UNIVERSITI PUTRA MALAYSIA

MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF ETHANOLIC NEEM (AZADIRACHTA INDICA) LEAF EXTRACT IN AN IN VIVO BREAST CANCER MODEL

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By

LAM TSUEY PENG

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

October 2007

Specially dedicated to,

My beloved mother, sister, brother, David Chieng, and all my family members

For their invaluable love, understanding, encouragement and patience

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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Chairman : Professor Fauziah Othman, PhD

Faculty : Institute of Bioscience

Breast cancer is the commonest cause of cancer death in women worldwide and Malaysia in all ethnic groups and all age groups. Neem's (*Azadirachta indica*) ability as a medicinal herb is traced as far back as 4500 years ago. Some of the impressive therapeutic qualities have been discovered such as anti-viral, anti-microbial, anti-inflammatory, anti-tumour, anti-bacterial, anti-fungal and anti-hyperglycemic; however the anticancer effect of ethanolic Neem leaves extract against breast cancer has not been documented. Besides this, Neem was found to induce apotosis in MCF-7 breast cancer cell line in local study recently. Thus, this study was done to evaluate the effect of ethanolic Neem leaves extract as apoptosis inducer in *in vivo* 4T1 breast cancer model. Two different concentrations of Neem, 250 mg/kg and 500

mg/kg were tested on 4T1 breast cancer model. The 4T1 breast cancer models were evaluated by light microscopy, transmission electron microscopy for morphological changes, TUNEL assay for apoptotic cell labeling and *in situ* RT-PCR for c-myc, c-erbB2 and c-fos oncogene expressions. All treatment groups exhibited a higher incidence of apoptosis compared to untreated group from morphological analysis and TUNEL assay. The cancerous mice treated with both different concentration of Neem showed significantly higher value ($p<0.05$) in mean body weight, mean apoptotic index and mean apoptotic score compared to the control group. At the same time both group were showing a significantly lower value of mean mitotic index in histological evaluation. The mean tumour volume and mass proved that there was evidence of tumour regression in Neem treated mice. However, the overall observation showed that 500 mg/kg of Neem has more significant effect ($p <0.05$) of inducing apoptosis in the 4T1 breast cancer cells compared to 250 mg/kg of Neem. Furthermore, the 500 mg/kg Neem concentration has significantly lengthened the mean survival time by 44.62% in the 4T1 breast cancer model ($p <0.05$). Neem 500 mg/kg group also showed a better suppression of c-myc, c-erbB2 and c-fos oncogenes expression in mean distribution and intensity score ($p< 0.05$) in the 4T1 breast cancer model. By considering all the three down regulated oncogenes (c-myc, c-erbB2 and c-fos) under effect of Neem 500 mg/kg together, it becomes clearer that Neem 500 mg/kg was effective in inducing apoptosis in the 4T1 breast cancer

model. In conclusion, the Neem 500 mg/kg treatment was effective in inducing cell death via apoptosis and regulates cell proliferation in 4T1 breast cancer model. Its effectiveness was proportional to the concentration of Neem treatment given.

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

KAJIAN MORFOLOGI DAN MOLEKULAR EKSTRAK ETHANOL DAUN NEEM (*Azadirachta indica*) KE ATAS MODEL KANSER PAYU DARA *IN VIVO*

Oleh

LAM TSUEY PENG

Oktober 2007

Pengerusi : Profesor Fauziah Othman, PhD

Fakulti : Institut Biosains

Kanser payu dara ialah kanser terkenal yang mengakibatkan kematian bagi wanita sedunia dan Malaysia bagi semua kaum dan kumpulan umur. Keberkesanan Neem (*Azadirachta indica*) sebagai herbal perubatan telah dikaji semenjak 4500 tahun yang lalu. Antara terapeutik kualitinya yang kagum yang telah dijumpai adalah seperti anti-viral, anti-mikrobial, anti-radang, anti-tumor, anti-bakteria, anti-fungus dan anti-hiperglisemic; tetapi kesan anti-kanser dari ekstrak etanol daun Neem terhadap kanser payu dara belum pernah didokumentasi. Di samping itu, Neem telah dikesani bahawa mendorong apoptotis pada MCF-7 kanser sel payu dara oleh kajian tempatan kebelakangan ini. Jadi, kajian ini dijalankan untuk menilai kesan etanol ekstrak daun Neem sebagai pemangkin apoptosis ke atas model kanser payu dara 4T1 kanser payu dara secara *in vivo*. Dua kepekatan Neem

yang berlainan, 250 mg/kg and 500 mg/kg telah diuji ke atas model kanser payu dara 4T1. Model kajian yang diuji telah dinilai melalui mikroskop cahaya, mikroskop transmisi elektron untuk mengkaji perubahan morfologi, ujian TUNEL untuk label sel apoptosis dan *in situ* RT-PCR untuk mengkaji ekspresi c-myc, c-erbB2 dan c-fos. Semua kumpulan rawatan mempamerkan insiden apoptosis yang lebih tinggi berbanding kepada kumpulan tanpa rawatan di bawah bukti uraian morfologi dan ujian TUNEL. Tikus kanser yang diubati dengan dua jenis penumpuan Neem yang berlainan menunjukkan nilai yang lebih tinggi dan ketara dari segi purata berat badan, purata indeks apoptasis dan purata markah apoptosis berbanding dengan kumpulan kawalan. Dalam masa yang sama, kedua-dua kumpulan tersebut menunjukkan nilai yang lebih rendah dengan ketaranya bagi purata indeks mitotic dalam penilaian histologi. Purata kandungan dan berat tumor telah membuktikan bahawa adanya kemunduran tumor bagi tikus kanser yang menerima perubatan Neem. Tetapi, pemerhatian keseluruhan menunjukkan bahawa 500 mg/kg Neem mempunyai kesan yang lebih ketara ($p < 0.05$) dalam memangkin apoptosis dalam 4T1 sel kanser payu dara berbanding kepada 250 mg/kg Neem. Tambahan pula, 500 mg/kg Neem telah memanjangkan purata masa hidup sebanyak 44.62 % dalam model kanser payu dara 4T1 dengan ketara ($p < 0.05$). Kumpulan Neem 500 mg/kg juga menunjukkan penindasan yang lebih bagus bagi ekpresi onkogen c-myc, c-erbB2 dan c-fos bagi purata markah taburan dan kekuatan ($p < 0.05$) di

dalam model kanser payu dara 4T1. Dengan menimbangkan kesemua tiga onkogen (c-myc, c-erbB2, c-fos) yang ditindas di bawah kesan 500 mg/kg Neem sekali, adalah lebih jelas bahawa 500 mg/kg Neem berupaya untuk menuju ke arah apoptosis di dalam model kanser payu dara 4T1. Kesimpulannya, rawatan 500 mg/kg Neem adalah berkesan dalam mendorong kematian sel melalui apoptosis dan pengawalan pembahagian sel dalam model kanser payu dara 4T1. Tahap keberkesanan tersebut adalah bergantung kepada kepekatan Neem yang diberi dalam rawatan.

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