

# Protective and Therapeutic Effects of Nanoliposomal Quercetin on Acute Liver Injury in Rats

**Xiangyan Liu**

Xiangya Hospital Central South University

**Yang Zhang**

Xiangya Hospital Central South University

**Ling Liu**

Xiangya Hospital Central South University

**Yifeng Pan**

Xiangya Hospital Central South University

**Yu Hu**

Central South University Third Xiangya Hospital

**Pu Yang** (✉ [puyang@csu.edu.cn](mailto:puyang@csu.edu.cn))

Xiangya Hospital Central South University

**mingmei liao** (✉ [mingmeiliao@csu.edu.cn](mailto:mingmeiliao@csu.edu.cn))

Xiangya Hospital Central South University

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## Research article

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# Abstract

**Background** Quercetin, a pigment (flavonoid) found in many plants and foods, has good effects on protecting liver function but poor solubility and bioavailability in vivo. A drug delivery system can improve the accumulation and bioavailability of quercetin in liver. **Objective** In this study, we used liposomal nanoparticles to entrap quercetin and evaluated its protective and therapeutic effects on drug-induced liver injury in rats. **Design** The nanoliposomal quercetin was prepared by a thin film evaporation-high pressure homogenization method and characterized by morphology, particle size and drug content. Acute liver injury was induced in rats by composite factors, including carbon tetrachloride injection, high-fat corn powder intake and ethanol drinking. After pure quercetin or nanoliposomal quercetin treatment, liver function was evaluated by detecting serum levels of glutamic-pyruvic transaminase (GPT), glutamic-oxal acetic transaminase (GOT) and direct bilirubin (DBIL). Histology of injured liver tissues was evaluated by hematoxylin and eosin staining. **Results and discussion** On histology, liposomal nanoparticles loading quercetin were evenly distributed spherical particles. The nanoliposomal quercetin showed high bioactivity and bioavailability in rat liver and markedly attenuated the liver index and pathologic changes in injured liver tissue. With nanoliposomal quercetin treatment, the serum levels of GPT, GOT and DBIL were significantly better than treated with pure quercetin. Using liposomal nanoparticles to entrap quercetin might be an effective strategy to reduce hepatic injury and protect hepatocytes against damage. **Conclusions** Liposomal nanoparticles may improve the solubility and bioavailability of quercetin in liver. Furthermore, nanoliposomal quercetin could effectively protect rats against acute liver injury and may be a new hepatoprotective and therapeutic agent for patients with liver diseases.

## Background

Hepatitis virus or toxin exposure leads to liver injury, eventually resulting in cirrhosis and liver cancer. [1,2,3] Therapy choices for liver injury are few. Quercetin, a pigment (flavonoid) found in many plants and foods such as wine, apple, onions, green tea, berries and some herbs, has wide bioactivity, including antioxidative, anti-fibrotic and anti-inflammatory effects.[4,5,6,7] Its protective effect on liver injury was previously identified.[8,9,10,11] However, quercetin has poor water solubility, so it cannot accumulate well in the liver.

Previous studies showed that various carriers such as chitosan or liposome could enhance the solubility of quercetin and improve its bioactivity for treating tumor[12] and diabetes.[13,14,15] A drug delivery system is needed to improve the accumulation and bioavailability of quercetin in liver.

Here, we prepared liposomal nanoparticles loading quercetin and detected their effect on rat liver injury induced by complex factors, which is relatively close to the naturally occurring process. We identified the protective and therapeutic effects of nanoliposomal quercetin on drug-induced liver injury in rats. Liposomal nanoparticle-entrapped quercetin may be a potential therapeutic agent for hepatic diseases.

## Methods

# Materials

Refined soy lecithin Lipoid S100 was from Germany Lipoid GmbH (No.790495–1). Cholesterol was from Beijing Chemical Reagent Co. Quercetin was from Hunan nine modern Chinese Medicine Co. The hematoxylin and eosin (H&E) staining kit was from Shanghai Huyu Biological Technology Co. GPT/GOT ELISA kit was from Shanghai Ximei Biological Technology Co. Bilirubin assay kit was from Shanghai Hengyuan Biological Technology Co. Carbon tetrachloride was from Beijing Jinhuitaiya Chemical Reagent Co. All other reagents such as anhydrous alcohol, ether, and methanol were of analytical grade.

