UNIVERSITY TEKNOLOGI MARA

THE ANTI-PROLIFERATIVE PROPERTIES OF TINOSPORA CRISPA ON TRIPLE NEGATIVE BREAST CANCER CELL LINES

REYADH RADHI AL-RASHIDI

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AUTHOR’S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulation of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student : Reyadh Radhi Al-Rashidi

Student I.D. No. : 2009582183

Programme : Master of Science (Molecular Medicine)

Faculty : Medicine

Thesis Title : The anti-proliferative properties of *Tinospora crispa* and possible role in treatment of triple negative breast cancer

Signature of Student : 

Date : December 2013
ABSTRACT

*Tinospora crispa* is a traditional medicinal plant in Malaysia with anti-cancer properties as shown in recent studies. The main objective of this study was to determine the anti-proliferative effect of *T. crispa* methanol extract on triple negative breast cancer. MTT assay was performed to determine the cell viability of triple negative breast cancer cell lines (MDA-MB-231 and HCC1806) and normal breast cell line (MCF-10A). The type of cell death was determined using flow cytometry and cellular DNA fragmentation ELISA while Comet assay was used to determine the genotoxicity. qPCR was used to investigate the mRNA expression levels of the caspases 3, 8, 9 and NF-κB. The present results showed that *T. crispa* decreased the cell viability in triple negative breast cancer cells in a dose dependent manner with an IC₅₀ of 66±3µg/ml and 60±4µg/ml in MDA-MB-231 and HCC1806 cells respectively. While for MCF-10A, the IC₅₀ was 248±4µg/ml. The type of cell death in MDA-MB-231, HCC1806 and MCF-10A cells was mainly due to apoptosis. The comet assay data for *T. crispa* did not detect any DNA damage on MDA-MB-231, HCC1806 and MCF-10A cell lines. Our results also showed that cisplatin significantly up-regulated NF-κB gene expression. Many studies reported that the up-regulation of NF-κB increases the resistance of cancer cells to apoptosis. Unlike cisplatin, *T. crispa* did not show any significant change in the mRNA expression levels of NF-κB. Furthermore, when used in combination *T. crispa* and cisplatin, the combination significantly down-regulated the gene expression of NF-κB and significantly up-regulated the gene expression of caspases 3, 8 and 9 in the cancer cells compared to single usage indicating more apoptotic activity. In Conclusion, *T. crispa* showed anti-proliferative effect on triple negative breast cancer cells with less toxicity to the normal breast cells. The cell death was mainly due to apoptosis and the combination of *T. crispa* and cisplatin significantly down-regulated the NF-κB gene expression which in turn increased apoptosis in the cancer cell lines.
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CHAPTER ONE
INTRODUCTION

1.1 OVERVIEW

Triple negative breast cancer (TNBC) is a subtype of breast cancer that accounts for approximately 15% - 20% of breast cancer cases. TNBC is negative for expression of progesterone receptor (PR), oestrogen receptor (ER) and erythroblastic leukaemia viral oncogene homolog 2 (ErbB2) [also known as human epidermal growth factor receptor 2 (HER2)]. It is characterized by its aggressive behaviour, unique molecular profile, lack of targeted therapies and distinct pattern of metastasis [1]. The pattern of metastasis of TNBC is more directed to an orderly progression from the primary site to the regional lymph node or the sentinel lymph node in the majority of cases with subsequent dissemination to the systemic sites. TNBC have higher tendency of visceral metastasis as compared to other types of breast cancer. This may be explained in part by altering the expression of Epidermal Growth Factor Receptor (EGFR) which in turn promotes aggressive pattern of metastasis in TNBC cells [2, 3].

Several studies showed the efficacy of cisplatin in treatment of TNBC [4-6]. Strategies to optimize the existing chemotherapy treatments have the potential to consolidate chemo-sensitivity and decrease their side effects. Recent studies suggested that a combined therapy using plant extracts and cisplatin can improve the anti-proliferative activity and reduce the side effects of cisplatin in several types of cancers [7-9].

Cancer chemotherapeutic agents can often provide prolongation of life, temporary relief from symptoms and occasionally, cures. An efficient chemotherapeutic agent should incapacitate or kill cancerous cells without causing unnecessary damage to the normal cells. Inducing apoptosis in cancerous cells could accomplish this ideal condition since apoptosis only involves one cell and it will not lead to inflammation, destruction, or scarring and fibrosis of adjacent tissues. The existence of both cancer and normal cells is considerably affected by the rate of apoptosis [10]. Therefore, modulating apoptosis may be valuable in the prevention or healing of cancer.