Acupuncture and Herbal Medicine

Research progress on the hepatoprotective effect, pharmacokinetic properties, and hepatotoxicity of geniposide

Songyuan Tang[1], Guangli Yan[1], Ling Kong[1], Hui Sun[1,*], Chang Liu[1], Ying Han[1], Xijun Wang[1,2,*]

[1]State Key Laboratory of Integration and Innovation of Classical Formula and Modern Chinese Medicines, National Chinmedomics Research Center, National TCM Key Laboratory of Serum Pharmacochemistry, Metabolomics Laboratory, Department of Pharmaceutical Analysis, Heilongjiang University of Chinese Medicine, Harbin, China; [2]State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Taipa, Macau.

Abstract

Liver disease (LD) is a global health problem caused by multiple factors. At present, there are still obvious problems with limited efficacy and strong side effects of drugs used in the clinical treatment of LD. Therefore, it is of great significance to search for effective hepatoprotective drugs from natural products. Geniposide (GS) is a cyclic ether terpenoid compound and a key component in the traditional Chinese medicine *Gardenia jasminoides*. It has a significant inhibitory effect on LD. However, there is currently no literature systematically analyzing its mechanism of action. To adapt to the environment of new drug research and the need for precision medication, this article summarizes the pathways and possible mechanisms of action discovered by GS in the treatment of LD, based on recent research literature: regulating bile stasis, antioxidant and anti-apoptosis, improving amino acid metabolism, improving energy metabolism, regulating lipid metabolism, anti-inflammatory and analgesic effects, etc. It also summarizes the pharmacokinetics of GS *in vivo* and discusses the liver toxicity of GS that is positively correlated with dosage. In addition, the existing problems in current research and possible future development directions were also discussed, to lay the foundation for the clinical development of natural product GS.

Keywords: Geniposide, Hepatotoxicity, Liver disease, Molecular mechanism, Pharmacokinetics

Graphical abstract: https://links.lww.com/AHM/A178

Introduction

Liver disease (LD) is a major global health problem caused by multiple factors, including (1) exogenous factors, such as alcohol abuse causing alcoholic liver disease (ALD)[1–2], drug-induced liver injury (DILI)[3–4], viral hepatitis (VH)[5–6], etc, and (2) endogenous metabolic abnormalities, such as non-alcoholic fatty liver disease (NAFLD)[7–8], metabolically abnormal fatty liver disease (MAFLD)[9], and autoimmune hepatitis (AIH)[10–11], among others. According to the World Health Organization (WHO), LD causes approximately 2 million deaths annually, of which about 50% are related to cirrhosis or hepatocellular carcinoma[12]. The pathological process of LD usually proceeds in three stages—the acute injury phase, the chronic inflammation phase, and the fibrosis phase—and can eventually develop into irreversible cirrhosis or even liver failure[13]. At present, commonly used hepatoprotective medicine in clinical practice mainly include bile acid modulators (such as ursodeoxycholic acid), insulin sensitizers (such as metformin and semaglutide), antioxidants (such as glutathione), lipid-lowering drugs (such as clopidogrel and atorvastatin), anti-inflammatory agents (such as glucocorticoids), angiotensin receptor antagonists (such as telmisartan), and so on[14]. However, these drugs do not significantly improve liver function or promote liver cell regeneration, and they have significant side effects, such as osteoporosis and muscle toxicity[15–16]. In recent years, research has indicated that some natural products have positive intervention effects on the development of diseases and minimal side effects when used as drugs or dietary supplements. However, determining the most suitable ingredient for the treatment of LDs is still a difficult problem.

Geniposide (GS) is one of the bioactive compounds in the fruit of *Gardenia jasminoides* Ellis (Fructus Gardeniae), which has been used to treat LDs and inflammation (Figure 1)[17]. Fructus Gardeniae is a component

*Corresponding author. Hui Sun, E-mail: sunhui7045@163.com; Xijun Wang, E-mail: xijunw@sina.com.

Received 10 January 2025 / Accepted 14 May 2025

How to cite this article: Tang SY, Yan GL, Kong L, Sun H, Liu C, Han Y, Wang XJ. Research progress on the hepatoprotective effect, pharmacokinetic properties, and hepatotoxicity of geniposide. Acupunct Herb Med 2025;5(2):136–146. doi: 10.1097/HM9.0000000000000160

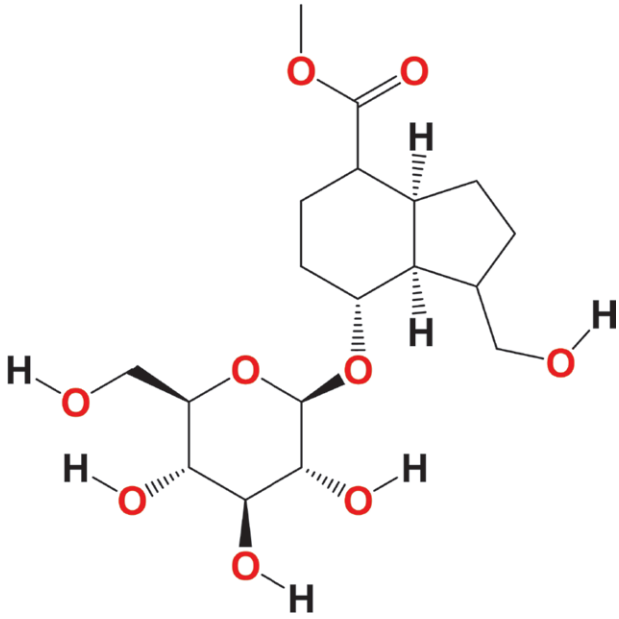

Figure 1. Chemical structural formula of GS. GS: Geniposide.

of Yinchenhao decoction, a famous prescription used in traditional Chinese medicine (TCM) to treat various LDs and jaundice[18]. Yinchenhao decoction can inhibit liver cell apoptosis and promote bile secretion and excretion, and it has the clinical effect of promoting liver regeneration and preventing liver failure[19]. Research has shown that one of the key pharmacological substances in Yinchenhao decoction that plays a crucial role in alleviating LD is GS[20–21]. However, the exact mechanism of action of GS on liver injury is still unclear, and further research is urgently needed. Currently, the efficacy and treatment concepts of TCM are receiving increasing attention from researchers around the world, but there are few systems that can clearly explain its theory and mechanism.

Multiple research studies and analyses have been conducted to characterize the pathogenesis of LD and explore the therapeutic pathways of GS for LD. Most phenotypes of LD include the accumulation of reactive oxygen species (ROS) and lipid peroxidation (LPO) in the liver, as well as bile acid stasis and inflammation[22–24]. To date, through in-depth research on the pathogenesis of LD, a series of potential therapeutic targets and pathways for LD have been widely studied, including the gut microbiota, cell apoptosis, oxidative stress, amino acid metabolism, and the tricarboxylic acid (TCA) cycle, all of which are considered to be related to the treatment of LD[25–29]. With the continuous deepening of research, GS is considered one of the most promising natural products for treating LD[30–31]. The possible molecular mechanism is shown in Figure 2. In addition, a large number of animal experiments have shown that GS not only has hepatoprotective effects but also causes significant liver toxicity when used in excess. Therefore, specific analyses of the liver toxicity of GS are necessary.

GS may exert hepatoprotective effects by activating glucagon-like peptide-1 receptor (GLP-1R)[32]: (1) inhibition of oxidative stress through nuclear factor E2-related factor 2 (Nrf2) pathway: research shows that Nrf2 is a key factor mediating the upregulation of antioxidant enzyme heme oxygenase-1 (HO-1)[33], GS can promote Nrf2 nuclear translocation and the expression of enzymes such as HO-1, inhibit ROS accumulation, and thus reduce Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and generation of inflammatory factors[34–36]; (2) inhibit apoptosis through multiple ways: ① activate protein kinase B (PKB) and protein kinase B (AKT) by increasing the level of phosphoinositide 3-kinase (PI3K), thereby inhibiting glycogen synthase kinase-3β (GSK-3β). Furthermore, it controls the AMP-activated protein kinase (AMPK) signaling pathway and mechanistic target of rapamycin (mTOR) to inhibit cell apoptosis[37–38]; ② regulating the TCA cycle through miR-144-5p: The TCA cycle is the foundation of mitochondrial energy production, and GS can regulate key enzymes IDH1 and IDH2 in the TCA cycle by controlling miR-144-5p, increasing ATP content in cells and slowing down liver cell damage[29];③ reduce Fas-mediated cell apoptosis and inhibit caspase-3 activity[28,39]; (3) inhibiting bile acid secretion by activating FXR and reducing CYP7A1 activity[29,40–41].

