Food Science of Animal Products

Research Article

Exploring the mechanism of galangin in alleviating alcoholic fatty liver disease based on the Gene Expression Omnibus database and network pharmacology

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Abstract: This study aimed to use the Gene Expression Omnibus (GEO) database combined with network pharmacology technology to investigate the positive effect of galangin on alcoholic fatty liver disease (AFLD) and explore its potential mechanism. The study first screened the differential genes in ALFD mice through the GEO database, obtained the possible targets of galangin through the SwissTargetPrediction and PharmMapper databases, and then obtained the intersection of drug and disease targets through a Venn diagram to build a "drug-target-pathway-disease" network relationship diagram, and its possible molecular mechanisms were analyzed through Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. The network pharmacology analysis identified 22 potential targets for galangin in the treatment of AFLD, and protein-protein interaction network analysis revealed that the top 5 targets were protein kinase B (AKT) 1, epidermal growth factor receptor, mitogen-activated protein kinase (MAPK) 3, myeloid cell leukemia 1, and KIT proto-oncogene receptor tyrosine kinase. The KEGG results showed that the treatment of AFLD by galangin may be related to the MAPK signaling pathway, cyclic adenosine monophosphate signaling pathway, Ras-related protein 1 signaling pathway and phosphoinositide 3 kinase/AKT signaling pathway. Therefore, the combination of GEO database and network pharmacology prediction results showed that galangin could alleviate alcoholic fatty liver and exert anti-inflammatory effects. It provides a theoretical basis for research on the mechanism of galangin in treating AFLD and other diseases.

Keywords: galangin; alcoholic fatty liver disease; network pharmacology; Gene Expression Omnibus database

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1 Introduction

The World Health Organization currently estimates that approximately three million people die from alcohol-related liver disease every year[1]. Moreover, in recent years, the incidence of liver damage caused by alcohol has increased year by year in our country, becoming the second largest liver disease after viral hepatitis, posing a serious threat to the health of our people[1]. Alcoholic liver disease is mainly a series of liver damaging lesions caused by long-term drinking or occasional heavy drinking. According to the pathological condition and course of the disease, it covers initial hepatic steatosis and alcoholic fatty liver, and then it develops into alcoholic hepatitis, liver fibrosis, and cirrhosis, which can induce large-scale liver cell necrosis, severe liver failure, and even liver cancer in the later stages[2]. The initial stage of alcoholic fatty liver disease (AFLD) can be completely recovered by quitting drinking. However, the persistence of steatosis will promote adverse reactions such as liver lipid peroxidation and oxidative stress. Especially in the case of excessive drinking, it will further develop into alcoholic hepatitis[3–4]. Although the negative impact of alcoholic liver disease on the health of our country's citizens is increasing, effective prevention and treatment methods are still very limited[5]. Therefore, it is necessary to understand the mechanisms of the pathogenesis of alcoholic liver disease and identify novel therapeutic targets. The formation mechanism of alcoholic liver disease is very complex, mainly because heavy drinking affects the synthesis and oxidation of fatty acids[6–7]. Additionally, alterations in intestinal permeability associated with alcohol consumption elevate portal endotoxins, which trigger innate immune reactions and liver inflammation by activating various cytokine pathways[8]. Despite substantial proceeds in the pathological processes of alcoholic liver disease, there are no effective treatments other than alcohol abstinence. Therefore, there is an urgent need to reveal the potential pathways and therapeutic targets in the pathogenesis of alcoholic liver disease to find new treatments.

Galangin is a flavonoid component found in honey and galangal root, has long been used in traditional medicine[9] with antioxidant and anti-obesity properties[10–12]. Moreover, galangin also exerts a critical effect in the intervention of liver diseases. It is reported that galangin could alleviate inflammation by down-regulating the liver inflammatory pathway in fructose-fed rats[10]. Galangin could alleviate acetaminophen-induced acute hepatotoxicity in mice,

mainly by reducing acetaminophen-induced hepatic oxidative stress, increasing hepatic glutathione levels, and reducing hepatic microsomal cytochromes P450 (CYP) 2E1 levels[13]. Galangin could reduce concanavalin A-provoked liver injury in mice, mainly by inhibiting nuclear factor κB (NF-κB)/signal transducer and activator of transcription 1 (STAT1) signaling pathways, thereby reducing the expression and secretion of inflammatory mediators[14]. Moreover, the study showed that galangin could improve non-AFLD by promoting autophagy[15]. Although the pharmacological effects of galangin have been extensively studied, its ability to protect against alcohol-induced liver injury remains unknown.

