Exploring the effect of Radix Bupleuri (Bupleurum chinense DC) on nonalcoholic fatty liver disease based on network pharmacology

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Abstract

Objective: To investigate the main effects of Radix Bupleuri (Chinese name called Chai Hu) in the prevention and improvement of nonalcoholic fatty liver disease using network pharmacology techniques. Methods: We used the Traditional Chinese Medicine Systematic Pharmacology database to query the main active ingredients of Radix Bupleuri; used the DisGenet database, Treatment Target Database, and DrugBank Database to screen the targets of nonalcoholic fatty liver disease; used the matching traditional Chinese medicine-disease targets to build the traditional Chinese medicine-component-target network system using Cytoscape software; used STRING software to build the protein-protein interaction system and visualized the data; DAVID database was used for gene ontology functional enrichment study and Kyoto Encyclopedia of Genes and Genomes pathway study. Results: Twelve major functional components and 175 targets have been obtained for the prevention and alleviation of nonalcoholic fatty liver disease in Radix Bupleuri; network pharmacology also confirmed the maximum degree value of kaempferol, the main active component of Radix Bupleuri; gene ontology functional enrichment analysis obtained the top 10 entries of biological process, cellular component, molecular function and Kyoto Encyclopedia of Genes and Genomes pathway analysis obtained the top 30 entries of the signalling pathway. Conclusion: Radix Bupleuri may use Fluid shear stress and atherosclerosis, Cancer, Advanced glycation end-(receptor of advanced glycation, interleukin 17, Hepatitis B, Toxoplasmosis, Relaxin, and tumor necrosis factor signalling pathway to regulate the inflammatory response of interleukin 6, tumor necrosis factor, and prostaglandin endoperoxide synthase2 targets and reduce extracellular matrix deposition to improve the therapeutic effect of Nonalcoholic fatty liver disease. And the active ingredient of traditional Chinese medicine Radix Bupleuri, kaempferol, may also play a significant role in this.

Keywords: network pharmacology; Radix Bupleuri; nonalcoholic fatty liver disease; traditional Chinese medicine
Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome characterized by diffuse hepatocellular spheroid steatosis and alcohol consumption, and other obvious causes of liver damage. It is associated with obesity, type 2 diabetes mellitus (T2DM), and atherosclerosis [1–2]. NAFLD has a broad spectrum of diseases, including simple steatohepatitis, nonalcoholic steatohepatitis (NASH), progressive liver fibrosis, liver stiffening and hepatocellular carcinoma. Due to the rapid increase in the incidence of obesity and metabolic syndrome in China, NAFLD has become one of the most prevalent metabolic liver diseases in the world and the most predominant chronic liver disease in China [3].

The pathogenesis and new theories of NAFLD include insulin resistance (IR), including oxidative stress, inflammatory response, mitochondrial dysfunction, fatty liver, and hyperinsulinemia [4]. The deposition of large amounts of lipids in hepatocytes, especially triglycerides, is a prerequisite for the formation and development of NAFLD and one of the most critical aspects of the disease [5]. The “second strike theory” can explain the formation mechanism of NAFLD more reasonably [6–7]. The first blow is that insulin resistance affects lipid metabolism, resulting in increased peripheral lipolysis, which leads to the continuous transport of free fatty acids (FFA) to the liver and increases fatty acid synthesis, while the synthesis or secretion of very low-density lipoproteins (VLDL) decreases, resulting in increased Triglyceride (TG) accumulation; the second blow is mainly due to oxidative stress and abnormal production of inflammatory cytokines such as tumor necrosis factor α (TNF-α), which causes inflammation, necrosis and even fibrosis in steatotic hepatocytes [8].

