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Development of a Quercetin-loaded nanostructured lipid carrier formulation for topical delivery

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ABSTRACT

The main objective of this study was to evaluate the potential of Quercetin-loaded nanostructured lipid carriers (QT-NLCs) as a topical delivery system. QT-NLCs were prepared by the method of emulsion evaporation–solidification at low temperature. The average entrapment efficiency and drug loading of the optimized QT-NLCs were 89.95 ± 0.16% and 3.05 ± 0.01%, respectively. Under the transmission electron microscope, the nanoparticles were spherically shaped. The average particle size was 215.2 nm, the zeta potential was −20.10 ± 1.22 mV and pH value of QT-NLCs system was 4.65. Topical delivery of QT in the form of NLCs was investigated in vitro and in vivo. The results showed that QT-NLCs could promote the permeation of QT, increase the amount of QT retention in epidermis and dermis, and enhance the effect of anti-oxidation and anti-inflammation exerted by QT. Then the mechanism of NLCs for facilitating drug penetration was further investigated through histological sections. In conclusion, NLCs could be a promising vehicle for topical delivery of QT.

1. Introduction

In modern life, the skin is often subjected to environmental insults including excess sun exposure and increased air pollution, which commonly leads to oxidative stress (OS). Under these conditions, excess reactive oxygen species (ROS) can form, overwhelming the diminishing capability of skin, and induce skin disorders (Wheeler et al., 1986; Fuchs et al., 1989).

Quercetin (QT, 3, 3′, 4′, 5, 7-pentahydroxyflavone) is a natural flavonoid (Fig. 1) which has a variety of biological activities and pharmacological actions, such as anti-cancer, anti-oxidation, anti-inflammation, decreasing blood lipid, dilating coronary arteries, anti-platelet aggregation, anti-anemic action, and anti-anaphylaxis effects (Hollman and Kata, 1999). Recently, studies have shown that natural flavonoids possess the potential against OS-induced skin damage (Fuchs, 1998; Aquino et al., 2002). QT, the most commonly investigated flavonoid, presents the highest antiradical property compared to other flavonoids. It has been verified that QT can scavenge free radicals and inhibit lipid peroxidation (Formica and Regelson, 1995; Skaper et al., 1997). Therefore, topical application of QT has received considerable attention for the ability of against ROS-mediated damage in the skin (Casagrande et al., 2006, 2007; Vicentini et al., 2008). However, QT has a low solubility (7.7 μg/mL in water, 5.5 μg/mL in simulated gastric fluid and 28.9 μg/mL in simulated intestinal fluid), which contributes to a low absorption in vivo (Li et al., 2009; Khaled et al., 2003; Gugler et al., 1975). As a result, clinical application of QT is greatly restricted, and developing a novel vehicle that can transport sufficient QT into skin and exert its bioactivity is urgently needed.

Nanostructured lipid carriers (NLCs) are second generation lipid-based nanoparticles which are developed based on solid lipid nanoparticles (SLN) (Müller et al., 2002). In order to overcome the drawbacks of SLN including relatively low drug payloads and potential drug expulsion, liquid lipid which can disturb the highly regular lattice structure and form an imperfect matrix structure and further increase the space for accommodating drugs is introduced into NLCs (Saupe et al., 2006). In recent years, many studies have been focus on topical application of NLCs for their unique properties (Pardeike et al., 2010; Mitri et al., 2011; Nikolićć et al., 2011). Further, NLCs can enhance the apparent solubility of embedded drugs, which can form high concentration gradient on skin to facilitate drug permeation. The nano-sized particles can tightly adhere to the skin surface and transport the drugs in a more controlled

Abbreviations: QT, Quercetin; QT-NLCs, Quercetin-loaded nanostructured lipid carriers.

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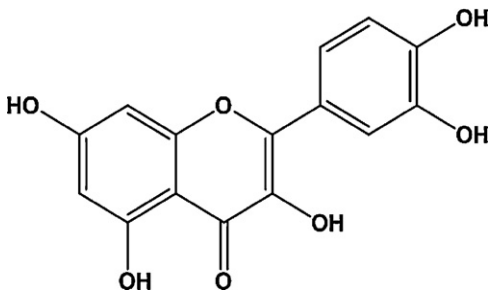

Fig. 1. The chemical structure of QT.

fashion. The occlusive effect exerted by NLCs can improve skin hydration and promote the penetration of drugs. Additionally, the components of NLCs including lipid and surfactants can be acted as permeation enhancers to loosen or fluidize the lipid bilayers of stratum corneum (SC). For example, Fang et al. (2008) prepared psoralen-loaded NLCs and evaluated the potential use in topical application. Results indicated that NLCs could significantly promote the permeation of psoralen compared to the drug suspension and lipid emulsion, showing the usefulness of NLCs as carriers for topical administration. Additionally, NLC has been considered as a novel and safe carrier for skin application of antioxidant. Ruktanonchai et al. (2009) prepared the NLCs for topical administration of alpha-lipoic acid (LA) as an effective antioxidant, found that the NLCs formulation showed lower cytotoxicity (more than 80% of cell survivals were found up to 1 μM of LA concentrations) and the similar antioxidant activity to pure LA. Coenzyme Q10 (CoQ10) as a powerful antioxidant can protect skin from ROS, recent study showed that CoQ10 loaded NLC had greater antioxidant properties and topical skin penetration than the CoQ10 loaded emulsion (Yue et al., 2010).

