UNIVERSITI TEKNOLOGI MARA

DRUG-FREE" OLIGOCHITOSAN NANOPARTICLES AND THEIR FATTY ACID DERIVATIVES AS INHALABLE LUNG CANCER THERAPEUTICS

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ABSTRACT

Lung cancer has the highest mortality incidence about 1.8 million deaths in 2020. Use of chemotherapeutics inhibits the growth of proliferative cancer cells but causes severe adverse systemic effects. Oligochitosan has been shown to exert anti-proliferative activities in several cancers *in vitro* and *in vivo.* Fatty acids, on the other hand, could be a cancer promoter or inhibitor as a function of its physicochemical attributes. Both chitosan/oligochitosan and fatty acid are pharmaceutical excipients which receive a widespread application in the pharmaceutical sector as binder, mucoadhesive, permeation enhancer and/or controlled-release agent. The conjugation of oligochitosan with fatty acids and their translation into nanoparticles is envisaged to promote the delivery aspects of a nanoparticulate system for cancer treatment such as binding to cancer cells and increased membrane permeability for transcellular transport of nanoparticles into the cancer cells. Their anti-cancer potential however can be dubious. This study aimed to investigate oligochitosan and oligochitosan-fatty acid conjugates in the form of nanoparticles as a potential anti-non-small cell lung cancer nanotherapeutic from the perspectives of pulmonary delivery and bioactivity. The conjugates of oligochitosan-fatty acid were synthesised by carbodiimide reaction with the conjugation status being evaluated by FTIR and ^{1}HNMR spectroscopy techniques. They were developed into nanoparticles by means of nanospray drying method. Their size, zeta potential, morphology, crystallinity and contact angle were characterized. When required, oligochitosan-polyethylene glycol (PEG) conjugate nanoparticles were produced as a control sample. The nanoparticles were subjected to two studies: (1) soft agglomeration of nanoparticles with chitosan microparticles for pulmonary aerosolization and inhalation; (2) cancer cytotoxicity, cellular internalization, cancer cells-nanoparticles interaction, endocytic pathway, cancer molecular biology and *in vivo* pharmacodynamics performance assessment. The oligochitosan nanoparticles and their fatty acid conjugate nanoparticles were deliverable into the lung in the form of soft agglomerates with low molecular weight chitosan microparticles (size = 1.18 ± 0.01 urn, span = 1.48 ± 0.10, aspect ratio = 1.00 ± 0.14, surface roughness = 57.12 ± 10.77 nm, crystallinity = 7.40 ± 0.26 %) as the nanoparticle carrier that admixed the nanoparticles by gentle blending. The inhaled fine particle fraction of the nanoparticles was promoted by their hydrophobicity, surface roughness, off-spherical shape and amorphous particulate structure. The oligochitosan nanoparticles were an effective anti-cancer therapeutic *in vitro* and *in vivo.* Conjugation of fatty acid/PEGonto oligochitosan generally reduced its cancer cytotoxicity. The oligochitosan linoleate conjugate nanoparticles and oligochitosan linoleneate conjugate nanoparticles however expressed tumour suppressor potential and increased immune activity that could benefit cancer control. Oligochitosan nanoparticles induced cell death through inhibiting cell growth *via* cell cycle arrest at the Go/Gi phase, intrinsic and extrinsic apoptosis, mitigating metastasis tendency and sustaining immune responses. The anti-cancer effects of oligochitosan nanoparticles were mediated *via* the suppression of MAPK/ERK and Akt/mTOR/4E-BPl signalling pathways. The oligochitosan nanoparticles were taken up predominatly by lung cancer cells *via* caveolae- and lipid-raft mediated endocytic pathways following their interaction with the domains of the plasma membrane of the cancer cells. The oligochitosan nanoparticles were able to reduce the tumour burden, repair damaged lung tissues and restored deteriorated liver function in rats. They are potentially a therapeutic agent for lung cancer.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Chitosan has received massive attention in biomedical, food, pharmaceutical, agriculture and environmental industries because of its unique nature namely biodegradability, biocompatibility, abundant availability and bioactivity (Ul-Islam et al., 2024). Chitosan and chitosan derivatives have been shown to exert anti-cancer activity against several types of cancer *in vitro* and *in vivo* (Adhikari & Yadav, 2018). The neat oligochitosan and low molecular weight chitosan (LMWC) are more toxic against cancer cells *in vitro* and *in vivo* than the higher molecular weight chitosan (Iskandar et al., 2024). Chitosan can be converted into nanoparticles by means of ionotropic gelation, spray drying, freeze drying and other techniques (Sazali et al., 2023). The size, morphology, zeta potential and crystallinity of the formed nanoparticles are primarily governed by the interplay of formulation, processing and equipment variables (Khan et al., 2019). Transformation of chitosan into nanoparticles generally raises its anti-cancer efficacy in a particle size dependent manner (Iskandar et al., 2024). Prashanth & Tharanathan (2005) observed over 90 % tumour volume reduction (7.5 ± 0.5 mL to 0.7 ± 0.2 mL) *in vivo* after injecting 50-100 mg of oligochitosan and LMWC to 6-8-week old Swiss albino mice on every alternate day for 6 days. Ahmed et al. (2018), on the other hand, reported that chitosan nanoparticles at a much lower dose (11-12.5 mg for 7 days) bring about 85 % tumour volume reduction (300 mm^3 to 45 ± 5.1 mm^3) in the 8-week old adult female Swiss albino mice weighing 22 - 25 g/mouse.

One intriguing area of research involves modifying the functional group of chitosan through incorporating hydrophobic moiety, such as fatty acid to improve the physicochemical and biological properties of chitosan for pharmaceutical, medical and biomedical engineering applications (Abdulsalam et al., 2024). Conjugation of fatty acid onto chitosan and conversion of such conjugate into nanoparticulate systems are deemed able to prolong systemic circulation time, improve nanoparticle bioavailability, decrease risk of adverse reactions, and eventually enhance therapeutic responses