Hindawi
Journal of Nanomaterials
Volume 2019, Article ID 7140132, 10 pages
https://doi.org/10.1155/2019/7140132

Research Article

Nanoencapsulation of Curcumin and Its Protective Effects against CCl_4-Induced Hepatotoxicity in Mice

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Received 3 August 2018; Revised 10 November 2018; Accepted 15 November 2018; Published 4 February 2019

Academic Editor: P. Davide Cozzoli

Curcumin is a natural phenolic compound extracted from the herb *Curcuma longa* L. rhizome and has received much attention on account of its biological properties. However, its poor solubility and low bioavailability limit its use. The purpose of this study was to synthesize curcumin-loaded nanoliposomes (Cur-NLs) to improve their bioavailability and evaluate the hepatoprotective effect of Cur-NLs against tetrachloromethane- (CCl_4-) induced acute liver injury in mice. We prepared Cur-NLs by thin film dispersion method, and the characterizations of Cur-NLs were measured by transmission microscope, laser particle size analyzer, infrared spectrometer, and X-ray diffraction. After 14 days pretreatment of Cur-NLs, free curcumin, silybin, or PBS, the models of acute liver injury were established by CCl_4 intraperitoneal injection in mice. The organ index, biochemical liver function parameters, histopathology, and antioxidant enzyme activities of liver tissues were measured further to evaluate the protective effects of Cur-NLs on liver injury. Compared with the CCl_4 model control group, pretreatment of Cur-NLs effectively reduced the serum levels of ALT, AST, and ALP and attenuated the hepatic necrosis induced by CCl_4 intoxication. Furthermore, Cur-NL pretreatment remarkably exhibited decreased MDA level and increased SOD, GPx, and CAT activities compared to CCl_4 model control group. Compared with the free curcumin group, the Cur-NLs also showed a better hepatoprotective effect. These observations imply that Cur-NLs act as a promising hepatoprotective agent in reducing liver oxidative stress produced by different stress factors.

1. Introduction

The liver is a vital organ which detoxifies various toxic substances, chemicals, and microbiological agents to which it is exposed. The morphological changes in the liver have a tendency to affect the metabolic events of the whole body, which is often associated with dysfunction of the detoxification process [1]. Various factors including hepatitis, acute hepatic failure, various types of cancer, toxins, cytokines, and drugs have been reported to cause liver damage [2]. Hepatotoxic substances including tetrachloromethane (CCl_4), acetaminophen, lipopolysaccharides, D-galactosamine (D-GalN), and TNF-α are widely used for experimentally induced liver damage [3].

CCl_4 is a well-known hepatotoxin that is widely used to induce toxic liver injury in a range of laboratory animals. CCl_4-induced hepatotoxicity is believed to include two phases. The initial phase involves the metabolism of CCl_4 by cytochrome P450 to the trichloromethyl radicals ($CCl_3^{\cdot}$ and/or $CCl_3OO^{\cdot}$), which leads to membrane lipid peroxidation and finally to cell necrosis [4, 5]. The second phase of CCl_4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators.

Curcumin is a natural phenolic compound extracted from the herb *Curcuma longa* L. rhizome [6]. It is widely used as a traditional herbal medicine for a variety of diseases. Curcumin contains methoxy groups and phenols which are responsible for its biological and pharmacological properties [7]. Besides being anti-inflammatory and antimicrobial, curcumin can inhibit tumor growth, thus lowering cancer risks [8–10]. However, the therapeutic use of curcumin has been limited due to its low solubility in aqueous solutions (≤0.125 mg/L) and its high decomposition rate at neutral or basic pH as well as its susceptibility to photochemical degradation [11]. Optimal pharmacological effects require an oral dose of >8.0 g/day [12]. Improving bioavailability of curcumin is a major challenge.

The nanoparticle is one of the novel drug carriers for therapeutic and diagnostic objectives which have several potential effects in improving accumulation and bioavailability of drug in target side, thereby suppressing immunogenicity and finally reducing adverse effects. Additionally, nanoparticles also promote drug solubility, controlled and sustained drug release, decreased drug elimination, and delivered more drug combination treatment for synergistic effect [13–15]. Also, nanoparticles are ideal probes for the determination of mass transport laws in tumors, acting as imaging contrast enhancers, and can be employed for the lesion-selective delivery of therapy. Their size, shape, density, and surface chemistry dominate convective transport in the blood stream, margination, cell adhesion, and selective cellular uptake as well as subcellular trafficking and localization [15]. Among the most promising drug delivery systems, liposomes are an attractive option for advantageous drug transport [16]. They are self-assembled nanoparticles and have been used to encapsulate hydrophobic and hydrophilic drugs [17]. Liposomes have many advantages, including good biocompatibility, biodegradability, low toxicity, and controlled release of the entrapped drug [18]. In particular, small unilamellar vesicles enter into cells easily and are beneficial for drug uptake.

Nanoparticles such as liposomes, micelles, nanogels, and polymeric nanoparticles can be used to deliver therapeutic concentration of curcumin that enhances the therapeutic efficacy of curcumin. Grama et al. [19] demonstrated that nanocurcumin could more effectively delay diabetic cataract in experimental rats compared with dietary curcumin. Chereddy et al. [20] demonstrated that in a full-thickness excisional wound-healing mouse model, poly lactic-co-glycolic acid- (PLGA-) curcumin nanoparticles than curcumin due to efficient drug delivery improved solubility and bioavailability. To increase the anticancer efficiency of curcumin, Bisht et al. [21] designed a polymeric nanoparticle encapsulated formulation of curcumin having size distribution in the 50 nm range using the micellar aggregates of cross-linked and random copolymers of N-isopropylacrylamide (NIPAAM) and found that this nanocurcumin prevents the growth of pancreatic cancer cell lines. Abdel-Wahhab et al. [22] found that curcumin nanoparticle-loaded hydrogels possessed protective effects on AFB1-induced genotoxicity in rat liver.