The aim of the present study was to design and characterize Quercetin-loaded nanostructured lipid carriers (QT-NLCs). In vitro drug permeation through excised mouse skin and in vivo drug distribution in epidermis and dermis of mice were evaluated. The effect of QT-NLCs application on the skin surface was also discussed based on the observation under light microscope. In addition, anti-inflammatory and anti-oxidation effects were investigated to estimate the potential use of NLCs for topical delivery of QT.

2. Materials and methods

2.1. Materials

QT was supplied by Xi'an Senmu Biological Technology Co. Ltd. (Xi'an, China). TPGS was purchased from Wuhan Yuancheng Co. Ltd. (Wuhan, China). Soya lecithin (SL) was obtained from Shanghai Taiwei Pharmaceutical Co. Ltd. (Shanghai, China). Glyceryl monostearate (GMS) was provided by Shanghai Chemical Reagents Co. Ltd. (Shanghai, China) and stearic acid (SA) by Beijing Chemical Reagents Co. Ltd. (Beijing, China). Media chain triglyceride (MCT) was purchased from Tieling Beiya Medicinal Oil Co. (Tieling, China). All other chemicals and solvents used in the study were of analytical reagent grade.

2.2. Preparation of QT-NLC

QT-NLCs were prepared by the method of emulsion evaporation–solidification at low temperature according to the previous report (Jia et al., 2010; Li et al., 2009). In brief, based on the formulation compositions, certain amount of QT, GMS, SA and MCT were dissolved in chloroform, and SL was dissolved in acetone, and then the two organic phases were mixed and kept in a water bath at 70 °C. The aqueous phase was prepared by dissolving TPGS in distilled water at 70 °C. Subsequently, the organic phase was slowly injected into the aqueous phase with magnetic stirring (800 rpm) and the resulting solution was emulsified for 4 h at 70 °C. After removing the organic solvent, semi-transparent nanoemulsion was obtained, and then it was transferred into cold distilled water (0–2 °C) under stirring for 2 h in order to acquire QT-loaded NLCs.

2.3. Physicochemical characterization of QT-NLCs

2.3.1. Morphology

The microstructure of QT-NLCs was observed using transmission electron microscope (TEM, JEM-1200EX, JEOL, Tokyo, Japan). Initially, samples diluted with double-distilled water were deposited on a film-coated copper grid, following by stained with 1% aqueous solution of phosphotungstic acid, ultimately the superfluous phosphotungstic acid on the samples was wiped off by filer paper and the sample was allowed to dry before examined under the TEM.

2.3.2. Particle size, zeta potential and pH value

The mean diameter of QT-NLCs was measured by photon correlation spectroscopy using a particle sizer (Zetasizer 3000 HAS; Malvern Instruments Ltd., Worcestershire, UK) at a fixed angle of 90° with a He–Ne laser of 633 nm at 25 °C. Particle size was evaluated using volume distribution.

The zeta potential was determined using a microscopic electrophoresis system (DXD-II; Jiangsu Optics Co. Ltd., Jiangsu, China) at 25 °C.

The pH values were evaluated at 25 °C using a pHS-25 digital acidimeter (Shanghai Rex Instrument Factory, Shanghai, China).

2.4. Entrapment efficiency (EE) and drug loading (DL)

Based on the pre-experiment, mini column centrifugation technique was adopted to separated free drug from prepared NLCs (Fry et al., 1978). In brief, 0.5 mL of the prepared QT-NLCs suspension was placed in a minicolumn of Sephadex G-50 made from the barrel of 5 mL syringe (pre-saturated with drug-free NLCs prepared with the same composition and methods) and centrifuged at 500 rpm for 1 min and the column was subsequently eluted six times with 0.5 mL of distilled water under the same condition. Then a certain volume of ethanol was added to the collected elutes containing drug-loaded NLCs, and the resultant was vortexed for 3 min to ensure QT completely dissolve. After centrifugation at 10,000 rpm for 10 min, QT dissolving in the supernatant was measured using an ultraviolet–visible spectrophotometer (UV-2102, Shanghai Instrument Ltd., China) at a wavelength of 373 nm. EE and DL of QT-NLCs were calculated according to the following equations (Li et al., 2009):

$$\mathrm{EE}(\%) = \frac{W_{\mathrm{entrapped}}}{W_{\mathrm{total}}} \times 100\% \quad (1)$$

$$\mathrm{DL}(\%) = \frac{W_{\mathrm{entrapped}}}{W_{\mathrm{NLCs}}} \times 100\% \quad (2)$$

In above equations, $W_{\mathrm{entrapped}}$ showed the amount of QT entrapped in QT-NLCs, W_{total} was the total amount of QT in QT-NLCs, and W_{NLCs} presented the weight of QT-NLCs.

