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REVIEW

Silybin: A Review of Its Targeted and Novel Agents for Treating Liver Diseases Based on Pathogenesis

Jijiao Wu[1,2] | Lin Wen[1,2] | Xiaolian Liu[1,2] | Qiuxia Li[1,2] | Zihao Sun[1,2] | Chuipeng Liang[1,2] | Fan Xie[3] | Xiaofang Li[1,2]

[1]State Key Laboratory of Southwestern Chinese Medicine Resources, Chengdu University of Traditional Chinese Medicine, Chengdu, China | [2]School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China | [3]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China

Correspondence: Fan Xie (xiefan959@icloud.com) | Xiaofang Li (lixiaofang@cdutcm.edu.cn)

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ABSTRACT

Liver disease represents a significant global public health concern. Silybin, derived from *Silybum marianum*, has been demonstrated to exhibit a range of beneficial properties, including anti-inflammatory, antioxidative, antifibrotic, antiviral, and cytoprotective effects. These attributes render it a promising candidate for the treatment of liver fibrosis, cirrhosis, liver cancer, viral hepatitis, non-alcoholic fatty liver disease, and other liver conditions. Nevertheless, its low solubility and low bioavailability have emerged as significant limitations in its clinical application. To address these limitations, researchers have developed a number of silybin formulations. This study presents a comprehensive review of the results of research on silybin for the treatment of liver diseases in recent decades, with a particular focus on novel formulations based on the pathogenesis of the disease. These include approaches targeting the liver via the CD44 receptor, folic acid, vitamin A, and others. Furthermore, the study presents the findings of studies that have employed nanotechnology to enhance the low bioavailability and low solubility of silybin. This includes the use of nanoparticles, liposomes, and nanosuspensions. This study reviews the application of silybin preparations in the treatment of global liver diseases. However, further high-quality and more complete experimental studies are still required to gain a more comprehensive understanding of the efficacy and safety of these preparations. Finally, the study considers the issues that arise during the research of silybin formulations.

1 | Current Research on Liver Diseases

A multitude of the body's physiological processes occur in the liver, including the regulation of the immune system, the balance of lipids and cholesterol, and the metabolism of drugs. It is evident that the liver plays a pivotal role in human health (Trefts, Gannon, and Wasserman 2017). Consequently, liver damage or liver failure can have a profound impact on human health. Common liver diseases include liver cirrhosis (Ginès et al. 2021), liver cancer (Anna et al. 2020), viral hepatitis (Odenwald and Paul 2022), and metabolic dysfunction-related fatty degeneration liver disease (MASLD) (Ko, Yoon, and Jun 2023).

Global data from the year 2023 indicate that liver disease is the cause of more than two million deaths annually (liver cirrhosis, viral hepatitis, and hepatocellular carcinoma [HCC]), which represents 4% of all disease-related mortalities globally. According to data from global surveys, liver disease represents the 11th leading cause of death (Devarbhavi et al. 2023).

Jijiao Wu and Lin Wen contributed equally to this work and should be considered co-first authors.

The liver is composed of several cell types of different embryonic origins, including hepatocytes, bile duct cells, stellate cells, Kupffer cells, and hepatic sinusoidal endothelial cells (Trefts, Gannon, and Wasserman 2017). Hepatocytes represent the predominant epithelial cell population of the liver, comprising approximately 70% of the liver volume. When the liver is affected by pathogenic factors, interactions between hepatocytes and other liver cells result in the development of liver disease (Sato et al. 2019). Bile duct cells are the second largest epithelial cell group, which are the cells in the bile duct lumen. Hematopoietic stem cells are located in the intervertebral disk space, which is a dynamic cell population with two states: quiescent and activated. Injury to the liver causes the activation of hematopoietic stem cells, which in turn deposit collagen, and so forth, resulting in liver scarring and cirrhosis. Kupffer cells are a group of macrophages in the liver that play a role in the immune system daily. Different polarization of Kupffer cells in liver fibrosis aggravates the development of liver disease (Yang, Jia et al. 2023). Hepatic sinusoidal endothelial cells are endothelial cell groups with unique characteristics. They form sieve plates with pore sizes of 50–180 nm in the human hepatic sinus and have the function of a physiological barrier.

Increasing levels of liver fibrosis have a direct impact on increasing mortality from liver disease (Unalp-Arida and Ruhl 2017). The end stage of liver fibrosis develops into cirrhosis, and deaths due to cirrhosis have been increasing globally between 1990 and 2017, with cirrhosis causing more than 1.32 million deaths globally in 2017 (Sepanlou et al. 2020). Despite the incomplete understanding of the mechanism of liver fibrosis, studies have demonstrated that the activation and proliferation of hepatic stellate cells (HSCs) represent the most fundamental cause of liver fibrosis (Higashi, Friedman, and Hoshida 2017), as shown in Figure 1. Given that activated hematopoietic stem cells (aHSCs) represent the primary source of extracellular matrix (ECM) molecules and other proteins that constitute pathological fibrous tissue, various cells in the liver, when stimulated by appealing factors, secrete inflammatory factors, which in turn continuously activate HSCs, inducing the activation, proliferation, and secretion of a variety of fibrotic cytokines, resulting in excessive ECM accumulation, ultimately leading to liver fibrosis. An effective means of treating liver fibrosis is to introduce drugs that can reduce the progression of liver fibrosis. In recent years, the research and development of anti-hepatic fibrosis drugs for different targets have made significant progress (Shan et al. 2022).

According to the 2020 cancer survey data, primary liver cancer is the sixth most commonly diagnosed cancer in the world and the third leading cause of cancer death in the world (Bray et al. 2018). Primary liver cancers include HCC, cholangiocarcinoma (CCA), and hepatoblastoma (HB). A common cause of HCC is liver cirrhosis (Marquardt 2016), in addition to chronic liver disease, alcohol consumption, nonalcoholic fatty liver disease (AFL), environmental factors, and so forth. (Massarweh and El-Serag 2017). The formation

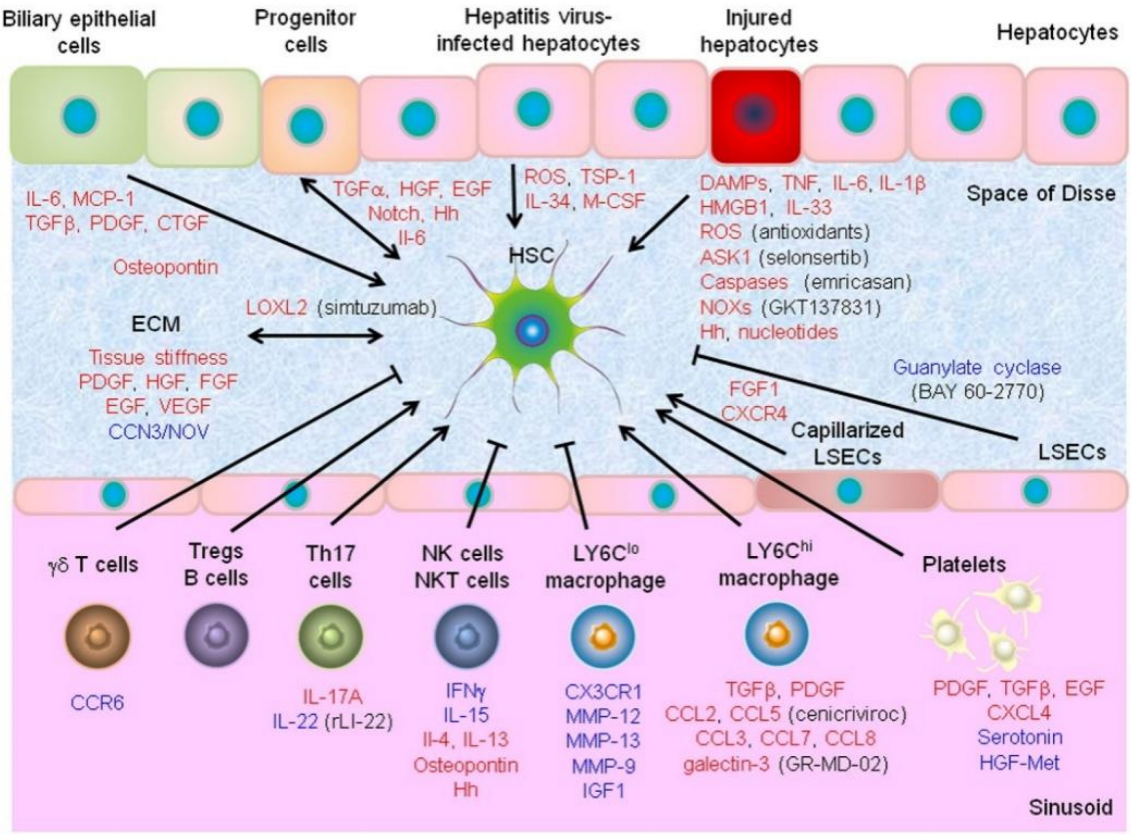

FIGURE 1 | Extracellular regulation of HSC activation. Hepatic resident cells, ECM, and circulating cells promote (sharp arrows) or inhibit (blocking arrows) HSC activation via paracrine factors. Red and blue fonts indicate positive and negative regulators of HSC activation, respectively. Pharmacological interventions for each candidate target are shown in parentheses.

and development of HCC is a complex process involving many stages, systems, and signaling pathways. Current cancer treatments include surgical interventions, radiotherapy, and chemotherapeutic drugs, but the drugs used often kill healthy cells. Therefore, the development of chemotherapeutic drugs that can passively or actively target cancer cells is desirable. Ossipov et al. believe that nanotechnology has the potential to revolutionize cancer diagnosis and treatment (Ossipov 2010). Liver cancer is treated with early liver transplantation, but liver transplantation carries risks and reinfection. Targeted therapy is a promising approach in oncology, and the study of molecular targets of cancer cells is becoming a hot topic, researchers are beginning to design relevant targeted agents based on the pathological developmental features of liver cancer.

Viral hepatitis is a major health problem worldwide. Hepatitis B, C, D, and occasionally E viruses (HBV, HCV, HDV, and HEV) develop in chronic infections, while hepatitis A virus (HAV) is often produced in acute self-limiting hepatitis. The most important goal in the treatment of viral hepatitis is to avoid chronic liver disease and complications (Leoni et al. 2022). Hepatitis B virus (HBV) and hepatitis C virus (HCV) affect more than 320 million people worldwide (Popping et al. 2019). HBV is the leading cause of chronic liver disease worldwide, with approximately 54% of HCC cases caused by HBV infection (Akinyemiju et al. 2017). Currently, the goal of HBV treatment is to suppress viral replication, halt disease progression, and prevent liver cirrhosis, liver failure, and HCC. HDV is a highly pathogenic virus that causes acute, fulminant, and chronic hepatitis, leading to cirrhosis in more than 70% of cases (Botelho-Souza et al. 2017). HCV is one of the most important causes of chronic liver disease and the main goal of treatment is to cure the infection. Direct-acting antivirals (DAAs) approved in 2014 have revolutionized the treatment of hepatitis C, making it possible to cure almost all patients, with high efficacy and tolerability, and are the gold standard for the treatment of chronically infected patients at all stages of liver disease (Pawlotsky et al. 2018).

MASLD is the most common liver disease, with the most recent data available for 2023 suggesting that the global prevalence is estimated to be around 25% (Ko, Yoon, and Jun 2023). With drastic lifestyle changes, MASLD has become the most common liver disease in China (Zhou et al. 2020). MASLD includes simple steatosis (non-AFL), steatohepatitis (nonalcoholic steatohepatitis [NASH]), and liver cirrhosis. Hepatic steatosis is the hallmark of MASLD and may progress to NASH, liver cirrhosis, and HCC. A large body of clinical evidence suggests that MASLD not only increases liver-related morbidity and mortality but also increases the risk of other extrahepatic diseases (Mantovani et al. 2020). MASLD is strongly associated with obesity and insulin resistance, and treatment goals include improving liver fat content, liver inflammation, and liver fibrosis, which can currently be treated with diet and exercise, as well as medications (Brunner et al. 2019).

In conclusion, liver disease persists as a significant global health concern, and researchers have been consistently engaged in efforts to develop efficacious pharmacological interventions and ensure their optimal delivery to affected regions.

2 | Mechanisms of Silybin in the Treatment of Liver Diseases

2.1 | History of Silybin in the Treatment of Liver Diseases

Looking back in history, humankind has been using natural botanicals to treat illnesses for a very long time. The use of the medicinal herb milk thistle (MT) to treat diseases began in ancient Rome and is still in use today (Abenavoli et al. 2010).

MT has been used to treat chronic liver disease since the fourth century BC. Clinical practice using it to treat liver disease has been documented in ancient Greece, India, and China (Abenavoli et al. 2010). With the development of medical technology, research on MT has been continuously updated (Abenavoli et al. 2018; Meier, Saller, and Brignoli 2001; Hawke et al. 2013). In the 1970s, MT was listed by the World Health Organization as an official drug with hepatoprotective properties (WesoŁOwska et al. 2007). In 1959, Biedermann et al. (2014) discovered and isolated the secondary metabolite of MT, silybin (SLB). Bijak (2017) described the molecular structure, bioavailability, and in vivo metabolism of SLB. Abenavoli et al. (2018) reviewed the chemical properties, pharmacokinetics, and pharmacological effects of SLB in the treatment of liver diseases, including anti-inflammatory, antioxidant, antifibrotic, immunomodulatory, and tissue metabolism, and illustrated the potential of SLB in alcoholic liver disease (ALD), non-AFL, and viral hepatitis.

With the development of medicine and pharmacy, researchers have gradually discovered and explained the mechanism and main active components (SLB) of plant MT in the treatment of liver diseases. As for the source of SLB, silymarin was first extracted from the seeds of *Silybum marianum*, which consisted of silybin A and silybin B, isosilybin A, isosilybin B, and polyphenol compounds (Hackett, Twedt, and Gustafson 2013). Among the various components of silymarin, SLB represents the primary bioactive constituent, accounting for approximately 70%–80% of the total content (Bijak 2017). Among the diseases that silymarin can treat, SLB is often the main medicinal ingredient (Dixit et al. 2007). With the help of modern technology, SLB can be well extracted and separated from silymarin (Yang et al. 2014).

