UNIVERSITI TEKNOLOGI MARA

MECHANISM OF ALOE EMODIN-INDUCED APOPTOSIS IN ER^{+}-BREAST CANCER CELLS, MCF-7

INDAH BINTI MOHD AMIN

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ABSTRACT

Aloe emodin, an anthraquinone exhibits higher cytotoxicity to hepatoma, prostate and cervical cancer cells through cell cycle arrest and apoptosis compared to normal cells. However, its underlying mechanism on ER^+-breast cancer cell death remains unclear. Therefore, this study was done to investigate aloe emodin cytotoxicity and its mechanism on estrogen receptor (ER)-positive (MCF-7), ER-negative breast cancer cells (MDA-MB-231) and control breast cells (MCF-10A) in comparison with tamoxifen. Cytotoxicity was determined using WST-1 proliferation assay and Trypan blue exclusion test. Apoptosis mechanism was investigated using Annexin V-FITC/PI staining and DNA fragmentation assay. Both genes and proteins involved in the regulation of cell cycle (p53, p21, CDK1, CDK2, cyclin B1 and cyclin E1) and apoptosis (Fas, FADD, Caspase-3, Caspase-8, Caspase-9, Bax, Bcl-2, and Cytochrome c) in aloe emodin-treated MCF-7 were determined using Quantigene 2.0 Plex and protein ELISA assays respectively. Maximum treatment time was set up to 72 hours in all assays. Aloe emodin inhibited the proliferation of MCF-7 with IC_{50} of 80μM. No IC_{50} value was obtained on MDA-MB-231 and MCF-10A, even up to 150μM. In contrast, tamoxifen was non-selective to all cells with IC_{50} of 27μM, 19μM and 42μM, respectively. IC_{50} values obtained were used in all the other assays. Results from Trypan blue exclusion test were in concordance with the proliferation assay. Study on Annexin/PI staining showed the presence of early and late apoptosis (18.42% ± 3.53 to 29.25% ± 0.55; $p<0.05$, n=3 and 28.45% ± 2.36 to 30.22% ± 0.56; $p>0.05$, n=3, respectively) in aloe emodin and tamoxifen-treated MCF-7 cells. Accordingly, DNA fragmentation was observed. Aloe emodin and tamoxifen enhanced MCF-7 cytotoxicity through apoptosis. In cell cycle signalling, aloe emodin upregulated the expression of p53 and p21 proteins; while downregulating CDK1. Only CDK1 protein is in accordance with gene expression. In intrinsic apoptosis signalling, Bax, Cytochrome c and Caspase-9 proteins were upregulated; while no change observed in Bcl-2 protein. Except for Caspase-9, these results are in accordance with gene expression. In extrinsic apoptosis, Fas and Caspase-8 were upregulated, contrary to gene expressions. These findings indicate that aloe emodin cytotoxic action on MCF-7 cells is through G2/M arrest; both extrinsic and intrinsic apoptosis pathways. Its actions on G2/M phase arrest and activation of intrinsic apoptosis pathways were p53-dependent, while extrinsic apoptosis was p53-independent. Data obtained suggests (i) aloe emodin has potential as a selective apoptotic inducer in ER^+-breast cancer management and (ii) and the present study could be used as a basic rationale for *in vivo* experiment setting.

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TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 RESEARCH BACKGROUND

Breast cancer is the most frequent type of cancer (18.1%) with an occurrence of 32.1% among Malaysian women as compared to other types of cancer (Omar & Tamin, 2011). The incidence and death among women remain a major concern not only for Asian countries, but also worldwide. Approximately two-thirds of breast cancer patients expressed estrogen receptor (ER), and received endocrine treatment with anti-estrogens such as tamoxifen, toremifene, raloxifene, and fulvestrant (Clarke et al., 2003; Baumgarten & Frasor, 2012). Despite these therapeutic advances treatment, the overall incidence rate is still unacceptable. Mortality reported from breast cancer is still alarming (Baumgarten & Frasor, 2012).

Over the past few years, the anti-cancer drugs available target on killing the most actively proliferating cell. It is a well-accepted concept as cancer is generally defined as uncontrolled cell growth. However, these drugs affect not only cancer cells but also destroy the normal active proliferating cells such as bone marrow, gastrointestinal epithelial and dermal papilla of hair follicle. The treatment outcome unfortunately worsens the patients' situation as increasing evidences of toxicity incidences were seen (Yang et al., 2013). Besides being non-selective, most of the drugs were reported to cause resistance in cancer patients with adverse effects after prolonged exposure (Fisher et al., 2001).

The prospect of phytochemicals extracted from potential medicinal herbs or plants to stimulate tumor growth arrest and death has recently been of interest to many researchers. Primarily, the natural plants have been practically used as traditional remedies and are believed to be able to heal wounds and cure many types of diseases such as diabetes, osteoarthritis, malaria, skin diseases, cancer and other critical diseases for many years. Moreover, the increasing interests and the use among the consumers contributed to the popularity of these approaches as