Based on this, this article summarizes and generalizes the possible protective mechanisms, pharmacokinetics, and liver toxicity of GS in the treatment of LD, providing theoretical support for its clinical application in the treatment of LD and new ideas for the development of TCM drugs using GS as the active ingredient.

Possible protective mechanism of GS on LD

The successful establishment of an animal model of LD is a crucial step in exploring the therapeutic mechanism of GS in the later stage. Depending on the damage mechanism, modeling methods can be mainly divided into the following categories: (1) CCl_4-induced model: CCl_4 is metabolized by CYP450 into trichloromethyl-free radicals, which cause LPO and liver cell necrosis and are suitable for studying liver oxidative stress and fibrosis mechanisms[42]; (2) ethanol-induced model: ethanol metabolites directly cause toxicity and oxidative stress, suitable for ALD[43]; (3) lipopolysaccharide (LPS) combined with D-galactosamine (D-GalN) model: an intraperitoneal injection of LPS and D-GalN induces explosive hepatitis[44]; (4) a high-fat diet or gene-deficiency model: high-fat diet (60% fat)-fed or ob/ob or db/db mice are suitable as an NAFLD model[45]; (5) a bile acid stasis model: bile duct ligation and ANIT gavage cause bile duct epithelial injury, suitable for a bile stasis liver injury model[46–47]. Accumulating evidence suggests that GS has a mitigating effect on LD both *in vitro* and *in vivo*. Currently, GS is considered a multi-target drug that can antagonize LD.

Regulating cholestasis

Regulating bile acids

When the liver is damaged, it can easily lead to bile stasis, causing secondary damage to the liver[48]. Bile acids are important components of bile and play a crucial role as signaling molecules and metabolic regulators in regulating lipid, glucose, and energy metabolism in the liver[49–50]. In the liver, taurine combines with bile acids to form taurocholic acid, which can increase the solubility of lipids and cholesterol, increase bile flow, and alleviate inflammation[51–52]. Experiments have shown that after

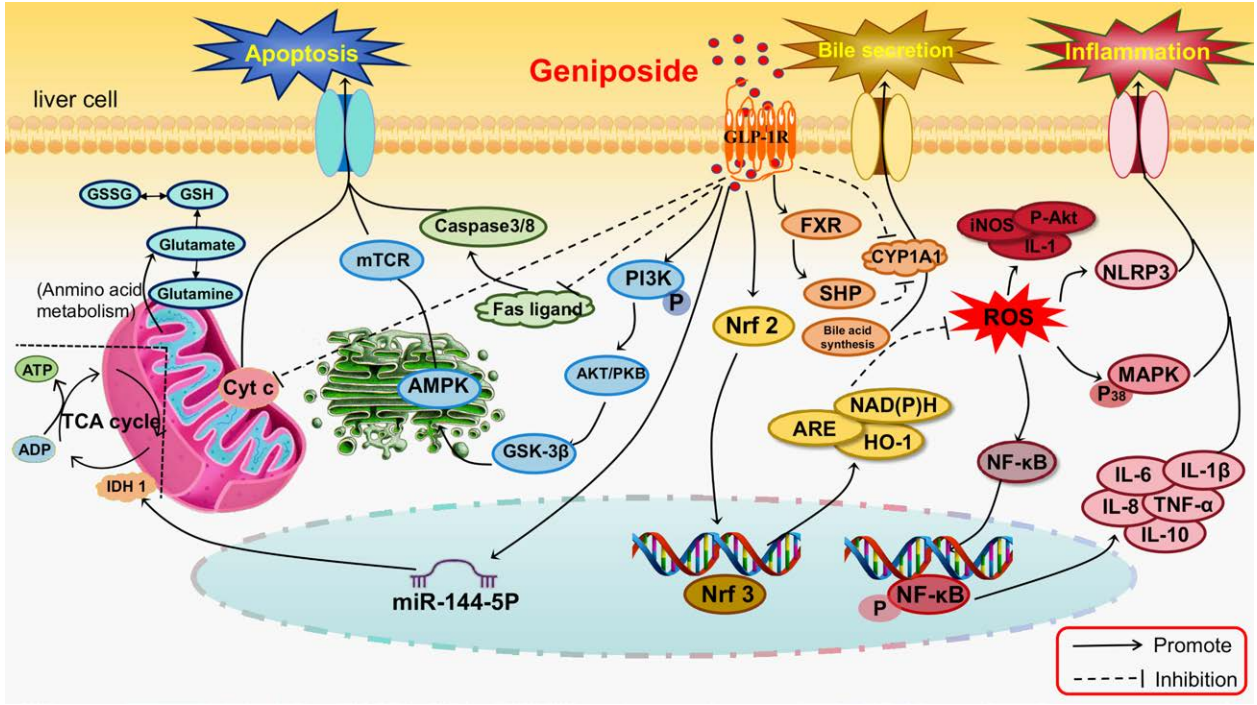

Figure 2. Possible mechanism of GS in the treatment of LD. AKT: Protein kinase B; AMPK: AMP-activated protein kinase; ARE: Antioxidant response element; FXR: Farnesol X receptor; GLP-1R: Glucagon-like peptide-1 receptor; GS: Geniposide; GSH: Glutathione; GSK-3β: Glycogen synthase kinase-3β; GSSG: Oxidized glutathione; HO-1: Heme oxygenase; IDH: Isocitrate dehydrogenase; IL: Interleukin; iNOS: Inducible nitric oxide synthase; LD: Liver disease; MAPK: Mitogen-activated protein kinase; mTOR: Mechanistic target of rapamycin; NAD(P)H: Nicotinamide adenine dinucleotide (phosphate)—reduced form; NLRP3: NLR family pyrin domain-containing 3; Nrf2: Nuclear factor E2-related factor 2; PI3K: Phosphoinositide 3-kinase; PKB: Protein kinase B; ROS: Reactive oxygen species; SHP: Small heterodimer partner; TCA: Tricarboxylic acid cycle; TNF: Tumor necrosis factor.

administering 11.25 mg/100 g GS to ALD model mice for 14 consecutive days, an increase in taurocholic acid levels can be observed in the mice, indicating that GS alleviates the decrease in taurocholic acid levels in the model mice by improving abnormal bile acid biosynthesis pathways and inhibiting inflammation in the body[29].

The silencing regulatory protein SIRT1 has been reported to regulate the activity of the farnesol X receptor (FXR), which is now known as a bile acid nuclear receptor and plays a core regulatory role in bile acid metabolism, lipid homeostasis, glucose metabolism, and liver protection[53–54]. Studies have shown that after 2 weeks of administration of 25/50 mg/kg GS, the NLR family pyrin domain-containing 3 (NLRP3) inflammasome was significantly inhibited and liver fibrosis was significantly improved in bile duct-ligated mice. Further research has shown that GS exerts its hepatoprotective effect by activating SIRT1 and deacetylating FXR[55].

Regulating the hepatic intestinal circulation of bile acids

The hepatic intestinal circulation of bile acids plays a crucial role in the absorption, distribution, and balance of nutrients in the human body[56–57]. LD can impair the circulation of bile acids between the intestine and liver, preventing bile acids from flowing back to the liver and leading to elevated levels of bile acids in the blood, resulting in a series of adverse reactions, such as liver failure[58]. Research has shown that if the key enzyme in bile acid synthesis, cholesterol 7α-hydroxylase (CYP7A1), is inhibited and sterol 12α- hydroxylase (CYP8B1) is upregulated, the content of bile acids in liver cells decreases, and bile acid stasis can be effectively alleviated[59–61]. In addition, FXR plays an important regulatory role in inhibiting cholesterol 7α-hydroxylase (CYP7a1). The activation of FXR indirectly inhibits CYP7A1 by inducing small heterodimer partners (SHPs), thereby reducing bile acid secretion and lowering bile acid levels[62–67]. Experiments have shown that after the oral administration of GS (25, 50, and 100 mg/kg) for 7 days, GS can reduce the concentration of bile acids in liver cells by inhibiting CYP7A1 and upregulating FXR activity, inducing liver cells to reabsorb bile acids from the intestine, thereby reducing the concentration of bile acids in the intestine and blood. At the same time, the model rats showed decreased levels of serum markers of bile stasis, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), and direct bilirubin (DBIL), indicating that GS alleviated bile acid stasis[68]. As a key component of Yinchenhao decoction, GS plays an irreplaceable role in the treatment of bile stasis with Yinchenhao decoction[69]. In addition, after oral administration of 21 and 42 mg/kg of GS for 5 days, GS could also alleviate ANTI-induced cholestasis by upregulating CYP8B1 and promoting bile acid excretion[70].

