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Preparation, characterization and *in-vitro* anti-cholestatic activity of swertiamarin-loaded galactosylated solid lipid nanoparticle

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Abstract: **Background**: Cholestatic liver injury (CLI) is a rapidly progressive liver disorder characterized by the accumulation of bile acids (BA). The therapeutic effects of current medicines used to treat CLI are unsatisfactory. **Objectives**: This study aimed to prepare galactosylated solid lipid nanoparticle (SLN) with swertiamarin (STM) by using a galactosylated lipid, N-hexadecyl lactobionamide (N-HLBA) and evaluate its anti-cholestasis effect *in-vitro*. **Methods**: The galactosyl-lipid N-HLBA was prepared via the lactone form intermediates of lactobionic acid and synthesized by anchoring galactose to hexadecylamine lipid. The STM-loaded galactosylated SLN (STM-GalSLN) was successfully prepared by a melt-emulsification-ultrasound method. The prescription was optimized by orthogonal test. The morphology was observed by transmission electron microscope. The particle size and zeta potential were determined by laser granularity equipment. The encapsulation efficiency (EE) and drug loading capacity (DL) were determined by ultrafiltration, centrifugation and HPLC method. **Results**: The optimized prescription was as follows: 50 mg of STM, 20 mg of N-HLBA, 500 mg of glyceryl behenate, 400 mg of soybean lecithin, 200 mg of poloxamer188 and 500 mg of Tween 80. The STM-GalSLN was spherical in shape and its particle size, zeta potential, EE and DL were 164.40±4.68 nm, -14.53±3.20 mV, 82.41±2.88 % and 0.32±0.02 %, respectively, and the EE of STM-GalSLN did not change significantly over 30 days at 4°C. Furthermore, STM-GalSLN alleviated cholestatic liver injury (CLI) in a concentration-dependent manner *in-vitro*. **Conclusion**: The findings from this study indicated that the melt-emulsification-ultrasound method was rational and reliable, which provided an experimental basis for developing a new nano preparation of STM for CLI.

Keywords: Cholestatic liver injury; Galactosylated lipid; Melt-emulsification-ultrasound method; Solid lipid nanoparticles; Swertiamarin

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INTRODUCTION

Cholestatic liver injury (CLI) is a hepatic disease caused by various factors that impair bile formation and excretion and is characterized by bile stasis. CLI is accompanied by changes in hepatocyte polarity and an imbalance in bile acid homeostasis. To date, there remains a lack of effective therapeutic agents for CLI (Lu, 2022). In traditional Tibetan medicine, swertiamarin (STM), a secoiridoid glucoside compound with high content and strong bioactivity from *Swertia mussotii* Franch (Gentianaceae), is a potential pharmacological compound for the treatment of liver diseases (Zhang *et al*., 2019). STM also has a significant therapeutic effect on experimental CLI (Shi, Tang *et al*., 2022), suggesting that it is a new candidate for the treatment of CLI and has broad clinical application prospects; however, its oral bioavailability is very low due to poor water solubility and extensive first-pass hepatic metabolism (Muhamad Fadzil *et al*., 2021), which greatly limits its clinical application. Improving the dosage form to enhance clinical efficacy is an urgent issue that must be addressed.

Solid lipid nanoparticle (SLN) is a type of colloidal nanodrug delivery system made by encapsulating drugs in a lipid core or adsorbing them on the surface of nanoparticles using natural or synthetic solid lipid materials, with a particle size range of 10–1000 nm. SLN has the advantages of both polymer nanoparticles and O/W fat emulsions, which can be administered intravenously for targeted or controlled release and can also be administered orally to improve the oral bioavailability of poorly soluble drugs (Fathi, Machado *et al*., 2024). Traditional drugs for treating liver disease often lack target selectivity and tissue specificity, leading to poor efficacy and even significant adverse drug reactions. The development of efficient and low-toxicity liver-targeted preparations has become a major topic of research. Asialoglycoprotein receptor (ASGPR) is highly expressed on the surface of hepatic parenchymal cells; it can specifically recognize and bind molecules bearing galactose or N-acetylgalactosamine residues at their ends, thereby mediating their entry into hepatocytes via clathrin-coated pits. Therefore, drugs and carriers with galactose or N-acetylgalactosamine terminal groups can target ASGPR-mediated hepatic parenchymal cells (Ramírez-Cortés and Ménová, 2025).

To improve the bioavailability and liver targeting of STM for the treatment of CLI, this study synthesized the galactose-ligand material, N-hexadecyl lactamide (N-HLBA), as reported in the literature (Wang *et al*., 2010). STM galactosylated solid lipid nanoparticle (STM-

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GalSLN) with active hepatocyte-targeting groups of galactose residues were subsequently prepared by using N-HLBA as a galactosylated lipid material and characterized. Its anti-cholestatic effect was evaluated using an *in-vitro* CLI model.