# Preparation and determination of nanoliposomal quercetin

Nanoliposomal quercetin was prepared by the thin film evaporation method as described.[15] The morphology of the nanoparticles encapsulated with quercetin was evaluated by using a S–3400N scanning electron microscope (SEM, Japan) and the size determination was detected by particle size analyzer (ZEN3600, Malvern, UK). The drug content was analyzed by high-performance liquid chromatography (HPLC). In the following experiments, the dose of nanoliposomal quercetin was based on the content of quercetin.

# Experimental animals

We obtained 40 healthy Sprague-Dawley (SD) rats, 80 to 120 g, from the experimental animal department of Xiangya Medical College of Central South University, and rats were kept under lab conditions (20–25°C, 50% relative humidity, 12-h light-dark cycle). The experiments were approved by the ethics committee of Xiangya Hospital of Central South University. All experimental animals were finally euthanized.

# Treatments in hepatic-injured rat models

Rats were randomly divided into 4 groups (10 rats per group): normal control and 3 groups of hepatic-injured rats that received saline, quercetin, or nanoliposomal quercetin. Liver injury was induced by composite factors,[16] including carbon tetrachloride injection, high-fat corn powder intake and ethanol drinking. First, rats were injected subcutaneously with a castor oil solution containing 40% carbon tetrachloride at 0.5 mL per 100 g body weight, then 0.3 mL per 100 g body weight every 3 days for 13 times. Simultaneously, rats were fed high-fat corn powder (79.5% pure corn powder with 10% lard and 0.5% cholesterol) and drank 10% ethanol as the only liquid every day. After the first subcutaneous injection, all groups were injected intraperitoneally with 0.9% normal saline for control and saline-treated rats and pure quercetin and nanoliposomal quercetin at 5 mg/100g body weight once a day for 1 week, then every 2 days for 4 weeks for quercetin- and nanoliposomal quercetin-treated rats. About 6 weeks later, all rats were killed after anesthesia, and blood and liver samples were collected for further analysis.

At 1 day after the last drug injection, 2 mL blood per rat was obtained from the inferior vena cava for liver function detection, including serum levels of glutamic-pyruvic transaminase (GPT), glutamic-oxal acetic transaminase (GOT) and direct bilirubin (DBIL). The livers of all groups were promptly harvested, weighed and fixed in 10% formalin, then processed for histology. The liver index was calculated by the ratio of liver weight and body weight (Liver index = liver weight/body weight×100).

## Histology

Liver tissues were fixed in 10% formalin overnight at room temperature, then underwent gradient alcohol dehydration, embedding in paraffin, and sectioning at a 5 µm thick. Sections were stained by H&E staining and observed under a microscope for gross pathology evaluation.

## Statistical analysis

Data are reported as mean ± SEM. SPSS for Windows 18.0, GraphPad Prism 7 and Microsoft Office 2010 Excel were used for data analysis. Student t-test was used for single variable comparisons. Differences were considered significant at  $P < 0.05$ .

## Results And Discussion

Many kinds of factors including biological damage such as with hepatitis virus,[17] chemical damage such as with drugs,[18,19] and physical damage such as with liver transplantation[20,21] can lead to liver injury. The use of carbon tetrachloride[22] and other composite factors such as a high-fat, high-cholesterol, and low-protein diet as well as alcohol drinking can induce liver injury models in animals, with pathological results very similar to human chronic liver diseases. Quercetin is a hepatoprotective drug<sup>9-11</sup>[23] but with poor solubility and bioavailability in liver.[14,24] In this study, we induced liver injury in rats by complex factors and used liposomal nanoparticles as the drug carrier loading quercetin to treat the rats.

