DEVELOPMENT & EVALUATION OF INTRA-NASAL LIPID NANOCARRIERS FOR LUNG CANCER

Thesis submitted in partial fulfilment for

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Doctor of Philosophy

By

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PREFACE

Lung cancer represents the most common root cause of deaths related to cancer around the world.According to Cancer Statistics, 2024, published in CA: A Cancer Journal for Clinicians, 2,001,140 new cases of cancer have been reported with 611,720 deaths due to cancer in the United States and nearly 1680 deaths per day. The greatest number of deaths are reported to be due to lung cancer which is reported to be more than breast cancer, prostate cancer, pancreatic cancer, and colorectal cancer. Chemotherapy has emerged as a boon for the management and treatment of cancer in conjunction with radiation therapy, surgery, and targeted therapy. But conventional synthetic drugs used as chemotherapeutic agents for the treatment of lung cancer have been reported to incite associated nonspecific and systemic cytotoxicity and this leads to a hunt for better biologically compatible compounds of natural origin-based chemotherapy that could be explored as alternative therapy for lung cancer.Solid lipid nanoparticles are a colloidal carrier system encompassing solid lipid, water, and surfactant.The obvious merits of SLNs over nanoemulsions and polymer-based nanoparticles are enhanced stability of the drug, accentuated drug entrapment efficiency and biocompatibility as these are mostly formulated from biodegradable excipients and generally regarded as safe (GRAS) certified excipients. Apart from these merits SLNs also command the encapsulated drug release and averts the premature degradation of the drug in the system[1][1](Radhakrishnan et al., 2016)(Radhakrishnan et al., 2016)(Radhakrishnan et al., 2016). Conventional treatment approaches of lung cancer offer not so satisfactory results, and a compromised life associated with potential side effects of chemotherapy, radiation therapy, and surgical resection. In the absence of targeted therapies, systemic administration of chemotherapy exposes normal cells to these potent chemotherapeutic drugs resulting in the inhibition of their growth making patient weak and could eventually leads to death. Therefore, intranasal administration of chemotherapeutic drugs could pave a way for the

efficient delivery of these potent drugs directly to lungs and avoiding first pass metabolism and other potential side effects associated with systemic route of drug administration.

The aim of our study was to design, optimize, develop, characterize, and evaluate the gemcitabine and epigallocatechin-3-gallate loaded solid lipid nanoparticles (GEM-EGCG SLNs) against lung cancer via intranasal route.The SLNs were prepared by double emulsification solvent evaporation method and characterized for micromeritics and physiochemical characterization with entrapment efficiency and drug release kinetics. Further, the GEM-EGCG SLNs were also evaluated for *in vitro* cell line studies against A549 lung cancer cell lines. A proportional decrease in the percent cell viability of A549 cells has been noticed with an increase in the concentration of GEM-EGCG SLNs, GEM SLNs, and EGCG SLNs with an IC_{50} value of approximately 10 µg/mL for GEM-EGCG SLNs. The wound healing (scratch) assay revealed that GEM-EGCG SLNs could significantly avert the migration of A549 cells, with 85 % of uncovered scratch wound area after 48 hours of incubation at 5 µg/mL which increased to around 90 % at 50 µg/mL. Cellular uptake studies revealed that SLNs were able to be internalized into the nuclei of cells which could be due to their small particle size in the nanometer range. The high apoptotic rate shown by GEM-EGCG SLNs depicts its better anti-tumor activity against A549 cells as compared to other treatment groups along with ROS production which mediated the apoptosis of A549 cells. We found that GEM-EGCG SLNs were stable for the observation period of 3 months with only slight changes in the values of particle size, PDI, zeta potential, and percent entrapment efficiency. Overall, the histopathological studies of the lungs of tumor bearing mice and safety assessment of GEM-EGCG SLNs postulates the synergistic anti-tumor potential of epigallocatechin-3-gallate co-loaded with gemcitabine in biocompatible solid lipid nanoparticles for use as an anti-cancer therapeutic agent against lung cancer.

LIST OF FIGURES

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DECLARATION BY THE CANDIDATE

I, ***Mohini Mishra***, certify that the work embodied in the thesis is my own bona fide work and carried out by me under the supervision of ***Dr. Ruchi Chawla, Associate Professor*** from ***July 2019 to March 2025***, at the ***Department of Pharmaceutical Engineering and Technology***, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

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CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of my/our knowledge.

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CERTIFICATE

It is certified that the work contained in the thesis titled *"**DEVELOPMENT & EVALUATION OF INTRA-NASAL LIPID NANOCARRIERS FOR LUNG CANCER**"* by ***Mohini Mishra*** has been carried out under my supervision and this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Course work, Comprehensive, Candidacy, SOTA, and Pre-submission seminar.

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LIST OF TABLES

List of abbreviations

GEM- Gemcitabine

EGCG- Epigallocatechin-3-gallate

SLNs- Solid lipid nanoparticles

GEM-EGCG SLNs- Gemcitabine epigallocatechin-3-gallate loaded solid lipid nanoparticles

DCM- Dichloromethane

HPLC- High-performance liquid chromatography

UV- Ultraviolet

MRT- Mean residence time

AUC- Area under curve

QbD- Quality by design

PVA- Poly vinyl alcohol

PF-127- Pluronic F-127

HSPC- Hydrogenated soy phosphatidylcholine

GMS- Glyceryl monostearate

B(a)P- Benzopyrene

FBS- Fetal bovine serum

MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

DMEM- Dulbecco's Modified Eagle Medium

DMSO- Dimethyl sulfoxide

OVAT- One variable at a time

rpm- Rotations per minute

W/O- Water in oil

W/O/W- Water in oil in water

PDI- Poly dispersity index

ATR- Attenuated total reflectance

DSC- Differential scanning calorimetry

TGA- Thermogravimetric analysis

pH- Potential of hydrogen

PBS- Phosphate buffer saline

CO_2- Carbon dioxide

Kg- Kilogram

mg- Milligram

mL- Milliliter

µL- Microliter

nm- Nanometer

µM- Micromolar

h- Hours

°C- Degree Celsius

min- Minutes

i.n.- Intranasal

i.v.- Intravenous

DAPI- 4',6-diamidino-2-phenylindole

Rh- Rhodamine

DCFDA- 2',7'-dichlorofluorescein diacetate

ROS- Reactive oxygen species

EDTA- Ethylenediamine tetra acetate

H_2O_2- Hydrogen peroxide

DTI- Drug targeting index

IVIS- *In vivo* imaging system

p- Probability coefficient

R^2- Correlation coefficient

NC- Negative control

PC- Positive control

Dt-Absorbance of the test sample

Dnc- Absorbance of the negative control

Dpc- Absorbance of the positive control

Dedicated to My Son

Ashwattha