Network pharmacology represents an innovative approach that combines chemical informatics, bioinformatics, traditional pharmacology, biological networks, and network analysis. Utilizing network pharmacology allows for the creation of an extensive network linking compounds, active targets, and disease targets[16]. As a powerful method for predicting the intricate mechanisms of multi-component and multi-target drugs, it has been widely used to clarify the multi-target effects of various diseases and combined with metabolomics or other databases[17–18]. The Gene Expression Omnibus (GEO) is an definitive genetics database with a large amount of sequencing data and clinical data on varied diseases[19]. Additionally, the analysis of data matrices through bioinformatics is extensively utilized to recognize genes that are expressed differently and to conduct a variety of assessments[19]. Integrating data from various independent studies through the combination of multiple databases allows for the acquisition of a larger sample size for analysis, thus enhancing the rigor and precision of the findings. Given this, more and more studies are integrating information through network pharmacology combined with GEO database and conducting further analysis through bioinformatics and experiments. In this study, GEO database combined with network pharmacology were used to investigate the mechanism of galangin in alleviating AFLD. It is of great significance for galangin as a new drug to alleviate AFLD.

2 Materials and methods

2.1 Database and analysis software

The database and software used in this article were shown in Table 1.

Table 1 Database and software used for network pharmacology.

Database and software	Website
PubChem database	https:/pubchem.ncbi.nlm.nih.gow/
National Center for Biotechnology Information (NCBI) database	https://www.ncbi.nlm.nih.gov/
GeneCards database	https://www.genecards.org
SwissTargetPrediction database	http://www.swisstargetprediction.ch/
UniProt database	https:/www.uniprot.org
String database	https://en.string-db.org
DAVID database	https://david.nciferf.gov/
Cytoscape software (v3.8.2)	https://cytoscape.org/
R-project software	https://www.r-project.org
Weishengxin software	http://www.bioinformatics.com.cn/

2.2 Structure and target acquisition of galangin

The PubChem database was subjected to obtain the simplified molecular input line entry system (SMILES) structure of galangin and inputted the obtained SMILES structure into the SwissTargetPrediction and PharmMapper databases. The species was selected as *Mus musculus* (mouse). After obtaining the potential targets of galangin, the obtained targets were merged and deduplicated to form a collection of potential targets of galangin's biological activity in mice.

2.3 Acquisition of differential genes in the liver of AFLD mice

The differential genes of the AFLD model mice were obtained from the sequencing data published in the data set GSE250473 downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE250473). This data set was liver transcriptome sequencing data 8 weeks after the establishment of AFLD model in male mice. The test mice were divided into normal diet group and alcohol treatment group according to different treatments. In this study, data from the normal diet group (GSM7979596, GSM7979597, GSM7979598, GSM7979599, and GSM7979600) and the alcohol treatment group (GSM7979601, GSM7979602, GSM7979603, GSM7979604, and GSM7979605) were selected to construct the gene expression matrix of male AFLD model mice. The DESeq2 package of R-project software was used for differential analysis, and the ggplot2 package was used to draw the volcano plot.

2.4 Screening of intersection targets

The obtained galangin targets and AFLD targets were sorted and counted, the intersection targets were screened. Then a Venn diagram was drawn using the online drawing tool of the Weishengxin website.

2.5 Construction of protein-protein interaction (PPI) network

The 22 intersection genes were imported into the String network platform to construct a galangin target PPI network. The intersection of disease-related genes and galangin target genes were organized into a file. In the search interface of the String network platform, we imported the organized file, selected the species as "*Mus musculus*" and increased the minimum interaction score to 0.900. This could delete nodes that have no connection with other proteins in the network, the galangin target PPI network could be obtained. Finally, we downloaded the network file in "tsv" format for the next step.

2.6 Enrichment analysis

The DAVID database was conducted for Gene Ontology (GO) biological function annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of the intersection core targets. The biological processes (BP), molecular functions (MF), and cellular components (CC) of GO were used to perform correlation analysis on the biological functions of the target and data visualization.

