UNIVERSITI PUTRA MALAYSIA

CYTOTOXIC EFFECTS OF ZERUMBONE ON OVARIAN AND CERVICAL CANCER CELL LINES

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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This thesis is dedicated to my loving family who has been supporting me.

Through the good and the bad times they have been whatever I needed.

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Chairman: Dr Ahmad Bustamam Abdul, PhD.
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Globally, ovarian cancer is the fifth most common cancer among women that affects approximately 1 in 75 women in the developed countries. Over 75% of cases were presented at an advanced stage, with disease spread beyond the ovaries. To date, cervical cancer in women remains a major problem with about 400,000 new cases per year and almost 250, 000 reported deaths. However, this disease affects predominantly poor women in underdeveloped countries. It is estimated that 60% of the global market for anticancer and anti-infectious drugs or those under clinical trial are of natural origin. Zerumbone, a sesquiterpene compound isolated from the rhizomes *Zingiber zerumbet* was shown to suppress TNF-α release and also induces apoptosis in a variety of human colonic adenocarcinoma cell lines. In this current study, the chemotherapeutic potential of zerumbone in cervical cancer (HeLa) and ovarian cancer (Caov-3) cell lines of human origin was evaluated together with cisplatin, a commercially used drug currently used for treating ovarian and cervical

cancers. Exposure of both cancer cells to a range of zerumbone concentrations demonstrated growth inhibition in both cancer cells at a dose-dependent manner. The IC_{50} values, determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) reduction assay were as follows: zerumbone; Caov-3, 24.0 μM (5.2 μg/ml), HeLa, 20.7 μM (4.5 μg/ml) and cisplatin; Caov-3, 3.7 μM (1.1 μg/ml), HeLa, 5.3 μM (1.6 μg/ml). Laser scanning confocal microscopy following AO/PI staining were used to examine morphological changes of both cancer cells after zerumbone and cisplatin treatment. Apoptotic features that included membrane blebbing and nucleus condensation were evident in both treated cancer cells. Following this, TUNEL (TdT-mediated dUTP Nick-End Labeling) assay was conducted to confirm apoptosis. The studies conducted seems to suggest that zerumbone induce cell death by stimulating apoptosis better than cisplatin, based on significantly higher percentage of apoptotic cells in zerumbone treated cancer cells as compared to cisplatin. In addition, zerumbone and cisplatin arrests cancer cells at G_2/M phase as analyzed by flow cytometry. Abnormal synthesis of IL-6 appears to contribute to the pathogenesis of several kinds of diseases and the constitutive production of IL-6 has been implicated in malignant diseases. Increased levels of IL-6 indicated the aggressiveness of a disease. IL-6 is suggested to provide prognostic value based on its role as a cancer cell growth factor. The effects of zerumbone on IL-6 levels were studied using a human base ELISA. The results indicated that zerumbone significantly decreased the levels of IL-6 secreted by both cancer cells. However, membrane-bound IL-6 receptor is still intact after zerumbone treatment as demonstrated using immunofluorescence technique. This study concludes that the

compound, zerumbone inhibits both cancer cells growth through the induction of apoptosis, arrests cell cycle at G_2/M phase and inhibits the secretion levels of IL-6 in both cancer cells. Therefore, zerumbone is a potential candidate as a useful chemotherapeutic agent in treating both cervical and ovarian cancers in future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN SITOTOKSIK ZERUMBONE TERHADAP SEL-SEL SELANJAR KANSER OVARI DAN KANSER SERVIKS