In the experimental study of nonalcoholic fatty liver, there is no specific effective drug to prevent and treat nonalcoholic fatty liver. In the early days, it was believed that nonalcoholic fatty liver disease did not require special treatment and could be prevented and treated by dietary modification. Now, the main moral measures are still dietary modification, exercise therapy and weight loss. Therefore, drug treatment is very critical. Current clinical treatment studies show regular use of hepatoprotective, fat-reducing drugs and antioxidants such as vitamins B, C, E, lecithin, ursooxychoic acid, silymarin, inosine, coenzyme A, reduced glutathione, taurine, lactated carmin, liver Tailor and some lipid-lowering drugs. These drugs not only lack specificity in the treatment of fatty liver, but some of them have potential liver toxicity and even aggravate the lesions of fatty liver. The ideal animal model should be similar to the disease characteristics, with a specific disease development process, high formation rate, low mortality, good repeatability, simple and easy modelling method, and adopt new technologies and new indicators to make the model more accurate, consistent with the actual situation of the disease. Wang Jianqiang et al. established a model of nonalcoholic steatohepatitis in rats. Male SD rats were randomly divided into standard and model groups. Serum Alaninetransaminasine (ALT), Aspartate Transaminasine (AST), total cholesterol (TC), TG, High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C) and liver TC and TG were measured after 6 weeks of experimental duration and examined histologically. The results showed that serum ALT, AST, TC, HDL-C, LDL-C, and liver TC and TG were significantly increased in the model group; the pathological histological examination indicated that hepatocytes in the model group showed diffuse steatosis with small liver size [9]. Yang Ling et al. observed the effect of lipid elimination and liver protection formula on the recovery of blood lipid and liver function in rats with a fatty liver model and its mechanism. The content of leptin and leptin long-type receptor Obesity-ectoper Leptin (OB-RL) in the hypothalamus was measured by immunohistochemistry. It was shown that liver protection could restore the elevated blood lipids and damaged liver function to normal in rats with fatty liver. The possible mechanism is related to increased Leptin and OB-RL expression in hypothalamus tissue [10].

Although the pathogenesis of NAFLD is still being carefully studied, there are no specific and effective western drugs for the treatment of NAFLD. Traditional Chinese medicine (TCM) has gained increasing attention in providing drug candidates for NAFLD treatment. Numerous experimental and clinical studies have also reported that TCM has unique efficacy in the treatment of NAFLD. The Chinese literature on clinical studies of fatty liver in the past 10 years was searched through CNKI-Chinese journal database, and the prescriptions and drugs in 102 papers that met the requirements were classified and counted, and frequency analysis was performed with statistical software. Results: a total of 100 Chinese compound prescriptions with 173 medicinal flavours were collected, and the drugs used more frequently were Crapeae Folium, Radix Salviae, Alissma Orientale, Cassiae Semen, Radix Bupleuri, etc. [11]. Based on our current research on the mechanism of Radix Bupleuri single herbal medicine, Radix Bupleuri was selected as the discussion point of this paper.

Materials and methods

Screening of the main active ingredients and their targets of action
All chemical constituents of Radix Bupleuri were collected from the Traditional Chinese Medicine Systematic Pharmacology (TCMSP) database (https://old.tcmsp.e.com/). According to the method of the database and the latest literature recommendations, the chemical constituents with oral bioavailability (OB) ≥ 20% and drug-like (DL) properties ≥ 0.1 were selected as active substances in this study [12–14].

NAFLD disease target collection
We searched for disease targets from the DisGenet database (https://www.disgenet.org/home/) using the search “NAFLD”. Other disease target databases can also be searched by searching the Online Mendelian Inheritance in Man (OMIM; https://omim.org/) database, the PharmGkb platform (https://www.pharmgkb.org/), the Therapeutic Target Database (TTD; http://db.idrblab.net/ ttd/) and DrugBank database (https://www.drugbank.ca/) to specify NAFLD disease targets [15].

