



UNIVERSITI PUTRA MALAYSIA

**NEUROPROTECTIVE EFFECTS OF TOCOTRIENOL-RICH FRACTION
AND α -TOCOPHEROL OF VITAMIN E AGAINST GLUTAMATE
TOXICITY ON ASTROCYTE-NEURONAL MONO-CULTURE
AND COCULTURE SYSTEMS**

YAP HUI MIN

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By

YAP HUI MIN

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

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

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January 2013

Chair : Huzwah Binti Khaza'ai, PhD

Faculty : Medicine and Health Sciences

Elevated concentration of glutamate, also known as glutamate neurotoxicity, is the major contributor to pathological cell death in nervous system. It has been suggested to play a key role in neurodegenerative diseases. In recent studies, palm tocotrienol-rich fraction (TRF) has been shown to exhibit better neuroprotection than α -tocopherol against glutamate toxicity. It was also shown to exert potent antioxidant, anti-inflammatory, anticancer and cholesterol-lowering properties. The main objective of this study is to elucidate the effects of TRF and α -tocopherol pre-treatment and post-treatment against glutamate toxicity in astrocyte and neuronal cell. Besides, the synergism between astrocyte and neuronal cell in protecting glutamate neurotoxicity with the supplementation of TRF and α -tocopherol were assessed through co-culture model. Astrocyte and neuronal cell in this study were exposed to high concentration of glutamate. The behavior of cell lines responding to glutamate toxicity was determined through dose-response and time course study.

Astrocyte and neuronal cell were subjected to glutamate injury before or after TRF and α -tocopherol treatment. The effects of TRF and α -tocopherol against glutamate toxicity were assessed through MTT cell viability assay, glutathione production, neuron-specific enolase (NSE) expression study and mode of cell death study. The expression of NSE was examined through reverse-transcriptase real time polymerase chain reaction (RT- qPCR) while mode of cell death was determined through acridine orange/propidium iodide (AOPI) assay. The morphological changes due to glutamate toxicity and TRF and α -tocopherol treatments were observed under fluorescence microscope. Statistical analysis was performed using one way ANOVA with SPSS 17.0. The concentration of glutamate needed to cause 50% cell death for astrocyte and neuronal cell were 230 mM and 80 mM, respectively. The concentrations of glutamate used throughout this study were only meant to cause injury to the cells. Glutamate with concentration of 60 mM and 180 mM were used to cause injury in neuronal cell and astrocyte respectively. Generally, TRF and α -tocopherol improved the cell viability of glutamate-injured neuronal cell and astrocyte by approximately 10%. In co-culture model study, TRF and α -tocopherol post-treatments provided nearly complete protection toward glutamate toxicity. Besides, TRF and α -tocopherol post-treatments were showed to restore the glutathione content upon glutamate injury. In astrocyte, TRF pre-treatment inhibited the decrease of glutathione content. In the presence of astrocyte, TRF and α -tocopherol pre-treatments inhibited decrease of glutathione content in neuronal cell which was not observed in mono-culture model. In addition, 300 ng/mL TRF and α -tocopherol completely restored glutathione production in glutamate-injured neuronal cell in co-culture model. TRF and α -tocopherol generally increased the percentage of healthy cell and decreased the percentage of necrotic cell in both cell lines as well

as in co-culture model. TRF and α -tocopherol post-treatments with concentration of 100 to 300 ng/mL decreased the percentage of necrotic cell in glutamate-injured astrocyte and neuronal cell more than 10%. Downregulation or suppression of NSE expression was observed in glutamate induced astrocyte and neuronal cell as well as in co-culture model. In conclusion, TRF and α -tocopherol provided protection and recovery properties toward astrocyte and neuronal cell against glutamate toxicity. Similar effects between TRF and α -tocopherol were found in both astrocyte and neuronal cell against glutamate toxicity. Co-culture model in this study has demonstrated synergistic properties of astrocytes and neuronal cell. Supplementation of TRF and α -tocopherol in co-culture system further improved the recovery process and protection of astrocytes and neuronal cells compared to mono-culture.