2.5. Superoxide radical scavenging activity in vitro

The autoxidation of pyrogallol could occur in weak alkaline environment, and the autoxidation products had UV absorption at a wavelength of 325 nm. When the antioxidant was added into this system, the autoxidation rate of pyrogallol was reduced. Based on the previous report (Huang et al., 2011), the anti-oxidation effects of

Table 1
Amount of reagent added for determination of autoxidation rate of pyrogallol.

	Control	QT-NLCs	QT	Vitamin C
50 mmol/L of tris–HCl buffer (pH = 8.22) (mL)	4.5	4.5	4.5	4.5
Distilled water (mL)	3.2	3.2	3.2	3.2
50 mmol/L of pyrogallol (mL)	0.3	0.3	0.3	0.3
Ethanol (mL)	1	0.8	1	1
QT-NLCs (mL)	–	0.2	–	–
QT (μg)	–	–	200	–
Vitamin C (μg)	–	–	–	200

QT, QT-NLCs were evaluated with vitamin C as control in this study. Briefly, the reagents listed in Table 1 are mixed. The obtained mixture was incubated at 25 °C for 30 s and then the absorbance was immediately measured at 325 nm. This process was repeated for 8 times. Then the autoxidation rate of pyrogallol and the inhibition rates to pyrogallol autoxidation could be calculated.

2.6. In vitro permeation studies

2.6.1. Preparation of skin

Male Kunming mice weighting 20 ± 2 g were provided by Experimental Animal Center of Shandong University (Shandong, China) for the in vitro permeation studies. The dorsal skins were obtained after hair was carefully removed with a razor, and then the subcutaneous fat and connective tissue were trimmed. Subsequently, the excised skins were washed with physiological saline solution and examined for integrity, and then preserved in a refrigerator at 4 °C for later use (Zhu et al., 2009).

2.6.2. In vitro skin permeation studies

This experiment was performed using Franz diffusion cells with an effective diffusion area of 3.14 cm^2. The skin samples were mounted carefully on Franz diffusion cells with SC facing the donor chamber. 0.5 mL of QT-NLCs suspension (1.0 mg/mL) or QT propylene glycol solution (1.0 mg/mL) as control was applied on the skin surface, respectively. The receptor chamber was filled with 15 mL of physiological saline containing 1% Tween 80 to obtain the sink condition. The diffusion cells were maintained at 32 ± 0.5 °C with stirring at 600 rpm throughout the experiment. At predetermined time intervals (1–3, 6, 9 and 12 h), 0.5 mL sample of receptor fluid was withdrawn and the same volume of blank receptor fluid was immediately added to the receptor chamber. 3 mL of acetic ether was added to the sample. The resulting mixture was vortexed for 3 min, and then centrifuged at 4000 rpm for 15 min. The supernatant was collected and dried in nitrogen atmosphere. The obtained product was redissolved with 200 mL mobile phase and then filtered through 0.22 μm pore size cellulose membrane filter, and the filtrate was analyzed by HPLC.

2.7. In vivo permeation study in mice

All the experimental mice were housed in cages, with access to food and water ad libitum until use. 24 h prior to the experiment, the dorsal skins of mice were washed with physiological saline solution after their hair was removed with a razor. 0.5 mL of QT-NLCs (1.0 mg/mL) or QT propylene glycol solution (1.0 mg/mL) was applied on the dorsal surface (3.14 cm^2), respectively. At 3, 6, 9 and 12 h after dorsal administration, the mice were sacrificed and the administrated skins were stripped. The excised skins were thoroughly washed using physiological saline and cleaned with ethanol, and subsequently placed in water bath at 60 °C for 60 s to separate epidermis and dermis (Puglia et al., 2001, 2008). Epidermis and dermis were cut into pieces, and then homogenized for 3 min after adding 1 mL of the physiological saline solution. The procedure of extracting QT from the skin homogenate was the same as that from the receptor fluid described above. The amount of QT was analyzed by HPLC.

