UNIVERSITI PUTRA MALAYSIA

EFFECT OF GAMMA-ORYZANOL, ORYZANOL RICH FRACTION AND FRACTIONED COMPONENTS ON COLORECTAL CANCER CELL LINE (HT-29) AND CYTOKINE PRODUCTION BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

SIMA ALISHAVANDI

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By

SIMA ALISHAVANDI

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

June 2011

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DEDICATION

THIS THESIS IS DEDICATET TO

My parents, although my time in Malaysia was not easy for them, they always supported me in my aims and goals. Today I can say that what I really learnt from all my experiences in the field is that I had grown up in a privileged position with loving and caring parents, who always put me first. You are like the sunshine, always giving me the feeling of warmth, hope and peace.

My husband, I don't know what my life would be without you. It is so wonderful to have him beside me, in the past, present, and future. So many new ideas I have received from him, which kept me going. You always take my problems as your own, help me to overcome them, and encourage me to aim high level. Thank you so much for your faithful love and endless help. I could say, without you, this thesis wouldn't exist.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfillment of the Requirements for the degree of Master of Science

EFFECT OF GAMMA-ORYZANOL, ORYZANOL RICH FRACTION AND FRACTIONED COMPONENTS ON COLORECTAL CANCER CELL LINE (HT-29) AND CYTOKINE PRODUCTION BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

By

SIMA ALISHAVANDI

June 2011

Chairman: Professor Maznah Ismail , PhD

Faculty : Medicine and Health Sciences

A number of reports reveal that cells use antioxidants as part of the signaling process, responsible for activating an important mechanism for eliminating cancer cells, via programmed cell death (apoptosis). Some study also focused on the capability of antioxidants to inhibit cancer cell growth through modulation of immune system. The present study was to evaluated the Cytotoxicity effect of gamma-oryzanol (OR), oryzanol rich fraction (ORF) and major components of OR, i.e. cycloartenyl ferulate, 24-methylene cycloartanyl ferulate, Campesteryl ferulate

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and sitosteryl ferulate, on colorectal cancer cell line (HT-29), and immunomudulatory effects of those components on human peripheral blood mononuclear cell (PBMC). Cytoxicity study was performed on HT-29 by using MTS assay .The result showed that OR , ORF, cycloartenyl ferulate and 24-methylene cycloartanyl ferulate significantly inhibited cell growth with the IC_{50} value of 98, 80, 75 and 22µg/ml after 72h respectively. The results of AO/PI staining showed that after treated cells in IC_{50} value of OR, ORF, cycloartenyl ferulate and 24-methylene cycloartanyl ferulate for 72h percentages of apoptotic cell increased to 18, 38, 19 and 43 % compared to untreated group (1.63%) and the results of cell cycle assay showed an arrest in all treated group by increase in G2/M phase and a decrease in G0/G1 phase respectively. The results of AO/PI and cell cycle was confirmed by Annexin V-FITC/PI and data showed that OR, ORF, cycloartenyl ferulate and 24-methylene cycloartanyl ferulate after 72h in IC_{50} value induced 21, 30, 18 and 40% apoptosis in HT-29 cell respectively as compared to untreated group (2%). The results demonstrated that caspase -3 activities of HT-29 cells after treated with IC_{50} value of those components in 72h increased up to 0.64, 0.73, 0.51 and 0.76 % compared to untreated group (0.17%) respectively. And caspase -9 activity also increased up to 2.17, 3.75, 2.23 and 6.21 % as compared to untreated group (0.06%).Immunomodulatory study revealed that OR, ORF, cycloartenyl ferulate and 24-methylene cycloartanyl ferulate were able to stimulated the proliferation of PBMC even at low concentration (1 µg/ml) and did not inhibit

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on at higher concentration (400 µg/ml) in all treated group. The results also demonstrated that those component stimulated interferon-gamma (IFN-γ) and interleukin-2 (IL-2), production in PBMC after 72h. Data revealed that OR, ORF, cycloartenyl ferulate and 24-methylene cycloartanyl ferulate stimulated production of IL-2 up to 51, 155, 120 and 162 pg/ml respectively as compared to untreated group (2.23 pg/ml) and the production of IFN-γ also increased up to 32, 43, 45 and 47 pg/ml as compared to untreated group (6.6 pg/ml). Based on the result presented, the components OR, ORF, cycloartenyl ferulate and 24-methylene cycloartanyl ferulate can act as sytotoxic and immunomodulatory agent which are very useful in treating cancer and enhancing the immune system.