2.2 | Mechanisms of Silybin Treatment of Liver Disease

SLB can treat chronic liver disease, liver cancer, liver fibrosis, and other liver diseases because of its anti-inflammatory, antioxidation, and antifibrosis effects (Abenavoli et al. 2018). Federico, Dallio, and Loguercio (2017) reviewed the pharmacological mechanisms of SLB in the treatment of liver diseases and clearly described the molecular mechanisms, signaling pathways, and so forth. (Federico, Dallio, and Loguercio 2017). For example, in the treatment of viral hepatitis, SLB reduces its damage by softening the inflammatory cascade and modulating the immune system, and intravenous administration of SLB can directly interfere with the life cycle of HCV. For the treatment of AFL, SLB increases cell viability under the

influence of ethanol. In the treatment of MASLD, SLB antagonizes progression by intervening in therapeutic targets such as oxidative stress (OS), insulin resistance, hepatic fat accumulation, mitochondrial dysfunction, and so forth. Hackett, Twedt, and Gustafson (2013) review the role of SLB in chronic liver disease. In liver fibrosis, SLB restricts the conversion of HSCs to myofibroblasts (the key to the pathogenesis of liver fibrosis) by interrupting cell signaling. At the same time, SLB reduces stellate cell DNA synthesis, proliferation and migration, and fibrotic tissue production.

2.2.1 | Liver Fibrosis and Cirrhosis

As previously stated, the inhibition of HSC activation energy represents a fundamental approach to the treatment of liver fibrosis (Baghaei et al. 2022). HSCs are activated by growth factors, cytokines, OS, and other factors to become myofibroblasts (Friedman 2003). In liver injury, factors that can activate HSCs include growth factor-β (TGF-β), platelet-derived growth factor (PDGF-B), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), reactive oxygen species (ROS), platelet-activating factor (PAF), and so forth. TGF-β and PDGF-B are both factors that stimulate the proliferation of aHSCs and induce collagen deposition (Bissell 2001; Czochra et al. 2006). Trappoliere et al. (2009) found that SLB, directly and indirectly, resists fibrosis by reducing PDGF-induced cell proliferation and migration and reducing TGF-β-induced new synthesis of type I collagen in an in vitro human liver fibrosis model. The inflammatory response is intimately associated with the development of liver fibrosis. TNF-α is a pro-inflammatory immune mediator that induces tissue damage and produces other cytokines that replenish inflammatory cells; IL-6 is a crucial pro-inflammatory cytokine that has the capacity to activate HSCs. SLB has been demonstrated to reduce the release of IL-6 and effectively disrupt the vicious cycle of inflammation-related liver fibrosis. TNF-α, IL-6, and ROS promote the initiation of HSC activation (Brenner et al. 2013). In addition, ROS is also a part of PDGF signal transduction. The increase in intracellular ROS production is related to the direct profibrotic effect, that is, the increased expression and secretion of type I procollagen (Casini et al. 1997). SLB significantly reduces ROS production, thus exerting anti-inflammatory and antifibrotic effects (Trappoliere et al. 2009). PAF is produced in large quantities in inflammatory environments and promotes the ability of HSCs to produce large amounts of collagen. The two different types of LPCAT1 and LPCAT2 of lysophosphatidylcholine acyltransferase can reduce PAF levels, but their expression is decreased when liver fibrosis and cirrhosis occur. SLB restored the activity and expression of LPCAT1 and LPCAT2 in liver cirrhosis by increasing the genes encoding LPCAT1 and LPCAT2, significantly reducing the level of pro-inflammatory lipids and exerting anti-inflammatory effects (Stanca et al. 1832).

In addition to the key role of stellate cells and myofibroblasts in ECM production, the generation and regression of hepatic fibrosis also involves other resident cells such as macrophages, which have different subpopulations, some profibrotic and some antifibrotic, such as specific fibrotic subpopulations of macrophages ("Ly-6C+ lo" cells) directly promote fibrosis regression (Lee, Wallace, and Friedman 2015). Prior research has demonstrated that SLB can influence macrophage polarization and reduce liver fibrosis (Federico, Dallio, and Loguercio 2017).

2.2.2 | Liver Cancer

In the treatment of HCC, a variety of cell cycle proteins are involved in the development of the etiology of HCC (Chetty 2003). Some nontoxic drugs can be used to play the role of cell cycle regulators, inhibit cell growth, or make cell apoptosis, so as to achieve the purpose of treating liver cancer. SLB is a drug that is consistent with this approach and has been shown to treat HCC by regulating cyclins (Varghese et al. 2005). In addition, SLB is a broad-spectrum anticancer drug with therapeutic effects on a variety of cancers, such as lung cancer (Verdura et al. 2021), breast cancer (Si et al. 2019), prostate cancer (Anestopoulos et al. 2016), skin cancer (Singh and Agarwal 2005), colon cancer (Shafiei et al. 2023; Agarwal et al. 2003), bladder cancer (Tyagi 2004), ovarian cancer (Wei et al. 2022), and liver cancer (Varghese et al. 2005; Lah, Cui, and Hu 2007).

In human HCC HepG2 and Hep3B cells, SLB exerted growth inhibitory, cytotoxic, and apoptotic effects, and was more cytotoxic to Hep3B cells. SLB decreased the levels of cell cycle protein D1, cell cycle protein D3, cell cycle protein E, cell cycle protein-dependent kinase (CDK)-2, and CDK4 in both HCC cell lines. In HepG2 cells SLB caused G1 block, and in Hep3B cells SLB caused G1 and G2-M block, as well as a decrease in protein levels of G2-M regulators. These experimental results establish for the first time the biological efficacy of SLB on HCC cells to support the clinical application of SLB in HCC (Varghese et al. 2005). Zhang and co-workers found that silymarin-induced apoptosis in HepG2 cells via the mitochondrial pathway, downregulating the expression of Bcl2 protein (an anti-apoptotic protein) and upregulating the expression of Bax protein (a pro-apoptotic protein) and the activity of caspase3 (a key step in the apoptotic process) (Lee et al. 2013). For apoptosis induced by ethanol or acetaldehyde in human HCC HepG2 cells and immortalized liver HL7702 cells, SLB increased cell viability, inhibited apoptosis, and restored mitochondrial function. SLB protects cells from apoptosis induced by ethanol or acetaldehyde-induced mitochondrial fission (Song et al. 2022).

In addition to cell cycle regulation, SLB may also inhibit HCC by inhibiting HCC cell growth and proliferation, blocking invasion and metastasis, among other ways. Yassin et al. (2022) proved that Sb significantly inhibited HCC cell proliferation, Wnt/β-catenin, and PI3K/Akt/mTOR signaling pathways, hepatocyte growth factor (HGF)/cMET, and OS. The formation and development of HCC involve many stages, systems, and signaling pathways (Zou et al. 2016). Abnormal Wnt-β-catenin signaling promotes the development and progression of different liver diseases, such as HCC and CCA. Meanwhile, the Wnt/β-catenin pathway is associated with poor prognosis in HCC patients and plays a key role in promoting HCC development by increasing cell proliferation and inhibiting cell adhesion (Takigawa and Brown 2008). SLB treatment significantly reduced mRNA expression of Wnt, which in turn inhibited cell proliferation and tumorigenicity (Perugorria

et al. 2018). Activation of the phosphatidylinositol-3 kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway is involved in the pathogenesis of many tumor types, such as HCC pathogenesis (Meric-Bernstam et al. 2012). Dysregulation of HGF and its receptor c-MET (HGF/c-Met) has been associated with the promotion and progression of HCC, and blockade of the c-Met receptor inhibits tumor growth, cell proliferation, angiogenesis, and migration in liver cancer (Xie et al. 2010). OS plays an important role in hepatocellular carcinogenesis by disrupting normal cell function or genetic material and interfering with signal transduction pathways (Wang et al. 2016). Similarly, Lah, Cui, and Hu (2007) reported that SLB significantly inhibited the growth of human HCC cells HuH7, HepG2, Hep3B, and PLC/PRF/5. Momeny et al. (2008) found that silymarin effectively inhibited the growth and proliferation as well as the invasive properties of HepG-2 cells.

In HepG2 cells, the cytochrome P450 isoform CYP1A1 is the only CYP enzyme that can be induced to be expressed and is involved in the induction of carcinogenesis by chemicals such as polycyclic aromatic hydrocarbons, and Dvořák, Vrzal, and Ulrichová (2006) have reported that SLB inhibits the catalytic activity of CYP1A1, favoring its cytoprotective role in liver metabolism. In addition, when the concentration of SLB is 200 mM, it can induce DNA migration of HepG2 cells, produce oxidized DNA bases, reduce cell viability, reduce cell replication index, and induce OS, that is, high-concentration (200 IM) SLB has antigenotoxic activity (Angeli et al. 2009).

2.2.3 | Viral Hepatitis

SLB has potent antiviral activity against HCV infection (Ferenci et al. 2008). SLB has been used in several clinical trials for the treatment of hepatitis C. Intravenous SLB inhibits viral replication by directly interfering with the HCV life cycle, inhibiting protein expression in polymorphonuclear cells and HCV viral replication at 20 μmol/L in patients with chronic HCV infection (Morishima et al. 2010). Polyethylene glycolated interferon (IFN) plus ribavirin therapy is a treatment for HCV (Fried et al. 2002), but it remains ineffective in some patients and interferon therapy is expensive with significant side effects. SLB can inhibit HCV RNA-dependent RNA polymerase function independently of the intracellular interferon (IFN)-induced antiviral pathway (Ahmed–Belkacem et al. 2010). Ferenci et al. (2008) also analyzed the effect of intravenous administration in inhibiting viral replication and confirmed that SLB is an effective antiviral agent in patients with chronic hepatitis C for whom polyethylene glycolated interferon/ribavirin therapy is ineffective. Meanwhile, in the case of limited therapeutic efficacy of triple therapy with first-generation protease inhibitors in the treatment of HIV/HCV co-infected patients with advanced hepatic fibrosis, and the lack of patient response to pegylated interferon-ribavirin, the introduction of silymarin before triple therapy was evaluated as beneficial to the treatment of this condition. The results showed that the introduction of silybin before triple therapy was safe and effective for patients with HIV/HCV co-infection who were difficult to treat. It was found that HCV-RNA decreased significantly in the introduction stage (George et al. 2015). In addition, it also inspires the possibility of introducing SLB into standard-of-care therapy in selected patients with refractory HCV in the future, which deserves continued exploration by investigators.

The family of signal transducer and activator of transcription (STAT) proteins are transcription factors, response cytokines, and growth factors, of which transcription activator-3 (STAT3) is the most studied, and it plays an important role in all aspects of the HCV life cycle (Kuchipudi 2015). STAT3 directly interacts with and is activated by HCV, which in turn increases HCV replication (Fried et al. 2002). SLB is a natural STAT3 downregulator and blocks the constitutive activation of STAT3 through its role as a hyperphosphorylated STAT3 inhibitor (Bosch-Barrera, Queralt, and Menendez 2017; Bosch-Barrera and Menendez 2015).

2.2.4 | Non-AFL

Mitochondrial dysfunction and OS are decisive events in the pathogenesis of NASH. Haddad et al. (2011) analyzed the results of SLB treatment in a rat model of NASH and concluded that SLB ameliorates hepatic steatosis and inflammation by decreasing NASH-induced lipid peroxidation, plasma insulin, and TNF-α. Baldini et al. (2020) proved that SLB improved the fatty acid profile of lipid droplets, stimulated mitochondrial oxidation, upregulated microRNA miR-122 related to liver fat metabolism, and restored aquaporin-9 (AQP9) and glycerol permeability levels. At the same time, it reduces the activation of OS-dependent transcription factor NF-κB and the conversion of autophagy. Excessive intake of fat and sugar is the main cause of MASLD. Fructose and fatty acids positively interfere with the adipogenesis pathway, leading to increased steatosis and liver dysfunction, decreased cell viability, increased apoptosis, OS, and mitochondrial respiration. Treatment with SLB completely alleviates hepatocellular abnormalities. Grasselli and coworkers reported that the hepatoprotective effect of SLB mainly affects mitochondrial function, downregulates the expression of PPARγ (the main transcription factor of lipogenesis gene), reduces intracellular ROS production, lipid peroxidation, and apoptosis rate, and treats MASLD together (Baldini et al. 2019).

2.2.5 | Other Liver Diseases

In addition to these four categories of liver diseases mentioned above, ALD and drug-induced liver injury (DILI) can also be treated with SLB. However, there are few studies on related preparations, and it is expected that researchers will further explore them.

ALD is the most common type of chronic liver disease worldwide and can progress from AFL to alcoholic steatohepatitis (ASH), with the potential for fibrosis cirrhosis, and HCC (Seitz et al. 2018). Alcohol abstinence is the basic treatment of ALD. ALD is related to the changes in cell redox potential caused by lipid peroxidation and ethanol metabolism, and mitochondrial dysfunction caused by direct toxic effects caused by acetaldehyde accumulation. SLB can treat or assist in the treatment of ALD by regulating OS and improving mitochondrial function (Detaille et al. 2008).

Liver damage can accompany common liver diseases and can also be caused by toxins or drugs. Some toxic mushrooms can cause hepatotoxicity and liver damage, and SLB has been used clinically in this regard for a long time. Pharmacological liver injury occurs when patients use medication, and studies have shown that herbal medicines have become an important cause of pharmacological liver injury, herbal medicine-induced hepatotoxicity is based on mechanisms such as mitochondrial homeostasis, oxidative damage, apoptosis, and specific responses (Pan et al. 2020). SLB is a hepatoprotective agent that ameliorates DILI as well as experimental liver injury.

