Chapter 6

SUMMARY, LIMITATIONS AND FUTURE PERSPECTIVES

6.1 Summary

The results of the study of optimization, development, characterization, and evaluation of intranasally administered gemcitabine solid lipid nanoparticles co-loaded with epigallocatechin-3-gallate postulates effective lungs targeted treatment modality against lung cancer. The results of the study are enlisted below:

- The study aimed to develop and evaluate QbD-based solid lipid nanoparticles (SLNs) of gemcitabine (GEM) co-loaded with epigallocatechin-3-gallate (EGCG) that can be administered through the nose against lung cancer.
- The SLNs were prepared by a double emulsification solvent evaporation method.
- The average particle size and polydispersity index (PDI) of GEM-EGCG SLNs, GEM SLNs, and EGCG SLNs were determined to be 122.8 ± 8.02 nm, 0.1738 ± 0.02; 118.9 ± 12.71 nm, 0.257 ± 0.03; and 117.4 ± 9.37 nm, 0.202 ± 0.05, respectively.
- The zeta potential of GEM-EGCG SLNs, GEM SLNs, and EGCG SLNs was found to be -35.9 ± 1.6 mV, -33.9 ± 2.1 mV, and -24.0 ± 2.7 mV, respectively.
- The entrapment efficiency of GEM-EGCG SLNs was calculated to be 95.035 ± 8.6% for GEM and 92.406 ± 11.4% for EGCG.
- ATR studies were conducted for GEM-EGCG SLNs, GEM, EGCG, GMS, Pluronic F-127, PVA, HSPC, and a physical mixture of GEM and EGCG to evaluate the compatibility of excipients and drugs.
- TGA weight loss curve and DSC graph were also plotted for GEM-EGCG SLNs.
- The weight loss curve of GEM-EGCG SLNs formulation depicts a two-step degradation pattern that shows 5 % weight loss from 20 °C to 250 °C which could be attributed to the loss of adsorbed or bound water in the formulation.
- DSC studies enable us to gain knowledge about interactions between excipients, GEM, and EGCG and its resultant effect on thermal properties.

- The endothermic peak of GEM, at 280 °C which corresponds to its melting point, and also, a baseline shift at a temperature of around 135°C is observed (DSC curve for GEM represents crystallization (depicted by exothermic peak) after glass transition (or baseline shift) and subsequent melting).
- When entrapped in SLNs the original peaks corresponding to the melting of GEM and EGCG and glass transition temperature were lost in the DSC curve for GEM-EGCG SLNs
- The drug release study demonstrated a sustained drug release profile for GEM-EGCG SLNs that followed the Higuchi model.
- It could be comprehended from the drug release data that the fabrication of gemcitabine and epigallocatechin-3-gallate loaded solid lipid nanoparticles showed a sustained release profile for GEM and EGCG.
- The drug release profile for GEM and EGCG from GEM-EGCG SLNs follows a release mechanism based on diffusion following the 'Fick's Law'
- MTT assay revealed an IC_{50} value of 10 µg/mL for GEM-EGCG SLNs against A549 cell lines.
- 85% of the scratch wound area was uncovered at 5 µg/mL, which was increased to 90% at 50 µg/mL by GEM-EGCG SLNs.
- SLNs were able to internalize into the nuclei of cells and the anti-proliferative action was mediated by ROS production.
- GEM-EGCG SLNs were found to be stable for 3 months. Results depicted that GEM-EGCG SLNs have potential as a therapeutic approach against lung cancer.
- We analyzed the formulation for its effectiveness in terms of anti-cancer activity in the benzopyrene-induced Swiss albino mice lung cancer model.
- We also assessed the pharmacokinetics, biodistribution, biocompatibility, and hemocompatibility of GEM-EGCG SLNs.

