THE EFFECT OF *Bacopa monnieri* EXTRACT ON TAU PROTEIN IN NEURONAL CELLS

KANCHANAT TERNCHOOCHEEP

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy Degree
in Biochemistry
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This thesis entitled "The effect of *Bacopa monnieri* extract on Tau protein in neuronal cells" submitted by Kanchanat Ternchoocheep in partial fulfillment of the requirement for the Doctor of Philosophy Degree in Biochemistry is hereby approved.

S. Kup
..... Chair
(Sarawut Kumphune, Ph.D)

..... Committee
(Associate Professor Sukkid Yasothornsrikul, Ph.D)

K. Ingkaninan
..... Committee
(Associate Professor Kornkanok Ingkaninan, Ph.D)

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Nuanchawee Wetprasit
..... Committee
(Associate Professor Nuanchawee Wetprasit, Ph.D.)

Sittiruk Roytrakul
..... Committee
(Sittiruk Roytrakul, Ph.D)

Approved

K. Pupatwibul
.....
(Assistant Professor Kanungnit Pupatwibul, Ph.D)
Dean of the Graduate School
11 May 2012

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to Venerable Pradhammashinghaburajan (Luangpor Jarun Thitadhammo) for his instruction I followed during working on my dissertation and to my parents for their loves, supports, understanding, and encouraging throughout my study.

I am greatly indebted to "The Commission of Higher Education of Thailand (CHE)" for subsidizing me the fellowship for the shortage program in 2006 and for the exchange program in 2007.

I would like to deeply express my gratitude to Associate Professor Dr. Sukkid Yasothornsrikul, my supervisor, for sacrifying his times and valuable advices on this study, Associate Professor Dr. Kornkanok Ingkaninan, for her generously providing for Bacopa monneiri crude extract and all of its beneficial information, Associate Professor Dr. Suthisa Thanoi for her kindness to offer me an opportunity to learn how immunocytochemistry technique is done and Assistant Professor Dr. Nanteetip Limpeanchob for her kindliness gift the SH-SY5Y cells for all of my study.

Unforgetably, I am very grateful to all staffs in Department of Biochemistry, Faculty of Medical Science, Naresaun University, especially Associate Professor Dr. Dawan Shimbhu for her valuable suggestion and encouraging, Assistant Professor Dr. Jiraporn Tocharus, Dr. Damratsamon Surangkul and Dr. Jongrak Attarat for their friendly technical guides during my research work.

I am also sincerely thank to all members in Associate Professor Dr. Sukkid's laboratory, especially Miss Kanoktip Petcharat, for their warmly collaboration.

The last but not least, I express my especially gratitude to "The Graduated School Naresaun University" for assisting in my dissertation preparation.

Kanchanat Ternchoocheep

Title	THE EFFECT OF *Bacopa monnieri* EXTRACT ON TAU PROTEIN IN NEURONAL CELLS
Author	Kanchnat Ternchoocheep
Advisor	Associate Professor Sukkid Yasothornsrikul, Ph.D.
Co-advisor	Associate Professor Kornkanok Ingkaninan, Ph.D. Associate Professor Suthisa Thanoi, Ph.D.
Academic Paper	Thesis Ph.D. in Biochemistry, Naresaun University, 2011
Keywords	Alzheimer's disease, Bacopa monnieri, Tau protein, Phosphorylated tau protein, NGF-deprived PC12 cells, SH-SY5Y cells, Apoptosis, Neurodegenerative disorders

ABSTRACT

Bacopa monnieri is a revered ancient Indian Ayurvedic herb specific for cognition enhancing benefit. This research is aimed to measure the effect of the alcoholic BM extract on the viabilities and the amount of Tau protein expression of NGF-deprived PC12 cells or human SH-SY5Y neuroblstoma cells brought up in apoptotic-induced media. The PC12 cells were cultured in normal-serum medium for a week before transferred to low-serum medium for the same period. After that the cultures were raised in normal-serum (NR) or serum-free (SF) medium and each treated with either 0 (control), 50, 100, 150, 200, 250, and 300 µg/ mL BM extract for up to 7 days for MTT viability assay or 0, 50, and 100 µg/ mL BM extract for 2 days for immunoblot assay. The SH-SY5Y cells were brought up in normal-serum medium for a week before differentiation in low-serum medium containing 20 µM Retinoic acid further for a week. Then, the cultures were raised in low-serum medium and treated with absolute 0.025% DMSO, absolute 5µM camptothecin, and either BM extract at final concentration 50-25 µg/ mL for up to 12, 24, and 48 hours for MTT viability assay or 0, 50, and 100 µg/ mL BM extract for 12, 24, and 48 hours for immunoblot assay and Tau gene expression determination. The MTT viabilities were measured and the average cellular viability percentages of each treatment were compared to their corresponding controls daily. Immunoblot assay of the proteins

were performed by using total tau (Tau 5), dephosphorylated tau (Tau 1), and glyceraldehydes dehydrogenase (GAPDH) antibodies and then quantified by Scion Image software. Tau 1 immunoreactivity was normalized by total tau. All data in the present study were analyzed by one-way ANOVA with $\alpha = 0.05$. RT-PCR of Tau and GAPDH gene from each condition was performed. The findings of this study revealed that (1) BM extract could improve the cellular viability of the normal neuronal cells and apoptotic-induced neuronal cells, (2) BM extract could be able to abate down both the amount of total Tau (Tau 5) and phosphorylated Tau (at Tau-1 site) expression in apoptotic-induced neuronal cells, and (3) BM extract at both 50 and 100 μg/ mL concentration could be able to reduce the 3R tau mRNA to express in differentiated SH-SY5Y cells, apoptotic-induced by camptothecin. These results offer a supportive document advocating to the benevolent property of BM extract to be an alternative therapy for neurodegenerative diseases such as Alzheimer's disease.

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