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Curcumin-Loaded Long-Circulation Liposomes Ameliorate Insulin Resistance in Type 2 Diabetic Mice

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Introduction: Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterised by insulin resistance, hyperglycaemia, and inflammation, with oxidative stress contributing to its progression. Curcumin (CUR), known for its anti-inflammatory, antioxidant, and insulin sensitising effects, has shown potential for the treatment of T2DM but is limited by low solubility and bioavailability. This study investigated long-circulating curcumin-loaded liposomes (CUR-LPs) to improve curcumin stability, solubility, and circulation and assessed their effect on insulin resistance in a murine model of T2DM.

Methods: CUR-LPs were prepared using the ethanol injection method and characterized for morphology, particle size, zeta potential, encapsulation efficiency, drug-loading capacity, and in vitro release. Cell viability was tested on murine L929 cells. In a T2DM murine model, after four weeks of CUR-LP treatment, inflammatory markers TNF-α and IL-6 were measured by real-time polymerase chain reaction, and liver tissues were analyzed for glutathione (GSH) and superoxide dismutase (SOD) via colorimetry.

Results: CUR-LPs were spherical, with an average diameter of (249 ± 2.3) nm and a zeta potential of (−33.5 ± 0.8) mV. They exhibited an encapsulation efficiency of (99.2 ± 0.5) %and a drug-loading capacity of (1.63 ± 0.02) %. CUR embedding in liposomes significantly maintained CUR release. In L929 cells, over 80% viability was maintained at 12 uM CUR concentration after 24 h. In HFD/STZ-induced T2DM mice, CUR-LPs improved blood glucose and insulin levels more efficiently than free CUR, and CUR-LPs also reduced hepatic inflammation (TNF-α, IL-6), enhanced hepatic GSH and SOD, and attenuated liver injury.

Conclusion: CUR-LPs improved glucose metabolism and insulin resistance in HFD/STZ-induced T2DM mice, which may be associated with a decrease in liver inflammation and oxidative stress.

Keyworks: T2DM, curcumin, liposomes, insulin resistance, glucose metabolism, oxidative stress

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action, with Type 2 diabetes mellitus (T2DM) accounting for 90–95% of diabetes patients.[1] T2DM is a metabolic disease characterized by hyperglycemia and occurs due to a variety of complex physiological and pathological processes, with insulin resistance being a hallmark characterizing.[2] Insulin resistance has a complex pathology, which is promoted by multiple factors, including inflammation and oxidation stress.[3] Inflammation and insulin resistance are closely related, and inflammatory cytokines such as TNF-α and IL-6 can inhibit insulin signaling through various mechanisms.[4] Along with inflammation, oxidative stress is also a key factor in the development of insulin resistance and T2DM. High blood glucose levels produce reactive oxygen species, which are key contributors to oxidative stress.[5]

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Curcumin (CUR) is a polyphenol with a relatively small molecular mass and is the most effective ingredient in turmeric, can activate multiple cell signal pathways in the body, inhibit the formation of advanced glycation end products, reduce the generation of inflammatory factors, and show antioxidant stress effects, thereby protecting islet βcells, improving glucose metabolism, and combating insulin resistance.[6] In addition, previous studies have shown that CUR can improve blood lipid levels, stimulate the antioxidant defense mechanism, and improve mitochondrial dysfunction.[7] Studies have shown that the main active ingredients of turmeric have a significant treatment effect on diabetes.[8] However, CUR has poor water solubility, is easily oxidized in vitro, has low bioavailability, and is metabolized rapidly. Therefore, CUR as a therapeutic agent has greatly limited its clinical application. In this study, liposomes were used as delivery carriers for curcumin, to improve the solubility and stability of CUR. The incorporation of poly(ethylene glycol) 2000 (PEG2000) into the bilayers of liposomal vesicles to obtained the long-circulation CUR-loaded liposomes (CUR-LPs), was assumed to extend the blood-circulation time of CUR by avoiding reticuloendothelial system (RES) uptake.[9] This safe, effective, and stable nano drug delivery system was constructed to explore the efficacy and related mechanisms of CUR in improving insulin resistance in type 2 diabetes mice.

Materials and Methods

Materials

Curcumin (purity 98%, Lot No. J14HS188388) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Hydrogenated soybean phosphatidylcholine (HSPC) and cholesterol (CHOL) were from AVT (Shanghai) Pharmaceutical Tech Co., Ltd. (Shanghai, China). 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine-conjugated poly(ethylene glycol) 2000 (DSPE-PEG2000) was purchased from Xi'an Ruixi Biological Technology Co., Ltd. (Xi'an, China). Streptozocin (STZ) was from Sigma–Aldrich, Co. (MO, USA). TRIzol reagent was purchased from Yeasen Biotechnology Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and were used as received.

Cells

Murine epithelioid fibroblast L929 cells were from Hysigen Bioscience Tech Co., Ltd. (Suzhou, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)-high glucose supplemented with 10% fetal bovine serum (Yeasen Biotechnology Co., Ltd., Shanghai, China) at 37°C in a humidified 5% CO_2 atmosphere.

