Bioinformatics-based Prediction of Schaftoside Remission in Liver Disease with Cholestasis and Steatosis

Yueping ZHI, Kefeng ZHANG, Ya GAO, Bo LI*, Houkang CAO*

Key Laboratory of Pharmacology for Prevention and Treatment of High Incidence Diseases in Guangxi Higher Education Institutions, Guilin Medical University, Guilin 541199, China

Abstract [**Objectives**] To explore the target and mechanism of Schaftoside on cholestasis and steatosis based on network pharmacology and molecular docking. [**Methods**] The targets of "cholestasis" and "steatosis" were predicted using databases (OMIM and GeneCards), and the key targets were obtained after screening the retrieval data. The binding relationship between Schaftoside and key targets was analyzed by molecular docking. [**Results**] There were 3 370 and 4 433 targets for "cholestasis" and "steatosis", respectively, and 1 767 overlapping genes were obtained. The results of molecular docking showed that Schaftoside had high binding energy with key targets. [**Conclusions**] Schaftoside can alleviate cholestasis and steatosis by regulating SREBP-1, CYP7, PPAR-gamma and other key targets to protect liver.

Key words Cholestasis, Steatosis, Schaftoside

1 Introduction

Schaftoside, as a flavonoid carbon compound, exists in many plants, including *Glycyrrhiza uralensis* Fisch, sugarcane, *Isodon serra* (Maximowicz) Kudo, *Artemisia argyi* Levl. et Vant., *Arisaema erubescens* (Wall.) Schott, *etc*[1]. Modern pharmacological studies have revealed multiple pharmacological activities of Schaftoside, including anti-inflammatory[2-3], inhibition of gallbladder, bladder and kidney stone formation[4], protection against acetaminophen-induced liver injury[5] and inhibition of liver lipid accumulation[6]. Although Schaftoside has a variety of pharmacological effects, its alleviation effects and mechanisms on cholestasis and steatosis are still not clear. Cholestasis is a pathological condition in which bile flow is blocked inside and outside the liver, resulting in bile accumulation inside and outside the liver cells, which may be associated with a variety of liver diseases[7]. Steatosis involves the abnormal accumulation of intracellular fat and is often associated with metabolic diseases such as nonalcoholic fatty liver disease (NAFLD)[8]. The anti-inflammatory and antioxidant properties of Schaftoside may have a positive therapeutic effect on these pathological conditions, especially in reducing inflammation and protecting the liver from further damage[9]. Therefore, Schaftoside, as the key active component in *Desmodium styracifolium*, has important scientific and clinical value in the treatment of cholestasis and steatosis. This study will further clarify the mechanism of action of Schaftoside in alleviating cholestasis and steatosis, and provide a scientific basis for the development of new therapeutic strategies.

Received: January 15, 2025 Accepted: April 6, 2025

Supported by Qingmiao Talent Funding Research Project of Guangxi Province; National College Student Innovation and Entrepreneurship Training Program (202310601031).

Yueping ZHI, master's degree candidate. * Corresponding author. Bo LI, doctoral degree, associate professor; Houkang CAO, doctoral degree, assistant researcher.

2 Methods

2.1 Software and database Software: Autodock 1.5.6 software; Chem3D; PyMOL 2.1.1 software; Discovery Studio 4.5, *etc*. Database: OMIM database (http://www.omim.org/), GeneCards database (https://www.genecards.org/), Lianchuan biological cloud platform (https://www.omicstudio.cn/tool), PubChem database (https://pubchem.ncbi.nlm.nih.gov/), UniProt database (https://www.uniprot.org/), PDB database (http://www.rcsb.org/), *etc*.

2.2 Potential target prediction The keywords "cholestasis" and "steatosis" were searched in OMIM and GeneCards databases, and the search results were sorted out to obtain the targets.