As research into the pathogenesis of SLB in the treatment of various liver diseases becomes increasingly comprehensive and precise, it can assist researchers in the development of more effective drug delivery systems for the treatment of these diseases.

3 | Limitations of Silybin in the Treatment of Liver Disease

Despite the promising efficacy of SLB in the treatment of liver disease, its use is still limited. The limitations are mainly due to SLB's low solubility, and low bioavailability (Abenavoli et al. 2010, 2018; Meier, Saller, and Brignoli 2001). According to the analysis of chemical structure, SLB is a flavonoid component, and the solubility of SLB in water is about 0.5 g/L. The solubility of silymarin in water is about 1.5 g/L (Gazak, Daniela, and Vladimir 2007). It is insoluble in polar proton solvent (methanol), insoluble in nonpolar solvent (chloroform), but highly soluble in polar nonproton solvent (acetone) (WesoŁOwska et al. 2007). Analyzing the metabolic processes in vivo, the low oral bioavailability of flavonoids is due to extensive first-pass metabolism in the intestine and liver (Zhang, Zuo, and Lin 2007). Wu et al. (2007) demonstrated that the absolute oral bioavailability of SLB in rats is about 0.95% and discussed that the poor bioavailability may be due to high phase II coupling reactivity and poor absorption. After administration to rats, SLB rapidly binds to sulfate and glucuronide in the liver and is excreted through the bile. Due to the rapid distribution and equilibrium of SLB between the blood and the hepatobiliary system, the levels of unconjugated and total silymarin in the bile are higher than those in the plasma (Wu et al. 2007; Yanyu et al. 2006; Han et al. 2004).

In the current application, intravenous SLB prevents reinfection of liver grafts and is used in the HCV liver transplant setting (Neumann et al. 2010). Long-term intravenous SLB has shown potent anti-HCV activity with no apparent toxicity, but this application involves the specialized medical care requirements of intravenous infusion, making the treatment economically burdensome and of limited availability.

With the development and application of new technologies of traditional Chinese medicine preparations, new preparations or drug delivery systems such as nanopreparations continue to improve the solubility and bioavailability of SLB, and the research on SLB-targeted preparations is also increasing. This article reviews the preparations of SLB in the treatment of liver diseases from two aspects. On the one hand, it is a targeted preparation, that is, based on the mechanism of liver disease, the preparation is ingeniously designed to target the diseased tissue. On the other hand, it is a new preparation, such as the application of nanotechnology to improve the solubility and bioavailability of SLBs.

4 | Silybin Preparations for the Treatment of Liver Diseases

4.1 | Liver Fibrosis and Cirrhosis

HSCs account for about 15% of the total number of resident cells in normal liver. It is located in the subendothelial space between the basolateral surface of hepatocytes and the anti-lumen side of sinusoidal endothelial cells so that the drug is easily internalized by Kupffer cells and hepatic sinusoidal endothelial cells before reaching hematopoietic stem cells (Yin et al. 2013). Many small pores with an average diameter of 100 nm are present on the surface of hepatic sinusoidal endothelial cell LSECs, so the particle size of the drug and carrier must be small enough (< 100 nm) to pass through these pores to further reach the activated stellate cells. SLB itself can not actively target the liver and can achieve the purpose of active targeting through appropriate carriers. For example, SLB is targeted to the liver by the CD44 receptor and retinol-binding protein receptor, which are highly expressed on the surface of HSCs, or the particle size is reduced by nanotechnology to achieve passive targeting (Evangelopoulos and Tasciotti 2017; Bertrand et al. 2014). Bonepally et al. state that various nanoparticle systems are currently focused on targeting HSCs to treat liver fibrosis (Bonepally et al. 2013).

4.1.1 | Targeted Agents

4.1.1.1 | CD44 Receptor. The CD44 receptor is a cell surface glycoprotein widely expressed in human lymphocytes, fibroblasts, and smooth muscle cells (Weng et al. 2022). When hepatic fibrosis occurs, the expression of the CD44 receptors on the surface of hematopoietic stem cells in the liver is significantly increased. At present, chondroitin sulfate (CS) and hyaluronic acid (HA), through their binding to the CD44 receptor, mediate drug targeting to the liver.

CS is a sulfated polysaccharide found on the surface of mammalian cells and in the ECM (Li, Su, and Liu 2017). It not only protects collagenase from premature inactivation but also recognizes the targeted membrane receptor CD44, which is used in the field of drug delivery carriers (Lee et al. 2021). Luo et al. (2021) first prepared nanoparticles loaded with collagenase and SLB by thin-film hydration, and then combined CS based on charge neutralization to form COL + SLB-MLPs, as shown in Figure 2. CS mediated the targeting of COL + SLB-MLPs to activated HSCs by specifically recognizing and binding to the CD44 receptor, followed by the release of collagenase and SLB from the nanoparticles to break down the dense collagen matrix and inhibit activated HSCs, respectively, which synergistically inhibited liver fibrosis.

HA is a nonsulfonated glycosaminoglycan composed of *n*-acetylglucosamine and glucuronic acid, which is the main component of ECM (Berdiaki et al. 2023). HA is an endogenous

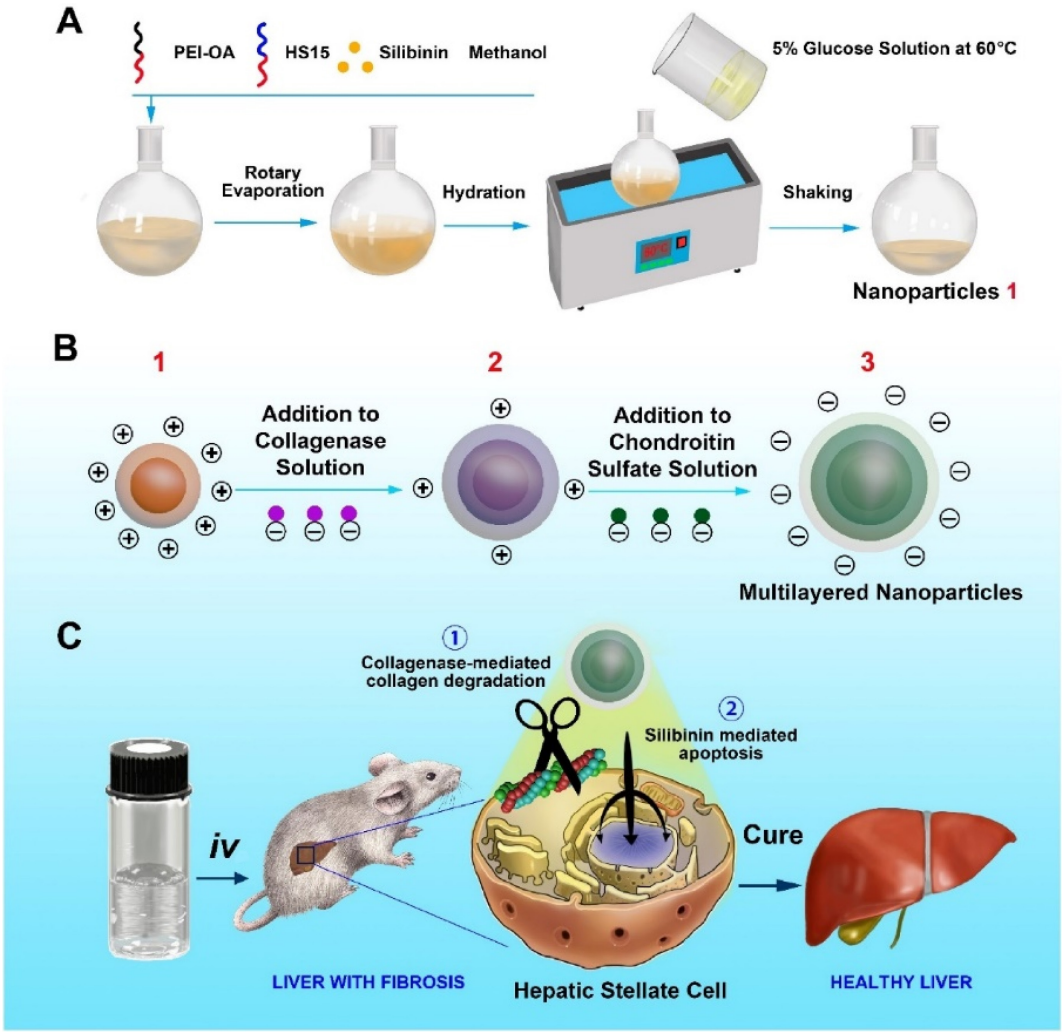

FIGURE 2 | Schematic illustration of the preparation of multilayered nanoparticles containing collagenase and silybin, and their synergistic effects in fibrotic liver. (A) Preparation of precursor nanoparticle 1 by thin-film hydration. (B) Encapsulation of collagenase and silybin into nanoparticle 1 by electrostatic attraction, followed by surface coating with chondroitin sulfate to generate the final nanoparticle 3. (C) Targeting of hepatic stellate cells by final nanoparticle 3 to treat liver fibrosis through a two-pronged mechanism. HS15, solutol HS15; iv, intravenous injection; OA, oleic acid; PEI, polyethyleneimine.

substance and is also considered a biodegradable, biocompatible, nonimmunogenic, and nontoxic biomaterial (Massarweh and El-Serag 2017). HA specifically binds to the CD44 receptor overexpressed on the surface of various tumor cells (Zhang, Sun, and Jiang 2018). Li et al. (2020) designed HA (SLB-HA) micelles loaded with SLB, as shown in Figure 3. The HA micelles exhibited specific uptake by hematopoietic stem cells and significant hepatic targeting through CD44 receptor-mediated endocytosis, avoiding drug distribution in normal hepatocytes and phagocytosis by macrophages. The SLB-HA micelles selectively killed activated HSCs and exhibited excellent anti-hepatic fibrosis and significant slow-release effects in vivo. It also represents a novel nanomicellar system with great potential in anti-hepatic fibrosis drug delivery.

Yang, Tan et al. (2023) prepared homogeneous SLB nanohybrids (NS-SLB) by self-assembly and modified them with HA-cholesterol coupling (NS-SLB-HC) to enhance the ability of NS-SLB to target CD44, as shown in Figure 4. NS-SLB-HC was shown to target aHSCs through receptor-ligand interactions between HA and CD44. It reversed hepatic fibrosis in vivo by downregulating TGF-β and inhibiting the secretion of α-SMA and type I collagen. In this study, SLB-M, a medical excipient analog, was found to construct a minimal carrier drug delivery system with high drug delivery efficiency, safety, and anti-hepatic fibrosis efficacy. SLB-M and SLB are self-assembled by π–π stacking to form a homogeneous nanosuspension (NS-SLB) with an average particle size of 37.9 ± 7.2 nm. Due to its small size, it accumulates in the liver, crosses the Disse barrier, is removed from the mononuclear phagocyte system, and reaches aHSCs.

4.1.1.2 | Secreted Protein, Acidic, and Cysteine-Rich. Secreted protein, acidic and cysteine-rich (SPARC), is a multifunctional glycoprotein and a typical albumin-binding protein, highly expressed in aHSCs when liver fibrosis occurs (Nakatani et al. 2002). It induces tissue reconstruction and growth, regulates the interaction between cells and ECM, and mediates the internalization of albumin by cells. It is a promising tumor-specific drug delivery target (Zhao et al. 2018). SPARC binds to human serum albumin (HSA), a versatile drug carrier with

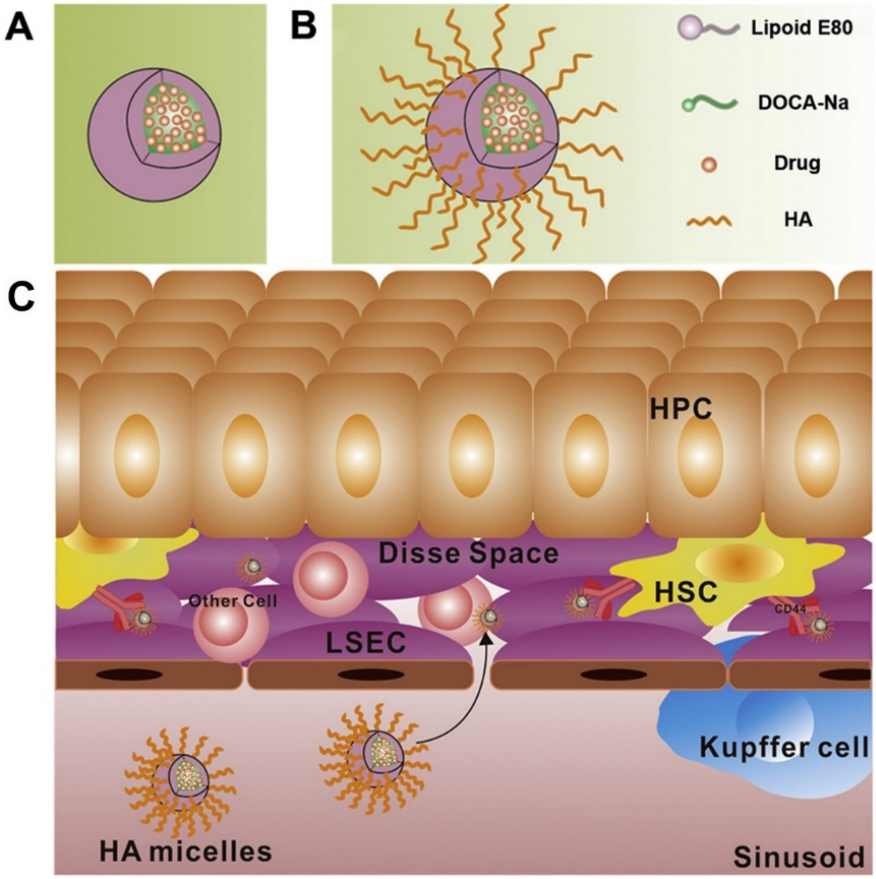

FIGURE 3 | (A) Phospholipid bile salt micelles and (B) HA micelles fabrication. (C) Strategic illustration for the application of HA micelles to target HSCs. Targeted delivery of hyaluronic acid 695.

intrinsic biocompatibility for the construction of nanomedicines and nanotherapeutics (Zhang et al. 2020).