Animals

This study was conducted in accordance with the ARRIVE 2.0 guidelines and approved by the Ethics of Animal Experiments Committee of Southern Medical University (No. SYXK-2021-0167). All the processes were in strict accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Animals in laboratory experiments. Four-week-old male C57BL/6J mice (20–22 g) were purchased from the Laboratory Animal Center, Southern Medical University (Guangzhou, China) and housed in 24°C with a 12 h light/dark cycle in animal facilities of Southern Medical University. They had free access to food and water.

Methods

Preparation of Curcumin Loaded Liposomes

The curcumin-loaded liposomes (CUR-LPs) were prepared using ethanol injection method. Briefly, Curcumin, HSPC, CHOL, and DSPE-PEG2000 (molar ratio=7:122:65:11) were accurately weighed and dissolved in an absolute ethanol solvent. Then, the lipid solution was injected into an aqueous solution of 5% glucose (volume ratio=1:10) and kept in a water bath at 60°C for 1 h, followed by ultrasonication in an ice water bath (200W, 2 min). The prepared vesicles were then dialyzed in 5% glucose solution (volume ratio=1:2000) for another 2 h at room temperature. Finally, the CUR-LPs were obtained. Conventional liposomes (Empty LPs) were prepared with the same protocol, without the addition of CUR.

Characterization of CUR-LPs

Morphology of CUR-LPs was observed by transmission electron microscopy (TEM) (JEM-2100, JEOL, Tokyo, Japan). Particle size and polydispersity index (PDI) of CUR-LPs were measured by Zetasizer Nano ZS (ZS ZEN3690, Malvern, UK) by dynamic light scattering and electrophoresis. Drug loading (DL)% and encapsulation efficiency (EE)% were calculated as described earlier.[10,11] Free drugs (WF) and encapsulated drugs were separated by an ultrafiltration method, and the WF in filtrate were sampled as previously mentioned.[12] The same volume of liposomes was dissolved in methanol, and the total drug (WT) was obtained. WF and WT were determined by HPLC analysis on an Elite C_{18} column (250 mm × 4.6 mm, 2.5 μm) at 430 nm. The mobile phase was 4% acetic acid solution−acetonitrile (52:48, v/v) with a flow rate of 1.0 mL/min. The column temperature was maintained at 30°C, and the injection volume was 10 μL. DL% and EE% were calculated according to the following equations:

$$DL\% = \frac{WT-WF}{\text{Mass of LPs}} \times 100$$

$$EE\% = \frac{WT-WF}{WT} \times 100$$

where the mass of LPs was weighed after lyophilization of the equal volume of CUR-LPs.

In vitro Release of CUR-LPs

The dialysis method was employed to detect the in vitro release behavior of CUR from liposomes.[13] Briefly, 1.0 mL of CUR-LPs were placed into a dialysis bag (cutting MW 10KD, Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China). Then, these bags were placed in 500 mL of physiological saline containing 1.0% Tween 80 with pH adjusted to 7.0. The experiment is conducted at 37°C under stirring at 250 rpm. The CUR-contained suspension (Free CUR) was prepared as control and subjected to the same protocol. At decided time intervals of 0.5, 1, 2, 4, 6, 12, and 24 h, 100 μL of sample was collected from these bags and diluted with 4% acetic acid solution−acetonitrile (52:48, v/v). The concentration of CUR at every time point was measured by the HPLC condition as described above. The cumulative release of CUR from preparations was calculated as following:

$$\text{Cumulative release of CUR } (\%) = \frac{CUR_t - CUR_i}{CUR_t} \times 100$$

Where CUR_t was the total CUR concentration at t = 0, and CUR_i was the CUR concentration in the dialysis bags at different time points.

Cell Viability Assays

Cell growth and viability of L929 murine fibroblasts treated with different CUR-loaded preparations were determined in vitro by using Cell-Counting Kit 8 (CCK-8) assays as previously reported.[14] Cells were seeded in 96-well plates at 5×10^3 cells per well and cultured overnight. Then, the cells were incubated with a series of concentration of CUR-LPs (Concentration of CUR = 1.5, 3.0, 6.0, and 12 μM). The negative control was treated with culture medium. After culturing for 24 h, the viability of cells in each well was determined by CCK-8 kit as the manufacturer's protocol (Meilunbio Biotechnology, Dalian, China). The relative cell viability was calculated compared to that of negative control.

Construction of Type 2 Diabetes Mice

Male C57BL/6J mice were divided into a normal diet group (Normal diet, D12450B, 10% calories from fat, n = 6) and T2DM groups. The T2DM groups were fed with high fat feed (HFD) (D12492, 60% calories from fat) during the experimental period, and were intraperitoneal (i.p.) injected with STZ (40 mg/kg, dissolved at 0.1 mm cold citrate buffer, pH 4.4) for 5 days. The weight, fasting blood glucose, serum insulin, and blood lipid levels of the mice were monitored weekly, and when fast blood glucose levels were over 16.7 mmol/L, the model of type 2 diabetes mice was successfully established and the animals were randomly grouped.