The aims of the current study were to synthesize and characterize Cur-NLs and to evaluate its protective role against CCl_4-induced liver damage in mice. We employed thin film hydration method to prepare Cur-NLs with high entrapment efficiency and drug loading efficiency. Experiment in vivo demonstrated that Cur-NLs display a better hepatic protective effect on CCl_4-induced acute liver injury in mice compared with free curcumin administration. Cur-NLs exhibiting better hepatic protective effect might be due to the slow and regular release of curcumin by nanoparticles.

2. Materials and Methods

2.1. Materials. Curcumin was purchased from Xi'an Tianbao Biotechnology Co. Ltd. Soybean phosphatidylcholine (SPC) and cholesterol (CHOL) were purchased from AVT Pharmaceutical Technology Co. Ltd. Dichloromethane, methanol, and CCl_4 were purchased from Tianjin Fenghua Chemical Reagent Technology Co. Ltd. Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) measurement kits were purchased from Nanjing Jiancheng Bioengineering Institute.

2.2. Animals. Male Balb/c mice (SPF) were purchased from the Laboratory Animal Center in the Academy of Military Medical Sciences (Beijing, China) at 6 to 8 weeks old with an average weight of 18~22 g. The animals were housed in a standard environmental condition and were provided ad libitum access to food and water. All procedures with animals were in strict accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH publications no. 8023, revised 1978) and approved by the Animal Care and Use Committee of Yanshan University, China (Ethics number: YD2017011).

2.3. Preparation of Cur-NLs and Nanoliposomes (NLs). Cur-NLs were prepared according to an established method [23]. The lipid phase was prepared by dissolving SPC, CHOL, and curcumin (8 : 2 : 1) in dichloromethane-methanol mixture (2 : 1, V/V), then the mixture was removed by rotary evaporation at 60°C to form a thin film of lipids on the wall of the eggplant-shaped bottle. After being purged with nitrogen for 5 minutes, the lipid film was hydrated with ultrapure water at 60°C, followed by sonication for 15–30 minutes at a frequency of 40000 Hz to reduce particle size. The resulting suspension was passed through filter membranes of 0.45 μm and 0.22 μm successively to remove the nonincorporated curcumin. Finally, the deep yellow Cur-NLs were obtained and stored at 4°C. NLs were also prepared without curcumin in the same manner described above.

2.4. Characterization of Cur-NLs

2.4.1. Transmission Electron Microscopy. A TEM (JEM-100CX/II) was used to observe the size and the morphology of the Cur-NLs. The samples for TEM were prepared by a standard procedure. 2% of ammonium molybdate was used as a staining agent. Then the carbon film-coated copper grid was placed on the samples for 10 min, and the excess solution

was removed with a filter paper. The samples were air-dried and then observed under the TEM.

2.4.2. Particle Size and Zeta Potential. Malvern Zetasizer ZS (Malvern Instruments, UK) was used to measure the sizes and surface zeta potentials of the Cur-NLs. The mean liposome diameters and zeta potentials were determined by dynamic light scattering (DLS) and electrophoretic mobility measurement, respectively. All characterization measurements were repeated three times at 25°C.

2.5. The Interaction between Drug and Excipients

2.5.1. X-Ray Diffraction. X-ray diffraction was performed using X-ray diffractometer (Model PW 1710) control unit Philips Anode material Cu, 40 kV, 30 MA, which Cu Kα radiation $\lambda = 1.5405\,\text{Å}$ over a wide range of Bragg angles. The curcumin, physical mixture of curcumin excipients, and Cur-NLs were measured using X-ray diffractometer [24].

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR). Potassium bromide (KBr) technique was used for FTIR analysis. First, KBr was dried at 105°C and grounded finely. The curcumin, physical mixture of curcumin excipient, and Cur-NL samples were added to it (sample: KBr = 1 : 3), respectively, and were finely grounded again. They were compressed under high pressure to prepare pellets of 10.0 mm and 1-2 mm thick. The pellets were scanned over a range of 4000 cm^{-1} to 400 cm^{-1} and spectra were recorded using FTIR (Jasco FTIR 460 Plus, Japan) [25].

2.6. Determination of Entrapment Efficiency (EE) and Drug Loading (DL). Firstly, the regression curve of concentration vs absorbance in 425 nm was obtained to analyze the concentration of resulting curcumin solution after ultrafiltration. Then Cur-NLs were put in ultrafiltration centrifuge tube (Millipore, 10 KD) and centrifuged to separate the untrapped curcumin from Cur-NLs (5000 rpm, 15 min). Subsequently, 1 mL of resulting free curcumin solution was transferred into a new centrifuge tube. The curcumin concentration was determined as described above after 2 mL of methanol was added into this tube. EE and DL were calculated with the following formulas [26].

$$\text{EE\%} = (W_{\text{total}} - W_{\text{free}})/W_{\text{total}} \times 100, \quad (1)$$

$$\text{DL\%} = (W_{\text{total}} - W_{\text{free}})/W_{\text{lipid}} \times 100, \quad (2)$$

where W_{total} is the analyzed weight of the drug in the dispersions and W_{free} is the analyzed weight of free drug in the supernatant and W_{lipid} is the total weight of the lipid content.

