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Preliminary Screening a Potential AChE Inhibitor in Thai Golden Shower (*Leguminosae mimosoideae*) Extracts

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Abstract

Pesticides are used to control pests of agriculture products in many countries including Thailand. Since they can exert harmful effects not only on target pests but also on other useful organisms, alternative agents are investigated. We studied the capacity of the Thai golden shower (*Leguminosae mimosoideae*) extracts (root and pod) to inhibit acetyl cholinestarese (AChE) in the golden apple snail (*Pomacea canaliculata*) as a pest representative. The results showed that the percentage of AChE inhibition increased with increasing in exposure times. The inhibition expressed the same trend in both male and female apple snails. AChE inhibition was higher in extracts from root than from pod. Chromatography-Mass Spectrometer (GC-MS) chromatograms demonstrated anthraquinone, an AChE inhibitor, in extracts of golden shower. Our data indicate that a potential AChE inhibitor tends to accumulate more in the root part than in the pod.

Keywords: AChE inhibitor; ethanolic plant extracts; golden shower (*Leguminosae mimosoideae*); golden apple snail (*Pomacea canaliculata*)

1. Introduction

Endosulfan, which is a GABA-gated chloride channel antagonist, and a Ca^{2+}, Mg^{2+} ATPase inhibitor, is frequently used in Thai paddy fields. It has a range of harmful effects, not only on target pests, but also on other useful organisms (Cooper and Bidwell, 2006; Reinecke and Reinecke, 2007; Falfushinska *et al*., 2013; Kristoff *et al*., 2010; Schweikert and Burritt, 2012). Endosulfan attacks the central nervous system, causing overstimulation (Karatas *et al*., 2006). Moreover, it passes through the food chain and then eventually reaches human being which is the top consumer. Therefore, alternative substances with pest controlling capabilities have been searched for which are less less toxicity than endosulfan.

In some parts of Thailand, the farmers use a local herb; the golden shower (*Leguminosae Mimosoideae*), for controlling and eliminating pests in their paddy fields. It is extracted, dissolved in water and then sprayed in paddy field. It is effective in pest control, especially on the golden apple snail (*Pomacea canaliculata*). Some plants in the family of Leguminosae, such as *Glycyrrhiza glabra* L. and *Mimosa pudica* L., have been used in traditional medicine (Rastogi *et al*., 2011), as free radical-scavengers, as H^+, K^+-ATPase inhibitors (Megala and Geetha, 2010), and for biosorption (Hanif *et al*., 2007). Moreover, they also inhibit AChE activity (Vinutha *et al*., 2007). However, the specific AChE inhibiting substance in Thai golden shower has not previously been identified and scientifically studied.

The aim of this study was to provide the scientific evidence of AChE inhibitory capability of golden shower (*L. Mimosoideae*) extracts on golden apple snail (*P. canaliculata*) and to identify a potential AChE inhibitor in the extracts.

2. Materials and Methods

2.1. Plant materials collection and preparation

Plant material used in this study was golden shower (*L. Mimosoideae*) collected from the demonstrating fields of Rajamangala University of Technology, Isan Sakon Nakhon Campus, Thailand, in December 2011-February 2012. After collection, the plant material was washed to remove all the external contaminants and unwanted materials. Next, mature pod and root were separated, shade dried for 72 h, and then crushed into a coarse powder. Subsequently, 100 g of dried powder of each part (pod and root) was separately extracted with 250 ml of 95% ethyl alcohol by using Soxhlet extraction (Ingkaninan *et al*., 2003). The extracted solutions were kept at room temperature until further use.

2.2. AChE inhibition testing

Golden apple snails (*P. canaliculata*) were collected from the demonstration field of Rajamangala University of Technology, Isan Sakon Nakhon Campus, in January-February 2012. They were acclimatized in plastic boxes (14x40x48 cm) (each box contained 5 snails) with 20 L of aerated clean freshwater for 3 days. Subsequently, 5 snails were placed in each treatment box (separating male from female snails), containing rice stalks and paddy clay soil, thus simulating real paddy fields. Four groups of snails were studied: by adding: (1) extracts of mature pod (50% v/v of mature pod extract/ethanol), (2) extracts of root (50% v/v of root extract/ethanol), (3) 1 mg/L of endosulfan (98% technical grade from Micro Flo Co., Sparks ,GA, USA), and (4) 95% ethyl alcohol (50% v/v of ethyl alcohol/distilled water) as a control group. The exposure times were: 0, 10, or 20 min, and 1, 6, 12, 24, 48, 72, or 96 h.

