



UNIVERSITI PUTRA MALAYSIA

**PROTECTIVE EFFECTS OF PALM VITAMIN E ON GLUTAMA
TE-INDUCED INJURY OF ASTROCYTES**

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**PROTECTIVE EFFECTS OF PALM VITAMIN E ON GLUTAMATE-
INDUCED INJURY OF ASTROCYTES**

By

IBRAHIM BIN MUSA

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**PROTECTIVE EFFECTS OF PALM VITAMIN E ON GLUTAMATE-INDUCED
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Chair : Huzwah Khaza'ai, PhD

Faculty : Faculty of Medicine and Health Sciences

Glutamate toxicity is a major contributor to neurodegeneration in the nervous system. In the past few years, palm tocotrienol-rich fraction (TRF) has been shown to provide neuroprotection to neurons against glutamate excitotoxicity. Palm TRF is an extract of palm oil and consists of 25% α -tocopherol and 75% tocotrienols. TRF has been shown to possess potent antioxidant, anti-inflammatory, anticancer, neuroprotection and cholesterol-lowering activities. The main objective of the present study is to observe the effects of vitamin E when given to astrocytes before (pre-treatment) and after (post-treatment) glutamate excitotoxicity. A few parameters were selected; cell viability, mRNA expression of neuron specific enolase (NSE), concentration of glutathione (GSH) in the astrocytes and morphology of the cell. Cell viability was measured by using the MTT assay. NSE which is a type of neurobiological marker was observed by using the Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Finally, cell morphology was monitored under fluorescence microscope by using the acridine

orange/propidium iodide (AO/PI) assay. The concentration of glutamate (180mM) used throughout this study was only meant to cause injury to the astrocytes. Three types of vitamin E were used for the cell viability assay; tocotrienol rich fraction (TRF), tocotrienol enriched fraction (TEF) and α -tocopherol. Exposure to 180 mM glutamate resulted in 20% of cells death. There was no significant difference between the viability of the cells that were pre- and post-treated with various concentrations of TRF, TEF and α -tocopherol upon glutamate exposure. In contrast, the mRNA expression of NSE was reduced significantly when treated with TRF, but not tocopherol. At 300 ng of TRF, the NSE expression for both pre- and post-treatment was reduced by 50%. In addition, the concentration of GSH in cells treated with TRF was higher compared to the cells treated with tocopherol. Further results from the histology studies also showed that TRF not only provide a better protection against glutamate, but also able to reduce the number of necrotic and apoptotic cells. When 300 ng of TRF was given to the astrocytes, the percentage of healthy cells increased to 60% for pre-treatment and 30% for post-treatment which indicated that even at nanogram concentration, TRF protects the astrocytes against glutamate induced oxidative stress. On the other hand, high concentration of α -tocopherol showed the pro-oxidant effects which promoted cells death at 300 ng of α -tocopherol which increased the expression of NSE. The percentage of apoptotic and necrotic cell remained high upon tocopherol treatment. The results from the present study demonstrate that tocotrienols, but not α -tocopherol, protect astrocytes against glutamate-induced cells death.