2.8. Effect of QT-NLCs on skin surface

The effect of QT-NLCs on skin surface was evaluated by observing the microstructural changes of skin in the form of tissue sections after application of QT-NLCs (Fang et al., 2008; Vicentini et al., 2008). Kunming mice were divided into three groups (normal group, QT-NLCs treated group and QT propylene glycol solution treated group). According to Section 2.7, mice were sacrificed after application of QT-NLCs and QT propylene glycol solution for 24 h, and the corresponding skins were stripped. Afterwards, the skin samples were dissected and immersed in 4% formalin for histological study. About 3–4 μm of paraffin tissue sections were cut and subjected to hematoxylin and eosin stain, and then these samples were observed using light microscope (BX51, Olympus Instruments Ltd., Japan).

2.9. Effect of QT-NLCs on xylene-induced ear edema in mice

For ease of topical application, QT-NLCs were uniformly mixed with glycerin and carbomer 940. The final composition of the preparation was 85% QT-NLCs, 5% glycerin and 0.5% carbomer 940. Male Kunming mice (weight 20 ± 2 g) were employed as animal models of ear edema caused by xylene to evaluate the anti-inflammatory effect exerted by QY-NLCs (Adeyemi et al., 2008). Additionally, the commercial indomethacin gel was acted as positive control. In this study, empty matrix, QT-NLCs (0.2 mg QT/ear) and indomethacin gel (1 mg indomethacin/ear) were applied on the left and right ears of mice once every hour in 8 h. 30 min after the last administration, the preparations were washed off, and then 30 μL of xylene was applied on the right ear in order to induce edema. 1 h later, the mice were put to death by dislocating neck. Subsequently, the ears were perforated by punching bear (9 mm diameter), and the resulting tissues were accurately weighted. The edema weight and inhibition percentage are assessed according to the following equation (Fan et al., 2011):

$$W = W_{\text{right}} - W_{\text{left}} \tag{3}$$

$$\text{Inhibition}\,(\%) = \frac{W_{\text{control}} - W_{\text{treated}}}{W_{\text{control}}} \tag{4}$$

where W was edema weight, W_{right} or W_{left} stood for weight of the right or left ear, and W_{control} or W_{treated} showed edema weight of control or treated group.

2.10. HPLC analysis of QT

The samples were analyzed using an HPLC system (1200 series; Agilent Technologies, Palo Alto, CA) and an HPLC column (C18, 4.6 × 250 mm, 5 μm). The mobile phase was a mixture of methanol–water at a ratio of 50:50 (v/v) containing 3% acetic acid. The flow rate was 1 mL/min, and the detective wavelength was 373 nm. Aliquots of 20 μL of each sample were injected into the column, and all operations were performed at ambient temperature. The peak area correlated linearly with QT concentration in the range of 1.5–150 μg/mL and the lowest detection limit at 0.5 μg/mL.

2.11. Statistical analysis

Data were shown as mean ± S.D. (n = 5). Statistical data were analyzed by the Student's t-test at the level of p = 0.05.

Table 2
Uniform experiment design and the results.

	A (mg)	B (%)	C (mg)	D (w/w)	E (%)	F (°C)	S
1	20	3	200	2:1	40	80	12.41
2	20	4	400	1:2	30	75	11.08
3	20	4	200	4:1	20	70	11.38
4	25	5	400	1:1	50	65	11.59
5	25	6	200	1:2	30	80	11.62
6	25	6	400	2:1	20	75	8.94
7	30	3	100	1:1	50	70	12.02
8	30	3	300	4:1	40	65	15.56
9	30	4	100	2:1	20	80	15.49
10	35	5	300	1:2	50	75	14.61
11	35	5	100	4:1	40	70	13.91
12	35	6	300	1:1	30	65	12.15

3. Results and discussion

3.1. Formulation optimization and physicochemical characterization of QT-NLCs

Based on the single factor experiment, the amount of drug, the concentration of surfactant, the amount of cosurfactant, the ratio of GMS to SA, the amount of liquid lipid and emulsifying temperature were proved to have great effects on the properties of QT-NLCs. What is more, EE (S_1) standing for the drug loaded efficiency or production efficiency and DL (S_2) presenting the loading capacity of carrier for drug are two important indexes to evaluate the preparation technologies and prescriptions of nanoparticles (Subedi et al., 2009). With the higher EE and DL, the nanoparticle system will have higher production efficiency and higher drug concentration gradient beneficial to the skin permeation of drug. So the uniform design method was used to optimize the formulation and preparation technology of QT-NLCs with $S(S = \sqrt{S_1 \times S_2})$ as evaluation index. The U_{13} (13^{12}) uniform design was established as shown in Table 2. The data were analyzed with SPSS (Statistical Product and Service Solutions) 16.0, and the experimental designs resulted in a mathematical expression (multiple linear regression equation):

$$Y = 2.09176 + 0.7391A + 1.44420B - 0.00506C - 0.36769D - 0.00327E - 0.00828F - 0.10465AB \quad (5)$$