Recovery of AChE activity was studied in snails from each of the 4 treatments (after 96 h of exposure) following transfer to clean water. The time periods for recovery were 5, 30 min., and 3, 12, 48, 96 h., and 15 and 20 d. AChE activity was measured by the method described by Ellman *et al*. (1961). There were 5 snails in each experimental group. One way analysis of variance (ANOVA) was used to test the differences between AChE activities from each treatment.

2.3. Instrumental analysis

The potential AChE inhibitor in crude plant extracts (mature pod and root) was identified by using Gas Chromatography-Mass Spectrometer (GC-MS) (Agilent Technologies 5973 Inert, Thailand) with an Agilent DB-5ms Capillary GC Column (Agilent Technologies 122-5532). Standard of anthraquinone (9,10-Anthraquinone) was purchased from Sigma-Aldrich (analytical grade). For GC-MS measuring condition, the initial temperature in the oven was increased to 90°C and maintained for 1.0 min, then sequentially increased at 10°C per minute to reach 200°C maintaining for 5.0 min, 3°C per minute to reach 230°C maintaining for 5.0 min, 10°C per minute to reach 280°C maintaining for 8.5 min, and 30°C per minute to reach 290°C, after which it was maintained for 1.0 min.

3. Results

3.1. AChE inhibition testing

After AChE inhibitory capability of golden shower extracts was tested, the results showed that there was no significant different in effect ($p>0.05$) between male and female snails (Fig. 1). The results demonstrated that the strongest AChE inhibitor was endosulfan accompanied by root and pod extracts, respectively. Percentage of AChE inhibition (male/female) after 96 h of exposure with endosulfan, root, and pod extracts were 65.0±1.89 / 63.5±0.31, 64.8±2.15 / 56.9±7.70, and 58.4±1.02 / 55.6±0.39, respectively.

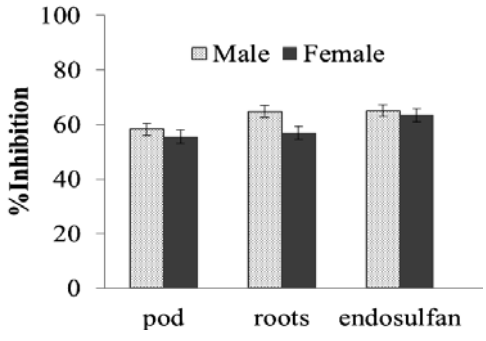

Figure 1. AChE inhibition percentage (means ± S.D; *n=5*) in male and female of golden apple snail

Considering the effect of exposure time, we found that AChE inhibition increased with increasing exposure times (Fig. 2). The highest inhibition was observed in the snails exposed to endosulfan. The inhibition increased sharply during the first 10 min and remained quite stable until 96 h after exposure. Percentage of AChE inhibition after 20 d of exposure to endosulfan, root, and pod extracts were 94.41±2.60, 96.52±2.58, and 89.14±2.90, respectively.

For AChE recovery, AChE activity in golden apple snails remained quite low until 48 h after transfer to the new box filled with clean freshwater. During the following recovery period up to 20 days, the activity increased from 5.33±1.62 to 19.5±7.07 nmoles ACTC/min/mg protein which was higher than that in the groups exposed to endosulfan and roots extracts. AChE activities in endosulfan and root extacts treatment increased from 2.71±2.53 to 8.54±8,28 nmoles ACTC/min/mg protein and 1.73±0.50 to 7.15±3.33 nmoles ACTC/min/mg protein, respectively (Fig. 3).