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

KESAN GAMMA-ORIZANOL, FRAKSI KAYA ORIZANOL DAN KOMPONEN FRACTIONED PADA JUJUKAN SEL KOLOREKTAL KANSER (HT-29) DAN PENGHASILAN PRODUK SITOKIN OLEH MONONUKLEAR DARAH PERIFERI MANUSIA

Oleh

SIMA ALISHAVANDI

Jun 2011

Pengerusi: **Profesor Maznah Ismail, PhD**

Fakulti: **Perubatan dan Sains Kesihatan**

Beberapa laporan mendedahkan sel-sel yang menggunakan antioksidan sebagai sebahagian daripada proses isyarat, bertanggungjawab untuk mengaktifkan mekanisme penting untuk menghapuskan kanser sel, melalui program (apoptosis). Sesetengan kajian juga memfokuskan pada keupayaan antioksidan untuk merencat pertumbuhan sel kanser melalui modulasi sistem imun. Kajian ini telah menilai kesan sitotoksisiti orizanol-gamma (OR), fraksi kaya orizanol (ORE) dan beberapa komponen utama dari OR seperti ferulat sikloartenil, 24- ferulat sikloartanil metilin,

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ferulat kampesteril dan ferulat sitosteril pada jujukan sel kanser kolorektal (HT-29), dan kesan imunomudalatori sesetengah komponen pada sel mononuklear darah periferi manusia (PBMC). Kajian sitotoksik telah dilakukan pada HT-29 dengan menggunakan asai MTS. Keputusan menunjukkan OR, ORF, ferulat sikloartenil dan ferulat 24-metilin sikloartenil secara signifikan merencat petumbuhan sel dengan niai IC_{50} aitu 98, 80, 75 dan 22 µg/ml selepas 72 jam. Keputusan pewarnaan AO/PI menunjukkan selepas sel dirawat dalam nilai IC_{50} OR, ORF, ferulat sikloartenil dan ferulat sikloartenil 24-metilin selama 72 jam, peratus sel apoptotik meningkat kepada 18, 38, 19 dan 43 dibandingkan kepada kumpulan yang tidak dirawat (1.63%) dan keputusan asai kitaran sel menunjukkan terdapat penahanan dalam semua kumpulan rawatan dengan peningkatan dalam fasa G2/M dan pengurangan dalam fasa G_0/G_1. Keputusan AO/PI dan kitaran sel disahkan dengan analisis Annexin V-FITC/PI dan data menunjukkan OR, ORF, ferulat sikloartenil dan ferulat sikloartenil 24-metilena selepas 72 jam dalam nilai IC_{50} yang diaruh dengan 21, 30, 18 dan 40% apoptosis dalam sel HT-29 apabila dibandingkan dengan kumpulan tidak dirawat (2%). Keputusan menunjukkan aktiviti caspase-3 pada HT-29 selepas dirawat dengan nilai IC_{50} sesetengah komponen dalam 72 jam meningkat sehingga 0.64, 0.73, 0.51 dan 0.76% dibandingkan dengan kumpulan yang tidak dirawat (0.17%). Aktiviti caspase-9 juga meningkat sehingga 2.17, 3.75, 2.23 dan 6.21% apabila dibandingkan dengan kumpulan yang tidak dirawat (0.06%). Kajian modulatori-imun juga mendedahkan OR, ORF, ferulat sikloartenil

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dan ferulat sikloartanil juga boleh menggalakkan proliferasi PBMC walaupun pada kepekatan rendah (1 µg/ml) dan tidak dapat merencat pada kepekatan lebih tinggi (400µg/ml) dalam semua kumpulan rawat Keputusan juga menunjukkan sesetengah komponen menggalakkan gama-interferon (IFN-γ) dan interleukin-2 (IL-2) dihasilkan dalam PBMC selepas 72 jam. Data mendedahkan OR,ORF, ferulat sikloartenil dan ferulat sikloartenil 24-metilin mengaruh penghasilan IL-2 sehingga 51, 155, 120 dam 162 pg/ml apabila dibandingkan dengan kumpulan yang tidak dirawat dengan nilai (2.23 pg/ml) dan penghasilan IFN-γ juga meningkat sehingga 32, 43, 45 dan 47 pg/ml apabila dibandingkan dengan kumpulan tidak dirawat (6.6 pg/ml). Berdasarkan keputusan yang didapati, komponen OR, ORF, ferulat sikloartenil boleh bertindak sebagai sitotoksik dan agen modulatori-imun yang sangat berguna untuk merawat kanser dan meningkatkan sistem imun.

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I certify that an Examination Committee met on to conduct the final examination of Sima Alishavandi on her Master of Science thesis entitled "Effect of gamma-oryzano, orytzanol rich fraction and oryzanol components on colorectal cancer cell line (HT-29) and cytokine production of human peripheral blood mononuclear cells (PBMC)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Chairman
Universiti Putra Malaysia
(Chairman)

Examiner 1
Universiti Putra Malaysia
(Member)

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

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DECLARATION

I declare that the thesis is my original work except for 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 other institutions.

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Date: 16 June 2011

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