



UNIVERSITI PUTRA MALAYSIA

***IN VIVO* ANTI-TUMOR EFFECTS OF ANDROGRAPHOLIDE IN 4T1
BREAST CANCER CELLS-CHALLENGED MICE**

KHOO MUN HONG

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***IN VIVO* ANTI-TUMOR EFFECTS OF ANDROGRAPHOLIDE IN 4T1 BREAST
CANCER CELLS-CHALLENGED MICE.**

By

Khoo Mun Hong

161480

A THESIS SUBMITTED TO

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DECLARATION

Declaration by Undergraduate Student:

It is certified that I, Khoo Mun Hong (161480), have carried out and finished this final year project under the title of "*In vivo* Anti-tumor Effects of Andrographolide in 4T1 Breast Cancer Cells-Challenged Mice". Throughout this project, my supervisor, Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen from the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, have supervised the entirety of the project.

I hereby declare the permission to my supervisor to write and prepare the manuscript of this research to be published in any form, if I have not done so within six months from the date with a condition that my name will be included as one of the authors of this article. However, the order of the names is depend on the discretion of my supervisor.

Sincerely,

.....

(Khoo Mun Hong)

Undergraduate Student of

Degree of Bachelor Science (Honors) of Cell and Molecular Biology

Date:

Declaration by Supervisor:

The research is conducted under the supervision of me, Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen from the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia.



Sincerely,

.....

(Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen)
Supervisor of undergraduate student, Khoo Mun Hong,
Department of Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Science,
Universiti Putra Malaysia.

Date:.....

APPROVAL

The thesis titled "*In vivo* Anti-tumor Effects of Andrographolide in 4T1 Breast Cancer Cells-Challenged Mice" was prepared by Khoo Mun Hong and submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia as fulfillment of the requirement for the Degree of Bachelor Science (Honors) of Cell and Molecular Biology. The project was conducted under the supervision of Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen, from the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia.

Approved by:

.....
(Prof. Madya Dr. Janna Ong Abdullah)

Head of Department of Cell and Molecular Biology,
Faculty of Biotechnology and Biomolecular Science,
Universiti Putra Malaysia.

Date:.....

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfillment of the requirement the Degree of Bachelor Science (Honors) of Cell and Molecular Biology

***In vivo* Anti-tumor Effects of Andrographolide in 4T1 Breast Cancer Cells-Challenged Mice.**

By

Khoo Mun Hong (161480)

June 2015

Supervisor: Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen

Faculty of Biotechnology and Biomolecular Science

The traditional therapies of cancer, such as physical surgery, radiation and chemotherapy, is often associated with various unwanted side effects. Breast cancer in particular, which is among one of the most common diseases with very high mortality rate all around the world, has been the central of several studies for the discovery of alternative viable treatment method. With this in mind, we have discovered that the compound andrographolide, which is derived from a common herbal plant found widely in Asian Tropical (*Andrographis paniculata*), is a potent alternative for breast cancer treatment. The advantage of this compound is that there is no requirement for direct administration into the bloodstream for the compound to be active, as oral intake in edible hydrophobic liquid is possible. The study of the effects of andrographolide was conducted on 4T1 breast cancer cell-challenged BALB/c mice, and it showed that there was 2.0-4.3% decrease in tumor weight between treated and untreated mice. The significance in reduction in metastasis was notable

in excised lung tissue with as much as 56.4% of decrease in colony-count by clonogenic assay. The potential of andrographolide is not limited to anticancer activity, as frequent studies of the compound have report the capability of andrographolide as an antioxidant, which is potent for other application such as liver-protection activity. As such, further study of the compound is required to further elucidate the implication and toxicity of this compound.

Keywords: 4T1 breast cancer cells, metastasis, andrographolide, *Andrographis paniculata*, BALB/c mice.



Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai keperluan untuk memenuhi syarat ijazah Bachelor Sains (Kepujian) (Biologi Sel Dan Molekul)

Kesan anti-tumor *in vivo* Andrografolid dalam 4T1 Tikus Dicabar Oleh Sel Barah Payudara.

Oleh

Khoo Mun Hong (161480)

Jun 2015

Penyelia: Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen

Fakulti Bioteknologi dan Sains Biomolekul

Terapi tradisional kanser, seperti pembedahan fizikal, terapi radiasi dan terapi kimia, sering dikaitkan dengan pelbagai kesan sampingan yang tidak diingini. Khususnya kanser payudara yang merupakan antara penyakit yang kadar kematiannya paling tinggi di seluruh dunia, telah menjadi perhatian kajian untuk penemuan kaedah rawatan alternatif yang berkesan. Dengan ini, kami mendapati bahawa kompaun Andrografolid, yang berasal dari tumbuhan herba yang biasa didapati secara meluas di Asia Tropika (*Andrographis paniculata*), adalah alternatif berpotensi untuk rawatan kanser payudara. Kompaun ini mempunyai kelebihan seperti tidak perlu administrasi langsung ke dalam aliran darah untuk mengaktifkannya. Pengambilan oral dengan larutan kompaun tersebut dalam cecair hidrofobik boleh dilaksanakan. Kajian kesan andrografolid telah dijalankan ke atas tikus BALB /c yang dicabar sel kanser payudara 4T1 telah menunjukkan bahawa terdapat 2,0-4,3% penurunan berat tumor antara tikus dirawat dan tidak dirawat. Pengurangan metastasis adalah

signifikan dalam tisu paru-paru tikus tersebut dengan penurunan sebanyak 56.4% daripada analisa pengiraan koloni dalam assay klonogenik. Potensi Andrografolid tidak terhad kepada aktiviti anti-kanser, kerana kajian lain yang berkaitan dengan kompaun tersebut dilaporkan bahawa andrografolid berupaya sebagai antioksidan yang kuat untuk aplikasi lain seperti aktiviti perlindungan organ. Oleh itu, kajian-kajian yang lanjut diperlukan untuk menjelaskan implikasi dan ketoksikan kompaun ini.

