EVALUATION OF ACETYLCHOLINESTERASE ACTIVITY AND CYTOTOXICITY OF DIFFERENT PARTS OF NELUMBO NUCIFERA GAERTN ON HUMAN NEUROBLASTOMA CELL LINE (SH-SY5Y)

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ABSTRACT

Objective: Cognitive deterioration occurring in patients with probable Alzheimer’s disease is associated with a progressive loss of cholinergic neurons and a consequent decline in levels of acetylcholine in the brain. This study aimed to evaluate the acetylcholinesterase (AChE) inhibitory effects and cytotoxicity in SH-SY5Y cells of different parts of three lotus extracts.

Methods: AChE activity was quantified by spectrophotometry and cytotoxicity by flow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in SH-SY5Y cells exposed to extracts.

Results: All of the extracts had inhibitory effects to AChE at p<0.05, but Roseum Plenum stem extract could inhibit AChE more than 30% (p<0.05). The all of the extracts could be an increase SH-SY5Y cell proliferation, while Album Plenum flower extract could be cytotoxic on SH-SY5Y cells.

Conclusion: The extracts of lotus could be supplemented compound for cognitive deterioration or Alzheimer’s patients.

Keywords: Nelumbo nucifera Gaertn., Acetylcholinesterase activity, Human neuroblastoma cell line.

INTRODUCTION

The most common cause of dementia in the elderly associated with probable Alzheimer’s disease (AD) is one of the most common types of dementia, a chronic, progressive, disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity, and language [1]. Patients with AD often have cholinergic deficits in association with the disease [2]. The symptoms of all types of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the brain areas affected [3]. Cognitive deterioration occurring in patients with probable AD is associated with a progressive loss of cholinergic neurons and a consequent decline in levels of acetylcholine (ACh) in the brain.

The neurons can be subdivided into subclasses based on location, function, or chemistry. Neurons express a wide array of molecular components that may result in lethality if they, or their function, are adversely affected. To survive, a functioning nervous system is crucial. Therefore, neurons, synapses, signal molecules, and brain are needed. The endogenous signal molecules in the nervous system are called neurotransmitters, and their functions are chemically induced or attenuate the activity of other neurons or other organs in the body [4]. The storage of neurotransmitter in the vesicles has two main functions to protect the neurotransmitter from degradation and the first release of the neurotransmitter. Neuronal transmission, parasympathetic signaling and cholinergic signaling in the central nervous system are dependent on ACh. ACh is rapidly hydrolyzed in the brain by acetylcholinesterase (AChE). Although AChE is found in higher concentrations in the brain tissue of patients with AD, there is evidence that known to receive cholinergic innervations [5].

The expression of both muscarinic and nicotinic ACh receptors has been reported in SH-SY5Y cells. The G-protein coupled muscarinic receptors are present on membranes of both undifferentiated and differentiated cells, though the levels and binding properties are differentially regulated according to the method of differentiation.

METHODS

Plant materials and extraction

The N. nucifera materials including of N. nucifera cv. Roseum Plenum (RP), N. nucifera cv. Album Plenum (AP), and N. nucifera cv. Hindu Lotus were collected from lotus museum, Rajamangala University of Technology Thanyaburi, Pathum Thani, Thailand. The different parts of lotus (leaf, stem, and flower) were macerated in 95% ethanol for 7 days. The ethanolic extracts were evaporated and dried in a rotary evaporator.

Reagents

Dulbecco’s Modified Eagle Medium (DMEM), F12 medium, penicillin/streptomycin, trypsin/EDTA, and 3-(4,5-dimethythiazol-2-yl)-2,5-
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Determinations of AChE activity
The activity of AChE in red blood cell (RBC) was determined according to slightly modified Ellman’s method as previously described [28-30]. The mixture was prepared by mixing 10 μL of each aliquot of 1:10 RBC and extracts added in 1.0 mL of 0.25 mM DTNB in phosphate buffer pH 7.4. This was pre-incubated for 5 min at room temperature, and the reaction was started with the addition 25 μL of acetylthiocholine iodide. The absorbance per minute (ΔA/min) of thiocholine product was determined by spectrometric absorption at 405 nm. The data were converted into the standardized units of nanomoles substrate hydrolyzed/min x mL, using the extinction coefficient for the yellow product (ε= 13.6 mM⁻¹cm⁻¹) to find the concentration. The AChE activity was calculated and expressed as U/L (AChE factor = 76,838).

Statistical analysis
All data were analyzed with a mean ± standard deviation of three determinations. The AChE activity was calculated and expressed as U/L (AChE factor = 76,838). The experiment was run in triplicate. The AChE activities and AChE inhibitions were calculated by the following:

\[
\text{AChE activity} = \frac{\Delta A}{\text{min} \times \text{factor}}
\]

\[
\% \text{AChE inhibition} = \left(1 - \frac{\text{Ac}}{\text{As}}\right) \times 100
\]

Where
- \( \Delta A \) = the absorbance per minute of thiocholine product
- \( \text{Ac} \) = the delta absorbance per minute of control
- \( \text{As} \) = the delta absorbance per minute of sample

MTT reduction assay
SH-SYSY cells were seeded in 6-well culture plate (2.5 × 10⁴ cells per well) for 24 h. After SH-SYSY cells were pre-treated with extracts. The end of the treatment, SH-SYSY cells were incubated with 100 μL of MTT solution (5 mg/mL in PBS) for 2 h. Then, the medium was discarded and added formazan crystal products. The formazan crystal products were dissolved in 200 μL DMSO and stirred for 10 min. Absorbance was measured at 570 nm using a microplate reader. The results were compared with the untreated control, expressed as the percentage of cell viability. The data were obtained from five replicates. The percentage of cell viability was calculated from the following:

\[
\text{Cell viability (}) = \left(\frac{\text{Ac}}{\text{As}}\right) \times 100
\]

Where
- \( \text{Ac} \) = the absorbance of SH-SYSY treated with vehicle medium
- \( \text{As} \) = the absorbance of SH-SYSY treated with sample medium

CONCLUSION
The extracts promoted a moderate cytotoxic effect against SH-SYSY cell and did not show a significant AChE inhibitory activity. According to the study, we found that the ethanolic extracts of AP showed a significant AChE inhibitory activity against SH-SYSY cell. Further study on the effect of phytochemical composition in the different parts of N. nucifera on the activity of AChE and cytotoxicity on SH-SYSY cell.
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CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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