Matching and protein protein interaction (PPI) network construction between Radix Bupleuri and liver fibrosis targets
The screening targets of active components and NAFLD-related protein targets of Radix Bupleuri’s actions were imported into the Venn diagram webtool (http://bioinformatics.psb.ugent.be/webtools/Venn/) and the intersection was said to be the target of Radix Bupleuri for the treatment of NAFLD disease. Then the interactions between NAFLD active ingredients and their target proteins were studied using the interaction database platform STRING v.11.0 (https://string-db.org/) to search for drug-disease crossover target genes PPI networks and finally poured into Cytoscape v3.9.2 mapping optimization [16].

Chinese herbal-component-target network construction
The Radix Bupleuri herbal-component-target relationships and the list were entered into Cytoscape software for mapping to determine the parameters such as network nodes, edges, and length values.

Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis
The list of common targets was imported into the DAVID (https://www.david.ncifcrf.gov) database, GO analysis was performed, and potential NAFLD-related genes targeted by the active ingredients of Radix Bupleuri were identified using KEGG (https://www.genome.jp/kegg/kegg2.html) pathway enrichment analysis [17–18].

Results
Screening and targeting of the main active ingredients of Radix Bupleuri
A total of 12 active ingredients of Radix Bupleuri were obtained after screening by TCMSP database method. A total of 20 drug targets were predicted by two-dimensional and three-dimensional structure comparison (Table 1).

NAFLD disease target screening
Removing the duplicate values, we get a total of 1,060 NAFLD-related targets were searched in the DisGenet database and Treatment Target Database and DrugBank Database database.

Common target screening and interactions network construction
By mapping Venn images, 75 intersecting targets between Radix Bupleuri and NAFLD could be obtained (Figure 1). Then, a PPI network could be built for the 75 targets (Figure 2). In this network, each node represents a target, and each edge represents a target-target interaction relationship. In addition, the three nodes with the highest degree values are Tumor Protein (TP53) (degree 68), TNF (degree 54) and signal transducer and activator of transcription 1 (STAT1) (degree 36) (Figure 3). The values of the points can indicate the influence of a node on the system to which it belongs.

Herbal-component-target network
The network was constructed for the main active ingredients and their corresponding targets in Radix Bupleuri (Figure 4). The red nodes note-Chai Hu (CH) nodes, represent the single Chinese herbal medicine of Radix Bupleuri, green nodes represent the main activities (in order of abbreviation CH1-CH12), and blue nodes represent the corresponding targets. The three main activities with the highest degree of activity in this network are kaempferol (degree 60), isorhamnetin (degree 29), and stigmasterol (degree 26) (Figure 5).

GO analysis and KEGG pathway analysis
DAVIDv6.8 can be used to analyze and visualize the enrichment of GO and KEGG pathways. The results of the GO enrichment analysis were plotted in bar graphs for the entries in biological process (BP), cellular component (CC), and molecular function (MF); the smaller the value, the more significant the enrichment (Figure 6). Important terms for the biological processes are positive regulation of blood vessel endothelial cell migration, positive regulation of transcription from Ribonucleic Acid (RNA) polymerase II promoter, and regulation of ossification. Transcription from RNA polymerase II promoter, regulation of ossification, etc. Essential terms for cellular components are cytoplasm, basal dendrite, neuron reflex, etc. The essential terms of the cellular components were cytoplasm, basal dendrite, neuron projection, etc. The critical parts of the molecular functions were enzyme binding, sequence-specific DNA binding, steroid hormone receptor activity, etc. More than 30 signal transduction channels were obtained from KEGG channel analysis, shown in Figure 7. The main information channels are Fluid shear stress and atherosclerosis information channel, Cancer information channel, Advanced glycation end-receptor of advanced glycation (AGE-RAGE) information channel, interleukin 17 (IL-17) information channel Hepatitis B information channel, Toxoplasmosis information channel, Relaxin signalling pathway, TNF signalling pathway, etc. The diagram of the main signalling pathways is shown in Figure 8.