MATERIALS AND METHODS

Materials

STM (Lot 2023061125, purity ≥98%) was obtained from Beisheng Biotechnology (Shanxi, China) and soybean lecithin (Lot S30870) was obtained from Yuanye Biotechnology (Shanghai, China). Tween 80 (Lot A2312256) was obtained from Aladdin Biochemical Technology (Shanghai, China). Glyceryl behenate (Compritol 888) was obtained from Gattefosse (Saint-Priest, France). N-HLBA (self-synthesized), Poloxamer 188 (Lot BD105932) was obtained from Yuanye Biotechnology (Shanghai, China) and α-naphthylisothiocyanate (ANIT, N4525) was purchased from Sigma (St. Louis, USA). Methanol (HPLC grade) was obtained from Bodie Chemical Reagents (Tianjin, China) and ultrafiltration centrifuge tubes (Briscale UF ULRT0030040P-X) were obtained from Cobetter Biotechnology (Hangzhou, China).

Quantification of STM with HPLC analysis

An HPLC system (Waters 1525, Waters, USA) coupled with a photodiode array detector (Waters 2998) and an RP-18 column (Agilent ZORBAX Eclipse XDB-C18 (4.6 mm×250 mm, 5μm)) was employed under the following chromatography conditions: a UV wavelength of 237 nm (Wang, *et al*., 2007), a mobile phase consisting of methanol:water acidified with orthophosphoric acid (0.05%, v/v) at a 25:75 (v/v) ratio, a flow rate of 1.0 mL/min, an injection sample volume of 10 μL and an oven compartment temperature maintained at 35 °C throughout the analysis. Analytical concentrations of STM were prepared at 0.5, 1.0, 2.0, 5.0, 10.0, 40.0, 50.0 and 80.0 μg/mL. The selectivity, linearity range, precision and recovery rate of the HPLC method were accordingly validated. The intermediate precision (interassay) and repeatability (intra-assay) of STM were determined at concentrations of 0.5, 40.0 and 80.0 μg/mL.

Preparation of STM-GalSLN

SLN was prepared by a melt-emulsification-ultrasound method(Li, Ji *et al*., 2011). Briefly, the prescribed amounts of STM, N-HLBA and Compritol 888 were mixed with absolute ethanol and heated to 81°C for complete dissolution. The prescribed amounts of soybean lecithin, Poloxamer 188 and Tween 80 were dispersed in distilled water by using a magnetic stirrer (DF101S, Kobei Instruments, China) at the same temperature. After the ethanol had completely evaporated, the water phase was added to the oil phase dropwise at 81 °C, followed by 10 min of magnetic stirring. The coarse premix was subjected to ultrasonic treatment at 600 W for 10 min using an ultrasound cell pulverizer (JY92-IIN; Xinyi Equipment Inst., China). Afterward, the sonicated emulsion was immediately cooled in an ice-water bath. After that, the cooled emulsion was filtered through a 0.45 μm microporous membrane to obtain the final preparation.

Transmission electron microscopy (TEM)

The morphology of the SLN was examined by using a transmission electron microscope (Tecnai G2 Spirit, FEI, USA). After being diluted 50-fold in the preparation's original dispersion medium, the samples were negatively stained with a 2% (w/v) osmium tetroxide solution for observation.

Particle size and zeta potential

The mean diameter and zeta potential of SLN in the dispersion were determined using a nanoparticle size and zeta potential analyzer (Zetasizer Nano ZS90, Malvern, UK) at 25 °C. Before measurement, SLN dispersions were diluted 50-fold with the original dispersion preparation medium for size determination and zeta potential measurement.

Encapsulation efficiency (EE) and drug loading capacity (DL) evaluation

The SLN dispersions were centrifuged using an ultrafiltration centrifuge tube at 4000×g by a refrigerated centrifuge (BY-R20, Baiyang Devices, China) for 30 min at 4 °C under vacuum. The filtrate containing the free drug was collected for HPLC analysis as described above. The equations for EE and DL are as follows:

EE= (amount of STM in SLN/amount of STM used in formulation) × 100%;

DL= (amount of STM in SLN/amount of lipid used in formulation) × 100%.

Orthogonal test

An orthogonal test was conducted to optimize the prescription of STM-GalSLN, with EE as the index and four main influencing factors were selected: (A): drug–lipid ratio, (B): N-HLBA concentration, (C): soy lecithin/Poloxamer 188 ratio and (D): Tween 80 concentration. Each factor was divided into three levels. An L9(34) orthogonal design table was used for prescription screening. All the factors and levels are shown in Table 1.