Experimental Group

These HFD/STZ-induced T2DM mice were then randomly divided into five groups and given different treatments: a diabetic model control group (0.9% sodium chloride, 5mg/kg, i.v., T2DM, n = 6), a low-dose free curcumin treatment

group (T2DM+Free CUR-L; 5mg/kg, i.g., n = 6), a high-dose free curcumin treatment group (T2DM+Free CUR-H; 50mg/kg, i.g., n = 6), an empty liposome group (T2DM+Empty LPs; 5mg/kg, i.v., n = 6), a long-circulation liposomes loaded with CUR group (T2DM+CUR-LPs; 5mg/kg, i.v., n = 6). The mice in the normal control group were fed with normal diet (0.9% sodium chloride, 5mg/kg, i.v., n = 6). All groups were given the treatment every two days for a duration of four weeks.

Detection of Weight and Fasting Blood Glucose

Body weights were measured weekly during the experimental period. After fasting for 12 h every week, the blood was collected from the tail vein, and the fasting blood glucose was detected by a standard glucose meter (Roche, Basel, Switzerland).

Measure of Insulin Levels and Assessment of Insulin Resistance

Mice were fasted overnight. After the mice were fully anaesthetized, the blood samples were collected from the abdominal aorta and centrifuged at 12000 × g for 20 min at 4°C. The serum was collected and stored at −80°C until use. Serum glucose was measured with a standard glucose meter, and insulin was determined using ELISA (Proteintech, America) according to the instructions from the manufacturer. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: HOMA-IR = [fasting plasma glucose (mmol/L) × fasting plasma insulin (mU/L)]/22.5.[13,15]

Detection of Liver Inflammatory Factors and Injury Assays

Liver samples were collected in all animal experiments for quantitative real-time polymerase chain reaction (qRT-PCR) to analyze hepatic gene expression. Total RNA was extracted with TRIzol reagent, and cDNA was synthesized from 3 mg RNA with random primers using a reverse transcription kit from Thermo Fisher Technology (China) Co., Ltd. (Shanghai, China). RT-PCRs were performed with SYBR green PCR mix (Thermo Fisher) in a 7500 real-time PCR system (Applied Biosystems, CA, USA). The levels of TNF-α and IL-6 were measured using β-actin as internal reference. All primer sequences used in the study are shown in Table 1. Liver tissues were fixed in 4% neutral-buffered formalin for 24 h. Then, the tissues were embedded in paraffin after dehydration in a graded ethanol series (75–100%). Embedded samples were sectioned using a rotary microtome and mounted on slides. For histochemical analysis, the slides were dewaxed and stained with hematoxylin and eosin, and then hepatic steatosis was analyzed by microscopy (Leica Application Suite, version 4.0).

Assessment of Oxidative Stress Factors

The liver tissues were collected and washed with pre-cooled PBS (0.01 M, pH = 7.4). The residual blood was removed. The tissues were accurately weighed, and PBS was added in a certain proportion. The tissues were fully broken by ultrasound on ice and centrifuged at 5000 × g for 10 min at 4°C. After absorbing supernatant, SOD activity and GSH content were measured by colorimetry.

Table 1 PCR Primer Sequences of Genes Used in RT-PCR

Gene	Primer Sequences (5'-3')	
β-actin	Forward primers	CCTCTATGCCAACACAGTGC
	Reverse primers	CCTGCTTGCTGATCCACATC
TNF-α	Forward primers	AACTGGCAGAAGAGGCACTC
	Reverse primers	GGAATGAGAAGAGGCTGAGAC
IL-6	Forward primers	TGATGCACTTGCAGAAAACA
	Reverse primers	ACCAGAGGAAATTTTCAATAGGC
IL-1β	Forward primers	GGTCAAAGGTTTGGAAGCAG
	Reverse primers	TGTGAAATGCCACCTTTTGA

Statistical Analysis

The data is presented as mean ± SEM from at least three independent experiments. Statistical analysis was performed using SPSS 22.0 Software. ANOVA analysis of variance was used for comparison between multiple groups. Differences were considered to be statistically significant at *p < 0.05.

Results

Preparation and Characterization of CUR-LPs

In our current study, we designed PEG-modified stabilized liposomes encapsulating curcumin using the ethanol injection method. The TEM images of the CUR-LPs are shown in Figure 1, indicated that the particles were spherical in shape. Shadow can be seen around the vesicles, and it could be the modification of PEG chains distributed on the surface of the vesicle membrane. The CUR-LPs show nanometer sizes at (249 ± 2.3) nm with a PDI of 0.15 ± 0.01. Zeta potential of CUR-LPs was (−33.5 ± 0.8) mV. The EE% and DL% of CUR-LPs were (99.2±0.5) % and (1.63±0.02) %, respectively, which indicated the high encapsulation of CUR by liposomal vesicles.

