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Lecithin-Based Novel Cationic Nanocarriers (Leciplex) II: Improving Therapeutic Efficacy of Quercetin on Oral Administration†

Abhijit A. Date,[‡] Mangal S. Nagarsenker,*[,‡] Shilpa Patere,[‡] Vivek Dhawan,[‡] R. P. Gude,[§] P. A. Hassan,[⊥] V. Aswal,[⊥] Frank Steiniger,[∥] Jana Thamm,[#] and Alfred Fahr[#]

[‡]Department of Pharmaceutics, Bombay College of Pharmacy, Kalina, Santacruz (East), Mumbai-400098, India

[§]Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai-410210, India

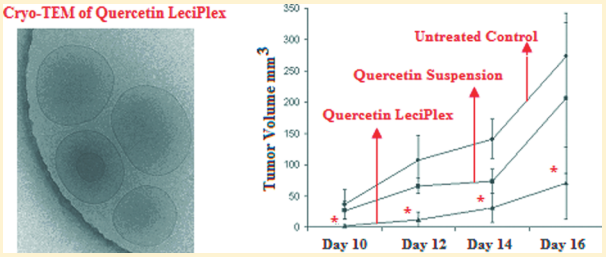
[⊥]Bhabha Atomic Research Center, Mumbai 400 085, India

[∥]Center for Electron Microscopy of the Medical Faculty, Friedrich-Schiller-University Jena, Ziegelmühlenweg 1, D-07740 Jena, Germany

[#]Department of Pharmaceutical Technology, Friedrich-Schiller-University Jena, Lessingstr. 8, D-07743 Jena, Germany

Supporting Information

ABSTRACT: The objective of the present investigation was to evaluate ability of the novel self-assembled phospholipid- based cationic nanocarriers (LeciPlex) in improving the therapeutic efficacy of a poorly water-soluble natural polyphenolic agent, quercetin (QR), on oral administration. Quercetin loaded LeciPlex (QR-LeciPlex) were successfully fabricated using a biocompatible solvent Transcutol HP. The QR-LeciPlex were characterized for particle size, encapsulation efficiency, zeta potential, and particle morphology by cryo-TEM. UV and fluorescence spectral characterization was carried out to find out the association of QR with LeciPlex. Small angle neutron scattering studies (SANS) were carried out to understand the internal structure of Leciplex and to evaluate the influence of the incorporation of QR in the LeciPlex. Anti-inflammatory and antitumorigenic activity of QR-LeciPlex was determined in comparison to QR suspension to evaluate the potential of LeciPlex in improving oral delivery of QR. QR-LeciPlex exhibited a particle size of ~400 nm and had excellent colloidal stability. The QR-LeciPlex had a zeta potential greater than +30 mV and exhibited very high encapsulation efficiency of QR (>90%). UV and fluorescence spectral characterization indicated the interaction/association of QR with LeciPlex components. Cryo-TEM studies showed that LeciPlex and QR-LeciPlex have a unilamellar structure. SANS confirmed the unilamellar structure of LeciPlex and indicated that the incorporation of QR does not have any effect on the internal structure of the LeciPlex. QR-LeciPlex exhibited significantly higher anti-inflammatory and antitumorigenic activity ($p < 0.01$) as compared to that of QR suspension on oral administration.



KEYWORDS: quercetin, poor solubility, phospholipids, lipid nanoparticles, lecithin, oral delivery, antitumorigenic activity

1. INTRODUCTION

Phytochemicals are naturally occurring substances found in plants. There has been a great public and scientific interest in use of phytochemicals derived from dietary components to treat various ailments, especially cardiovascular diseases and cancer. More particularly, the focus has been on various dietary polyphenols, a large group of natural antioxidants that are widely present in the vegetables and fruits consumed by human in a normal diet. Dietary polyphenols are being extensively studied for their potential in the treatment of cancer and various other diseases. Quercetin (QR, 3,3′,4′,5,7-pentahydroxyflavone) is one such dietary polyphenol which is commonly found in fruits and vegetables such as apples and onions. QR belongs to the flavonoid class and is a major representative of the flavonol subclass.[1] QR has a long history of intake as a part of human diet and has been shown be to nontoxic, noncarcinogenic, and nonmutagenic on long-term consumption.[2] A recent study has revealed that QR, at the dose of 3 g/kg, does not have any toxic and harmful effect in mice.[3] Over the past few years, QR has been shown to exhibit antioxidant, anti-inflammatory, antianxiety, and anticarcinogenic activities.[1,4−6]

QR is gaining popularity in the prevention and treatment of cancer. Various studies have demonstrated that QR exhibits an antiangiogenic effect and can also induce apoptosis in the various cancer cell lines.[1,7] The utility of QR has been shown in the treatment of various types of cancers such as lung cancer, colon cancer, melanoma, and hepatic cancer by various *in vitro* and/or *in vivo* studies.[7−9] However, despite its demonstrated safety and efficacy, the use of the QR as a therapeutic agent is mainly hampered due to its poor water solubility, short biological half-life, and low oral bioavailability. The oral bioavailability of the QR in rats is <17%, whereas in case of humans, the bioavailability is

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even lower (around 1%).[10] It has been observed that oral administration of the QR (4 g) did not result in any detectable QR levels in plasma and urine.[10] The poor oral bioavailability of QR is due to its poor solubility in gastric fluids (5.5 μg/mL) and intestinal fluids (28.9 μg/mL) and also due to rapid metabolism in the gastrointestinal tract.[11]

In view of this, it is necessary to design novel delivery approaches which would help in improving oral bioavailability of QR. Kale et al. prepared a QR-cyclodextrin inclusion complex which improved the aqueous solubility of QR and showed higher antitumorigenic activity in mice.[12] Recently, the utility of lipid-based novel delivery systems such as microemulsions and solid lipid nanoparticles in improving oral bioavailability and/or therapeutic efficacy of QR has been established.[11,13] Azuma et al. reported significant increase in the bioavailability of QR after coadministration of phospholipids and emulsifiers.[14] In view of this, it is worthwhile to investigate potential of phospholipid-based nanocarriers in improving oral delivery of QR. The application of phospholipid-based nanocarriers such as liposomes, microemulsions, and solid lipid nanoparticles in improving permeability and/or oral bioavailability of various drugs has been established.[15,16]

We have recently developed novel self-assembled phospholipid based cationic nanocarriers (LeciPlex) using biocompatible solvents for the improved delivery of hydrophobic drugs.[17] The objective of the investigation was to fabricate and characterize QR loaded LeciPlex and to evaluate their *in vivo* efficacy on oral administration in suitable disease conditions such as inflammation and cancer.

2. EXPERIMENTAL SECTION

2.1. Materials. Transcutol HP (diethylene glycol monoethyl ether; Gattefosse India Ltd., Mumbai, India), trehalose (Gangwal Chemicals Ltd., Mumbai, India), mannitol (Signet Chemicals, Mumbai, India), and Phospholipon 90 G, (soy lecithin, Lipoid GmBH, Germany) were received as gift samples. Acetonitrile (HPLC grade), dipotassium hydrogen orthophosphate, butylated hydroxyl anisole (BHA), and phosphoric acid (Qualigens Fine Chemicals, Mumbai, India), didodecyldimethylammonium bromide (DDAB; Fluka Chemicals, NY, USA), and quercetin dihydrate (Sigma Chemicals, NY, USA) were purchased for the study. All the chemicals used for the study were of analytical grade unless specified. Double-distilled water was freshly prepared whenever required.

