



UNIVERSITI PUTRA MALAYSIA

**ENHANCEMENT OF *IN VITRO* EFFECTS OF POLYMERIC
NANOPARTICLE ENCAPSULATED TAMOXIFEN COMPARED TO
TAMOXIFEN IN MCF-7 BREAST CANCER CELL LINE**

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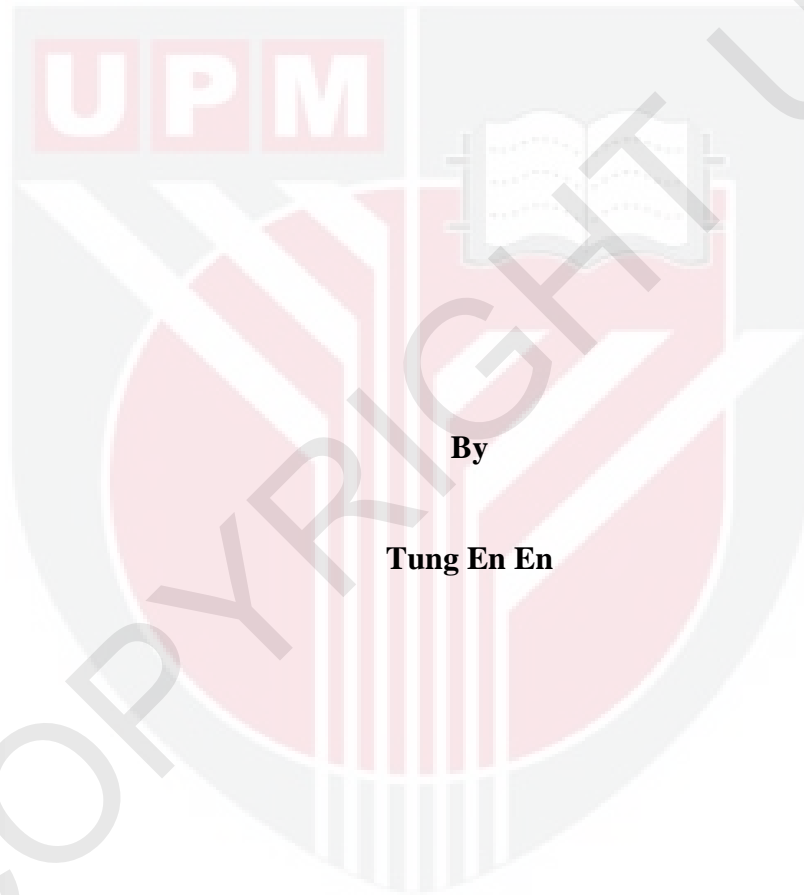


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**MASTER OF SCIENCE
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ENCAPSULATED TAMOXIFEN COMPARED TO TAMOXIFEN IN MCF-7
BREAST CANCER CELL LINE**



By

Tung En En

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Master of Science**

May 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Masters of Science

ENHANCEMENT OF *IN VITRO* EFFECTS OF POLYMERIC NANOPARTICLE ENCAPSULATED TAMOXIFEN COMPARED TO TAMOXIFEN IN MCF-7 BREAST CANCER CELL LINE

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May 2012

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Tamoxifen (TMX) is one of the common hormone therapies for breast cancer treatments. It acts as an anti-estrogen on breast cancer tissues by inducing apoptosis which is regulated by a variety of cellular signalling pathways such as tumour suppressor protein p53 and caspase-9. However, it is associated with side effects at high doses. This suggests the use of nanoparticles (NP) to deliver a lower dose of TMX with an enhance efficiency. Thus, the objective of this research was to assess and compare the *in vitro* effects of synthesized polymeric NP-encapsulated TMX to TMX toward MCF-7 breast cancer cell line. NP composed mainly of N-isopropylacrylamide (NIPAAm) were synthesized and loaded with TMX. Photon Cross Correlation Spectroscopy Nanophox (PCCS-Nanophox), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR) were used to determine the size, morphology and infrared spectrum of the NP, respectively. The drug release pattern of the NP was investigated through dialysis. To study the cytotoxicity properties, MTT assay was performed on