FXR is also an important factor in improving the hepatic intestinal circulation of bile acids. Molecular mechanism studies have shown that GS can promote hepatic bile acid synthesis by inhibiting the FXR-mediated negative feedback regulation of bile acids, thereby enhancing the reverse transport and catabolism of cholesterol. It can also reduce FXR-dependent ileal bile acid reabsorption and increase bile acid excretion[71]. In addition, after administering 90 mg/kg of GS and 1.34 mg/kg of chlorogenic acid to NAFLD mice for

4 consecutive weeks, it was found that GS could induce FXR signaling, that is, regulate the expression of SHP in the liver tissue of mice, and this effect could be reversed by antibiotics. This indicates that GS can improve bile stasis through gut microbiota and bile acid signaling[20].

Antioxidant and cell apoptosis

Improve oxidative stress

Although many organisms have evolved complex antioxidant systems to alleviate oxidative stress, there are still many factors that can cause elevated levels of ROS, leading to cell and tissue damage[72–73]. The liver is the main organ attacked by ROS, and high levels of ROS can lead to LPO, resulting in liver damage and fibrosis[74–75]. Oxidative stress has been confirmed as a key pathological mechanism of liver injury induced by various pathogenic factors, such as alcohol, drugs, viral infections, environmental pollutants, and dietary components. It can cause enzyme activity disorders in liver tissue and consume high levels of enzyme removers, such as glutathione peroxidase (GSH-PX), ultimately affecting collagen metabolism in liver tissue[76–77]. As a widely present "free radical scavenger" in the body, GSH binds with ROS to produce oxidized glutathione (GSSG), which protects cells. The value of GSH/GSSG is usually constant but also changes accordingly when cellular function is impaired. A decrease in this ratio, to some extent, indicates a decrease in antioxidant capacity[78]. Research has shown that after administering GS to ALD mice, GSH levels significantly increased, the GSH/GSSG ratio slightly increased, malondialdehyde (MDA, an important marker of cell membrane LPO) levels decreased, CuZn superoxide dismutase (SOD) and catalase (CAT) levels increased, and oxidative stress and ethanol-induced liver injury were alleviated[29]. In addition, gastric administration of 20, 40, or 80 mg/kg of GS can significantly reverse the excessive elevation of ALT/AST and hepatic LPO levels induced by alcohol in rats and increase the liver mRNA expression of CuZn SOD and CAT, indicating that GS can protect against acute alcohol-induced liver injury by upregulating the expression of major antioxidant enzymes[79]. There is also evidence to suggest that GS can increase the activity of SOD and GSH-PX in the liver and alleviate the oxidative stress response in mice with liver fibrosis[80].

Anti-apoptosis mechanism

Along with oxidative stress, cell apoptosis also occurs in liver tissue, and apoptosis inhibition has become an effective strategy to combat LD[81–84]. Caspase-3 is a terminal cleavage enzyme in the process of cell apoptosis and has become a key biomarker for determining apoptosis. Its high expression is closely related to cell apoptosis[85]. Terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) is a method of examining cell apoptosis using fluorescence microscopy. In this method, apoptotic cells are labeled as positive cells, and the larger the area and density of positive cells, the higher the degree of cell apoptosis[86]. In our study, we found that the model group rats had higher caspase-3 activity and larger and more obvious positive cell areas. However, treatment with GS reversed caspase-3 overexpression, and the area and density of TUNEL-positive cells decreased, indicating that GS can be used as an anti-apoptotic agent to protect the liver of rats with ALD[29]. In addition, studies have shown that the daily administration of 50 mg/kg GS to mice with liver fibrosis for four consecutive weeks can increase the activity of SOD and GSH-PX in the liver while reducing the level of MDA. Furthermore, GS treatment leads to a decrease in the concentrations of interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α in liver tissue homogenate. TUNEL staining further shows that GS is able to reduce the apoptosis of liver cells in mice with liver fibrosis[80].

Another noteworthy factor is the methylation/demethylation of anti-apoptotic genes, which can have a profound impact on gene expression[87]. B-cell lymphoma 2 (BCL-2) is a key protein regulating apoptosis and belongs to the core member of the anti-apoptotic protein family (BCL-2 family). Overexpression of BCL-2 may prevent apoptosis and protect tissues[88]. Research has shown that GS can reduce specific DNA methylation levels in the BCL-2 promoter region during the H_2O_2-induced regulation of the human liver cell line L-02, inhibit cell apoptosis, and protect human liver cells[89].

Regulation of amino acid metabolism

Except for branched-chain amino acids, the liver is the main organ for the metabolism of a vast majority of amino acids in the body[90–91]. When the liver is damaged or liver function is impaired, amino acid metabolism becomes insufficient, leading to an increase in the concentration of amino acids to be metabolized in the blood and a lack of amino acids in the body[92]. The excessive concentration of phenylalanine in the blood can lead to mental disorders, phenylketonuria, albinism, and damage to the liver[93–94]. Glutamine is a rich and versatile amino acid in the body, which is easily converted into glutamate and produces the antioxidant glutathione[95–97]. It can also generate an arginine precursor through citrulline transport. Arginine is crucial for cardiovascular function, skeletal muscle, and fat metabolism and participates in the ornithine cycle, which promotes growth hormone release and immune system maintenance[98–99]. Ornithine is further converted into proline, which helps balance the redox state, reduce oxidative stress, regulate protein synthesis, and more[100–101].

Metabolomics studies have shown that GS can improve liver damage caused by high levels of phenylalanine and enhance phenylalanine metabolism by regulating the biosynthesis of phenylalanine, tyrosine, and tryptophan in the liver and the metabolic pathways of phenylalanine. In addition, GS can promote the metabolism of arginine, ornithine, and proline in the liver, thereby reducing the concentrations of arginine, ornithine, and proline in ALD model mice, as well as increasing the level of glutamine in mice and improving amino acid metabolism disorders and hepatic fat accumulation[29]. In addition, after 19 potential biomarkers of type II diabetes were identified using mass spectrometry-based fecal metabolomics technology, GS was found to regulate phenylalanine metabolism, tryptophan metabolism, and the diabetes-induced secondary bile acid biosynthesis pathway in type II diabetes rats, which also confirms its ability to protect the liver and intestine by improving amino acid metabolism[102].

Improve energy metabolism

Relieve mitochondrial dysfunction

Abnormal energy metabolism in liver cells occurs after LD and is one of the important causes of liver damage[103–105]. Mitochondria are the main structures that provide energy to liver cells. They perform cellular biological oxidation and provide ATP to liver cells so that they can maintain their normal biological activity[106]. Damage to the liver can cause changes in mitochondrial dynamics and impaired adaptability and flexibility, directly leading to a decrease in ATP synthesis[107–109]. Our experiment showed that after being exposed to alcohol for 24 hours, liver cells treated with 0.9 μg/mL GS had significantly increased mitochondrial respiratory capacity and ATP content[110]. There is also evidence to suggest that gastric administration of GS can significantly alleviate mitochondrial dysfunction: GS has a significant protective effect on mitochondrial dysfunction in APP/PS1 mice by inhibiting oxidative damage processes in mitochondria, increasing mitochondrial membrane potential, and enhancing cytochrome C oxidase activity[111].

Regulating the TCA cycle

The TCA cycle is the basis for mitochondrial energy production and can directly reflect the functional status of mitochondria[112]. One of the key enzymes guiding the TCA cycle is isocitrate dehydrogenase (IDH), which catalyzes the oxidative decarboxylation of isocitrate and maintains cellular homeostasis[113]. IDH exists in the cytoplasm in the form of IDH1, while in mitochondria, it exists in the form of IDH2. IDH1 and IDH2 mutations can cause TCA cycle dysfunction, and multiple researchers have discovered IDH1 and IDH2 mutants in various cancers[114–117]. Research has shown that MiR-144-5p is highly involved in the regulation of IDH1/IDH2 and oxoglutarate (key organic enzymes in the TCA cycle) in the TCA cycle. Through the study of proteomics and metabolomics data, we found that GS can regulate IDH1/IDH2 and oxoglutarate in the TCA cycle by reducing MiR-144-5p levels, thereby improving the TCA cycle and promoting the recovery of liver cells affected by alcohol[113]. In addition, there is evidence to suggest that after a systematic analysis of the therapeutic effect of GS on liver injury using biochemistry, metabolomics, and proteomics, it was found that the TCA cycle is one of the nine therapeutic differentiation metabolites, and the identified differential proteins are also related to energy metabolism, indicating that the molecular therapeutic mechanism of GS on liver injury rats is closely related to regulating energy metabolism[118].

