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Exploring anti-nonalcoholic fatty liver disease mechanism of Gardeniae Fructus by combining an animal model with network pharmacology and molecular docking

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Research

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Abstract

Background

Gardeniae Fructus (GF), a traditional Chinese medicine in clinic for the treatment of nonalcoholic fatty liver disease (NAFLD). However, the mechanisms of action of GF was still margin. To explore the efficacy and mechanism of action of GF for the treatment of NAFLD, we proposed a strategy combined *in vivo* efficacy verification, network pharmacology analysis, molecular docking, and validity assay of target protein.

Methods

Firstly, an animal model induced by the high fat diet feed was established, then orally administrated with GF, the mRNA expression levels of lipogenesis was performed by RT-PCR, the liver tissue specimens were stained by hematoxylin and eosin (H&E), then observed by light microscopy. Secondly, network pharmacology studies clarified the relationship among the active constituents, target protein, and pathways, and then explored by the molecular docking. Finally, validity assay of target protein was performed in surface plasmon resonance (SPR) test.

Results

GF protected against NAFLD in rats. Network pharmacology showed that quercetin, oleanolic acid, and geniposide, targeted on PPARα, PPARγ, and CA2 genes, through regulating PPAR, AMPK, and cGMP-PKG signal pathways, to protect against NAFLD. And the

Conclusion

GF could alleviate NAFLD through the molecular mechanisms explored by network pharmacology, molecular docking, and surface plasmon resonance, those method can be effective tools to clarify the mechanisms of actions of traditional Chinese medicine from a holistic perspective.

Background

Nonalcoholic fatty liver disease (NAFLD) is a prevalent hepatic manifestation of the metabolic syndrome disorder, characterized by ectopic accumulations of triglycerides in the absence of excessive alcohol consumption [1]. NAFLD causes severe and prolonged damage to liver tissue, which may progress into fibrosis, cirrhosis, and eventually hepatocellular carcinoma. About 30% Chinese adult population have fatty liver, the incidence of NAFLD is increasing in the world every year. However, the precise mechanisms of NAFLD and effective treatment strategy continue to lag behind. Thus, the development of novel

strategies for preventing and treating such diseases is in urgent need [2]. Recently, system medicine was found to prevent or treat NAFLD [3].

Gardeniae Fructus (GF), derived from the dried fruits of *Gardenia jasminoides* Ellis, widely used as a common traditional Chinese medicine (TCM), recorded in the Chinese Pharmacopoeia in 2015. It had been also used for the treatment of acute or chronic hepatic diseases [4], icteric hepatitis, diabetes [5], depression, and cancer [6]. Chemical investigation suggested that GF contained iridoid glycosides, diterpenes, triterpenes, flavonoids, and other chemical components [7]. Network pharmacology emerged as a new field that integrated chemical, pharmacology, bioinformatics, and genomics, to construct a model based on the analysis of system-level network, and can shed insight into the complicated mechanisms of TCM [8, 9]. Molecular docking, an increasingly important tool for structural molecular biology, used to identify the binding modes and predict binding affinity of molecules that fit together with target proteins [10].

However, no reports have been reported regarding the therapeutic effects of GF for the treatment of NAFLD. Thus, we attempted to evaluate the potential efficiency of GF in attenuating the development of NAFLD induced by the oral administration of a HFD in rats as a model animal that reflect human NAFLD, and explored the mechanism of action of anti-NAFLD of GF by the network pharmacology and molecular docking analysis.

Methods

Chemical reagents

Gardenoside, geniposide, Genipin 1-gentiobioside, and 6α-Hydroxygeniposide were purchased from EFEBIO (Shanghai, China). Acetonitrile, ethanol, and formic acid of HPLC grade were purchased from Merck KGaA (Darmstadt, Germany). The deionized water purified by Milli-Q water purification system (Millipore, Billerica, MA, United States) was applied to prepare and extract plasma samples. Other reagents and chemicals were all analytical grade.

Preparation of Gardeniae Fructus Extract (GFE)

GF was purchased from Shanghai Kang Qiao Herbal Pieces Co.Ltd. Morphological, microscopic authentications, thin layer chromatography, and HPLC were performed in accordance to Chinese Pharmacopoeia (2015) by one of the author Dr. Zhengxiang Xia. GFE was prepared as follows, the herbal materials (200 g) was crushed into small pieces and mixed, the mixture was then soaked in 70% ethanol for 0.5 h before decocting for 1 h. The filtrates were collected and the residues were then refluxed in water (1:5, *w/v*) for 1 h. GFE (equal to 1.0 g/mL GF) could be obtained by mixing the two-stage filtrates and concentrating the volume to 200 mL. The chemical profile was characterized by HPLC (Milford, MA, USA).

Analysis of mRNA Expression

Total RNA was extracted by the TRizol reagent based on the manufacturer's instructions. One microgram of RNA was used to synthesize cDNA. Real-time PCR was performed using an AccuPower® GreenStar qPCR Master Mix (Bioneer, Daejeon, Korea) on a C1000 Touch Thermal cycler with the CFX manager software (Bio-Rad laboratories, Hercules, CA, USA). Primer sequences were exhibited in (Table 1).

Table 1
Primer sequences used in quantitative real-time PCR.

Gene	Primers	Sequence (5' to 3')
SREBP-1c	Forward	TGCATTTTCTGACACGCTTC
	Reverse	CCAAGCTGTACAGGCTCTCC
FAS	Forward	CCCCTGATGAAGAAGGATCA
	Reverse	ACTCCACAGGTGGGAACAAG
SCD-1	Forward	TTGGAGAAGCGGTGGATAAC
	Reverse	AAAAATCCCACCCAATCACA
CD36	Forward	TGGAACAGAGGCTGACAACT
	Reverse	TTGATTTTTGATAGATATGGG
PPAR-*α*	Forward	ACGATTCGACTCAAGCTGGT
	Reverse	GTTGTGTGACATCCCGACAG
CPT-1	Forward	CCT CCG TAG CTG ACT CGG TA
	Reverse	GGA GTG ACC GTG AAC TGA AA
β-action	Forward	CTCTTCCAGCCTTCCTTCCT
	Reverse	AGCACTGTGTTGGCGTACAG

SREBP-1c, sterol regulatory element binding protein1-c; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase-1; CD36, cluster of differentiation 36; PPAR-*α*, peroxisomeproliferator-activated receptor-alpha; CPT-1, carnitine palmitoyltransferase-1.

