



UNIVERSITI PUTRA MALAYSIA

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

LAM TSUEY PENG.

IB 2007 4



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BREAST CANCER MODEL**

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

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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 apoptosis 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

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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 apoptotik 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 apoptosis 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 ekspresi 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.

ACKNOWLEDGEMENTS

I would like to acknowledge a number of people, without their support the completion of this dissertation would not be possible. First, I would like to thank my research advisor, Prof. Dr Fauziah Othman, for revealing to me the beauty of science and teaching me many valuable scientific techniques; for her great guidance, support, and endless optimism and smile, which have been a constant source of inspiration and encouragement. Special thanks to my graduate committee: Assoc. Prof. Dr. Asmah Rahmat, Dr. Sharida Fakurazi, and Dr. Chong Pei Pei for their critical remarks, guidance and invaluable discussions of my project.

Thanks to my friends and colleagues: Mun Yee, Hanim, Pele, Shahrul, Hernani, Mahani, Lee Yean and Phelim for their unconditional help, support, and friendship throughout all these years. Thanks to everyone in the Microscopy Imaging and Nanoscience Unit, Institute of Biosciences: (Mr. Ho, Azilah, Aini, Rafi, Ida) sharing their priceless experiences and unsparing assistance throughout the entire microscopy work of this research.

My sincere gratitude is also accorded to Prof. Dr Nordin Hj. Lajis, Laboratory of Natural Products, Institute of Biosciences for his kindness in providing the facilities of leaves extraction to start on this study. I would also like to



acknowledge and thank Prof. Datin Paduka Dr Khatijah Mohd. Yusoff from Faculty of Biotechnology, Universiti Putra Malaysia for her generosity in providing the animal house facility to make this study possible.

I would also like to express my love and gratefulness to my mother, Tan Yook Lin, my sister, Lam Tsuey Yun and my brother, Lam Boon Chin, for their patience, love, and support. Not forgetting also my dear colleague at National Blood Centre for their kind understanding and support during my thesis writing. Most importantly, I would like to thank my boy friend, David Chieng for his love, understanding, and encouragement for being a constant source of joy and surprises.

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