Verification and stability experiments

Based on the prescription process optimized by the orthogonal test, three batches of STM-GalSLN samples were prepared as described above. The particle size, zeta potential, EE and DL were determined. To investigate the stability of the SLN, three batches of STM-GalSLN were stored at 4 °C and sampled at 0, 15 and 30 days to observe the appearance and determine the EE.

Table 1: Factors and levels of the orthogonal test.

Levels	Factors			
	A	B	C	D
1	1:50	0.025	1:1	1.5
2	1:25	0.05	1.5:1	2.0
3	1:10	0.1	2:1	2.5

A: STM/CP (%, w/w); B: N-HLBA (%, w/w); C: SL/Flu68 (%, w/w); D: Tween 80 (%, w/w)

Cell culture and treatment

In-vitro experiments (including modeling of dosage and administration) were conducted using the human hepatoblastoma cell line HepG2 to establish an ANIT-induced CLI model based on published studies (Ma *et al*., 2024, Mao *et al*., 2022). HepG2 cells (Cat No: CL-0103) were obtained from Wuhan Pricella Biotechnology Co., Ltd. (Wuhan, China). The cells were cultured in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO_2. After digestion with 0.25% trypsin (BL526A, Biosharp), the cells were passaged 2-3 times, and cells in the logarithmic growth phase were used for the experiment. Hepatocytes were plated at a density of 5×10^3 cells per well into 96-well plates and treated with 100 µM ANIT and different concentrations of STM-GalSLN (10, 25, 50, 100 and 200 µM) for 12, 24 and 48 h, respectively. Furthermore, the influence of the STM solution (dissolved in DMSO; the final concentration of DMSO in the culture system was less than 0.1% v/v), STM-GalSLN and STM-CSLN (N-HLBA was not added to the STM-CSLN preparation) treatments on ANIT-induced hepatocytes was also evaluated.

Measurement of cytotoxicity in ANIT-induced HepG2 cells

ANIT-induced cholestatic cytotoxicity was determined using a CCK-8 test kit (BCCK0500, Bioswamp).

Measurement of apoptosis in ANIT-induced HepG2 cells

Apoptosis was detected by flow cytometry using an Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, USA). After treatment, cells were double-stained with 10 µL Annexin V-FITC and PI in the dark for 30 min at 4 °C. Apoptotic cells were subsequently analyzed using a flow cytometer (ACEA NovoCyte, ACEA Biosciences). NovoExpress 1.5 (ACEA Biosciences Inc.) was used to measure the total apoptosis rate.

Statistical analysis

The data are presented as the mean ± SD. At least three replicates were used for each statistical analysis. Multiple-group comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

RESULTS

Synthesis and identification of N-HLBA

Lactobionic acid (4 g) was added to 40 mL of methanol and the solvent was evaporated three times to obtain approximately 3.4 g of a white solid (lactobiono-1,5-lactone) with a yield of approximately 90%. Afterward, hexadecylamine (1.6 g) was added and the solid was dissolved in 50 mL of anhydrous ethanol, stirred at 40 °C for 36 hours and then recrystallized three times after filtration to obtain approximately 3 g of solid (N-HLBA) with a yield of approximately 75%. The chemical structure was confirmed by ^{1}H NMR and ^{13}C NMR (Fig. 1C-D): ^{1}H NMR (400 MHz, DMSO) δ 7.56 (s, 1H), 5.16 (s, 2H), 4.73 (d, *J* = 46.5 Hz, 3H), 4.49 (s, 2H), 4.26 (s, 1H), 4.04 (d, *J* = 29.8 Hz, 3H), 3.79–3.45 (m, 7H), 3.35 (d, *J* = 29.1 Hz, 3H), 3.05 (s, 2H), 1.22 (s, 28H) and 0.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 172.5 (C-1), 105.07 (C-4'), 83.44 (C-4), 76.17(C-3'), 73.69 (C-5'), 72.44 (C-2'), 71.85 (C-3), 71.57 (C-5), 70.90 (C-2), 68.70 (C-4'), 62.81 (C-6'), 61.15 (C-6), 38.78 (C-1"), 31.81, 29.73, 29.59, 29.36, 29.24, 26.92, 22.60 (C-4"-15"), 14.38 (C-16"). The chemical structure of STM and N-HLBA was shown in figs. 1A and 1B, respectively.

Validation of the HPLC method for the quantification of STM

To assess potential interference, different formulations were injected individually into the HPLC system (Fig. 2). No chromatographic peaks were observed at the STM retention time (approximately 9.78 min), suggesting that the components in SLN did not interfere with STM detection. To examine the linearity, an analytical curve was constructed spanning the concentration range of 0.5 to 80 µg/mL. The linear correlation coefficient (r^2) was evaluated. The responses exhibited direct proportionality to sample concentration within the specified range, with an r^2 of 0.9991, indicating a linear range of 0.5 to 80 µg/mL. The results of the HPLC method revealed a linear correlation between the STM concentrations and the chromatographic peak area, as expressed by the regression equation: y = 14454x + 2148.1. The proposed STM quantification method exhibited precision, as indicated by CV values below 5% for both inter- day and intraday precision. Furthermore, the method demonstrated accuracy, with experimental results (expressed as % recovery) aligning with the original concentrations (Table 2).