Regulating lipid metabolism

Improve lipid breakdown

The liver plays a central role in regulating systemic lipid circulation. LD can affect the liver TCA cycle and reduce fatty acid oxidation, and if accompanied by a high-fat diet, it can easily lead to hyperlipidemia and exacerbate liver steatosis[119–121]. Research has shown that lipid accumulation and the loss of insulin function can lead to an increase in TCA oxidation flux, accelerating the formation of fatty liver[122–123]. In our experiment, we found that after the administration of GS, the levels of citric acid, isocitric acid, and D-glucose involved in the TCA cycle and starch, sucrose, and acetaldehyde metabolism increased in ALD model mice, alleviating symptoms such as loss of appetite and hypoglycemia[110]. In addition, evidence suggests that GS can inhibit lipid accumulation in the rat liver by activating peroxisome proliferator-activated receptor α (PPAR α) and regulating the AMPK/mTOR complex signaling pathway[124–126]. There are also studies showing that GS can control cholesterol synthesis in the liver by inhibiting HMG CoA reductase (HMGCR, the rate-limiting enzyme for cholesterol synthesis), steroid regulatory element binding protein 1c (SREBP-1c), and their downstream target genes, thereby inhibiting lipid synthesis in the liver[127–128]. The above evidence indicates that GS can reduce the risk of liver steatosis by regulating lipid metabolism.

Adjust the content of lysophosphatides

Glycerol phospholipid (GP) is the most common phospholipid in the body. In addition to forming biofilms, it is also one of the components of membrane surfactants, with functions such as transporting substances and acting as receptors. Phosphatidylcholine has a good protective effect on liver damage caused by toxic effects and is an important nutrient in the liver. The changes in the content and metabolic pathways of one of the metabolites of glycerophospholipids, lysophosphatidylcholine (LysoPC), are the focus of many disease studies[129–131]. Research has shown that rapid updates of phosphatidylcholine in the early stages of liver damage are associated with LysoPC[132]. In our study, the levels of LysoPC (15:0) and LysoPC (16:0) in ALD model mice treated with GS tended to be normal, indicating that GP metabolism in mice improved after treatment with GS and the GP metabolic pathway was improved. At the same time, it was found in the study that the serum levels of sphingolipids in ALD model mice were significantly increased, and sphingolipids are considered highly diverse lipids with multiple biological activities. Therefore, sphingolipids are also suspected to affect the pathogenesis of ALD[110].

Anti-inflammatory and analgesic

Arachidonic acid (AA) is a liquid essential omega-6 fatty acid, which only accounts for a small portion in the human body[133], it is important for the production of prostaglandins, thromboxanes, and leukotrienes, as well as for infant brain development[134]. The main effect of AA in the body is to stimulate inflammation[135–137]. AA is a precursor form of bioactive derivatives of cyclododecanoic acid such as prostaglandins and thromboxanes, which play important roles in lipoprotein metabolism, vasodilation, coagulation enhancement, and regulation of body temperature[138]. As a central metabolic organ, when the liver is damaged, it can lead to impaired AA metabolism and elevated levels of AA in the body, resulting in increased levels of active derivatives such as prostaglandin E2. Prostaglandin E2 can cause vasodilation and increased permeability, leading to redness,

fever, swelling, and pain[139], and this is closely related to liver pain in ALD patients in clinical practice, and experiments have also shown that the levels of AA and prostaglandin E2 in ALD model mice are higher, and the inflammatory response is obvious. Treatment with GS can alleviate these symptoms, indicating that the effect of GS on inflammation may be related to regulating AA metabolism[110].

In addition, the level of inflammatory factors can also indicate the fact. Studies show that the levels of IL-1 β, IL-6, and TNF-α in diabetes db/db mice are significantly inhibited after the administration of GS. Western blot results show that the expression levels of proinflammatory proteins such as serine kinase ROK1/2 and p-NF-κB p65 are reversed, indicating that GS has a good inhibitory effect on diabetes hepatitis[140].

Pharmacodynamics

While the aforementioned mechanisms elucidate GS's therapeutic potential at the molecular level, its clinical efficacy is inherently constrained by its pharmacokinetic behavior *in vivo*. A thorough understanding of GS's absorption, distribution, metabolism, and excretion (ADME) properties is critical for optimizing dose regimens, mitigating off-target effects, and tailoring individualized therapeutic strategies for LD. First, the absorption of GS after entering the body is a complex process. In a rat model, after oral administration of GS, a bimodal phenomenon and the first-pass effect were observed, which may be related to the hepatic intestinal circulation and water solubility of GS[141]. In the intestine, the absorption of GS exhibits first-order kinetic characteristics, especially in the duodenum and jejunum, where the absorption effect is better[142]. In addition, GS also has transdermal absorption properties, and pathological conditions may affect its absorption in the body[143]. There are certain differences in the distribution of GS among various tissues in the body. Research has shown that the GS content is highest in the kidneys, followed by the spleen, liver, heart, lungs, and brain. This indicates that GS has a certain tissue-specific distribution *in vivo*[144]. It is worth noting that after oral administration of GS for 0.5 hours, Cmax (0.46 ± 0.26 μg/mL) appeared in the liver, and area under the curve (AUC) 0.58 ± 0.21 μg·h/mL, indicating that GS can rapidly accumulate in liver tissue at significant levels, consistent with GS exerting multiple effects in protecting the liver, including enhancing liver antioxidant capacity, improving lipid metabolism disorders, and eliminating inflammatory reactions. This specific distribution and significant pharmacological activity provide a solid pharmacokinetic basis for its development as a novel hepatoprotective drug. However, GS cannot penetrate the blood–brain barrier. To improve its poor brain-targeting ability, researchers have prepared GS in liposomes to prolong its half-life or used borneol to promote its transfer to the hippocampus, thereby transporting GS to the brain[145–146]. Regarding the metabolic process, the pathway of GS metabolism in the body is relatively complex and currently not fully understood. Previous studies have shown that GS may be metabolized through steps such as deglycosylation of iridoid glycosides, glucuronidation, and ring cleavage to produce compounds such as sulfates or glucuronic acids. In addition, GS is also converted into genipin in the body, which is also a part of its metabolic process[147–151], as shown in Figure 3. Finally, the process of GS excretion in the body has not been fully elucidated. However, based on its distribution and metabolic characteristics, it can be inferred that GS may be excreted from the body through pathways such as urine and feces. It should be noted that the pharmacokinetic properties of GS may be influenced by various factors, such as the administration route, dosage, individual differences, etc. Therefore, in practical applications, the appropriate administration method and dosage need to be chosen according to the specific situation to ensure its safety and effectiveness.

In summary, the pharmacokinetic study of GS is of great significance in guiding its rational use as a drug. However, the current research on its metabolism and excretion is insufficient, and further research is needed to improve its pharmacokinetic properties.

Hepatotoxic effects and coping strategies of GS

Although GS exhibits significant multi-target effects in LD protection, its potential hepatotoxicity still needs to be carefully evaluated. Existing studies have shown

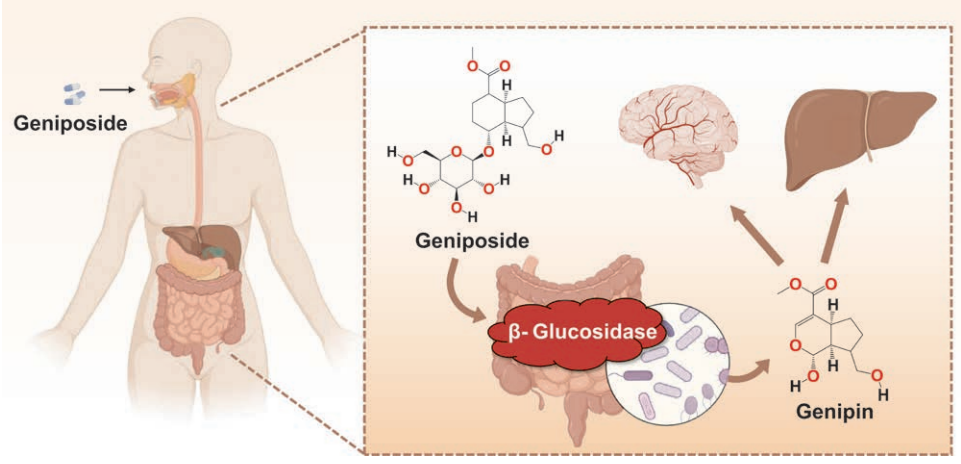

Figure 3. Metabolism of GS. GS: Geniposide.

that the hepatotoxicity of genipin is closely related to its metabolic processes: after oral administration, genipin is hydrolyzed by intestinal microbiota β-glucosidase to produce the active metabolite genipin, which can induce LD through various pathways[152]. First, genipin can covalently bind with glutathione (GSH), significantly reducing the level of GSH in liver cells and weakening the antioxidant defense ability of cells[153]. Second, genipin can also activate apoptosis-related signaling pathways, such as caspase-3 and Bax/Bcl-2 pathways, to induce hepatocyte apoptosis[154]; and according to positive tests for *in vitro* micronuclei and chromosomal aberrations, genipin has genetic toxicity[155]. It is worth noting that these toxic effects exhibit a clear dose and time dependence, particularly significant in long-term high-dose administration[156–159].

