UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF AEROSOLIZED PALM-BASED NANOEMULSION SYSTEM CONTAINING QUERCETIN FOR PULMONARY DELIVERY OF LUNG CANCER

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By

NOOR HAFIZAH BINTI ARBAIN

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Chair : Professor Mohd Basyaruddin Abdul Rahman, PhD
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Quercetin (QT) is an attractive natural compound, has been extensively investigated for its pharmacological effects towards lung cancer. However, clinical applications of QT as chemotherapeutic agent are limited due to low water solubility and low bioavailability. A new nanoemulsion system to enhance the solubility of QT in the dispersed phase and its bioavailability was developed for pulmonary delivery of lung cancer.

Aerosolized palm-based nanoemulsion system containing QT was carried out using high energy emulsification method by dissolving QT in oil phase and then it was added into aqueous phase. Screening of oils and surfactants were done by solubility and emulsification test. From the results, it showed that the combination of palm oil esters (POE), ricinoleic acid (RC) with ratio 1:1 (wt. / wt.) and Tween 80 gave the highest solubility (0.66 mg/mL) of QT compared to other oil mixtures and showed the smallest droplet size was obtained (131.5 nm). These compositions were used for further optimization of nanoemulsion formulation. The formulation was optimized using Mixture Experimental Design (MED) and Artificial Neural Network (ANN).

The composition effects of the mixture of POE:RC (1.50–4.50 wt. %), lecithin (1.50–2.50 wt. %), Tween 80 (0.50–1.00 wt. %), glycerol (1.50–3.00 wt. %), and water (88.00–94.95 wt. %) towards the droplet size and volume median diameter (VMD) as the responses were studied. The mathematical model from MED suggested three optimized formulations named OPT 1, OPT 2 and OPT 3 with specific amount of POE:RC (1.50, 3.40 and 4.50 wt. %), lecithin (1.50 and 2.50 wt. %), Tween 80 (1.50 wt. %), glycerol (1.50, 3.00, and 2.43 wt. %) and water (93.95, 89.56, and 89.02 wt. %) gave predicted response values of

droplet size (110.42 nm, 132.95 nm and 146.04 nm) and VMD (5.959 μm, 4.576 μm and 4.378 μm). These values showed good correlation with the actual values of droplet size (110.30 nm, 131.40 nm and 150.60 nm) and VMD (5.882 μm, 4.557 μm and 4.266 μm). The results from ANN analysis gave no significant differences between the actual and predicted values of VMD with lower residual standard error than MED.

From the physicochemical characterizations, the optimized formulations (OPT 1, OPT 2 and OPT 3) possessed suitability for pulmonary application. The droplet size measured in Transmission Electron Microscopy (TEM) was consistent with the size obtained using Zetasizer analysis and showed the droplets of nanoemulsion were spherical. These optimized formulations exhibited good stability against phase separation and remained in nano-sized under storage. Stability evaluation shows these formulations were stable under centrifugation test, freeze thaw cycle test and storage at 4 °C for three months.

The evaluation of aerosol nanoemulsion showed efficient delivery with more than 90% aerosols output, higher percent dispersed and percent inhaled of drug formulation. The aerosols delivery properties for OPT 1, OPT 2 and OPT 3 yielded mass median aerodynamic diameter (4.25 ± 0.38 μm, 3.20 ± 0.07 μm and 3.09 ± 0.05 μm), fine particle fraction (70.56 ± 6.33%, 89.01 ± 1.37% and 90.52 ± 0.10%) and geometric standard deviation (1.96 ± 0.07, 1.76 ± 0.03 and 1.77 ± 0.03) that suitable for aerosolization to be inhaled in the lung.

The optimized nanoemulsions demonstrated the sustained QT release of about 18.33 ± 0.32%, 24.15 ± 1.68% and 26.75 ± 2.20% within 48 hours and there were in adherence to Korsmeyer's Peppas mechanism. Cytotoxicity analysis showed the developed formulation has a better cytotoxicity action on human lung cancer cells (A549) compared to human lung fibroblast cells (MRC5). In conclusion, a stable palm-based nanoemulsion system containing QT was successfully developed in this study and shows potential for pulmonary delivery of lung cancer.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN SISTEM AEROSOL NANOEMULSI BERASASKAN SAWIT MENGANDUNGI QUERCETIN UNTUK PENGHANTARAN PULMONARI KANSER PARU-PARU

Oleh

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Quercetin (QT) adalah sebatian semulajadi yang menarik, telah dikaji secara meluas untuk aktiviti farmakologinya sebagai ubat kanser paru-paru. Walau bagaimanapun, aplikasi klinikal QT sebagai ejen kemoterapi adalah terhad kerana kelarutannya di dalam air dan bioavailabiliti yang rendah. Sistem nanoemulsi yang baru untuk meningkatkan kelarutan QT dalam fasa penyebaran dan bioavailabilitinya telah dibangunkan untuk penghantaran pulmonari kanser paru-paru.