| Ingredients      | Mol ID    | Gene names          | Molecular weight | OB (%) | DL  |
|------------------|-----------|---------------------|------------------|--------|-----|
| Radix Bupleuri   | MOL001645 | Linoleyl acetate    | 308.56           | 42.1   | 0.2 |
| Radix Bupleuri   | MOL002776 | Baicalin            | 446.39           | 40.12  | 0.75|
| Radix Bupleuri   | MOL000449 | Stigmasterol        | 412.77           | 43.83  | 0.76|
| Radix Bupleuri   | MOL000354 |isorhamnetin         | 316.28           | 49.6   | 0.31|
| Radix Bupleuri   | MOL000422 | kaempferol          | 286.25           | 41.88  | 0.24|
| Radix Bupleuri   | MOL004598 | tetramethoxy        | 432.46           | 31.97  | 0.59|
| Radix Bupleuri   | MOL004609 | Areapillin          | 360.34           | 48.96  | 0.41|
| Radix Bupleuri   | MOL013187 | Cubebin             | 356.4            | 57.13  | 0.64|
| Radix Bupleuri   | MOL004624 | Longikaurin A       | 348.48           | 47.72  | 0.53|
| Radix Bupleuri   | MOL004628 | Octalupine          | 264.41           | 47.82  | 0.28|
| Radix Bupleuri   | MOL004644 | Sainfuran           | 286.3            | 79.91  | 0.23|
| Radix Bupleuri   | MOL004648 | Trozerutin          | 346.56           | 31.6   | 0.28|
| Radix Bupleuri   | MOL004653 | ( + )-Anomalain     | 426.5            | 46.06  | 0.66|
| Radix Bupleuri   | MOL004702 | saikosaponin c qt   | 472.78           | 30.5   | 0.63|
| Radix Bupleuri   | MOL004718 | α-spinasterol       | 412.77           | 42.98  | 0.76|
| Radix Bupleuri   | MOL000490 | petunidin           | 317.29           | 30.05  | 0.18|
| Radix Bupleuri   | MOL000998 | quercetin           | 781.1            | 20.45  | 0.13|
| Radix Bupleuri   | MOL004623 | NOS2                | 232.3            | 21.36  | 0.11|
| Radix Bupleuri   | MOL004657 | ACHE                | 220.39           | 29.06  | 0.13|
| Radix Bupleuri   | MOL001600 | ADHE                | 204.39           | 29.47  | 0.12|

OB, oral bioavailability; DL, drug-like.

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Figure 1 Venn diagram of intersection of Radix Bupleuri and NAFLD gene targets. NAFLD, nonalcoholic fatty liver disease.

Figure 2 PPI network diagram. PPI, protein protein interaction.
Figure 3 Analysis chart of the common target of Radix Bupleuri-NAFLD. NAFLD, nonalcoholic fatty liver disease; TPS3, Tumor Protein; TNF, tumor necrosis factor; STAT1, signal transducer and activator of transcription 1.

Figure 4 Radix Bupleuri-component-target analysis network diagram
Figure 5 Radix Bupleuri component degree value analysis bar chart

Figure 6 Top 10 bar charts of Radix Bupleuri BP, CC, MF analysis. BP, biological process; CC, cellular component; MF, molecular function; P38, protein 38; RNA, Ribonucleic Acid; GO, gene ontology.
Discussion

Radix Bupleuri is the medicinal part of the dried rhizome of the weedy plant Bupleurum of the family Umbelliferae. It is also known as ground fumigation, mountain vegetable, fire mushroom herb, and wood grass, bitter in taste, slightly cold in nature, and belongs to the liver and gall bladder meridian. It has the special function of harmonizing the table and promoting Yang in the liver. It treats flu, cold and fever, malaria, liver stagnation, chest and rib distension. Some scientific studies have confirmed that the Radix Bupleuri agent has hepatoprotective and choleretic, hypolipidemic function and anti-atherosclerosis, and also has anti-allergic, anti-inflammatory, analgesic, and therapeutic effects in hepatitis, fatty liver, and hyperlipidemia [19]. In addition, some studies have shown the potential of Radix Bupleuri cypress for the treatment of NAFLD [20]. Therefore, this paper used a network pharmacological approach to investigate the possible mechanism of the treatment of NAFLD by Radix Bupleuri. It has a complex composition. In this study, the database was used to screen the explored active ingredients of Radix Bupleuri. A total of 12 ingredients were extracted that were easily available for human use. In subsequent trials, 175 functional targets of these components were also identified, in addition to 75 potential targets for the treatment of NAFLD.