breast cancer cell line, MCF-7. The apoptotic effects were determined qualitatively through acradine orange and propidium iodide (AO/PI) stains with fluorescent microscopy, and quantitatively through FITC Annexin V and PI staining with flow cytometry. The expression levels of tumour suppressor protein p53 and caspase-9 were determined through ELISA. Finally, the data collected were analyzed by One Way Analysis of Variance (ANOVA), Tukey's test. In this study, the polymeric NP were successfully prepared by gamma irradiation, forming spherical NP at a size of 49.89 ± 0.55 nm in diameter. TEM images showed that the particles were spherical in shape with a distinct core-shell structure. It was demonstrated the void polymeric NP were non-toxic, and were able to release the drug in a sustained manner with 50.99 ± 1.21 % entrapment efficiency, underscoring the potential of these NP as a carrier for drugs. The proliferation of MCF-7 cells was significantly inhibited by the TMX-NP with a lower 50% inhibitory concentration (IC_{50}) value of 24.63 ± 1.56 μ M at 48 hr. It also possessed a greater apoptotic effect, resulting in a percentage of $68.53 \pm 3.81\%$ at 32.0 μ M. Furthermore, higher levels of p53 (23.22 ± 2.79 U/ml) and caspase-9 (85.35 ± 11.1 ng/ml) were detected in a dose-dependent manner. In conclusion, the therapeutic effects of the synthesized TMX NP were enhanced when compared to TMX. Its potential is not limited to anti-cancer drugs, but may also be applied in other drugs and diseases.

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

**PENINGKATAN KESAN-KESAN *IN VITRO* NANOPARTIKEL POLIMER
TAMOXIFEN DIBANDINGKAN DENGAN TAMOXIFEN DALAM
SEL KANSER PAYUDARA MCF-7**

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Tamoxifen (TMX) merupakan salah satu terapi hormon yang biasa digunakan dalam rawatan kanser payudara. Ia bertindak sebagai anti-estrogen pada tisu kanser payudara melalui apoptosis, di mana apoptosis dikawal oleh pelbagai laluan selular isyarat seperti protein p53 penekan tumor dan caspase-9. Walau bagaimanapun, ia boleh mengakibatkan kesan-kesan sampingan pada dos yang tinggi. Ini mencadangkan penggunaan nanopartikel (NP) menyampaikan TMX pada dos yang lebih rendah dengan meningkatkan kecekapannya. Justeru, objektif kajian ini adalah untuk membandingkan kesan *in vitro* NP polimer yang mampu memuatkan TMX dengan TMX asal ke atas sel kanser payudara, MCF-7. NP yang terdiri daripada N-isopropylacrylamide (NIPAAm) telah disintesis dan dimuatkan dengan TMX. *Photon Cross Correlation Spectroscopy Nanophox* (PCCS-Nanophox), *Transmission Electron Microscopy* (TEM), dan *Fourier Transform Infrared Spectroscopy* (FTIR) digunakan untuk menentukan saiz, morfologi dan spektrum inframerah NP tersebut, masing-masing. Dialisis dilakukan

untuk menentukan ciri-ciri pelepasan TMX dari NP. MTT dijalankan ke atas sel MCF-7 untuk mengkaji ciri-ciri sitotoksiti. Kesan kualitatif apoptosis ditentukan dengan pewarnaan acradin oren dan propidium iodida melalui mikroskop floresen, manakala kesan kuantitatif ditentukan dengan FITC Annexin V dan propidium iodida melalui flositometer. Tahap ekspresi p53 dan caspase-9 ditentukan melalui ELISA. Akhir sekali, data yang diperoleh dianalisis dengan menggunakan Analisis Varian Satu Hala (ANOVA), uji Tukey. Dalam kajian ini, NP polimer telah berjaya disediakan melalui sinaran gama, dengan bentuk sfera yang bergaris pusat $49.89 \pm 0.55\mu\text{m}$. Imej TEM menunjukkan bahawa partikel-partikel tersebut berstruktur sfera dan mempunyai lapisan luar dan dalam. NP tersebut adalah tidak bertoksik dan TMX yang dimuatkan dilepaskan secara perlahan-lahan dengan kecekapan pengkapsulan $50.99 \pm 1.21\%$. Ciri-ciri ini menjadikan NP tersebut sebagai penghantar ubat yang efektif. Proliferasi sel MCF-7 dihalang oleh TMX-NP dengan nilai perencatan 50% (IC_{50}) yang lebih rendah, iaitu $24.63 \pm 1.56\ \mu\text{M}$ pada 48 hr. Ia juga mempunyai kesan apoptosis yang lebih besar dengan $68.53 \pm 3.81\%$ pada $32.0\ \mu\text{M}$. Selanjutnya, tahap p53 ($23.22 \pm 2.79\ \text{U/ml}$) and caspase-9 ($85.35 \pm 11.11\text{ng/ml}$) juga adalah lebih tinggi dalam sel-sel yang dirawat dengan TMX-NP. Kesimpulannya, kesan terapeutik TMX-NP yang disintesis adalah lebih tinggi apabila dibandingkan dengan TMX. Potensi NP ini tidak terhad kepada ubat antikanser sahaja, tetapi boleh diaplikasikan juga dalam ubat-ubatan dan penyakit-penyakit lain.

