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Acetylcholinesterase Inhibitory Effect of *Pseuderanthemum palatiferum* in Albino Rats

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Abstract

Alzheimer's disease is commonly treated by Acetylcholinesterase inhibitors (AChEIs) drug therapy. Because of the varying side effects of Alzheimer's drugs, interest in the exploitation of medicinal plants as an alternative to AChEIs has greatly increased in recent years. This study aimed to determine whether the aqueous extract form the medicinal plant, *Pseuderanthemum palatiferum*, could inhibit AChE in normal rats. Male albino rats were given an oral extract at the doses of 0.3, 0.7 and 1.0 g/kg BW for 30 days. These were compared to controls which received only distilled water. AChE activity was then evaluated from the brains, serum, and red blood cells of the rats. The results showed that only AChE activity in the hippocampus was significantly inhibited by the extract at the doses of 0.7 and 1.0 g/kg BW ($p < 0.05$). However, AChE activity in the serum and red blood cells of treated rats showed no significant differences from that of controls. These results suggest that the extract could reduce the synthesis of AChE in the rat's brain. The marked inhibitory effect of aqueous extract from *P. palatiferum* against AChE indicates the potential of this plant in the treatment of Alzheimer's disease.

Key words : Acetylcholinesterase, Inhibition, *Pseuderanthemum palatiferum*, Rat, Brain, Blood

Introduction

Acetylcholine (ACh) is an important neurotransmitter of the brain cholinergic system involved in memory formation. ACh is hydrolyzed by acetylcholinesterase (AChE) enzyme at the central and peripheral cholinergic synapses into its two component parts, acetic acid and choline [3,19]. Loss of ACh activity in the brain correlates with the severity of Alzheimer's disease (AD), the common cause of dementia in elderly people worldwide [13]. AChE inhibitor drugs have been designed to inhibit the breakdown of ACh in the brain and thus to increase cholinergic neurotransmitter activity in AD patients. Due to the high costs and the varying side effects of the synthetic drugs, interest in the exploitation of medicinal plants as an alternative AChE inhibitor has greatly increased in the recent years. A number of researches have been conducted to evaluate the AChE inhibitory activity of various plant species, for instance, Tabernaemontana divaricata, Green tea, *Fumaria* sp., *Vaccinium angustifolium, Corydalis spp., Lavandula pedunculata, Mentha suaveolens* and *Hypericum undulatum* [1,8,11,16,20-21]. Nevertheless, until now no plant species has scored full marks for its potential to replace the AD drugs. In the study reported here we were interested in determining the AChE inhibitory effect of *Pseuderanthemum palatiferum*, a native Vietnam plant which is popularly used in Thailand for curing various ailments. The reputed medicinal properties of *P. palatiferum* includes wound healing, and the treatment of trauma, stomachache, colitis, high blood pressure, nephritis and diarrhea [9]. In view of its broad spectrum of reported therapeutic properties, but with only limited scientific reports on the effective activity of this plant, we aimed to determine whether or not the extract from *P. palatiferum* could inhibit AChE activity in albino rats. The results obtained from our study might facilitate the development of a new herbal medicine for AD treatment.

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Materials and Methods

Plant materials and extraction

Fresh leaves of *P. palatiferum* were collected from Chiang Mai Province, Chiang Mai, Thailand, during the summer of 2009. They were cut into small pieces

and extracted with distilled water to yield concentrations of 50, 100 and 150 mg/ml, respectively. The aqueous extracts were filtered before being used.

Animals

Male albino rats *(Rattus Norvegigus)*, 4-5 weeks of age, with weights between 130 - 150 g, were purchased from the National Laboratory Animal Center, Mahidol University, Salaya campus, Thailand. The animals were kept in cages with five animals per group. They had access to water and a standard diet (C.P. 082). The room temperature was controlled at 24 - 26 o C in a 12 hour light/dark cycle. All procedures involving the animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Animal Care and Use Committee of Biology Department, Faculty of Sciences, Chiang Mai University, permission number Re 004/09.

Animal treatments

The rats were divided into 4 groups (n = 5). The animals in each group were orally given the extracts of *P. palatiferum* at concentrations of 50, 100 and 150 mg/ml/day which were comparable to doses of 0.3, 0.7 and 1.0 g/kg.BW, respectively. Control rats received only distilled water. After the 30 days of the treatment period, the animals were sacrificed and blood samples were collected by cardiac puncture technique. The brain was immediately removed and the hippocampus was dissected and stored in a normal saline solution. All processes were conducted at -4 °C.

