UNIVERSITI PUTRA MALAYSIA

MECHANISMS OF NORDAMNACANTHAL-INDUCED APOPTOSIS IN BREAST CANCER CELL LINES (MCF-7) AND ACUTE T-LYMPHOBLASTIC LEUKEMIA (MOLT-4)

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By

NORSYAFINI BINTI ISHAK

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Trustful humanity placed its faith in the belief that there exist cures in the plants of field and forests for every ill (James et al., 2007).

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the Degree of Master of Science

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Chairperson : Latifah Saiful Yazan, PhD

Faculty : Faculty of Medicine and Health Sciences

Nordamnacanthal, an anthraquinone extracted from the root of *Morinda elliptica* from Rubiaceae family has cytotoxic properties towards cancer cell lines and antitumor promoting activities. This study was conducted to determine the mechanisms of nordamnacanthal-induced apoptotic cell death in the breast cancer (MCF-7) and acute T-lymphoblastic leukemia (MOLT-4) cell lines at the concentration that reduced 50% of number of viable cells as compared to the untreated sample (IC_{50}) and that caused 50% of the total cell population underwent apoptosis (50% Apoptosis), inclusive of analysis on the cell viability, cell cycle, and the expression of Bcl-2, Bax, caspases (caspase-2,-3,-6,-8 and -9) and p53. Nordamnacanthal was found to be more cytotoxic towards MOLT-4 than MCF-7 with the IC_{50} value of 3.8 µg/ml and 54 µg/ml, respectively, as detected by using the trypan blue dye exclusion test. However, by using staining with acridine orange (AO) and propidium iodide (PI) nordamnacanthal caused 50% of the total cell population of MCF-7 and MOLT-4 to undergo apoptosis (50% Apoptosis) at 70 µg/ml and 15 µg/ml, respectively, as analyzed by using a fluorescence microscope. The nordamnacanthal-treated MCF-7 and MOLT-4 cells at both concentrations employed (IC_{50} and 50% Apoptosis) showed characteristics of apoptosis such as membrane blebbing, chromatin condensation in the nucleus and the formation of

apoptotic bodies observed under an inverted light microscope. Cell cycle analysis by flow cytometry indicated that nordamnacanthal did arrest MCF-7 cells at the G2/M phase at both IC_{50} and 50% Apoptosis values. For MOLT-4, the cell cycle was arrested at G0/G1 and S phases. In the treatment at the IC_{50} value, significant downregulation (p<0.05) in the expression of Bcl-2 and Bax was only observed in MCF-7 as detected by using commercial kits of Enzyme-linked Immunosorbent Assay for Quantitative Detection of Human Bcl-2 and Human Bax Enzyme Immunometric Assay, respectively. All the caspases were not activated in both cell lines following the treatment (IC_{50}) as analyzed by using Apo*Target*™ Caspase Colorimetric Protease Assay kit. The expression of p53 reduced significantly (p<0.05) in MCF-7 but increased significantly (p<0.05) in MOLT-4. In the treatment at the 50% Apoptosis, significant downregulation (p<0.05) in the expression of Bcl-2 and Bax was also only observed in MCF-7. Caspase-2,-3 and -8 were found to be activated only in MCF-7. The expression of p53 reduced significantly (p<0.05) in MCF-7 only. Whereas, the expression of p53 increased significantly (p<0.05) in MOLT-4. Nordamnacanthal was found to be more cytotoxic towards MOLT-4 than MCF-7 cell line. At the IC_{50} value, induction of apoptosis involved down-regulation of Bcl-2 in MCF-7 and up-regulation of p53 in MOLT-4 cells, with no caspase activity detected for both cell lines. Meanwhile, at the 50% Apoptosis, incidence of the programmed cell death in MCF-7 involved down-regulation of Bcl-2 and activation of caspase-2, -3 and -8. On the other hand in MOLT-4, the apoptotic pathway is still unclear because it involved up-regulation of p53 protein only.