Y is the dependent variable (indicated by the synthetic criteria (S) to evaluate the formulation and preparation technology of QT-NLCs in Table 2, A (the amount of QT), B (content of TPGS), C (the amount of SL), D (the weight ratio of GMS to SA), E (percentage of liquid lipid in total lipid) and F (the emulsifying temperature) are the independent variables. This expression gives an insight into the effect of the different independent variables on Y. The larger the optimized value (Y), the better the level was. The independent variables with larger coefficients should show the more significant influence on Y. From Eq. (5), the coefficients for A and B were 0.7391 and 1.44420, respectively, which indicated that they had the greater effects on Y. While C–F with smaller coefficients than that of A or B exerted the less influence. According to the above equation, in order to get a larger optimized value (Y), the variables C–F should choose the lowest levels in the set ranges based on the preliminary tests and uniform design. Based on the above formula, Fig. 2 is designed to show the relationship between Y and A, B. In order to improve the stability of QT-NLCs and enhance the DL, the optimized formulation and technology were as follows: the amount of QT was 35 mg, the content of surfactants (TPGS) in the system was 4% (w/v), the amount of co-surfactant was 200 mg, the ratio of GMS to SA was at 1:1, the amount of liquid lipid was 20%, with the emulsifying temperature at 70 °C and 4 h for the emulsifying time.

The result of TEM imaging of QT-NLCs as shown in Fig. 3 indicated that the particles presented uniformly nano-sized

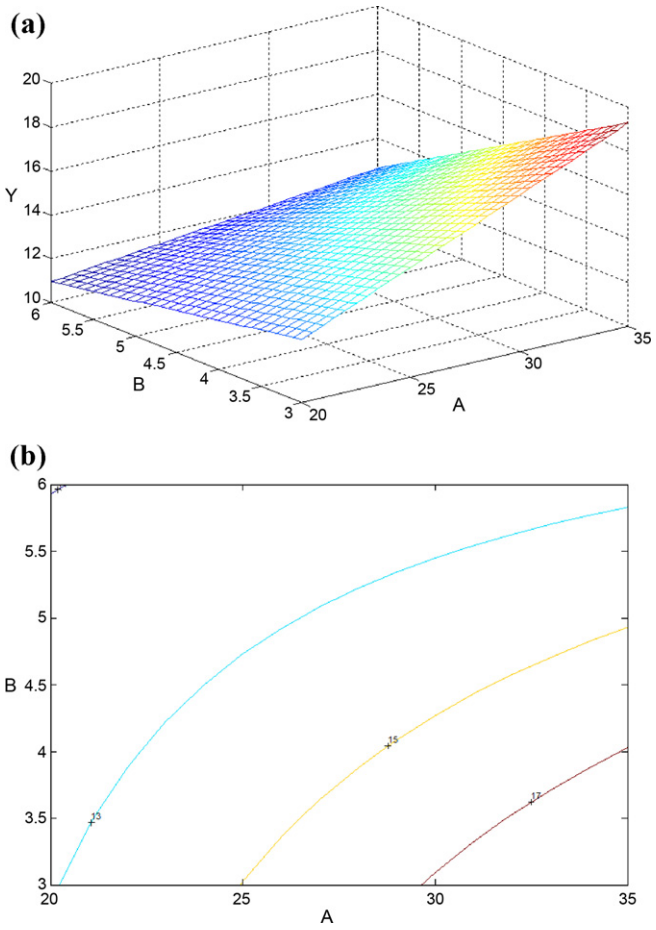

Fig. 2. Three dimensional map (a) or contour map (b) of variation of dependent variable (Y) follows independent variables (A and B).

spherical shapes. The average particle size and the zeta potential of QT-NLCs were 215.2 nm and -20.10 ± 1.22 mV, respectively. The small particle size as well as uniform size distribution of QT-NLCs nanoparticles was suitable for the development of nanomedicines. In addition, the pH value for the nanoparticle system was 4.65, which was benefit to stability of QT.

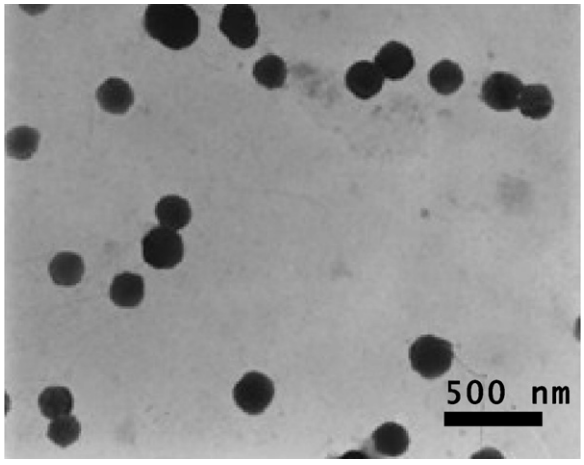

Fig. 3. TEM photograph of QT-NLCs.