In vitro Release

The release behaviors of CUR from different preparations were detected and shown in Figure 2. The release rate of free CUR reached 95.7% within 24 h, while the release rate of CUR-LPs was 52.4%. Within the first hour, only 13.6% of CUR were released from liposomes, which was about 24% of that released from Free CUR. Thanks to the extreme insolubility of CUR in water, it is conveniently to be entrapped in liposomes. The liposomal bilayer was assumed to improve the drug stability and solubility, at the same time, to work as a barrier hindering contact between CUR and the release medium. Therefore, the obviously sustained-release of CUR was exhibited for CUR-LPs.

Cell Viability Assays

The relative cell viability of CUR-treated cells was shown in Figure 3. According to the viability of L929 cells incubated with CUR-LPs, more than 80% of cells were alive with an applied concentration of CUR up to 12 μM was observed. It was previously reported that the viability of L929 cells would still reach more than 60% at concentration up to 27.1 μM.[16] Taking the total CUR in liposomes and the sustained release of CUR from preparations (Figure 3), under the range

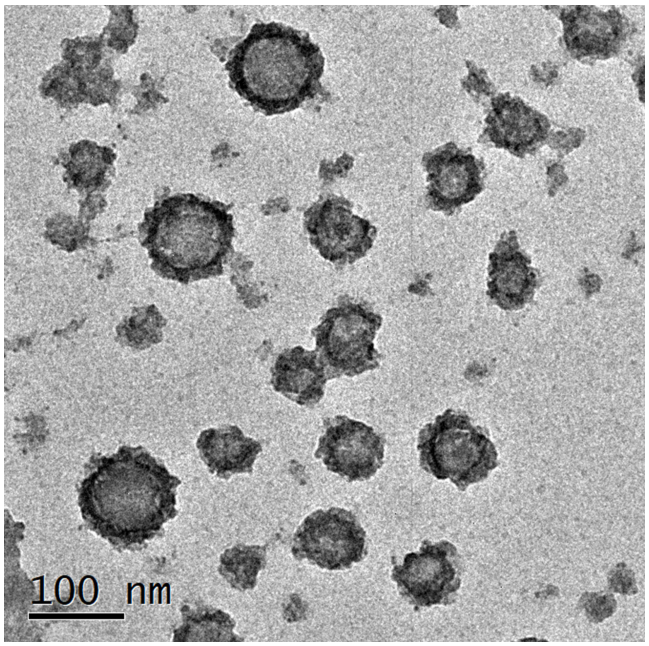

Figure 1 The transmission electron microscope images of CUR-LPs (×40000 Magnification, scale bar: 100 nm).

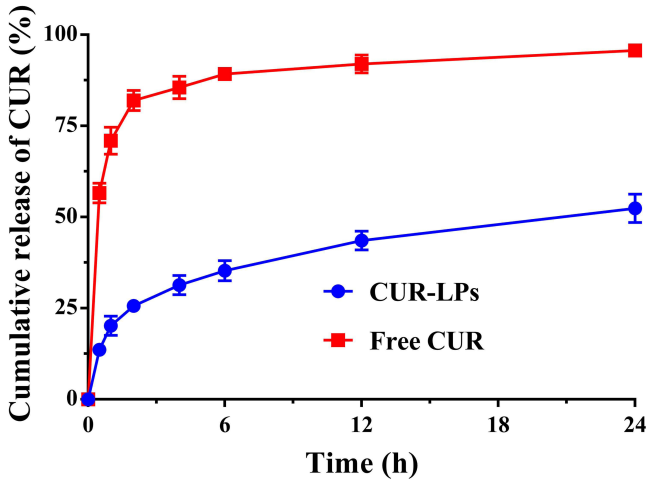

Figure 2 In vitro release of CUR from different preparations detected by dialysis method (n=3). The release medium was physiological saline containing 1.0% Tween 80 with pH adjusted to 7.0.

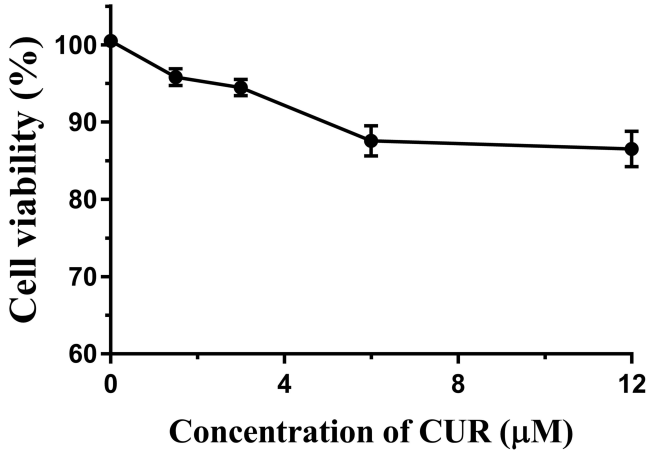

Figure 3 CCK-8 assays of CUR-LPs against L929 cells within 24 h (n=3).

of concentration of CUR, which is applicable for CUR-LPs and much lower than 27.1 μM, therefore, there was no significant cytotoxicity for CUR-LPs.