2.7. Animal Experiment

2.7.1. Animals and Experiment Design. Male Balb/c mice were randomly divided into five experimental groups with 8 mice in each, including normal control group (PBS, 0.2 mL), CCl_4 model control group (PBS, 0.2 mL), free curcumin treatment group (free-Cur, 2 mg/kg), curcumin nanoliposome treatment group (Cur-NLs, 20 mg/kg ≈ 2 mg/kg pure curcumin), and positive control (silybin, 50 mg/kg). All the grouped animals were administrated with above different drugs or PBS by tail vein injection for 14 days. Except the normal group, mice in the other 4 groups received single dose of 2% CCl_4 in peanut oil (v/v, 0.3 mL) intraperitoneal injection on the 14$^{\text{th}}$ day. Normal control received equal amount of vehicle instead of CCl_4.

2.7.2. Measurement of Organ Index. On the 15th day, the mice were anesthetized and sacrificed. The liver, kidney, thymus, and spleen tissues were immediately removed and weighed. The organ index was calculated according to the following formula.

$$\text{Organ index(mg/10g)} = [\text{organ weight(mg)/body weight(g)}] \times 10. \quad (3)$$

2.7.3. Measurement of Liver Function Parameter Test. To test liver function, we examined the serum levels of ALT, AST, and ALP. Before execution, the blood of the experimental mice of control and treated groups was collected in Eppendorf tubes, deposited for 1 h at 4°C, and centrifuged for 15 min at 3000 rpm, and then the serum was collected. ALT, AST, and ALP activities were assayed according to the instructions of the kits.

2.7.4. Histopathological Study of Liver Tissues. The liver tissues of mice were fixed by 10% formaldehyde and embedded in paraffin. The slices were cut into 4 μm thick slices and stained with hemotoxylin and eosin (HE). The pathological changes of liver tissues were observed under light microscope [27].

2.7.5. Measurement of the Antioxidant Enzyme Activities in Liver Tissues. After execution, the livers of the experimental mice in all groups were removed. The liver homogenates (10.0%, w/v) were prepared in the normal saline. The resulting suspensions were centrifuged at 4000 rpm for 15 min, and the supernatants were collected for further measurement. All treatments were conducted in an ice bath or at 4°C. The SOD, GPx, and CAT were then determined by commercial reagent kits according to the instructions. Lipid peroxidation in liver tissues was measured by the formation of MDA and it was estimated by commercial reagent kit as well.

2.8. Statistical Analysis. All experiments were performed in three groups of parallel experiments and analyzed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Multiple comparisons were performed using variance analysis and considered $P < 0.05$ significant.

3. Results and Discussion

3.1. Characterization of Cur-NLs

3.1.1. Morphology Observation of Cur-NLs. The morphology of Cur-NLs was observed by TEM. Figure 1(a) shows that Cur-NLs were spherical. The liposomes in the field of view were intact and the particle size was uniform. The size of Cur-NLs was between 50 and 150 nm.