Sistem aerosol nanoemulsi berasaskan minyak kelapa sawit mengandungi QT disediakan menggunakan kaedah pengemulsi tenaga tinggi dengan melarutkan QT di dalam fasa minyak dan kemudian ia dimasukkan ke dalam fasa berair. Pemilihan minyak dan surfaktan dilakukan melalui ujian keterlarutan dan pengemulsian. Daripada keputusan tersebut, ia menunjukkan gabungan ester minyak sawit (POE) dan asid risinolik (RC) dengan nisbah 1:1 dan Tween 80 memberikan kelarutan QT yang tertinggi (0.66 mg/mL) berbanding campuran minyak yang lain dan menunjukkan saiz zarah yang lebih kecil telah diperolehi (131.5 nm). Komposisi ini seterusnya telah digunakan untuk pengoptimuman penghasilan nanoemulsi. Formulasi ini dioptimumkan dengan menggunakan Reka Bentuk Eksperimen Campuran (MED) dan Rangkaian Saraf Tiruan (ANN).

Kesan komposisi campuran POE:RC (1.50-4.50 wt. %), lesitin (1.50-2.50 wt. %), Tween 80 (0.50-1.00 wt. %), gliserol (1.50-3.00 wt. %) dan air (88.00-94.95 wt. %) kepada respon iaitu saiz zarah dan isipadu median diameter dikaji. Model matematik daripada MED mencadangkan tiga formulasi yang dioptimumkan iaitu OPT 1, OPT 2 dan OPT 3 dengan jumlah POE:RC (1.50, 3.40 dan 4.50 wt. %), lesitin (1.50 dan 2.50 wt. %), Tween 80 (1.50 wt. %), gliserol (1.50, 3.00, dan 2.43 wt. %) dan air (93.95, 89.56, dan 89.02 wt. %) memberikan nilai respon

yang diramalkan bagi saiz zarah (110.42 nm, 132.95 nm and 146.04 nm) dan isipadu median diameter (5.959 μm, 4.576 μm and 4.378 μm). Nilai-nilai ini menunjukkan hubungkait yang baik dengan nilai sebenar bagi saiz zarah (110.30 nm, 131.40 nm dan 150.60 nm) dan isipadu median diameter (5.882 μm, 4.557 μm dan 4.266 μm). Analisis ANN menunjukkan tiada perbezaan ketara untuk isipadu median diameter di antara nilai yang diramalkan dengan nilai sebenar dengan baki ralat piawaian yang lebih rendah daripada MED.

Dari pencirian fizikokimia, formulasi yang optimum (OPT 1, OPT 2 dan OPT 3) mempunyai kesesuaian untuk aplikasi pulmonari. Saiz zarah yang diukur dalam Transmisi Elektron Mikroskopi (TEM) adalah selaras dengan saiz yang diperoleh menggunakan analisis Zetasizer dan menunjukkan bahawa titisan nanoemulsi adalah sfera. Formulasi yang dioptimumkan ini menunjukkan kestabilan yang baik terhadap pemisahan fasa dan kekal dalam saiz-nano semasa penyimpanan. Penilaian kestabilan ini menunjukkan formulasi tersebut stabil di bawah ujian pengemparan, ujian kitaran beku- cair dan penyimpanan pada ± 4 °C selama 3 bulan.

Penilaian aerosol nanoemulsi menunjukkan penghantaran yang efektif dengan lebih daripada 90% pengeluaran aerosol, peratus formulasi dadah tersebar dan disedut yang lebih tinggi. Ciri-ciri penyerapan aerosol bagi OPT 1, OPT 2 dan OPT 3 menghasilkan median jisim diameter aerodinamik (4.25 ± 0.38 μm, 3.20 ± 0.07 μm dan 3.09 ± 0.05 μm), pecahan zarah halus (70.56 ± 6.33%, 89.01 ± 1.37% dan 90.52 ± 0.10%) dan sisihan piawai geometri (1.96 ± 0.07, 1.76 ± 0.03 dan 1.77 ± 0.03) yang sesuai untuk aerosolisasi untuk disedut dalam paru-paru.