Animal Experiment

Five-week-old male Sprague–Dawley (SD) rats weighing 180–220 g were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). The scheme of animal experimental method exhibited (Fig. 1). After a one-week acclimation period, the rats were divided into six groups (n = 6 each), the control group was fed a normal diet (Con, 10 kcal% fat, D12450B, Research Diet, Inc., New Brunswick, NJ, USA), the others were fed with a HFD (60 kcal% fat, D12492), following six weeks, the NFLD model was established, four groups were supplemented with GFE (25, 50, and 100 mg/kg, daily oral administration) and metformin (100 mg/kg, daily oral administration), respectively, for six weeks. At the end of 13-week treatment period, the rats were sacrificed with 10% chloral hydrate. Blood was collected

from the portal vein, and then centrifuged at 4℃, 500 g for 20 min. Plasma was collected and stored at -80 ℃ in a centrifuge tube until ready for biochemical analysis. Thereafter, the liver was removed, rinsed with cold normal saline solution and snap frozen in liand stored at -80 ℃ in a centrifuge tube until ready for biochemical analysis. The ethical aspects of animal experimentation studies were approved by the Ethics Committee on Animal Research in School & Hosipital of Stomatology, Tongji University. All surgeries were performed under 10% chloral hydrate and all efforts were made to minimize suffering.

Biochemical Assays

Serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were purchased from Jiancheng Company (Nanjing, PRC). Glucose was determined by enzymatic colorimetric methods using an Erba XL-200 analyzer (ERBA diagnostics Mannheim GmbH, Mannheim, Germany).

Histopathological Analysis

The liver tissue specimens were fixed in 10% formalin, embedded in paraffin, and serially sectioned. Hematoxylin and eosin (H&E) staining was used to visualize liver cells and matrices. Histological changes were observed by light microscopy (Olympus CX31/BX51, Olympus Optical Co., Tokyo, Japan) and photographed (Olympus DP70).

Molecular docking

The crystal structure of PPARγ was gained from the Protein Data Bank (PDB ID: 3K8S) [11]. The crystal structure of PPAR*α* was also obtained from the Protein Data Bank (PDB ID: 3ET1) [12]. The crystal structure of CA2 was also received from the Protein Data Bank (PDB ID: 1IWO) [13]. The docking exercise was conducted through AutoDock sofware.

SPR Assay

In order to prove the validity of the network pharmacological method, different protein-molecule interactions were selected to participate in this surface plasmon resonance (SPR) test. In the SPR test, the target proteins were first combined with the CM5 chip. After correction through standard solutions, a series of different concentrations of molecules (0, 15.6, 31.2, 62.5, 125, 250, and 500 *μmol*/l) were tested for their binding to the protein. Finally, the affinity between the protein and molecule was determined by a of array of parameters.

Statistical Analysis

All results were shown as the mean standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance with Dunnett's multiple comparisons test for multiple comparisons, and $p < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism Software version 6.0 for Windows (GraphPad Software, La Jolla, CA, USA).

Construction and screening of active components in GF

To obtain active components from GF, First of all, *Gardenia jasminoides* was searched from the literature and public databases, such as, PubMed, Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) (Available online: http://lsp.nwsusaf.edu.cn/), and TCM Database@Taiwan. 98 compounds were collected. Secondly, the values of OB and DL were screened using absorption, distribution, metabolism, and excretion (ADME) models provided by TCMSP. The threshold values for these screening models were set to OB ≧ 30% and/or DL ≧ 0.18. Finally, seven compounds were selected as candidates for subsequent analysis.

Target fishing

To get the target information of active compounds from GF for the treatment of NAFLD, a comprehensive method of chemoinformatics and text-mining database was applied. Firstly, the information obtained from the Search Tool for Interactions of Chemicals and Proteins database were used, such as, STITCH (http://stitch.embl.de/), TCMSP (http://lsp.nwu.edu.cn/tcmsp.php), and PharmMapper (http://lilab.ecust.edu.cn/pharmmapper/index.php) [14]. 112 target genes were collected after the removal of duplicates. Secondly, the key words including nonalcoholic fatty liver disease, obesity, and lipid metabolism on NAFLD-associated target genes were searched from the therapeutic targets database (TTD; http://bidd.nus.edu. sg/BIDD-Databases/TTD/TTD.asp, updated 11th January 2018) [15], Pubmed: (https://www.ncbi.nlm.nih.gov/pubmed/), and DRUGBANK (https://www.drugbank.ca/). Finally, 15 target genes were got as candidates for subsequent analysis.

Construction of target protein-protein interaction (PPI) network

To explore the relationship among these target proteins, the core target proteins of the active compounds were enriched by the STRING (http://string-db.org).

Network construction and analysis

To facilitate the visualization of bio-active compounds from GF and their potential target genes related to NAFLD, networks were constructed using network visualization software Cytoscape ver. 3.2.1. In networks, nodes represented compounds or target genes or pathways, and edges indicated the interactions compound-target or compound-pathway. To illustrate the mechanism of action of the active compounds from GF and their roles in signal transduction, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to analyze the KEGG pathway enrichment of their target protein genes. The cellular components involved in the target protein and the pathways involved were also described.

