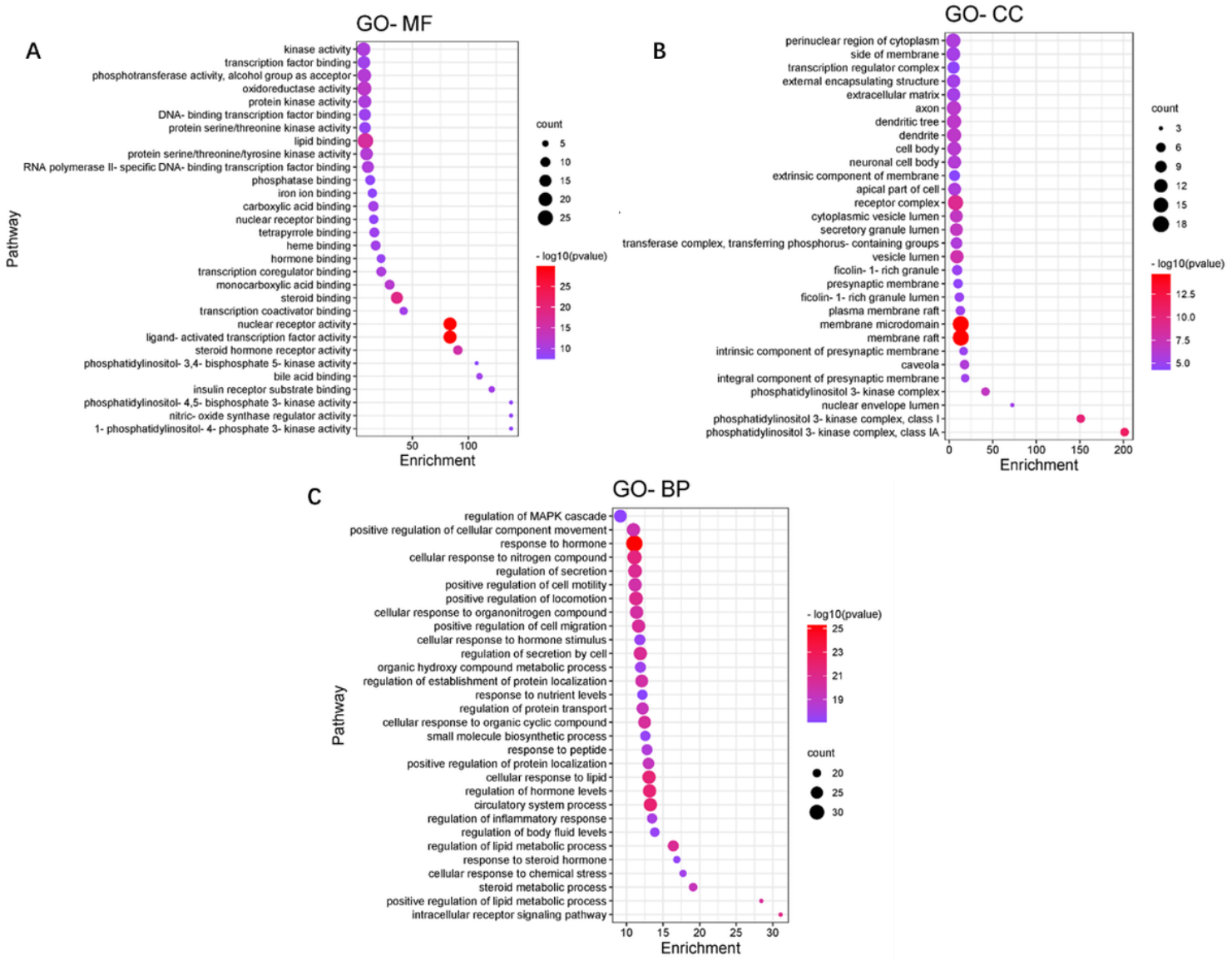

Figure 10

Analysis results of GO enrichment of the Gynostemma pentaphyllum treatment of hyperlipidaemia