Nanoemulsi yang dioptimumkan menunjukkan pelepasan QT yang berterusan kira-kira 18.33 ± 0.32%, 24.15 ± 1.68% dan 26.75 ± 2.20% dalam masa 48 jam dan ia berpegang kepada mekanisme pelepasan Korsmeyer Peppas. Analisis ketoksikan menunjukkan formulasi yang dibangunkan ini mempunyai tindakan ketoksikan yang lebih baik pada sel-sel kanser paru-paru manusia (A549) berbanding dengan sel fibroblas paru-paru manusia (MRC5). Kesimpulannya, sistem nanoemulsi berasaskan sawit mengandungi QT yang stabil berjaya dibangunkan dalam kajian ini dan menunjukkan potensi untuk penghantaran pulmonari kanser paru-paru.

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I certify that a Thesis Examination Committee has met on 14 August 2018 to conduct the final examination of Noor Hafizah Binti Arbain on her thesis entitled "Development of Aerosolized Palm-Based Nanoemulsion System Containing Quercetin for Pulmonary Delivery of Lung Cancer" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AmB	Amphotericin B
ANOVA	Analysis of variance
ACI	Andersen cascade impactor
ANN	Artificial Neural Network
bEGF	Biotinylated epidermal growth factor
DMSO	Dimethyl sulfoxide
DOX	Doxetacel
ELISA	Enzyme linked immunosorbent assay
FPF	Fine particle fraction
FDA	Food and Drug Administration
FTIR	Fourier transforms infrared
GP	Gelatin particles
GEM	Gemcitabine
GRAS	Generally recognized as safe
GSD	Geometric standard deviation
IC_{50}	Half maximal inhibitory concentration
HPLC-UV	High-performance liquid chromatography- Ultra-violet
h	Hour
A549	Human lung cancer cell line
MRC5	Human lung fibroblast cell line
HA–Pt	Hyaluronic-cisplatin
HLB	Hydrophile lipophile balance
Iv	Intravenous
MMAD	Mass median aerodynmic diameter
Min	Minutes
MED	Mixture experiment design
O/W	Oil-in-water nanoemulsion
POE	Palm oil esters
PD	Percent dispersed
PIP	Percent inhaled
PIC	Phase inversion composition
PIT	Phase inversion temperature
PBS	Phosphate buffer solution
PDI	Polydispersity index
PEG	Polyethylene glycol
Tween 80	Polyoxyethylene (20) sorbitan monooleate
PRESS	Prediction error sum of squares
QT	Quercetin
RSE	Residual standard error
RC	Ricinoleic acid
RMSE	Root means squared error
rpm	Rotation per minute
TEM	Transmission electron microscopy
v/v	Volume per volume
VMD	Volume median diameter
W/O	Water-in-oil nanoemulsion
wt.	Weight
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Globally, lung cancer is among the main cause of cancer death and the most regular type of cancer cases. More than one million people killed by lung cancer a year. Lung cancer was rarely occurs in the last decades in which a survey shows only a small number of lung cancer cases reported from the whole cancer cases. However, the percentage has increased after several years afterwards (Witschi, 2001). In addition, only about 17% of people suffering from lung cancer surviving for five years which may indicates that the available treatment are associated with significant limitations in efficacy of treatment for the disease. Hence, it showing an important need for choices in more effective treatment (Jaggi, 2017; Burris 2009).

The current treatment of lung cancer is chemotherapy. Mostly, the chemotherapeutics are given by oral or intravenous therapies. However, the efficacy of these therapies are limited by constrains of systemic side effects due to non-localize delivery of drugs to the target site (Shah *et al*., 2017; Tseng *et al*., 2008). The chemotherapeutics that delivered directly to the lungs is an interesting strategy to improve the efficacy of lung cancer therapy.

Pulmonary delivery system, a non-invasive administration is a new concept especially for lung cancer treatment. Pulmonary delivery offers many advantages than other routes of administration including its ability to deliver high concentrations of drugs locally at the target site while minimizing the side effects and enhancing patient compliance. These advantages are due to the lungs provides large surface area through which molecules can be absorbed and go direct into the bloodstream (Laouini *et al*., 2014).

