UNIVERSITI PUTRA MALAYSIA

IN VITRO ANTIPROLIFERATIVE ACTIVITY OF HT-29 AND HL-60 CELLS AND PROLIFERATIVE ACTIVITY OF MSC TREATED WITH CHANNA STRIATUS BLOCH CRUDE EXTRACTS

NUR SYAMSYIAH BINTI MOHD JAMIL

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MASTER OF SCIENCE

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IN VITRO ANTIPROLIFERATIVE ACTIVITY OF HT-29 AND HL-60 CELLS, AND PROLIFERATIVE ACTIVITY OF MESENCHYMAL STEM CELLS TREATED WITH *CHANNA STRIATUS* BLOCH CRUDE EXTRACTS

By

NUR SYAMSYIAH BINTI MOHD JAMIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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

IN VITRO ANTIPROLIFERATIVE ACTIVITY OF HT-29 AND HL-60 CELLS, AND PROLIFERATIVE ACTIVITY OF MESENCHYMAL STEM CELLS TREATED WITH _CHANNA STRIATUS_ BLOCH CRUDE EXTRACTS

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NUR SYAMSYIAH BINTI MOHD JAMIL

October 2012

Chair: Professor Abdul Manan Bin Mat Jais, PhD

Faculty: Faculty of Medicine and Health Sciences

Channa striatus (Haruan) has been traditionally used for wound healing especially for post-natal women and after surgery. It is scientifically proven to possess analgesic properties. Until today, the effectiveness of anticancer drugs is limited due to its toxicity to cancer cells as well as normal cells and bone marrow. The present study was carried out to determine the haruan crude extracts (Haruan traditional extract liquid phase [HTE_A] and solid phase [HTE_S], haruan chloroform extract [HCE] and haruan methanol extract [HME]) *in vitro* cytotoxic activity on promyelocytic leukemia (HL-60) cells and colorectal adenocarcinoma (HT-29) cells and proliferative activity on mesenchymal stem (MSC) cells. The extracts were found to induce anti-proliferative effect on HL-60 and HT-29 cells and proliferative effect on MSC cells after tested using MT assay. HTE_S most effective to inhibit cell growth of HL-60 cells at 50% of cell population (IC_{50}) values were 44.0 ± 0.37 µg/ml, 38.0 ± 0.52 µg/ml and 5.0 ± 0.83 µg/ml respectively, after treated for 24, 48

and 72 hours. HCE and HME also able to inhibit the growth of HL-60 cells which the IC_{50} values ranged from 78.0 ± 0.25 µg/ml at 48 hours up to 18.0 ± 0.32 µg/ml at 72 hours. However, only HME shows inhibition effect effectively against HT-29 which IC_{50} value was 78.0 ± 0.28 µg/ml at 72 hours. For comparative purposes, the IC_{50} values of several commercial anticancer drugs against HL-60 and HT-29 cells were tested. Doxorubicin was more significant to inhibit HL-60 cells with the IC_{50} was <5.0 ± 0.2 µg/ml at 24 hours of incubation period, while the 5-fluorouracil treated on HT-29 cells showed the IC_{50} values at 84.0 ± 0.19 µg/ml and 5.0 ± 0.71 µg/ml respectively, after 48 and 72 hours of incubation period. Interestingly, all extracts were found to induce proliferation of the MSC cells. HTE_A shows the greatest value of cell proliferation which ranged from 115.4% ± 0.07 up to 148.0% ± 0.07 at 100 µg/ml. Furthermore, observation on morphological alterations indicating apoptosis was evaluated by using phase contrast and fluorescent microscopes. Analyses of AO/PI staining, DNA content and cell cycle have confirmed that the haruan crude extracts have ability in promoting apoptosis. However, the event is time-dependent. At the IC_{50} value, HTE_S, HCE and HME were able to induce apoptosis in HL-60 cells, and it also induced necrosis in HT-29 cells. Based on the results obtained, HTE_S, HCE and HME were found promoted better inhibitory effect compared to HTE_A. As a result, this study demonstrated the antiproliferative activity of haruan crude HTE_A, HTE_S, HCE and HME extracts against the HL-60 and HT-29 cell lines, as well as its ability to induce proliferation of MSC cells, which is in line with traditional claims that haruan promotes tissue growth.