Physical characterization of the SLN

The mean particle size, zeta potential and EE of the STM-GalSLN were presented in Fig. 3. The mean diameter and zeta potential of the STM-GalSLN were 164.40 ± 4.68 nm and -14.53 ± 3.20 mV, respectively. TEM micrographs revealed that the STM-GalSLN had spherical or quasi-spherical morphology and uniform size.

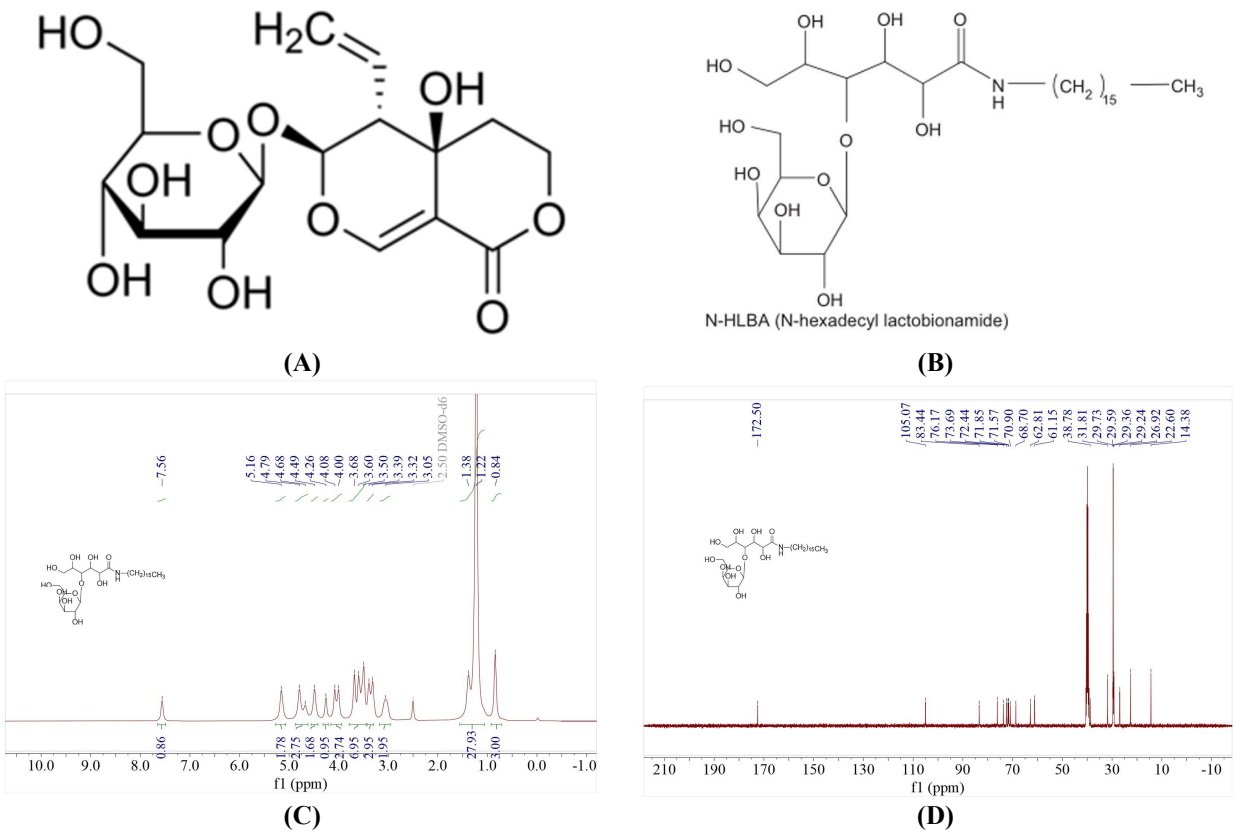


Fig. 1: Chemical structure of STM and N-HLBA. (A) Chemical structure of STM; (B) Chemical structure of N-HLBA; (C and D) ^{1}H NMR and ^{13}C NMR of N-HLBA.

Table 2: Precision and recovery rate of the analytical method validated for the quantification of STM by HPLC (n = 3).

Concentration (μg/mL)	Intraday precision (%)	Interday precision (%)	Recovery (%)
0.50	1.04	2.00	95.13 ± 1.27
40.00	0.15	0.97	104.74 ± 0.15
80.00	0.66	1.20	101.72 ± 0.67

Table 3: Design and results of L9 (3^4) orthogonal test.