2.7 Construction of drug-target-pathway-disease network

Through the above analysis and search, we have obtained the intersection targets of galangin and AFLD. At the same time, the

intersection targets point enrichment pathways was obtained through KEGG pathway enrichment analysis. A drug-target-pathway-disease network map was established based on the previous analysis and the therapeutic targets of galangin in AFLD. The entire map was constructed using Cytoscape software (v3.8.2, Boston, MA) and Java analytically software.

3 Results

3.1 Galangin target prediction

Based on the principle of chemical structure similarity, there were 100 targets were predicted based on the SwissTargetPrediction database. Then, 56 of them were obtained through screening based on the condition of "probability > 0". And the amount of 290 targets was predicted by the PharmMapper database. Then, we used the UniProt database to convert the targets obtained after screening the PharmMapper database from the "UniPlot" form into "Gene Name" and then removed the names containing "none" and "human", and finally got 172 targets. Finally, the targets from the two databases were combined and deduplicated to obtain 228 targets.

3.2 Differentially expressed genes in the liver of AFLD model mice

Differential gene analysis was performed on the normal and AFLD model groups of male mice in GSE250473. Compared with the normal group, the gene expression of AFLD model mice showed significant differences. The results showed that there was obtained 2 473 differentially expressed genes, with 1 521 up-regulated genes and 952 down-regulated genes. Among them, the up-regulated genes mainly included *GPNMB*, *CYP2B13*, and *SLC7A11*, and the down-regulated genes mainly included *MUP7*, *TFF3*, and *SLC22A28* (Figure 1).

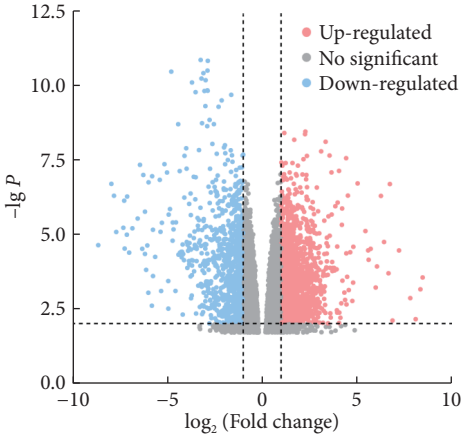

Figure 1 Volcano plot of differently expressed genes in the liver of AFLD mice.

3.3 Intersection targets between galangin and AFLD in mice

The above-obtained targets of galangin were mapped to differential genes in the livers of AFLD model mice, and the results of galangin treatment of AFLD were obtained. There were 22 potential targets in mice, as shown in Figure 2.

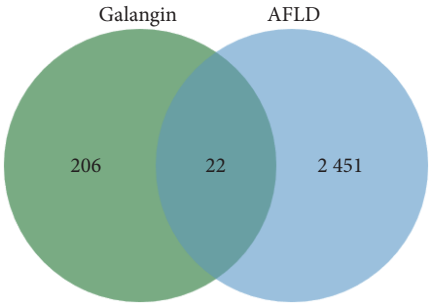

Figure 2 Venn diagram of targets of galangin for alleviating AFLD in mice.

Using Cytoscape CytoNCA plug-in, targets whose betweenness and degree were greater than the average of all points were selected as core targets, and these core targets were used to draw PPI network diagrams. As shown in Figure 3, the PPI network data was obtained through the String database, and the PPI network diagram was drawn and analyzed using Cytoscape software (v3.8.2). The network consisted a total of 22 nodes (representing interacting targets) and 41 edges (representing the relationships between targets). The "Network analysis" module was used to analyze and obtain the betweenness centrality, closeness, and degree attribute values of each node of the intersection network. A network diagram was drawn based on the attribute value of each node. The larger the node shown in the figure and the darker the color, the higher the importance of the node. The thicker the connection line and the darker the color, the closer the correlation. As shown in the Figure 3, the core targets obtained mainly included protein kinase B (AKT) 1, epidermal growth factor receptor (EGFR), mitogen-activated protein kinase (MAPK) 3, myeloid cell leukemia 1 (MCL1), and KIT proto-oncogene receptor tyrosine kinase (KIT), etc. The core target attribute values were shown in Table 2.