2.2. Fabrication of QR Loaded Phospholipid-Based Cationic Nanocarriers (QR-LeciPlex). Soybean lecithin (Phospholipon 90G) was used for the fabrication of LeciPlex. Briefly, Phospholipon 90G at a concentration of 0.24 mM (or 0.186 g), BHA (10 mg), and didodecyldimethylammonium bromide (DDAB) at a concentration of 0.24 mM (or 0.111 g) were weighed accurately and transferred to a test tube. Transcutol HP, 0.5 mL, was added to this test tube, and the phospholipid and DDAB were dissolved by heating test tube at 70 °C in a constant temperature water bath (Superfit Ltd., Mumbai, India). QR (25 mg) was added to the phospholipid phase maintained at 70 °C, and the heating was continued until the formation of homogeneous yellow clear solution. To this solution, double-distilled water, 9.5 mL (maintained at 70 °C), was added at once under cyclomixing (~1200 rpm). The cyclomixing was continued until the uniform pale yellow colored dispersion is formed. All the experiments were done in triplicate. The particle size of the dispersions was evaluated by photon correlation spectroscopy.

2.3. Particle Size Analysis. The average particle size and polydispersity index of the QR-Leciplex were determined in triplicate by the photon correlation spectroscopy (PCS; Beckman Coulter N4 plus, Wipro, India). Measurements were performed at an angle of 90° at 25 °C. Dispersions were diluted with double-distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range intensity (between 4e + 006 to 1e + 006). Double-distilled water was filtered through 0.45 μm membrane filters (Pall Life Sciences, Mumbai) prior to its use for particle size determination.

2.4. UV Analysis of QR. For the UV method, a standard solution of QR (100 μg/mL) was prepared by dissolving accurately weighed quantity of QR in methanol, and working standards were prepared by dilution of this standard solution with methanol. The absorbance of the resulting solutions was recorded at 374 nm in a 10 mm quartz cell on a Shimadzu UV-1650 UV−vis double beam spectrophotometer (Shimadzu, Japan). The assay was linear (r^2 = 0.999; % CV = 1.12) in the concentration range of 1−12 μg/mL.

2.5. HPLC Analysis of QR. The amount of QR in the LeciPlex was determined by the reverse-phase HPLC method developed in house. The HPLC apparatus consisted of Jasco PU-2080 Plus Intelligent HPLC pump (Jasco, Japan) equipped with a Jasco UV-2075 Intelligent UV−vis detector (Jasco, Japan), a Rheodyne 7725 injector (Rheodyne, USA), a Jasco Borwin Chromatography Software (version 1.50) integrator software, and a Hi-Q-Sil C_{18} (4.6 mm × 250 mm and 10 μm particle size) column. The mobile phase consisted of a mixture of acetonitrile and pH 2.5 phosphate buffer (35:65 v/v) at a flow rate of 0.8 mL/min that led to retention time of 10.3 min when detection was carried out at 254 nm. The assay was linear (r^2 = 0.999; % CV = 1.22) in the concentration range of 1−20 μg/mL with the lowest detection limit of 300 ng/mL of QR. The method was validated in terms of accuracy (% CV = 1.31) and precision (% CV = 1.19).

2.6. Evaluation of QR Loaded LeciPlex (QR-LeciPlex). *2.6.1. Encapsulation Efficiency.* The encapsulation efficiency (EE), of QR in the LeciPlex, was determined by measuring the concentration of free QR in the dispersion medium. QR-LeciPlex dispersion (1 mL) was centrifuged at 14000 rpm (Eppendorf Mini-centrifuge) for 20 min to separate nanoparticles. For facilitating the separation of the nanocarriers, electrolytes such as NaCl was used. After suitable dilution, the supernatant was analyzed at 374 nm for unencapsulated QR by using a validated UV-spectrophotometric method.

The entrapment efficiency was calculated by the following equation:

$$\%\mathrm{EE} = \left[\frac{M_{\text{initial drug}} - M_{\text{free drug}}}{M_{\text{initial drug}}}\right] \times 100$$

where $M_{\text{initial drug}}$ is the total amount of QR used for the fabrication of LeciPlex and the $M_{\text{free drug}}$ is the amount of free QR detected in the supernatant after centrifugation of the QR-LeciPlex dispersion.

2.6.2. Evaluation of the Zeta Potential. For evaluation of the zeta potential, QR-LeciPlex dispersion was suitably diluted with double-distilled water. The zeta potential value was evaluated in triplicate by using Malvern Zetasizer (NY, USA).

2.6.3. QR Content of QR-LeciPlex. Quantity of QR-LeciPlex equivalent to 2.5 mg of QR was transferred to 50 mL volumetric

flask. The volume was made up to 50 mL with methanol in volumetric flask to obtain a stock solution of 50 μg/mL. From this stock solution, 2 mL was withdrawn accurately and diluted to 10 mL with methanol to get a QR solution of concentration 10 μg/mL. This solution in quantity of 50 μL was injected in HPLC, and the QR content was determined using a validated HPLC method described earlier.

2.6.4. Evaluation of Functional Stability of QR-LeciPlex (DPPH Assay). The functional stability of QR in LeciPlex was assessed by evaluating *in vitro* antioxidant activity of the QR. The *in vitro* antioxidant activity of QR solution and QR-LeciPlex was determined by evaluating the ability of the QR-LeciPlex and QR-solution to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. The procedure reported by Wu and co-workers for evaluating functional stability of QR polymeric nanoparticlces was employed for this purpose with suitable modifications.[18]

Briefly, a calculated amount of DPPH was dissolved in methanol to yield 200 μM DPPH solution. A calculated amount of QR was dissolved in a suitable amount of Transcutol HP to yield QR solution of concentration 2.5 mg/mL. QR-LeciPlex were fabricated as per the procedure reported earlier (final QR concentration: 2.5 mg/mL). BHA was not included during preparation of QR-LeciPlex as it can interfere with determination antioxidant activity of QR. Blank LeciPlex (LeciPlex without QR) were also prepared for comparison.

For the determination of antioxidant activity, 500 μL of DPPH methanolic solution was mixed with 500 μL of QR solution or QR-LeciPlex or blank LeciPlex. A control sample was prepared by mixing 500 μL of DPPH methanolic solution with 500 μL of distilled water. All the samples were stored at room temperature for 30 min. After 30 min, the absorbance of samples was measured at 517 nm against blank (MeOH: water 1:1). The percent antioxidant activity (or free radical scavenging activity) was calculated by the formula

$$\%\text{antioxidant activity} = \left(\frac{A_c - A_t}{A_c}\right) \times 100$$

where A_c = absorbance of the control sample and A_t = absorbance of the test sample (QR solution or blank Leciplex or QR-Leciplex).

The statistical significance of differences in the data was analyzed by Student's paired t test (GraphPad InStat demo version). Differences were considered statistically significant at $p < 0.05$.

2.6.5. UV−vis Spectral Characterization of QR-LeciPlex. Briefly, QR-Leciplex and blank LeciPlex (without QR) were fabricated. The QR-LeciPlex was diluted with distilled water to yield a concentration of QR equivalent to 5 μg/mL. Blank Leciplex were also diluted with the same quantity of double-distilled water that was used for the dilution of QR-LeciPlex. A plain QR solution of concentration equivalent to 5 μg/mL of QR was also prepared using a hydroalcoholic mixture. The UV spectra of plain QR solution and QR-LeciPlex were recorded from 220 to 600 nm. The blank LeciPlex was kept as a control while recording the UV spectrum of QR-LeciPlex to negate absorption of LeciPlex components. The wavelength of maximum absorption (λ_{max}) of QR was determined.

2.6.6. Fluorescence Spectroscopy. Fluorescence spectra of methanolic QR solution and QR-LeciPlex were recorded on a Varian Cary Eclipse fluorescence spectrophotometer with emission and excitation slit width of 10 nm. The excitation wavelength was 430 nm, and emission spectra were recorded from 440 to 650 nm. The concentration of the QR in the solution and LeciPlex was 10 μg/mL.