2.3 Construction of molecular docking model The structure of Schaftoside was obtained from PubChem database and transformed into Chem3D, then saved in MOL2 format. After that, the protein with ligand molecules was downloaded from the PDB database, the solvent was deleted by PyMOL 2.1.1, and the protein and component data were further hydrogenated and dehydrated by Autodock 1.5.6 and saved in pdbqt format, and docked by Vina-1.1.1. Then, PyMOL 2.1.1 and Discovery Studio 4.5 were used to visualize the results.

3 Results and analysis

3.1 Prediction and screening of disease targets By searching the disease database, 3 370 targets of "cholestasis" and 4 433 targets of "steatosis" were obtained; by taking the intersection of the Wayne diagram, 1 767 targets were obtained (Fig. 1). The obtained intersection targets are combined with literatures to screen and obtain SREBF1 (encoding protein SREBP-1), CYP7A1 (encoding protein CYP7), PPARG (encoding protein PPAR-gamma), ABCB4 (encoding protein MDR3), NR1H4 (encoding protein FXR), ABCB11 (encoding protein BSEP), NR0B2 (encoding protein SHP), FGF19 (encoding protein FGF-

19), PPARA (encoding protein PPAR-alpha), PRKAA1 (encoding protein AMPK) key targets.

3.2 Molecular docking In order to further clarify the binding effect between Schaftoside and key targets, molecular docking was carried out by using Autodock 1.5.6 software with Schaftoside as ligand and key target protein as receptor. The results showed that the binding energy of Schaftoside with key target proteins was less than −6 kcal/moL, and it had good binding activity, among which SREBP-1, CYP7, PPAR-gamma, MDR3, FXR and BSEP had better binding activity. The docking demonstration is shown in Fig. 2.