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

MEKANISMA APOPTOSIS YANG DIARUH OLEH NORDAMNAKANTAL PADA JUJUKAN SEL KANSER PAYUDARA (MCF-7) DAN SEL AKUT T-LIMFOBLASTIK LEUKEMIA (MOLT-4)

Oleh

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Pengerusi : Latifah Saiful Yazan, PhD

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Nordamnakantal, sejenis antrakuinon yang diekstrak dari akar pokok *Morinda elliptica* daripada keluarga Rubiaceae mempunyai ciri sitotoksik terhadap beberapa jujukan sel kanser dan aktiviti penggalak antitumor. Kajian ini dilaksanakan untuk menentukan mekanisma apoptosis yang diaruh oleh nordamnakantal pada jujukan sel kanser payudara (MCF-7) dan sel akut T-limfoblastik leukemia (MOLT-4) pada kepekatan yang mengurangkan 50% bilangan sel hidup berbanding sampel tanpa rawatan (IC_{50}) dan yang menyebabkan 50% dari populasi keseluruhan sel mengalami apoptosis (50% Apoptosis) termasuklah analisis ke atas kemandirian sel, kitaran sel dan penzahiran protin Bcl-2, Bax, caspase (caspase-2,-3,-6,-8 dan -9) dan p53. Nordamnakantal didapati lebih sitotoksik terhadap MOLT-4 daripada MCF-7 dengan nilai IC_{50} 3.8 μg/ml dan 54 μg/ml, masing-masing, seperti dikesan menggunakan ujian penyahwarnaan tripan biru. Akan tetapi, selepas pewarnaan dengan akridina oren (AO) dan propidium iodida (PI) nordamnakantal telah menyebabkan 50% daripada populasi keseluruhan sel MCF-7 dan MOLT-4 mengalami apoptosis (50% Apoptosis) pada 70 μg/ml dan 15 μg/ml. Sel MCF-7 dan MOLT-4 yang dirawat dengan nordamnakantal pada kedua-dua kepekatan (IC_{50} dan 50% Apoptosis) menunjukkan ciri-ciri apoptosis seperti bleb pada membran, pemadatan kromatin di dalam nukleus dan pembentukan badan apoptotik yang dilihat melalui mikroskop cahaya songsang. Analisis kitaran sel menggunakan sitometri alir menunjukkan nordamnakantal menahan sel MCF-7 pada fasa G2/M pada kedua-dua nilai IC_{50} dan 50% Apoptosis. Untuk MOLT-4, penahanan kitaran sel adalah pada fasa G0/G1 dan S. Dalam rawatan pada nilai IC_{50}, penurunan yang signifikan ($p<0.05$) pada penzahiran protin Bcl-2 dan Bax hanya dilihat berlaku pada MCF-7 yang dikesan menggunakan kit komersil *Enzyme-linked Immunosorbent Assay for Quantitative*

Detection of Human Bcl-2 dan *Human Bax Enzyme Immunometric Assay*, masing-masing. Caspase tidak diaktifkan dalam kedua-dua jujukan sel pada IC_{50} yang dianalisis dengan menggunakan kit *ApoTarget™ Caspase Colorimetric Protease Assay*. Penzahiran protin p53 menurun secara signifikan (p<0.05) pada MCF-7 dan menaik secara signifikan (p<0.05) pada MOLT-4. Dalam rawatan pada 50% Apoptosis, penurunan yang signifikan (p<0.05) penzahiran protin Bcl-2 dan Bax juga hanya berlaku pada MCF-7. Caspase-2, -3 dan -8 didapati hanya diaktifkan dalam MCF-7. Penzahiran protin p53 hanya menurun secara signifikan (p<0.05) pada MCF-7, akan tetapi menaik dengan signifikan (p<0.05) pada MOLT-4. Nordamnakantal adalah lebih sitotoksik terhadap jujukan sel MOLT-4 berbanding MCF-7. Pada nilai IC_{50}, aruhan apoptosis melibatkan penurunan dan peningkatan penzahiran protein Bcl-2 dan p53 yang signifikan (p<0.05) dalam MCF-7 dan MOLT-4, masing-masing. Sementara itu, pada nilai 50% Apoptosis, kematian sel yang terancang dalam MCF-7 melibatkan penurunan penzahiran Bcl-2 dan pengaktifan caspase-2, -3 dan -8. Tapak jalan apoptosis pada MOLT-4 masih kurang pasti kerana melibatkan peningkatan penzahiran p53 sahaja.

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I certify that a Thesis Examination Committee has met on **4 March 2011** to conduct the final examination of **Norsyafini Binti Ishak** on her thesis entitled **"The Mechanisms of Nordamnacanthal-Induced Apoptotic Cell Death in the Breast Cancer (MCF-7) and Acute T-Lymphoblastic Leukemia (MOLT-4) Cell Lines"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Degree of Master of Science.

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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

NORSYAFINI BINTI ISHAK

Date: 4 March 2011

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