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I certify that a Thesis Examination Committee has met on the 25th May 2012 to conduct the final examination of Tung En En on her thesis entitled "**Enhancement of *in vitro* effects of polymeric nanoparticle encapsulated tamoxifen compared to tamoxifen in MCF-7 breast cancer cell line**" 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 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 at any other institution.



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Date: 25 May 2012

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 INTRODUCTION	1
1.1 Introduction	1
1.2 Hypothesis	4
1.3 Objectives	4
1.3.1 General Objective	4
1.3.2 Specific Objectives	4
2 LITERATURE REVIEW	5
2.1 Breast Cancer	5
2.1.1 Definition	5
2.1.2 Overview of Breast Cancer in the World	5
2.1.3 Overview of Breast Cancer in Malaysia	6
2.1.4 Breast Cancer Treatment	7
2.2 Apoptosis	9
2.2.1 Tumour Suppressor Gene p53	9
2.2.2 Caspase-9	10
2.3 Tamoxifen	11
2.3.1 Function of Tamoxifen	11
2.3.2 Limitation of Tamoxifen	13
2.3.3 Breast Cancer Cell Lines Responsive to Tamoxifen	14
2.4 Nanotechnology and Nanoparticle Systems	16
2.4.1 Recent Development of Nanotechnology	16
2.4.2 Types of Nanoparticle Systems in Drug Delivery	17
2.4.3 Nanoparticle Systems in Anti-cancer Drug Delivery	18
2.4.4 Recent development of Tamoxifen-Nanoparticles	18
2.5 Polymeric Nanoparticle	19
2.5.1 Background	19
2.5.2 N-isopropylacrylamide	20
2.5.3 Preparation of Polymeric Nanoparticles	21

3	METHODOLOGY	25
3.1	Introduction	26
3.2	Synthesis of Nanoparticles	26
3.2.1	Chemically Induced Polymerization	26
3.2.2	Gamma Radiation Induced Polymerization	26
3.3	Characterization of Nanoparticles	27
3.3.1	Photon Cross Correlation Spectroscopy	27
3.3.2	Transmission Electron Microscopy (TEM)	27
3.3.3	Fourier Transform Infrared (FTIR)	28
3.3.4	Loading of Tamoxifen	28
3.2.5	Encapsulation Efficiency and <i>In vitro</i> Drug Release	28
3.4	Maintenance of Cancer Cell Line	29
3.4.1	Media Preparation	29
3.4.2	Recovery and Thawing	30
3.4.3	Changing Media	31
3.4.4	Subculture	31
3.4.5	Cell Counting	32
3.5	Cytotoxicity Assay	32
3.5.1	Cell Seeding	32
3.5.2	Treatment	33
3.5.3	MTT Assay	33
3.6	AO/PI Fluorescent Staining	34
3.6.1	Seeding	34
3.6.2	Treatment	34
3.6.3	Acridine Orange and Propidium Iodide (AO/PI) Staining	34
3.7	Flow Cytometry	35
3.7.1	Cell Seeding	35
3.7.2	Treatment	36
3.7.3	FITC Annexin V and Propidium Iodide Staining	36
3.8	p53 and Caspase-9 Expression	37
3.8.1	Cell Lysis	37
3.8.2	p53 ELISA	37
3.8.3	Caspase-9 ELISA	38
3.8.4	Data Interpretation	39
3.9	Statistical Analysis	39
4	RESULTS AND DISCUSSIONS	40
4.1	Characterization of Nanoparticles	40
4.1.1	Size and Stability	40
4.1.2	Morphological Study	50
4.1.3	Infrared Spectrum	52
4.1.4	Drug Loading	56
4.1.5	Encapsulation Efficiency and <i>In vitro</i> Drug Release	58
4.2	Cytotoxicity	60
4.2.1	Void Nanoparticles	60
4.2.2	Tamoxifen-Nanoparticles and Tamoxifen	61
4.3	Analysis of Apoptosis	66

4.3.1	Microscopy	66
4.3.2	FITC Annexin V and Propidium Iodide Staining	70
4.4	Expression of p53 and Caspase-9	73
5	CONCLUSION	77
5.1	Summary	77
5.2	Recommendation	78
	REFERENCES	80
	APPENDICES	91
	BIODATA OF STUDENT	114

