ALOE EMODIN AS AN AGENT TO ENHANCE THE CYTOTOXIC EFFECTS OF TAMOXIFEN IN BREAST CANCER CELLS

DAYANG ZAHIDAH BINTI ABANG OTHMAN

UNIVERSITI TEKNOLOGI MARA

AUGUST 2012

ACKNOWLEDGEMENT

I would like to extend my deepest gratitude to the Faculty of Medicine, Universiti Teknologi MARA (UiTM) for giving me the opportunity to be part of the MBBS with Advanced Medical Science (AMS) programme, which has given me the most fruitful and wonderful experiences in my life.

My special thanks in particular to Dr Rosfaiizah Siran for being a very helpful, supportive, understanding and lovely supervisor along this one-year programme. Her constructive suggestions, views and guidance have been greatly helpful to me not only in producing this dissertation but also as a good researcher and a better person. Not to forget my co-supervisor, Dr Narimah Hamid Hasani, thank you so much for the suggestions, views and guidance that have been helpful in order to make my study a success.

My gratitude also goes to my co-research teammates, Melor Mohd Daud and Mohd Ridzuan bin Hamid for being so helpful and cooperative along my study.

I would also like to thank A/P Dr Gabrielle Ruth Anisah Froemming, the Director of Institute of Medical Molecular Biotechnology (IMMB), UiTM for giving me the opportunity to work in their laboratories. I also wish to thank all parties; especially my colleagues in the Cell Culture Unit and Imaging Unit of IMMB who have willingly helped me out with their abilities in making this research a success.

To all my friends and course mates, thank you very much for the support and understanding.

Last but not least, to both my lovely and understanding parents, and thank you so much for the prayers, patience and su pport. Not to forget my siblings; I will always love all of you and thank you so much for the support and understanding.

TABLE OF CONTENTS

ALOE EMODIN AS AN AGENT TO ENHANCE THE CYTOTOXIC EFFECTS OF TAMOXIFEN IN BREAST CANCER CELLS

ABSTRACT

Tamoxifen is a well-established therapy of choice for breast cancer management. However, there are growing evidences indicate the emergence of tamoxifen resistances in treating the breast cancer. Aloe emodin (1,8-dihydroxy-3-hydroxymethyl-anthraquinone) is a herbal anthraquinone derivative of aloe vera which has been suggested to induce apoptosis in cancer cells by efficiently limiting various tumour cell proliferation as well as restricting cancer growth without affecting the normal cells. Hence, the objectives of this study are to determine the threshold doses of aloe emodin and tamoxifen in the MCF7 breast cancer cells and to clarify the effect of aloe emodin, tamoxifen and combination of both treatments on the MCF7 breast cancer cells as compared to the MCF10A normal breast cells using the threshold doses. Cell proliferation assay was initially conducted to determine the threshold doses of tamoxifen and aloe emodin on MCF7 cells. The threshold doses for MCF10A cells have been determined earlier by other co-researcher in different project. There were three main treatments in this study: aloe emodin, tamoxifen or combination of both which were conducted in both MCF7 and MCF10A cells. The Trypan blue exclusion assay was then conducted in time dependent manner after each treatment for the MCF7 and MCF10A cells using the determined threshold doses. The viable cells were identified and counted at the end of 18, 24, 36 and 48 hours. Apoptosis morphology evaluation assay was performed by viewing the cells under the inverted light microscope after each treatment at the end of 18, 24, 36 and 48 hours. Further morphological changes were studied using the acridine orange and propidium iodide dual staining on both cells at the 48 hours post treatment using a fluorescence microscope. Aloe emodin and tamoxifen exerted a significant threshold effect on the MCF7 cells by significantly halved the number of the viable cells after the 24 hours post treatment at the concentrations of 30 μM and 50 μM, respectively. Aloe emodin induced significant reduction on the percentage of viable cells in

CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

Breast cancer is reported to be the most common cancer among women (Agarwal *et al.*, 2007; Parsa *et al.*, 2006) and appears to be the second leading cause of cancer-related death in the world (Agarwal *et al.*, 2007; Hortobagyi *et al.*, 2005). Furthermore, breast cancer is an unpredictable and fast growing disease. In 2004, the National Cancer Registry of Malaysia has suggested that one in twenty Malaysian women have a risk towards breast cancer (Yip *et al.*, 2006). Tamoxifen, a well-established therapy of choice for breast cancer management, has been remained as the endocrine treatment of choice in hormone receptor positive breast cancer for the past 30 years (Gonzalez-Angulo *et al.*, 2007; Karn *et al.*, 2010; Singh Ranger, 2005; Swaby *et al.*, 2007) either in primary or metastatic disease stages as well as a form of adjuvant therapy (Kurebayashi, 2005). Interestingly, tamoxifen has also been suggested to have chemopreventive properties on breast cancer cells (Kurebayashi, 2005; Swaby *et al.*, 2007). However, it is accepted that hormone receptor positive breast cancer demonstrates some degree of resistances to endocrine therapy including tamoxifen (termed as *de novo* resistance). Furthermore, metastatic breast cancer which initially responds well to endocrine therapy also develops some degree of resistances after prolonged treatment (termed as acquired resistance) (Gonzalez-Angulo *et al.*, 2007; Kurebayashi, 2005). Both *de novo* and acquired resistances are the vital emerging problems in the breast cancer management. Several potential mechanisms of action on the development of endocrine therapy resistances have been extensively studied and proposed; however, a common signalling transduction pathway has not yet been verified (Kurebayashi, 2005).