
**UNIVERSITI PUTRA MALAYSIA**

# *PROTECTIVE EFFECTS OF BIOCHANIN A ON PC12 CELLS AGAINST Aβ AND L-GLUTAMATE NEUROTOXICITY*

**TAN JI WEI**

**IB 2013 13**

# PROTECTIVE EFFECTS OF BIOCHANIN A ON PC12 CELLS AGAINST Aβ AND L-GLUTAMATE NEUROTOXICITY

By

TAN JI WEI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

August 2013

## COPYRIGHT

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

**PROTECTIVE EFFECTS OF BIOCHANIN A ON PC12 CELLS AGAINST Aβ AND L-GLUTAMATE NEUROTOXICITY**

By

**TAN JI WEI**

**August 2013**

**Chairman: Prof. Daud Ahmad Israf Ali, PhD**

**Faculty: Institute of Bioscience**

Alzheimer's disease (AD) is characterised by the production of the β-amyloid protein (Aβ) and progressive loss of neurons in the brain. It is considered one of the major neurodegenerative diseases and there is often an association with an excessive amount of glutamate that can lead to excitotoxicity. Estrogen has been previously studied and reported to decrease the risk and delay the onset and progression of Alzheimer's disease. However, estrogen intake increases the risk of unexpected side effects such as heart disease and breast cancer. As such, more attention is being paid towards the usage of phytoestrogens as an alternative solution to replace estrogen. Biochanin A, a phytoestrogen compound found mainly in *Trifolium pretense* (commonly known as red clover), is a commercial nutraceutical that is available for women who suffer from postmenopausal symptoms. However, its beneficial potential towards human health still remains unexplored. Therefore, biochanin A was used in the

present study as a potential alternative to estrogen replacement therapy by investigating its neuroprotective ability and its potential mechanisms in cultured PC12 cells. MTT cell viability assay and LDH release test was used to evaluate the cytotoxic effect of $A\beta_{25-35}$ and L-glutamate on the cells. Flowcytometry and fluorescent microscopy were performed for qualitative and quantitative analysis of $A\beta_{25-35}$-induced and L-glutamate-induced cell death. Measurements of caspase-3, 8 and 9 activity were made using caspase colorimetric kits. The mitochondrial membrane potential (MMP) was accessed using spectrofluorometric assessment of rhodamine dye and Western blot analysis was used to measure the pro- and anti-apoptotic protein levels. The results showed that exposure of PC12 cells to either $A\beta_{25-35}$ or L-glutamate alone caused significantly lower cell viability and higher LDH release and apoptotic activity. However, all of these adverse effects were markedly inhibited in the presence of biochanin A. In addition, biochanin A also recovered the loss of MMP in $A\beta_{25-35}$-treated PC12 cells by regulating the level of pro-apoptotic (Bax and cytochrome c) and anti-apoptotic (Bcl-2) proteins. In conclusion, the protective effect of biochanin A was demonstrated in the inhibition of apoptosis induced by $A\beta_{25-35}$ and L-glutamate. Specifically, the apoptosis inhibition of biochanin A involved the prevention of mitochondrial mediated apoptotic processes in the cell. Hence, the present study can serve as a basic platform to further study the effects of biochanin A as it could be developed into a potential treatment agent for AD and other related neuronal degenerative diseases.

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

**KESAN PERLINDUNGAN BIOCHANIN A DALAM SEL-SEL PC12 TERHADAP KENEUROTOKSIKAN Aβ DAN L-GLUTAMAT**

Oleh

**TAN JI WEI**

**Ogos 2013**

**Pengerusi: Prof. Daud Ahmad Israf Ali, PhD**

**Fakulti: Institut Biosains**

Penyakit Alzheimer (PA) merupakan sejenis penyakit neurodegeneratif utama yang dicirikan dengan penghasilan protein β-amiloid (Aβ) serta kehilangan neuron otak secara progresif. PA juga sering dikaitkan dengan kelebihan glutamat yang mengakibatkan kesan ketoksikan dalam otak. Walaupun estrogen pernah dilaporkan dapat mengurangkan risiko dan perkembangan PA, pengambilannya dapat meningkatkan risiko kesan sampingan yang lain seperti penyakit jantung dan kanser payudara. Oleh itu, tumpuan telah mula diberikan dalam penggunaan fitoestrogen sebagai penyelesaian alternatif untuk menggantikan estrogen. Biochanin A, sejenis kompaun fitoestrogen yand boleh didapati terutamanya dalam *Trifolium pretense* (Semanggi merah) merupakan sejenis ubat komersil nutraseutikal bagi wanita yang mengalami gejala menopaus. Akan tetapi manfaatnya terhadap kesihatan manusia masih belum diterokai dengan sepenuhnya. Oleh sebab itu, biochanin A telah digunakan dalam kajian ini untuk menerokai kesan perlindungannya