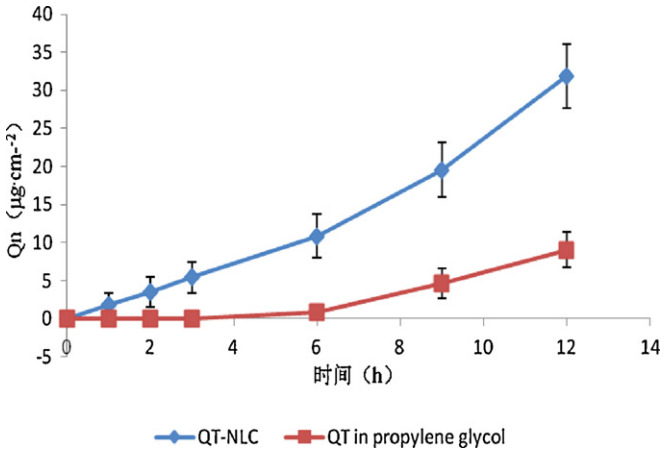

Fig. 4. Percutaneous permeation profiles of QT from QT propylene glycol solution and QT-NLCs through the excised rat skins (mean ± S.D.; $n = 5$).

3.2. Drug encapsulation efficiency and drug loading

The samples were determined spectrophotometrically at 373 nm. The blank sample had no disturbance at this wavelength in the concentration range of 2.0–10.0 μg/mL, the following standard regression equation was obtained: $A = 0.0683C - 0.0244$, $R^2 = 0.9997$ ($n = 5$). An average EE of 89.95 ± 0.16% and an average DL of 3.05 ± 0.01% were achieved for the optimized QT-NLCs formulation.

3.3. Superoxide anion radical scavenging activity in vitro

Damage produced by free radicals is involved in various patho-physiological processes and the pivotal role of radicals in patho-physiology forms the rational for antioxidant intervention. The drugs with higher clearance rate to free radicals showed greater antioxidant capacity. The drugs' clearance to free radical of O_2^- was investigated with the pyrogallol autoxidation method in our study. The results showed that the clearance rates of QT-NLC, QT and vitamin C were 18.79%, 11.15% and 100%, respectively. From the result we could find that the antioxidant capability of vitamin C was 5.3 times that of QT, the reason might be that at the same weight, vitamin C has a greater number of —OH group which is related to the activity of antioxidant (Fang et al., 2011) than QT does. The clearance rate of QT-NLCs was significantly higher than that QT ($p < 0.05$). The reason might be that vitamin E existing in TPGS played an actor as antioxidant (Van Acker et al., 1993), and enhanced the antioxidant capacity of the QT-NLC system. In brief, the result was confirmed the retention of complete functional architecture of QT after nanoencapsulation.

3.4. Skin permeation studies

In vitro studies, the cumulative amounts of QT at different time in the receptor solution are shown in Fig. 4, and the steady-state flux (J_s), permeation coefficient (K_p) for 12 h according to Eqs. (6)–(9) (Lv et al., 2009) are shown in Table 3. It indicated that J_s of QT in propylene glycol solution and loaded in NLC were 1.153 ± 0.11 μg/(cm^2 h) and 3.503 ± 0.27 μg/(cm^2 h), respectively. The permeation of QT in the two preparations followed zero order release kinetics, and the equations are expressed in Table 2. The cumulative amounts of QT from propylene glycol solution and NLCs at 12 h after dosing were 9.00 ± 1.19 μg cm^{-2} and 31.89 ± 3.15 μg cm^{-2}, respectively. In other words, the cumulative amount of QT from NLCs penetrating through the mouse skins was more than 2.54 times that from propylene glycol solution at 12 h.

Table 3
Skin permeation parameters of QT propylene glycol solution and QT-NLCs ($n = 6$).

Preparation	Permeation equation	J_s (μg/(cm^2 h))	R
QT propylene glycol solution	$Q_n = 1.153t - 3.330$	1.153 ± 0.11	0.997
QT-NLC	$Q_n = 3.503t - 0.241$	3.503 ± 0.27	0.990

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i}{A} \quad (6)$$

$$Q_n = AKLC_0 \left[\left(\frac{D_t}{L^2} \right) - \left(\frac{1}{6} \right) - \frac{2}{\pi^2} - \sum \left(\frac{(-1)^n}{n^2} \right) \exp\left(\frac{D^n 2\pi^2 t}{L^2} \right) \right] \quad (7)$$

$$\frac{Q_n}{A} = KLC_0 \left[\left(\frac{D_t}{L^2} \right) - \left(\frac{1}{6} \right) \right] \quad (8)$$

$$J_s = C_0 \frac{KD}{L} = C_0 K_P \quad (9)$$

where C_n stood for the QT concentration of the receiver medium at each sampling time, C_i for the QT concentration of the ith sample, C_0 for the QT concentration which remains constant in the donor vehicle, A was the effective diffusion area, V_0 and V_i showed the volumes of the receiver solution and the sample, respectively. D was the diffusion coefficient, L was the thickness of the membrane, K stood for the partition coefficient of QT between membrane and vehicle, and K_P was the permeability coefficient.