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

**KESAN PERLINDUNGAN VITAMIN E DARIPADA KELAPA SAWIT
TERHADAP ASTROSIT YANG DICEDERAKAN OLEH GLUTAMAT**

Oleh

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Februari 2012

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Keracunan glutamate merupakan penyebab utama kepada neurodegenerasi di dalam system saraf. Beberapa tahun kebelakangan ini, fraksi kaya tocotrienol (TRF) daripada kelapa sawit didapati mampu melindungi neuron daripada keracunan glutamate. TRF merupakan sejenis ekstrak daripada minyak sawit yang mana ianya terdiri daripada 25% tokoferol dan 75% tokotrienol. TRF telah terbukti memiliki sifat antioksidan yang kuat, anti-keradangan (anti-inflamasi), anti-kanser, memberi perlindungan saraf serta berupaya mengawal kolestrol. Objektif utama kajian ini adalah bertujuan untuk mengenalpasti pengaruh vitamin E yang diberikan sebelum (pra-rawatan) dan selepas (pasca rawatan) pengeksitoksikan glutamat. Beberapa parameter telah digunakan, antaranya ujian kebertahanan sel, ekspresi mRNA untuk neuron spesifik enolase (NSE), kandungan glutathione (GSH) di dalam astrosit, dan morfologi sel. Kebertahanan sel ditentukan melalui ujian MTT. NSE yang merupakan sejenis penanda neurobiologi pula dipantau dengan menggunakan Reaksi Berantai Polimerase Transkripsi Songsang (RT-PCR).

Akhir sekali, morfologi sel diteliti melalui mikroskop pendafluoran dengan menggunakan ujian acridine oren/propidium iodide (AO/PI). Amon glutamate (180mM) yang digunakan sepanjang kajian ini hanya bertujuan untuk menyebabkan kecederaan kepada astrosit. Terdapat tiga jenis Vitamin E yang digunakan untuk ujian kebertahanan sel iaitu; fraksi kaya tokotrienol (TRF), fraksi tokotrienol yang diperkaya (TEF) dan juga α -tokoferol. Menerusi ujian MTT, 20 peratus sel didapati mati akibat terdedah kepada 180 mM glutamate. Tiada perbezaan yang signifikan (ketara) ke atas kebertahanan sel bagi sel-sel yang dirawat oleh TRF, TEF atau α -tocopherol sebelum atau selepas pendedahan kepada glutamat. Walau bagaimana pun, ekspresi mRNA neuron enolase spesifik (NSE) mengalami penurunan yang ketara apabila dirawat dengan TRF, tetapi tidak dengan tokoferol. Apabila 300 ng TRF diberikan kepada astrosit, ekspresi NSE berkurangan sebanyak 50% untuk pra- dan pasca-rawatan. Kajian histologi pula mendapati bahawa TRF bukan sahaja memberikan perlindungan yang lebih baik terhadap glutamat, bahkan ianya juga mampu bertindak untuk memulihkan sel akibat kecederaan glutamat. Selain daripada itu, jumlah GSH di dalam sel yang dirawat dengan TRF juga lebih tinggi berbanding dengan sel yang dirawat dengan tokoferol. Keputusan daripada kajian histologi menunjukkan bahawa TRF bukan sahaja memberikan perlindungan yang lebih baik terhadap glutamate, malah mampu mengurangkan bilangan sel nekrotik dan apoptotic. Apabila 300 ng TRF diberikan kepada astrosit, bilangan sel sihat telah bertambah sebanyak 60% untuk pra-rawatan dan 30% untuk pasca-rawatan, yang mana menunjukkan bahawa walaupun pada kepekatan serendah nanogram, TRF mampu melindungi astrosit daripada tekanan oksidatif yang disebabkan oleh glutamat. Kadar α -tokoferol yang tinggi pula sebaliknya menunjukkan kesan tindakan pro-oksidan yang mana akan merangsang kematian sel berbanding

bertindak sebagai perawat dengan peningkatan ekspresi NSE pada aras 300 ng α -tokoferol. Peratusan sel-sel apoptik dan nekrotik pula kekal tinggi apabila dirawat dengan 300 ng α -tokoferol. Daripada hasil kajian ini, didapati hanya tokotrienol memberikan perlindungan kepada astrosit terhadap kesan kematian sel yang dirangsang oleh glutamat tetapi tidak bagi α -tokoferol.



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I certify that a Thesis Examination Committee has met on 27th February 2012 to conduct the final examination of Ibrahim bin Musa on his thesis entitled "**Protective Effects of Palm Vitamin E on Glutamate-Induced Injury of Astrocytes**" 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 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.

IBRAHIM BIN MUSA

Date: 27 February 2012



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