UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ZERUMBONE FROM ZINGIBER ZERUMBET ON CERVICALCANCER-INDUCED FEMALE BALB/C MICE

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EFFECTS OF ZERUMBONE FROM *ZINGIBER ZERUMBET* ON CERVICAL CANCER-INDUCED FEMALE BALB/C MICE

By

NIRMALA DEVI TAILAN

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

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This thesis is especially dedicated to:

My loving grandmother & Aunty, who are infinitely precious to me,

&

Kaviyarasu and Vasanth, who have filled my life with joy and happiness,

&

My friends, who were there for me!

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Ahmad Bustamam Hj Abdul, PhD

Faculty : Medicine and Health Sciences

In the present study, the chemotherapeutic potential of zerumbone towards HeLa cell line and cervical cancer induced in female Balb/c mice were investigated. The chemotherapeutic potential of zerumbone was compared with cisplatin, a commercial drug used to treat cervical cancer. The cytotoxicity of both zerumbone and cisplatin towards HeLa cell line were determined using MTT assay. The findings showed that the IC_{50} value (± S.E.M) of zerumbone towards HeLa cell line was 11.3 ± 0.2 μM, whilst the IC_{50} value of cisplatin was 7.5 ± 0.3 μM. Both IC_{50} values for zerumbone and cisplatin fall within the very significant group based on the National Cancer Institute Standard. All the values are significant ($P < 0.01$). The HeLa cells were treated with IC_{50} concentration of zerumbone and cisplatin respectively for morphological analysis using inverted microscopy. The results showed significant growth retardation in HeLa

cells exposed to zerumbone and cisplatin at 24, 48 and 72 hours, whilst the control cells are well spread and confluent. Pregnant female Balb/c mice were exposed to Diethylstilbestrol (DES) at 13^{th} to 18^{th} day of gestation. The progeny of the DES-exposed mothers developed cervical intra-epithelial neoplasia. These progenies were divided into 4 groups and were either given treatment with normal saline, 8 mg/kg zerumbone, 16 mg/kg zerumbone and 10 mg/kg cisplatin. The mice were sacrificed following the treatments and their cervical tissues subjected to histological examination, TUNEL Assay and immunohistochemistry. The histological examination revealed that both zerumbone and cisplatin treatments were able to inhibit the progression of cervical dysplasia from becoming more severe dysplasia (CIN 3). In the mice treated with normal saline, the dysplasia had progressed to CIN 3 (severe dysplasia). The TUNEL assay micrographs showed that there was no apoptosis in the cervical tissue of the normal saline treated mice compared to the cervical tissue of mice treated with zerumbone and cisplatin, where abundant apoptotic cells were noticed. The levels of serum IL-6 were suppressed in mice treated with zerumbone and cisplatin. In contrast, mice treated with normal saline showed elevated level of serum IL-6. Immunohistochemistry study demonstrated that the production level of membrane bound IL-6 receptor had been suppressed by the treatment of zerumbone and cisplatin compared to mice treated with normal saline which had a higher concentration of membrane bound IL-6 receptors. This showed that both zerumbone and cisplatin act in a similar manner *in vivo* and *in vitro*. In conclusion, it is suggested that zerumbone, a plant derived compound, could be explored as a new anti cancer agent for treating cervical cancer in the future.

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

KESAN ZERUMBONE DARIPADA *ZINGIBER ZERUMBET* KE ATAS TIKUS MENCIT YANG TELAH DIINDUKSI DENGAN KANSER SERVIKS

Oleh

NIRMALA DEVI TAILAN

MAC 2007

Pengerusi: Ahmad Bustamam Hj Abdul, PhD

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Dalam kajian ini, keupayaan kemoterapeutik zerumbone terhadap sel selanjar kanser serviks (HeLa) dan tikus mencit Balb/c telah dikaji. Keupayaan terapeutik zerumbone dibandingkan dengan cisplatin, dadah komersial bagi merawat kanser serviks. Kesan sitotoksik zerumbone dan cisplatin terhadap sel HeLa dikaji dengan menggunakan asai MTT. Keputusan menunjukkan bahawa nilai IC_{50} (± S.E.M) bagi zerumbone ialah 11.3 ± 0.2 μM, manakala bagi cisplatin ialah 7.5 ± 0.3 μM. Nilai IC_{50} bagi zerumbone dan cisplatin adalah sangat signifikan berdasarkan garis panduan Institut Kanser Nasional. Semua nilai IC_{50} adalah signifikan ($P < 0.01$). Sel HeLa dirawat dengan zerumbone dan cisplatin dengan nilai IC_{50} masing-masing dan kajian morfologi dijalankan dengan bantuan mikroskopi inverted. Keputusan menunjukkan pertumbuhan sel pada jam ke-24, 48 dan 72 rawatan zerumbone dan cisplatin telah terbantut, manakala sel kawalan