In vivo studies, the cumulative amounts of QT (Q_n, μg/cm^2) in epidermis and dermis from the QT propylene glycol solution and QT-NLCs at 3, 6, 9 and 12 h after administration are shown in Fig. 5. The amount of QT penetrated into epidermis and dermis from NLCs at 12 h was 1.52 and 3.03 times that of propylene glycol solution ($p < 0.05$), respectively. The small size and close interaction between NLCs and the stratum corneum are the possible reasons why NLCs can increase the drug amount penetrating into the viable skin. NLCs with small diameter could closely contact to skin surface and form adhesion of thin films on skin which could improve the hydration of stratum corneum and enhance the penetration of drug. In addition, carrier materials (e.g. lecithin) as percutaneous absorption enhancers could also facilitate the permeation of drug to skin. Furthermore, previous studies (Zhao et al., 2010) showed that nanoparticles between 20 and 200 nm could locate in skin "furrows", so the drugs from NLCs might have more opportunities to tightly contact with the skin, and more drugs could enter skin or stratum corneum.

3.5. Effect of QT-NLCs on skin surface

Skin histological sections of untreated animals, animals treated with QT propylene glycol solution and QT-NLCs were visualized by conventional light microscopy. The results as shown in Fig. 6 revealed that the skin stratum corneum treated by QT-NLCs was more scattered and loose compared with that treated by propylene glycol solution. Meanwhile, the stratum corneum treated by QT-NLCs appeared more swollen and overall thickness of the skin obviously increased. Moreover, cell conjunction was broken and intercellular space increased. In a conclusion, QT-NLCs could weaken the barrier function of stratum corneum and promote drug permeation. The reasons were as follows, NLCs could increase the apparent solubility of QT in the preparation, and then a high concentration gradient was formed, which was beneficial to promote

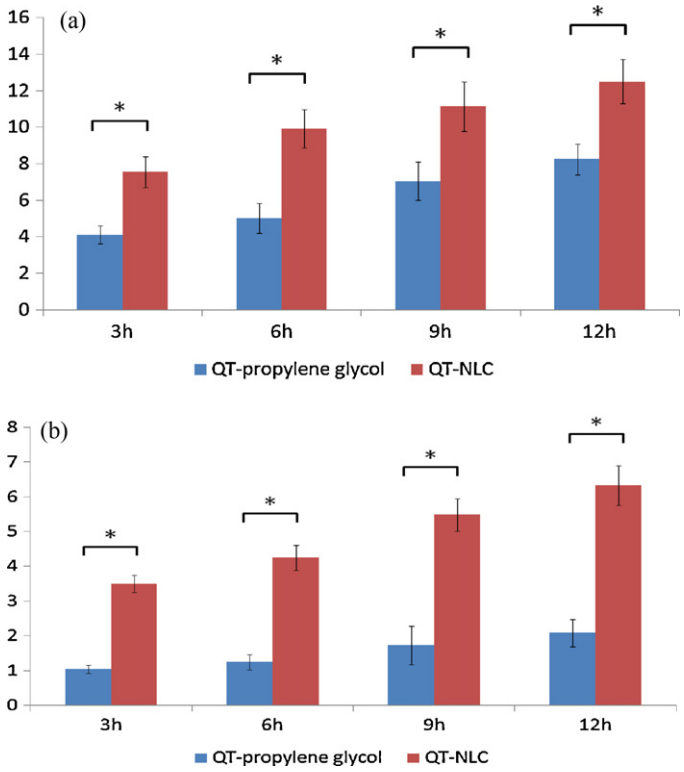

Fig. 5. The amount of QT in epidermis (a) and dermis (b) after 3, 6, 9 and 12 h, respectively ($n = 6$).

drug permeation. The nano-sized particles of QT-NLCs could tightly adhere to the skin surface and transport the drugs in a more controlled fashion. The occlusive effect exerted by NLCs could improve skin hydration and promote the penetration of drugs. In addition, the components of QT-NLCs including TPGS and SL could be acted as permeation enhancers to loosen or fluidize the lipid bilayers of SC.