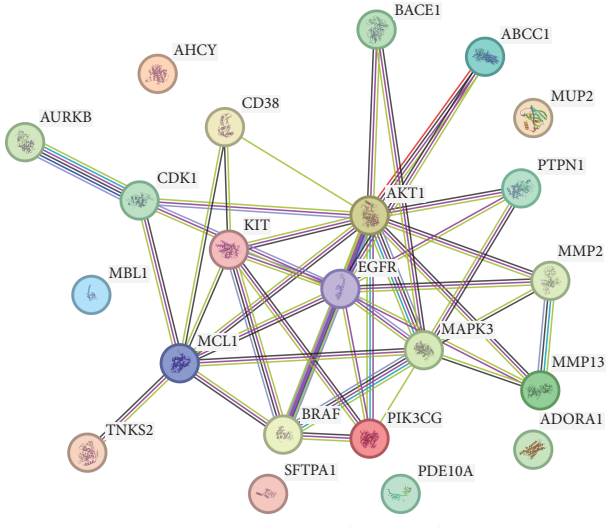

Figure 3 PPI network diagram of targets of galangin for alleviating AFLD in mice. ABCC1, multidrug resistance-associated protein 1; ADORA1, adenosine receptor A1; AHCY, adenosylhomocysteinase; AURKB, aurora kinase B; BACE1, *β*-secretase 1; BRAF, serine/threonine-protein kinase B-raf; CD38, ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1; CDK1, cyclin-dependent kinase 1; MBL1, mannose-binding protein A; MMP13, matrix metalloproteinase 13; MMP2, matrix metalloproteinase 2; MUP2, major urinary protein 2; PDE10A, cyclic adenosine monophosphate (cAMP) and cAMP-inhibited cyclic guanosine monophosphate 3′,5′-cyclic phosphodiesterase 10A; PIK3CG, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit γ isoform; PTPN1, tyrosine-protein phosphatase non-receptor type 1; SFTPA1, pulmonary surfactant-associated protein A; TNKS2, poly(ADP-ribose) polymerase tankyrase-2.

Table 2 Topology properties of the core targets.

Target	Degree	Betweenness centrality	Biological function
AKT1	26	0.323 174 603	Related to cell growth and survival
EGFR	22	0.173 968 254	Regulated cell growth, survival, adhesion, migration, and differentiation
MAPK3	18	0.086 349 206	Related to biotic and abiotic stress signaling
MCL1	16	0.174 920 635	Maintained normal cellular homeostasis
KIT	12	0.014 920 635	Related mast cell proliferation, survival, differentiation, mediator release, adhesion, and chemotaxis

3.4 GO biological process and KEGG signaling pathway enrichment analysis

At the same time, GO biological process and KEGG signaling pathway enrichment analysis were performed on the twenty-two intersection genes obtained. GO biological process enrichment analysis resulted in 118 GO items, including 82 BP items, 20 MF items, and 16 CC items. GO biological process enrichment analysis was sorted by count according to the three modules of BP, CC and MF, and the top 10 were selected to draw a schematic diagram, as shown in Figure 4. BP items were involved in protein phosphorylation, signal transduction, negative regulation of apoptotic process, cellular response to reactive oxygen species, and response to hypoxia. CC items were involved in membrane, cytoplasm, nucleus, mitochondrion, and extracellular space. MF items were involved in protein binding, nucleotide binding, ATP binding, enzyme binding, protein kinase activity, and protein serine/threonine kinase activity.

On the other hand, through KEGG signaling pathway enrichment analysis of intersection genes, the top 20 significantly enriched signaling pathways were screened using $P \leq 0.01$ as filter conditions. As shown in Figure 5, the KEGG pathway in intersecting genes was arranged by the number of related genes and displayed in the form of a bubble chart. Among them, MAPK signaling pathway, cAMP signaling pathway, Ras-related protein 1 (Rap1) signaling pathway and PI3K/AKT signaling pathway had significant statistical significance. There were also pathways for pancreatic cancer, breast cancer, bladder cancer, leukemia, and endocrine related diseases. Moreover, as shown in Figure 6, we also obtained the drug-target-pathway-disease network map of galangin.