2.6.7. Cryo-Transmission Electron Microscopy (cryo-TEM) of QR-LeciPlex. Appropriately diluted QR-Leciplex dispersion (5 μL) was placed on a copper grid covered by a holey carbon film (Quantifoil R 1.2/1.3, pore size 1.2 μm, 400 mesh, Quantifoil Micro Tools, D-Jena). An excess of sample was blotted for 3 s between two strips of filter paper, and the grid was rapidly plunged into liquid ethane (cooled to about −180 °C with liquid nitrogen) in a cryo box (Carl Zeiss NTS GmbH, D-Oberkochen). After blotting excess ethane on the grid with filter paper, the frozen specimen was transferred with a cryo-transfer unit (Gatan 626-DH) into the precooled cryo-electron microscope (Cryo-TEM, Philips CM 120) operated at 120 kV and viewed under low dose conditions. The images were recorded with a 1k CCD camera (FastScan F114, TVIPS, Gauting, Germany).

2.6.8. Small Angle Neutron Scattering (SANS) Studies on QR-LeciPlex. Small angle neutron scattering (SANS) measurements were carried out to identify internal structure of the LeciPlex and the influence of QR loading on the internal structure of LeciPlex. Plain DDAB dispersion, blank phospholipid (Phospholipon 90 G) dispersion, blank LeciPlex (without QR), and QR-LeciPlex were analyzed by SANS. SANS measurements were carried out at room temperature using a SANS instrument at Dhruva reactor BARC, Mumbai, India. The protocol reported by Redkar et al. was used with slight modifications.[19] The scattering intensities were measured as a function of the scattering vector $\mathbf{Q}$ [$= (4\pi/\lambda)\sin(\theta/2)$, where λ is the neutron wavelength and θ is the scattering angle]. A rectangular quartz cell of 2 mm thickness was used for measurements. The mean wavelength of the incident neutron beam was 5.2 Å with a wavelength resolution of approximately 15%. The angular divergence of the incident beam was $\pm 0.5°$, and the beam size at sample position was 1.5 × 1.0 cm. The scattered neutrons are detected in an angular range of 1−15° using a linear He^3 position sensitive gas detector. The $\mathbf{Q}$ range of the diffractometer was 0.018−0.32 $Å^{-1}$. The data were corrected for background and empty cell contribution and placed on absolute scale using standard procedures. All samples were prepared in D_2O.

2.6.9. Evaluation of Ability of QR-LeciPlex To Protect QR from NaOH Mediated Degradation. The ability of QR-LeciPlex to protect QR from alkaline hydrolysis (NaOH mediated degradation) was evaluated in comparison to QR solution. Briefly, a calculated amount of QR was dissolved in methanol to yield solution (QR concentration 1 mg/mL). QR-LeciPlex was prepared as described earlier and was diluted with water to yield a QR concentration of 1 mg/mL. QR solution or QR-LeciPlex (1.5 mL) was transferred to a clean test tube, and 0.01 N NaOH (1.5 mL) was added to the test tube. The NaOH was allowed to react with QR solution or QR-LeciPlex for 1 min. Immediately after 1 min, 100 μL of reaction mixture from QR solution and QR-LeciPlex was diluted to 5 mL with distilled water. The absorbance of the QR solution and QR-LeciPlex was monitored at 374 nm for the period of 45 min. The absorbance of the samples at the beginning of the experiment was denoted as A_0, and absorbance at time t was denoted as A_t. The percent QR remaining in the sample was calculated by the following equation

$$\%\text{QR remaining} = (A_t/A_0) \times 100$$

The % QR remaining was plotted against time to show the difference in the degradation of QR in solution and LeciPlex.

2.6.10. In Vitro Release of QR from LeciPlex and Solution. *In vitro* release studies were performed to evaluate ability of LeciPlex to sustain release of the QR. The *in vitro* release of QR from QR-LeciPlex and QR solution was studied using the dialysis bag method as per the procedure reported by Li and co-workers with suitable modifications.[13] A mixture of 35% PEG 400 and 65% double-distilled water was employed as a dissolution medium to maintain sink conditions. The dialysis bags were soaked (MWCO 12 KD, HiMedia, Mumbai, India) in double-distilled water for 12 h prior to *in vitro* dissolution experiments. 1.5 mL of QR solution (solvent: Transcutol HP; QR conc.: 2.5 mg/mL) or QR-LeciPlex (QR conc.: 2.5 mg/mL) were introduced in the dialysis bag. Each dialysis bag was placed in a beaker containing 45 mL of dissolution medium. The beakers were placed in a shaker bath maintained at temperature of 37 °C and speed of 50 rpm. An aliquot of dissolution medium (10 mL) was withdrawn at each time interval and was replaced with same volume of fresh dissolution medium to maintain the sink conditions. The amount of QR released in the medium was analyzed by a validated UV spectrophotometric method described earlier. The amount of QR released was plotted as a function of time.

2.6.11. Stability of QR-LeciPlex. The stability of QR-LeciPlex was evaluated at 5 ± 3 °C. The QR-LeciPlex were subdivided into three 5 mL glass vials with rubber stoppers and aluminum-crimped tops. The vials were stored upright for a period of 1 month at 5 ± 3 °C. The parameters evaluated during the study were colloidal stability of LeciPlex, particle size, and chemical stability of QR in LeciPlex. The chemical stability of QR was evaluated by using a stability-indicating HPLC method described earlier. The samples were analyzed on day 0, day 15, and day 30 for QR content and particle size. All the experiments were carried out in triplicate. The data obtained at various time points about the QR content and particle size of LeciPlex were subjected to statistical analysis. The statistical significance of differences in the data was analyzed utilizing analysis of variance (ANOVA) followed by Bonferroni's test (GraphPad InStat demo version). Differences were considered statistically significant at $p < 0.05$.

2.7. Evaluation of Anti-inflammatory Activity. Anti-inflammatory activity of orally administered QR suspension and QR loaded LeciPlex was determined in rats using a carrageenan-induced paw edema model. Adult female Sprague−Dawley rats with a body mass of 200−250 g were obtained from Piramal Life Sciences Ltd. (formerly Nicholas Piramal Research Centre), Mumbai, India at the beginning of the experiments. Rats were maintained under an artificial 12 h light−dark cycle (lights on from 08:00 to 20:00 h) and at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available, and the animals were acclimatized for >7 days before use. Experiments were performed between 08:00 and 14:00 h. Animal care and handling throughout the experimental procedure were performed in accordance to the CPCSEA (Committee for the Purpose of the Control and Supervision on Experiments on Animals) guidelines. The experimental protocol was approved by the Animal Ethical Committee of Bombay College of Pharmacy. The overnight fasted animals were divided into three groups of six rats each as follows: group 1: control (no treatment); group 2: QR suspension in 0.2% sodium carboxy methyl cellulose; and group 3: QR-LeciPlex.

Group 2 and 3 were orally administered with QR suspension and QR-LeciPlex (both equivalent to 20 mg/kg of QR), respectively, whereas Group 1 received 0.2% (w/v) sodium sodium carboxy methyl cellulose solution.

After 30 min, rats of all three groups were challenged by a subcutaneous injection of 0.1 mL of a 1% (w/v) carrageenan solution, into the plantar site of the left hind paw. The paw volumes were measured using Ugo basile 7140 Plethysmometer, just before and after 1, 1.5, 2, and 3 h of carrageenan administration. The percent inhibition of edema at any time for each rat was calculated as

$$\%\text{inhibition} = 100[1 - (A - x)/(B - y)]$$

where A is the paw volume after the administration of carrageenan at time t, and x is the paw volume before the administration of carrageenan. B is the mean paw volume of control rats after administration of carrageenan at time t, and y is the mean paw volume of control rats before the administration of carrageenan.