3. Results

Quility control of GF

The GF was purchased from Shanghai Kang Qiao Herbal Pieces Co. Ltd. (Shanghai, China). HPLC was used to identify the active components of GF. And the compound was finally confirmed by the comparison with the authentic compound. The chromatographic separation was carried out on a Diamonsil C_{18} column (150 × 4.6 mm I.D., 5 mm) at 25 °C. The mobile phase consisted of acetonitrile (solvent A) and water (0.1% formic acid) (solvent B). The optimized elution condition was applied as follows: 0–60 min, 5–95% A. The solvent flow rate and injection volume was kept as 0.5 mL/min and 5 μL, respectively. Then the result was exhibited (Fig. 2), compounds **1**–**4** were identified as geniposide, genipin 1-gentiobioside, 6α-hydroxygeniposide, and gardenoside, respectively.

Regulation of the genes involved in lipogenesis and fat oxidation by GF

NAFLD is a chronic disease affecting liver tissues that characterized by an increasing the mRNA expression of lipogenesis (*SREBP-1c*, *FAS*, *SCD-1*, *CD36*, *PPAR-α*, and *CPT-1*). As shown (Fig. 3), they were significantly reduced following the supplement of GF on HFD-fed rats compared to the non-treatment HFD-fed rats, and also in a dose-dependent manner. Furthermore, the high dosage (100 mg/kg) of GF administrated on the HFD-fed rats showed comparative effects to the positive control drug metformin owing to they had similar values of mRNA expression of lipogenesis. These data suggested that GF protected against NAFLD through regulating the mRNA expression of lipogenesis in liver tissue.

GF reduced lipids, liver Enzymes, and MDA Levels in serum

To further explore the preventive effects of GF on HFD-induced liver steatosis, we measured the serum biochemical indices in different groups of rats. As exhibited (Fig. S1), the serum TC, LDL-C, and TG levels significantly increased in the model rats fed a HFD diet compared with the rats fed a normal diet. However, while the HFD-fed rats orally administrated with GF, serum TC, LDL-C, and TG levels in those rats decreased significantly, and also in a dose-dependent manner. The serum ALT, AST, and LDH levels (hepatic damage markers) exhibited the similar trends. Besides, serum MDA levels, the products of lipid peroxidation, an oxidative marker used as an indicator of oxidative damage, also showed the similar trends. Therefore, the results showed that GF had comparative effects to metformin through resisting oxidative stress and reducing serum lipids.

GF decreased serum glucose and insulin in HFD-fed rat

Serum glucose and insulin levels are important biomarkers in metabolic diseases especially in NAFLD. They changed in different groups of rats as displayed (Fig. S2), the serum glucose and insulin levels were increased in HFD-induced rats compared to the control rats, whereas they reduced after intragastric administration on the HFD-fed rats with GF obviously compared with the HFD-fed rats without any treatments. Moreover, the high dosage (100 mg/kg) of GF treatment showed the comparative effects to the positive control metformin against the HFD-induced rats with NAFLD. Collectively, GF may protect against NAFLD by reducing serum glucose and insulin levels.

GF improved the liver steatosis in HFD-fed model rats

Liver fat accumulation during the ingestion of HFD can lead to NAFLD. As shown (Fig. S3), the histological evaluation of liver samples exhibited obviously increases in lipid droplets in hepatocytes in the HFD-fed rats compared those without any treatment, while these lipid droplets were reduced significantly following the GF and positive control metformin supplement on HFD-fed groups. In the other side, according to the above biochemical data analysis, liver lipid contents, liver TC, and TG levels in the HFD group were significantly increased compared with those in the control group, which was consistent with the histological data. Thus, GF showed the similar effects to the positive control metformin for the treatment of NAFLD.

In silico network analysis and prediction of target genes and pathways related to NAFLD

Several compounds in GF may play a synergistic manner to protect against NAFLD. To further illustrate which ingredients from GF may attribute to the hepatoprotective effects, a network pharmacology experiment was carried out. According to the analysis of this network, seven components in Table 2 were selected and linked to 15 potential target genes were present (Fig. 4). Among these compounds, quercetin (7), oleanolic acid (3), kaempferol (3), and geniposide (3) were linked to three or more genes with higher degrees. In addition, the core genes including peroxisome proliferator-activated receptor alpha (PPARα) regulated by oleanolic acid and quercetin, Peroxisome proliferator-activated receptor gamma (PPARγ) targeted by quercetin and kaempferol, and carbonic anhydrase II (CA2) linked by genipin and geniposide. In all, the above compounds and genes might play myriad roles in the NAFLD development and progression.

Due to technical limitations, Table 2 is provided in the Supplementary Files section.

PPI Network analysis

Through the analysis of the interaction between target genes, the result was shown (Fig. S4), proliferator-activated receptor alpha (PPARα), Peroxisome proliferator-activated receptor gamma (PPARγ) and carbonic anhydrase II (CA2) have greater degrees than others, which indicated that these proteins might play important roles in NAFLD. Interestingly, it was consisted with the conclusion from the above network analysis.

To understand the hepatoprotective mechanism of GF against NFALD, we performed functional enrichment analysis of target genes (Fig. 5) of bio-active compounds from GF using DAVID software and the KEGG database, potential target genes were functionally associated with various signal transduction pathways such as peroxisome proliferator-activated receptor (PPAR), glucagon signaling pathway, AMPK signaling pathway (AMPK) and cGMP-PKG signaling pathway (cGMP-PKG) shown in Table 3. Interestingly, many of the potential target genes appeared to be connected to the PPAR signaling pathway. Subsequently, GF protected against NAFLD through multiple pathways especially the PPAR signaling pathway.

Table 3
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and target genes of compounds in GF extract (GFE) potentially responsible for the therapeutic activities against NAFLD.