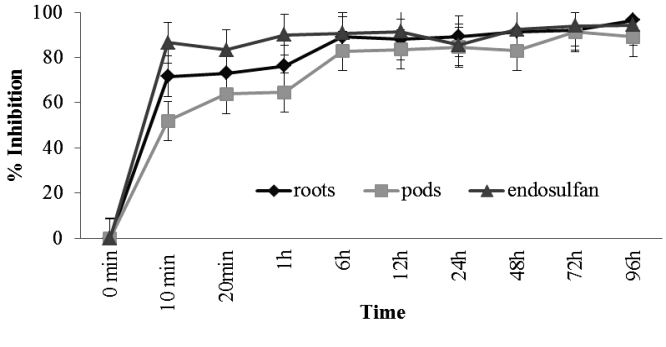

Figure 2. AChE inhibition percentages (means ± S.D; *n=5*) in golden apple snail after exposure

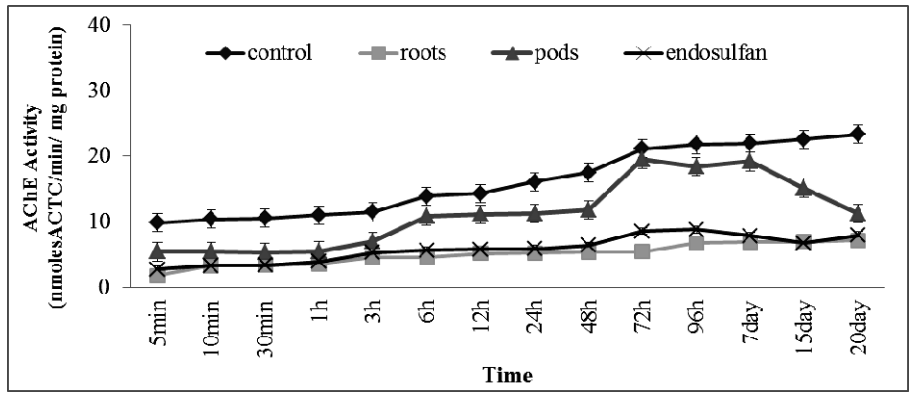

Figure 3. AChE activities (means ± S.D; *n=5*) in golden apple snail in recovery period

3.2. Screening a potential AChE inhibitor identification

After crude extract solution expressed AChE inhibitory capability, it was tested by using GC-MS for identifying the potential AChE inhibitor. The results showed that there was anthraquinone in the crude extracts. Anthraquinone which is an oxygenated polycyclic aromatic hydrocarbon has been identified as weak AChE inhibitor (Kang and Fang, 1997), thus it might be a substance causing AChE inhibition in the ethanolic golden shower extracts.

4. Discussions

We found no difference between male and female golden apple snails with regard to AChE inhibition. This is in agreement with the results of Kopecka *et al*. (2004) who showed that AChE activity inhibition in fish was not affected by the difference of sex. Moreover, Yi *et al*. (2006) also found that AChE levels in the brain of goldfish (*Carassius auratus*) exposed to carbamate were not different between male and female.

The results indicated that AChE inhibition percantage increased with an increasing exposure time in all treatmentst. The highest inhibition was found in snails exposed to endosulfan. Comparing both plant extracts, AChE inhibition in root extracts was higher than that in the pod extracts. This finding confirms the result of Ingkaninan *et al*. (2003) who measured AChE inhibitory activity using Ellman's colorimetric method in 96-welled microplates. They studied the AChE inhibitory potencies of extract solutions from plants in the family of Leguminosae; *Cassia fistula* L. (root) and *Mimosa pudica* L. (whole plant) and exerted higher AChE inhibitory capability in root extracts (54.13±3.90%) compared to the whole plant (21.40±6.68%). This finding indicates that the plant constituent having AChE inhibitory capacity may tend to accumulate in the root. This finding is in agreement with the study of Rajasree *et al*. (2012) who found that methanolic extracts of Cassia fistula roots caused 60 - 65 % AChE inhibition.

For AChE recovery, the results showed that AChE activity in the snail exposed to pod extracts recover faster than that in root extracts and endosulfan exposure. This result might be caused by the lower level of AChE inhibitor in pod as compared to root.