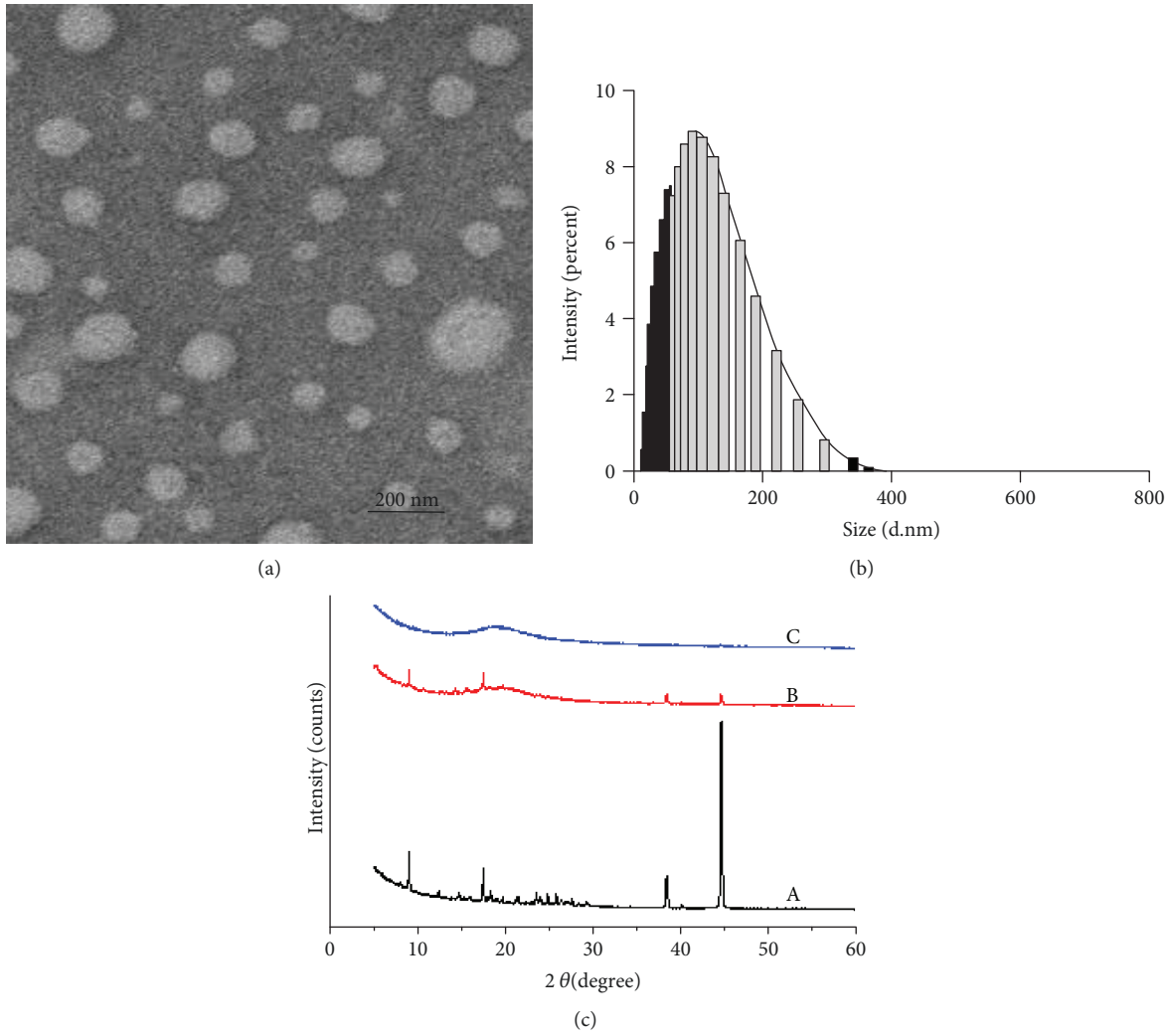

Figure 1: Characterization of curcumin-loaded nanoliposomes. (a) TEM images of curcumin-loaded nanoliposomes. (b) Size distribution of curcumin-loaded nanoliposomes assessed by dynamic light scattering (DLS). (c) XRD of curcumin, physical mixture of curcumin and excipients, and curcumin-loaded nanoliposomes. (A) Curcumin. (B) Physical mixture of curcumin and excipients. (C) Curcumin-loaded nanoliposomes.

3.1.2. Measurement of Particle Size and Zeta Potential of Cur-NLs. The particle size of Cur-NLs was measured by laser particle sizer, and the average size of Cur-NLs was 72.26 ± 20 nm (Figure 1(b)). The particle size distribution of Cur-NLs was normal distribution, and the polydispersity coefficient showed that the particle size distribution was relatively concentrated and reached the requirements of nanosize drugs. The zeta potential of liposomes determines the stability of liposomes and also affects some characteristics of liposomes, such as passive targeting. The results showed that the zeta potential of Cur-NLs was around -30.9 ± 3.1 mV, which ensured that the liposomes exhibit certain stability and could be stored for a long time without the phenomenon of precipitation. The polydispersity index (PDI) was to examine the level of uniformity of particle size and the PDI of Cur-NLs was 0.140 ± 0.002, which indicated that the particle was uniform and further prevented the Que-NLs to agglomerate.

3.2. The Interaction between Curcumin and Excipients

3.2.1. X-Ray Diffraction of Cur-NLs. When X-rays are diffracted by crystals, each crystalline material has its own unique diffraction pattern, and their characteristics can be characterized by the relative intensity of the diffraction I/I_0.

The diffraction peaks at different angles of the curcumin monomer crystals can be observed in the X-ray diffraction pattern and the peaks of the physical mixture of curcumin and excipients were reduced, but these diffraction peaks disappeared in the Cur-NLs. This result proved that curcumin had been highly dispersed in liposomes (Figure 1(c)).

3.2.2. Infrared Spectroscopy of Cur-NLs. In order to determine the interaction between the drug and the excipients, the compatibility of the drug and the excipients was further determined by infrared spectroscopy. The infrared spectra