Pathway Classification	Pathway ID	Term	Target Gene
Signal transduction	hsa03320	PPAR signaling pathway	PPARA, PPARD, PPARG
Signal transduction	hsa04970	Salivary secretion	ADRB2, ADRA1B, ADRA1A
Signal transduction	hsa04080	Neuroactive ligand-receptor interaction	ADRB2, ADRA1B, ADRA1A, GLP1R
Signal transduction	hsa04922	Glucagon signaling pathway	GCG, PPARA, ACACA
Signal transduction	hsa04152	AMPK signaling pathway	PPARG, ACACA, ADRA1A
Signal transduction	hsa04261	Adrenergic signaling in cardiomyocytes	ADRB2, ADRA1B, ADRA1A
Signal transduction	hsa04022	cGMP-PKG signaling pathway	ADRB2, ADRA1B, ADRA1A
Signal transduction	hsa04020	Calcium signaling pathway	ADRB2, ADRA1B, ADRA1A
Signal transduction	hsa04024	cAMP signaling pathway	PPARA, ADRB2, GLP1R

Docking exercises of binding the main ingredients and protein

Computational docking exercises were conducted to mimic the characteristic of ingredient-target binding mode. The results showed that oleanolic acid and quercetin formed stronger or comparative interactions with PPARα to the native ligand (Fig. 6) featuring higher or similar docking score. The results were similar to genipin and geniposide formed interactions with CA2, and quercetin and kaempferol formed interactions with PPARγ. Taken together, GF protected against NAFLD *via* multi-compounds regulating multi-targets, for example, oleanolic acid and quercetin targeted on PPARα, genipin and geniposide targeted on CA2, quercetin and kaempferol targeted on PPARγ.

Validity Assay of Target Protein

Quercetin and PPARγ were selected to take part in the SPR test, The results showed that they had a high response and affinity with the Kd value of 71.5 μM (Fig. S5).

4. Discussion

NAFLDs progression and development are complicated processes involving multiple factors that alter the homeostasis of liver tissue. In the other hand, TCM contains of tens or thousands of compounds that can act on multiple target proteins through multiple pathways to exert their synergism or compatible pharmacological effects on complex diseases. Thus, TCM may have myriad advantages on the treatment of NAFLD. In this paper, we combined the HFD-induced rat model, network pharmacology, and molecular docking to explore the efficacy and mechanism of action of GF for the treatment of NAFLD.

After the HFD-induced rat model established, then supplemented with GF, the histopathological analysis exhibited that GF exerted potential protective effects against NAFLD. Specifically, we observed significant changes in serum glucose, insulin, the levels of lipids, liver function enzymes (ALT, AST, and LDH), and MDA Levels in serum and the mRNA expression of lipogenesis in liver tissue among different groups of rats. ALT, AST, and LDH are the important of biochemical indicators of liver function, they were widely used in clinic. As we expected, this study showed that they changed differently in the control, HFD-fed rats treated with or without GF and metformin. Although the detailed pathogenesis of NAFLD has not resolved completely, lipogenesis is believed to have a significant role in the triglyceride accumulation. Lipogenesis including *SREBP-1c*, *FAS*, *SCD-1*, *CD36*, *PPAR-α*, and *CPT-1* have been implicated in the NAFLD pathogenesis, their mRNA expression levels of liver tissues were significantly decreased following the HFD-fed rats treated with GF compared the non-treated ones, also in a dose-dependent manner. Moreover, serum glucose, insulin resistance, dyslipidemia and metabolic syndromes have played important roles in the NAFLD [16], insulin resistance inhibited the anti-lipolytic activity of insulin in the adipose tissue and increased free fatty acids (FFAs) in the serum and liver, leading to mitochondrial dysfunction as well as cardiac fat accumulation. As expected, the serum glucose, TC, LDL-C, and TG levels and insulin were decreased in HFD-fed rats supplemented GF compared with those untreated ones [17]. Furthermore, oxidative stress is essential risk factors for NAFLD, and promotes the production of reactive oxygen species (ROS) that stimulate an inflammatory process in hepatic tissues [18]. Our research showed that the MDA levels in serum was decreased in the HFD-induced rats treated with GF compared with those without any supplement. Moreover, the high dosage (100 mg/kg) of GF showed similar effects to the positive control drug metformin on the treatment of rats with NAFLD. Consequently, GF may play an important role in preventing or slowing the progression of NAFLD by regulating multi-factors, such as, serum glucose, insulin resistance, oxidative stress, free fatty acids, liver enzyme, and lipogenesis.

Based on the network pharmacology analysis, the main active compounds in GF including quercetin, oleanolic acid, kaempferol, and geniposide played key roles in the treatment of NAFLD by targeted on genes including PPAR*α*, PPAR*γ*, and CA2. Moreover, these main protein genes exerted their effects concentrated on the PPAR pathway. Next, we will discuss their roles in NAFLD from three molecular levels, such as, compounds, protein genes, and pathways.

Firstly, quercetin and kaempferol are natural flavonoids widely distributed in herbal medicine, vegetable, and edible fruits, featuring a variety of biological functions, studied primarily for their potential roles to combat oxidative and inflammatory processes. They showed potential for the treatment of fatty liver.

Previous studies showed that both single quercetin and contained in herbal medicine could ameliorate NAFLD in HFD-induced mice [19]. Moreover, mice treated with up to 3000 mg/kg quercetin did not show any toxic effects [20]. Kaempferol, chemical structural analogues of quercetin, with less than a hydroxyl, also exhibited hepatoprotective effect [21], and suppressed hepatic gluconegeonesis [22] and inhibited hepatocellular carcinoma cell [23]. Geniposide, a major characteristic constituent in GF, which exerted protective effects against hepatic steatosis in rats fed with a HFD, the underlying mechanism might be associated with its antioxidant actions or regulation of adipocytokine release and expression of PPARα [24]. Oleanolic acid attenuated the subsequent development of high fructose diet-induced non-alcoholic fatty liver disease in rats [25].

Secondly, we identified PPAR*α*, CA2, and PPAR*γ* as potential target genes. PPAR*γ* was a regulator of adipocyte differentiation. Additionally, it had been implicated in the pathology of numerous diseases including obesity, diabetes, atherosclerosis, and cancer [26]. Previous study showed that PPAR*α* could inhibit fatty liver disease by activating the periostin-dependent JNK signaling pathway and modulating fatty acid oxidation [27]. Peroxisome proliferator-activated receptors (PPARs) are binder-activated nuclear receptors that are involved in the transcriptional regulation of lipid metabolism, energy balance, inflammation, and atherosclerosis [28].

