

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47335

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

May 2012

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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.

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### ABSTRACT

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*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 neuroblastoma 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  $\mu\text{g}/\text{mL}$  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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