3.6. Effect of QT-NLCs on xylene-induced ear edema in mice

In this study, xylene which could cause acute inflammation was used as inflammatory agent. And the result showed that the inhibition rates of QT-NLCs gel and indomethacin gel were 32.3% and 69.4%, respectively (Table 4). In addition, Rotelli et al. (2003) had studied the effect of QT suspended in normal saline administered with an intraperitoneal injection (0.25 M/kg) on xylene-induced ear edema, and the inhibition rate was 12%. Based on the above results, we could conclude that the topically applied QT-NLCs showed higher ability of inhibiting ear edema induced by xylene than QT suspended in normal saline by intraperitoneal injection, which indicated the advantages of QT-NLCs for topical delivery.

Table 4
Effect of QT-NLCs on edema induced by xylene in mice ($n = 6$).

Group	Edema (W/mg)	Inhibitory (%)
Control (empty matrix)	13.45 ± 2.86	
Indomethacin gel (1 mg/ear)	4.72 ± 3.82*	69.4
QT-NLCs gel (0.2 mg/ear)	9.1 ± 1.64**	32.3

* $p < 0.01$, compared with the control group.
** $p < 0.05$, compared with the control group.

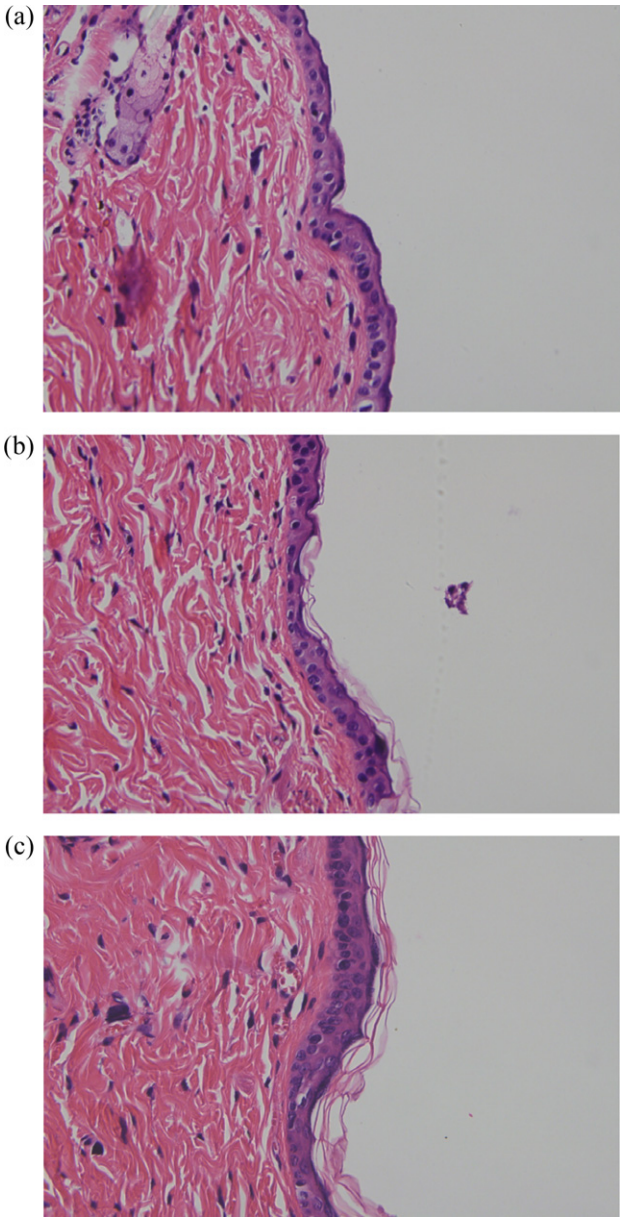

Fig. 6. Photomicrographs (400×) of skin sections of untreated animals (a), animals treated with QT propylene glycol solution (b) and QT-NLCs (c).

4. Conclusion

In the study, we have successfully incorporated QT, a poorly soluble drug, into NLCs by an emulsion evaporation–solidification at low temperature method. The obtained results confirmed the potential of NLCs as carriers for topical administration. In vitro and in vivo skin permeation studies showed that QT-NLCs could obviously increase the amount of drug retention in epidermis and dermis compared to QT propylene glycol solution. Studies on effect of QT-NLCs on skin surface confirmed that QT-NLCs could weaken the barrier function of stratum corneum and facilitate drug permeation in skin. In vivo anti-inflammatory experiment indicated that QT-NLCs could suppress ear edema induced by xylene in rats, and the in vitro studies of superoxide anion radical scavenging activity confirmed the retention of complete functional architecture of QT after nanoencapsulation. This study provides supplementary evidences that NLCs have a targeting and prolonged release effect with great potentials in dermal delivery.

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