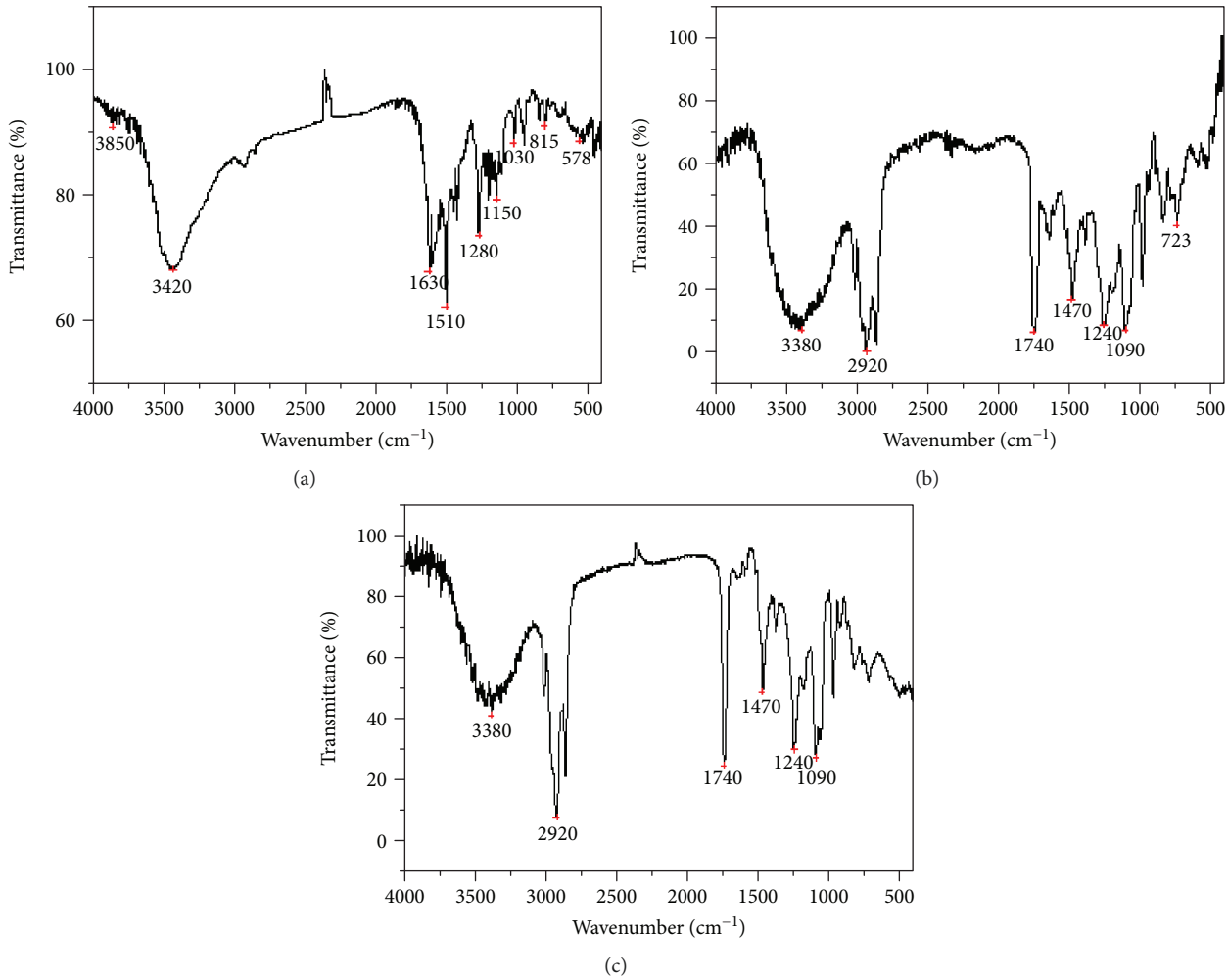

Figure 2: FTIR spectrum of curcumin, physical mixture of curcumin and excipients, and curcumin-loaded nanoliposomes. (a) FTIR spectra of curcumin. (b) FTIR spectra of physical mixture of curcumin and excipient. (c) FTIR spectra of curcumin-loaded nanoliposomes.

of curcumin, physical mixture of curcumin and excipients, and Cur-NLs are shown in Figures 2(a)–2(c), respectively.

The infrared spectra showed that characteristic peak of curcumin appears at 3420 cm^{-1} for phenolic hydroxyl groups, 1630 cm^{-1} for C = O symmetrically stretch, 1510 cm^{-1} for the C = O and C = C stretch, 1280 cm^{-1} for -C-O of the aromatic ring stretch. Compared with the characteristic peaks of curcumin, the characteristic peaks of physical mixture of curcumin and excipients and Cur-NLs both showed 1510 cm^{-1} and 1280 cm^{-1} red shift to 1470 cm^{-1} and 1240 cm^{-1}. This suggested that the olefin bond and the aromatic ring of curcumin have a chemical bond with the phospholipid [28]. These minor shifting also may be caused by the creation of hydrogen bonds or dipole moment or Van der Waals forces among phospholipids and the polar functional groups of drug. These interactions may favor the creation of vesicular shape, stability, and slower drug release [25].

3.3. Entrapment Efficiency (EE) and Drug Loading (DL) of Cur-NLs. The Cur-NLs were prepared by ultrasound membrane dispersion method. After ultrafiltration, the solution was determined at 425 nm using spectrophotometer. The EE of Cur-NLs was 92.33 ± 2.11% and the DL was 8.6 ± 0.47%, respectively, which was calculated by formulas (1) and (2). High EE and DL indicated that curcumin was almost completely encapsulated in liposomes. A certain proportion of CHOL could reduce the fluidity of the membrane and increase the stability of the membrane and thus improve the EE.

3.4. Effect of Cur-NLs on Organ Index. Livers and kidneys are important organs of body metabolism. The results in Figure 3(a) showed that the liver index in CCl_4 model control group increased significantly compared with the index of the normal control group ($P < 0.05$), which was due to the congestion of the liver caused by the CCl_4. The other administration groups were not different from the normal group ($P > 0.05$), which indicated that the liver index of drug treatment could be restored to the normal range. The liver index of the free curcumin group was not significantly different from that of the Cur-NL group, indicating that the difference of protective effects between free curcumin and Cur-NLs on