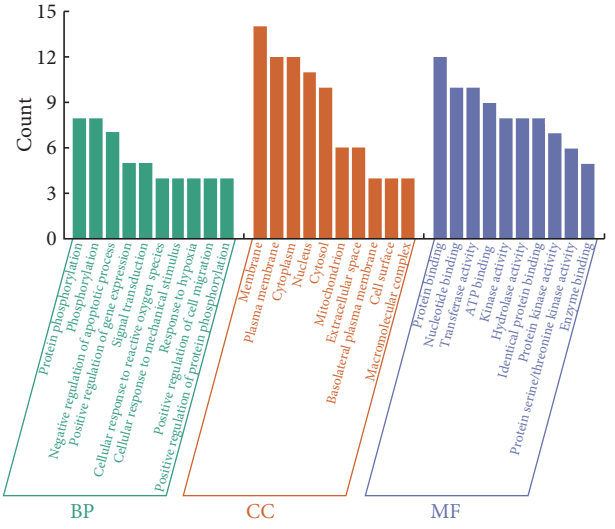

Figure 4 Histogram of GO bioprocess enrichment analysis in intersecting genes.

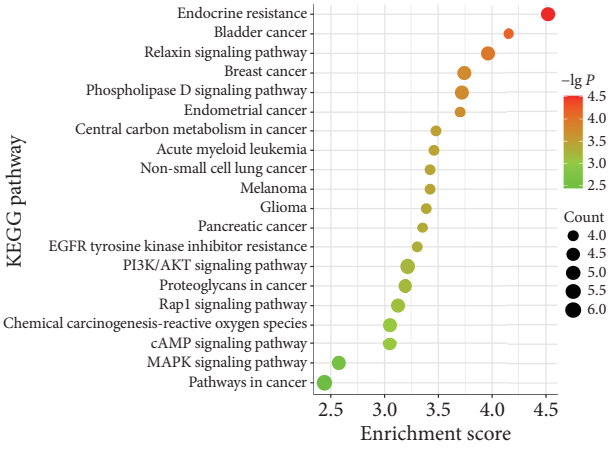

Figure 5 Bubble graph of KEGG pathway in intersecting genes.

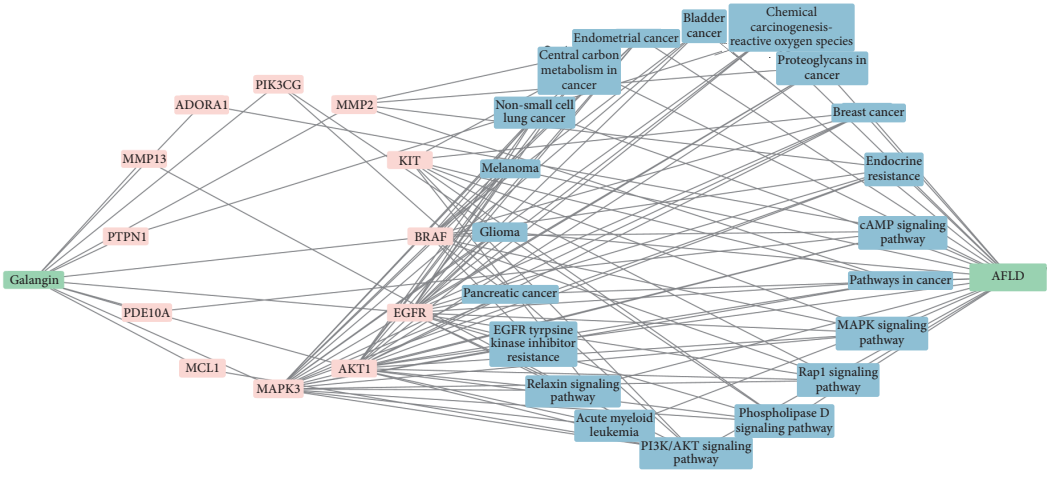

Figure 6 Network map of drug-target-pathway-disease of galangin.

4 Discussions

Alcoholic liver disease is a common chronic liver disease with high morbidity and mortality worldwide[1,20–21]. As a flavonoid, galangin has been shown the properties of antioxidant, anti-obesity, and antiviral properties[10–12]. Galangin could reduce ConA-induced liver injury in mice by blocking the NF-κB and STAT1 signaling pathways[14]. Moreover, our previous studies revealed that galangin could alleviate alcohol-induced intestinal barrier dysfunction in mice via the NF-κB/MAPK signaling pathway[22]. Network pharmacology, as a new method that combines system network analysis and pharmacology. Moreover, network pharmacology can decipher the mechanisms of interactions between organisms and diseases based on protein abundance, gene expression, and small molecule (metabolite) levels[23]. As a public genomics database, the GEO database has been widely used in disease research[24]. Therefore, the study used the GEO database and network pharmacology to screen the key targets of galangin in alleviating AFLD and construct its disease target network diagram, thereby conducting a preliminary study on the mechanism of galangin in alleviating AFLD.