However, it is not rational to completely deny the hepatoprotective value of GS due to its potential toxicity. From the perspective of drug development, almost all effective drugs have certain toxic side effects, and the key is how to control risks and exert therapeutic effects through scientific means. For GS, there are at least the following strategies that can significantly reduce its liver toxicity risk: in terms of dose control, adopting a low-dose divided administration regimen (such as <50 mg/kg/d) can effectively avoid toxic reactions; In terms of structural optimization, chemical modification of hydroxyl or sugar groups can reduce the intestinal production rate of genipin. In terms of combination therapy, combining with antioxidants (such as N-acetylcysteine) or hepatoprotective drugs (such as silymarin) can significantly reduce their adverse reactions. More importantly, the unique pharmacological mechanism of GS, including regulating FXR-mediated bile acid metabolism, inhibiting NF-κB inflammatory pathway, activating Nrf2 antioxidant pathway, etc, gives it irreplaceable advantages in the treatment of LD.

Conclusion

The liver, as the largest metabolic organ in the human body, plays multiple important physiological functions. The prevention and treatment of LD is not only related to the health of a single organ but also the fundamental guarantee for maintaining overall life activities. GS is a natural product with various pharmacological activities as a cyclic terpenoid glycoside. Improving LD is one of the important pharmacological activities. GS, as a candidate drug for treating liver injury, has enormous potential as it can alleviate symptoms from the root. Due to the water-soluble nature of GS, it is easily converted into GS and absorbed by the intestine when taken orally, making it easier to exert its pharmacological effects. Despite the risk of liver toxicity, with scientific utilization, it can still exert the maximum effect of liver protection. This review is based on existing research progress and summarizes the possible mechanisms by which GS improves LD. At the same time, the pharmacokinetics of GS *in vivo* are systematically reviewed, and the mechanism of its liver toxicity is elucidated and solutions are proposed.

Although there are many studies on the alleviation of LD by the natural product GS, there are still many challenges waiting to be overcome. At present, most research is still in the primary stage of animal experiments, with limited clinical data support. In the future, appropriate clinical trials should be conducted to ensure the safety of GS in large-scale applications. In addition, current research mainly focuses on searching for LD biomarkers and possible pathways but neglects the verification process. In the future, research should focus on targeted studies of biomarkers and pathways to demonstrate their reliability. Moreover, the mechanism of action of GS in alleviating LD remains not clearly explained at present, although metabolomics research has made considerable efforts in this area. In the future, we can try to combine metabolomics with multi-omics and use multiple methods, such as mass spectrometry imaging and magnetic resonance imaging, to identify specific mechanisms through a "point-to-point" approach.

In summary, GS has great potential for development and research as a new drug for treating LD.

Conflict of interest statement

Xijun Wang is an editorial board member of this journal. The other authors declare no conflict of interest.

Funding

This study was supported by the National Natural Science Foundation of China Key Project (81830110).

Author contributions

Songyuan Tang analyzed the data and wrote the manuscript. Guangli Yan, Ling Kong, Hui Sun, Chang Liu, Ying Han, and Xijun Wang revised the manuscript. All the authors read and approved the final manuscript.

Ethical approval of studies and informed consent

Not applicable.

Acknowledgments

None.

Data availability

All data generated or analyzed during this study are included in this published article.

References

[1] Lucey MR, Mathurin P, Morgan TR. Alcoholic hepatitis. *N Engl J Med* 2009;360(26):2758–2769.
[2] Avila MA, Dufour JF, Gerbes AL, et al. Recent advances in alcohol-related liver disease (ALD): summary of a Gut round table meeting. *Gut* 2019;69(4):764–780.
[3] Andrade RJ, Chalasani N, Björnsson ES, et al. Drug-induced liver injury. *Nat Rev Dis Primers* 2019;5(1):58.
[4] Björnsson HK, Bjöörnsson ES. Drug-induced liver injury: pathogenesis, epidemiology, clinical features, and practical management. *Eur J Intern Med* 2022;97:26–31.
[5] Zeng DY, Li JM, Lin S, et al. Global burden of acute viral hepatitis and its association with socioeconomic development status, 1990–2019. *J Hepatol* 2021;75(3):547–556.
[6] Dubuisson J, Cosset FL. Virology and cell biology of the hepatitis C virus life cycle—an update. *J Hepatol* 2014;61(1):S3–S13.