By GC-MS Kang and Fang (1997) demonstrated that the crude extracted solution of golden shower was anthraquinone which is classified as AChE inhibitor. This is in agreement with the study of Duraipandiyan *et al*. (2011) who found anthraquinone in *C. fistula* samples. Anthraquinone was found in both root and pod; however, the results of AChE inhibition and recovery indicate that anthraquinone may accumulate more in roots resulting in the higher AChE inhibition in the snail exposed to root extracts as compared to pod extracts.

5. Conclusion

We found that AChE inhibition in golden apple snail exposed to ethanolic extracts of golden shower increased with an exposure time. In root extracts the inhibition was higher than that in the pod. This may indicate that AChE inhibitor tends to accumulate to a higher degree in the root part. Preliminary screening using GC-MS, the chromatogram showed that ethanolic extracts of golden shower contain anthraquinone which is classified as AChE inhibitor

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References

Cooper NL, Bidwell JR. The use of cholinesterase activity and ecologically-relevant behavioral parameters to indicate chlorpyrifos exposure in the Asian clam, *Corbicula fluminea*, Aquatic Toxicology 2006; 76: 258-67.

Duraipandiyan V, Ignacimuthu S, Paulraj MG. Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L., Saudi Journal of Biological Sciences 2011; 18: 129-33.

Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical Pharmacology 1961; 7: 88-95.

Falfushynska HI, Gnatyshyna LL, Stoliar OB. In situ exposure history modulates the molecular responses to carbamate fungicide Tattoo in bivalve mollusk, Ecotoxicology 2013; 22(3): 433-45.

Hanif MA, Nadeem R, Zafar MN, Akhtar K, Bhatti HN. Kinetic studies for Ni(II) biosorption from industrial wastewater by *Cassia fistula* (Golden Shower) biomass, Journal of Hazardous Materials 2007; 145(3): 501-05.

Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem Y, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. Journal of Ethnopharmacology 2003; 89: 261-64.

Kang JJ, Fang HW. Polycyclic aromatic hydrocarbons inhibit the activity of acetylcholinesterase purified from electric eel, Biochemical and Biophysical Research Communications 1997; 238(2): 367-69.

Karatas AD, Aygun D, Baydin A. Characteristics of endosulfan poisoning: a study of 23 cases. Singapore Medical Journal 2006: 47(12); 1030-32.

Kopecka J, Rybakowas A, Baršienė J, Pempkowiaw J. AChE levels in mussel and fish collected of Lithuania and Poland (Southern Baltic). Oceanology 2004; 43(3): 405-18.

Kristoff G, Guerrero NR, Cochón AC. Inhibition of Cholinesterases and carboxylesterases of two invertebrate species, *Biomphalaria glabrata* and *Lumbriculus variegates*, by the carbamate pesticide carbaryl. Aquatic Toxicology (Amsterdam, Netherlands) 2010; 96(2): 115-23.

Megala J, Geetha A. Free radical-scavenging and H^+, K^+-ATPase inhibition activities of *Pithecellobium dulce*. Food Chemistry 2010; 121(4): 1120-28.

Rajasree PH, Ranjit S, Sankar C. Screening for acetylcholinesterase inhibitory activity of methanolic extract of *Cassia fistula* roots. International Journal of Pharmacy & Life Sciences 2012; 3(9): 1976-78.

Reinecke SA, Reinecke AJ. The impact of organophosphate pesticide in orchards on earthworms in the Western Cape, South Africa. Ecotoxicology and Environmental Safety 2007; 66: 244-57.

Rastogi S, Pandey MM, Rawat AK. An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L) DC. and *Desmodium adscendens* (Sw) DC., Journal of Ethnopharmacology 2011; 136(2): 283-96.

Schweikert K. and Burritt DJ. The organophosphate insecticide Coumaphos induces oxidative stress and increase antioxidant and detoxification defence in the green macroalgae *Ulva pentusa*. Aquatic Toxicology. 2012; 122-123: 86-92.

Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, Radhika S, Amit A, Venkateshwarlu K, Deepak M. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity, Journal of Ethnopharmacology 2007; 109(2): 359-63.

Yi MQ, Liu HX, Shi XY, Liang P, Gao XW. Inhibitory effect of four carbamate insecticides on acethylcholinesterase of male and female *Carassiua auratus* in vitro, Comparative Biochemistry and Physiology 2006; 143: 113-11.

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