Thirdly, the functions of potential target genes identified from KEGG enrichment analysis were associated with multiple signal transduction pathways, the most important one is the PPAR pathway, which was consistent with the conclusion of the PPI analysis. Which contribute to the complex regulation of NAFLD progression, and was the therapeutic target of anti-NAFLD compounds. Recent research found that PPARs were closely related to metabolic syndrome and its relevant complications [29]. PPARs pathway had protective effect on the NAFLD development because it could regulate the lipid metabolism [30]. Therefore, our results suggested that these signal pathways might be coordinated during NAFLD progression, and the effects of GF could be mediated through multiple signaling pathways concentrated on PPAR.

Finally, computational docking exercises showed that the main ingredients from GF formed comparative interactions with their according predicted proteins compared to the native ligands with those. Furthermore, quercetin had a high response and affinity with PPARγ were validated in the SPR test with the Kd value of 71.5 *μM*. The regulatory mechanisms of the processes were likely to be valid targets for modulating lipid metabolism and inflammation in the treatment of NAFLD. However, further studies on the relevance to NAFLD are needed.

5. Conclusion

In conclusion, our results revealed that GF had the protective effects on the HFD -induced rats, restored triglycerides in the HFD-induced rats by alleviating the histopathological features and suppressing the levels of serum glucose, ALT, AST, LDH, MDA, TC, LDL-C, TG, and mRNA of lipogenesis in the liver. GF showed comparative effect to the positive control metformin against NAFLD. Our subsequent network

analysis suggested that quercetin, oleanolic acid, and geniposide targeted on PPARγ, PPARα, and CA2 through various signaling pathways concentrated on the PPAR signaling pathway for the treatment of NAFLD, the molecular docking further confirmed the above results. GF featured multi-components, targeted on multi-targets, and through multi-signal pathways, protected against NAFLD. Further research is needed to identify the individual components responsible for the therapeutic activities, to establish their mechanisms of action, and to confirm the target genes and signaling pathways involved. To our knowledge, this is first report that integration the model animal of network pharmacology and molecular docking to illustrate the pharmacological mechanism of TCM. The results not only provide evidence for the therapeutic mechanism of GF but also shed new insights on the molecular mechanism analysis of other complex drugs.

Abbreviations

GF: Gardeniae Fructus; TCM: traditional Chinese medicine; NAFLD: nonalcoholic fatty liver disease; HFD: high fat diet; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; MDA: malondialdehyde; PPARA: Peroxisome Proliferator Activated Receptor Alpha; PPARG: Peroxisome Proliferator Activated Receptor Gamma; CA2: Carbonic Anhydrase 2; HPLC: high performance liquid chromatography; SD: Sprague–Dawley; OB: oral bioavailability; DL: drug-likeness; H&E: Hematoxylin and eosin; PDB: Protein Data Bank; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database; ADME: absorption, distribution, metabolism, and excretion; TTD: therapeutic targets database; AMPK: Adenosine 5’-monophosphate (AMP)-activated protein kinase

Declarations

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was reviewed and approved by the ethics committee of Hosipital of Stomatology, Tongji University. Professor Qi Zhang is the chairman of the committee.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests.

Author’ s contributions

X-ZX and L-LH conceived and designed the research methods. L L and L-LH collected and analyzed the data. X-ZX wrote the paper. All authors read and approved the final manuscript.

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Figures

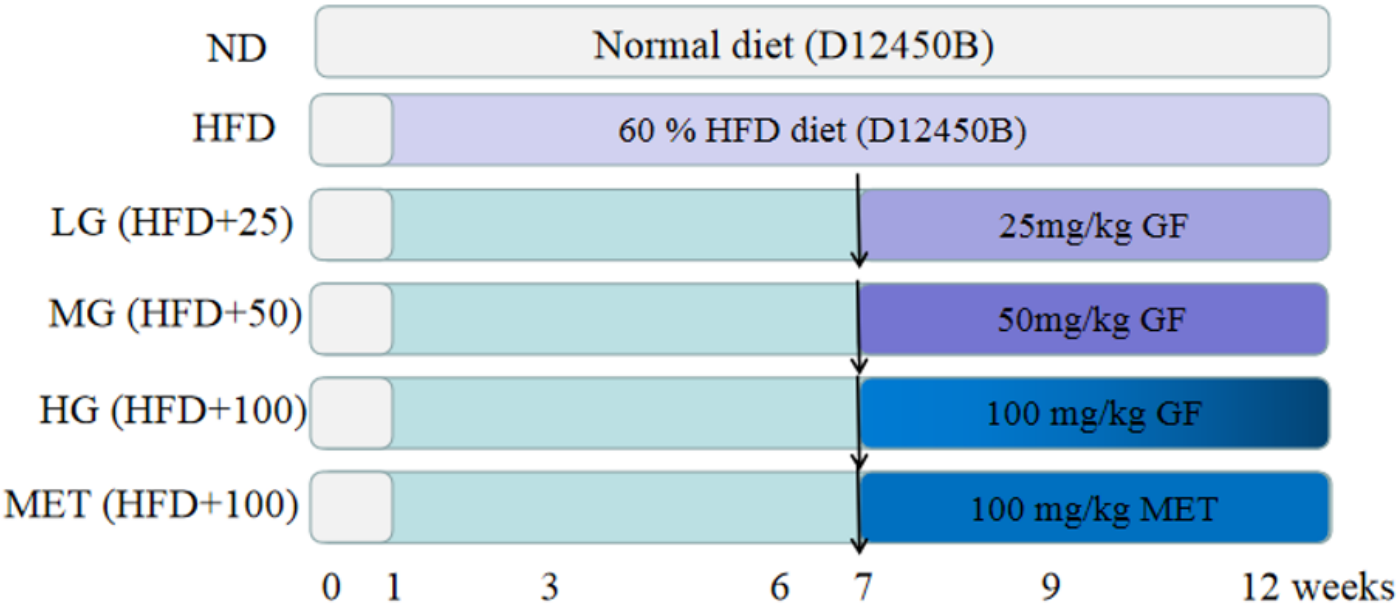

Figure 1

Schematic overview of the experimental design.