Serum and tissue preparation

Blood samples were centrifuged at 3,000 rpm for 10 min at room temperature. Sera and red blood cells (RBC) were separately stored at - 40°C until further use for AChE assay.

Homogenated samples of hippocampus or RBC and serum were mixed in a 0.1 M phosphate buffer solution (~20 w/v or v/v), pH 8.0 at - 4oC and centrifuged at 3,500 rpm for 10 min. The clear supernatant was stored at - 40°C for the examination of AChE activity within 24 hours.

Acetylcholinesterase assay

The AChE activity was measured by following the increase of yellow color produced by the reaction between the substrate (acetylthiocholine) and 5,5'-Dithiobis (2-nitrobenzoic) acid (DTNB). The method for determining AChE activity was modified from [9]. Briefly, 20 µl of samples were mixed with 1,300 µl of 0.2 mM DTNB and 63 µl of 0.75 mM acetylthiocholine iodide. 250 µl of the mixed solutions were added to 96 well plates and a kinetic reaction of the mixture was measured by ELISA reader (GDV, DV 990BV4, Italy) at 405 nm for 10 min with readouts taken every 2 min. The rate of AChE activity was calculated using the following formula:

$$R = \frac{\text{changed in absorbance/min}}{1.36 \text{ xø } 104} \quad x \quad \frac{1}{20(130/1300+130+63)}$$

where R is the rate of AChE activity in moles of substrate hydrolyzed/minute/g of tissue or ml serum, 1.36 x 104 is constant of activity, 20 is the weight of samples and 130, 1300 and 63 are the values of tissue extracts, DTNB and ATChI, respectively. AChE inhibitory effect was expressed as a percentage of AChE activity in comparison to control values.

Statistical analysis

For statistical analysis, data were analyzed by one – way ANOVA by SPSS statistical software version 16 for windows. In all the tests, a level of p value less than 0.05 was considered of statistical significance.

Results

The results of AChE activity in hippocampus, serum and RBC of male albino rats treated with *P. palatiferum* extract at the doses of 0.3, 0.7 and 1.0 g/kg BW for 30 days are presented in Fig. 1, 2 and 3. The values of AChE activity significantly decreased at the doses of 0.7 and 1.0 g/kg BW ($p \leq 0.05$) only in the hippocampus. Nevertheless, in serum and RBC, there were no significant differences between the extracts treated groups and the control group.

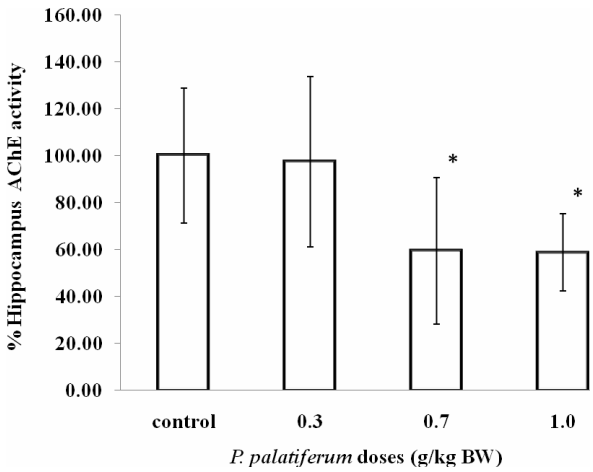

Figure 1. Hippocampus acetylcholinesterase activity of rats treated with P. palatiferum extract at doses of 0.3, 0.7 and 1.0 g/kg BW for 30 days as compared to the control group.

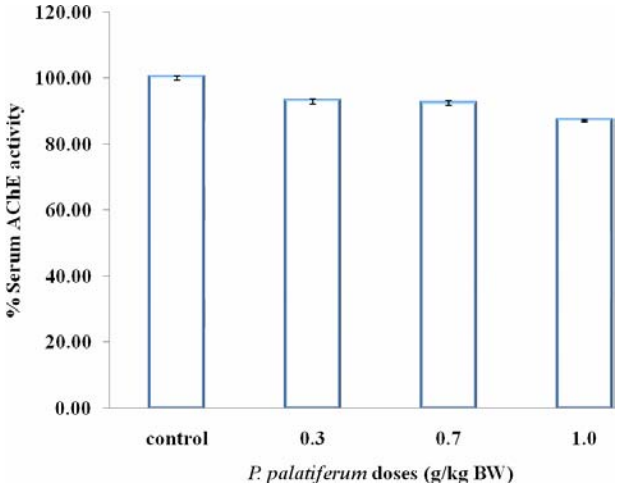

Figure 2. Serum acetylcholinesterase activity of rats treated with P. palatiferum extract at doses of 0.3, 0.7 and 1.0 g/kg BW for 30 days as compared to the control group.