disamping menyiasat mekanisme yang terlibat dalam sel-sel PC12. Ujian MTT dan LDH telah digunakan untuk meguji kesitotoksikan bagi $A\beta_{25–35}$ dan L-glutamat terhadap sel. Flowcitometri dan mikroskopi kependarfluoran telah digunakan untuk analisis kualitatif dan kuantitatif kematian sel yang diakibatkan oleh $A\beta_{25–35}$ dan L-glutamat. Pengukuran aktiviti caspase-3, 8 dan 9 telah dinilai dengan menggunakan kit caspase. Potensi membran mitokondria (MMP) telah diakses menerusi penilaian spectrofluorometrik rhodamine manakala teknik Western blot telah digunakan untuk mengukur tahap pro- dan anti-apoptotic protein. Hasil kajian menunjukkan bahawa pendedahan sel-sel PC12 terhadap sama ada $A\beta_{25-35}$ atau L-glutamat menyebabkan penurunan yang ketara dalam kebolehhidupan sel serta peningkatan dalam pembebasan LDH dan juga aktiviti apoptosis. Walau bagaimanapun, semua kesan-kesan negatif yang ketara ini dapat direncatkan dengan perawatan biochanin A. Selain itu, biochanin A dapat memulihkan penurunan MMP sel-sel PC12 yang didedahkan dengan $A\beta_{25-35}$ menerusi kawalan protein pro-apoptotic (Bax dan cytochrome c) serta protein anti-apoptotic (BCL-2). Sebagai kesimpulan, hasil kajian ini telah menunjukkan bahawa biochanin A dapat memainkan peranan perlindungannya menerusi pencegahan apoptosis yang dicetuskan oleh $A\beta_{25-35}$ dan L-glutamat. Jika dipandang dengan lebih teliti, pencegahan apoptosis ini melibatkan peranan mitokondria dalam sel. Justeru, kajian ini dapat dijadikan sebagai platform asas dalam pengajian kesan biochanin A pada masa hadapan agar fitoestrogen ini dapat dijadikan sebagai ejen rawatan yang berpotensi bagi penyakit Alzheimer dan penyakit-penyakit degeneratif yang lain.

## ACKNOWLEDGEMENTS

First and foremost, I would like to express my utmost gratitude to my project supervisor, Dr. Kim Min Kyu for his countless guidance, priceless advice and unflinching encouragement throughout the course of this research. The inspiration and motivation given by him always keep me in high spirit throughout the difficult time of my research. His dedication in the research field and constant oasis of ideas has enriched my growth as a future outstanding researcher and scientist-to-be. Without him, this particular thesis and research project would not have been completed. No word can describe how I am indebted to him.

Apart from that, I would also like to express my appreciation towards my main supervisor, Prof. Dr. Daud Ahmad Israf Ali and co-supervisor, Dr. Tham Chau Ling for their constructive criticism, generous input and invaluable advice in my research project. I am also very grateful to the Institute of Bioscience (IBS), University Putra of Malaysia for supporting my research proposal and giving me the green light to proceed my research project.

Many thanks also go in particular to all of my lab mates, Mr. Lee Yu Zhao, Ms. Revathee Rajajendram, Ms. Tee Yee Sim and Mr. Muhammad Hafiq Hashim for their constructive advices, ideas and tremendous help during the completion of

my research project. Not to forget to express my appreciation to the other laboratory colleagues and friends for sharing their wealth of technical knowledge and continuous support. Sincere gratitude also channel to the lab staff from Cell Signaling Laboratory including Mr. Zulkhairi bin Zainol, Madam Nora Asyikin Mohd Salim and Madam Norazren Ismail for their patience and excellent technical support during the use of the lab equipments.

Not forgetting to express my greatest gratitude and innumerous blessing to my beloved family members for their sacrifice, constant love and continuous moral support. Because of their teaching since I was young, I now have the wisdom and courage to face all the challenges in my life with a positive mind and open heart. Finally, I would like to express my deepest appreciation to those have ever contributed to the successful realization of this thesis. May God bless all of you. Thank you.

Tan Ji Wei

I certify that a Thesis Examination Committee has met on 2 August 2013 to conduct the final examination of Tan Ji Wei on his thesis entitled "Protective Effects of Biochanin a on PC12 Cells against Aβ and L-Glutamate Neurotoxicity" 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.

Members of the Examination Committee were as follows:

**Mohd Roslan bin Sulaiman, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Syahrilnizam bin Abdullah, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Cheah Yoke Kqueen, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Young-Il Hwang, MD, PhD**
Professor
Seoul National University
Republic of Korea
(External Examiner)

**NORITAH OMAR, PhD**
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Daud Ahmad Israf Ali, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Tham Chau Ling, PhD**
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

**Kim Min Kyu, PhD**
Senior Research Felo
Institute Of Bioscience
Universiti Putra Malaysia
(Project Leader)

**BUJANG BIN KIM HUAT, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for the 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 institutions.

Tan Ji Wei

**TAN JI WEI**

Date: 2 August 2013

## TABLE OF CONTENTS