Kata kunci: Sel kanser payudara 4T1, metastasis, andrografolid, *Andrographis paniculata*, tikus BALB/c.



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LIST OF ABBREVIATIONS

~%	percentage
PCD	Programmed cell death
ROS	Reactive oxygen species
$O_2^{\bullet-}$	Superoxide radical
$\bullet OH$	Hydroxyl radical
$NO\bullet$	Nitric oxide radical
NO	Nitric oxide
$R\bullet$	Organic radical
$ROO\bullet$	Peroxy radicals
$RO\bullet$	Alkoxy radicals
$RS\bullet$	Thiyl radicals
$ROS\bullet$	Sulfonyl radicals
$RSOO\bullet$	Thiyl peroxy radicals
RSSR	Disulfides
ROOH	Hydrogen peroxide
HOCl	Hypochloride
ONO^-	Peroxynitrite
$O=NOOCO^{2-}$	Nitrosoperoxycarbonate anion
O_2NOCO^{2-}	Nitrocarbonate anion
N_2O_2	Dinitrogen dioxide
NO^{2+}	Nitronium
IMU	International Medical University
~mg	milligram

~ml	millilitre
~ μ l	microlitre
UPM	University Putra Malaysia
~ $^{\circ}$ C	degree Celsius
N	Normal (BALB/c mice treatment group)
UT	Untreated control (BALB/c mice treatment group)
Andro	Andrographolide-treated (BALB/c mice treatment group)
H ₂ O	Liquid water
PBS	Phosphate buffered saline
~rpm	revolutions per minutes
~nm	nanometre (wavelength)
BHT	Butylated hydroxytoluene
TCA	Trichloroacetic acid
EDTA	Ethylenediaminetetraacetic acid
TBA	Thiobarbituric acid
~M	molar mass
IL-2	Interleukin-2
IL-1 β	Interleukin-1 β (beta)
IL-10	Interleukin-10
IFN- γ	Interferon- γ (gamma)
HRP	Horseshoe peroxidase
~g	gram
NH ₄ Cl	Ammonium chloride
Na ₂ EDTA	Ethylenediaminetetraacetate
KH ₂ PO ₄	Monopotassium phosphate

~pH	power of hydrogen
H&E	Hematoxylin and eosin
~ μ M	micromolar mass
RPMI	Roswell Park Memorial Institute
~pg	picogram
CD3	Cluster determinant 3 cell marker
CD4	Cluster determinant 4 cell marker
CD8	Cluster determinant 8 cell marker
NK1.1	Natural killer antigen 1.1
MDA	malondialdehyde

(Note: ~ numerical values)

CHAPTER 1

INTRODUCTION

In recent years, extracts or compounds derived from traditional medicinal plants have regained attention in the therapeutics for the discovery of novel and alternative drugs for treatment of many disease and the trend is on the rise. Malaysia, being located within the tropical rainforest region, has enormous diversity in flora, which is the source of many naturally-derived products. Such diversity is not restricted to the species of higher plants that are readily discovered in the rain forest, but may apply to the variability of secondary metabolites of higher plants due to biological or environmental variations (eg. seasonal variation) (Scogings *et al.*, 2015).

Breast cancer is among one of the diseases that have poor survival rate in Malaysia with more than half of the reported cases are preventable. Factors such as the poor access to information regarding risk factors, symptoms, and methods of early detection among Malaysian women are responsible for such high mortality. (Yip *et al.*, 2014). Reliance to the alternative medicines for cancer prevention and recovery has always been an awareness of the community but the lack of proper assistance and guidance from well-trained medical professionals might lead to misuse of chemicals.

The use of plant secondary metabolites in cancer therapeutics and as cancer preventives have been actively explored by oncologists and phytochemists alike in the last few decades (Kinghorn *et al.*, 2000). Andrographolide, which is derived from *Andrographolis paniculata*, a common herbal plant that is widely planted and used in most tropical Asian countries in traditional local medical practice, is a lipid-soluble molecule that is reported to be a potent antioxidant that is likely to confer anticancer effects.

In previous studies, the use of crude extracts and the pure compound of *A. paniculata* is reported to have similar pharmacological effects (Sermkaew *et. al.*, 2013). To elucidate the mechanism of andrographolide delivery and the impeding effects on growth and proliferation of cancerous cells were not known. In this study, dissolved andrographolide is administered to BALB/c and the effectiveness of andrographolide will be monitored. Different assays were conducted to test for the effects of andrographolide on biological samples. For instance, cytokine assays were used to detect the variation in different cytokine levels in different groups of biological samples and clonogenic assay are conducted to monitor the metastasis of the cancer cells to other organs, i.e. lungs and bone marrows.

Objectives

- 1) To determine the anti-tumor effects of andrographolide on 4T1 breast cancer-induced BALB/c mice.
- 2) To evaluate the effects of andrographolide in impeding metastasis of cancer cells to other organs.
- 3) To elucidate the effects of andrographolide on different cytokine levels in treated BALB/c mice.

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