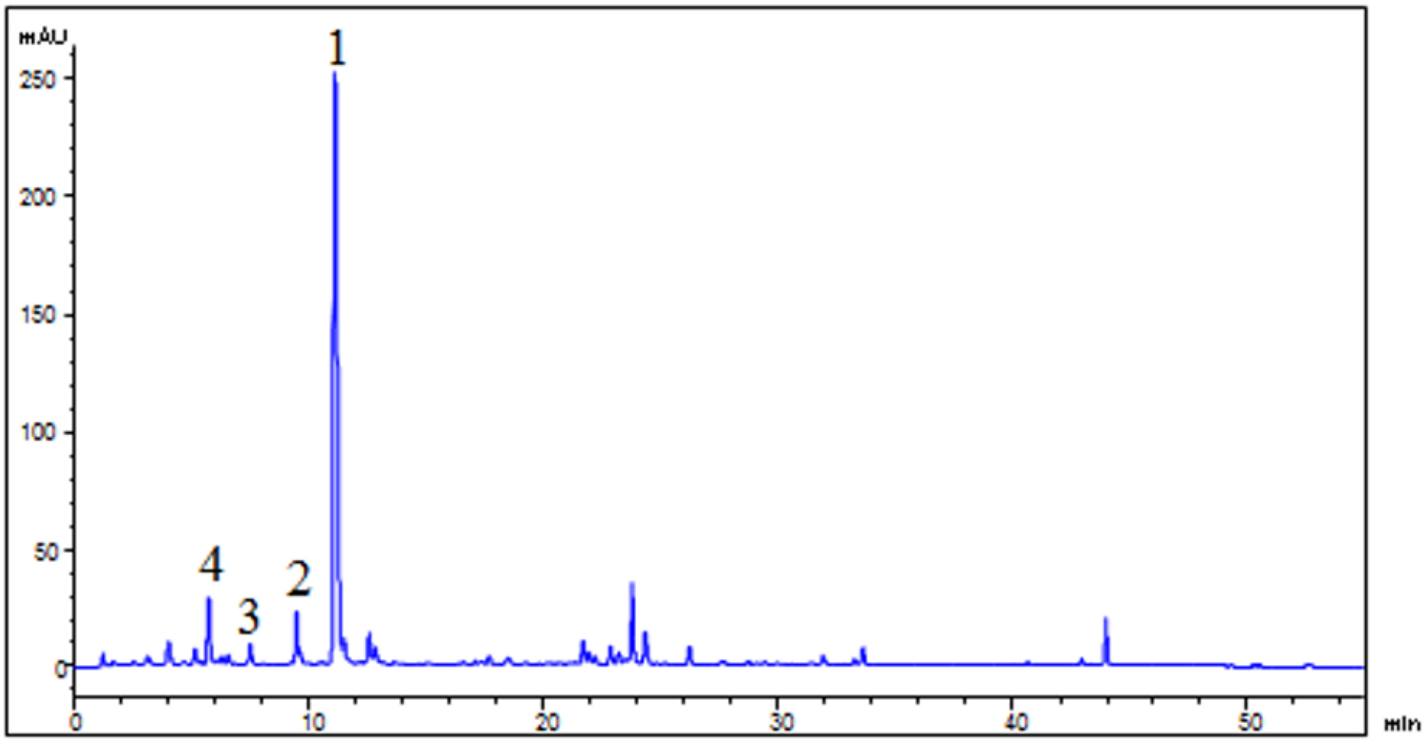

Figure 2

HPLC chromatography of GF.