## Characteristics of nanoliposomal quercetin

Quercetin, present in fruits and vegetables, is one of the most common flavonoidal compounds, with non-toxic, non-lethal, non-carcinogenic, non-teratogenic and non-mutation effects in humans. It possesses anticancer effects,[25,26] prevents the development of hepatic fibrosis,[27,28] and reduces toxicant-induced liver injury.[8,11] However, quercetin is not soluble in water and has poor bioavailability in liver. Our study used a drug carrier, liposome, to entrap quercetin. The main component of liposome is phospholipids, which has a typical feature of both hydrophilicity and hydrophobicity. It can be used as a drug carrier to bind with a variety of drugs and has many advantages, such as good biocompatibility, no toxic effects and good bioavailability.[9,13] Furthermore, we nanoformulated the liposome-entrapped

quercetin compound to improve the utilization of quercetin and targeting to the liver.[13,15] Nanoparticles can change the transport mechanism of the cell membrane and increase the accumulation of quercetin in the liver to improve its bioavailability.[29] As shown in Figure 1B, the nanoparticles were evenly distributed spherical particles. The size of nanoliposomal quercetin was  $142\pm 19$  nm (Fig. 1A). The drug loading was  $5.08\pm 0.17$  mg/mL.

## Effect of quercetin treatments on body weight and liver index of the hepatic-injured rats

As compared with the other groups, hepatic-injured rats with saline treatment showed significantly decreased food intake, body weight and activity accompanied by poor light reactions. Furthermore, two hepatic-injured rats in the saline treatment group died of liver failure before tissue harvesting. As shown in Figure 2A, simultaneous treatment of pure quercetin and nanoliposomal quercetin significantly increased the body weight of hepatic-injured rats when compared with the hepatic-injured rats with saline treatment ( $P<0.01$ ). However, the liver weight of hepatic-injured rats with saline treatment was heavier with fat accumulation in liver than the other groups ( $9.85\pm 0.32$ g in saline treatment group,  $9.12\pm 0.26$ g in normal control group,  $9.36\pm 0.15$ g in pure quercetin group and  $9.27\pm 0.14$ g in nanoliposomal quercetin group, respectively). As shown in Figure 2B, the liver index of hepatic-injured rats with saline treatment was  $4.07\pm 0.13\%$ , and quercetin treatment can markedly decreased the liver index of hepatic-injured rats ( $3.28\pm 0.14\%$  in pure quercetin group and  $2.80\pm 0.09\%$  in nanoliposomal quercetin group). Compared with the pure quercetin group, nanoliposomal quercetin was more effective on decreasing liver index of hepatic-injured rats ( $P=0.005$ ). Moreover, the liver index showed no significant difference in nanoliposomal quercetin group with the normal control group ( $P>0.05$ ). The results indicate that the protective effect of nanoliposomal quercetin in the injured liver was stronger than that of pure quercetin.

## Effect of nanoliposomal quercetin on serum liver function of experimental rats

Under physiological conditions, the content of transaminase in liver cells is about 100 times that in peripheral blood. The hepatic cellular membranes can be damaged by various pathogenic factors, with some enzymes secreting to peripheral blood. Thus, transaminases (GPT and GOT) in serum are sensitive indicators of hepatocyte damage and can reflect the severity of liver injury to a certain extent.[30] The liver is the main organ of bilirubin metabolism, and liver cells are the only cells that can form direct bilirubin (DBIL). Therefore, the level of serum bilirubin, especially DBIL, can comprehensively reflect the functional status of liver cells including their intake, transport, binding and excretion. To determine the effect of nanoliposomal quercetin on liver function, we detected serum levels of GPT, GOT and DBIL by using GPT/GOT ELISA kit and bilirubin assay kit. As shown in Table 1, GPT, GOT and DBIL values for hepatic-injured rats with saline treatment were higher than for control rats ( $P<0.001$ ). With simultaneous treatment of pure quercetin and nanoliposomal quercetin in rats with hepatic injury, the values of GPT,

GOT and DBIL markedly decreased as compared with saline treatment. Liver function did not differ between nanoliposomal quercetin and control treatment ( $P>0.05$ ), which indicates the hepatoprotective and treatment effects of nanoliposomal quercetin on liver function of rats.