The SLB-albumin nanocrystals prepared by Luo et al. (2023) have two features compared to previous findings, as shown in Figure 5. The first feature is that the previous silymarin-albumin nanocrystals had a large particle size (up to 188 nm) (Sohrabi et al. 2019), and the inability to reach the aHSCs in the Disse space via LSECs. To reduce the size of the SLB nanocrystals, Luo et al. cleverly added SLB-methylamino (SLB-M), an amphiphilic material that can reduce the surface tension of the nanocrystals and decrease the nanocrystals' particle size (Yang et al. 2019). The second feature is that based on the fact that SLB is insoluble in aqueous solution but soluble in strongly alkaline solution, Luo et al. prepared SLB nanocrystals with high-loading capacity by using the precipitation method of an acid-base neutralization reaction. The change of acid-base conditions led to the rapid nucleation of SLB and its development into nanocrystals, and the amphiphilic SLB-M adhered to the crystal surface and reduced the particle size. After this, HSA is adsorbed on the crystal surface to provide sufficient spatial stability and electrostatic repulsion to inhibit particle aggregation and maintain the particle size. In summary, SLB-HSA nanoparticles with a particle size of 60.78 ± 1.51 nm were able to passively target aHSCs by smoothly passing through the 100 nm-diameter pores on LSECs, and actively target aHSCs by the HSA on the surface in combination with SPARC-mediated endocytosis.

4.1.1.3 | Retinol-Binding Protein Receptors. Vitamin A, a series of retinoid compounds (retinol, retinoic acid, and retinaldehyde), is stored in the HSCs of the liver (Romeo and Valenti 2016). HSCs have retinol-binding protein receptors (Saxena and Anania 2015). The retinol-modified carriers can be specifically targeted to the liver, and Pan et al. (2016) designed retinol-modified lipid nanoparticles loaded with SLB, which were found to accumulate rapidly in the liver and spleen, and the nanoparticles' inclusion of SLB significantly reduced pulmonary deposition and increased hepatic uptake.

The occurrence of hepatic fibrosis is closely related to HSCs and Kupffer cells. When a liver injury occurs, macrophages release ROS, profibrotic factors, and aHSCs (Sato et al. 2016). Therefore, targeting macrophages is also a method for treating liver fibrosis, that is, targeting Kupffer cells in the liver. However, only SLB can not target Kupffer cells or HSCs, and appropriate carrier materials are needed.

Hayashi et al. (2018) synthesized multifunctional organic–inorganic hybrid hollow nanoparticles (HNPs) of SLB, as shown in Figure 6. It can be taken up by Kupffer cells via phagocytosis after entering the body, which were able to target HSCs by modifying the surface of HNPs with retinol. SLB reduces fibrotic tissue and improves liver function by being released from the cracks formed by the deformation of HNPs in vivo. The experimental results show that the therapeutic effect of HNPs containing SLB is greater than that of SLB injection alone.

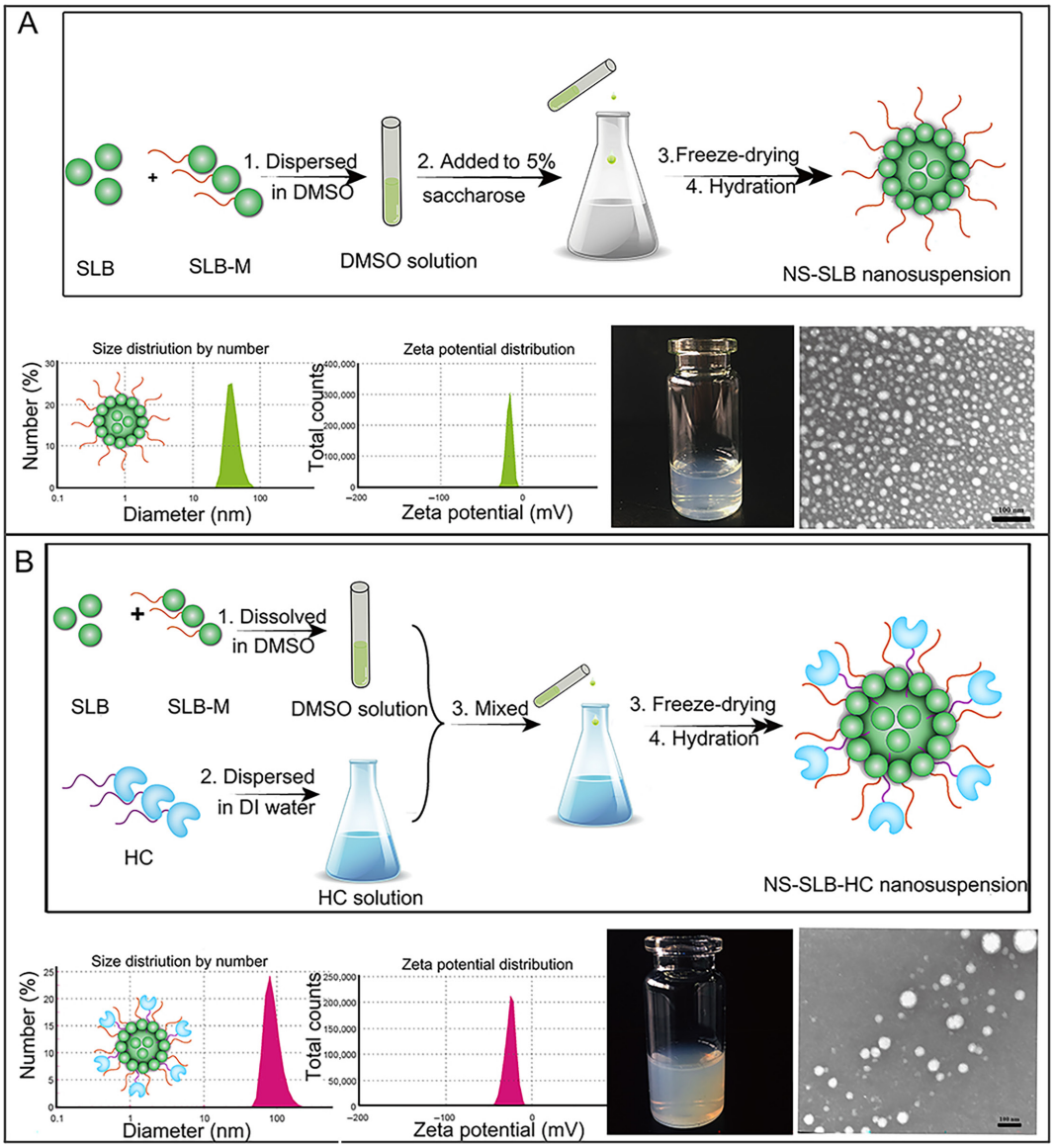

FIGURE 4 | Preparation, dynamic light scattering measurements, photographs in solution, and transmission electron micrographs of (A) NS-SLB and (B) NS-SLB-HC. Scale bar, 100 nm.

4.1.1.4 | **Vitamin A.** Although HSCs lose the deposition of vitamin A during activation, they have significant vitamin A uptake capacity as static HSCs (Zhang et al. 2015). Therefore, vitamin A has the specificity to target HSC and can be used to modify nanoparticles (Sato et al. 2008). (Lactic acid-co-g lycoside)-poly(spermidine)-poly(ethylene glycol)-vitamin A (PLGA-PSPE-PEG-VA) self-assembled into core-shell polymer micelles at low concentrations. After SLB entered the PLGA hydrophobic core, the genetic drug siCol1α1 bound to PSPE via electrostatic interactions to give the resulting chemically/genetically drug-loaded (CGPYM), as shown in Figure 7. It effectively accumulates in the fibrotic liver and specifically targets activated HSCs (Qiao et al. 2018). PEG located at the periphery of the polymeric micelles helped to reduce protein adsorption, decrease nonspecific uptake, and prolong circulation time, and exposed vitamin A provided specific targeting of activated HSCs. Upon internalization of the polymeric micelles, PSPE buffers the acidic endosomes, disrupting the membrane by increasing internal osmotic pressure, and polymeric micelle endosomes escape. Subsequent release of SLB and siCol1α1 from polymeric micelles into the cytoplasm inhibits collagen I expression.

4.1.1.5 | **Glycyrrhetinic Acid Receptor.** The glycyrrhetinic acid (GA) receptor is highly expressed in hepatocytes. Lipid nanoparticles are modified with GA, which can specifically bind to improve the targeting efficiency, and then encapsulate the traditional hepatoprotective drug SLB, thereby eliminating excessive ROS and promoting hepatocyte regeneration.

During liver fibrosis, capillaries hepatic sinusoidal endothelial cells (LSECs), aHSCs, and dysfunctional hepatocytes crosstalk with each other to form a vicious cycle that aggravates the disease. Zhang et al. (2024) proposed a vicious cycle-breaking strategy by targeting and repairing pathological cells, respectively, to stop the malignant progression of liver fibrosis. They first

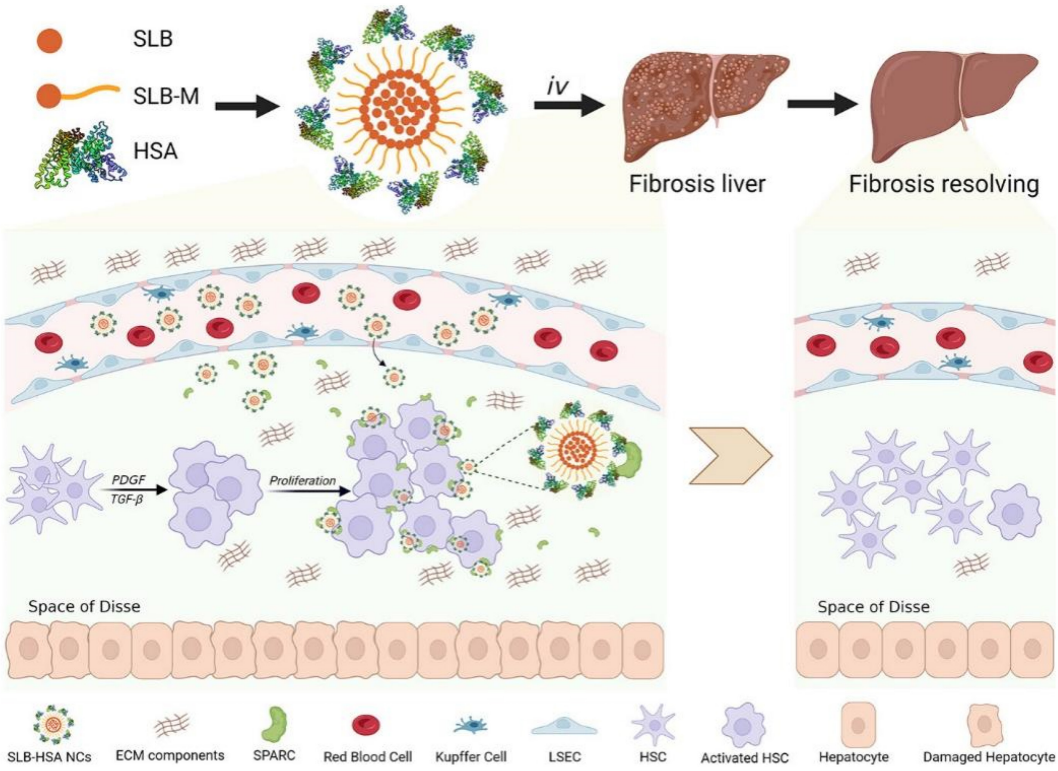

FIGURE 5 | Summary map of literature SLB-HSA nanoparticles. Copyright 2023 Wiley Online Library (Luo et al. 2023).

designed CS-modified and viscosity-loaded nanoparticles (CS-NPs/VDG) to effectively normalize LSECs and restore aHSCs to static. At the same time, GA-modified and SLB-loaded nanoparticles (GA-NPs/SIB) were prepared to restore hepatocyte function by alleviating OS, as shown in Figure 8. The results of the study demonstrated that the simultaneous multiple modulation of pathological cells by the two preparations successfully blocked the vicious cycle and showed significant fibrosis regression.

In summary, SLB targets hepatic agents via biological targets such as CD44 receptors, SPARC, retinoid receptors, and several other pathways for antifibrotic purposes (Schuppan et al. 2018).

4.1.2 | Nanopreparations

The targeted preparation of SLB can effectively deliver the drug to the diseased site. In addition, nanotechnology has a unique role in solving the problems of low water solubility, poor bioavailability, high metabolism, and poor permeability of intestinal epithelial cells. In the treatment of liver fibrosis and cirrhosis, nanotechnology also has good results in improving the low solubility and low bioavailability of SLB.

Nanoparticles have the advantages of maximum drug loading and longer shelf life to improve hydrophobic drug solubility. Bonepally et al. (2013) successfully prepared SLB nanoparticles using an o/w emulsion solvent evaporation technique with an average particle size of 130–430 nm, which sustained the release of the drug for up to 10 days in vivo and in vitro and had better pharmacokinetic properties than the free drug. It is useful for the treatment of cirrhosis and fibrosis.

Di Sario et al. (2005) evaluated the hepatoprotective and antifibrotic properties of a novel SLB-phosphatidylcholine-vitamin E complex with high bioavailability and lipophilicity, therapeutic effect on dimethylnitrosamine and cholangiocolitis-induced hepatic fibrosis in rats, reduction of HSC proliferation and activation, and reduction of collagen deposition and type I collagen mRNA expression.