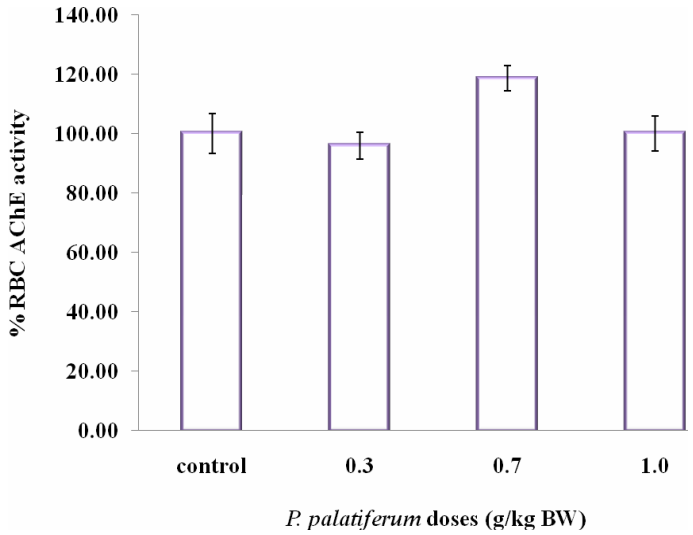

Figure 3. Acetylcholinesterase activity of red blood cells in rats treated with *P. palatiferum* extract at doses of 0.3, 0.7 and 1.0 g/kg BW for 30 days as compared to the control group.

Discussion

The ability of *P. palatiferum* extracts to inhibit AChE activity in rats hippocampus, serum and RBC was assessed. It was found that oral administration of the extract at doses of 0.7 and 1.0 g/kg BW for 30 days could inhibit AChE activity in the hippocampus (Fig. 1). The AChE enzyme, which resembles a globular subunit is responsible for hydrolyzing ACh at the cholinergic synaptic clefts [2]. AChE was found in brain tissue, muscle, plasma and RBC [4,6]. Previously reported research reported on AD patients has shown that the reduction of neurons in the hippocampus and cerebral cortex resulted in a low level of ACh [14], while AChE levels remained constant. The inhibition of AChE activity, thus, helped to slow down the destruction rate of ACh, and this concept was used to develop of the drugs for AD patients [18]. The AChE inhibitory effect of *P. palatiferum* extracts found in the hippocampus, the important site for memory and recognition, of rats may indicate the usefulness of this plant as an alternative AD drug.

The AChE activity in the serum and RBC of treated rat with *P. palatiferum* extract were not different from the control group (Fig 2,3). This indifference might be explained by the following 3 reasons; 1). The level of AChE in RBC might be less than that in the brain tissue where AChE actively breakdown ACh, an essential neurotransmitter in cholinergic system [13,21]. The method employed in this study was highly specific for AChE activity. Nevertheless, the main type of cholinesterase (ChE) in serum is in the form of butyrylcholinesterase (BuChE) [2,5,22]. It was reported that average adult human plasma contains 3300 ng of BuChE/ml and 8 ng of AChE/ml [5]. The low activity of AChE detected in serum, thus, was not surprising., 3). P. palatiferum extract may inhibit only the certain type of AChE. The study of [7] and [15] revealed that AChE type found in the brain was G1 AChE-S, while that found in RBC was G1 AChE-E. The results of our study demonstrated that the extract inhibited only G1 AChE-S in the hippocampus, but not G1 AChE-E in RBC. The low activity of AChE in the hippocampus indicated that the *P.palatiferum* extract specifically inhibit the synthesis of AChE in the brain.

In recent years, the study of medicinal plants as an alternative AChE inhibitor has greatly increased. The ability of *P. palatiferum* extracts to inhibit AChE activity in the brain may come from the chemical elements contained in the leaves of this plant. It is currently reported that several plants species contained flavonoids, such as tiliroside, 3-methoxy quercetin, quercitrin and quercetin and those flavonoids exhibited AChE inhibitory activity [16]. The results

of our study may shed new light on alternative herbal products for AD treatment. Further investigation on the chemical composition of *P. palatiferum* involved in AChE inhibition is needed.

Conclusion

P. palatiferum extracts can inhibit AChE activity in the hippocampus, but not in serum and RBCs. Considering its potent AChE inhibitory effect, this plant species may be of great interest for future studies in the treatment of Alzheimer's Disease.

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