Abstrak tesis yang dikemukakan kepada Senat Unversiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

RAWATAN EKSTRAK MENTAH BLOCH *CHANNA STRIATUS* TERHADAP AKTIVITI ANTI-PEMBIAKKAN SEL HT-29 DAN HL-60 DAN AKTIVITI PEMBIAKKAN SEL STEM MESENKIMAL SECARA *IN VITRO*

Oleh

NUR SYAMSYIAH BINTI MOHD JAMIL

Oktober 2012

Pengerusi: Profesor Abdul Manan Bin Mat Jais, PhD

Fakulti: fakulti Perubatan dan Sains Kesihatan

Channa striatus (Haruan) digunakan secara tradisional untuk merawat luka khususnya untuk wanita selepas melahirkan dan selepas pembedahan. Ia terbukti secara saintifik memiliki kesan analgesik. Sehingga hari ini, keberkesanan ubat antikanser adalah terhad berdasarkan sifat toksiknya terhadap sel kanser serta sel normal dan tulang sum-sum. Kajian ini dilaksanakan untuk menentukan kesan ekstrak mentah haruan (Ekstrak tradisional haruan; fasa cecair [HTE_A] and fasa pepejal [HTE_S], ekstrak kloroform haruan [HCE] dan ekstrak metanol haruan [HME]) secara *in vitro* terhadap aktiviti sitotoksik ke atas sel sel leukemial promyelositik (HL-60) dan sel usus adenokarsinoma (HT-29) dan aktiviti pembiakkan ke atas sel stem mesenkimal (MSC). Ekstrak tersebut menunjukkan kesan penggalak anti-pembiakkan ke atas sel HL-60 dan HT-29 dan kesan pembiakkan ke atas sel MSC selepas diuji menggunakan asai MTT. HTE_S paling berkesan merencat pertumbuhan sel bagi sel HL-60 pada 50% dari sel populasi

(IC_{50}) pada nilai 44.0 ± 0.37 µg/ml, 38.0 ± 0.52 µg/ml dan 5.0 ± 0.83 µg/ml masing-masing, selepas dirawat selama 24, 48 dan 72 jam. HCE dan HME juga mampu merencat pertumbuhan sel HL-60 di mana nilai IC_{50} berbeza dari 78.0 ± 0.25 µg/ml pada 48 hours sehingga 18.0 ± 0.32 µg/ml pada 72 jam. Walaubagaimanapun, hanya HME menunjukkan kesan perencatan paling berkesan terhadap HT-29 di mana nilai IC_{50} ialah 78.0 ± 0.28 µg/ml pada 72 jam. Bagi tujuan perbandingan, nilai IC_{50} dari beberapa ubat antikanser komersial terhadap sel HL-60 and HT-29 diuji. Doxorubicin lebih signifikan merencat sel HL-60 dengan nilai IC_{50} ialah <5.0 ± 0.2 µg/ml pada 24 jam tempoh inkubasi, sementara rawatan 5-fluorouracil ke atas sel HT-29 menunjukkan nilai IC_{50} pada 84.0 ± 0.19 µg/ml dan 5.0 ± 0.71 µg/ml masing-masing, pada 48 dan 72 jam tempoh inkubasi. Menariknya, semua ekstrak didapati merangsang pembiakkan MSC. HTE_A menunjukkan nilai pembiakkan sel paling ketara di antara 115.4% ± 0.07 sehingga 148.0% ± 0.07 pada 100 µg/ml. Selain itu, pemerhatian ke atas perubahan morfologi yang menunjukkan apoptosis dinilai menggunakan mikroskop fasa perbezaan dan fluoresent. Analisis pewarnaan AO/PI, kandungan DNA dan kitaran sel telah menunjukkan ekstrak mentah haruan mempunyai kebolehupayaan untuk menggalakkan apoptosis. Walaupun demikian, kesan apoptosis ini adalah bergantung pada masa rawatan. Pada nilai $IC_{50,}$ HTE_S, HCE dan HME merangsang apoptosis pada sel HL-60, dan ia juga merangsang necrosis pada sel HT-29. Berdasarkan keputusan yang diperolehi, HTE_S, HCE dan HME didapati merangsang kesan perencatan yang lebih baik berbanding HTE_A. Kesimpulannya, kajian ini menunjukkan aktiviti anti-pembiakkan ekstrak mentah haruan (HTE_A, HTE_S, HCE dan HME) terhadap sel HL-60 dan HT-29, serta kebolehupayaannya untuk merangsang pembiakkan sel MSC, sejajar dengan dakwaan tradisional iaitu haruan merangsang pertumbuhan tisu.

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I certify that a Thesis Examination Committee has met on 5th October 2012 to conduct the final examination of Nur Syamsyiah Binti Mohd Jamil on her thesis entitled "*In Vitro* Antiproliferative Activity of HT-29 and HL-60 Cells, and Proliferative Activity of Mesenchymal Stem Cells Treated With *Channa Striatus* Bloch Crude Extracts" in accordance with the Universities and University Colleges Act 1971 and the Constitution on 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.

Members of the Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

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Date:

DECLARATION

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

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NUR SYAMSYIAH MOHD JAMIL

Date:

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