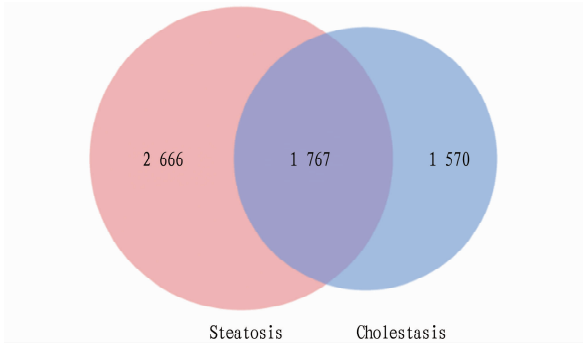

Fig. 1 Venn diagram

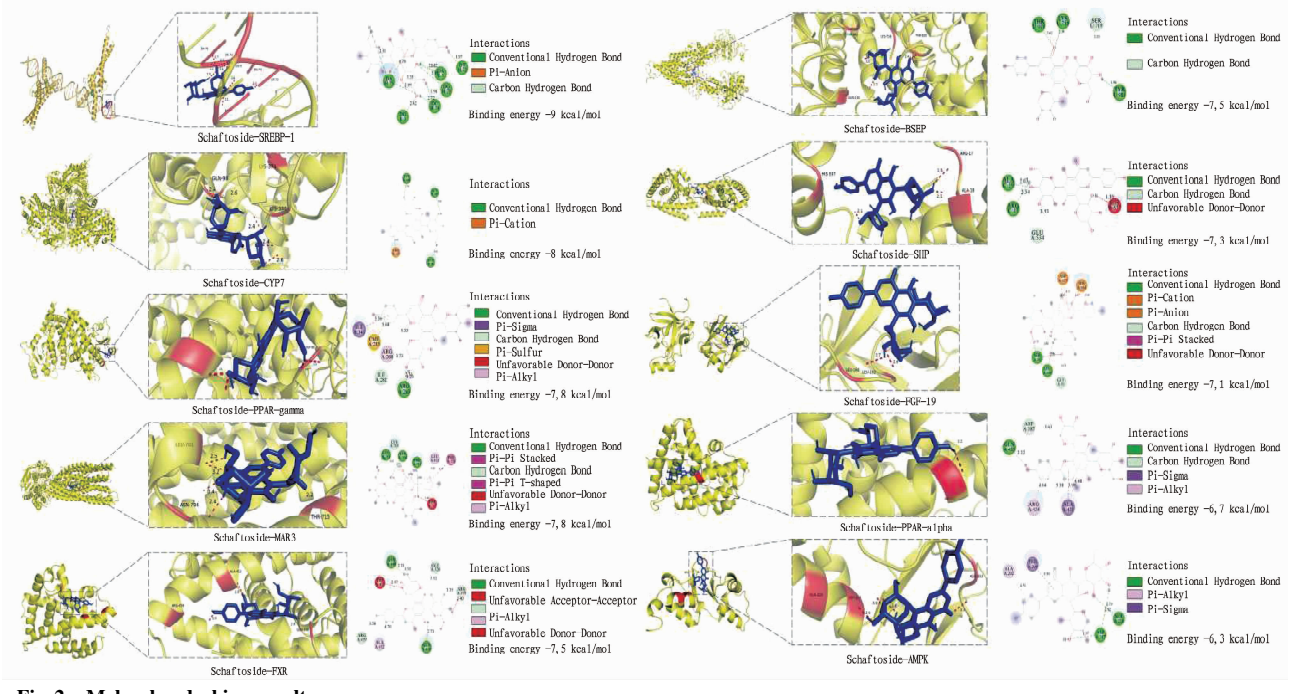

Fig. 2 Molecular docking results

4 Discussion

Cholestasis is a pathological condition in which bile accumulates in liver cells and blood due to obstruction of intrahepatic and extrahepatic bile ducts or dysfunction of liver cells, resulting in the failure of bile to reach the duodenum normally[10]. Steatosis, particularly NAFLD, is a growing health problem worldwide, characterized by the abnormal accumulation of fat in the liver, which is closely related to metabolic diseases such as metabolic syndrome, type 2 diabetes, and cardiovascular disease[11]. NAFLD is associated with the development of biliary tract diseases, including cholestasis. In patients with NAFLD, fatty deposition in liver tissue not only causes liver cell damage, but also may lead to bile duct cell damage, manifested as cholangitis, bile duct swelling, bile duct loss and cholestasis[12-13]. The role of bile acids in NAFLD is increasingly recognized. The imbalance of bile acid metabolism in adult patients with NAFLD increases the risk of liver injury. Studies have found that the level and composition of bile acids in patients with NAFLD are significantly different from those in healthy controls, and are closely related to the severity of liver disease[14-15]. In summary, cholestasis and steatosis are interrelated in liver diseases, and they may interact with each other to affect liver health by affecting bile acid metabolism, cholesterol clearance and liver fat accumulation. Further research will help to understand the complex relationship between these two pathological conditions and provide guidance for clinical treatment.

After the database search, and combining with the relevant literature screening, we obtained key targets: SREBF1, CYP7A1, PPARG, ABCB4, NR1H4, ABCB11, NR0B2, FGF19, PPARA, and PRKAA1. Farnesoid X receptor (FXR), as a bile acid regulatory receptor, can be activated by PPARG to reduce hepatic lipid accumulation by increasing fatty acid oxidation and reducing triglyceride synthesis, indicating that PPARG has a potential therapeutic effect on cholestasis[16]. FXR is a key regulator of bile acid metabolism, which maintains bile acid homeostasis by controlling the transcription of genes related to bile acid synthesis, transformation, transport and signal transduction[17]. FXR activation can

