1. Introduction

Memory impairment has been recognized as one important problem as the age advances. This problem is still increasing its important due to the increased life span. Therefore, it is critical to minimize the memory deficit in the elderly people. Numerous mechanisms are implicated in the development of age-related dementia and neurodegeneration. Recent studies showed that the excess reactive oxygen species contributed the important role on both neurodegeneration and memory impairment (Harman 1994; Villeponteau et al., 2000; Glad, 2010). The neurdgeneration and hypofunction of cholinergic system has been reported to play the important role on cognitive impairment associated with aging and Alzheimer's disease (Bartus, 2000; Terry, Buccafusco, 2003). The cholinergic degeneration and memory impairment are attenuated by the substance possessing (Lockrow et al., 2009; Chonpathompikunlert et al., 2010; Uabundit et al., 2010).

Mango or *Mangifera indica*, a plant in a family of Anacardiaceae, is one of Thailand's premier tropical fruits. "Namdokmai" has been recognized as the most popular variety and its production ranks first among commercial mango varieties. Mango is not eaten as fruit but also as medicine for numerous ailments such as asthma, cough, diarrhea, leucorrhea, jaundice, pain (Madunagu et al., 1990; Gilles, 1992) and diabetes (Ojewole et al., 2005). Moreover, it also possesses anti-inflammatory (Garrido et al., 2001) and anti-oxidant (Sanchez et al., 2003) activities. Several findings have also confirmed that *Mangifera indica* is rich in antioxidants such as vitamin C, vitamin E, carotenoids and phenolics (Ornelas-Paz et al., 2007; Rocha-Ribeiro et al., 2007). These substances were also previously reported to attenuate memory impairment (Harrison et al., 2009; Takatsu et al., 2009; Suganuma et al., 2004; Frautschy et al., 2001). Therefore, the neuroprotective and cognitive enhancing effects of mango pulp extract have been considered. To elucidate this issue, the current study aimed to determine the protective effect of aqueous extract of ripened mango pulp against neurodegeneration and memory impairment in cognitive deficit condition induced by a cholinotoxin, AF64A..

2. Materials and methods

2.1 Animals

Adult male Wistar rats (180±20 g, 8 weeks old) were obtained from National Animal Center, Salaya, Nakhon Pathom, and they were housed in group of 5 per cage in standard metal cages at 22 ± 2°C on 12:12 h light - dark cycle. All animals were given access to food and water ad libitum. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC).

The experimental protocols were approved by the Institutional Animal Care and Use Committee.

2.2 Drugs and chemicals

Donepezil hydrochloride (Aricept 5 mg/tablet) (Pfizer pharmaceuticals Inc.) and vitamin C (500 mg/tablet) (Government Pharmaceutical Organization) were used as standard drugs in this

study. They were dissolved in propylene glycol and administered via oral route. The animals were received donepezil hydrochloride and vitamin C at doses of 1 and 250 mg/kg BW respectively. All chemical substances used in this study were analytical grade.

2.3 Mango pulp extracts preparation

Ripe fruits of Namdokmai were purchased from local market in Amphoe Maeng Khon Kaen, Khon Kaen Province, Thailand. All mangoes were free from physical and pathological defects and were processed immediately after their arrival at the laboratory. They were cleaned and dried. Then, the pulp of ripened mango fruit was homogenized with distilled water at a ratio of 20 g : 100 ml. The resulting homogenate was filtered through four layered muslin cloth and centrifuged at 4000×g for 5 minutes at room temperature. The supernatant was then collected for further study. The obtained extract was approximate 40% yield. All preparations of mango pulp extract were freshly prepared and adjusted to the designed dose with vehicle by calculating the volume of mango extract (ml) into mg unit

2.4 Mango extract treatment

After acclimatization, rats were divided into 6 groups comprising 8 animals in each group as following:

Group I — Vehicle+AF64A: Rats had been treated with vehicle for 2 weeks before and 1 week after the administration of AF64A, a cholinotoxin, in order to induce a cholinergic deficit as found in age- related cognitive impairment and in Alzheimer's disease.

Group II — Donepezil+AF64A: Animals were orally treated with donepezil, a cholinesterase inhibitor which used as standard drug for dementia treatment. The animals were treated as same as that mentioned in group I and served as positive control in this study, .

Group III — Vitamin C+AF64A: Rats were orally given vitamin C, a standard antioxidant which previously reported to enhance memory and attenuate neurodegeneration. This group was also trated as mentioned in group II and served as positive control.

Group IV-VI — MJ+AF64A: The animals were orally treated with the aqueous extract of ripened mango pulp extract at doses of 50, 100 and 200 mg/kg BW respectively (These doses were selected according to the preliminary data of our lab which showed the cognitive enhancing effect of mango pulp extract).

The animals were determined the spatial memory 1 week after AF64A administration then they were sacrificed and determined the density of survival neurons and in various subregions of hippocampus.