Levels	Factors					
	A	B	C	D	EE (%)	DL (%)
1	1	1	3	2	81.64	0.08
2	2	1	1	1	68.53	0.10
3	3	1	2	3	75.57	0.31
4	1	2	2	1	77.07	0.10
5	2	2	3	3	89.17	0.16
6	3	2	1	2	78.77	0.39
7	1	3	1	3	79.01	0.08
8	2	3	2	2	78.15	0.12
9	3	3	3	1	90.90	0.32
K1	79.24	75.25	75.44	78.83		
K2	78.62	81.67	76.93	79.52		
K3	81.75	82.69	87.24	81.25		
R	3.13	7.44	11.80	2.42		

Prescription optimization

The results of the orthogonal experiments are shown in table 3. An orthogonal test range analysis of the results revealed that the order of influence of the four factors on EE, based on the magnitude of the R values, was C>B>A>D. These findings indicated that the effects of soy lecithin/Poloxamer 188, the N-HLBA concentration, the drug–lipid ratio and the Tween 80 concentration on EE decreased in that order. The level analysis results for each factor were A3>A1>A2, B3>B2>B1, C3>C2>C1 and D3>D2>D1. Therefore, the optimal combination of factors was A3B3C3D3. According to the variance analysis results in table 4, factors A, B, C and D significantly influenced the EE of the STM-GalSLN ($P < 0.05$). Therefore, by selecting the highest level of each factor and integrating the results of the orthogonal test and variance analysis, the optimal prescription determined with EE as the evaluation index was as follows: 50 mg swertiamarin, 20 mg N-HLBA, 500 mg glyceryl behenate, 400 mg soy lecithin, 200 mg Poloxamer 188 and 500 mg Tween 80.

Stability of SLN

As shown in table 5, the three batches of samples prepared by the hot-melt ultrasonic method showed good reproducibility, which demonstrates that the preferred prescription process was stable and feasible. There were no significant changes in the appearance of the three batches of STM-GalSLN stored at 4 °C or obvious flocculation or precipitation and there was no significant difference in the EE among the samples examined at 0, 15 and 30 days from each batch (Table 6). The results indicated that the prepared STM-GalSLN was stable when stored at 4 °C for up to 30 days.

Anti-cholestasis effect evaluation

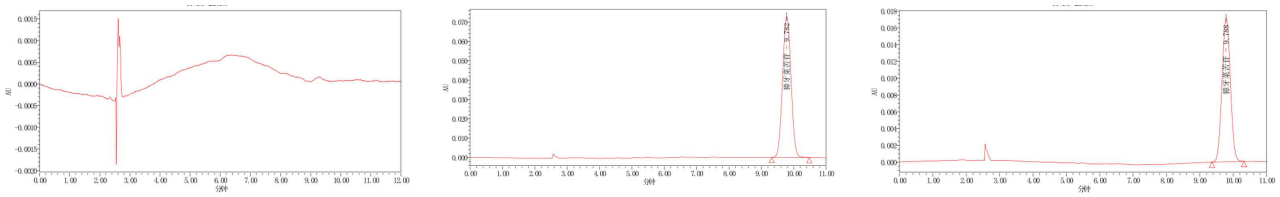
The results of the CCK-8 assay indicated that ANIT (100 μM) exposure significantly inhibited hepatocyte survival at 12, 24 and 48 h ($P < 0.01$). STM-GalSLN treatment increased cell survival in a concentration-dependent manner compared with the ANIT group (Fig. 4A-C). However, the protective effect of STM-GalSLN was weakened at concentrations of 100 and 200 μM. Based on the percentage of cell survival rate, compared with ANIT, 50 μM STM-GalSLN seemed to be the most effective protective concentration at 12, 24 and 48 h ($P < 0.01$) and was suitable as an intervention concentration for further experiments. The cell survival rate in the STM-GalSLN (50 μM) group was significantly higher than that in the STM (50 μM) group ($P < 0.01$) after 24 h of treatment, which indicated the superior protective activity of STM-GalSLN (Fig. 4D). And STM-GalSLN (50 μM) significantly inhibited the ANIT-induced increase in the rate of hepatocyte apoptosis ($P < 0.01$), which might have contributed to its anti-cholestatic activity *in-vitro* (Fig. 4E-I).

DISCUSSION

Currently, one approach to achieve liver targeting in pharmaceutical preparations is to use lipid nanoparticles (LNPs), particularly via receptor-mediated endocytosis. ASGPR is found mainly on the cell membranes of liver parenchymal cells, laying the foundation for ASGPR-mediated liver-targeted drug delivery. Thus, liver-targeted drug delivery can be achieved by binding bioactive compounds to Gal or GalNAc (Zhang *et al*., 2025). In this study, we successfully prepared a novel STM-loaded galactosylated SLN and verified its *in-vitro* anti-cholestatic activity.

