Preparation, characterisation and biological applications of Rutin loaded liquid crystalline nanoparticles in targeting airway diseases

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INTRODUCTION

Airway diseases including lung cancer, chronic obstructive pulmonary disease (COPD) and asthma affect millions of people all over the world. Cigarette smoke results in 90% of lung cancer deaths [1]. About 1.04 million cases of lung cancer are recorded each year worldwide, with the highest prevalence observed in North America and Europe. Apart from smoking, environmental pollution, rapid development and urbanization are the major causes of lung cancer worldwide [2]. These factors results in lung inflammation, wherein matrix metalloproteinase interact with growth factors, cytokines and cell adhesion factors leading to tumour progression and metastasis. Conventional treatment methodologies are not sufficient to cure or prevent the disease. With the advent of nanotechnology, drug delivery to specific target site is still challenging, but targeted delivery can be achieved by physiochemical properties of the nanoparticles [3,4].

Rutin is a natural flavonoid found in medicinal plants, food and other products. Due to its high antioxidant potential, it can be a potent therapeutic for inflammatory diseases including airways diseases. However, rutin is poorly soluble in water and low oral bioavailability limits it as a therapeutic agent [5]. But with the advancement of nanotechnology, the development of nanoparticles have improved the pharmacokinetics and bioavailability of drug to the target site compared to traditional drug delivery. However, there is an urgent need for translational studies to validate for clinical administration in the pulmonary clinic.

AIM

The main aim of the research was to formulate rutin loaded LCNs followed by characterisation and evaluation of *in vitro* release. The effect of rutin loaded LCNs has been evaluated for adenocarcinomic human lung alveolar basal epithelial cells(A549) respectively.

METHODS

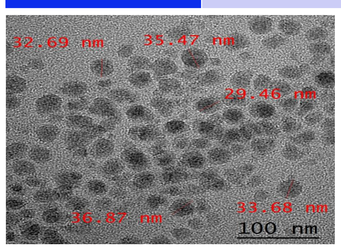
The rutin LCNs was prepared by ultra-sonication method and Zetasizer Nano ZS was used to measure particle size, polydispersity index (PDI) and zeta potential. The entrapment efficiency was determined by UV-spectrophotometer.

The anticancer activity was performed using A549 cell line and was analysed for proliferation assay using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypan blue staining. Migration of the cells was studied by wound healing assay and Boyden's/transwell chamber assay.

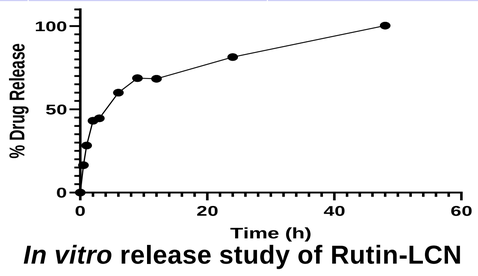
RESULTS

1. Characterization parameter of the Rutin-LCNs

	Z- average	Polydispersity index (PDI)	Zeta potential	Entrapment efficiency (EE) %
Rutin LCNs	160.46±2 nm	0.229± 0.02 nm	28.6±0.2 nm	68.9±0.37