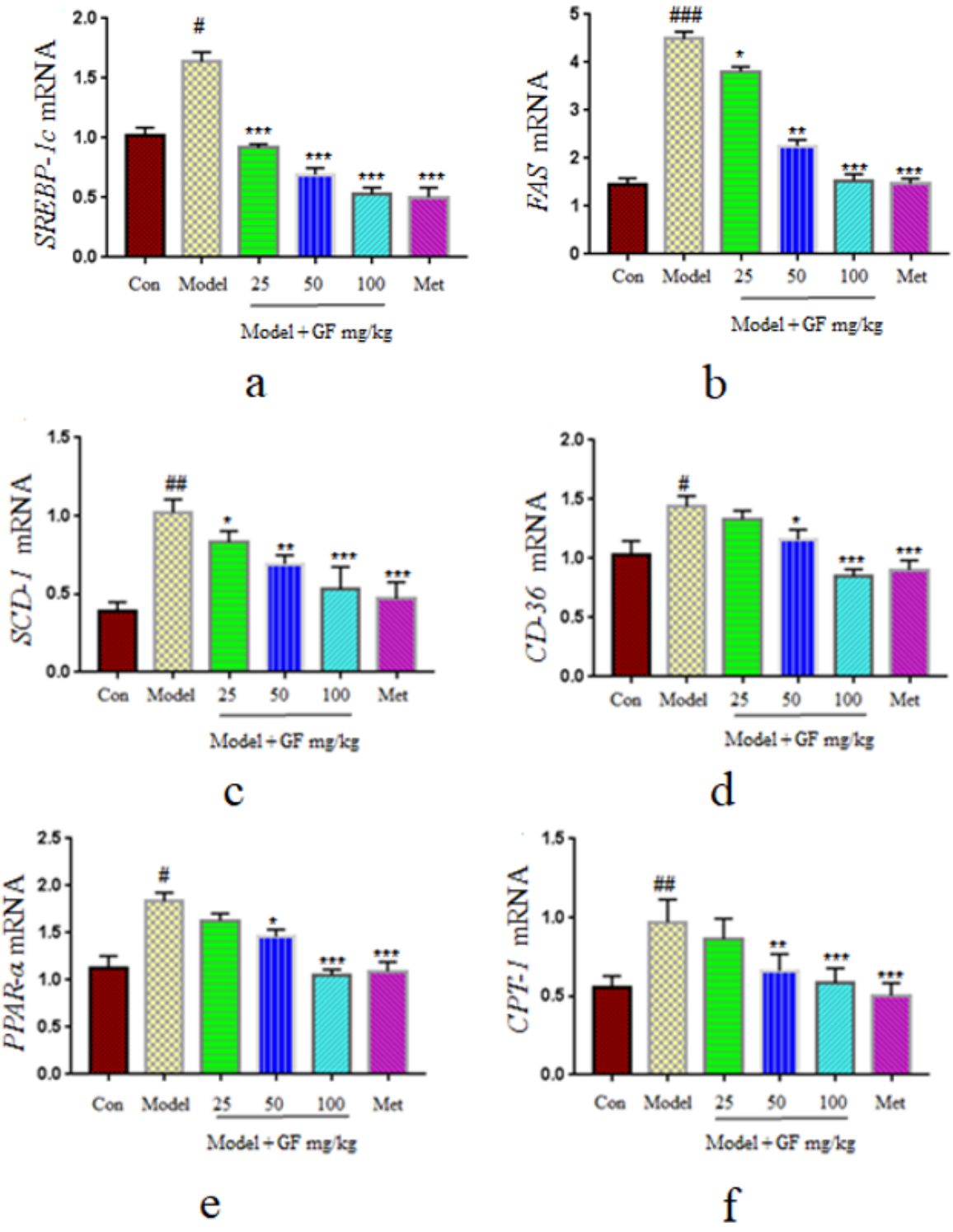

Figure 3

Effects of GF on the lipogenesis markers in liver.

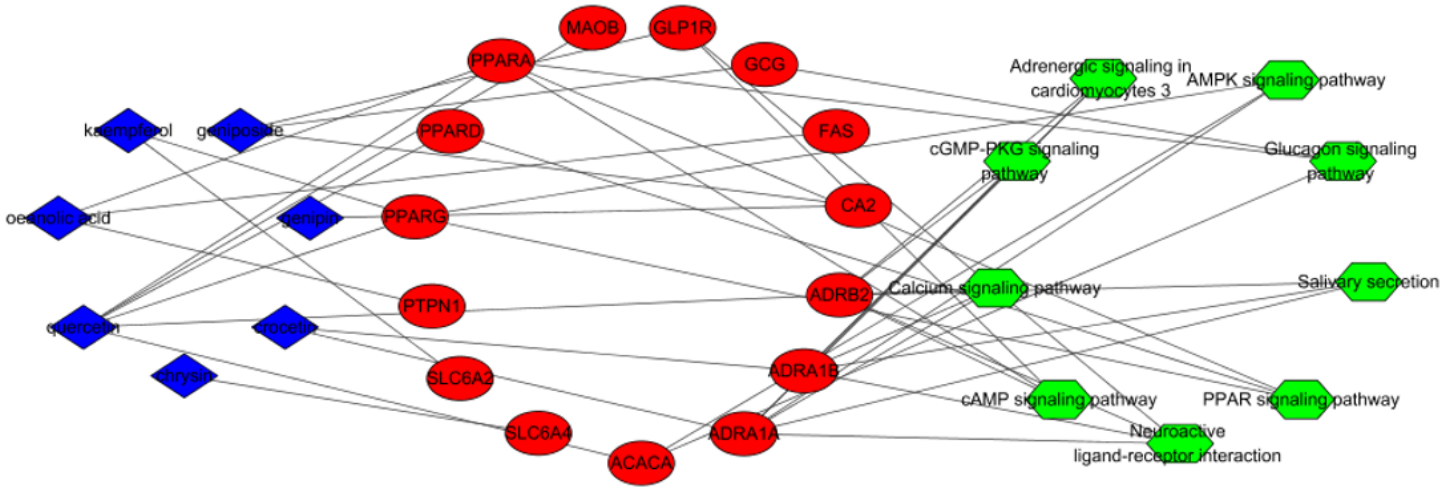

Figure 4

Compound-target gene-pathway network clarified the mechanism of GF against NAFLD.