[7] Byrne CD, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015;62(1):S47–S64.
[8] Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64(1):73–84.
[9] Kawaguchi T, Tsutsumi T, Nakano D, et al. MAFLD: Renovation of clinical practice and disease awareness of fatty liver. *Hepatol Res* 2021;52(5):422–432.
[10] Muratori L, Lohse AW, Lenzi M. Diagnosis and management of autoimmune hepatitis. *BMJ* 2023;380:e070201.
[11] Sharma R, Verna EC, Simon TG, et al. Cancer risk in patients with autoimmune hepatitis: a nationwide population-based cohort study with histopathology. *Am J Epidemiol* 2021;191(2):298–319.
[12] Devarbhavi H, Asrani SK, Arab JP, et al. Global burden of liver disease: 2023 update. *J Hepatol* 2023;79(2):516–537.
[13] Torbenson M, Washington K. Pathology of liver disease: advances in the last 50 years. *Hum Pathol* 2020;95:78–98.
[14] Służały P, Paśko P, Galanty A. Natural products as hepatoprotective agents—a comprehensive review of clinical trials. *Plants* 2024;13(14):1985.
[15] GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet* 2018;392(10159):1789–1858.
[16] Oaklander AL, Lunn MPT, Hughes RC, et al. Treatments for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP): an overview of systematic reviews. *Cochrane Database Syst Rev* 2017;2017(1):CD010369.
[17] Chen LP, Li MX, Yang ZQ, et al. *Gardenia jasminoides* Ellis: Ethnopharmacology, phytochemistry, and pharmacological and industrial applications of an important traditional Chinese medicine. *J Ethnopharmacol* 2020;257:112829.
[18] Zhang AH, Sun H, Qiu S, et al. Advancing drug discovery and development from active constituents of yinchenhao tang, a famous traditional Chinese medicine formula. *Evid Based Complement Alternat Med* 2013;2013:1–6.
[19] Ogasawara T, Morine Y, Ikemoto T, et al. Beneficial effects of Kampo medicine Inchin-ko-to on liver function and regeneration after hepatectomy in rats. *Hepatol Res* 2008;38(8):818–824.
[20] Li HS, Xi YF, Xin X, et al. Geniposide plus chlorogenic acid reverses non-alcoholic steatohepatitis *via* regulation of gut microbiota and bile acid signaling in a mouse model *in vivo*. *Front Pharmacol* 2023;14:1148737.
[21] Wang XJ, Lv HT, Sun H, et al. Metabolic urinary profiling of alcohol hepatotoxicity and intervention effects of Yin Chen Hao Tang in rats using ultra-performance liquid chromatography/electrospray ionization quadruple time-of-flight mass spectrometry. *J Pharm Biomed Anal* 2008;48(4):1161–1168.
[22] Rinella ME, Neuschwander Tetri BA, Siddiqui MS, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology* 2023;77(5):1797–1835.
[23] Xian YX, Weng JP, Xu F. MAFLD vs. NAFLD: Shared features and potential changes in epidemiology, pathophysiology, diagnosis, and pharmacotherapy. *Chin Med J (Engl)* 2020;134(1):8–19.
[24] Fuchs CD, Trauner M. Role of bile acids and their receptors in gastrointestinal and hepatic pathophysiology. *Nat Rev Gastroenterol Hepatol* 2022;19(7):432–450.
[25] Huang WF, Kong DS. The intestinal microbiota as a therapeutic target in the treatment of NAFLD and ALD. *Biomed Pharmacother* 2021;135:111235.
[26] Kitade H, Chen G, Ni Y, et al. Nonalcoholic fatty liver disease and insulin resistance: new insights and potential new treatments. *Nutrients* 2017;9(4):387.
[27] Katsiki N, Mikhailidis DP, Mantzoros CS. Non-alcoholic fatty liver disease and dyslipidemia: an update. *Metabolism* 2016;65(8):1109–1123.
[28] Zhang TL, Zhang AH, Qiu S, et al. High-throughput metabolomics approach reveals new mechanistic insights for drug response of phenotypes of geniposide towards alcohol-induced liver injury by using liquid chromatography coupled to high resolution mass spectrometry. *Mol Biosyst* 2017;13(1):73–82.
[29] Qiu S, Zhang AH, Guan Y, et al. Functional metabolomics using UPLC-Q/TOF-MS combined with ingenuity pathway analysis as a promising strategy for evaluating the efficacy and discovering amino acid metabolism as a potential therapeutic mechanism-related target for geniposide against alcoholic liver disease. *RSC Adv* 2020;10(5):2677–2690.
[30] Liu LP, Wu Q, Chen YP, et al. Updated pharmacological effects, molecular mechanisms, and therapeutic potential of natural product geniposide. *Molecules* 2022;27(10):3319.
[31] Zhou YX, Zhang RQ, Rahman K, et al. Diverse pharmacological activities and potential medicinal benefits of geniposide. *Evid Based Complement Alternat Med* 2019;2019:4925682.
[32] Song N, Xu H, Liu J, et al. Design of a highly potent GLP-1R and GCGR dual-agonist for recovering hepatic fibrosis. *Acta Pharm Sin B* 2022;12(5):2443–2461.
[33] Yin F, Liu JH, Zheng XX, et al. Geniposide induces the expression of heme Oxygenase-1 *via* PI3K/Nrf2-signaling to enhance the antioxidant capacity in primary hippocampal neurons. *Biol Pharm Bull* 2010;33(11):1841–1846.
[34] Wang JJ, Chen L, Liang ZB, et al. Genipin inhibits LPS-induced inflammatory response in BV2 microglial cells. *Neurochem Res* 2017;42(10):2769–2776.
[35] Tu YY, Li LL, Zhu LL, et al. Geniposide attenuates hyperglycemia-induced oxidative stress and inflammation by activating the Nrf2 signaling pathway in experimental diabetic retinopathy. *Oxid Med Cell Longev* 2021;2021(1):9247947.
[36] Seo MJ, Hong JM, Kim SJ, et al. Genipin protects d-galactosamine and lipopolysaccharide-induced hepatic injury through suppression of the necroptosis-mediated inflammasome signaling. *Eur J Pharmacol* 2017;812:128–137.
[37] Liu JH, Yin F, Xiao H, et al. Glucagon-like peptide 1 receptor plays an essential role in geniposide attenuating lipotoxicity-induced β-cell apoptosis. *Toxicol In Vitro* 2012;26(7):1093–1097.
[38] Zhang ZH, Wang XJ, Zhang D, et al. Geniposide-mediated protection against amyloid deposition and behavioral impairment correlates with downregulation of mTOR signaling and enhanced autophagy in a mouse model of Alzheimer's disease. *Aging (Milano)* 2019;11(2):536–548.
[39] Yamamoto M, Miura N, Ohtake N, et al. Genipin, a metabolite derived from the herbal medicine Inchin-ko-to, and suppression of Fas-induced lethal liver apoptosis in mice. *Gastroenterology* 2000;118(2):380–389.
[40] Kringen MK, Piehler AP, Grimholt RM, et al. Serum bilirubin concentration in healthy adult North-Europeans is strictly controlled by the UGT1A1 TA-repeat Variants. *PLoS One* 2014;9(2):e90248.
[41] Feldman AG, Sokol RJ. Neonatal cholestasis: emerging molecular diagnostics and potential novel therapeutics. *Nat Rev Gastroenterol Hepatol* 2019;16(6):346–360.
[42] Dong S, Chen QL, Song YN, et al. Mechanisms of CCl_4-induced liver fibrosis with combined transcriptomic and proteomic analysis. *J Toxicol Sci* 2016;41(4):561–572.
[43] Hong X, Huang S, Jiang H, et al. Alcohol-related liver disease (ALD): Current perspectives on pathogenesis, therapeutic strategies, and animal models. *Front Pharmacol* 2024;15:1432480.
[44] Farghali H, Kemelo MK, Canová NK. SIRT1 modulators in experimentally induced liver injury. *Oxid Med Cell Longev* 2019;2019:1–15.
[45] Recena Aydos L, Aparecida Do Amaral L, Serafim De Souza R, et al. Nonalcoholic fatty liver disease induced by high-fat diet in C57bl/6 models. *Nutrients* 2019;11(12):3067.
[46] Pan PH, Wang YY, Lin SY, et al. Plumbagin ameliorates bile duct ligation-induced cholestatic liver injury in rats. *Biomed Pharmacother* 2022;151:113133.
[47] Zhao YL, Ma X, Zhu Y, et al. *Paeonia lactiflora* Pall. protects against ANIT-induced cholestasis by activating Nrf2 *via* PI3K/Akt signaling pathway. *Drug Des Devel Ther* 2015;9:5061.
[48] Li M, Cai SY, Boyer JL. Mechanisms of bile acid mediated inflammation in the liver. *Mol Aspects Med* 2017;56:45–53.
[49] Chiang JYL, Ferrell JM. Bile acid metabolism in liver pathobiology. *Gene Expr* 2018;18(2):71–87.
[50] Cai JW, Rimal BP, Jiang CT, et al. Bile acid metabolism and signaling, the microbiota, and metabolic disease. *Pharmacol Ther* 2022;237:108238.
[51] Liu ZM, Zhang ZF, Huang M, et al. Taurocholic acid is an active promoting factor, not just a biomarker of progression of liver cirrhosis: evidence from a human metabolomic study and in vitro experiments. *BMC Gastroenterol* 2018;18(1):112.
[52] Salhab A, Amer J, Lu YY, et al. Sodium+/taurocholate cotransporting polypeptide as target therapy for liver fibrosis. *Gut* 2021;71(7):1373–1385.
[53] Chiang JYL, Ferrell JM. Discovery of farnesoid X receptor and its role in bile acid metabolism. *Mol Cell Endocrinol* 2022;548:111618.