## Effect of nanoliposomal quercetin on histopathology of injured liver

Although the liver index and liver function are important indexes to reflect the severity of liver injury, the gold standard is still histopathological examination. It is always used to diagnose liver inflammation activity, evaluate the degree of liver injury and determine drug efficacy. To evaluate the effect of nanoliposomal quercetin on histopathology of the injured liver, liver sections from all four groups were stained with H&E. In control rats, the structures of hepatic lobules were clear and intact (Fig. 3A). Hepatocytes were arranged around the central vein in a single line and had a radial shape with no lesions. Hepatic sinusoids, interlobular arteries, interlobular veins and interlobular bile ducts in portal areas were normal. However, in hepatic-injured rats with saline treatment, hepatic lobules lost their normal structure and boundaries were not clear. Most hepatic cords and hepatic sinusoids resolved and disappeared (Fig. 3B). Liver sinuses around the hepatic lobules were narrowed significantly, accompanied by focal fibrosis and collagen fiber hyperplasia in portal areas. In addition, many inflammatory cells were visible. With the formation of lipid droplets and diffuse vacuolar degeneration, fatty degeneration and necrotic hepatocytes were widespread. Parts of hepatocytes showed typical ballooning degeneration, nuclear condensation, and shrinkage of the nuclear membrane and widespread death. Thus, the rat model of complex hepatic injury was successfully established.

In hepatic-injured rats with quercetin and nanoliposomal quercetin treatment, the pathologic changes of liver tissues with saline treatment were attenuated (Fig. 3C-D). As compared with saline treatment in hepatic-injured rats, nanoliposomal quercetin treatment showed intact structures of the hepatic lobule and liver cells, accompanied by resolved fatty change, no obvious fibrosis formation and no obvious infiltrates of interstitial inflammatory cells (Fig. 3D), which still could be found in liver tissues of pure quercetin-treated rats (Fig. 3C). Our data indicate that treatment with nanoliposomal quercetin could reduce hepatic injury and protect hepatocytes against damage effectively.

## Conclusion

Liposomal nanoparticles may improve the solubility and bioavailability of quercetin in liver. Furthermore, nanoliposomal quercetin could effectively protect rats against acute liver injury and may be a new hepatoprotective and therapeutic agent for patients with liver diseases.

## Declarations

## Acknowledgements

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## Conflicts of Interest

The authors declare no potential conflicts of interest.

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## Tables

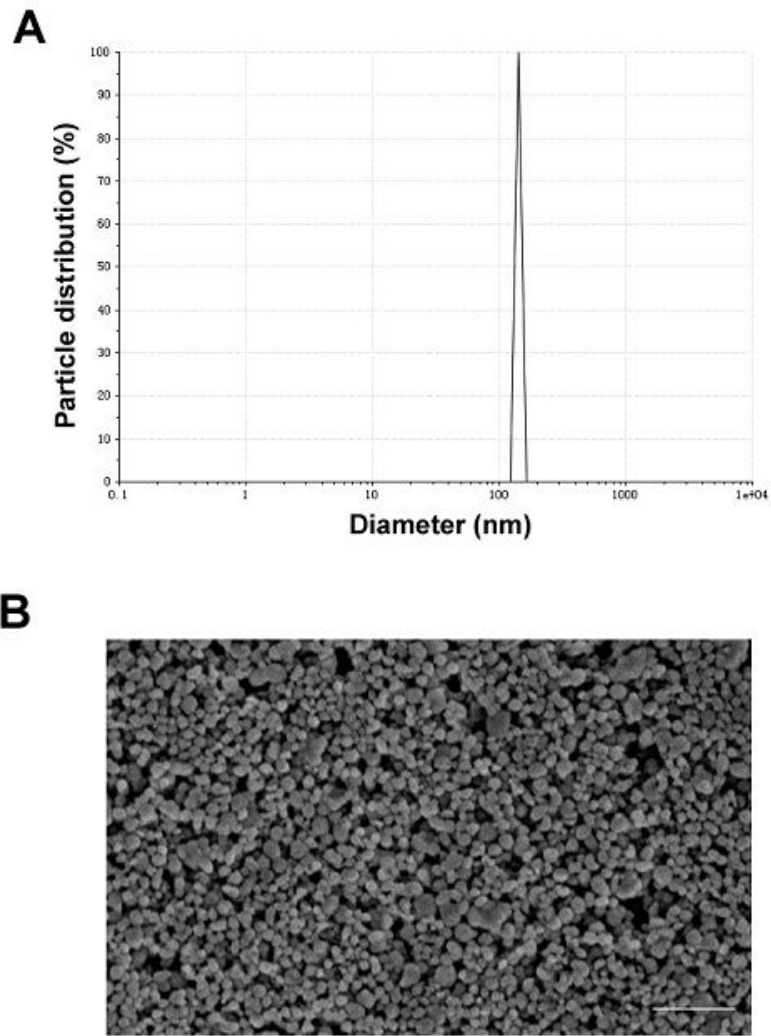
Table 1. Effect of nanoliposomal quercetin on liver function in rats