Effect of CUR-LPs on Body Weight, Blood Glucose of T2DM Mice

Male C57BL/6J mice were divided into a normal control group (normal diet, n = 6) and five HFD/STZ-induced T2DM groups, and then were given free CUR, CUR-LPs, or Empty LPs treatment, respectively (Figure 4A). As shown in Figure 4B, T2DM mice had a slightly lower body weight than the normal diet group. The body weight of T2DM mice increased with the treatment of CUR-LPs, while treatment with free CUR had no significant effects on body weight. As shown in Figure 4C, T2DM mice had significant up-regulated effects on the blood glucose, which was improved by CUR-LPs treatment.

Effect of CUR-LPs on Insulin and Blood Lipid Levels Level of T2DM Mice

As shown in Figure 5A, CUR-LPs and high dose of free CUR could reduce the insulin level in T2DM mice. And in Figure 5B, the HOMA-IR measurements showed that HFD combined with STZ injection increased insulin resistance, while CUR-LPs and high dose of free CUR could significantly reverse this effect. As shown in Table 2, liver lipid analysis showed that HFD/STZ-induced T2DM mice exhibited significantly elevated levels of total cholesterol (TC),

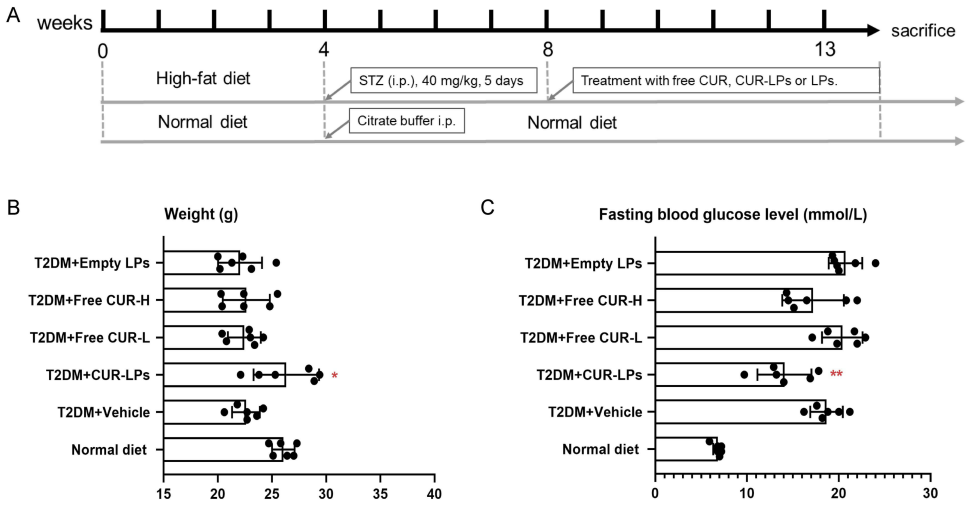

Figure 4 Effect of CUR-LPs on body weight and fasting blood glucose level of T2DM mice. Normal diet: a normal diet group (0.9% sodium chloride, 5mg/kg, i.v., n=6), T2DM +Vehicle: a diabetic model control group (0.9% sodium chloride, 5mg/kg, i.v., T2DM, n=6), T2DM+Free CUR-L: a low-dose free curcumin treatment group (5mg/kg, i.g., n=6), T2DM+Free CUR-H: a high-dose free curcumin treatment group (50mg/kg, i.g., n=6), T2DM+Empty LPs: an empty liposome group (5mg/kg, i.v., n=6), T2DM+ CUR-LPs: a long-circulation liposomes loaded with CUR group (5mg/kg, i.v., n=6). (**A**) Description of the whole experimental process. (**B**) The body weight of T2DM mice treated with different ways. (**C**)The blood glucose level of T2DM mice treated with different ways. *p < 0.05 and **p < 0.01 vs T2DM+Vehicle.

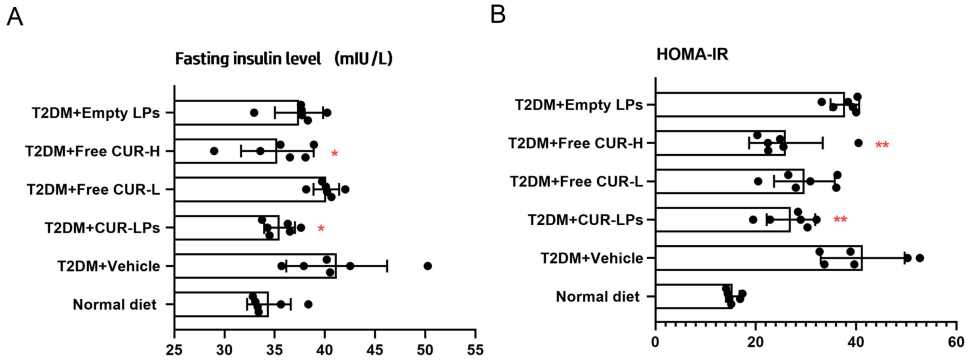

Figure 5 Effect of CUR-LPs on insulin and HOMA-IR level of T2DM mice. (**A**)Evaluation of blood glucose levels in mice treated with different treatments. (**B**)The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: HOMA-IR = [fasting plasma glucose (mmol/L) × fasting plasma insulin (mU/L)]/22.5. *p < 0.05, **p < 0.01 vs T2DM+Vehicle.

low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) in the liver, whereas CUR-LPs treatment had a beneficial improvement effect.