2.4 Determination of the total phenolic compound

The total phenolic compound in mango pulp extract was measured using Folin-Ciocalteu colorimetric method (Singleton et al., 1999). Mango extract was oxidized with Folin Ciocalteu reagent at a ratio of 1:5 (30 µl: 150 µl). After 5 minutes, the reaction was neutralized with 120 µl sodium carbonate. The absorbance was measured at 620 nm after 2 h using a microplate reader. Gallic acid was used as standard, and the results were expressed as l g of gallic acid equivalents/ml.

2.5 Determination of beta carotene in mango extract

The level of beta carotene in mango pulp extract was performed using reversed phase column of high performance liquid chromatography. The mobile phase used in this study was methanol: acetonitrile: dicholormethane at a ratio of 85.5:9.5:5. Moreover, 9 µM triethylamine or TEA was also added to the mobile phase in a final step in order to improve peak shape. Beta carotene was monitored at 450 nm. The peak of beta carotene was observed at retention time of 13.3 minutes.

2.6 AF64A administration

AF64A was prepared as described previously by Hanin et al. (1996). Briefly, an aqueous solution of acetylethylcholine mustard HCl (Sigma, St. Louis, MO) was adjusted to pH 11.3 with NaOH. After stirring for 30 min at room temperature, the pH was lowered to 7.4 with the gradual addition of HCl and stirred for 60 min. The amount of AF64A was then adjusted either to 2 nmol/2 µl. The vehicle of AF64A was distilled water prepared in the same manner as the AF64A and recognized as ACSF. In order to administer AF64A bilaterally via intracerebroventricular (i.c.v.) route, the animals were anesthetized with the intraperitoneal injection of sodium pentobarbital at dose of 60 mg/kg BW. Then, AF64A (2 nmol/2 µl) was infused bilaterally via intracerebroventricular (i.c.v.) route with a 30-gauge needle inserted through a burr hole drilled into the skull into both the right and left lateral ventricles. Stereotaxic coordinates were (from the bregma): posterior 0.8 mm, lateral ± 1.5 mm, and ventral (from dura) 3.6 mm. The rate of infusion was 1.0 µl/min and the needle was left in place for 5 min after infusion and then slowly withdrawn.

2.7 Morris water maze test

The water maze consisted of a metal pool (170 cm in diameter × 58 cm tall) filled with tap water (25°C, 40 cm deep) divided into 4 quadrants. In the center of 1 quadrant was a removable escape platform below the water level and covered with a nontoxic milk powder. The pool was divided into 4 quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the center of the pool. For each animal, the location of invisible platform was placed at the center of one quadrant and remained there throughout training. The rats must memorize the platform location in relation to various environmental cues because there was nothing directly showed the location of the escape platform in and outside the pool. Therefore, the placement of the water tank and platform were the same in all acquisition trials. Each rat was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim

until it found and climbed onto the platform. During training session, the rat was gently placed on the platform by experimenter when it could not reach the platform in 60 S. In either case, the subject was left on the platform for 15 s and removed from the pool. The time for animals to climb on the hidden platform was recorded as escape latency or acquisition time. In order to determine the capability of the animals to retrieve and retain information, the platform was removed 24 hr later and the rat was released into the quadrant diagonally opposite to that which contained the platform. Time spent in the region that previously contained the platform was recorded as retention time. In each trial, the animal was quickly dried with towel before being returned to the cage. All tests were carried out within 45 minutes after the administration of vehicle or plant extract or donepezil, a cholinesterase inhibitor, which served as positive control.

2.8 Histological procedure

Following anesthesia with sodium pentobarbital (60 mg/kg BW), fixation of the brain was carried out by transcardial perfusion with fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3. The brains were removed after perfusion and stored over a night in a fixative solution that used for perfusion. Then, they were infiltrated with 30% sucrose solution for approximately 4° C. The specimens were frozen rapidly and 30 µM thick sections were cut on cryostat. They were rinsed in the phosphate buffer and picked up on slides coated with 0.01 % of aqueous solution of a high molecular weight poly L-lysine.

2.9 Nissl staining

The duplicate coronal sections of brains were stained with 0.75% cresyl violet, dehydrated through graded alcohols (70, 95, 100% 2×), placed in xylene and coverslipped using DPX mountant.

2.10 Acetylcholinesterase and lipid peroxidation assays

The rats were divided into various groups as previously described in 2.3. After the last dose of administration, all rats were sacrificed. Hippocampus was isolated, prepared as hippocampal homogenate and the determination of the lipid peroxidation product level and acetylcholinesterase activity in hippocampus were performed. Lipid peroxidation was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS) in the hippocampal homogenate whereas acetylcholinesterase activity was performed using the colorimethod.

2.11 Statistical analysis

Data are presented as mean ± standard error of mean (S.E.M). One-way analysis of variance (ANOVA), followed by Tukey post hoc test. A probability level less than 0.05 was accepted as significance.