The percent edema inhibition (or percent anti-inflammatory activity) observed with the QR suspension and QR-LeciPlex solution was compared using Student's two tailed paired t test (GraphPad InStat demo version). Differences were considered statistically significant at $p < 0.05$.

2.8. Evaluation of Antitumorigenic Activity. The ability of QR suspension and QR-LeciPlex to inhibit progression of tumor (antitumorigenic activity) was also evaluated to confirm the ability of the QR-LeciPLex to improve therapeutic efficacy of QR on oral administration. The method and protocol reported by Kale and co-workers was employed with suitable modifications.[12] C57BL/6 mice (female; weight range: 20−25 g) were received from Animal Breeding Centre of Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Tata Memorial Centre, at the beginning of the experiments. Mice were maintained under an artificial 12 h light−dark cycle (lights on from 08:00 to 20:00 h) and at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available, and animals were acclimatized for >7 days before use. Experiments were performed between 08:00 and 14:00 h. Animal care and handling throughout the experimental procedure were performed in accordance to the CPCSEA (Committee for the Purpose of the Control and Supervision on Experiments on Animals) guidelines. The experimental protocol was approved by the Animal Ethical Committee of ACTREC.

All mice each injected subcutaneously on the dorsal right flank with 10^6 B16F10 melanoma cells in sterile PBS, and this day was considered day zero. The mice were divided into three groups of four mice each as follows: group 1: control (no treatment); group 2: QR suspension in 0.2% sodium carboxy methyl cellulose; and group 3: QR-LeciPlex.

Group 2 and 3 were orally administered with QR suspension and QR-LeciPlex (both equivalent to 50 mg/kg of QR), respectively, on days 3, 5, 7, 9, 11, 13, and 15, whereas Group 1 received 0.2% (w/v) sodium sodium carboxy methyl cellulose solution throughout the study.

Tumor diameters were measured every alternate day with a slide calliper and tumor volume calculated using the formula:

$$\text{tumor volume } (V) = 0.4ab^2$$

where a is the length and b is the breadth of the tumor implant.

The tumor volumes obtained for various groups were statistically evaluated by using one way ANOVA followed by Dunnet's test. The differences in tumor volume were considered statistically significant at $p < 0.05$.

2.9. Freeze-Drying of the QR-LeciPlex. The feasibility of freeze-drying was evaluated using mannitol and trehalose as

cryoprotectants. These cryoprotectants are commonly used in the pharmaceutical research. Briefly, QR-LeciPlex (5 mL) was prepared as per the method described earlier. The concentration of the QR in LeciPlex was 2.5 mg/mL. A suitable amount of mannitol or trehalose was dissolved in QR-LeciPlex to yield a cryoprotectant concentration of 20% (w/v). The QR-LeciPlex containing cryoprotectant (5 mL) was transferred to 10 mL glass vials, and the systems were freeze-dried using an automated system (AdVantage, VirTis, USA) that was previously optimized for the system. In brief, the conditions were as follows: condenser temperature of −60 °C and pressure applied during each step was 200 Torr. After freeze-drying, all the glass vials containing solid QR-LeciPlex were stored over anhydrous calcium chloride in a desiccator. The QR content in the freeze-dried QR-LeciPlex was evaluated by the HPLC method. The solid QR-LeciPlex was reconstituted with double-distilled water, and the particle size of the QR-LeciPlex was evaluated using PCS.

3. RESULTS

3.1. Fabrication of QR Loaded Phospholipid Based Cationic Nanocarriers (QR-LeciPlex). QR-LeciPlex could be easily fabricated in a single step by using biocompatible solvent Transcutol HP. It was observed that 25 mg of QR could be easily incorporated in LeciPlex (10 mL) without any signs of drug precipitation during or after fabrication. The particle size of QR-LeciPlex was 403.1 ± 12.1 nm, whereas the polydispersity index was 0.54. The QR-LeciPlex had excellent colloidal stability and did not show any increase in the particle size after 24 h of storage.

3.2. Evaluation of QR-LeciPlex. *3.2.1. Determination of Encapsulation Efficiency and Zeta Potential and QR Content.* The encapsulation efficiency of the QR-LeciPlex was 91.3 ± 1.9% indicating excellent association of QR with the LeciPlex. The zeta potential of the QR-LeciPlex was +31.1 ± 1.6 mV. This indicates that QR-LeciPlex would have good colloidal stability on storage. The positive charge of the QR-LeciPlex is due to the presence of cationic agent DDAB. HPLC analysis indicated that the content of QR in LeciPlex was 93.3 ± 1.9%. This indicates that QR did not undergo any significant degradation during the fabrication process.

3.2.2. Evaluation of Functional Stability of QR-LeciPlex (DPPH Assay). QR is known to have an excellent antioxidant property and is shown to have ability to scavenge free radicals (like DPPH).[1,4−6,18] The antioxidant activity of QR has major role in the therapeutic activity of QR in various ailments.[1,4−6] Hence, it was important to evaluate functional stability of the QR in LeciPlex to confirm that the method of preparation does not affect the inherent antioxidant capability of the QR. The results of *in vitro* antioxidant activity are shown in Table 1. It is evident from Table 1 that *in vitro* antioxidant activity of QR-LeciPlex (>90%) was significantly higher ($p < 0.01$) than that of QR solution (<50%). It is also evident from Table 1 that the blank LeciPlex also showed *in vitro* antioxidant activity which is a reason for very high antioxidant activity of QR-LeciPlex. It has been reported that soy lecithin has antioxidant potential *in vitro* and QR has been shown to act in synergy with soy lecithin.[20] In view of this, it can be assumed that the functional (*in vitro*) stability of QR in LeciPlex would be not only be retained but might also be enhanced due to presence of soy lecithin. However, the advantage of antioxidant potential of soy lecithin may not be available for nanocarriers (LeciPlex) delivered orally as the enzymes in the gastrointestinal tract would diminish inherent activity of lecithin. It is noteworthy that the QR-LeciPlex showed very high *in vitro* antioxidant activity despite the fact that QR is encapsulated in LeciPlex. This could be due to the fact that the methanol present in the DPPH solution can perturb LeciPlex structure.

Table 1. *In Vitro* Antioxidant Activity of Various Samples (n = 3; Data Expressed as Mean ± SD)

sample	% antioxidant activity
QR solution	55.31 ± 6.1
blank LeciPlex	33.14 ± 4.2
QR-LeciPlex	91.1 ± 6.2[a]

[a] ($p < 0.01$) as compared to QR solution; % antioxidant activity was calculated by measuring DDPH scavenging potential of the samples; QR solution was prepared in Transcutol HP.

3.2.3. Spectral Characterization of QR-LeciPlex. The spectral characterization was carried out to study the interaction/association of QR with LeciPlex components. The UV spectrum of QR solution and QR-LeciPlex in water was recorded (Figure 1). The concentration of QR was same in both the cases, and the blank LeciPlex was kept as a control while recording the spectrum of QR-LeciPlex. The plain QR solution showed absorption maxima at 374 nm which is in accordance with the literature values.[21] The absorption of QR in this region is due to the presence of cinnamyl system in the QR molecule. Interestingly, QR present in the QR-LeciPlex showed absorption maxima at 391 nm indicating a bathochromic shift. This indicates that the QR interacts with LeciPlex and it may be present in different forms.