Oleh

ZETTY NADIA BINTI MOHD ZAIN

November 2004

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Secara global, kanser ovari ialah kanser yang kelima biasa berlaku di kalangan wanita dan dialami oleh 1 daripada 75 wanita di negara maju. Lebih daripada 75% kes adalah di peringkat akhir di mana kanser telah merebak ke semua bahagian ovari. Kanser serviks pada wanita merupakan masalah besar dengan 400, 000 kes baru dilaporkan setiap tahun dan menyebabkan 250, 000 kematian, tetapi dialami oleh lebih ramai wanita miskin di negara kurang membangun. Dijangkakan lebih kurang 60% drug antikanser dan anti-jangkitan baik yang sudah berada di pasaran mahupun percubaan klinikal berasal dari alam semulajadi. Zerumbone, sebatian seskuiterpen yang diasingkan daripada rizom *Zingiber zerumbet* telah menunjukkan perencatan pengeluaran TNF-α dan merangsang apoptosis pada sel-sel selanjar adenokarsinoma kolon manusia. Di dalam kajian ini, dengan menggunakan sel selanjar kanser serviks (HeLa) dan kanser ovari (Caov-3) manusia, potensi kemoterapeutik zerumbone untuk kanser ovari dan serviks dikaji bersama cisplatin, drug antikanser komersil yang sekarang digunakan untuk kanser ovari dan serviks. Pendedahan sel-sel kepada julat

kepekatan zerumbone yang berbeza menghasilkan perencatan pertumbuhan bagi kedua jenis sel kanser dengan bergantung kepada nilai kepekatan. Nilai IC_{50} yang didapati daripada asai penurunan MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) adalah seperti berikut: zerumbone; Caov-3, 24.0 μM (5.2 μg/ml), HeLa, 20.7 μM (4.5 μg/ml) and cisplatin; Caov-3, 3.7 μM (1.1 μg/ml), HeLa, 5.3 μM (1.6 μg/ml). Kajian morfologi menggunakan mikroskop fasa terbalik dan mikroskop imbasan laser konfokal selepas perwarnaan dengan AO/PI dijalankan untuk melihat perubahan morfologi bagi kedua sel kanser selepas rawatan zerumbone dan cisplatin. Ciri-ciri apoptosis seperti gelembung membran dan kondensasi nukleus telah didapati bagi kedua-dua sel selanjar yang dirawat. Seterusnya, asai TUNEL (TdT-mediated dUTP Nick-End Labeling) dijalankan untuk menentukan apoptosis. Menariknya, zerumbone didapati menyebabkan kematian sel dengan merangsang apoptosis lebih baik daripada cisplatin berdasarkan peratusan sel-sel apoptotik yang menunjukkan perbezaan yang signifikan berbanding sel-sel kanser dengan rawatan zerumbone. Tambahan pula, zerumbone dan cisplatin menahan sel-sel kanser di fasa G_2/M seperti yang dianalisis dengan menggunakan 'flow cytometry'. Sintesis IL-6 yang luar biasa dikatakan menyumbang kepada patogenesis beberapa penyakit dan penghasilan berterusan IL-6 telah diimplikasikan dalam penyakit malignan. Peningkatan tahap IL-6 menunjukkan perebakan penyakit secara agresif. IL-6 dicadangkan mempunyai nilai prognostik berdasarkan fungsinya sebagai faktor pertumbuhan sel kanser. Kesan zerumbone terhadap tahap IL-6 dikaji menggunakan ELISA. Keputusan yang didapati menunjukkan zerumbone menurunkan tahap IL-6 yang dirembes oleh kedua jenis sel kanser dengan signifikan. Walaubagaimanapun,

reseptor IL-6 yang terdapat di membran masih ada selepas rawatan zerumbone seperti yang telah didemonstrasikan melalui teknik immunofluoresen. Daipada kajian ini, didapati zerumbone merencat pertumbuhan sel-sel kanser dengan merangsang apoptosis, menahan pada fasa G_2/M dan merencat tahap IL-6 sel HeLa dan sel Caov-3. Oleh itu, zerumbone dicadangkan mempunyai potensi perubatan untuk terapi kanser serviks dan ovari.

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LIST OF ABBREVIATIONS

BCNU	Carmustine
CDK	Cyclin dependent kinases
CIN	Cervical intraepithelial neoplasia
COX-2	Cyclooxygenase-2
DC	Dendritic cells
DMSO	Dimethyl Sulphoxide
DSS	Dextran Sodium Sulfate
EDTA	Ethylenediaminetetraacetic Acid
ER	Estrogen receptor
FCS	Foetal Calf Serum
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HRT	Hormone Replacement Therapy
HSV	Herpes Simplex Virus
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
IVF	*In vitro* fertilisation
NCI	National Cancer Institute, USA
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
sIL-6R	soluble interleukin-6 receptor
STD	Sexual Transmitted Diseases
TNF	Tumor Necrosis Factor