promote the transcription of NR0B2 (SHP) in the liver, and SHP, as a gene regulator of CYP7A1 expression[18-19], can regulate the expression of CYP7A1. In addition, FXR can regulate FGF19 to selectively bind to and activate the hepatic FGF receptor 4 complex (FGFR4-β-klotho), which ultimately leads to the inhibition of the transcription of *Cyp7a1* and *Cyp8b1* genes encoding key enzymes of hepatic bile acid synthesis[17]. Among them, CYP7A1 plays an important role in cholestasis and steatosis. As a key cholesterol 7α-hydroxylase, it is responsible for the first step of cholesterol conversion to bile acid. It is the rate-limiting enzyme of bile acid biosynthesis and plays a central role in cholesterol metabolism and bile acid synthesis[20]. Once hepatic bile acid is excessive, activated FXR/SHP induces ABCB11 (BSEP) expression and increases bile acid efflux. Both BSEP and ABCB4 (MDR3) are transport proteins responsible for transporting phospholipids from hepatocytes to bile[21], so their dysfunction leads to bile acid accumulation in hepatocytes, causing cholestasis[22]. In the case of cholestasis, the activity of CYP7A1 may be affected, resulting in increased bile acid synthesis, further exacerbating cholestasis[23]. In NAFLD, the expression of CYP7A1 may be affected, affecting the metabolism of cholesterol and the synthesis of bile acids, which in turn affects the accumulation of liver fat[24]. AMPK, as a master switch regulating the homeostasis of energy metabolism *in vivo*, can regulate lipid metabolism-related genes and keep lipid metabolism and synthesis at a relatively stable level[25]. Activated AMPK can inhibit ACC activity by down-regulating the expression of SREBP-1, thereby inhibiting the synthesis of cholesterol and fatty acids, reducing hepatic lipid synthesis and improving hepatic steatosis[26]. PPARG is a nuclear receptor that regulates lipid metabolism and inflammatory response, and is involved in the regulation of fatty acid metabolism and storage[27-28]. It reduces the incidence of hepatic steatosis by activating and enhancing the expression of genes related to fatty acid oxidation. In NAFLD, PPARG transfers fat from liver tissue by regulating downstream genes, reducing the accumulation of triglycerides in cells and increasing cholesterol efflux, which inhibits steatosis of liver tissue and reduces liver cell damage[29]. PPARA is involved in peroxisomal beta oxidation, mitochondrial beta oxidation, fatty acid transport, and hepatic glucose production as a regulator of gene transcription. It plays a key role in regulating fatty acid transport and oxidation in liver cells by regulating the transcriptional activity of related genes in the nucleus[30]. Therefore, there is a complex interaction between cholestasis and steatosis. The results of molecular docking show that the above key targets have good binding activity with Schaftoside, indicating that Schaftoside has a potential regulatory effect on cholestasis and steatosis, thus alleviating cholestasis and steatosis to protect the liver.

In summary, the key targets of cholestasis and steatosis were obtained by searching and screening the disease database. As a potential hepatoprotective active component, the binding activity of Schaftoside with these key targets was further verified by molecular docking experiments, showing strong binding ability. These results preliminarily confirm that Schaftoside may play a hepatoprotective role by regulating key targets to alleviate cholestasis and steatosis. However, although molecular docking experiments provide preliminary evidence, the specific regulatory mechanism and pathway of Schaftoside still need to be clarified through further experimental studies. Future studies can focus on the direct effects of Schaftoside on key targets and its effects at the cellular and molecular levels, as well as the verification of its hepatoprotective effects and safety in animal models or clinical trials.

References

[1] ZHANG HY, GAO YN, ZHANG M, *et al*. Schaftoside improves HFpEF through regulation the autophagy-lysosome pathway by allosterically targeting CaMKII-δ[J]. Redox Biology, 2024, 78: 103424.

[2] ZHOU KC, WU JY, CHEN J, *et al*. Schaftoside ameliorates oxygen glucose deprivation-induced inflammation associated with the TLR4/Myd88/Drp1-related mitochondrial fission in BV2 microglia cells[J]. Journal of Pharmacological Sciences, 2019, 139: 15-22.