Leung et al. reported that natural polyphenols such as curcumin can exist in an anionic state in the solution in the presence of cationic surfactants such as CTAB and can show a bathochromic shift.[22] A similar possibility was thought to occur in case of QR-LeciPlex initially. Hence, to understand this phenomenon, the pH of the QR-LeciPlex was measured. It was observed that the QR-LeciPlex has pH of 4.3. It is known that QR has two pK_a values, namely, 7.3 and 8.4.[21,23] Thus, at pH 4.3, QR would exist in un-ionized form. Hence, bathochromic shift observed in the QR-LeciPlex is unlikely to be due to the presence of anionic species of QR. It has been reported in the literature that QR shows a bathochromic shift in the presence of Triton X-100 micelles. The authors concluded that the bathochromic shift of QR observed in TritonX-100 micelles is due to hydrophobic interaction of QR with core of Triton X-100.[21] Hence, it can be anticipated that the bathochromic shift of QR observed in LeciPLex could be due to hydrophobic interaction of QR with the soybean lecithin present in the LeciPlex. Further investigations are required to understand the exact location and mode of interaction of QR in LeciPlex.

3.2.4. Fluorescence Spectroscopy. It has been reported that QR has intrinsic fluorescent properties.[24] It has been observed that association of flavonoids such as QR and curcumin with micelles or lipid nanovehicles can significantly alter their fluorescence behavior.[21,25] In view of this, we evaluated the fluorescence of QR in solution and in LeciPlex. The results of the study are shown in Figure 2. It is clearly evident from Figure 2 that the QR in solution shows maximum emission at 488.05 nm (fluorescence intensity: 347.13), whereas QR in LeciPlex shows a maximum emission at 528.95 (fluorescence intensity: 368.38) indicating a bathochromic shift and a small enhancement in the fluorescence. This indicates that the QR interacts with LeciPlex components. Liu et al. and Khumsupan et al. have clearly demonstrated similar

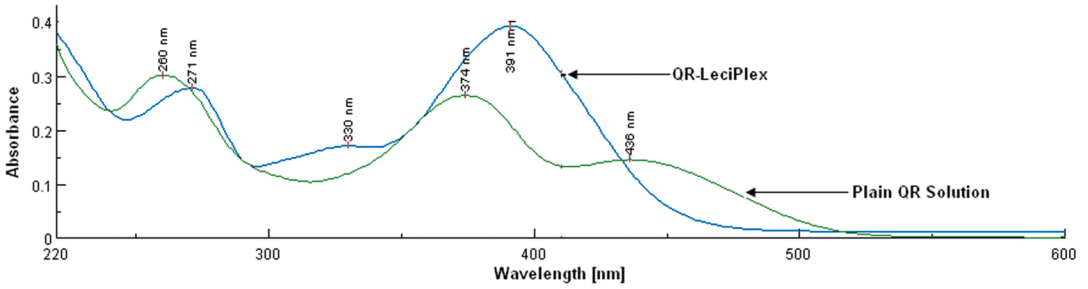

Figure 1. Absorption spectra of QR hydroalcoholic solution and QR-LeciPlex (QR concentration: 5 μg/mL).

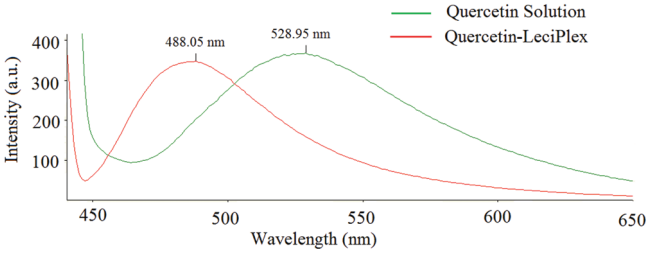

Figure 2. Fluorescence spectra of QR methanolic solution and QR-LeciPlex (QR concentration: 10 μg/mL).

results (bathochromic shift) in the emission spectrum of micelle associated QR and lipid nanovehicle associated curcumin, respectively,[21,25] and our results are in accordance with their observations. It should also be noted that the bathochromic shift observed in the case of QR-LeciPlex with respect to QR solution could also be in part due to difference in the dielectric constants of the vehicles used for obtaining the QR solution (methanol) and QR-LeciPlex (water). Because of extreme water insolubility of QR in water, it was not possible to prepare QR solution of 10 μg/mL concentration. Khumsupan et al. observed that the increase in the dielectric constant (or polarity) of the solvent resulted in the bathochromic shift in the emission spectrum of curcumin with concomitant decrease in the fluorescence intensity of curcumin (fluorescence quenching).[25] Furthermore, our preliminary experiments showed a significant fluorescence quenching of QR in the case of hydroalcoholic solution of QR as well as bathochromic shift (data not shown) which is in accordance with the reports by Liu et al. and Sengupta et al.[26,27] Hence, QR methanolic solution was used (dielectric constant of methanol: 33) for the study. On the contrary, QR-LecipPlex was fabricated in and diluted with the water (dielectric constant: 80) to obtain a QR concentration of 10 μg/mL.

It has been demonstrated by Liu et al. and Sengupta et al. that QR has a very weak fluorescence in aqueous solution and hydroalcoholic solutions due to strong interamolecular hydrogen binding of the polar groups of QR and water.[26,27] On the contrary, QR-LeciPlex (prepared and diluted with water) showed greater fluorescence as compared to that of QR methanolic solution. This clearly indicates that QR is associated with the (hydrophobic) components of the LeciPlex and the hydrophobic environment around QR (or encapsulation of QR in the LeciPlex) prevents the intermolecular hydrogen bonding of QR with the water resulting in preservation of the fluorescence of QR. This study clearly indicates the interaction (and encapsulation) of QR in the LeciPlex.

3.2.5. Cryo Transmission Electron Microscopy (TEM) of QR-LeciPlex. It is evident from Figure 3 that QR-LeciPlex have a

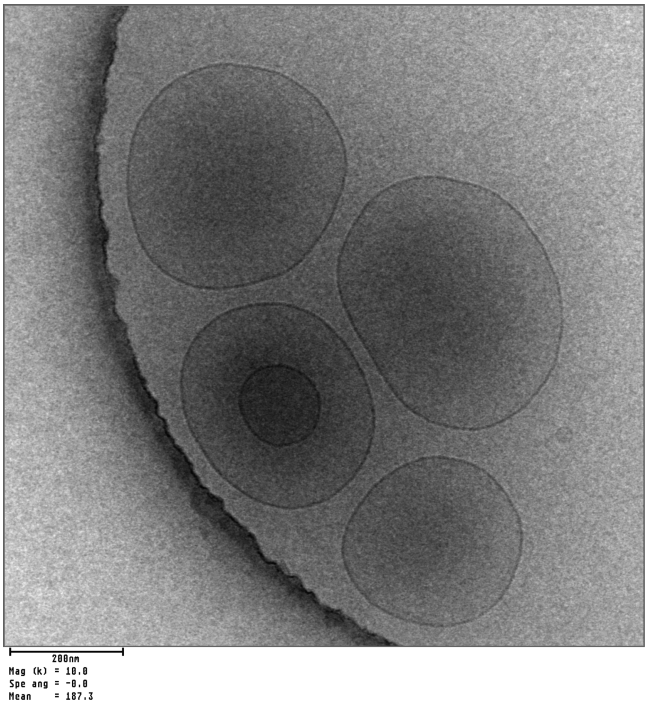

Figure 3. Cryo transmission electron microscopy of QR-LeciPlex.

unilamellar structure and spherical shape. The particle size of the QR-LeciPlex obtained with TEM was in accordance with that obtained with photon correlation spectroscopy.