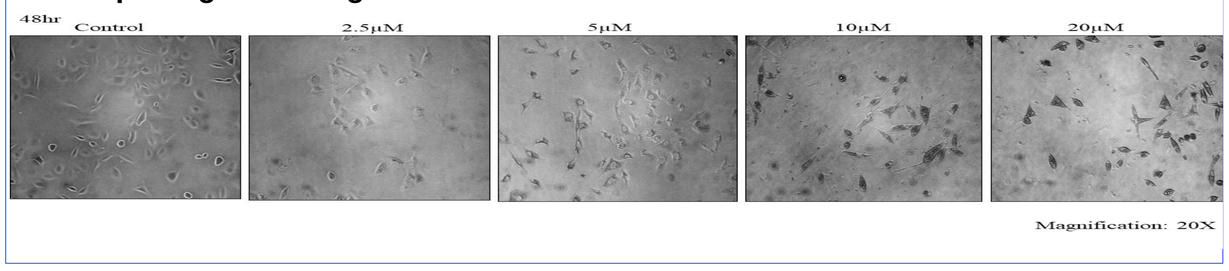


TEM image of Rutin-LCN with scale bar of 100 nm

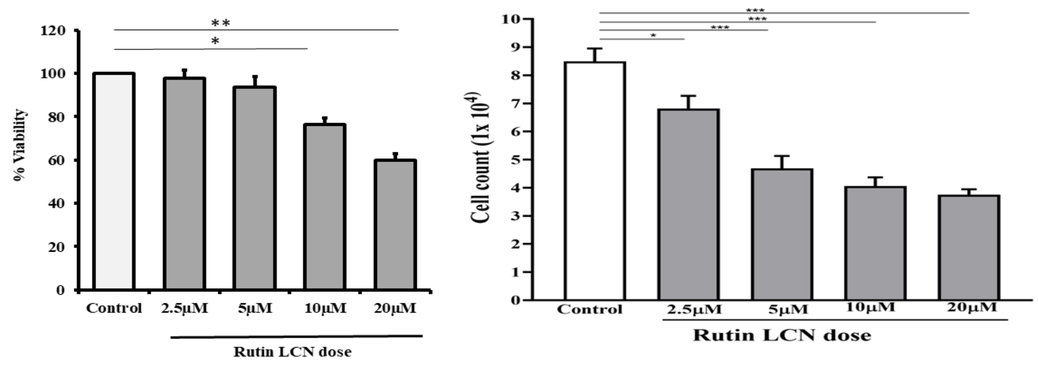


In vitro release study of Rutin-LCN

2. Morphological changes in A549 cell line after treatment of Rutin-LCNs



3. Effect of Rutin-LCNs on A549 proliferation



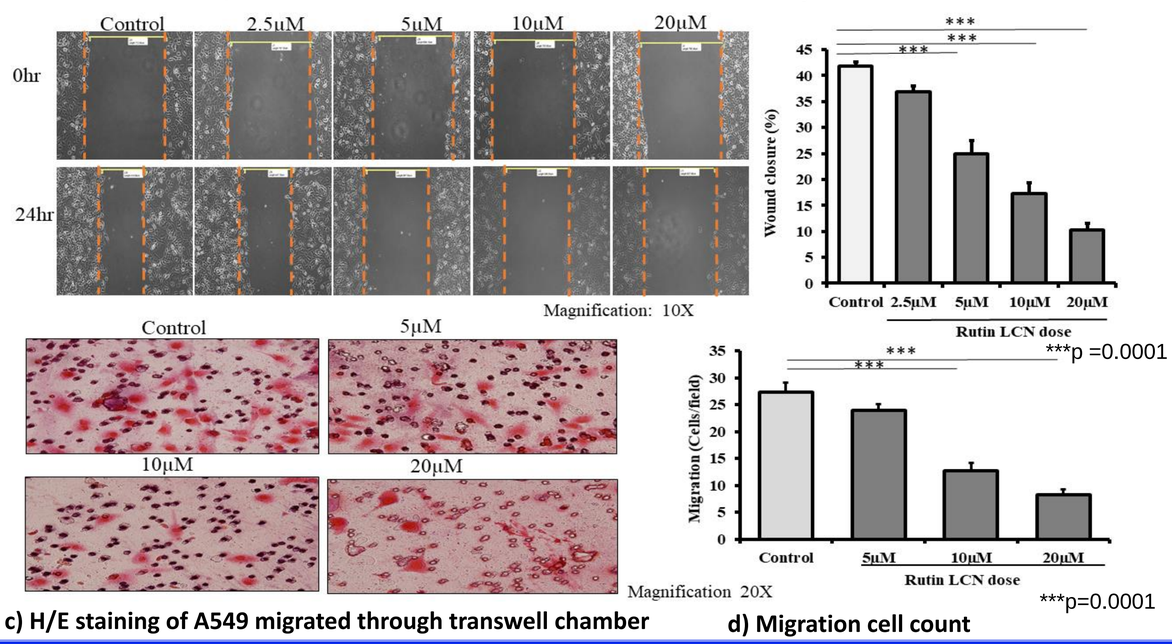
a) MTT assay **p=0.0001 *p=0.0002

b) Trypan blue staining ***p<0.0001 *p=0.01

4. Rutin-LCNs inhibits A549 migration

a) Wound healing assay

b) Wound closure



***p =0.0001

c) H/E staining of A549 migrated through transwell chamber

d) Migration cell count ***p=0.0001

DISCUSSION

In the current study, we made an effort to fabricate the rutin-LCNs and explore their efficacy *in vitro* in lung cancer cell line A549. The size of the Rutin-LCNs indicated the encapsulation drug into the nanoparticle. Drug encapsulation can be attributed to physical entrapment or chemical conjugation. Polydispersity index (PDI) represents the size distribution of and uniformity of the rutin encapsulated LCNs. The positive reading of zeta potential indicates that there is stable dispersion between particles which reduces the chances of aggregation. It is assumed that the hydrophobic part of the polypropylene oxide chain adheres to the surface of the nanoparticles whereas the hydrophilic portion of the chain extends out to the surrounding environment to provide steric shielding, therefore, avoiding agglomeration. Rutin-LCNs showed a moderated entrapment efficiency of 68.9%. Evaluation of *in vitro* anti-cancer activity was determined by proliferation and migration assay. Proliferation of the A549 cells was studied by MTT assay and trypan blue staining which showed a significant decrease in proliferation & cell count respectively. While the migration assay was studied by wound healing assay and transwell chamber assay. It was found that with increase in the concentration of drug upto 20µM inhibit the migration of cells. At 10 µM and 20µM, accumulation of the rutin-LCNs inside the cancer cells was microscopically seen, suggesting the formation of apoptotic bodies and degeneration of cytoskeleton.

CONCLUSIONS

Rutin was successfully encapsulated into liquid crystalline nanoparticles revealing their sustained release behaviour. Further, rutin-LCNs has shown to be anti-cancerous agent. This demonstrates that the rutin LCN can be potentially employed for various other airway diseases such as asthma and COPD. Moreover, the delivery of rutin in nanoparticles can overcome the issues associated with rutin such as the low solubility, poor bioavailability and short half-life.

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