[3] YU Y, LIANG J, YUAN Z, *et al*. Bioactive compound schaftoside from *Clinacanthus nutans* attenuates acute liver injury by inhibiting ferroptosis through activation the Nrf2/GPX4 pathway[J]. Journal of Ethnopharmacology, 2024, 328: 118135.

[4] LIU M, LIU C, CHEN H, *et al*. Prevention of cholesterol gallstone disease by schaftoside in lithogenic diet-induced C57BL/6 mouse model[J]. European Journal of Pharmacology, 2017, 815: 1-9.

[5] LIU MJ, ZHANG GH, SONG M, *et al*. Activation of farnesoid X receptor by schaftoside ameliorates acetaminophen-induced hepatotoxicity by modulating oxidative stress and inflammation[J]. Antioxidants & Redox Signaling, 2020, 33: 87-116.

[6] LIU X, PAN Y, SHEN Y, *et al*. Protective effects of Abrus cantoniensis Hance on the fatty liver hemorrhagic syndrome in laying hens based on liver metabolomics and gut microbiota[J]. Frontiers in Veterinary Science, 2022, 9: 862006.

[7] FUCHS CD, SIMBRUNNER B, BAUMGARTNER M, *et al*. Bile acid metabolism and signalling in liver disease[J]. Journal of Hepatology, 2025, 82(1): 134-153.

[8] GARCIA-MATEO S, RONDINELLA D, PONZIANI FR, *et al*. Gut microbiome and metabolic dysfunction-associated steatotic liver disease: Pathogenic role and potential for therapeutics[J]. Best Practice & Research Clinical Gastroenterology, 2024, 72: 101924.

[9] LIU M, ZHANG G, WU S, *et al*. Schaftoside alleviates HFD-induced hepatic lipid accumulation in mice via upregulating farnesoid X receptor[J]. Journal of Ethnopharmacology, 2020, 255: 112776.

[10] LLEO A, LEUNG PSC, HIRSCHFIELD GM, *et al*. The pathogenesis of primary biliary cholangitis: A comprehensive review[J]. Seminars in Liver Disease, 2020, 40: 34-48.

[11] RONG F, MAI Y, SHOU L, *et al*. Analysis of the association between non-alcoholic fatty liver disease and mortality in United States adults [J]. Frontiers in Nutrition, 2024, 11: 1502671.

[12] LONGO M, CROSIGNANI A, BATTEZZATI PM, *et al*. Hyperlipidaemic state and cardiovascular risk in primary biliary cirrhosis[J]. Gut, 2002, 51: 265-269.

[13] WALKER DI, JURAN BD, CHEUNG AC, *et al*. High-resolution exposomics and metabolomics reveals specific associations in cholestatic liver diseases[J]. Hepatology Communications, 2022, 6: 965-979.

[14] GAO Y, LI L, LI B, *et al*. Response rate and impact on lipid profiles of obeticholic acid treatment for patients with primary biliary cholangitis: A meta-analysis[J]. Canadian Journal of Gastroenterology & Hepatology, 2021, 2021: 8829510.

[15] SERGI CM. NAFLD (MASLD)/NASH (MASH): Does it bother to label at all? A comprehensive narrative review[J]. International Journal of Molecular Sciences, 2024, 25(15):8462.

(To page 61)