CUR-LPs Attenuated Liver Inflammation and Liver Injury in T2DM Mice

Liver inflammatory factors in mice were detected by RT-PCR, as shown in Figure 6, the pro-inflammatory cytokines TNF-α and IL-6 were significantly increased in the liver of HFD/STZ-induced T2DM mice, while the pro-inflammatory cytokines TNF-α

Table 2 Effects of Free CUR and CUR-LPs on Liver Profile Levels in HFD/STZ-Induced T2DM Mice

	Normal Diet	T2DM+Vehicle	T2DM+CUR-LPs	T2DM+Free CUR-L	T2DM+Free CUR-H	T2DM+Empty LPs
Total cholesterol (mmol/g prot)	0.083±0.010**	0.202±0.035	0.109±0.0193*	0.211±0.081	0.145±0.0277	0.160±0.061
Total triglyceride (mmol/g prot)	0.099±0.016***	0.289±0.071	0.137±0.030**	0.250±0.070	0.272±0.058	0.345±0.123
LDL cholesterol (mmol/g prot)	0.045±0.012**	0.195±0.084	0.096±0.017*	0.157±0.050	0.164±0.055	0.178±0.063
HDL cholesterol (mmol/g prot)	0.023±0.011*	0.010±0.003	0.016±0.009	0.019±0.008	0.028±0.014	0.018±0.020

Notes: *p<0.05, **p<0.01, ***p<0.001 vs T2DM+Vehicle.

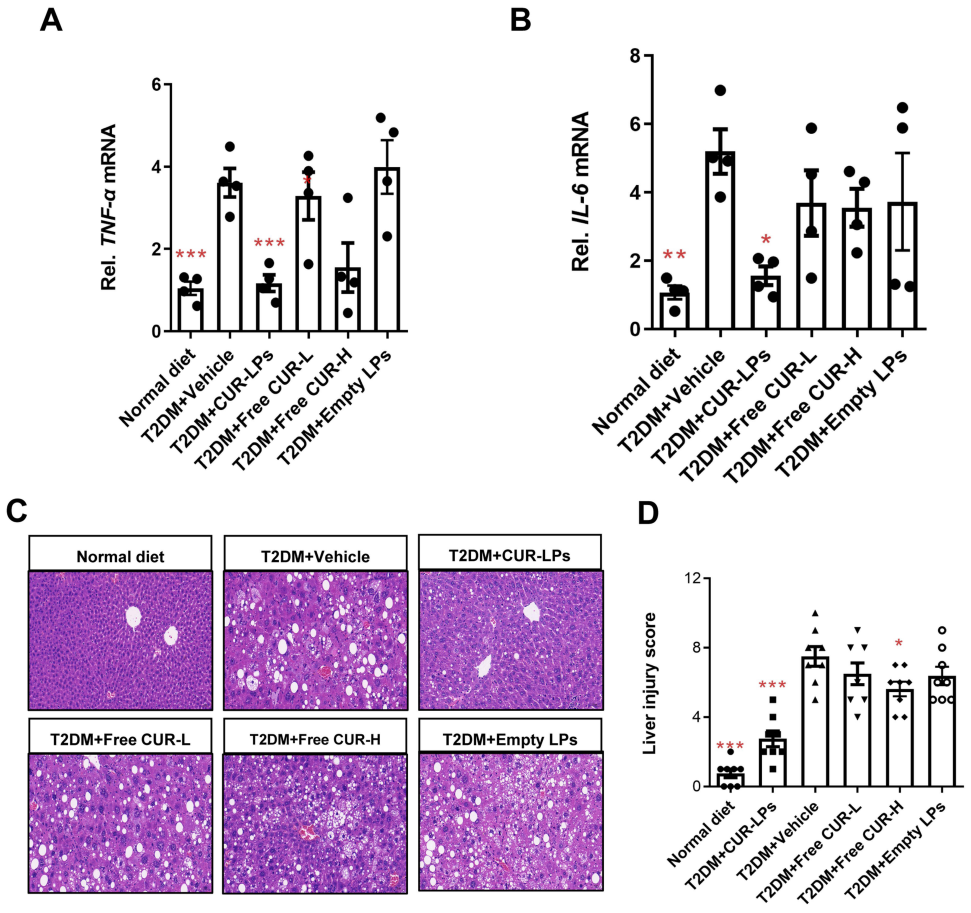

Figure 6 CUR-LPs attenuated liver inflammation and injury in T2DM mice. Mice with diabetes, induced by HFD/STZ, were treated with Free CUR, CUR-LPs, or Empty LPs. The expression of inflammatory cytokines TNF-α (**A**) and IL-6 (**B**) in the liver of HFD/STZ-induced T2DM mice were measured by RT-PCR. Liver tissues were fixed and sectioned for further analysis. (**C** and **D**) Hematoxylin and eosin staining was conducted for histochemical examination of liver tissue (original magnification 20×). *p < 0.05 and **p < 0.01, ***p < 0.001 vs T2DM+Vehicle.