- GEM-EGCG SLNs had better pharmacokinetics than other treatment groups.
- The increase in the $t_{1/2}$ (which is defined as the time required for half of the drug to be excreted out from the body) could be accounted to the sustained release of GEM and EGCG into the plasma from the SLNs.
- An escalation in the values of MRT for GEM-EGCG SLNs could be attributed to the long circulation of these nanoparticles in the systemic circulation due to the enhanced permeability and retention (EPR) effect and sustained and regulated release of these drugs from the SLNs into the bloodstream.
- A better pharmacokinetic profile can improve the therapeutic activity of the drugs.
- High drug targeting index value of 17.605 for GEM and 2.118 for EGCG, indicating their effectiveness in targeting the lungs.
- The IVIS images at 24 and 48 hours showed the presence of GEM-EGCG SLNs in the lungs, indicating a sustained drug release profile provided by GEM-EGCG SLNs. The results confirm that the intranasal route can be used to target the lungs effectively.
- SLNs are present in greater concentration in lungs as compared to pure GEM and pure EGCG because of the EPR effect of tumor cells which allows greater penetration of nanosized SLNs into lung tumor cells owing to their leaky vasculature and reduced drainage by the lymphatic system.
- Images from treatment group GEM-EGCG SLNs show very few pathological lesions which support the potential of anti-tumor efficacy of GEM-EGCG SLNs.
- Blank SLNs showed no pathological lesions in the liver, kidney, and nasal region validating the safety of SLNs.
- Histological images of the kidney show clear glomerular cells and Bowman's capsule without any pathological lesions.

- The histological investigation postulates the safety of the formulation to be used in the study.
- GEM-EGCG SLNs also showed fewer pathological lesions than other treatment groups.
- GEM-EGCG SLNs depicted a lower hemolysis rate of 1.62 ± 0.10 %. These results suggest that GEM-EGCG SLNs could effectively treat lung cancer.
- The reduction in the rate of hemolysis for GEM-EGCG SLNs as compared to pure drug GEM and EGCG could be attributed to the presence of lipids and phospholipids in SLNs which provides a layer that acts as a shield on the surface and leads to attenuation of the hemolysis rate.
- The columnar goblet cells and basal cells of the nasal epithelium were visible and had no distortion in morphology or shape.
- Moreover, the lamina propria region of the treatment group appeared normal with no distortion or pathological lesions in its structure, as compared to the histopathological image of the normal group.
- These results indicate that GEM-EGCG SLNs are safe for use in the nasal region of mice.

6.2 Limitations

- While *in vitro* studies using A549 lung cancer cells provide valuable insights into cytotoxicity and mechanism of action, they may not fully replicate the complex tumor microenvironment observed *in vivo*.
- The use of a benzopyrene-induced lung cancer mouse model, though relevant, may not capture the full heterogeneity of human lung cancers, especially regarding genetic and phenotypic variations.
- Patient-related variability in intranasal drug absorption (e.g., due to mucociliary clearance, nasal pathologies, or breathing patterns) may affect therapeutic outcomes.

- Long-term safety and potential immunogenicity of repeated intranasal administration of SLNs need to be thoroughly investigated.

6.3 Future Perspectives

- Future studies should explore orthotopic lung cancer models or genetically engineered mouse models (GEMMs) that better mimic human lung cancer progression and metastasis.
- Detailed investigation into the molecular pathways modulated by GEM-EGCG combination therapy would help establish the mechanism of synergism and identify potential biomarkers of response.
- Conducting comprehensive PK/PD studies using radiolabeled or fluorescently tagged SLNs can provide insights into drug distribution, clearance, and retention time in lung tissue and other organs.
- Future formulations may include ligand-mediated targeting (e.g., transferrin, folate, or EGFR-targeting ligands) to further improve site-specific uptake by cancerous cells.
- Moving toward clinical application will require addressing regulatory concerns, performing toxicity profiling in higher species, and refining the formulation for human use (e.g., nasal spray or inhaler-compatible dosage forms).
- Other bioactive phytochemicals with proven anticancer potential can be co-formulated with standard chemotherapeutic agents to create new synergistic combinations with fewer side effects.