Phytosome is the conversion of water-soluble plant constituents into lipid-compatible complexes, which improves the bioavailability of the active ingredient by enhancing the ability to traverse lipid-rich biofilms and enter the circulating (Rossi et al. 2009). SLB-phytosome inhibits collagen accumulation and ROS production, thereby inhibiting HSC activation and halting the progression of cirrhosis through its antioxidant and antifibrotic effects (Ali, Darwish, and Ismail 2014).

In summary, the above-mentioned targeted agents based on the pathogenesis and the conventional nanopreparations using nanotechnology to improve the limitations, both of which are well designed and applied to help SLB treat liver fibrosis and cirrhosis, and the future research on these two types of preparations still has great potential for development.

4.2 | Liver Cancer

4.2.1 | Targeted Agents

4.2.1.1 | **Folate Receptors.** The folate receptor (FR) is a glycosylphosphatidylinositol (GPI)-anchored membrane protein, which previous studies have demonstrated to be overexpressed in breast, ovarian, and colorectal cancers, and low-expressed

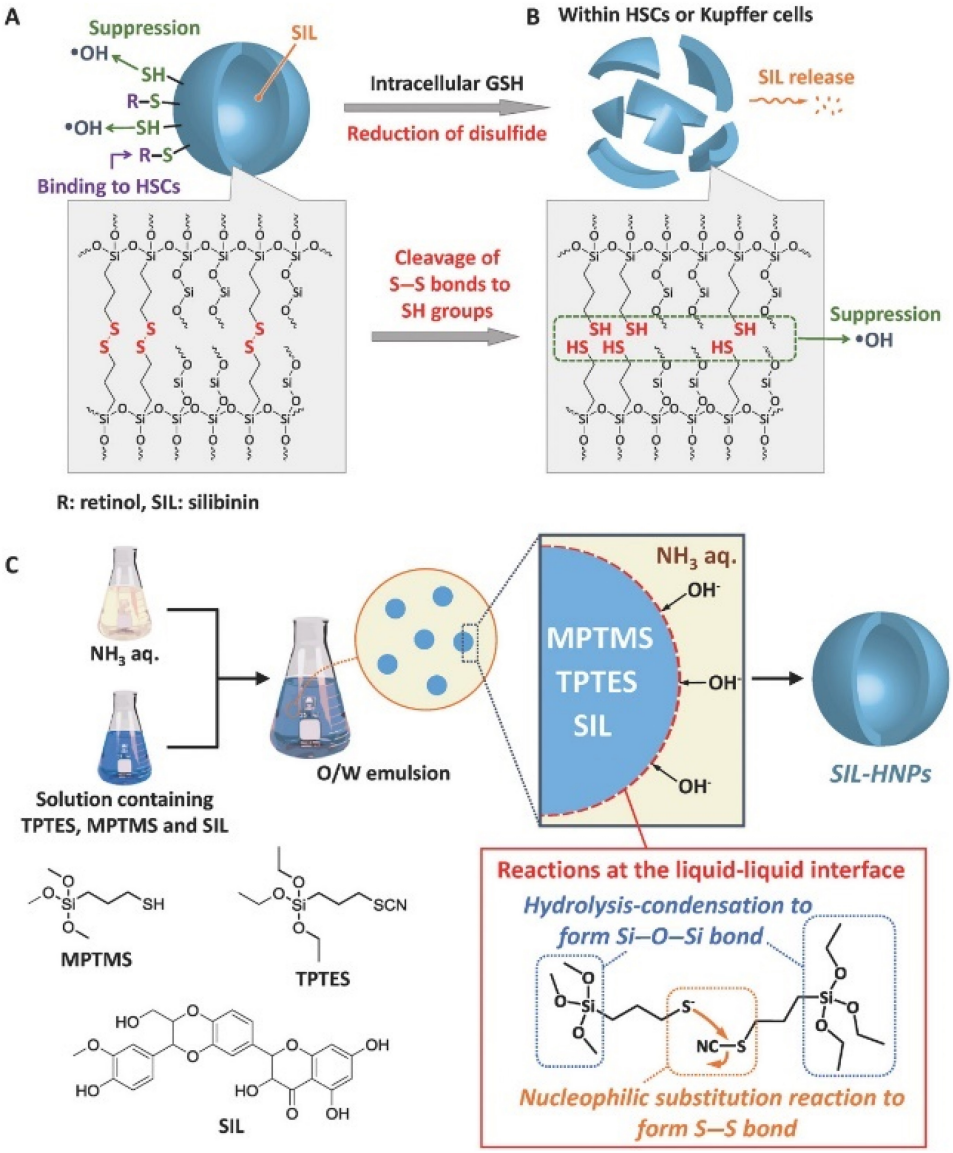

FIGURE 6 | Summary plot of (HNPs) of literature silymarin. Copyright 2023 Wiley Online Library (Hayashi et al. 2018).

and sparsely distributed in normal tissues (Cheung et al. 2016). The FR is an attractive therapeutic target, not only for localizing cancerous tissues but also for enabling selective drug delivery, which can be applied to personalized diagnosis and treatment of cancer (Ledermann, Canevari, and Thigpen 2015).

Koirala et al. (2019) performed immunohistochemistry with FRs in normal and HCC human and rat liver tissue samples and found that FR expression was significantly upregulated in HCC tissues, based on this they chose to target HCC with FR delivered drugs. They designed and evaluated the specificity and toxicity of folate-containing drug delivery vectors (DVDs) in a model of HCC, and their study. The results suggest that polyethylene glycol (PEG)-conjugated and folic acid-targeted via the intra-arterial route is an effective strategy for targeted delivery in the treatment of HCC.

SLB-containing folic acid-targeted nanomicelles, namely SLB-F127-FA nanomicelles, were prepared by Ghalehkhondabi, Soleymani, and Fazlali (2021). First, folic acid was coupled to the hydrophilic chain of Pluronic F127 copolymer using the Steglich esterification technique. Second, SLB was encapsulated in self-assembled FA-conjugated F127 hydrophobic cores to obtain SLB-F127-FA nanocolloid micelles. The SLB-F127-FA was approximately spherical with an average particle size of 17.7 nm. The drug loading and encapsulation efficiency were 2.36% and 79.43%, respectively. The in vitro release demonstrated a gradual and sustained release profile. In vitro cytotoxicity studies revealed that the cytotoxicity of SLB-F127-FA to HepG2 cancer cells was markedly elevated in comparison to nontargeted and free SLB. Consequently, it can be regarded as a promising liver cancer-targeted delivery platform, as shown in Figure 9.

4.2.2 | Nanopreparations

Nanotechnology has many advantages in cancer treatment, including the ability to encapsulate poorly soluble drugs, protect therapeutic molecules, alter their blood circulation and tissue distribution, and promote the combination therapy commonly

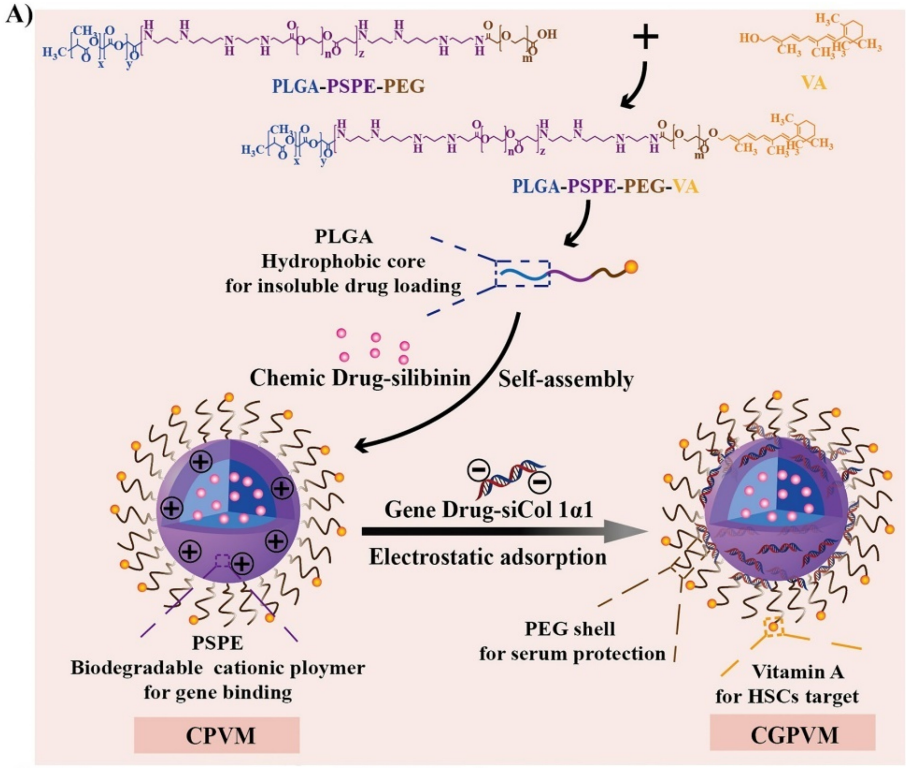

FIGURE 7 | (A) Reaction scheme for the synthesis of PLGA-PSPE-PEG-VA and illustration of the formation of CGPVM.

used in cancer treatment. As a result, the use of nanotechnology in cancer drug delivery and development has grown exponentially since the early 2000s (Bertrand et al. 2014).

Shete, Deshpande, and Shende (2022) review the mechanism of anticancer action of SLB and its synergistic combinations, focusing on the application of nanotechnology in the treatment of different cancers with SLB.

Zhang, Wang, and Liu (2016) used a central composite rotatable design response surface methodology to optimize the prepared SLB nanoparticles. Normal saline, SLB 30 mg/kg body weight, and nanopreparation equivalent to the dose of SLB were orally administered to three groups. Under the optimized conditions, the encapsulation efficiency of SLB NPs was 88%, the drug loading was 15%, and the average diameter was 216 nm. Finally, according to the results of liver nodule count and H&E staining data of tissue sections, it was concluded that oral administration of SLB NPs was more effective and safe than SLB in the treatment of liver cancer in rats.

Nanoplant liposome is an innovative preparation that enhances the bioavailability of hydrophilic flavonoids. It is a drug carrier with a lipid membrane. Nanoplant liposome vesicles, formed by the hydrogen bond interaction between lipid membrane phospholipids and plant molecules, are utilized to improve the delivery of therapeutic agents. For instance, when flavonoids are combined with phospholipids, such as phosphatidylcholine, nanoplant liposomes are created. These liposomes have improved absorption spectra and more appropriate lipid solubility, allowing them to penetrate biofilms (Shete, Deshpande, and Shende 2022).

Ochi et al. (2016) prepared co-encapsulated nanoliposomes of SLB and GA using the HEPES buffer thin layer membrane hydration method. The liposomes were prepared using dipalmitoyl phosphatidylcholine (DPPC), cholesterol (CHOL), and methoxy-polyethylene glycol 2000 (PEG2000) derived distearoyl phosphatidylethanolamine (mPEG2000-DSPE) in a specific molar ratio. The in vitro experiments demonstrated that nanoliposomes containing SLB and GA enhanced the biological activity of the drugs and improved the stability of SLB. The average particle size of the nanoliposomes was 46.3 nm. The encapsulation efficiency of SLB was 24.37%, and the encapsulation efficiency of GA was 68.78%. The half maximal inhibitory concentration (IC50) for co-encapsulated pegylated nanoliposomal herbal drugs was 48.68 μg/mL, while the IC50 for free SLB with GA was 485.45 μg/mL on the HepG2 cell line. Moreover, the co-loaded nanocarrier demonstrated a cytotoxicity that was threefold higher than that of the conventional herbs.

SLB can easily cross the lipophilic pathway of the enterohepatic cell membrane after tightly wrapping the phospholipid (Wellington and Jarvis 2001). Phytoliposomes composed of natural soy lecithin and its SLB-phospholipid complex delivered the drug in a nano shuttle, and SLB was found to be fully incorporated into the liposomes by UV–visible absorption spectroscopy, and its solubility in water was about 20 times its solubility, thus the phytoliposomes have the potential to serve as an SLB nanocarrier system (Angelico et al. 2014).