[54] Tian SY, Chen SM, Pan CX, et al. FXR: structures, biology, and drug development for NASH and fibrosis diseases. *Acta Pharmacol Sin* 2022;43(5):1120–1132.
[55] Qin T, Hasnat M, Wang Z, et al. Geniposide alleviated bile acid-associated NLRP3 inflammasome activation by regulating SIRT1/FXR signaling in bile duct ligation-induced liver fibrosis. *Phytomedicine* 2023;118:154971.
[56] Chen XL, Su SL, Liu R, et al. Chemical constituents and pharmacological action of bile acids from animal: a review. *China J Chin Mater Med* 2021;46(19):4898–4906.
[57] Ocvirk S, O'keefe SJD. Dietary fat, bile acid metabolism and colorectal cancer. *Semin Cancer Biol* 2021;73:347–355.
[58] Simbrunner B, Trauner M, Reiberger T. Review article: therapeutic aspects of bile acid signalling in the gut-liver axis. *Aliment Pharmacol Ther* 2021;54(10):1243–1262.
[59] Rizzolo D, Kong B, Taylor RE, et al. Bile acid homeostasis in female mice deficient in Cyp7a1 and Cyp27a1. *Acta Pharm Sin B* 2021;11(12):3847–3856.
[60] Yan Y, Niu ZM, Sun C, et al. Hepatic thyroid hormone signalling modulates glucose homeostasis through the regulation of GLP-1 production *via* bile acid-mediated FXR antagonism. *Nat Commun* 2022;13(1):6408.
[61] Zaborska KE, Cummings BP. Rethinking bile acid metabolism and signaling for type 2 diabetes treatment. *Curr Diab Rep* 2018;18(11):109.
[62] Kong LN, Dong RC, Huang K, et al. Yangonin modulates lipid homeostasis, ameliorates cholestasis and cellular senescence in alcoholic liver disease *via* activating nuclear receptor FXR. *Phytomedicine* 2021;90:153629.
[63] Clifford BL, Sedgeman LR, Williams KJ, et al. FXR activation protects against NAFLD *via* bile-acid-dependent reductions in lipid absorption. *Cell Metab* 2021;33(8):1671–1684.e4.
[64] Kosters A, Felix JC, Desai MS, et al. Impaired bile acid handling and aggravated liver injury in mice expressing a hepatocyte-specific RXRα variant lacking the DNA-binding domain. *J Hepatol* 2014;60(2):362–369.
[65] Pawlak M, Lefebvre P, Staels B. General molecular biology and architecture of nuclear receptors. *Curr Top Med Chem* 2012;12(6):486–504.
[66] Hasegawa Y, Kishimoto S, Takahashi H, et al. Altered expression of nuclear receptors affects the expression of metabolic enzymes and transporters in a rat model of cholestasis. *Biol Pharm Bull* 2009;32(12):2046–2052.
[67] Kong B, Wang L, Chiang JYL, et al. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology* 2012;56(3):1034–1043.
[68] Wang LL, Wu GX, Wu FH, et al. Geniposide attenuates ANIT-induced cholestasis through regulation of transporters and enzymes involved in bile acids homeostasis in rats. *J Ethnopharmacol* 2017;196:178–185.
[69] Chen Z, Ma X, Zhao Y, et al. Yinchenhao decoction in the treatment of cholestasis: a systematic review and meta-analysis. *J Ethnopharmacol* 2015;168:208–216.
[70] Qin SS, Tian JZ, Zhao Y, et al. Gardenia extract protects against intrahepatic cholestasis by regulating bile acid enterohepatic circulation. *J Ethnopharmacol* 2024;319:117083.
[71] Liu JX, Li Y, Sun C, et al. Geniposide reduces cholesterol accumulation and increases its excretion by regulating the FXR-mediated liver-gut crosstalk of bile acids. *Pharmacol Res* 2020;152:104631.
[72] Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 2014;94(3):909–950.
[73] Yang SS, Lian GJ. ROS and diseases: role in metabolism and energy supply. *Mol Cell Biochem* 2020;467(1):1–12.
[74] Prieto I, Monsalve M. ROS homeostasis, a key determinant in liver ischemic-preconditioning. *Redox Biol* 2017;12:1020–1025.
[75] Li S, Tan HY, Wang N, et al. The role of oxidative stress and antioxidants in liver diseases. *Int J Mol Sci* 2015;16(11):26087–26124.
[76] Seitz H, Mueller S. Die Bedeutung von Cytochrom P4502E1 bei der alkoholischen Lebererkrankung und bei der alkoholmediierten Karzinogenese. *Z Gastroenterol* 2019;57(1):37–45.
[77] Cichoż Lach H. Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol* 2014;20(25):8082.
[78] Liu T, Sun L, Zhang YB, et al. Imbalanced GSH/ROS and sequential cell death. *J Biochem Mol Toxicol* 2021;36(1):e22942.
[79] Wang JM, Zhang YY, Liu RX, et al. Geniposide protects against acute alcohol-induced liver injury in mice *via* up-regulating the expression of the main antioxidant enzymes. *Can J Physiol Pharmacol* 2015;93(4):261–267.
[80] Yang L, Bi LP, Jin LL, et al. Geniposide ameliorates liver fibrosis through reducing oxidative stress and inflammatory respose, inhibiting apoptosis and modulating overall metabolism. *Front Pharmacol* 2021;12:772635.
[81] Roehlen N, Crouchet E, Baumert TF. Liver fibrosis: mechanistic concepts and therapeutic perspectives. *Cells* 2020;9(4):875.
[82] Miyata T, Nagy LE. Programmed cell death in alcohol-associated liver disease. *Clin Mol Hepatol* 2020;26(4):618–625.
[83] Schwabe RF, Luedde T. Apoptosis and necroptosis in the liver: a matter of life and death. *Nat Rev Gastroenterol Hepatol* 2018;15(12):738–752.
[84] Zhou Y, Wu RM, Wang XQ, et al. Roles of necroptosis in alcoholic liver disease and hepatic pathogenesis. *Cell Prolif* 2022;55(3):e13193.
[85] Eskandari E, Eaves CJ. Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. *J Cell Biol* 2022;221(6):e202201159.
[86] Mirzayans R, Murray D. Do TUNEL and other apoptosis assays detect cell death in preclinical studies? *Int J Mol Sci* 2020;21(23):9090.
[87] Gopisetty G, Ramachandran K, Singal R. DNA methylation and apoptosis. *Mol Immunol* 2006;43(11):1729–1740.
[88] Ashkenazi A, Fairbrother WJ, Leverson JD, et al. From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. *Nat Rev Drug Discov* 2017;16(4):273–284.
[89] Peng X, Tan L, Song J, et al. Geniposide alleviated hydrogen peroxide-induced apoptosis of human hepatocytes *via* altering DNA methylation. *Food Chem Toxicol* 2023;182:114158.
[90] Paulusma CC, Lamers WH, Broer S, et al. Amino acid metabolism, transport and signalling in the liver revisited. *Biochem Pharmacol* 2022;201:115074.
[91] Trefts E, Gannon M, Wasserman DH. The liver. *Curr Biol* 2017;27(21):R1147–R1151.
[92] Tedesco L, Corsetti G, Ruocco C, et al. A specific amino acid formula prevents alcoholic liver disease in rodents. *Am J Physiol Gastrointest Liver Physiol* 2018;314(5):G566–G582.
[93] Elhawary NA, Aljahdali IA, Abumansour IS, et al. Genetic etiology and clinical challenges of phenylketonuria. *Hum Genomics* 2022;16(1):22.
[94] Schlegel G, Scholz R, Ullrich K, et al. Phenylketonuria: direct and indirect effects of phenylalanine. *Exp Neurol* 2016;281:28–36.
[95] Cruzat V, Macedo Rogero M, Noel Keane K, et al. Glutamine: metabolism and immune function, supplementation and clinical translation. *Nutrients* 2018;10(11):1564.
[96] Coqueiro AY, Rogero MM, Tirapegui J. Glutamine as an anti-fatigue amino acid in sports nutrition. *Nutrients* 2019;11(4):863.
[97] Achamrah N, Déchelotte P, Coëffier M. Glutamine and the regulation of intestinal permeability. *Curr Opin Clin Nutr Metab Care* 2017;20(1):86–91.
[98] Ho YY, Nakato J, Mizushige T, et al. l-Ornithine stimulates growth hormone release in a manner dependent on the ghrelin system. *Food Funct* 2017;8(6):2110–2114.
[99] Paßlack N, Kohn B, Zentek J. Effects of arginine and ornithine supplementation to a high-protein diet on selected cellular immune variables in adult cats. *J Vet Intern Med* 2020;34(2):852–856.
[100] Alvarez ME, Savouré A, Szabados L. Proline metabolism as regulatory hub. *Trends Plant Sci* 2022;27(1):39–55.
[101] Zhu JJ, Schwörer S, Berisa M, et al. Mitochondrial NADP(H) generation is essential for proline biosynthesis. *Science* 2021;372(6545):968–972.
[102] Zhou Y, Men LH, Pi ZF, et al. Fecal metabolomics of type 2 diabetic rats and treatment with gardenia jasminoides ellis based on mass spectrometry technique. *J Agric Food Chem* 2018;66(6):1591–1599.
[103] Siggins RW, Mcternan PM, Simon L, et al. Mitochondrial dysfunction: At the nexus between alcohol-associated immunometabolic dysregulation and tissue injury. *Int J Mol Sci* 2023;24(10):8650.
[104] Reinke H, Asher G. Circadian clock control of liver metabolic functions. *Gastroenterology* 2016;150(3):574–580.
[105] Rui L. Energy metabolism in the liver. *Compr Physiol* 2014;4(1):177–197.
[106] Xu FB, Li YF, Cao Z, et al. AFB1-induced mice liver injury involves mitochondrial dysfunction mediated by mitochondrial biogenesis inhibition. *Ecotoxicol Environ Saf* 2021;216:112213.
[107] Fromenty B, Roden M. Mitochondrial alterations in fatty liver diseases. *J Hepatol* 2023;78(2):415–429.