Group	GPT( $\mu$ /L)	GOT( $\mu$ /L)	DBIL( $\mu$ mol/L)
Normal control	33.63 $\pm$ 2.87**	59.88 $\pm$ 7.07**	0.21 $\pm$ 0.03**
Saline treatment	494.88 $\pm$ 6.53	765.50 $\pm$ 9.79	2.18 $\pm$ 0.06
Quercetin treatment	67.63 $\pm$ 6.49**	124.88 $\pm$ 14.76**	0.56 $\pm$ 0.07**
Nanoliposomal quercetin treatment	42.88 $\pm$ 3.39** $\Delta\Delta$ #	78.75 $\pm$ 8.64** $\Delta$ #	0.28 $\pm$ 0.04** $\Delta\Delta$ #

*Note.* Data are mean $\pm$ SEM (n=8-10). \*\* $P$ <0.01, compared with Group B;  $\Delta$  $P$ <0.05,  $\Delta\Delta$  $P$ <0.01, compared with Group C; # $P$ >0.05, compared with Group A, by  $t$  test.

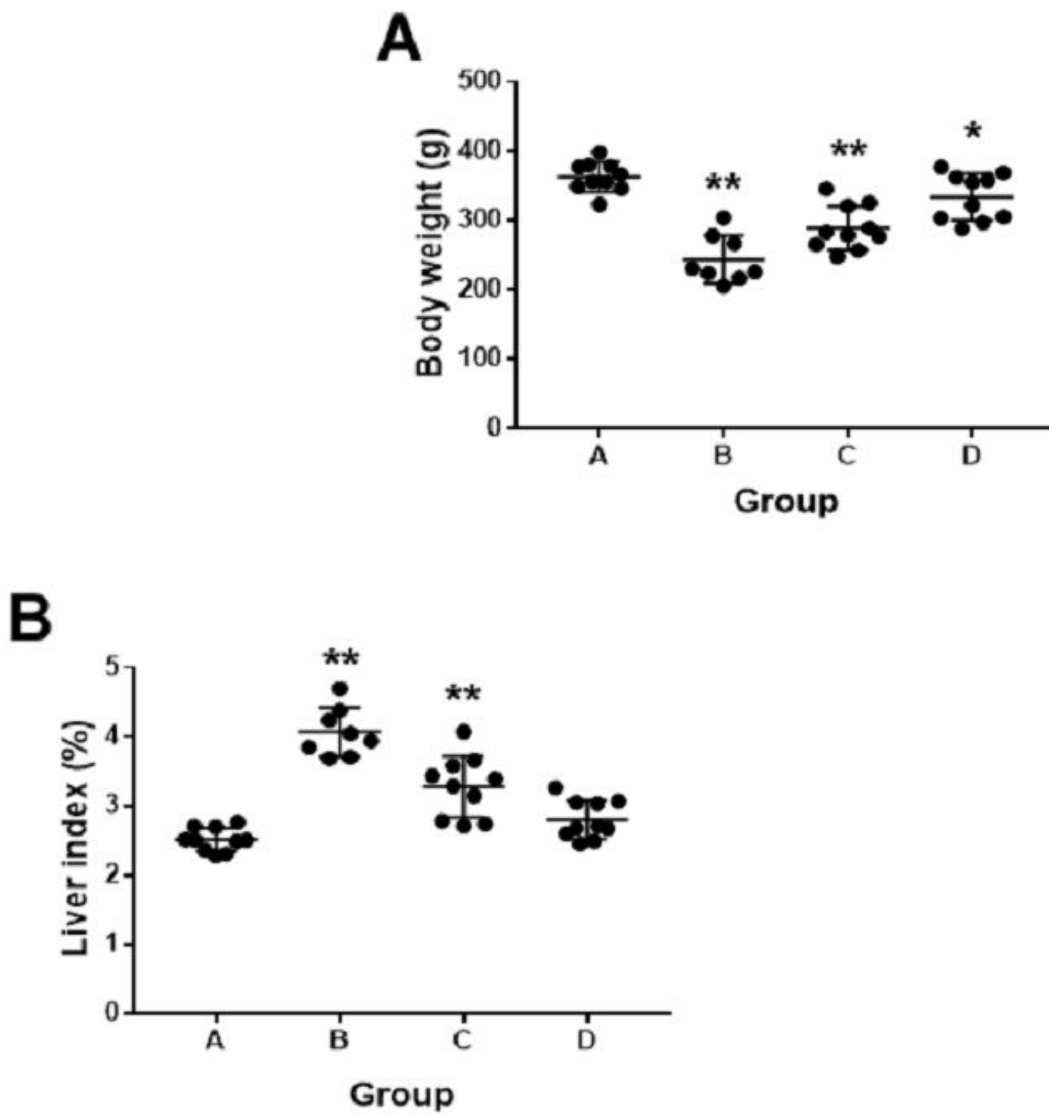
GPT: glutamic-pyruvic transaminase, GOT: glutamic-oxal acetic transaminase, DBIL: direct bilirubin.