In the PPI analysis, the important targets were TP53 (degree 68), TNF (degree 54) and STAT1 (degree 36), an oncogene located on human chromosome 17, named for a protein with a molecular weight of 53 kDa (P53 protein). The message-transfer and token activator 1 (STAT1) is a key protein linking receptor binding in the bacterial cell wall to information transfer between effectors. STAT1 in the cytoplasm, upon extracellular stimulation, can produce phosphorylation effects and polymerize to produce homo- or heterodimers, which then enter the nucleus to promote target gene transcription. It has been reported that STAT1 activation can induce apoptosis in cancer cells and thus have anti-tumor effects [21]. The mechanism of NAFLD is that TNF plays an important role. TNF is also a highly efficient bacterial factor formed by macrophages and immune cells, which can contribute to the development of inflammatory...
Figure 8 Demonstration of major signaling pathways. IL-1, interleukin 1; JNK, Jun N-terminal kinase; P38, protein38; MAPK, Mitogen-activated protein kinase; NF-κB, Nuclear factor kappa B; TNF-α, tumor necrosis factor α; IL-1R, Interleukin 1 receptor.

chemical reactions, etc. [22]. The development of this inflammatory response will cause excessive liver damage and produce large amounts of excess extracellular matrix, leading to lipid deposition in NAFLD [23]. Therefore, Radix Bupleuri may regulate the effect of NAFLD by improving the inflammatory response in the liver through its corresponding targets. Notably, kaempferol had the highest degree value among the active ingredients in Radix Bupleuri, suggesting that kaempferol may play a major role in the improvement of NAFLD. Kaempferol has significant anti-inflammatory, antioxidant, anti-fibrotic, and hepatoprotective effects [24]. Previous studies have shown that kaempferol can modulate the regulation of oxidative stress to improve NAFLD induced by high cholesterol food in zebrafish larval disease and the Human hepatocellular carcinoma gene 2 (HepG2) cell model [25]. In addition, kaempferol plays an important role in attenuating lipid accumulation and preventing NAFLD [26].

The results of GO analysis also confirmed the important role of shiborneol in the directional control of vascular endothelial cell migration, directional control of RNA endonuclease II promoter transcription, regulation of osification, and DNA binding at cytoplasmic, basal dendritic, and neuronal-specific sequences, and steroid hormone receptor activity. The results of the KEGG pathway analysis suggest that the mechanism of action of Radix Bupleuri cypress against NAFLD is related to the information pathway of Fluid shear stress and atherosclerosis signalling channel. The results of the KEGG pathway analysis showed that the mechanism of the effect of Radix Bupleuri on NAFLD was related to the information pathways of fluid shear stress and atherosclerosis signalling channels. This channel may be involved in the control of Jun N-terminal kinase (JNK), protein 38 (P38), tumor necrosis factor receptor 1 (TNFR1), Nuclear Factor erythroid 2-Related Factor 2 (NRF2) and other nodes in this channel. This channel also controls the regulation of multicycle hormones such as Interleukin 1 receptor (IL-1R) and is associated with Mitogen-activated protein kinase (MAPK), Apoptosis, and Nuclear factor kappa B (NF-κB) signalling channels. These cytokines and channels play a major role in NAFLD and its associated inflammatory response [27–28]. In conclusion, the herbal medicine Radix Bupleuri has the prospect of effectively controlling NAFLD. Its specific mechanism can be said to alleviate the hepatitis response and reduce hepatic fatty lipid deposition. The most important herbal medicine and the active ingredient that plays the main role in the control effect may be kaempferol.