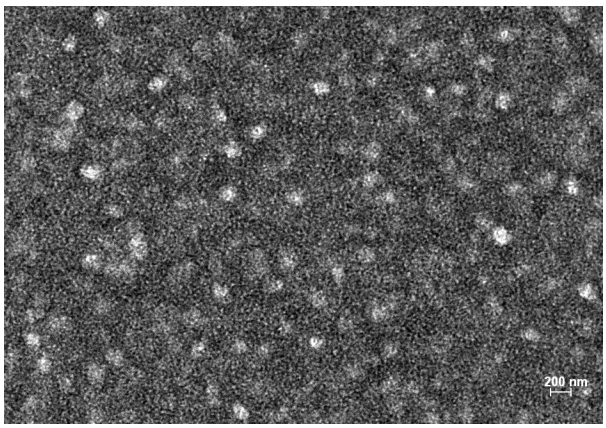
SLN is a promising nanodrug delivery system with diverse preparation methods and administration routes (Rehman *et al*., 2024). The main preparation methods for SLN include ultrasonication of a film, emulsion–solvent evaporation at low temperature, high-pressure homogenization, double emulsion–solvent diffusion, hot-melt ultrasonication, solvent emulsification–ultrasonication and microemulsion methods (M NK *et al*., 2024). Each of these methods has advantages and disadvantages. High-pressure homogenization is a common and reliable technique for SLN preparation but requires a specialized high-pressure homogenizer. The microemulsion method is simple, but the concentration of the resulting suspension is relatively low. Ultrasonication of a film is easy, but forming a film can be difficult. Given the equipment condition and operational difficulty, the hot-melt ultrasonication method was adopted to prepare SLNs in this study.

The determination methods for the EE of SLN include Sephadex gel column chromatography, ultracentrifugation, dialysis and ultrafiltration–centrifugation methods, which have similar principles. They involve first separating the free drug from the encapsulated drug and then calculating the EE by measuring the concentration of the free or encapsulated drug. In this study, Sephadex gel column chromatography failed to elute and separate the free drug from STM-GalSLN; thus, it was not suitable for determining the EE of STM-GalSLN. The ultrafiltration–centrifugation method separates drugs and nanocarriers based on differences in particle size under centrifugal force. The solvent and free drugs pass through the ultrafiltration membrane and are enriched in the collection tube, while the nanoparticles with larger relative molecular masses are retained in the inner tube (Wolska and Brach, 2022). By measuring the drug concentration in the collection tube (filtrate), the concentration of free drugs can be determined. In this study, the ultrafiltration–centrifugation method was proven to be suitable for determining the EE of STM-GalSLN. Lipid materials play a key role in SLN formation. Common lipid materials used for SLN preparation are physiologically compatible and biodegradable, including high-melting-point natural or synthetic solid lipids such as glyceryl monostearate and glyceryl behenate. In this study, we attempted to use glyceryl monostearate as the lipid material and found that, although the resulting SLN had a small particle size, its stability was poor, with flocculation occurring after a few hours.

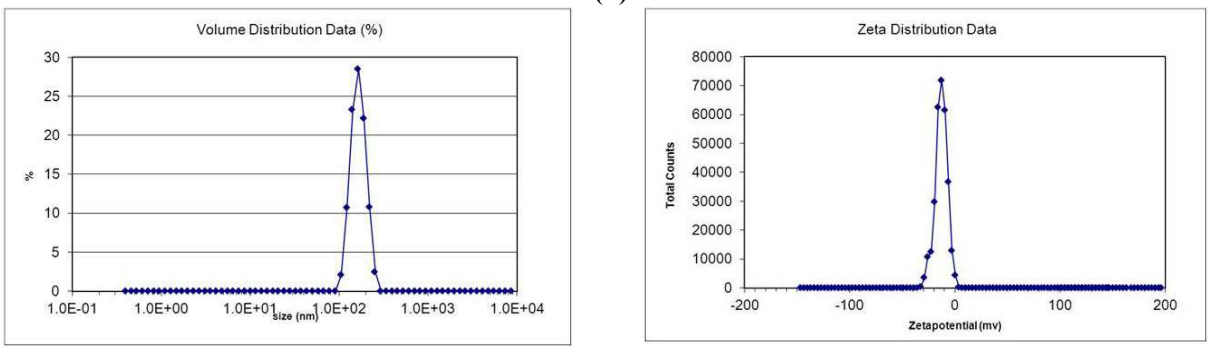


(A) (B) (C)

Fig. 2: HPLC chromatograms of STM: (A): blank; (B): STM solution; (C): STM-GalSLN.