For the treatment of HCC, studies have shown that oral liver-targeted liposomes co-loaded with SLB and doxorubicin (DOX) are a promising method. Distearoyl phosphatidylethanolamine,

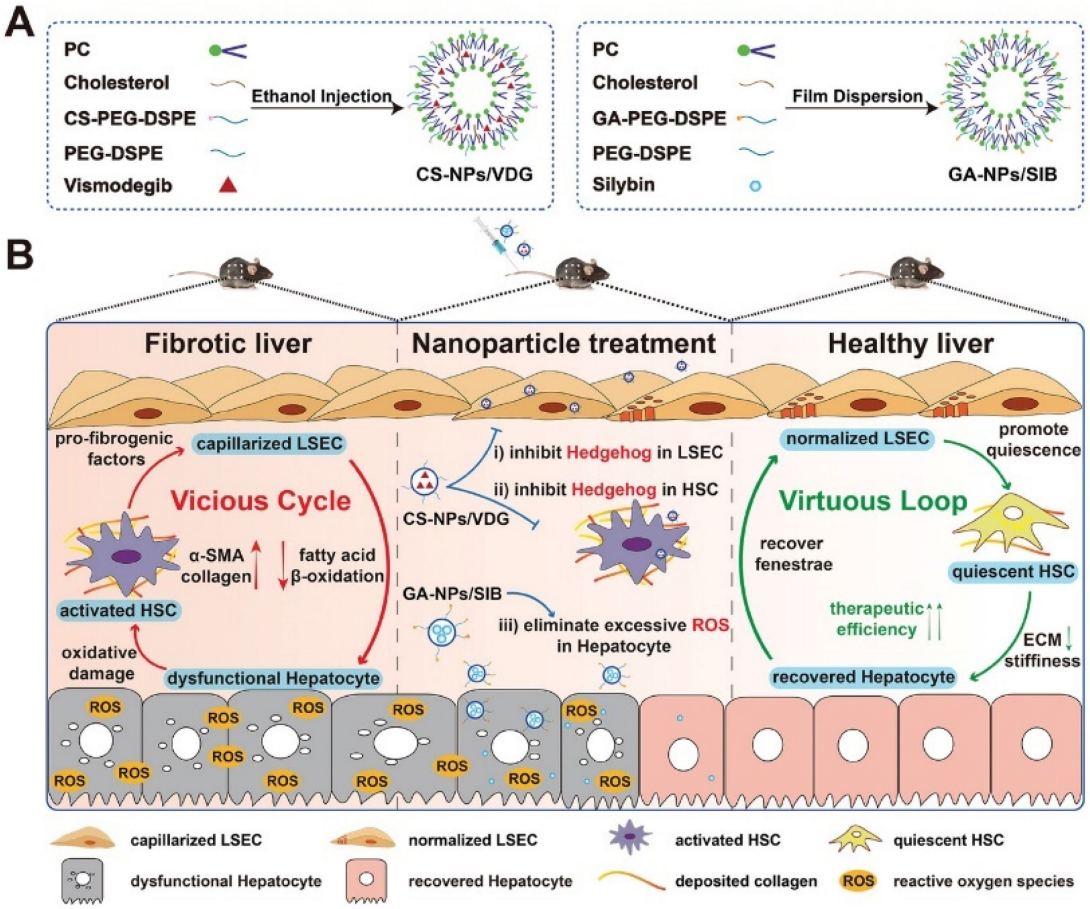

FIGURE 8 | Preparation process, in vivo mechanisms of the vicious cycle-breaking system, and the brief illustration of the virtuous loop after treatment. The restored cells were expected to exhibit positive reciprocal regulation and reverse the malignant progression of liver fibrosis.

polyethylene glycol, and bile acid were combined to form liposomes. The hydrophobic lipid bilayer of the nanoliposomes was loaded with SLB by ethanol injection, while DOX was actively loaded into the hydrophilic core of the preformed liposomes by ammonium sulfate gradient. The final hepatic-targeted nanoliposomes, which co-delivered SLB and DOX, were obtained (CA-LP-DOX/SLB). In vitro cellular experiments showed that CA-LP-DOX/SLB inhibited HepG2 cell proliferation and HCC97H cell migration. The in vivo experiments demonstrated that CA-LP-DOX/SLB had superior hepatic accumulation and targeting in H22-homozygous mice and HepG2-homozygous nude mice. Additionally, it was more effective in inhibiting liver tumor growth (Li et al. 2018).

Carbon nanotubes (CNTs) are carbon-based nanoparticles that are chemically modified to improve biocompatibility and solubility for effective drug delivery without causing any harm to target cells (Bhirde et al. 2009). Tan et al. (2014) attempted to improve the bioavailability of SLB by binding the SLB to multiwalled CNTs (SLB-MWCNTs). The results showed that SLB was released from the nanocarriers in a sustained and pH-dependent manner, suggesting that the nanohybrids could be developed as sustained and controlled release formulations of SLB. Meanwhile, the proliferation assay of SLB-MWCNTs on HepG2 showed that lower doses of SLB-MWCNTs significantly inhibited the proliferation of cancer cells compared to the free drug.

In summary, nanotechnology offers distinctive and noteworthy benefits in the delivery of SLB for the treatment of liver cancer. These include passive targeting, the high-concentration aggregation of drugs in cancerous sites, and other advantages. As the mechanism of carcinogenesis becomes increasingly elucidated, the combination of targeting and nanotechnology can be employed to achieve the most efficacious therapeutic effect.

4.3 | Viral Hepatitis

4.3.1 | Nanopreparations

Targeted formulations of SLB in the treatment of viral hepatitis are still under investigation and no known findings are available.

Current treatment for HCV involves the use of pegylated interferon and ribavirin, either alone or in combination with HCV protease inhibitors (Lin et al. 2014). Alternative drugs based on effective molecules with fewer side effects are being studied. Legalon SIL, a commercially available SLB intravenous preparation, is a water-soluble succinate dihydro mixture of SLB A and SLB B at a ratio of about 1:1. It is an antidote for death cap (poison umbrella) poisoning and inhibits HCV replicon and JFH1 replication in cell culture (Ahmed–Belkacem et al. 2010).

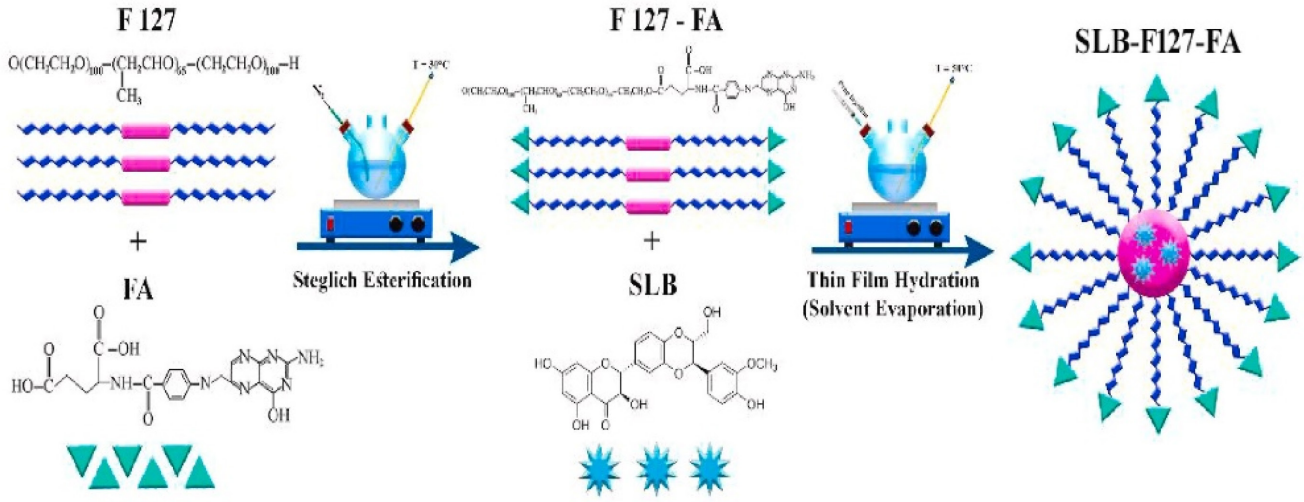

FIGURE 9 | Synthesis process for the preparation of silybin encapsulated folic acid-conjugated Pluronic F127.

Liu et al. (2017) prepared nanoparticles of solid lipid bilayer (SLB-NPs) using the nanoemulsification technique. The study found that the average particle size was 166.1 ± 5.5 nm, with an encapsulation efficiency of over 97% and a 75% increase in solubility. Pharmacokinetic studies demonstrated that SB-NPs had higher serum levels and better biodistribution in the liver compared to unmodified SLB. SB-NPs have enhanced bioavailability, effective anti-HCV activity, and overall liver protection, making them a potentially cost-effective anti-HCV drug.

Lutsenko et al. (2018) investigated the antihepatotoxic activity of SLB liposomes in mice using two mouse models of acute toxic hepatitis induced by carbon tetrachloride or paracetamol. The mean particle size of SLB liposomes was 293 ± 54 nm, with an encapsulation efficiency of 83% ± 4%. Following the administration of SLB liposomes, the blood transaminase and total protein levels of the experimental animals were found to be within the normal range. Furthermore, compared with natural SLB gavage, intravenous injection of SLB liposomes demonstrated a significantly enhanced bioavailability and more pronounced liver protection.

Plant liposomes are formed by encapsulating an SLB-phospholipid complex (SLB-phospholipid body) in liposomes. Ripoli et al. conducted a comparative analysis of the cell delivery and antiviral activity of SLB-coated plant liposomes and uncoated plant liposomes. The findings indicated that the cellular absorption efficiency of SLB-encapsulated plant liposomes was 2.4 times higher than that of free molecules, exhibiting 300 times the effective pharmacological activity. It can thus be concluded that plant liposomes represent an effective delivery system for the promotion of SLB-mediated anti-hepatitis C virus activity (Ripoli et al. 2016). Furthermore, liver cells enhance the absorption of plant liposome envelope molecules, and liposomes tend to accumulate naturally in the liver. This makes liposomes an optimal drug delivery system for the treatment of liver diseases (Torchilin 2005).

In conclusion, SLB has been demonstrated to have a favorable therapeutic impact on HCV in the context of viral hepatitis. In clinical applications, its nanopreparations are employed as a monotherapy or in combination with other antiviral drugs. These two methods remain viable avenues for future investigation.

4.4 | Non-AFL

The SLB-phospholipid complex has been demonstrated to prevent the development of severe OS and to safeguard hepatic mitochondrial bioenergetics in animal models where methionine and choline deficiency has been induced through the administration of an MCD diet, resulting in the development of NASH (Serviddio et al. 2010). Suguro et al. found that tangeretin (TG) can significantly increase the bioavailability of SLB by inhibiting efflux transporters. They also discovered that SLB and TG inhibit the ab initio synthesis and transport of fatty acids, as well as the uptake and hydrolysis of lipoproteins. Furthermore, TG potentiates the effects of SLB on NASH through modulation of OS, inflammatory response, and lipid metabolism, resulting in an increased therapeutic effect of SLB (Suguro et al. 2020). SLB capsules improve NASH induced by a high-fat diet in hamsters by modifying hepatic de novo lipogenesis and fatty acid oxidation (Cui et al. 2017). Gut microbiota plays a critical role in the pathophysiology and disease control of MASLD. Ren et al. established the SLB-2-hydroxypropyl-β-cyclodextrin inclusion complex (SHβCD), which improved the therapeutic effect of SLB. In hamsters fed a high-fat diet (HFD), SHβCD regulates gut health by restoring gut microbiota and gut integrity. Compared to SLB alone, SHβCD exhibits stronger anti-lipid accumulation and antioxidant and anti-inflammatory effects (Ren et al. 2022).

4.5 | Other Liver Diseases

4.5.1 | Nanopreparations

Next, we will introduce the preparation of SLB for ALD and liver injury. However, there is limited research in this area. Future researchers are expected to develop more

therapeutic agents based on the results of the targeted and new preparations.

To enhance the oral bioavailability and liver-targeted delivery of SLB, HA-deoxycholic acid (HA-adh-DOCA) and HA-glycyrrhetinic acid (HA-adh-GA) conjugates were designed and synthesized. Subsequently, SLB was successfully loaded into two micelles, with a drug loading of 20.3% ± 0.5% and 20.6% ± 0.6%, respectively. The mean particle size of the two drug-loaded micelles was 130 nm, and the stability was satisfactory. Additionally, the results of in vivo imaging analysis corroborated the findings of the preceding experiments, confirming the liver-targeted delivery of the micelles. The active liver-targeting properties of GA result in HA-adh-GA_{20} micelles exhibiting a higher targeting ability. In a mouse model of CCl4-induced liver injury, the therapeutic effect of silybin-loaded HA-adh-GA_{20} micelles was superior to that of the suspension and silybin-loaded HA-adh-$DOCA_{10}$ micelles (Han et al. 2015).

Lu et al. developed HSA nanoparticles encapsulated with SLB-phospholipid complexes (SLNPs) for hepatic targeting after intravenous administration. Firstly, they prepared SLB-phospholipid complexes (SLCs) to enhance the lipophilicity of SLB. Secondly, they encapsulated SLCs in albumin nanoparticles. The encapsulation rate of SLNPs was 96.2%, and the drug loading was 5.6%. In vivo experiments on tissue distribution and pharmacodynamics showed that SLNPs improved the accumulation of SLB in the liver through passive targeting, compared to SLB solution. Additionally, high doses of SLNPs restored ALT and AST enzyme activities to near-normal levels in CCL4 injury-induced mice. These findings suggest that SLNPs have great potential in the treatment of acute liver injury (Lu et al. 2019).

Lutsenko et al. (2018) prepared liposomes of SLB by the reverse transfer method and demonstrated that the liposomal drug could be injected directly into the bloodstream, significantly increasing its bioavailability compared to unmodified SLB. The study also found that intravenous SLB liposomes were significantly more effective than oral-free SLB in a chemically induced acute toxic hepatitis model.

Sahibzada et al. (2020) prepared SLB nanoparticles (SLB-APSP) using the antisolvent method with a syringe pump. The bioavailability of SLB-APSP was found to be approximately 6.8 times greater than that of untreated SLB, with the Cmax and AUC exhibiting a 15.6- and 6.9-fold increase, respectively. Furthermore, SLB-APSP demonstrated a more pronounced effect on CCl4-induced liver injury, with enhanced recovery and protection.

The hydroxyl group of SLB forms weak intermolecular interactions with the polar head of phospholipids, resulting in the formation of SLB-phospholipid complexes (SPCs). By combining SPCs with nanosuspension, a photosensitizer-nanosuspension (SPCs-NPs) with high bioavailability in vitro and in vivo was developed. The particle size was 223.50 ± 4.80 nm. This improved the dissolution rate in vitro and increased plasma concentration. SPCs-NPs were found to be safe and had a hepatoprotective effect on CCl4-induced oxidative hepatitis mice (Chi et al. 2020).

The use of nanosuspension has been shown to improve drug release in vitro, intestinal epithelial membrane permeability, and oral bioavailability in vivo. In a study by Wang et al. (2010), the pharmacokinetics of SLB nanosuspension in beagle dogs were investigated. The results of the experiment showed that the preparation improved the oral bioavailability and prolonged the half-life of the drug. Additionally, the oral SLB nanosuspension significantly improved its bioavailability. The SLB nanosuspension, with a smaller particle size of 127 ± 1.9 nm, has the potential to improve its oral bioavailability. For intravenous infusion, the drug release of the SLB nanosuspension produced by lower pressure is more durable. Based on this, a comparison of two SLB nanosuspensions with different particle sizes (637 ± 9.4 nm for SN-A and 132 ± 4.8 nm for SN-B) revealed that SN-A can be targeted to the liver and that the SN-A formulation may have better hepatoprotective effects and lower toxicity compared to SN-B and SLB solutions (Wang et al. 2012).