tumbuh dengan sihat dan tersebar luas. Dalam kajian *in vivo*, tikus betina yang hamil diberi suntikan DES (Diethylstibestrol) pada hari gestasi 13 hingga 18. Progeni betina bagi ibu-ibu yang terdedah kepada DES, telah menggalakan pertumbuhan CIN (Cervical Intra-epithelial Neoplasia). Tikus- tikus betina yang telah terdedah kepada DES dalam rahim ibu dibahagikan kepada 4 kumpulan dan diberi rawatan dengan normal saline, 8 mg/kg zerumbone, 16 mg/kg zerumbone dan 10 mg/kg cisplatin. Tikus-tikus tersebut dibunuh dan tisu serviksnya dianalisis melalui kaedah histologi, asai TUNEL dan kaedah immunohistokimia. Kajian histologi menunjukkan zerumbone dan cisplatin dapat menghalang pertumbuhan CIN 1 (displasia awal) kepada CIN 3 (displasia teruk) pada tisu serviks tikus. Manakala bagi tikus yang hanya diberi saline normal, penyakitnya (displasia) berkembang dan mencapai CIN 3. Hasil kajian TUNEL asai menunjukkan tiada sel apoptosis diperhatikan dalam tisu serviks tikus yang dirawat dengan normal saline, manakala tikus yang dirawat dengan zerumbone dan cisplatin menunjukkan sel apoptosis yang banyak. Paras IL-6 darah bagi tikus yang dirawat dengan zerumbone dan cisplatin adalah sangat rendah berbanding tikus yang dirawat dengan normal saline dimana paras IL-6 adalah amat tinggi. Kajian immunohistokimia menunjukkan penghasilan reseptor IL-6 menurun pada tikus yang dirawat dengan zerumbone dan cisplatin, manakala tikus yang dirawat dengan normal saline menunjukkan peningkatan dalam penghasilan reseptor IL-6 secara mendadak. Kedua-dua zerumbone dan cisplatin bertindak pada kadar yang sama dalam kajian *in vitro* dan *in vivo*. Kesimpulannya zerumbone, sebatian semulajadi daripada tumbuhan dicadangkan untuk digunakan bagi menhasilkan ubatan alternatif untuk merawat kanser serviks pada masa hadapan.

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AIDS	Acquired Immuno Deficiency Syndrome
ATCC	American Type Culture Collection
CIN I	Mild dysplasia
CIN II	Moderate dysplasia
CIN III	Severe dysplasia
CIN	Cervical Intra-epithelial Neoplasia
CIS	Carcinoma in situ
CNTF	Ciliary Neurotrophic Factor
COX-2	Cyclooxygenase-2
DDP	Diamminedichloroplatinum (cisplatinum)
DES	Diethylstilbestrol
DESAD	National Cooperative Diethylstilbestrol Adenosis
DESAD	National Cooperative Diethylstilbestrol Adenosis
DMSO	Dimethylsulphoxide
DNA	Deoxyribonecleic Acid
ELISA	Enzyme Link Immuno Sorbent Assay
FCS	Feotal Calf Serum
FDA	Food and Drug Administration
FIGO	Federation of Gynecology and Obstetrics
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography

HPV	Human Papilloma Virus
HSV-2	Herpes Simplex Virus type-2
HTC	Hepatome Tissue Culture
IL-6	Interleukin-6
IL-6R	Interleukin-6 Receptor
LCB	Liquid Base Cytology
LCMS	Liquid Chromatography Mass Spectrometry
LIF	Leukemia Inhibitory Factor
MT	Metallothionein
MTT	Micro Tetrazolium
NCI	National Cancer Institute
OC	Oral Contraceptives
OSM	Oncostatin M
PBS	Phosphate Buffer Saline
RNA	Ribonucleic Acid
TdT	Terminal Deoxynucleotidyl Transferase
TNF-α	Tumour Necrosis Factor-Alpha
TPA	Tetradecanoylphorbol-13-Acetate
TUNEL	**T**dT-mediated d**U**TP **N**ick-**E**nd-**L**abeling
UV	Ultra Violet
VEGF	Vascular endothelial Growth Factor

CHAPTER 1

INTRODUCTION

Cervical cancer remains the second common cancer among women worldwide. It is, however, the most common cancer in women in developing countries accounting for 80% of all cases. Majority of tumors in these women are diagnosed at advanced stages with a resulting high mortality (Rowlands and de Barros Lopez, 2001). Human papilloma virus (HPV) has been implicated as the primary causative agent for cervical squamous cancer, whereby 99.7% of all cervical cancers had been identified to have HPV DNA (Rowlands and de Barros Lopez, 2001).

In 1943, Papanicolaou and Traut published their experience on the role of cervical cytology in identifying cancer of the cervix. The technique known as Pap smear, was rapidly incorporated as a screening test for invasive and pre-invasive lesions (Rowlands and de Barros Lopez, 2001). Despite the success of the Papanicolou Smear (Pap smear), cervical carcinoma still causes high mortality but is treatable and curable if diagnosed at early stage. Moreover, the malignant transformation of cervical epithelial cells has been reported to have a long latency period of up to 10 years (Ho et al., 1998).

To date, the most effective single chemotherapeutic agent for cervical carcinoma is cisplatin, which normally achieves a response rate of between 23% and 50% (Ozols,

2003). Even though chemotherapy has been successful for treatment of some tumors such as testicular cancer and certain leukemia, its success rate for treatment of common epithelial tumors such as breast, colon, cervical, ovarian and lung has been less impressive (Johnstone et al., 2002). A chemotherapeutic drug used as an anti-cancer drug destroys the cancerous cells by interfering with the ability of the cancer cells to divide and reproduce. A drawback to this chemotherapeutic drug, however, is that these drugs also affect normal cells.

Apoptosis is also known as programmed cell death and is a mechanism for eliminating unwanted cells or damaged cells. The cells initiate a suicide sequence which results in a quick and systematic approach in triggering cell death. It is widely believed that tumor cells treated with anticancer agents, including radiation, will die by apoptosis and the tumors that do not readily undergo apoptosis are resilient towards treatment (Lowe and Lin, 2000). Furthermore, response to tumor treatment that employs plant derived-bioactive substances in cancer patients have been implicated by apoptosis induction in tumor cells (Smets, 1994; Paschka et al., 1998).

Most of the earliest pharmaceuticals were plant-derived compounds whereby plants have been used initially to treat diseases (Peter et al., 1998). The plant-derived drugs that are useful in clinical oncology include those of flavonoids, coumarins, cinnamates or phenolics.