(A)



(B) (C)

Fig. 3: TEM, size and zeta potential of STM-GalSLN: (A): TEM (68000×); (B): size distribution (nm); (C): zeta potential (mV). (scale bar = 100 nm). The final sample dilution was 50-fold.

Table 4: The results of variance analysis.

Sources of variance	*SS*	*df*	*MS*	*F*	*P*
A	48.810	2	24.405	13.162	.000
B	290.179	2	145.090	78.247	.000
C	749.630	2	374.815	202.137	.000
D	28.813	2	14.407	7.769	.004
Error	33.377	18	1.854		

Table 5: Mean size, zeta potential and encapsulation efficiency of STM-GalSLN (n = 3).

Samples	Particle size (nm)	Zeta potential (mv)	EE (%)	DL (%)
1	167.30	-13.10	85.25	0.34
2	166.90	-18.20	79.50	0.30
3	159.00	-12.30	82.48	0.31
Mean±SD	164.40±4.68	-14.53±3.20	82.41±2.88	0.32±0.02

Table 6: Stability of STM-GalSLN at 4 °C (n = 3).

EE (%)	0d	15d	30d
1	85.47±0.26	85.96±0.24	86.03±0.42
2	78.02±0.33	78.29±1.12	78.09±0.25
3	83.40±0.87	84.31±1.08	83.90±1.10