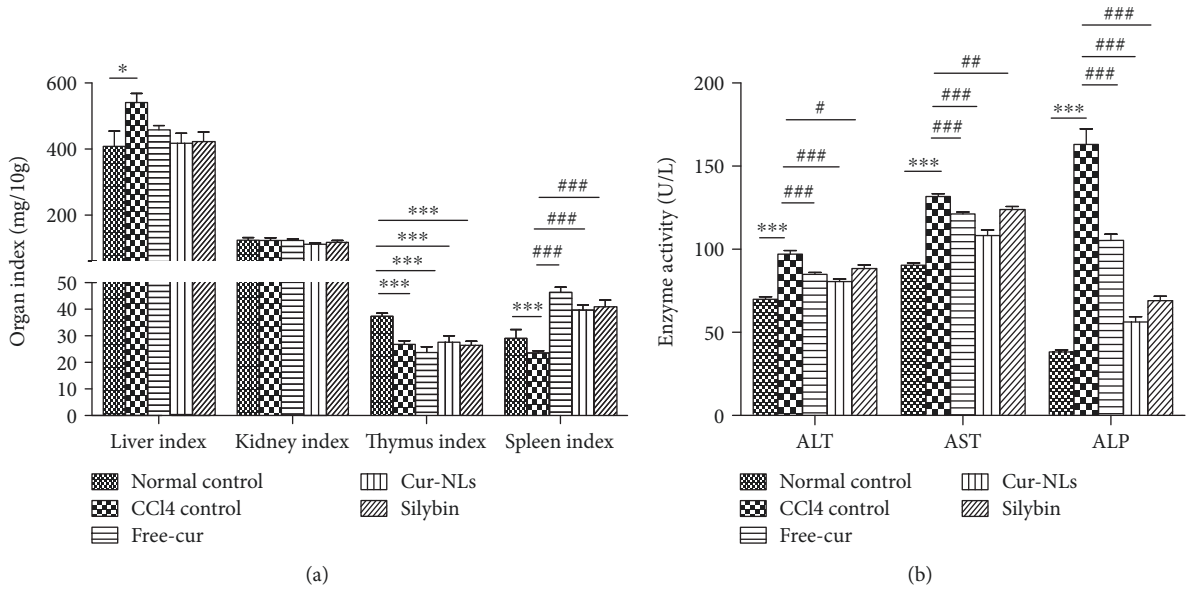

Figure 3: Effects of curcumin-loaded nanoliposomes on organ index and biochemical parameters of liver tissues. (a) Effect of curcumin-loaded nanoliposomes on organ index in mice. (b) Effect of curcumin-loaded nanoliposomes on serum ALT, AST, and ALP activities in mice. $^{*}P < 0.05$ compared to the normal control group; $^{***}P < 0.001$ compared to the normal control group; $^{\#}P < 0.05$ compared to the CCl_4 model control group; $^{\#\#}P < 0.01$ compared to the CCl_4 model control group; $^{\#\#\#}P < 0.001$ compared to the CCl_4 model control group.

the liver was not reflected in the liver index significantly. Compared with the normal group, the kidney index of mice after CCl_4-induced liver injury was not significantly different, showing that stimulation of CCl_4 did not affect the kidney index in mice remarkably. The thymus and spleen are important immune organs of the body. Compared with the normal control group, the thymus index decreased significantly ($P < 0.001$) after the treatment of CCl_4 in mice, indicating the atrophy of the thymus induced by CCl_4. The thymus index of free-Cur and Cur-NL treatment groups did not show significant changes compared to that of the CCl_4 treatment group ($P > 0.05$), which indicated that both of them showed little protective significant effects on the thymus organ. Compared with the normal control group, the spleen index of CCl_4 treatment group decreased dramatically, which is due to the atrophy of the spleen caused by CCl_4 treatment, while both Cur-NLs and silybin increased the spleen index to the normal value, which revealed that Cur-NLs showed significant protective effects on the spleen in mice. Interestingly, the spleen index of mice in the free-Cur group increased rapidly and exceeded the normal range. This might be attributed to the spleen hyperplasia under the stimulation of the free-Cur administration.

3.5. Effect of Cur-NLs on Biochemical Parameters of Liver Tissues in CCl_4-Induced Liver Damage. Hepatoprotective effects of Cur-NLs were determined by quantitative analysis of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) levels as shown in Figure 3(b). The current data showed that administration of CCl_4 resulted in hepatic enzyme (ALT, AST, and ALP) activities which increased significantly when compared to the normal mice ($P < 0.001$), while administration of both free-Cur and Cur-NLs reduced these enzymatic changes when compared to the CCl_4 control group ($P < 0.001$), indicating that curcumin suppressed the adverse effects of CCl_4 in mice. Furthermore, Cur-NLs showed better protective effect than free-Cur on liver injury in mice according AST and ALP levels.

3.6. Effect of Cur-NLs on Histopathology of Liver Tissues in CCl_4-Induced Liver Damage. After 14 days of treatment, liver tissues of all groups were taken from the animals of all groups and were subjected to histological analysis (Figure 4). The livers from the mice in the normal control group, Cur-NLs, and silybin had an overall smooth appearance and normal color. The livers in the normal control group were further found to have normal lobular morphology and hepatocytes with well-defined sinusoids (Figure 4(a)). The hepatic injury in the mice treated with CCl_4 manifested as inflammatory infiltration, swelling, hemorrhage, and necrosis involving mainly the centrilobular zone (Figure 4(b)). The livers of the mice treated with free-Cur appeared hyperemic, mottled, and were fragile (Figure 4(c)). Remarkably, the livers of the mice treated with Cur-NLs did not reveal any significant microscopic changes relative to normal control tissue (Figure 4(d)). Mild congestion of sinusoidal spaces was observed in the centrilobular area of the livers of silybin-treated mice (Figure 4(e)). These findings are in agreement with the biochemical parameter analysis.

3.7. Effect of Cur-NLs on Hepatic Antioxidant Biomarkers in CCl_4-Induced Liver Damage. In order to explore the protection mechanisms behind Cur-NLs on the liver toxicity