[108] Mansouri A, Gattolliat CH, Asselah T. Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology* 2018;155(3):629–647.
[109] Han D, Johnson HS, Rao MP, et al. Mitochondrial remodeling in the liver following chronic alcohol feeding to rats. *Free Radic Biol Med* 2017;102:100–110.
[110] Qiu S, Zhang AH, Zhang TL, et al. Dissect new mechanistic insights for geniposide efficacy on the hepatoprotection using multiomics approach. *Oncotarget* 2017;8(65):108760–108770.
[111] Lv C, Liu XL, Liu HJ, et al. Geniposide attenuates mitochondrial dysfunction and memory deficits in APP/PS1 transgenic mice. *Curr Alzheimer Res* 2014;11(6):580–587.
[112] Martínez Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun* 2020;11(1):102.
[113] Han S, Liu Y, Cai SJ, et al. IDH mutation in glioma: molecular mechanisms and potential therapeutic targets. *Br J Cancer* 2020;122(11):1580–1589.
[114] Dang L, Yen K, Attar EC. IDH mutations in cancer and progress toward development of targeted therapeutics. *Ann Oncol* 2016;27(4):599–608.
[115] Pirozzi CJ, Yan H. The implications of IDH mutations for cancer development and therapy. *Nat Rev Clin Oncol* 2021;18(10):645–661.
[116] Zarei M, Hue JJ, Hajihassani O, et al. Clinical development of IDH1 inhibitors for cancer therapy. *Cancer Treat Rev* 2022;103:102334.
[117] Wang Y, Agarwal E, Bertolini I, et al. IDH2 reprograms mitochondrial dynamics in cancer through a HIF-1α-regulated pseudohypoxic state. *FASEB J* 2019;33(12):13398–13411.
[118] Wang XJ, Zhang AH, Yan GL, et al. Metabolomics and proteomics annotate therapeutic properties of geniposide: targeting and regulating multiple perturbed pathways. *PLoS One* 2013;8(8):e71403.
[119] Xie ZF, Zhang M, Song Q, et al. Development of the novel ACLY inhibitor 326E as a promising treatment for hypercholesterolemia. *Acta Pharm Sin B* 2023;13(2):739–753.
[120] Jeon S, Carr R. Alcohol effects on hepatic lipid metabolism. *J Lipid Res* 2020;61(4):470–479.
[121] You M, Arteel GE. Effect of ethanol on lipid metabolism. *J Hepatol* 2019;70(2):237–248.
[122] Pal SC, Méndez Sánchez N. Insulin resistance and adipose tissue interactions as the cornerstone of metabolic (dysfunction)-associated fatty liver disease pathogenesis. *World J Gastroenterol* 2023;29(25):3999–4008.
[123] Satapati S, Sunny NE, Kucejova B, et al. Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. *J Lipid Res* 2012;53(6):1080–1092.
[124] Todisco S, Santarsiero A, Convertini P, et al. PPAR Alpha as a metabolic modulator of the liver: role in the pathogenesis of nonalcoholic steatohepatitis (NASH). *Biology* 2022;11(5):792.
[125] Ma TT, Huang C, Zong GJ, et al. Hepatoprotective effects of geniposide in a rat model of nonalcoholic steatohepatitis. *J Pharm Pharmacol* 2011;63(4):587–593.
[126] Kojima K, Shimada T, Nagareda Y, et al. Preventive effect of geniposide on metabolic disease status in spontaneously obese type 2 diabetic mice and free fatty acid-treated HepG2 cells. *Biol Pharm Bull* 2011;34(10):1613–1618.
[127] Leng EN, Xiao Y, Mo ZT, et al. Synergistic effect of phytochemicals on cholesterol metabolism and lipid accumulation in $HepG_2$ cells. *BMC Complement Altern Med* 2018;18(1):122.
[128] Shen D, Zhao D, Yang X, et al. Geniposide against atherosclerosis by inhibiting the formation of foam cell and lowering reverse lipid transport *via* p38/MAPK signaling pathways. *Eur J Pharmacol* 2019;864:172728.
[129] Kano K, Aoki J, Hla T. Lysophospholipid mediators in health and disease. *Annu Rev Pathol* 2022;17(1):459–483.
[130] Jia JJ, Zhang HY, Liang XY, et al. Application of metabolomics to the discovery of biomarkers for ischemic stroke in the murine model: a comparison with the clinical results. *Mol Neurobiol* 2021;58(12):6415–6426.
[131] Chin CF, Galam DLA, Gao L, et al. Blood-derived lysophospholipid sustains hepatic phospholipids and fat storage necessary for hepatoprotection in overnutrition. *J Clin Invest* 2023;133(17):e171267.
[132] Meyer JJ, Dreyhaupt J, Schwerdel D, et al. Blood-based targeted metabolomics discriminate patients with alcoholic liver cirrhosis from those with non-cirrhotic liver damage: an explorative study. *Dig Dis* 2021;40(2):223–231.
[133] Innes JK, Calder PC. Omega-6 fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 2018;132:41–48.
[134] Lucas GNC, Leitão ACC, Alencar RL, et al. Pathophysiological aspects of nephropathy caused by non-steroidal anti-inflammatory drugs. *J Bras Nefrol* 2019;41(1):124–130.
[135] Sonnweber T, Pizzini A, Nairz M, et al. Arachidonic acid metabolites in cardiovascular and metabolic diseases. *Int J Mol Sci* 2018;19(11):3285.
[136] Gai ZB, Visentin M, Gui T, et al. Effects of farnesoid X receptor activation on arachidonic acid metabolism, NF-kB signaling, and hepatic inflammation. *Mol Pharmacol* 2018;94(2):802–811.
[137] Melgar Lesmes P, Perramon M, Jiménez W. Roles of the hepatic endocannabinoid and apelin systems in the pathogenesis of liver fibrosis. *Cells* 2019;8(11):1311.
[138] Konishi T, Lentsch AB. Changes in arachidonic acid metabolism during liver ischemia triggers induction of inflammatory injury. *Hepatology* 2018;68(4):1642–1643.
[139] Kawahara K, Hohjoh H, Inazumi T, et al. Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. *Biochim Biophys Acta* 2015;1851(4):414–421.
[140] Hu XL, Yu DS, Zhuang LE, et al. Geniposide improves hepatic inflammation in diabetic db/db mice. *Int Immunopharmacol* 2018;59:141–147.
[141] Wu H, Wu H, Yuan MY, et al. Pharmacokinetics and tissue distribution of jasminoidin in normal rats. *J Anhui Tradit Chin Med Coll* 2011;30(2):57–60.
[142] Yu D, Zhang Y, Guo LW, et al. Study on the absorption mechanism of geniposide in the Chinese formula Huang-Lian-Jie-Du-Tang in rats. *AAPS PharmSciTech* 2017;18(4):1382–1392.
[143] Wang YG, Li LL, Li HY, et al. Transdermal permeation of geniposide in the herbal complex liniment in vivo and in vitro. *Int J Pharm* 2010;392(1-2):72–77.
[144] Wang FG, Cao J, Hao JF, et al. Pharmacokinetics, bioavailability and tissue distribution of geniposide following intravenous and peroral administration to rats. *Biopharm Drug Dispos* 2014;35(2):97–103.
[145] Yu B, Ruan M, Cui XB, et al. Effects of borneol on the pharmacokinetics of geniposide in cortex, hippocampus, hypothalamus and striatum of conscious rat by simultaneous brain microdialysis coupled with UPLC–MS. *J Pharm Biomed Anal* 2013;77:128–132.
[146] Wan JY, Long Y, Liu SY, et al. Geniposide-loaded liposomes for brain targeting: development, evaluation, and in vivo studies. *AAPS PharmSciTech* 2021;22(7):222.
[147] Chen CG, Han FX, Zhang Y, et al. Simultaneous determination of geniposide and its metabolites genipin and genipinine in culture of Aspergillus niger by HPLC. *Biomed Chromatogr* 2008;22(7):753–757.
[148] Han H, Yang L, Xu Y, et al. Identification of metabolites of geniposide in rat urine using ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2011;25(21):3339–3350.
[149] Gong GH, Zheng ZM, Liu H, et al. Purification and characterization of a β-glucosidase from Aspergillus niger and its application in the hydrolysis of geniposide to genipin. *J Microbiol Biotechnol* 2014;24(6):788–794.
[150] Ding Y, Hou JW, Zhang Y, et al. Metabolism of genipin in rat and identification of metabolites by using ultraperformance liquid chromatography/quadrupole time-of-flight tandem mass spectrometry. *Evid Based Complement Alternat Med* 2013;2013:1–13.
[151] Li Y, Cai W, Cai Q, et al. Comprehensive characterization of the in vitro and in vivo metabolites of geniposide in rats using ultra-high-performance liquid chromatography coupled with linear ion trap–Orbitrap mass spectrometer. *Xenobiotica* 2015;46(4):357–368.
[152] Li Y, Pan H, Li X, et al. Role of intestinal microbiota-mediated genipin dialdehyde intermediate formation in geniposide-induced hepatotoxicity in rats. *Toxicol Appl Pharmacol* 2019;377:114624.
[153] Zhao YL, Huang HY, Lv N, et al. Glutathione s-transferases mediate in vitro and in vivo inactivation of genipin: Implications for an underlying detoxification mechanism. *J Agric Food Chem* 2023;71(5):2399–2410.
[154] Khanal T, Kim HG, Choi JH, et al. Biotransformation of geniposide by human intestinal microflora on cytotoxicity against HepG2 cells. *Toxicol Lett* 2012;209(3):246–254.

[155] Hobbs CA, Koyanagi M, Swartz C, et al. Genotoxicity evaluation of the naturally-derived food colorant, gardenia blue, and its precursor, genipin. *Food Chem Toxicol* 2018;118:695–708.
[156] Qiu JN, Lin C, Ren GL, et al. Geniposide dosage and administration time: Balancing therapeutic benefits and adverse reactions in liver disease treatment. *Phytomedicine* 2024;132:155799.
[157] Zeng XY, Jiang JJ, Liu SM, et al. Bidirectional effects of geniposide in liver injury: preclinical evidence construction based on meta-analysis. *J Ethnopharmacol* 2024;319:117061.
[158] Tian JZ, Yi Y, Zhao Y, et al. Oral chronic toxicity study of geniposide in rats. *J Ethnopharmacol* 2018;213:166–175.
[159] Ding Y, Zhang T, Tao JS, et al. Potential hepatotoxicity of geniposide, the major iridoid glycoside in dried ripe fruits of *Gardenia jasminoides* (Zhi-zi). *Nat Prod Res* 2013;27(10):929–933.