Salimi-Sabour et al. (2023) prepared SLB-loaded nanostructured lipid carriers (Sili-NLCs) by emulsification-solvent evaporation technique and evaluated its hepatoprotective effect on diazinon (DZN)-induced liver injury in male mice. The average particle size of Sili-NLCs was 220.8 ± 6.35 nm, and the encapsulation efficiency was 71.83% ± 5.52%. DZN can cause liver cell damage, aspartic acid (AST), alanine (ALT), liver lipid peroxidation (LPO), and TNF-α levels are increased. Sili-NLCs are more effective than free SLB in improving liver enzyme function as well as inhibiting oxidative damage and DZN-induced histopathological damage.

4.6 | Preparations to Improve the Limitations of Silybin

Improving the solubility and bioavailability of SLB can effectively increase its application. There are two common strategies to achieve this. One is through the synthesis of SLB derivatives, such as SLB double hemisuccinate, β-cyclodextrin complex, and SLB-n-methyl-glucosamine. The second is the synthesis of glycosides through enzymatic reactions, such as SLB-b-galactoside, SLB-β-glucoside, SLB-b-maltoside, and SLB-b-galactoside (Loguercio 2011). In addition to the targeted agents for different liver diseases mentioned above, this text will also introduce the application of nanotechnology to improve the limitations of SLB.

Solid lipid nanoparticles (SLN) are formed by coupling SLB with lipid drugs of varying chain lengths. This increases their oral bioavailability by 5–7 times (Ma, He, Xia et al. 2017). ACP nanospheres and HAP nanorods made of amorphous calcium phosphate and hydroxyapatite, respectively, showed high silymarin loading (900 and 825 mg g^{-1}, respectively). The resulting SLB delivery system exhibited sustained drug release in simulated gastric and intestinal fluids (Chen et al. 2015). The particle size of SLB-encapsulated chitosan-trisphosphate nanoparticles was 263.7 ± 4.1 nm, and the decrease in particle size was favorable for the dissolution of SLB (Pooja et al. 2014). Yu et al. (2010) reported that sodium cholate/phospholipid hybrid micelles are a promising vehicle for oral administration of SLB with improved bioavailability and scale-up capability. The bioavailability of formulations loaded with SLB was 7–9 times higher after the lipid-based nanocarriers were converted into mixed micelles (MMs) during lipolysis compared to fast-release SLB solid dispersions. (Ma, He, Fan et al. 2017).

Zhu et al. (2019) designed a supersaturated polymer micelle, Soluplus-copovidone (Soluplus-PVPVA), loaded with SLB to improve the oral bioavailability of poorly water-soluble drugs. The micelle is based on the supersaturated drug delivery system (SDDS) and increases the gastrointestinal-free drug concentration, promoting oral administration. This formulation maintains the stability of the supersaturated solution and effectively enhances oral absorption.

Yi et al. (2017) prepared a novel SLB nanocrystalline self-stabilized Pickering emulsion (SN-SSPE) using a high-pressure homogenization method. The SN-SSPE emulsion droplets had a particle size of 27.3 ± 3.1 μm and showed high stability for over 40 days. The in vitro release rate of SN-SSPE was faster than that of SLB crude powder and similar to that of SLB nanocrystalline suspension (SN-NCS). Additionally, the nanocrystals of SLB stabilized SN-SSPE and improved the oral bioavailability of SLB. SLB can be successfully incorporated into physically stable nanoemulsions prepared from different oils (Calligaris et al. 2015).

After co-grinding with β-cyclodextrin, the powder characteristics of SLB changed significantly. The average diameter of the sample decreased, which improved the dissolution kinetics of the drug in vitro and increased oral bioavailability. In vivo studies in rats showed that the bioavailability of the formulation was 6.6 times higher than that of the Italian commercial product of *S. marianum* (Silrex1 200 capsules) used as a control (Voinovich et al. 2009).

In vitro, the dissolution of the SLB-β-cyclodextrin inclusion complex increased significantly (> 90% within 5 min). In vivo, after oral administration of the SLB complex, the concentration of SLB in rat bile was nearly 20 times higher than that in normal or conventional preparations (Arcari et al. 1992).

SLB nanoparticles were prepared using antisolvent precipitation and spray drying in the presence of a water-soluble matrix. The resulting water nanodispersion of SLB had an average particle size of 25 nm and a solubility 10 times higher than that of the original drug. This demonstrates the potential of water nanodispersions in addressing the issue of insoluble drugs (Wang et al. 2011). Later, Cui et al. (2013) used the t-type microchannel antisolvent precipitation method combined with the spray drying method to prepare SLB nanodispersions. When the average particle size of SLB nanodispersions was 26 nm, the dissolution rate was 10 times faster than that of the bulk drug.

The SLB-chitosan nanocomplexes showed 87% utilization, 63% yield, high loading, formation of a supersaturated delivery system, and good stability during storage under optimum conditions (pH 5.8, charge ratio 0.30) (Nguyen et al. 2016).

Nanohybrids were formed by SLB and carboxylated multiwalled CNTs (SB-MWCNTs). The maximum release rates of SLB in 1000 min were 96.6% and 43.1% when the pH was 7.4 and 4.8, respectively. The cytotoxicity of SB-MWCNTs to human cancer cell lines was enhanced compared to the low concentration of free SLB (Tan et al. 2014).

SLB-phosphatidylcholine complex (IdB 1016) has greater oral bioavailability compared to SLB in an animal model and in vivo, pharmacokinetic results after oral administration to nine healthy volunteers showed a much higher bioavailability than SLB (Barzaghi et al. 1990).

The SLB and phospholipids were dissolved in an ethanol medium, and the ethanol was removed under vacuum. This resulted in the formation of an SLB-phospholipid complex. The complex showed increased solubility in water and *n*-octanol and was found to be bioavailable in mice after oral administration (Yanyu et al. 2006).

The study investigated a high-loading supersaturated self-emulsifying drug delivery system (S-SEDDS) using hydroxypropyl methylcellulose (HPMC) as a precipitation inhibitor. The experimental results demonstrated an improvement in the oral bioavailability of SLB, indicating that supersaturated preparation is an effective method for enhancing the oral bioavailability of insoluble drugs (Wei et al. 2012).

Wang et al. (2021) first prepared silymarin into nanocrystals and then loaded them into adhesive microspheres to form adhesive microsphere nanocrystals encapsulating silymarin, which had an encapsulation efficiency of about 100% and a drug loading capacity of up to 35.41 ± 0.31%. Their results showed that SLB adhesive microsphere nanocrystals increased the dissolution rate and prolonged the retention time of SLB in the gastrointestinal tract, resulting in a significant 3-fold increase in its oral bioavailability compared to unprocessed SLB.

Wang et al. prepared SLB nanocrystals and loaded them into adhesive microspheres to create SLB-encapsulated adhesive microsphere nanocrystals. The encapsulation efficiency was approximately 100%, and the drug loading was as high as 35.41% ± 0.31%. The results indicate that SLB-adhered microsphere nanocrystals can enhance the dissolution rate and prolong retention time in the gastrointestinal tract. The oral bioavailability of microsphere nanocrystals adhered to SLB was three times higher than that of unprocessed SLB (Huang et al. 2011).

Xu et al. (2018) reported that baicalin inhibited the biliary excretion of SLB coupling and significantly improved the absorption and bioavailability of SLB. This provides a new combined therapeutic pathway for the treatment of chronic liver disease (Xu et al. 2018).

In general, two principal methods may be employed to enhance the solubility and bioavailability of SLB. One such method is the traditional approach, which involves the synthesis of SLB derivatives and glycosides. An alternative approach is the utilization of nanotechnology. Nevertheless, nanotechnology offers a multitude of advantages, including more efficacious enhancement of encapsulation efficiency, stability, solubility, and bioavailability. It is anticipated that nanotechnology will continue to offer distinctive advantages in these domains in the future.

5 | At Present, the Clinical Study of Silybin in the Treatment of Liver Disease

SLB has been approved by the US Food and Drug Administration (FDA) for the treatment of liver diseases due to its antioxidation

and antifibrosis effects (Dixit et al. 2007). Presently, SLB is undergoing continuous development and application as a therapeutic agent for the treatment of liver diseases on a global scale.

In Italy, a clinical trial of SLB in relation to nonalcoholic liver disease was conducted using Realsil (a tablet containing SLB 94 mg, phosphatidylcholine 194 mg, VE 90 mg), Realsil 100D (SLB-phospholipid complex 303 mg, VD 10 mg, and VE 15 mg). The combination of SLB with vitamin E and phospholipids has been demonstrated to enhance its bioavailability, antioxidant, and antifibrotic activity. In 2006, a study was conducted to investigate the therapeutic effect of SLB on patients with MASLD and HCV infection. A total of 85 outpatients were identified, including 59 patients with primary MASLD (Group A) and 26 patients with HCV-related chronic hepatitis C complicated with MASLD (Group B). The treatment group was administered Realsil for a period of 1 year. The preliminary results indicated that the SLB-VE-phospholipid complex tablets were effective in improving insulin resistance and liver injury in patients with MASLD. However, the study also highlighted two main limitations: the absence of a placebo treatment and the lack of a histological examination at the conclusion of the study (Federico et al. 2006). In 2012, a randomized controlled trial of SLB in combination with phosphatidylcholine and VE was conducted for the treatment of MASLD. The results of this multicenter, phase III, double-blind clinical trial were subsequently reported. A total of 179 patients with MASLD (of whom 36 were HCV positive) were treated for a period of 12 months. The results demonstrated that transaminase levels, insulin resistance, and liver histology exhibited improvement following administration. This study represents the inaugural systematic assessment of the role of SLB in patients with MASLD (Loguercio et al. 2012). In 2020, a systematic review and meta-analysis of a randomized controlled trial on the effect of SLB-phospholipid-VE complex on liver enzymes in patients with non-MASLD or NASH was conducted to systematically review the effect of Realsil on liver enzymes in patients. The findings indicate that Realsil can markedly diminish circulating GGT levels; however, it exerts no notable impact on AST and ALT levels. Nevertheless, it is imperative to conduct further trials to ascertain the optimal supplemental dose and frequency (Derakhshandeh-Rishehri 2020). In 2019, Federico et al. (2019) employed statistical methods to analyze the results of Realsil 100D administered orally for a period of 6 months to patients with MASLD. The findings indicated that patients with MASLD who received treatment exhibited a statistically significant improvement in metabolic markers, OS, endothelial dysfunction, and disease deterioration. The proportion of patients with MASLD who received treatment was greater than that of patients who did not receive treatment ($p < 0.05$). Furthermore, the findings of a prospective, randomized, placebo-controlled, double-blind clinical trial conducted from June 2010 to December 2012 indicated that the SLB-VE-phospholipid complex in conjunction with pegylated interferon α and ribavirin could potentially mitigate the incidence of liver fibrosis in patients with chronic hepatitis C caused by the latter treatment alone (Malaguarnera et al. 2015).

In China, in 2006, a study was conducted to examine the pharmacokinetics of the SLB-phosphatidylcholine complex (SLB capsule) in a cohort of healthy Chinese male volunteers. The results demonstrated that SLB was rapidly absorbed by 20 volunteers following the oral administration of an SLB capsule (equivalent to 280 mg SLB). The time to reach peak plasma concentration (Tmax) ranged from 0.67 to 2.67 h, with an average of 1.4 h. These doses were well tolerated, and no adverse reactions were observed. Nevertheless, pharmacokinetic parameters such as peak plasma concentration and $AUC_{(0-1)}$ exhibited considerable intersubject variability (Li et al. 2006). In 2015, Gu et al. (2015) proposed a novel treatment for tuberculosis based on the premise that the most common side effect of antituberculosis drugs is DILI. They combined SLB-phosphatidylcholine complex capsules in an effort to address this issue. A prospective, multicenter, randomized, open-label, controlled trial demonstrated that SLB can prevent DILI in the general population, including patients at high risk of non-DILI. In 2021, Lv et al. demonstrated that the SLB capsule (SC) in conjunction with lifestyle modifications could effectively alleviate hepatic steatosis in patients with chronic hepatitis B (Lv et al. 2021). In light of these findings, Su et al. demonstrated that the efficacy of SLB meglumine tablets and Ganshuang granules in combination with the antiviral drug tenofovir for the treatment of chronic hepatitis B with non-AFL (MASLD) was markedly superior to that of antiviral drugs administered alone (Su and Yang 2022). In 2022, Zhang et al. corroborated the efficacy and safety of SLB meglumine tablets in the treatment of DILI by employing the updated Roussel-Uclaf causality assessment method (Zhang et al. 2023).

In Mexico, the SLB-phosphatidylcholine complex (SPC) can reduce OS, lipid peroxidation, and collagen accumulation, thereby reducing liver injury. Méndez-Sánchez et al. (2019) compared the effects of SPC oil soft gel capsules (NeoCholal-S) and SM tablets (Legalon) in the treatment of liver fibrosis. The experimental results showed that in healthy Mexican volunteers, the plasma drug level of NeoCholal-S was higher than that of the conventional Legalon.

In Spain, some oral SLB products (e.g., Legalon, Sylarine) have been marketed, yet their bioavailability in humans is markedly low (Hoh et al. 2006; Zhu et al. 2013). Until January 2014, a new product containing SLB and VE was listed under the commercial name Legasil (Bosch-Barrera et al. 2014). Legasil has been demonstrated to possess hepatoprotective, anti-inflammatory, and antifibrotic properties, rendering it an efficacious treatment option for patients with liver disease (Falasca et al. 2008).