3.2.6. Small Angle Neutron Scattering (SANS) Studies on QR-LeciPlex. The SANS study was carried out to confirm the unilamellar structure of blank LeciPlex and the effect of the QR incorporation in LeciPlex. The SANS patterns for individual LeciPlex components, that is, DDAB and phospholipid (soybean lecithin), were also evaluated. The concentration of the individual components was same as that present in LeciPlex. It is evident from Figure 4 that the SANS patterns of individual components of LeciPlex, that is, DDAB solution and blank phospholipid dispersion, are considerably different from the SANS pattern of LeciPlex. Blank phospholipid dispersion showed a Bragg's peak, which is a characteristic of multilamellar vesicles (MLV).[19] Similar peaks have been observed with palmitoyl oleoyl phosphatidyl choline (POPC) multilamellar vesicles.[28] The SANS pattern of DDAB solution indicates the presence of micelles and vesicles. It is well-known that DDAB forms vesicles in solution at a concentration greater than 0.15% (w/v). The SANS pattern of DDAB solution obtained in the

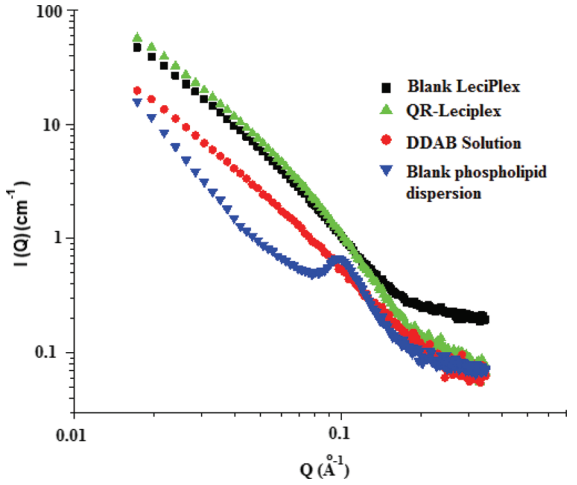

Figure 4. Small angle neutron scattering pattern for didodecyldimethylammonuim bromide (DDAB) solution, blank phospholipid (Phospholipon 90 G: soybean lecithin) dispersion, blank LeciPlex, and QR-LeciPlex.

present investigation is in line with the report by Grillo and coworkers.[29] The SANS pattern of LeciPlex was quite different from the individual components. The SANS data of LeciPlex showed a monotonic decay with a slope of −2, implying the existence of unilamellar vesicles (ULV) as explained by Yue et al.[30] This observation clearly showed interaction between DDAB and phospholipid in the LeciPlex. Furthermore, no Bragg's peak was seen in the LeciPlex, indicating absence of multilamellar structures. It is evident from cryo-TEM (Figure 3) that LeciPlex have a unilamellar structure. To further validate the internal structure of DDAB-LeciPlex, we fitted the SANS data into a unilamellar vesicle model as described by the Aswal et al.[31] The SANS data obtained with DDAB-LeciPlex was fitted into unilamellar vesicle model (Figure S1, Supporting Information), and the calculations indicated that the DDAB-LeciPlex have lamellar spacing of 2.56 nm. Thus, the SANS analysis validated the results of cryo-TEM studies.

The SANS pattern of the blank LeciPlex (without QR) and QR-LeciPlex was recorded to understand the interaction between QR and LeciPlex (Figure 4). Redkar et al. observed that the incorporation of drug in vesicular structures can result in the increase in the interlamellar distance which would reflect in the SANS pattern.[19] It is evident from Figure 4 that the SANS pattern of blank LeciPLex and QR-LeciPlex are almost overlapping. Interestingly, we did not observe any increase in the lamellar distance of blank LeciPlex and QR-LeciPlex (Figure S1 and S2, Supporting Information). This suggests that QR is not located at the lamella of the LeciPlex. We believe that QR could be dissolved/dispersed throughout the LeciPlex matrix. However, further studies are required to investigate the exact mode of interaction and location of QR in LeciPlex. Our mathematical analysis of SANS data showed that QR-LeciPlex also have a unilamellar structure. This study clearly indicates absence of internal structure alterations in the LeciPLex on encapsulation of QR.

3.2.6. Evaluation of Ability to QR-LeciPlex to Protect QR from NaOH Mediated Degradation. It has been reported that QR undergoes rapid hydrolysis in alkaline medium.[32,33] Our preliminary studies were in accordance with the reports. We observed that QR undergoes extremely rapid hydrolysis in 0.01 N NaOH and that the major degradation products of QR

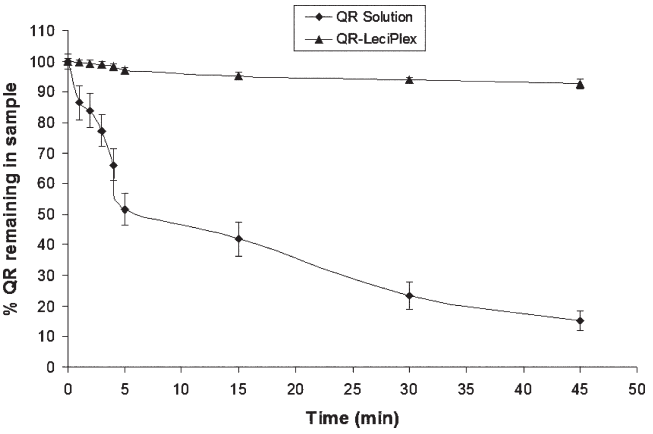

Figure 5. NaOH mediated degradation of QR solution and QR-LeciPlex (n = 3; data expressed as mean ± SD).

(protocatechuic acid, phloroglucinol, and 2,4,6-trihydroxybenzoic acid) do not absorb at 374 nm (λ_{max} of QR) (data not shown). We anticipated that QR-LeciPlex would be able to reduce/prevent alkaline hydrolysis of QR as QR is encapsulated in the LeciPlex. To validate this hypothesis, we monitored alkaline hydrolysis of QR in solution and in the LeciPlex at 374 nm. The results of the study are shown in Figure 5. It is evident from Figure 5 that QR was significantly hydrolyzed in solution at the end of 45 min (% QR remaining was ∼15%), whereas QR in LeciPlex underwent very less degradation at the end of 45 min (% QR remaining was ∼92%). This clearly indicates the ability of LeciPlex to improve stability of QR to alkaline hydrolysis. This study also proves significant encapsulation of QR in an indirect manner.

3.2.7. In Vitro Release of QR from LeciPlex and Solution. The *in vitro* release studies were carried out to evaluate the potential of LeciPlex to sustain the release of QR. The dialysis bag method is widely used for evaluating *in vitro* release of drug from nanocarriers. The QR has extremely low solubility in water, pH 1.2 buffer, and even pH 7.4 buffer (<30 μg/mL in all media). Hence, to maintain the sink condition, we used 35% PEG 400 solution as a dissolution medium. The *in vitro* release of QR from solution and LeciPlex is shown in Figure 6. It is evident from the figure that release of QR from the LeciPlex was significantly lower than that of QR solution. QR solution released more than 50% QR in first 2 h, whereas LeciPlex released less than 10% of QR at the end of 2 h. At the end of 24 h, QR solution released more than 90% of the QR whereas QR-LeciPlex released less than 25% of the QR at the end of 24 h. The sustained release observed with LeciPlex is due to encapsulation of QR in the LeciPlex. The sustained release properties of LeciPlex could be useful in maintaining therapeutic concentration of QR for a longer duration *in vivo*.

3.2.8. Stability of QR-LeciPlex. The short-term stability of QR-LeciPlex was evaluated at 5 ± 3 °C for the period of one month. The refrigeration conditions were chosen for the stability study of QR-LeciPlex as refrigeration is recommended as the storage condition for the phospholipids. The results of the stability study are shown in Table 2. It was observed that the QR content in the LeciPlex did not show any significant change at the end of the 1 month when stored at refrigerated conditions. The QR-LeciPlex maintained colloidal stability at the end of 1 month, and there was no significant change in the particle size and the polydispersity index at the end of 1 month.