Natural chemotherapeutics are becoming increasingly used for cancer treatment (Karadag *et al*., 2013: Verma *et al.*, 2013). Quercetin (QT) is one of the plant-based drugs with high accessibility and affordability (Gupta *et al*., 2010; Kennedy *et al*., 2009). QT showed potential to inhibit proliferation of various types of cancer cells, including lung cancer cells (Karadag *et al*., 2013). However, the use of QT is limited due to its hydrophobicity and low bioavailability (Scalia, *et al*., 2013; Gao *et al*., 2009). Many approaches have been introduced to increase the solubility of low-water soluble drugs through delivery systems. Previous study reported that the penetration of QT was complemented

significantly by the mixture of emulsifiers and lipids, which is afficiliated by its solubility in the carriers used (Azuma *et al*., 2002).

Nanoemulsions have the potential to deliver active drug compounds to the lungs because of their high efficiency in drug's solubilization in which enhance their bioavailability (Amani *et al*., 2010). Furthermore, they have the possibility to increase the drug deposition and reservation for a long period of time in the lung tissues (Nasr *et al*., 2012). With the advantage of solution-like physicochemical properties of nanoemulsions, nanoemulsions perform as a solution upon nebulization and will demonstrate suitability and improved aerosolisation performance for pulmonary delivery of lung cancer. Up to date, the delivery of nanoemulsion-based drugs via pulmonary administration for lung cancer treatment is still in its infancy, have not yet been fully expoited and published.

1.2 Problem Statement

Conventional treatment of drug delivery in the lung such as systemic administration is limited due to non-targeting nature which renders a higher drug doses needed to the target tissue and this results in increasing adverse effects to the normal cells (Akhter *et al*., 2015). To reduce such effects, it would clearly be preferable to administer therapeutic drugs by pulmonary delivery.

The favors of pulmonary delivery such as delivery of drugs to the lungs are localized, where the systemic side effects are reduced (Silva *et al*., 2013). Nevertheless, the pulmonary administration through aerosolization and inhalation is limited by a few challenges. The main challenge is the efficacy of inhaled aerosols, which is determined by their aerodynamic properties. It is generally preferred that aerosol droplets size of 1-5 μm are needed for an effective inhalation therapy (Laouini *et al*., 2014).

The properties of QT such as low water solubility and low bioavailability have limited its uses in pharmaceutical field (Amani *et al*., 2010) that may hinder effective pulmonary delivery (Nesamony *et al*., 2014). Hence, an efficient and biocompatible delivery system should be developed to increase its solubility and enhance its bioavailability. The major challenge in the development of nanoemulsion system is to maintain the droplet size in the nanometre range while remain physically stable for a period of time. Hence, it is important to find the right composition in the formulation for a stable nanoemulsion system with appropriate characteristics for pulmonary delivery.

1.3 Scope of Study

This study concentrated on the development of aerosolized palm-based nanoemulsion system containing quercetin for pulmonary delivery of lung cancer. The early stage was the selection of nanoemulsions composition. The formulation was then optimized using Multiple Experimental Design and Artificial Neural Network to determine the best composition in the formulation with respect to droplet size and volume median diameter. The physicochemical and aerodynamic performance of the developed formulations were examined. Thereafter, the drug release and cytotoxicity profiles were also determined.

1.4 Objectives of Study

The main objective of this research is to develop aerosolized palm-based nanoemulsion system containing QT for pulmonary delivery of lung cancer. Hence, the following objectives were targeted to assist in achieving main objective:

i) to formulate and optimize the compositions of the QT-loaded nanoemulsions using appropriate mathematical models
ii) to characterize the physicochemical properties and stability of the optimized nanoemulsions
iii) to characterize the aerosol performance of the optimized nanoemulsions
iv) to determine the *in vitro* release ability and cytotoxicity of nanoemulsion

1.5 Hypothesis of Study

i) Palm-based nanoemulsion system could increase the solubility and bioavailability of quercetin
ii) Palm-based nanoemulsion system containing quercetin could enhance the aerosolization performance in pulmonary delivery
iii) Aerosolized palm-based nanoemulsion system containing quercetin could demonstrate suitability for pulmonary delivery in lung cancer
iv) Palm-based nanoemulsion system containing quercetin could deliver high concentrations of drugs in the deep lung
v) Palm-based nanoemulsion system containing quercetin could enhance the drug deposition in the deep lung

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