UNIVERSITI PUTRA MALAYSIA

PREPARATION, CHARACTERIZATION, AND PRELIMINARY PHARMACOKINETIC STUDIES ON COMPLEX OF ZERUMBONE WITH HYDROXYPROPYL-β-CYCLODEXTRIN

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By

ELTAYEB ELAMIN MOHAMMED EID

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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PREPARATION, CHARACTERIZATION, AND PRELIMINARY PHARMACOKINETIC STUDIES ON COMPLEX OF ZERUMBONE WITH HYDROXYPROPYL-β-CYCLODEXTRIN

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Chairman: Ahmad Bustamam Abdul, PhD

Faculty: Institute of Bioscience

Zerumbone (ZER) is a mono-sesquiterpene compound derived from ginger zerumbet smith. It has been reported that, ZER showed a significant activity in both *in vivo/in vitro* studies for different types of cancers such as cervical, colon, liver and breast cancer.

To facilitate the *in vivo* characterization of ZER, a reversed-phase HPLC and UPLC-MS/MS methods were developed and validated according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and Food and Drug Administration (FDA) guidelines.

Due to the high lipophicity (logP > 5) and very limited water solubility of ZER, the bioavailability needs to be increased so that high blood levels can be obtained for moderate doses. In addition low water solubility contribute to its high protein binding that will leads to low therapeutic free ZER concentration and further successive doses will be imposed for the cancer therapy that will not preferable in clinical

environment, and preclude intravenous (i.v.) loading as well. Therefore, solubility and stability of ZER are essential physico-chemical properties that must be proved in early stages of drug development process.

Hydroxypropyl-β-cyclodextrin (HPβCD) was used in this study to increase the water solubility of ZER as well as its stability. The solubility of ZER was increased > 100 folds by 0.05M HPβCD in water. The ZER/ HPβCD complexes were characterized by phase solubility diagram, in which an A_L –isotherm type was investigated. The thermodynamic parameters: enthalpy change (ΔH°), entropy change (ΔS°) and Gibbs free energy (ΔG°) of the complexes were determined in the temperatures range (293-298 $^{\circ}$K) using Van't Hoff equation. Differential scanning calorimetry (DSC) was used to confirm the formation of the inclusion complexes. Fourier Transform infra-red spectroscopy (FT-IR), X-ray diffraction (XRD) and ^{1}H, ^{13}C nuclear magnetic resonance (NMR) were also used to estimate the stoichiometry of the complexes. Molecular mechanics (MM) was used to further scrutinize the mechanism of complexation between ZER and HPβCD, using theoretical semi-emprical calculation PM6.

Pure ZER and ZER/ HPβCD complex were found to decompose with acid, base, oxidation and reduction; however, ZER/ HPβCD complex was stable in dry heat compare with pure ZER that decomposed completely. The *in vitro* dissolution study of ZER from the complex was follow first order kinetics that is independent of concentration, as well as Hugchi model kinetics.

The second objective of the study was to determine the parenteral dosage form of ZER through *in vivo* studies. ZER suspended in carboxymethyl cellulose sodium salt (CMC) was for intraperitoneal while ZER/HPβCD inclusion complex for intravenous application. New Zealand white rabbits, Sprague-Dawley rats and BALB/c mice were dosed with ZER or ZER/ HPβCD complex at equivalent doses of 10, 20 and 40 mg ZER/kg body weight respectively. The study showed that ZER/HPβCD complex has improved the pharmacokinetic parameters over pure ZER. Thus the aqueous parenteral dosages of ZER are best achieved through the use of HPβCD in a complex form.

The allometric scaling approach is analyzed and the predictive performance for this scaling method in estimating human systemic clearance, volume of distribution, area under the curve and plasma half-life is evaluated. The results show that the formulation of zerumbone in hydroxypropyl-β-cyclodextrin as an intravenous preparation has a good correlation between the animal species and human pharmacokinetics based on the species body weight.

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

PERSEDIAAN, PENCIRIAN, DAN KAJIAN FARMAKOKINETIK AWAL KOMPLEKS ZERUMBON DENGAN HIDROKSIPROPIL-β-SIKLODEKSTRIN

Oleh

ELTAYEB ELAMIN MOHAMMED EID

Mac 2012

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Zerumbon (ZER) merupakan sebatian mono-seskuiterpena daripada halia zerumbet smith. Mengikut laporan, ZER menunjukkan kegiatan *in vivo* dan *in vitro* tererti terhadap pelbagai jenis kanser seperti kanser serviks, kolon, hati dan payudara. Untuk membantu dalam pencirian ZER, kaedah HPLC dan UPLC-MS/MS telah dikembangkan dan disahkan mengikut garis panduan International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) dan Food and Drug Administration (FDA).