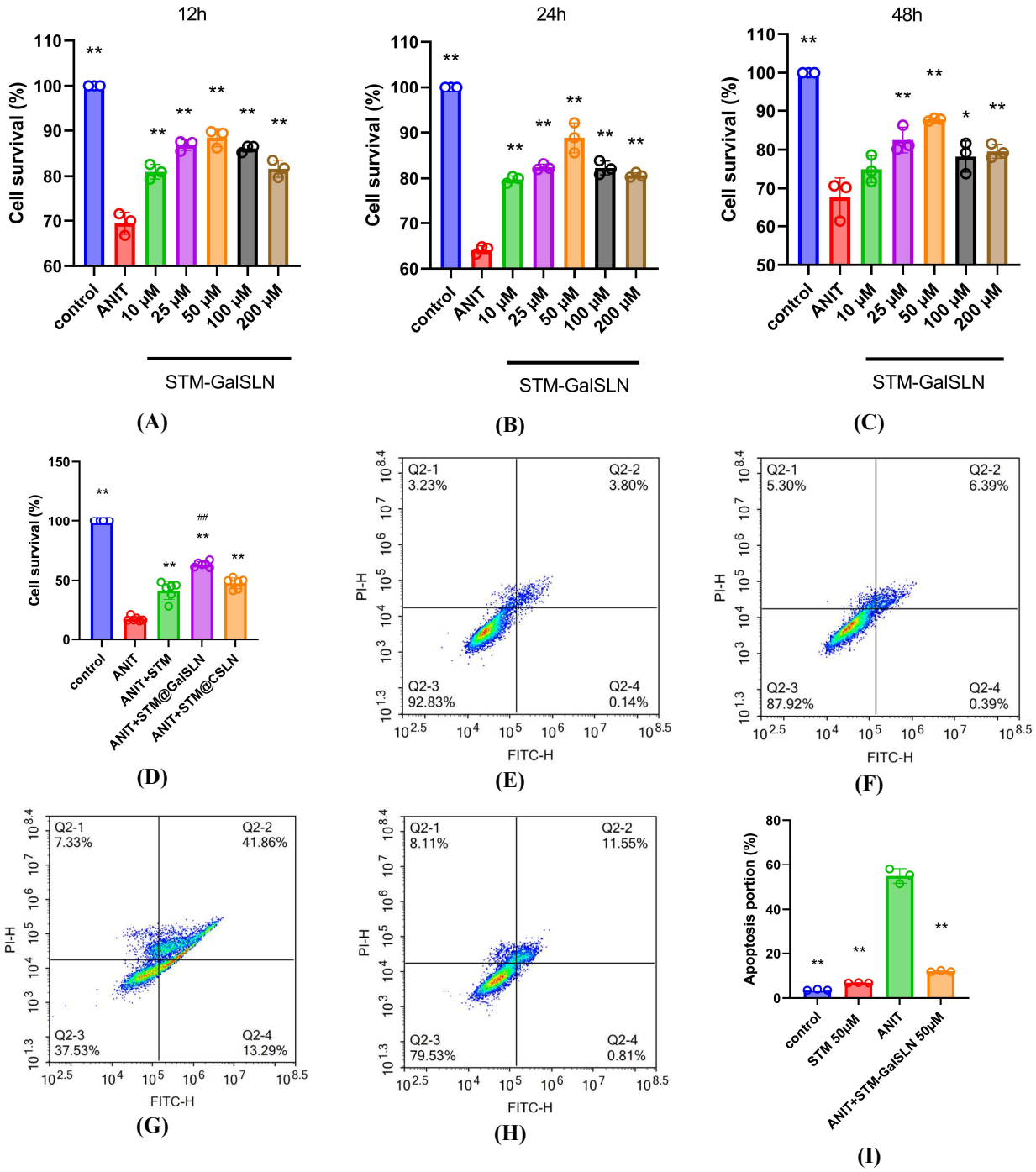


Fig. 4: Anticholestatic effect of STM-GalSLN on CLI in hepatocytes: (A–D): Cell survival rate; (E-H): Flow cytometry plot; (I): Quantitative analysis of apoptotic rate. Each column represents the mean±SD (n=3–6). * $p < 0.05$ versus the ANIT group, ** $p < 0.01$ versus the ANIT group, $^{\#\#}$ $p < 0.01$ versus the ANIT+STM group.

SLN prepared with Compritol 888 (glyceryl behenate) did not precipitate after storage at 4 °C for one month, with significantly improved stability. Compritol 888 was eventually used as the lipid material. Compritol 888 is a mixture of mono-, di- and triglyceryl behenates; it can form a defective lattice structure, which hinders the aggregation of solid colloidal particles and this may explain why SLN prepared with it have good stability (Rao, Rao *et al*., 2024). However, the use of Compritol 888 in nanodrug delivery systems, such as SLNs and nanostructured lipid carriers (NLCs), is prone to gelation during preparation and use. This study also revealed that when the concentration of Compritol 888 exceeded 4% (w/w), the prepared SLN was prone to gelation. If the concentration of Compritol 888 was too low, it would further reduce the DL of SLN. Thus, the concentration of Compritol 888 was limited to 2.5% (w/w).

In the entire SLN dispersion system, emulsifiers not only affect particle size and stability but also directly affect the structure or crystalline state of the matrix. Different types of emulsifiers significantly affect SLN stability and exhibit distinct characteristics. Common emulsifiers include mainly phospholipids, ionic surfactants (such as deoxycholate) and nonionic surfactants (such as Tween 80, poloxamer and span). The ratio of Tween 80/Span 20 surfactants could modulate the interfacial organization, caffeine encapsulation and release behavior of NLC. In addition, intermediate surfactant ratios resulted in the highest entrapment efficiency (Damayanti, Khong *et al*., 2025). Besides, poloxamer 188 and Tween 80 might prevent particle aggregation through steric hindrance effects, thus improving the stability of the SLN dispersion system (Sarhadi, Gholizadeh *et al*., 2020). Lecithin as a surfactant also allows more drugs to dissolve into the lipid matrix. On the other hand, although Tween 80 can reduce the particle size of SLN, excessive amounts also increase the amount of drugs dissolved in the aqueous phase, thereby reducing the EE of SLN. A mixed emulsifier composed of soy lecithin, poloxamer 188 and Tween 80 was used in this study. The results indicated that the prepared SLN had a small particle size and good stability, with no significant change in EE after storage at 4 °C for 30 days. In addition, the *in-vitro* drug release profiles of SLN is typically influenced by optimization parameters. The improved particle size and surface area of the nanoparticles led to superior performance of the optimized SLN. Additionally, the presence of surfactants could improve drug solubility and diffusion (Parihar, Prajapati *et al*., 2025). In this study, the formulation of STM-GalSLN was optimized using an orthogonal design, which may improve release kinetics and bioavailability.

Moreover, ANIT is known to be toxic to the gallbladder and liver and is commonly used to induce cholestasis-related hepatocyte injury *in-vitro*. ANIT exposure can decrease the cell survival rate, induce cholestatic injury and increase oxidative stress in hepatocytes (Li, Xu *et al*., 2022, Refat, Zhang *et al*., 2023). In this study, STM-GalSLN rescued the survival rate of ANIT-repressed cells in a dose-dependent manner. STM-GalSLN at 50 μM had the greatest beneficial effect on CLI *in-vitro* and significantly alleviated ANIT-induced hepatocyte apoptosis.

CONCLUSION

This study revealed that the prepared STM-GalSLN had a small particle size, high EE and good stability, providing an experimental basis for the subsequent development of new nanoformulations of STM. The prepared STM-GalSLN exhibited notable anti–cholestatic effects in cultured hepatocytes. Considering the convenience of clinical storage and use, as well as the long-term stability of drug formulations, we plan to develop a freeze-dried powder of SLN for injection to further improve its stability, liver targeting and pharmacokinetics.

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Authors' contributions
WT: Wrote the manuscript; HW: Collected data; GX: Collected data; ZX: Revised the manuscript; LL: Analyzed data. All authors read and approved the final submitted manuscript.

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Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval
Not applicable.

Conflict of interest
The authors declare no conflict of interest.

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