In addition, SLB is used in Europe as an adjuvant therapy for liver and kidney failure caused by fatal mushroom poisoning. For example, in acute liver poisoning caused by aflatoxin-containing mushrooms, SLB is the preferred antidote because it blocks the reuptake of aflatoxin by cells and interrupts the enterohepatic circulation of toxins. At the same time, intravenous injection of Legalon SIL can successfully prevent and reverse the manifestations of fulminant liver failure. More than 1300 cases have been recorded to support the clinical efficacy of Legalon SIL as an antidote for patients with acute aflatoxin poisoning (Mitchell, Mengs, and Pohl 2012).

Although SLB has initiated clinical trials for the treatment of various liver diseases, researchers have highlighted the necessity for more comprehensive and systematic trials, including tissue diagnostics and liver biopsy, to advance the field. Additionally, SLB is categorized as a dietary supplement or nutritional health

TABLE 1 | Silybin targeted agents based on the pathogenesis of liver disease.

Diseases	Preparations	Target head, target receptor, target cell	Mechanism	References
Liver fibrosis, cirrhosis	COL + SLB-MLPs	Chondroitin sulfate (CS), CD44 receptor, activated hepatic stellate cells	Nanoparticles release collagenase and SLB to decompose dense collagen matrix and inhibit aHSCs, respectively.	(Luo et al. 2021)
	SLB-HA	Hyaluronic acid (HA), CD44 receptor, activated hepatic stellate cells	Specific uptake of HSCs and significant liver targeting.	(Li et al. 2020)
	NS-SLB-HC		Downregulation of TGF-β inhibits the secretion of α-SMA and type I collagen, and reverses liver fibrosis in vivo.	(Yang, Tan et al. 2023)
	SLB-HSA nanocrystals	Human serum albumin (HSA), secreted protein, acidic and cysteine-rich, activated hepatic stellate cells	SLB-HSA nanoparticles can passively target aHSCs through the pores on LSECs, and actively target aHSCs through HSA-binding SPARC-mediated endocytosis.	(Luo et al. 2023)
	Retinol-modified lipid nanoparticles containing SLB	Retinol, retinol-binding protein receptors, hepatic stellate cells and Kupffer cells	Nanoparticles were found to accumulate rapidly in the liver and spleen, increasing liver uptake of SLB.	(Pan et al. 2016)
	HNPs of SLB		SLB is released through cracks formed by HNP deformation, reducing fibrotic tissue, and improving liver function.	(Hayashi et al. 2018)
	CGPYM	Vitamin A(VA), retinol-binding protein receptors, activated hepatic stellate cells	CGPYM was effectively accumulated in the fibrotic liver. PEG reduced nonspecific uptake and prolonged circulation time. After internalization of polymeric micelles, silybin and siCol1α1 were released into the cytoplasm and inhibited the expression of collagen I.	(Qiao et al. 2018)
	GA-NPs/SIB	Glycyrrhetinic acid (GA), glycyrrhetinic acid receptor hepatocyte	Relieve oxidative stress to restore liver cell function, block the vicious cycle, and have an obvious fibrosis regression effect.	(Zhang et al. 2024)
Liver cancer	SLB-F127-FA	Folic acid, folate receptor, hepatocellular carcinoma	The cytotoxicity of SLB-F127-FA on HepG2 cancer cells was significantly enhanced compared with nontargeted and free SLB.	(Ghalehkhondabi, Soleymani, and Fazlali 2021)

TABLE 2 | Nanotechnology is used for silybin in the treatment of liver diseases.

Diseases	Preparations	Animal and cell experimental models	Pharmacokinetics and pharmacodynamics	References
Liver fibrosis, cirrhosis	SLB NPs	In male Wistar rats (evaluation of pharmacokinetics and pharmacodynamics). Liver injury model induced by carbon tetrachloride (CCl4) (to evaluate its hepatoprotective effect).	The nanoparticles enable the release of the drug in vivo and in vitro for up to 10 days and have better pharmacokinetic properties than the free drug itself. Intravenous nanoparticle administration could reverse serum liver enzyme levels by 95%, while the drug solution could only reverse by 50%.	(Bonepally et al. 2013)
	SLB-phosphatidylcholine-vitamin E complex	Liver fibrosis induced by dimethylnitrosamine and cholangitis in rats.	It has high bioavailability and lipophilicity. It has a therapeutic effect on hepatic fibrosis induced by dimethylnitrosamine and cholangitis in rats. It can reduce the proliferation and activation of hepatic stellate cells, collagen deposition, and the expression of type I collagen mRNA.	(Di Sario et al. 2005)
	SLB-phytosome	Rat model of liver cirrhosis induced by thioacetamide.	Inhibition of collagen accumulation and ROS production, thereby inhibiting the activation of HSC, and through its antioxidant and antifibrotic effects prevent the progression of cirrhosis.	(Ali, Darwish, and Ismail 2014)
Liver cancer	SLB NPs	Diethylnitrosamine (DENA)-induced hepatocellular carcinoma in male Wistar rats.	Compared with silybin, oral administration of silybin NPs is more effective and safe in the treatment of rat liver cancer.	(Zhang, Wang, and Liu 2016)
	SLB and GA co-encapsulated nanoliposomes	HepG2 cells.	The IC50 value of SLB nanoliposomes on the HepG2 cell line was lower than that of free silybin combined with glycyrrhizic acid. The cytotoxicity of SLB nanoliposomes on HepG2 cells was 10 times higher than that of ordinary herbs.	(Ochi et al. 2016)
	CA-LP-DOX/SLB	H22 tumor-bearing mice and HepG2 tumor-bearing nude mice.	CA-LP-DOX/SLB not only showed better liver accumulation and liver targeting in H22 tumor-bearing mice and HepG2 tumor-bearing nude mice, but also more effectively inhibited liver tumor growth, HepG2 cell proliferation, and HCC97H cell migration.	(Li et al. 2018)
	SLB-MWCNTs	HepG2 cells.	The proliferation test of HepG2 cells showed that the lower dose of SB-MWCNTs could significantly inhibit the proliferation of cancer cells compared with free drugs.	(Tan et al. 2014)

(Continues)

TABLE 2 | (Continued)

Diseases	Preparations	Animal and cell experimental models	Pharmacokinetics and pharmacodynamics	References
Viral hepatitis C	SLB-NPs	Huh-7 cells, infectious HCV culture system (evaluation of the effect of SB-NP on viral life cycle).	SB-NPs have anti-HCV and antioxidant effects, as well as better solubility and bioavailability.	(Liu et al. 2017)
	SLB liposomes	Acute toxic hepatitis model induced by carbon tetrachloride or paracetamol.	Compared with natural SLB, intravenous injection of SLB liposomes showed significantly enhanced bioavailability and more significant liver protection.	(Lutsenko et al. 2018)
	SLB phytoliposomes	Huh7.5 permissive human hepatocellular carcinoma cells, Huh7.5-derived Con1/FL-Neo S2204I.	The cell uptake efficiency of SLB-encapsulated with phytoliposomes was 2.4 times higher than that of free molecules and showed 300 times more effective pharmacological activity.	(Ripoli et al. 2016)
Metabolic dysfunction-related fatty degeneration liver disease	SLB-phospholipid complex	Nonalcoholic steatohepatitis in male Wistar rats was induced by methionine and a choline-deficient MCD diet.	SLB-phospholipid complex has the effect of preventing severe oxidative stress and protecting liver mitochondrial bioenergy.	(Serviddio et al. 2010)
	SLB capsules	High fat diet (HFD)-induced metabolic dysfunction-related fatty degeneration liver disease (MASLD) in male hamsters.	Improvement of metabolic dysfunction-related fatty degeneration liver disease induced by high fat diet in hamsters by changing liver new fat production and fatty acid oxidation.	(Cui et al. 2017)
	SHβCD	High fat diet (HFD) induced metabolic dysfunction-related fatty degeneration liver disease (MASLD) in male hamsters.	The dissolution rate and transepithelial effect of SLB were increased, and bioavailability and liver drug deposition were increased. Regulating intestinal flora, reducing intestinal inflammation, and enhancing intestinal integrity to improve intestinal homeostasis. In HFD-fed hamsters, liver lipid accumulation and liver inflammation were effectively improved by regulating the gene expression of iκb and nfκb.	(Ren et al. 2022)

(Continues)

TABLE 2 | (Continued)

Diseases	Preparations	Animal and cell experimental models	Pharmacokinetics and pharmacodynamics	References
Liver injury	HA-GA-micelles loaded with SLB	Acute liver injury induced by CCL4 in mice.	The therapeutic effect of SLB-loaded HA-adh-GA20 micelles was better than that of SLB suspension and silybin-loaded HA-adh-DOCA10 micelles.	(Han et al. 2015)
	SLNPs		It was used for liver targeting after intravenous administration to restore ALT and AST enzyme activity to near-normal levels.	(Lu et al. 2019)
	SLB-APSP		Compared with untreated SLB, the bioavailability of SLB-APSP was increased by about 6.8 times, and the Cmax and AUC after treatment were 15.6 times and 6.9 times higher, respectively.	(Sahibzada et al. 2020)
	SPCs-NPs		SPCs-NPs have good safety and liver protection effects.	(Chi et al. 2020)
	SLB nanosuspensions		Improve oral bioavailability. During intravenous infusion, the drug release of SLB nanosuspensions produced by low pressure was more durable.	(Wang et al. 2010, 2012)
	Sili-NLCs	Diazinon (DZN)-induced liver injury in male mice.	Sili-NLCs are more effective than free SLB in improving liver enzyme function, inhibiting oxidative damage, and DZN-induced histopathological damage.	(Salimi-Sabour et al. 2023)

product (Deep and Agarwal 2010). Consequently, an investigation of SLB must take into account the disparate evaluation criteria prevalent in China and the West and must adopt a comprehensive approach to its use, pharmacology, clinical applications, and other pertinent trends (Williamson, Liu, and Izzo 2020; Izzo et al. 2016).

6 | Summary and Outlook

At present, liver disease is still one of the major problems that plague people's health, and the drugs and methods for its treatment have been explored. The plant *S. marianum* has been used to treat liver diseases for more than 2000 years, and SLB is extracted from it. Modern research shows that SLB has anti-inflammatory, antioxidant, antifibrosis, and other effects, so it can treat liver diseases and other diseases, such as lung cancer, breast cancer, prostate cancer, skin cancer, and so on. However, the low solubility and low bioavailability of SLB have always been the main factors limiting its application. The limitations can be improved by nanotechnology, and targeted agents can be designed and developed based on the mechanism of liver disease, so as to improve the bioavailability and effect.

In this study, we first reviewed that SLB can treat liver diseases such as liver fibrosis, HCC, viral hepatitis, and so forth, and described the specific mechanism of its treatment of liver diseases. Then, based on the limitations of SLB application, that is, low solubility and low bioavailability, the existing targeted preparations and nanopreparations of SLB for the treatment of liver diseases are emphatically introduced (Tables 1 and 2), in order to provide new ideas for the follow-up research and application of SLB or other drugs for the treatment of liver diseases.

Over the past 4 years, the study of the mechanism of liver disease has also led to advances in the system of delivering drugs to diseased cells and sites. As previously stated, SLB delivery is facilitated by novel targets, including CS and HA, which target the CD44 receptor in liver fibrosis (Luo et al. 2021; Li et al. 2020; Yang, Tan et al. 2023). HSA in conjunction with SPARC-mediated endocytosis to actively target aHSCs (Luo et al. 2023), and VA targeting HSCs and Kupffer cells (Hayashi et al. 2018). These targeted agents, based on the pathogenesis, not only improve the bioavailability and are more effective than nontargeted agents, but also inspire deeper research on SLB, including the inhibition of cell ferroptosis (Duan et al. 2023), the inhibition of mitochondrial fission (Song et al. 2022), and the inhibition of endoplasmic reticulum stress (Wu et al. 2023). Concurrently, as in previous studies, nanotechnology continues to play a distinctive role in enhancing solubility and bioavailability, as exemplified by organic–inorganic hybrid hollow nanoparticles (Hayashi et al. 2018) and CNTs (Bhirde et al. 2009). Furthermore, the investigation of SLB in the management of liver disorders encompasses a multifaceted approach, encompassing the utilization of network pharmacological analysis (Li et al. 2024), and the utilization of animal models of liver injury induced by diverse pharmaceutical agents (Salimi-Sabour et al. 2023). The etiology of liver disease is a complex and multifaceted phenomenon. It is therefore evident that further comprehensive, multifaceted, and step-by-step research is required to address this significant public health issue.

At the same time, when SLB is applied to the treatment of liver diseases, this study also considers some issues that need the attention of researchers. First, in the targeted preparation of SLB, whether there are some problems that can be more optimized, such as CD44 receptor, folic acid receptor, and so on, which are not only expressed in the liver but also expressed in other tissues. Therefore, when investigating the effect of the preparation, it should also be necessary to consider the drug concentration and its effect on these expression sites and explore whether it will affect the safety or efficacy of the drug. Second, whether SLB can be applied to different liver diseases can be considered in combination, just as liver fibrosis is a complex response of multifactor development, as well as other chronic diseases such as viral hepatitis and liver cancer. Therefore, single-target drugs or preparations have limitations, and multitarget anti-hepatic fibrosis drugs and combination therapy can better treat liver fibrosis. Third, the low solubility and low bioavailability of SLB are related to its own structure and in vivo metabolism. Therefore, these techniques, carriers, and complexes that improve the low bioavailability of SLB can be applied to drug research with similar limitations to SLB.

In general, in the treatment of liver diseases with SLB, it is still worthwhile to continue studying the existing good preparations in the hope that they can be put into clinical trials. At the same time, the specific mechanism of its treatment of liver diseases still needs to be explored more comprehensively to study the design of preparations and the combination of drugs. The current research results have proven that SLB has great potential in the future treatment of liver diseases.

Author Contributions

Jijiao Wu: writing – original draft, writing – review and editing. **Lin Wen:** writing – review and editing. **Xiaolian Liu:** conceptualization. **Qiuxia Li:** data curation. **Zihao Sun:** software. **Chuipeng Liang:** software. **Fan Xie:** conceptualization. **Xiaofang Li:** conceptualization, funding acquisition.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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