## Figures



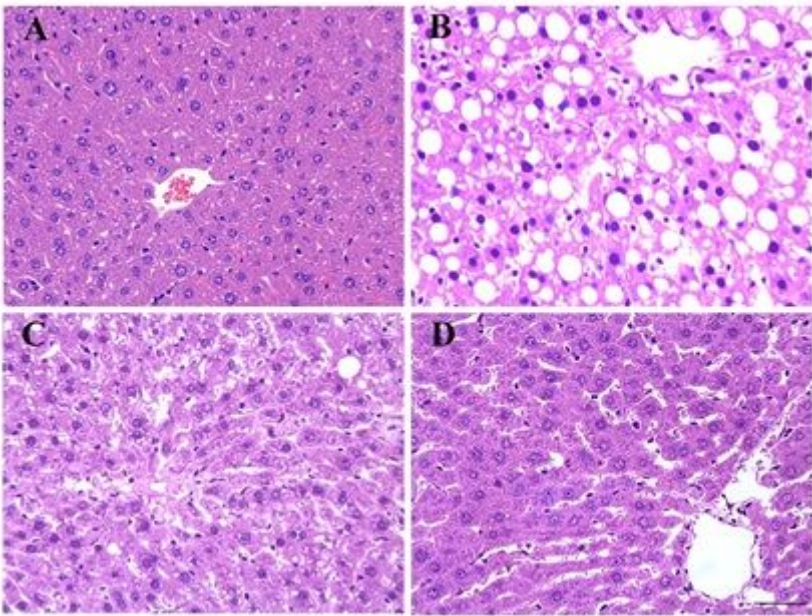
**Figure 1**

Fig. 1. Characteristics of nanoliposomal quercetin. Size distribution of nanoliposomal quercetin; B. Scanning electron microscopy image of nanoliposomal quercetin. Scale bar: 1 μm.



**Figure 2**

Fig. 2. Body weight and liver index of the rats after treatments. (A) Normal control; (B-D) hepatic-injured rats with B) saline treatment; C) quercetin treatment; and D) nanoliposomal quercetin treatment. Data are mean±SEM (n=8-10). \*P<0.05, \*\*P<0.01, compared with the normal control group, by t test.



**Figure 3**

Fig.3. Effect of nanoliposomal quercetin on histopathological changes in rat liver tissues. (Scale bar: 50µm; HE staining). (A) Normal control; (B-D) hepatic-injured rats with B) saline treatment; C) quercetin treatment; and D) nanoliposomal quercetin treatment.

## Supplementary Files

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