and IL-6 were significantly decreased in the liver of mice treated with CUR-LPs. The beneficial impact of CUR-LPs on the liver was corroborated by HE staining, as shown in Figure 6C. In HFD/STZ-induced T2DM mice, pathological changes in the liver tissue were observed, including hepatocyte swelling, cytoplasm rarefaction, scattered spotty necrosis in the hepatic tissue, and inflammatory cell infiltration. However, CUR-LPs treatment significantly alleviated these pathological changes (Figure 6C and D). Taken together, the findings unequivocally demonstrated that CUR-LPs could reverse liver inflammation and injury in HFD/STZ-induced T2DM mice.

CUR-LPs Attenuated Liver Oxidative Stress in T2DM Mice

The levels of GSH and SOD in the liver were detected by colorimetry. As shown in Figure 7A, the level of GSH in T2DM mice decreased, while the CUR-LPs and empty LPs achieved to increase GSH level in the liver of HFD/STZ-induced T2DM mice. As shown in Figure 7B, the SOD level also decreased in T2DM mice, while the CUR-LPs and free CUR treatment improved significantly.

Discussion

Diabetes mellitus is a multi-etiological metabolic disease characterized by insufficient insulin secretion and manifests as chronic hyperglycemia.[3] However, its etiology and pathogenesis remain incompletely understood. Insulin resistance is a crucial aspect in the pathogenesis of T2DM.[17] Previous studies have found that the pathogenesis of insulin resistance is

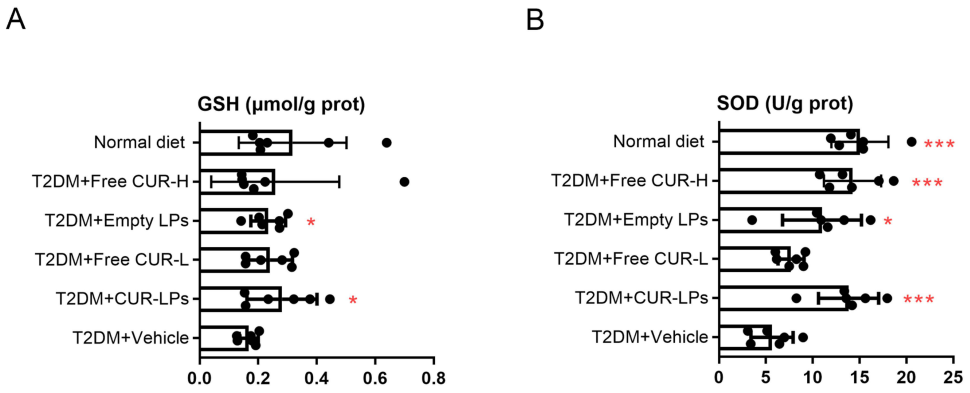

Figure 7 CUR-LPs alleviated oxidative stress in T2DM mice. The levels of GSH (**A**) and SOD (**B**) in liver of mice treated with different methods were detected. *p < 0.05, ***p < 0.001 vs T2DM+Vehicle.

related to inflammation and oxidative stress, which is generally considered the primary factor in the early stages of type 2 diabetes onset for most patients.[18]

Curcumin is a polyphenolic substance extracted from turmeric.[19] However, it is characterized by hydrophobicity, low water solubility, and poor stability in vivo, along with disadvantages such as rapid in-vivo metabolism, fast clearance, low gastrointestinal absorption, and low bioavailability, which significantly limit its clinical application[17,20] Previous studies have shown that curcumin has significant efficacy in the treatment of diabetes, and it may be more preferred over classical treatments in the case of prediabetes and insulin resistance[18,21] Nevertheless, some studies have found that compared to curcumin without a delivery system, curcumin liposomes exhibit reduced clearance, slower elimination rate, and increased absorption in the body[19,22] We prepared CUR-LPs using the ethanol injection method to establish a safe, effective, and stable nano-drug delivery system. These CUR-LPs possessed higher encapsulation efficiency and drug loading. The improved water solubility of CUR in CUR-LPs allows for their administration to diabetic mice via tail vein injection, thus greatly enhancing the body's absorption rate of CUR. By improving the physicochemical properties of curcumin-loaded long-circulation liposomes, we hypothesize that curcumin liposomes may exert more favorable effects in improving glucose levels, insulin sensitivity, liver inflammation, and oxidative stress compared to free CUR.

In this study, we constructed a type 2 diabetes mouse model and found that the HOMA-IR of T2DM mice was significantly higher than that of normal mice. Compared to free curcumin, blood glucose and HOMA-IR levels in T2DM mice decreased more significantly after treatment with CUR-LPs. Our results align with those of those of previous studies.[21,23] Surprisingly, even high doses (10-fold) of oral free curcumin therapy were still not as effective as CUR-LPs. This discrepancy may be attributed to the change in the route of administration, thereby avoiding the first-pass effect that oral therapy typically encounters. Since inflammation and oxidative stress are closely related to HOMA-IR, we further investigated the efficacy of curcumin in mitigating inflammation and oxidative stress in T2DM mice.