the prognosis of patients with post-intensive care syndrome[J]. Chinese Journal of Critical Care Nursing, 2021, 2(3): 216 – 220. (in Chinese).
[7] Chinese society of parenteral and enteral nutrition. Clinical application guidelines for parenteral and enteral nutrition in adult patients in China (2023 edition)[J]. Chinese Medical Journal, 2023, 103(13): 946 – 974. (in Chinese).
[8] TANG B, CHEN W, JIANG L, *et al*. Expert consensus on post-intensive care management[J]. Chinese Journal of Internal Medicine, 2023, 62(5): 480 – 493. (in Chinese).
[9] YUAN XF, ZHANG Y, ZHANG Y, *et al*. Reliability and validity of the brief assessment of impaired cognition (Chinese version) for stroke patients[J]. Applied Neuropsychology, 2021, 30(1): 27 – 33.
[10] SUN Y, LI L, YANG B, *et al*. Development and evaluation of a predictive model for post-intensive care syndrome in families of patients with hemorrhagic stroke[J]. Chinese Nursing Management, 2022, 22(10): 1491 – 1497. (in Chinese).
[11] YANG J, TANG L, ZOU S, *et al*. Construction and validation of a predictive model for prolonged hospitalization in elderly patients with stable coronary heart disease[J]. Nursing Research, 2021, 35(23): 4163 – 4168.
[12] RENNER C, JEITZINER MM, ALBERT M, *et al*. Guideline on multimodal rehabilitation for patients with post-intensive care syndrome[J]. Critical Care, 2023, 27(1): 301.
[13] ZHANG X, JIANG Z, LIU Q, *et al*. Analysis of fatigue trajectories and influencing factors in patients with post-intensive care syndrome[J]. Chinese Nursing Journal, 2022, 57(3): 272 – 278. (in Chinese).
[14] DUNN H, BALAS MC, HETLAND B, *et al*. Post-intensive care syndrome: A review for the primary care NP[J]. Nurse Practitioner, 2022, 47(11): 15 – 22.
[15] PAUL N, WEISS B. Post-intensive care syndrome after critical illness: An imperative for effective prevention[J]. Journal of Clinical Medicine, 2022, 11(20): 6203.
[16] MENG M, GUAN Y, GUO L, *et al*. Construction and validation of a risk prediction model for post-intensive care syndrome in cardiovascular surgery patients[J]. Chinese Nursing Journal, 2022, 57(12): 1486 – 1494. (in Chinese).
[17] MULKEY MA, BEACHAM P, MCCORMICK MA, *et al*. Minimizing post-intensive care syndrome to improve outcomes for ICU survivors[J]. Critical Care Nurse, 2022, 42(4): 68 – 73.
[18] KIM SJ, PARK K, KIM K. Post-intensive care syndrome and health-related quality of life in long-term survivors of the intensive care unit[J]. Australian Critical Care, 2023, 36(4): 477 – 484.
[19] MA H, HALINA, KUANG L, *et al*. A systematic review of the effects of virtual reality technology on interventions for ICU post-intensive care syndrome[J]. Chinese Nursing Management, 2022, 22(11): 1706 – 1712. (in Chinese).
[20] CAREL D, PANtet O, RAMELET AS, *et al*. Post-intensive care syndrome (PICS): Physical, cognitive, and mental health outcomes 6 months to 7 years after a major burn injury: A cross-sectional study[J]. Burns, 2023, 49(1): 26 – 33.
[21] DONG Z, YU B, ZHANG Q, *et al*. Early rehabilitation therapy is beneficial for patients with prolonged mechanical ventilation after coronary artery bypass surgery[J]. International Heart Journal, 2016, 57(2): 241 – 246.
[22] TELIAS I, WILCOX ME. Sleep and circadian rhythm in critical illness[J]. Critical Care, 2019, 23(1): 82.
[23] RAJA SN, CARR DB, COHEN M, *et al*. The revised international association for the study of pain definition of pain: Concepts, challenges, and compromises[J]. Pain, 2020, 161(9): 1976 – 1982.