3.3. Evaluation of Anti-inflammatory Activity. Rotelli et al. have demonstrated anti-inflammatory activity of QR and other

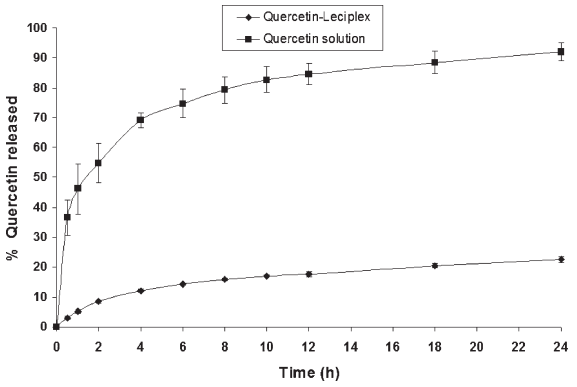

Figure 6. *In vitro* release profile of QR solution and QR-LeciPlex through dialysis membranes (n = 3; data expressed as mean ± SD).

Table 2. Physical and Chemical Stability of QR-LeciPlex Stored at 5 ± 3 °C (n = 3; Data Expressed as Mean ± SD)[a]

day	particle size (nm)	polydispersity index	% QR content
0	403.2 ± 11.2	0.53	95.1 ± 3.3
15	410.8 ± 9.1	0.61	93.2 ± 3.1
30	408.4 ± 10.3	0.55	92.8 ± 2.6

[a] % QR content was determined by a stability indicating HPLC method developed in-house.

flavonoids in the various models of inflammation including carageenan induced rat paw edema.[4] Hence, anti-inflammatory activity of QR-LeciPlex was evaluated for generating a preliminary proof-of-concept for the superiority of QR-LeciPlex over QR suspension. The results of anti-inflammatory study are shown in Figure 7. It was observed that QR-LeciPlex have significantly higher anti-inflammatory activity ($p < 0.01$) as compared to QR suspension at the end of 1 h. However, the anti-inflammatory activity of the QR-LeciPlex was not significantly higher than QR suspension after 1 h. It is also evident that the anti-inflammatory activity of QR-LeciPlex was highest at 1 h, whereas in the case of QR suspension, maximum activity was seen at 2 h. This indicates that LeciPlex may increase the absorption rate of QR and result in a quick onset of action. It is true that the dose of QR employed for the anti-inflammatory study proved to be lesser as the highest value of percent anti-inflammatory activity was not more than 40%. Due to constraints on the maximum volume of sample that can be administered to rats, it was not possible to administer higher dose of QR (greater than 20 mg/kg) to rats in case of QR-LeciPlex. However, the study provided preliminary proof of concept about the potential of QR-LeciPlex.

3.4. Evaluation of Antitumorigenic Activity. QR has also demonstrated good potential in the prophylaxis and treatment of various types of cancer. Hence, QR-LeciPlex could have good potential in the prevention, progression, and treatment of cancer. The utility of QR for the treatment of B16F10 melanoma cells has been demonstrated by *in vitro* and *in vivo* studies.[9,34] Kale et al. have demonstrated the superiority of QR-sulfobutylether-β-cyclodextrin complex in inhibiting growth B16F10 melanoma as compared to the QR suspension on oral administration.[12] Hence, in the present investigation, a similar study was designed to evaluate the efficacy of QR-LeciPlex. It is evident from the Figure 8 that the QR-LeciPlex group showed significantly lower tumor volumes ($p < 0.01$) as compared to that of the untreated

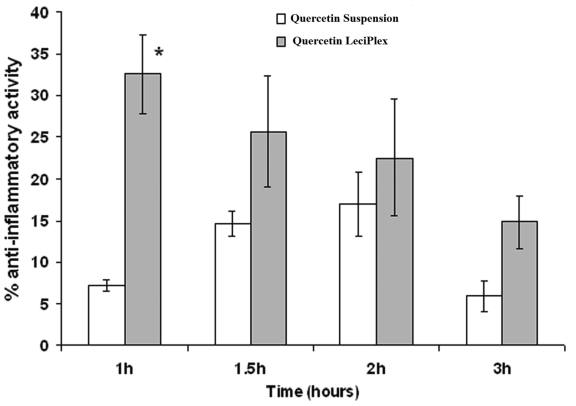

Figure 7. Anti-inflammatory activity of QR suspension and QR-LeciPlex in rats (n = 6; data expressed as mean ± SD); dose of QR: 20 mg/kg by the oral route (* = $p < 0.01$).

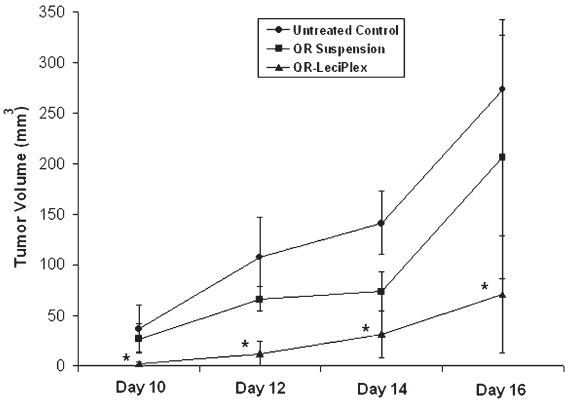

Figure 8. Antitumorigenic activity of QR suspension and QR-LeciPlex in C57BL/6 mice (n = 4; data expressed as mean ± SD); * = $p < 0.01$ as compared to QR suspension. Mice were subcutaneously injected with B16F10 melanoma cell lines on day 0 and QR suspension and QR Leciplex (both equivalent to 50 mg/kg of QR) were administered orally to mice on days 3, 5, 7, 9, 11, 13, and 15.

control, whereas QR suspension showed significantly lesser tumor volumes only on days 12 and 14. QR-LeciPlex showed significantly higher antitumorigenic activity at all time points as compared to that of untreated control and QR suspension. This clearly demonstrates the potential of QR-LeciPlex in improving the therapeutic efficacy of QR on oral administration.

3.5. Freeze-Drying of the QR-LeciPlex. Solid dosage forms have an edge over liquid dosage forms due to advantages such as ease of handling, greater patient compliance, and smaller chances of solution state mediated degradation. Hence, studies were undertaken to study the conversion of QR-LeciPlex to solid powder by means of freeze-drying. The process of freeze-drying has been employed for converting nanocarriers such as liposomes to the solid dosage forms.[35] In the present investigation, mannitol and trehalose were used as cryoprotectants. The cryoprotectants were employed at the concentration of 20% (w/v). The characteristic of the powders obtained after freeze-drying were considerably different for trehalose and mannitol. The trehalose containing QR-LeciPlex showed better powder characteristics with respect to flow whereas mannitol containing QR-LeciPlex yielded firm solid plug. However, the reconstitution of the QR-LeciPLex was relatively easier in case of

both the cryoprotectants. The particle size of the reconstituted QR-LeciPlex containing trehalose and mannitol was 545.3 ± 21.3 nm and 581.3 ± 19.6 nm, respectively. The polydispersity index was 0.71 and 0.79, respectively. There was increase in the particle size of QR-LeciPlex indicating the need for further optimization of the various parameters involved in freeze-drying process.