In this paper, the fundamental theories and methods of gene network pharmacology have been used to explore the effective active ingredients, essential targets, mechanism of action, and possible pathways for preventing and alleviating NAFLD. The main active ingredient, kaempferol, can also play a significant role in Radix Bupleuri. Therefore, Radix Bupleuri may be expected to be a single herbal medicine to improve and prevent NAFLD. It has become essential to find and develop relevant herbal and chemical drugs for the treatment of NAFLD. Also, understanding its pathogenesis helps researchers to target their drugs. It is believed that humanity will overcome this challenge under the guidance of TCM theory and based on modern clinical pharmacological techniques. With pharmacological approaches, humanity will overcome this challenge.
However, given the network pharmacology and database limitations, the mechanism of action of the single component of the herbal medicine cannot be fully demonstrated yet, and the subsequent animal and clinical experiments are still needed to observe and validate.

References

1. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA. 2015;313(22):2263–2273. https://doi.org/10.1001/jama.2015.5370
2. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015;62(1 Suppl):S47–S64. https://doi.org/10.1016/j.jhep.2014.12.012
3. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. J Hepatol. 2016;67(4):862–873. https://doi.org/10.1016/j.jhep.2017.06.003
4. Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28(4):370–379. https://doi.org/10.1055/s-0028-1091981
5. Muzurovíc E, Mikhailidis DP, Mantzoros C. Nonalcoholic fatty liver disease, insulin resistance, metabolic syndrome and their association with vascular risk. Metabolism. 2021;119:154770. https://doi.org/10.1016/j.metabol.2021.154770
6. Day CP, James OF. Steatohepatitis: a tale of two “hits”? Gastroenterology. 1998;114(4):842–845. https://doi.org/10.1016/s0016-5085(98)70599-2
7. Lee O, Bruce WR, Dong Q, Bruce J, Mehta R, O’Brien PJ. Fructose and carboyl metabolites as endogenous toxins. Chem Biol Interact. 2009;178(1–3):332–339. https://doi.org/10.1016/j.ceb.2008.10.011
8. Fabbriini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology. 2008;134(2):424–431. https://doi.org/10.1053/gastro.2007.11.038
9. Wang QJ, Li J, Zou YH, et al. The establishment of nonalcoholic steatohepatitis animal model. Acta Univ Med Anhui. 2006;41(1):61–63. (Chinese) https://doi.org/10.3969/j.issn.1000-1492.2006.01.020
10. Yang L, Gu KB, Zhang Z, et al. Effects of Xiaozhi Hugan Decoction on the recovery of blood lipid and hepatic function and on the expression of leptin and its receptor in the hypothalamus of rats with fatty liver. J Guangzhou Univ Tradit Chin Med. 2005;22(6):458–461. (Chinese) http://dx.chinaol.cn.10.3969/j.issn.1000-3213.2005.06.010
11. Liu MH, Wang SC, Zhang LH, Jia HT, Zhao WX. Analysis of the pattern of Chinese medicine evidence and medication use in fatty liver in the past 10 years. Chin Arch Tradit Chin Med. 2016;34(12):2852–2855. (Chinese) https://doi.org/10.13193/j.jis.1673-7717.2016.12.009
12. Guo W, Huang J, Wang N, et al. Integrating network pharmacology and pharmacological evaluation for deciphering the action mechanism of herbal formula Zuojin pill in suppressing hepatocellular carcinoma. Front Pharmacol. 2019;10:1185. https://doi.org/10.3389/fphar.2019.01185