Previous studies have demonstrated that curcumin liposomes are more effective than free curcumin in reducing systemic inflammation levels.[24] We further extracted liver tissue and analyzed the expression of representative inflammatory factors. The results indicated that the expression of TNF-α and IL-6 could be significantly decreased. Other studies have found that the inflammatory factor TNF-α alters glucose metabolism and may modulate glucose metabolism levels in fat, skeletal muscle, and the liver through glucose transporter (Glut4) mRNA mediating insulin resistance.[25] There is also evidence that TNF-α can increase insulin resistance by inducing Insulin Receptor Substrate 1(IRS-1) phosphorylation.[26] Recently, it has been shown that both IL-6 and TNF-α can increase insulin resistance, and both accomplish this by inhibiting transcription of the IRS-1 and Glut4 genes.[27] These inflammatory factors not only induce dysfunction in beta cells, leading to insufficient insulin secretion, but also interfere with insulin signaling pathways in peripheral tissues, ultimately resulting in insulin resistance.[28] Our research found that the expressions of liver inflammatory factors TNF-α and IL-6 in HFD/STZ-induced T2DM mice were significantly increased, and the levels of these inflammatory factors were improved after free CUR and CUR-LPs treatment. The CUR-LPs treatment was more effective than Free CUR. Our results align with those of previous reports,[24] demonstrating that CUR modified with long-

circulating liposomes is working in reducing the level of inflammatory factors, which may greatly improve its efficacy by increasing its absorption rate in the body. Our study also verified that the expression of liver representative inflammatory factors TNF-α, and IL-6 can be significantly downregulated after treatment with CUR-LPs in T2DM mice. The molecular mechanism of insulin resistance is complex, and the interplay between these inflammatory factor networks and insulin can explain the pathological occurrence of this mechanism. These cytokine networks could interfere with the molecular mechanisms of insulin resistance, thereby identifying potentially important targets for diabetes treatment.

Oxidative stress plays a key role in the development of diabetes complications.[29] There is evidence that SOD activity is negatively correlated with IR, and reduced SOD activity leads to increased insulin resistance.[30] In this study, SOD levels in the liver were detected, and the activity of SOD was significantly reduced in the T2DM group. However, in the CUR-LPs treatment group, SOD activity was restored. By encapsulating curcumin in long-circulating liposomes, its physicochemical properties were improved, enhancing its stability and promoting better absorption in the body. Given the relationship between SOD and HOMA, we speculated that after absorption of CUR-LPs in the body, it may reduce insulin resistance, restore SOD activity, and improve oxidative stress level of T2DM by reducing the HOMA index. T2DM is characterized by absolute or relative insulin deficiency with fasting and postprandial hyperglucagonemia. Glucagon can reduce hepatic GSH synthesis.[31] Studies have shown that if the body maintains high levels of glucagon for an extended period, it may decrease liver glutathione, resulting in reduced insulin sensitivity after meals and, thus, insulin resistance.[32] In this study, we assessed liver GSH levels and discovered a decrease in GSH in T2DM mice. Compared to the control group, the level of GSH in the T2DM group was decreased. As expected, CUR-LPs treatment increased GSH levels in comparison to the T2DM group. Curcumin was modified by using long-circulating liposomes to its water solubility. CUR-LPs was administered via intravenous injection into the tail vein enables it to swiftly reach the target organ and optimize systemic absorption and distribution. This results in an increased concentration of CUR-LPs in the liver, allowing for restoration of liver GSH levels, the reduction of liver oxidative stress, and the addressing of the insulin resistance component of T2DM. Insulin resistance is an early indicator of diabetes progression, and our findings highlight the need for further research to improve oxidative stress as a novel therapeutic target for the treatment of T2DM with insulin resistance.

However, there are still some deficiencies in this study. Firstly, the specific mechanism by which CUR-LPs improved insulin resistance in T2DM was still unclear, and we would conduct more research on its mechanism in future studies. Secondly, it is worth noting that in this study, we only conducted a one-month intervention and observation on type 2 diabetic mice. Because diabetes is a chronic condition, a longer period of time would be required to effectively improve glucose and lipid metabolism by improving insulin resistance.

Conclusion

In summary, the results of this study revealed that CUR-LPs significantly restored HFD/STZ-induced weight loss in mice and ameliorated blood glucose and insulin resistance, compared to that of free curcumin-treatment, which is thought to be the effect of the reduction of liver inflammation and oxidative stress.

Data Sharing Statement

The datasets generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

This study was conducted in accordance with the ARRIVE 2.0 guidelines and approved by the Ethics of Animal Experiments Committee of Southern Medical University (No. SYXK-2021-0167). All the processes were in strict accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Animals in laboratory experiments.

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The authors confirm that the study is reported in accordance with ARRIVE guidelines.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declared no conflict of interest.

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