In the study, a total of twenty-two interacting targets were obtained through GEO database combined with network pharmacology analysis. After PPI analysis, the core targets obtained were AKT1, EGFR, MAPK3, MCL1, and KIT. The AKT serine/threonine kinase family, consisting of AKT1, AKT2, and AKT3, is an integral part of PI3K protein and apoptosis pathways[25]. AKT1 has extensive tissue distribution and is related to cell growth and survival[26]. EGFR is one of four transmembrane receptor tyrosine kinases in the human epidermal growth factor receptor family, which regulates cell growth, survival, adhesion, migration, and differentiation[27–28]. MAPK3 is a member of the MAPK family and is closely related to biotic and abiotic stress signaling[29–30]. MCL1 is a key anti-apoptotic protein belonging to the B-cell lymphoma 2 (Bcl-2) protein family[31]. In order to maintain normal cellular homeostasis, cells must tightly regulate MCL1 protein expression, and overexpression of MCL1 protein result in tumorigenesis[31]. KIT is a member of the type III receptor tyrosine kinase family encoded by the proto-oncogene c-kit and its activation stimulates multiple cellular responses, including mast cell proliferation, survival, differentiation, mediator release, adhesion, and chemotaxis[32–34].

The KEGG results found that galangin alleviated AFLD mainly through several key signaling pathways: MAPK, cAMP, Rap1, and PI3K/AKT. It is reported that the MAPK signaling pathway involves in cell proliferation, differentiation, apoptosis, and inflammation by activating three different cascades: extracellular signal-related kinase, c-Jun N-terminal kinase, and p38 pathway[35–37]. Moreover, the MAPK signaling pathway is also related to inflammation and cellular stress[38]. The cAMP signaling pathway carries out signal transduction through cAMP-dependent protein kinase A (PKA)[39–40]. It is reported that cAMP can bind to and activate PKA mainly through cAMP/PKA signaling, thereby increasing the phosphorylation level of PKA[41–43]. The PI3K/AKT pathway is one of the most basic intracellular signal transduction pathways and is the main regulator of cell growth, apoptosis, migration, metabolism, etc.[44–45]. PI3K is an upstream effector of the PI3K/AKT signaling pathway, by which promoting the phosphorylation of Ser473 and Thr308 to activate the key downstream effector AKT[46] and can be triggered under physiological conditions such as insulin, cytokines, and growth hormones[47–48]. Rap1 is a member of the Ras GTPase family which regulates numerous cellular processes, such as cell growth, apoptosis, adhesion, and intracellular vesicle trafficking[49]. In addition, downstream signaling of the Rap1 pathway is also an activator of three signaling pathways: extracellular regulated protein kinase, MAPK, and PI3K/AKT[50]. These findings were consistent with some previous studies, which indicated that galangin could exert anti-inflammatory effects through the NF-κB/MAPK pathway[51], alleviate liver ischemia-reperfusion injury in a rat model by mediating the PI3K/AKT pathway[52], and increase the activity of cAMP response element-binding protein[53].

This study used the GEO database combined with network pharmacology to predict the effects and mechanisms of galangin in alleviating AFLD. Finally, it was found that its core targets involved oxidative stress, cell proliferation, differentiation, and apoptosis, as well as regulating lipid metabolism. The KEGG analysis showed enrichment of genes such as MAPK pathway, cAMP pathway, Rap1 pathway, and PI3K/AKT pathway. Combining GEO database and network pharmacology prediction results, it was shown that galangin could alleviate alcoholic fatty liver and exert anti-inflammatory effects. The study provides a research theoretical basis for the mechanism of galangin in treating AFLD and other diseases and provides a new basis for the application of galangin. It also provides new ideas for the development of functional activity of hormones.

Conflict of interest

The authors declare that they have no competing financial interests.

Acknowledgements

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