A: Molecular function; B: cell components; C: biological process

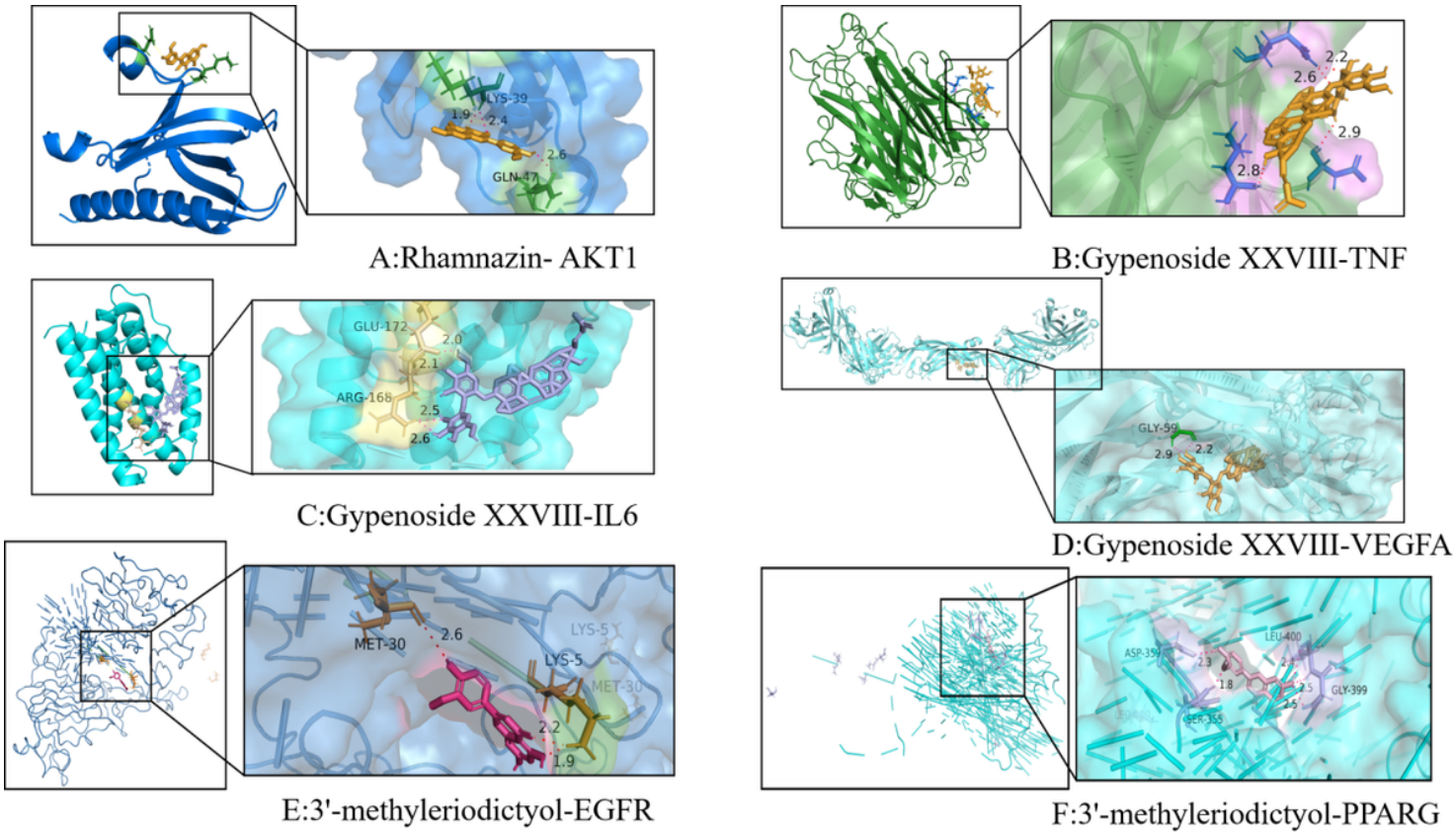

Figure 11

Molecular docking of six active components of Gynostemma pentaphyllum with corresponding core targets of hyperlipidaemia

Supplementary Files

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- 01Rawdataofbloodlipidinratssupplementaryfile1.xlsx
- 02rawdataofqPCRsupplementaryfile2.xlsx
- 03Networkdiagramofeffectiveactivecomponenttargetsof GPsupplementaryfile3.xlsx
- 04Waynediagramoftheintersectionofthetargetsof GPsupplementaryfile4.xlsx
- 05PPInetworkdiagramof GPsupplementaryfile5.xlsx
- 06Activeingredienttargethyperlipidaemianetworksupplementaryfile6.xlsx
- 07KEGGpathwaysupplementaryfile7.xlsx
- 08GOenrichmentsupplementaryfile8.xlsx
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- supplementaryfile3.xlsx
- supplementaryfile4.xlsx
- supplementaryfile5.tsv
- supplementaryfile6.xlsx
- supplementaryfile7.xlsx
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