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

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By

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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 enhanced 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 that 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 µM at 48 hr. It also possessed a greater apoptotic effect, resulting in a percentage of 68.53 ± 3.81% at 32.0 µM. Furthermore, higher levels of p53 (23.22 ± 2.79 U/ml) and caspase-9 (85.35 ± 11.11ng/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.
Peningkatan kesan-kesan in vitro nanopartikel polimer tamoxifen dibandingkan dengan tamoxifen dalam sel kanser payudara MCF-7

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untuk menentukan ciri-ciri pelepasan TMX dari NP. MTT dijalankan ke atas sel MCF-7 untuk mengkaji ciri-ciri sitotoksiti. Kesaran kualitatif apoptosis ditentukan dengan pewarnaan acradin oren dan popidium iodida melalui mikroskop floresen, manakala kesaran kuantitatif ditentukan dengan FITC Annexin V dan popidium 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 ± 0.55m. 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 ± 1.21%. Ciri-ciri ini menjadikan NP tersebut sebagai penhantar ubat yang efektif. Proliferasi sel MCF-7 dihalang oleh TMX-NP dengan nilai perencatan 50% (IC50) yang lebih rendah, iaitu 24.63 ± 1.56 µM pada 48 hr. Ia juga mempunyai kesaran apoptosis yang lebih besar dengan 68.53 ± 3.81% pada 32.0 µM. Selanjutnya, tahap p53 (23.22 ± 2.79 U/ml) and caspase-9 (85.35 ± 11.11ng/ml) juga adalah lebih tinggi dalam sel-sel yang dirawat dengan TMX-NP. Kesimpulannya, kesaran terapeutik TMX-NP yang disintesiskan 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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