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

KESAN PERLINDUNGAN SARAF OLEH FRAKSI KAYA TOKOTRIENOL DAN ALFA-TOKOFEROL DARI VITAMIN E TERHADAP KETOKSIKAN GLUTAMAT DALAM SISTEM MONO-KULTUR DAN KO-KULTUR SEL NEURON-ASTROSIT

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Kepekatan glutamat berlebihan juga dikenali sebagai neurotoksisiti glutamat merupakan penyumbang utama kepada patologi kematian sel dalam sistem saraf. Ketoksikan glutamat telah dicadangkan memainkan peranan penting dalam kebanyakan penyakit degeneratif saraf. Kebelakangan ini, fraksi kaya tokotrienol (tocotrienol-rich fraction/ TRF) telah didapati lebih berkesan daripada alfa-tokoferol dalam perlindungan saraf daripada ketoksikan glutamat. TRF juga telah dibuktikan mempunyai ciri-ciri yang berkesan sebagai anti-oksidan, anti-inflamasi, anti-kanser dan merendahkan kolesterol. Tujuan utama kajian ini adalah untuk mengenalpasti kesan sebelum dan selepas penggunaan TRF dan alfa-tokoferol terhadap ketoksikan glutamate astrosit dan sel neuron. Selain itu, sinergi antara astrosit dan sel neuron dengan penggunaan TRF dan alfa-tokoferol terhadap ketoksikan glutamat juga diketengahkan dalam kajian ini dengan sistem ko-kultur. Respon sel-sel terhadap ketoksikan glutamat telah ditentukan melalui respon terhadap dos dan masa inkubasi.

Dalam kajian ini, astrosit dan sel neuron telah didedahkan kepada glutamate dalam kepekatan yang tinggi sebelum atau selepas penggunaan TRF dan alfa-tokoferol. Beberapa parameter telah dikaji bagi melihat kesan TRF dan alfa-tokoferol terhadap ketoksikan glutamat dalam astrosit dan sel neuron, termasuk ujian MTT, penghasilan glutathione, ekspresi gen 'neuron-specific enolase (NSE)' dan mod kematian sel. Ekspresi NSE dikaji melalui reaksi polymerase berantai masa nyata (real-time PCR) manakala mod kematian sel diteliti melalui mikroskop fluorescence dengan ujian 'acridine orange/propidium iodide (AOPI)'. Analisis statistik 'one way ANOVA' telah digunakan dengan SPSS 17.0. Kepekatan glutamat yang diperlukan untuk menyebabkan 50% kematian sel (IC_{50}) adalah 230 mM bagi astrosit dan 80 mM bagi sel neuron telah digunakan. Dalam kajian ini, kepekatan glutamat yang digunakan hanya bertujuan untuk mengakibatkan kecederaan kepada astrosit dan sel neuron. Glutamat pada kepekatan 180 mM bagi astrosit dan 60 mM bagi sel neuron telah digunakan. Secara keseluruhan, TRF dan alfa-tokoferol berkesan menyihatkan lebih kurang 10% sel neuron dan astrosit yang dcederakan dengan glutamat. Dalam kajian model ko-kultur, penggunaan TRF dan alfa-tokoferol selepas pendedahan glutamat memberikan perlindungan yang hampir maxima kepada ketoksikan glutamat. Selain itu, rawatan TRF dan alfa-tokoferol selepas pendedahan glutamat juga berkesan memulihkan penghasilan glutathione dalam sel neuron. Pra-rawatan TRF juga didapati berkesan menghalang pengurangan kandungan glutathione dalam astrosit. Dalam kajian model ko-kultur, 300 ng/mL TRF dan alfa-tokoferol memulihkan penghasilan glutathione sepenuhnya dalam sel neuron yang telah dcederakan oleh glutamat. TRF dan alfa-tokoferol secara keseluruhannya meningkatkan peratusan sel sihat dan mengurangkan peratusan sel nekrotik dalam sel neuron, astrosit dan juga ko-kultur. Rawatan TRF dan alfa-tokoferol dengan

kepekatan 100 kepada 300 ng/mL selepas pendedahan glutamat dalam astrosit dan sel neuron menurunkan peratus sel nekrotik lebih daripada 10%. Diamati bahawa ekspresi NSE diturunkan atau dihalang dalam astrosit dan sel neuron yang dcederakan oleh glutamat. Kesimpulannya, TRF dan alfa-tokoferol memberikan perlindungan dan pemulihan bagi astrosit dan sel neuron daripada ketoksikan glutamat. Didapati kesan yang sama antara TRF dan alfa-tokoferol dalam sel astrosit dan neuron menentang ketoksikan glutamat. Model ko-kultur dalam kajian ini telah menunjukkan ciri-ciri sinergi antara sel astrosit dan neuron. Penggunaan TRF dan alfa-tokoferol dalam sistem ko-kultur meningkatkan lagi proses pemulihan dan perlindungan astrosit dan sel neuron daripada ketoksikan glutamat jika dibandingkan dengan sistem mono-kultur.

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