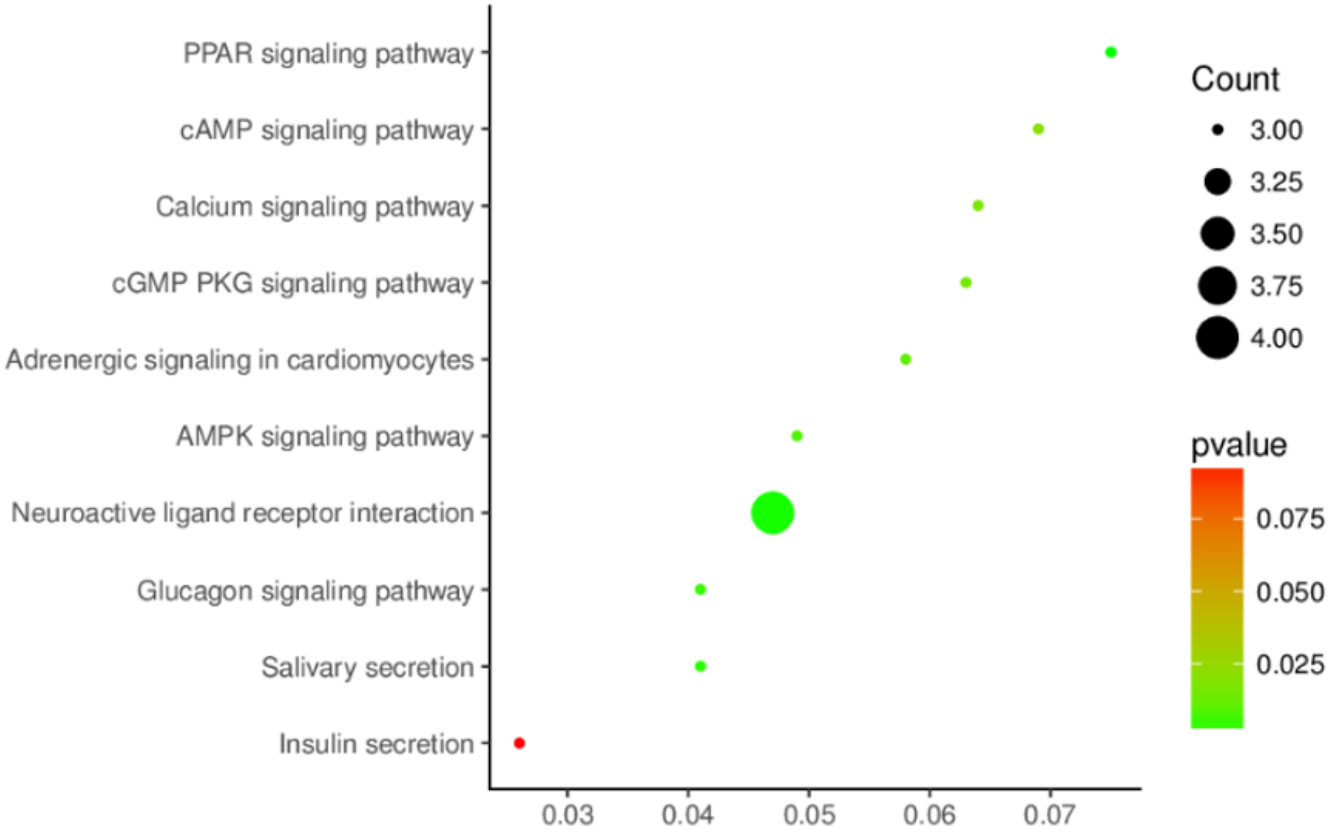

Figure 5

KEGG enrichment analysis map of potential pathways of GF treated on NAFLD.

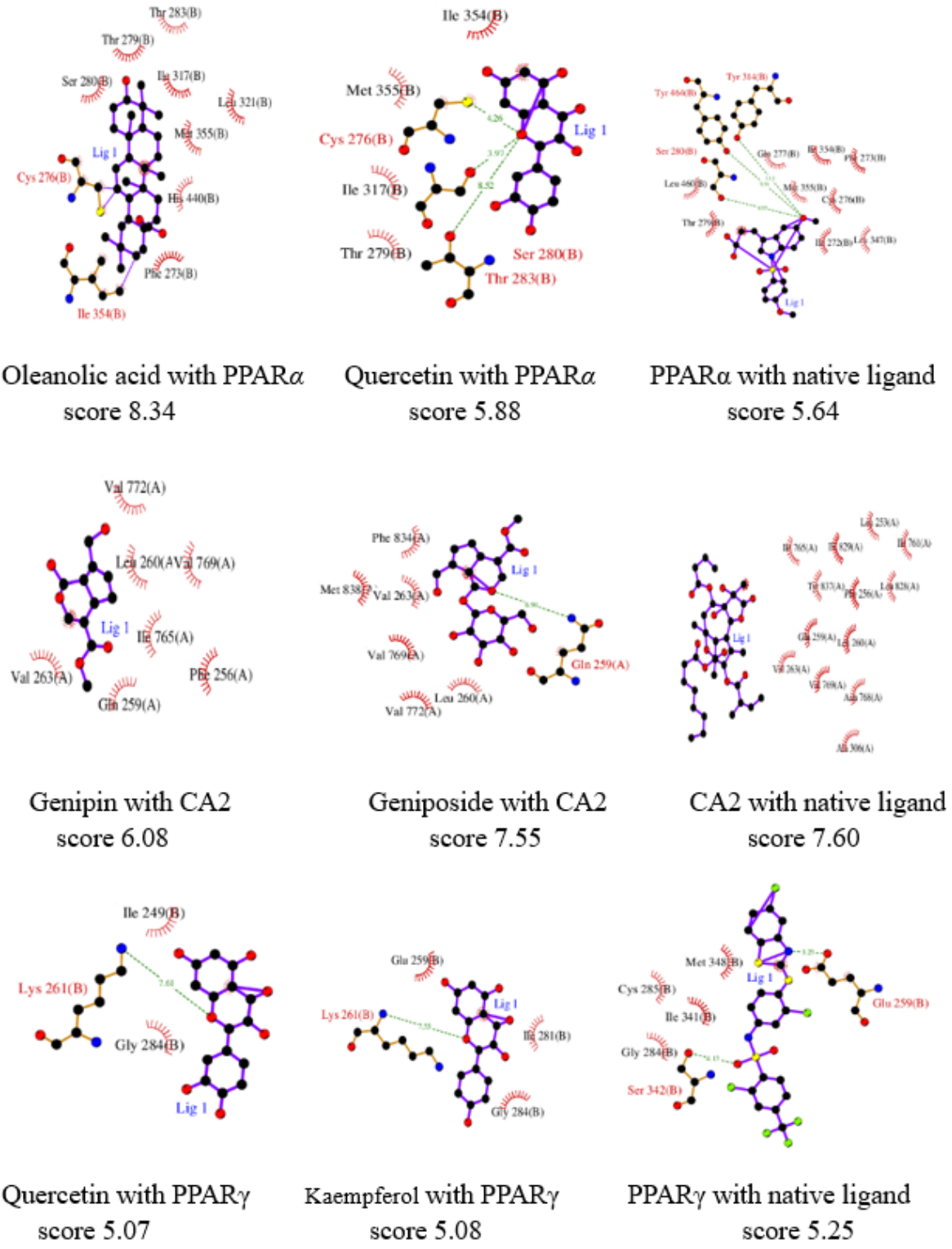

Figure 6

Docking exercises on ingredients of binding to PPARα, PPARγ, and CA2.

Supplementary Files

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