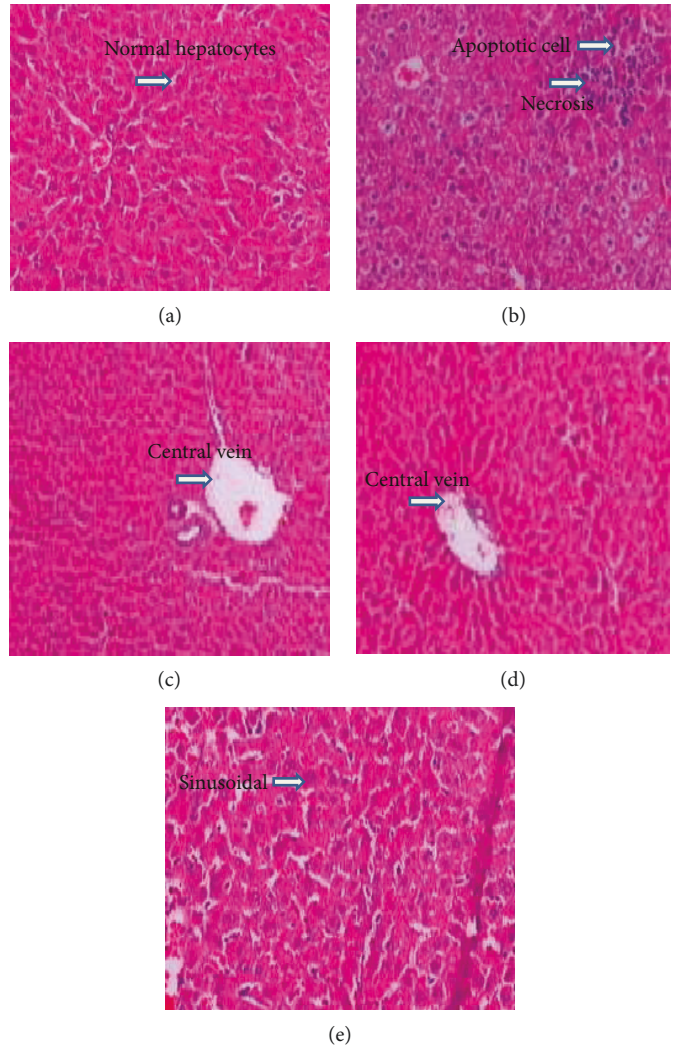

Figure 4: Photomicrographs of liver sections stained by HE from (a) normal control mice showing that the hepatocytes are polyhedral in shape, with central rounded vesicular nuclei and a acidophilic granular cytoplasm (×100); (b) CCl_4 model control mice showing disorganized hepatic architecture with multiple areas of necrosis with apoptotic cell and apoptotic body distinguished by dense eosinophilic cytoplasm and pyknotic nucleus and mononuclear cellular infiltration around the portal tract with branching of bile ductules and proliferation (×100); (c) free curcumin treatment mice showing mild mononuclear cellular infiltrate mildly dilated and congested portal vein and normal bile duct (×100); (d) curcumin-loaded nanoliposome treatment mice showing organized hepatic architecture with vesiculated nuclei and preserved central vein and a few numbers of hepatocytes show deeply stained acidophilic cytoplasm and dark nuclei (×100); (e) silybin treatment mice showing more or less normal portal vein, preserved bile ducts, and a few numbers of hepatocytes show deeply stained acidophilic cytoplasm and dark nuclei (×100).

induced by CCl_4 in vivo, the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) levels in the mice liver tissues were further determined.

The present data shown in Figure 5 indicated that giving CCl_4 initiated a significant augmentation in the hepatic MDA level as lipid peroxidation index ($P < 0.001$), weakening SOD, CAT, and GPx activity ($P < 0.05$) when compared to the normal mice, while pretreatment of free-Cur, Cur-NLs, or silybin produced a significant improvement in the hepatic antioxidant protection mechanism by increasing the hepatic GPx, SOD, and CAT activities ($P < 0.05$). At the same time, they induced a significant fall in the hepatic MDA level when compared with that of the CCl_4 administration group ($P < 0.001$). Curcumin, the potent antioxidants, can rapidly reduce the harmful effects of the hepatic toxicity through scavenging of the ROS, while curcumin nanocrystallization further improves the hepatoprotective effect as shown in our current study.

4. Discussion and Conclusion

Pharmaceutical nanocarriers such as liposomes have demonstrated enhanced in vivo stability, longer circulation times, better permeability, resistance to metabolic processes, and high efficiency of drugs. The benefits of nanoscale delivery

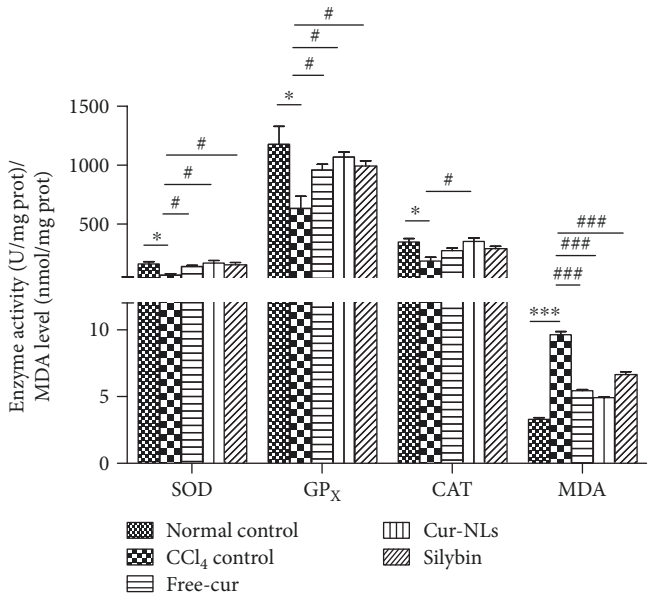

Figure 5: Effect of curcumin-loaded nanoliposomes on SOD, GPx, and CAT activities and MDA level in the mice liver tissues. $^{*}P < 0.05$ compared to the normal control group; $^{\#}P < 0.05$ compared to the CCl_4 model control group; $^{\#\#\#}P < 0.001$ compared to the CCl_4 model control group.

systems are supported by numerous preclinical and clinical data [29, 30].

