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 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
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-dimethylthiazol-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.
diphenyltetrazolium bromide (MTT) were purchased from Invitrogen (Invitrogen, USA). DMSO, phosphate-buffered saline (PBS), and dithiobisnitrobenzoic acid (DTNB) were purchased from ThermoFisher (Thermo Scientific, USA).

Determination 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 in 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−1 cm−1) to find the concentration. 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:

\[ \text{AChE activity} = \Delta A/\text{min} \times \text{factor} \]
\[ \% \text{AChE inhibition} = \frac{Ac - As}{Ac} \times 100 \]

Where \( \Delta A \) = the absorbance per minute of thiocholine product

\( Ac \) = the delta absorbance per minute of control

\( As \) = the delta absorbance per minute of sample

Cell culture and treatment

Human neuronal SH-SY5Y cells were cultured in DMEM/F12 with 10% fetal bovine serum (DMEM/F12) (Invitrogen, USA), and penicillin/streptomycin (100 IU/mL) at 37°C in a humidified incubator with 5% CO₂ atmosphere. SH-SY5Y cells were allowed to adhere at the bottom of a culture dish (60 mm) for 24 h before treatment with fresh medium containing 1 mg/mL of initial stock extracts for 24 h. SH-SY5Y cells were treated with a vehicle that served as a control. For all experiments were performed in five replicates.

MTT reduction assay

SH-SY5Y cells were seeded in 6-well culture plate (2.5 × 10⁴ cells per well) for 24 h. After SH-SY5Y cells were pre-treated with extracts. The end of the treatment, SH-SY5Y 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:

\[ \text{Cell viability} (%) = \frac{(As/Ac) \times 100}{1} \]

Where \( Ac \) = the absorbance of SH-SY5Y treated with vehicle medium

\( As \) = the absorbance of SH-SY5Y treated with sample medium

Statistical analysis

All data were analyzed with a mean ± standard deviation of three independent experiment, descriptive statistics, t-test, and one-way analysis of variance using GraphPad Prism 6 version 6.01 (GraphPad Software Inc. La Jolla, CA, USA). p=0.05 was considered a statistically significant difference.

RESULTS AND DISCUSSION

AChE inhibitory activities

The results of the AChE activity are shown in Fig. 1. The baseline AChE activity in 1:10 dilution of packed RBC was 10.32.19±379.03 U/L. At the 5 mg/mL concentration of leaf, stem, and flower ethanolic extracts of RP showed that ChE activity was 8,298.53±76.84, 6,480.02±319.90, and 9,476.72±247.00, respectively. The leaf, stem, and flower ethanolic extracts of AP showed that ChE activity was 9,579.17±117.37, 8,759.56±277.04, and 9,527.94±76.84, respectively. The leaf and stem ethanolic extracts of HL showed that ChE activity was 9,451.10±203.29 and 8,068.02±203.29, respectively. The extracts of Nelumbo nucifera (RP, AP, and HL) are shown mild AChE inhibition (14.36–27.64%) while the flower extract showed no symptoms of intoxication on AChE inhibition (10.57–28.86%), as shown in Fig. 1. The AChE activity decreased approximately to the one-third of the baseline activity (less than 33%), consistent with no symptoms of intoxication.

Cytotoxicity of SH-SY5Y cells

SH-SY5Y cells were incubated with extracts for 48 h. And then, MTT assay was carried out to determine the cell growth in response to extract treatment. The result showed that the growth of the cells was markedly inhibited from the extracts of flowers of AP and displayed a toxic response to the treatment, while the other samples could be increased SH-SY5Y cell proliferation, as shown in Fig. 2.

CONCLUSION

The extracts promoted a moderate cytotoxic effect against SH-SY5Y cell and did not show a significant AChE inhibitory activity. According to the study, we found that the ethanolic extracts of N. nucifera could be supplemented compound for cognitive deterioration or Alzheimer’s patients. However, further study on the effect of phytochemical composition in the different parts of N. nucifera on the activity of AChE and cytotoxicity on SH-SY5Y cell.
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CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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