13. Liu P, Xu HC, Shi YC, Deng L, Chen XY. Potential molecular mechanisms of plantain in the treatment of gout and hyperuricemia based on network pharmacology. Evid Based Complement Alternat Med. 2020;2020:3023127. https://doi.org/10.1155/2020/3023127
14. Chen Z, Lin T, Liao X, et al. Network pharmacology based research into the effect and mechanism of Yinchenhao Decoction against Cholangiocarcinoma. Chin Med. 2021;16(1):13. https://doi.org/10.1186/s13020-021-00423-4
15. Piñero J, Ramírez-Anguita JM, Sáuch-Pitarjch J, et al. The DisGeNET knowledge platform for disease genomics: 2019 update. Nucleic Acids Res. 2020;48(D1):D845–D855. https://doi.org/10.1093/nar/gkz1021
16. Achúcarro A, Avgoustidis A, López-Eiguren A, Martíns CJAP, Urestilla J. Cosmological evolution of semicircular string networks. Philos Trans A Math Phys Eng Sci. 2019;377(2161):20190004. https://doi.org/10.1098/rsta.2019.0004
17. Kanchisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017;45(D1):D353–D361. https://doi.org/10.1093/nar/gkw1092
18. Chen L, Zhang YH, Wang SP, Zhang YH, Huang T, Cai YD. Prediction and analysis of essential genes using the enrichments of gene ontology and KEGG pathways. Plazsa One. 2017;12(9):e0184129. https://doi.org/10.1371/journal.pone.0184129
19. Liang YJ, Zhang YP, Deng YJ, et al. Chaihu-Shugan-San Decoction modulates intestinal microbe dysbiosis and alleviates chronic metabolic inflammation in NAFLD rats via the NLRP3 inflammasome pathway. Evitl Based Complement Alternat Med. 2018;2018:9390786. https://doi.org/10.1155/2018/9390786
20. Nie H, Deng Y, Zheng CY, et al. A network pharmacology-based approach to explore the effects of Chaihu Shugan powder on a nonalcoholic fatty liver rat model through nuclear receptors. J Cell Mol Med. 2020;24(9):5168–5184. https://doi.org/10.1111/jcmm.15166
21. Martí-Rodrigo A, Alegre F, Moragrega AB, et al. Rilpivirine attenuates liver fibrosis through selective STAT1-mediated apoptosis in hepatic stellate cells. Gut. 2020;69(5):920–932. https://doi.org/10.1136/gutjnl-2019-318372
22. Ezquerra S, Mocha F, Frühbeck G, et al. Gherelin reduces TNF-α-induced human hepatocyte apoptosis, autophagy, and pyroptosis role in obesity-associated NAFLD. J Clin Endocrinol Metab. 2019;104(1):21–37. https://doi.org/10.1210/jc.2018-01171
23. Verhoeven F, Well-Verhoeven D, Prati C, Martino DV, Thevenot T, Wendling D. Safety of TNF inhibitors in rheumatic disease in case of NAFLD and cirrhosis. Semin Arthritis Rheum. 2020;50(4):544–548. https://doi.org/10.1016/j.semarthrit.2020.03.013
24. Tie FF, Ding J, Hu N, Dong Q, Chen Z, Wang HL. Kaempferol and kaempferide attenuate oleic acid-induced lipid accumulation and oxidative stress in HepG2 cells. Int J Mol Sci. 2021;22(16):8847. https://doi.org/10.3390/ijms22168847
25. Deng Y, Ma J, Weng X, et al. Kaempferol-3-O-glucuronide ameliorated non-alcoholic steatohepatitis in high-cholesterol-diet-induced larval zebrafish and HepG2 cell models via regulating oxidation stress. Life (Basel). 2021;11(5):445. https://doi.org/10.3390/life11050445
26. Lu YF, Shao MM, Xiang HJ, Zheng PY, Wu T, Ji G. Integrative transcriptomics and metabolomics explore the mechanism of kaempferol on improving nonalcoholic steatohepatitis. Food Funct. 2020;11(11):10058–10069. https://doi.org/10.1039/d0fo0123g
27. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signal pathways: insights into insulin action. Nat Rev Mol Cell Biol. 2006;7(2):85–96. https://doi.org/10.1038/nrm1837
28. Manco M. Insulin resistance and NAFLD: a dangerous liaison beyond the Genetics. Children (Basel). 2017;4(8):74. https://doi.org/10.3390/children4080074