In the current study, Cur-NLs were synthesized to enhance the water solubility of curcumin and to decrease the particle size which result in the formation of a thinner hydrodynamic layer around particles and increase the surface specific dissolution rate. The DLS analysis of the aqueous dispersion of the prepared Cur-NLs revealed the formation of nanoparticles with an average diameter of 72.26 ± 20 nm and the electron micrographs showed spherical shape. The particle size and shape reported herein were in agreement with those reported earlier using different preparation techniques [31–33]. The hyperbolic relation between the particle size and the surface specific dissolution rate increases the solubility due to the larger surface area which promotes dissolution [34]. Moreover, Kesisoglou et al. reported that reduction in the particle size of active ingredients to nanoparticles resulted in the improvement in the solubility and bioavailability [35]. The negative potential charge (-30.9 ± 3.1 mV) at natural pH also ensures the colloidal stability of the prepared Cur-NLs. In our previous study, we prepared quercetin-loaded nanoliposomes with the same method as this study and further found that quercetin-loaded nanoliposomes exhibit better tumor inhibition effect than free quercetin [23]. Nanomedicine is the use of nanotechnology to bring about improvements in healthcare. This involves the use of the properties of nanoscale materials, which may differ profoundly from those of the same material at a larger scale. Many biological mechanisms in the human body also occur at the nanoscale and nanoparticles, due to their small size, may potentially cross natural barriers and enter new sites different from the portal of entry into the body and interact with biomolecules in the blood or within organs, tissues, or cells; this may be highly advantageous for drug or gene delivery and imaging [36].

CCl_4 is an effective hepatotoxic agent, and even a single exposure can promote severe liver toxicity, including necrosis and steatosis. Hence, CCl_4 is widely used as a model for evaluating the hepatoprotective activity of new drugs or drug formulations [5, 37].

It is well known that AST, ALT, and ALP are sensitive indicators of liver injury. After the administration of CCl_4, reductive dehalogenation of CCl_4 catalyzed by cytochrome P450 will form a highly reactive trichloromethyl free radical (CCl_3) and then form the trichloromethyl peroxy radical (CCl_3OO) as the precursor of lipid peroxidation [38]. Increases in serum ALT, AST, and ALP levels by CCl_4 have been attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and released into the circulation after cellular damage has accrued [37]. Our study showed that serum ALT, AST, and ALP levels increased rapidly in parallel with CCl_4 injection, indicating the induction of acute hepatotoxicity by CCl_4. However, serum ALT, AST, and ALP activities significantly declined in free-Cur, Cur-NLs, and silybin treatment groups. In particular, the serum ALT, AST, and ALP activities in Cur-NL treatment group were much lower than those in the free-Cur treatment group, even lower than the positive control (silybin) treatment group, suggesting that Cur-NLs are the most beneficial in reversing liver injury.

There are several intrinsic antioxidative defenses of the cells, and one of which is the existence of antioxidant enzymes such as SOD, CAT, and GPx. In the present study, intraperitoneal injection of CCl_4 obviously downregulated the expressions of these enzymes and induced oxidative stress as one of the by-products of its metabolism. However, these enzymes were restored to their basal levels after the pretreatment with free-Cur, Cur-NLs, or silybin followed by CCl_4. Because of its polyphenolic structure and a β-diketone functional group, curcumin has stronger antioxidant inhibitory properties than other flavonoids with a single phenolic hydroxyl group [39]. Our results also demonstrated significantly better efficacy for Cur-NLs against lipid peroxidation compared to free-Cur. This is attributed to the longer circulation time of nanoparticles in the blood, which increases the bioavailability of curcumin.

We proved here that Cur-NLs successfully ameliorated CCl_4-induced hepatotoxicity in mice as demonstrated by decreased levels of the hepatic injury markers ALT, AST, and ALP. The observed protective effects of Cur-NLs may be due to antioxidant effects as shown by the reduced lipid peroxidation (MDA level), increased SOD, CAT, and GPx activities. The histopathological analysis conducted in this study also revealed dramatically protective effects on liver damage induced by CCl_4.

Cur-NLs were found to be more effective in curing liver damage than free-Cur. This might be due to the fact that lipid bilayers of liposomes improved the drug permissiveness into the cell membrane and enhanced the penetration of Cur-NLs and the slow and regular release of Cur by nanoparticles that provide Cur with a rise in bioavailability, which, in return, increases therapeutic effects.

Compared with the previous study about curcumin-loaded nanoliposomes, the drug loading, stability, and entrapment efficiency of Cur-NLs prepared in this study were enhanced markedly. Furthermore, we discussed the protective effect and possible mechanism of Cur-NLs on liver systematically. These observations imply that Cur-NLs act as a promising hepatoprotective agent in reducing liver oxidative stress produced by different stress factors.

Abbreviations

CCl_4: Tetrachloromethane
D-GalN: D-Galactosamine
Cur-NLs: Curcumin-loaded nanoliposomes
ALT: Alanine transaminase
AST: Aspartate transaminase
ALP: Alkaline phosphatase
SOD: Superoxide dismutase
CAT: Catalase
GPx: Glutathione peroxidase
MDA: Malondialdehyde
EE: Entrapment efficiency
DL: Drug loading
PDI: Polydispersity index.

Data Availability

No data were used to support this study.

Disclosure

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Conflicts of Interest

The authors declare that they have no direct financial relation with the commercial identities mentioned in this paper that might lead to a conflict of interests for any of the authors.

Acknowledgments

This work was financially supported by the Qinhuangdao Science and Technology Research and Development Plan (nos. 201101A132, 201705B024, 201701B044, and 201801B035) and by the Hebei Science and Technology Research and Development Support Program (no. 17272402D).

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