Oleh sebab lipofisiti tinggi (logP > 5) dan kelarutan air ZER rendah, maka bioperolehan perlu ditingkat untuk memastikan kepekatan darah tinggi sekalipun dos sederhana digunakan. Kelarutan air yang rendah menyumbang kepada pengikatan protein tinggi dan menghasilkan kepekatan ZER terapeutik rendah. Maka, dalam terapi kanser, dos tambahan diperlukan untuk memastikan kepekatan darah terapeutik tercapai. Dalam keadaan klinikal, pemuatan berlebihan sebatian ini mungkin tidak diingini. Dengan demikian, objektif pertama kajian ini ialah untuk

menentukan kelarutan dan kestabilan ZER, kerana ciri fizikokimia perlu ditentukan terlebih dahulu sebelum ianya boleh dikembangkan kepada drug berpotensi.

Hidroksipropil-β-siklodekstrin (HPβCD) telah digunakan dalam kajian ini untuk meningkatkan kelarutan dan kestabilan ZER. Kelarutan ZER telah meningkat > 100 kali ganda apabila 0.05 M HPβCD dalam air telah diguna sebagai pelarut. Kompleks ZER/HPβCD yang terbentuk dalam larutan dicirikan mengguna gambar rajah kelarutan fasa dan jenis A_I-isoterm diselidik. Parameter termodinamik: perubahan entalpi (ΔH^o), perubahan entropi (ΔS^o) dan tenaga bebas Gibbs (ΔG^o) ditentukan pada 293-298 ºK mengguna persamaan Van't Hoff. Kalorimetri imbasan pembeza (DSC) kemudian diguna untuk memastikan kompleks rangkuman telah terbentuk. Penganggarkan stiokiometri kompleks tersebut dilakukan melalui spekroskopi infra-merah transformasian Fourier (FT-IR), belauan sinar-X (XRD) dan resonans magnet nucleus ^1H, ^{13}C. Mekanik molecule (MM) diguna seterusnya untuk menentukan mekanisme pembentuk kompleks antara ZER dan HPβCD, dengan mengguna pengiraan separa empiric PM6.

ZER tulen dan kompleks ZER/HPβCD didapati tercerai selepas diperlakukan asid, bes, pengoksidaan dan penurunan. Bagaimanapun, kompleks ZER/HPβCD masih stabil dalam haba kering, sambil ZER tercerai sepenuhnya. Kajian penceraian *in vitro* terhadap kompleks ZER/HPβCD didapati menepati kinetik terbit pertama yang bersandar kepekatan dan kinetic model Hugchi.

Objektif kedua kajian ini ialah menentukan bentuk dos parenteral untuk ZER dan ini dilakukan melalui kajian *in vitro*. ZER diampaikan dalam garam natrium selulosa karboksimetil (CMC) untuk aplikasi intraperitoneum sambil kompleks rangkuman

ZER/HPβCD pula untuk intravena. Arnab New Zealand putih, tikus Sprague-Dawley dan mencit BALB/c disuntik dengan ZER atau kompleks ZER/HPβCD dengan dos setara ZER pada kadar 10, 20 dn 40 ZER/kg berat badan. Kajian ini telah menunjukkan yang parameter farmakokinetik kompleks ZER/HPβCD lebih baik daripada ZER tulen. Kesimpulannya, dos parenteral akueous ZER lebih mudah dicapai dengan menguna HPβCD dalam bentuk kompleks.

Prestasi ramalan ZER ditentukan melalui penskalaan alometri untuk menganggarkan pembersihan sistemik manusia, isipadu pengedaran dan separuh hayat plasma. Hasil kajian menunjukkan yang farmakokinetik ZER dalam rumusan HPβCD sebagai persedian intravena yang dilakukan dalam kajian haiwan, menyarankan ada perkaitan baik dengan farmakokinetik manusia berasas berat berat badan.

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I certify that a thesis Examination committee has met on 21. March.2012 to conduct the final examination of : **ELTAYEB ELAMIN MOHAMMED EID** on his Doctor of Philosophy thesis entitled "preparation, characterization and preliminary pharmacokinetic studies on complex of zerumbone with hydroxypropyl-β-cyclodextrin" in accordance with the Universities and University college 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.

Members of the Thesis Examination committee were as follows:

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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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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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ELTAYEB ELAMIN MOHAMMED EID
Date: 21 March 2012

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