4. DISCUSSION

Phospholipids or lecithins are components of cell membranes[36] and are being used in pharmaceutical research since decades due to their excellent biocompatibility, ability to interact with array of drugs (hydrophilic, amphiphilic, and hydrophobic), and acceptability for all major routes of administration such as parenteral, oral, and dermal. The ability of the phospholipid based nanocarriers such as liposomes, microemulsions, and solid lipid nanoparticles to improve oral bioavailability has been well-established in the literature. Cationic surfactant and/or lipids and cationic nanocarriers have gained importance in drug delivery since the last decade. It has been demonstrated that positively charged submicronic emulsions containing lipids such as olelylamine and sterylamine significantly improve Caco-2 cell permeability of the various hydrophobic drugs as compared to negatively charged submicronic emulsions.[37−39] It has recently been demonstrated that solid lipid nanoparticles containing a cationic surfactant cetyltrimethylammoniumm bromide (CTAB) improved oral bioavailability of 5-fluorouracil prodrug.[40] Ravi Kumar and co-workers have demonstrated that positively charged nanoparticles have greater uptake and transport across gastrointestinal tract as compared to drug and negatively charged nanoparticles.[41] Thus, cationic surfactants and/or nanocarriers are expected to have advantages in oral drug delivery.

Until now, there are no reports in the literature establishing the fabrication of nanocarriers based on phospholipids and cationic surfactants and their potential application in oral drug delivery. In view of this, we have recently reported single step fabrication of novel self-assembled phospholipid-based cationic nanocarriers (LeciPlex) with the use of biocompatible solvents like diethyleneglycol monoethylether (Transcutol HP).[17] The LeciPlex are composed of soybean lecithin, DDAB (dimethyldidodecylammonium bromide; a cationic surfactant), and Transcutol HP. The soybean lecithin and Transcutol HP have very good acceptability for oral and dermal delivery.[17,42−44] Cationic surfactants have limited utility in the oral drug delivery as they can result in toxicity, ulceration, and damage to gastrointestinal mucosa. However, these effects are dependent on structure as well as concentration. Recently, Kumar and co-workers have also established the tolerability of DDAB by Caco-2 cells. It is well-known that Caco-2 cells are widely used for studying oral delivery of drugs as well as delivery systems *in vitro*.[45] Furthermore, we have demonstrated that the toxic effects of cationic surfactants (to microbes) are greatly diminished after association with soy lecithin in LeciPlex.[17] Additionally, investigation reported by Cui et al. demonstrated that association of a cationic surfactant CTAB with lecithin greatly diminished the cytotoxicity of CTAB to TC-1 cells.[46] Hence, we believed that LeciPlex could have application in oral drug delivery. The major advantages associated with LeciPlex as compared to the other nanocarriers such as solid lipid nanoparticles, liposomes, and polymeric nanoparticles are (1) simplicity of fabrication and ease of scale up, (2) no need for solvent removal process due to use of biocompatible solvent (Transcutol HP), for the fabrication unlike polymeric nanoparticles and liposomes which require organic solvents such as acetone and chloroform for fabrication which need to be removed from the system, and (3) the ability to encapsulate an array of hydrophobic drugs with different log *P* values.[17]

QR is a dietary phytochemical and has shown a multitude of beneficial effects in the treatment of various ailments including cancer.[1,4−6] However, the clinical utility of QR is limited due to its poor aqueous solubility, poor gastrointestinal permeability,[47] and poor metabolic stability due to its polyphenolic nature.[48] To improve the therapeutic efficacy of the QR, a delivery strategy that would improve the permeability of QR with concomitant reduction in the metabolism is highly desirable. Until now, very few reports have focused on improving therapeutic efficacy of QR on oral administration. Although the potential of novel delivery systems such as microemulsions[11,49] and solid lipid nanoparticles[13] has been evaluated for improving oral delivery of QR, none of the reported investigations focus on utility of QR nanocarriers for the cancer chemoprevention and/or treatment. Furthermore, microemulsion and solid lipid nanoparticles could not achieve incorporation of QR in higher amounts (<0.05% w/v QR content in the final formulation). The low log *P* value of QR (∼1.8)[50] is responsible for poor solubility in oily vehicles and solid lipids (which are typically used for the fabrication of microemulsions and solid lipid nanoparticles) which results in lower QR incorporation. It is important to design a novel delivery strategy with ability to yield higher incorporation of QR in the formulation as it can reduce the total dose of the QR and intake of the formulation components.

In the present investigation, we could obtain considerably high (5-fold greater) QR incorporation in LeciPlex (0.25% w/v QR content in the final formulation) as compared to the reported nanocarriers. This clearly indicates the benefit of LeciPlex over the other nanocarriers. The association of QR with LeciPlex was confirmed by spectral characterization, and the chemical stability of QR in LeciPlex was established for 1 month.

Anti-inflammatory studies indicated that LeciPlex can improve the therapeutic efficacy of QR on oral administration as compared to that of the QR suspension. We also evaluated antitumorigenic potential of QR-LeciPlex to validate the results of the anti-inflammatory study. Although the study design for the antitumorigenic activity was similar to that reported by Kale et al., the results obtained with QR suspension were dramatically different than that reported by Kale and co-workers.[12] The difference in this observation could be due to the difference in the species of mice. QR-LeciPlex showed significantly higher antitumorigenic activity ($p < 0.01$) as compared to QR suspension and control at all the time points. This clearly indicated that LeciPlex can improve the therapeutic efficacy of QR on oral delivery.

The higher therapeutic efficacy of QR observed with LeciPlex could be a result of several attributes. Cationic nanocarriers and phospholipids are known to augment the permeability and uptake of the drug associated with them.[14,16,37−40] Furthermore, cationic nanocarriers have greater bioadhesive properties due to electrostatic interaction with gastrointestinal mucosa[14,16] which would result in sustained *in vivo* delivery and eventually a greater therapeutic effect. It can be assumed that LeciPlex are capable of protecting the entrapped drug (QR) from chemical and enzymatic degradation and/or presystemic metabolism of drug in the gastrointestinal tract which would result in greater bioavailability of the encapsulated drug. The significantly higher protection of QR in LeciPlex from NaOH mediated degradation as compared

to QR solution corroborates the aforementioned statement. It is well-known that lipid nanocarriers in the size range of 20−500 nm are preferentially taken up from lymphoid tissues associated with GIT which would circumvent the first pass metabolism of the entrapped drug.[17] It has also been reported that phospholipids can enhance the lymphatic transport of the coadministered triacylglycerols on enteral administration.[51] QR-LeciPlex are composed of soybean lecithin and have a particle size ~400 nm. Hence, enhanced lymphatic transport of QR encapsulated in LeciPlex could also be one of the reasons for greater therapeutic efficacy on oral administration.

The solid dosage forms have ease of handling and offer greater stability of drug and high degree of patient compliance. The success of QR-LeciPlex in oral delivery would be dependent on its amenability to offer avenues for development of solid dosage form. We have established that QR-LeciPlex could be converted to a solid powder with the help of freeze-drying. The sugars (cryoprotectants) employed in the freeze-drying would offer palatability to the oral formulation, and it may be possible to package QR-LeciPlex in a sachet for oral delivery. We are currently optimizing the freeze-drying conditions to obtain free-flowing powder with minimal impact on the particle size of QR-LeciPlex.

5. CONCLUSION

The ability of novel phospholipid based cationic nanocarriers (LeciPlex) to improve therapeutic efficacy of QR was successfully established. The LeciPlex have great potential in improving oral delivery of hydrophobic drugs including those with poor solubility in oils and solid lipids.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Figures S1 and S2: fittings of SANS data of blank LeciPlex and QR-LeciPlex in a unilamellar vesicle model. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Department of Pharmaceutics, Bombay College of Pharmacy, Kalina, Santacruz (East), Mumbai-400098, India. Tel.: 91-22-26670871. Fax: 91-22-26670816. E-mail address: mangal_nag511@yahoo.co.in; mangal@bcp.edu.in.

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■ ADDITIONAL NOTE

† Indian Patent Application No. 2167/MUM/2008.

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