(From page 57)

[16] SAVLA SR, BHATT LK. Exploration of anti-atherosclerotic activity of 1,8-cineole through network pharmacology, molecular docking, and *in vivo* efficacy studies in high-fat-diet-induced atherosclerosis in hamsters[J]. Molecular Diversity, 2024. DOI:10.1007/s11030-024-11015-3.
[17] FIORUCCI S, URBANI G, DI GIORGIO C, *et al*. Bile acids-based therapies for primary sclerosing cholangitis: Current landscape and future developments[J]. Cells, 2024, 13(19): 1650.
[18] SHI XD, SONG W, JIANG BY, *et al*. Highland barley alleviates high-fat diet-induced obesity and liver injury through the IRS2/PI3K/AKT signaling pathway in rats[J]. Nutrients, 2024,16(20): 3518.
[19] HERNÁNDEZ-MARTÍN M, GARCIMARTÍN A, BOCANEGRA A, *et al*. Silicon-enriched meat ameliorates diabetic dyslipidemia by improving cholesterol, bile acid metabolism and ileal barrier integrity in rats with late-stage type 2 diabetes[J]. International Journal of Molecular Sciences, 2024, 25(21): 11405.
[20] ZHANG W, WANG JH, YANG LY, *et al*. Hepatic SIRT6 protects against cholestatic liver disease primarily via inhibiting bile acid synthesis[J]. Journal of Biomedical Research, 2024, 25: 1 – 17.
[21] CAO LL, DONG Y, XU ZQ, *et al*. Clinical characteristics of ABCB4 gene variant-associated cholestatic liver disease in adults[J]. Zhonghua Gan Zang Bing Za Zhi, 2024, 32: 929 – 934.
[22] AGARWAL S, RAJVANSHI N, GOYAL JP, *et al*. A novel variant (p. Leu1054Arg) in ABCB11 presenting with progressive familial intrahepatic cholestasis (PFIC) with congenital hypothyroidism[J]. Indian Journal of Pediatrics, 2024, 91: 1194.
[23] HASSAN M, SALEM MB, HAMMAM OA, *et al*. Protective effects of cilostazol via the HNF1α/FXR signalling pathway and anti-apoptotic mechanisms in a rat model of estrogen-induced intrahepatic cholestasis[J]. Scientific Reports, 2024, 14: 22751.
[24] YU CAI LIM M, KIAT HO H. Pharmacological modulation of cholesterol 7α-hydroxylase (CYP7A1) as a therapeutic strategy for hypercholesterolemia[J]. Biochemical Pharmacology, 2024, 220: 115985.
[25] LU J, SHATAER D, YAN HZ, *et al*. Probiotics and non-alcoholic fatty liver disease: Unveiling the mechanisms of *Lactobacillus plantarum* and *Bifidobacterium bifidum* in modulating lipid metabolism, inflammation, and intestinal barrier integrity[J]. Foods, 2024, 13(18): 2992.
[26] YAN Q, LI CY, LI JF, *et al*. Protective effects of isostrictiniin against high-fat, high-sugar diet-induced steatosis in MASLD mice via regulation of the AMPK/SREBP-1c/ACC pathway[J]. Nutrients, 2024, 16(22): 3876.
[27] GONG M, YUAN Y, SHI X, *et al*. Compound Oolong tea ameliorates lipid accumulation through AMPK-PPAR pathway of hepatic lipid metabolism and modulates gut microbiota in HFD induced mice[J]. Food Research International, 2024, 196: 115041.
[28] YANG J, MA W, MEI Q, *et al*. Protective effect of Fuzi Lizhong Decoction against non-alcoholic fatty liver disease via anti-inflammatory response through regulating p53 and PPARG signaling[J]. Biological & Pharmaceutical Bulletin, 2020, 43: 1626 – 1633.
[29] CUI K, ZHANG LC, LA XQ, *et al*. Ferulic acid and P-coumaric acid synergistically attenuate non-alcoholic fatty liver disease through HDAC1/PPARG-Mediated free fatty acid uptake[J]. International Journal of Molecular Sciences, 2022, 23(23): 15297.
[30] NIU QQ, XI YT, ZHANG CR, *et al*. Potential mechanism of perillaldehyde in the treatment of nonalcoholic fatty liver disease based on network pharmacology and molecular docking[J]. European Journal of Pharmacology, 2024, 985: 177092.