Original article

Effects of longan seed extract on scopolamine-induced learning and memory deficit in mice

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Abstract:

Longan (Euphoria longana) seed is a traditional Chinese medicinal plant which has been claimed to have positive effect on deficit in learning and memory. In this study, the effects of crude ethanolic extracts of longan seed on learning and memory impairment induced by scopolamine were investigated in mice. With reference to saline-treated group, scopolamine-treated mice demonstrated impairment of learning and memory seen as an increment of the latency to find the platform in learning trial in Morris water maze as well as a reduction of latency and an increased numbers of error in step-down test. Performances of mice receiving longan seed extract at the dose of 300 mg/kg once daily were not different from those of the scopolamine-treated group. However, oral administrations of longan seed extract at the dose of 1,000 mg/kg significantly reduced the latency to find the platform in Morris water maze test but showed no improvement on the passive avoidance task. Additionally, injection of scopolamine depressed locomotor activity which were unaffected by the administration of longan seed extract. Furthermore, we had investigated the effect of longan seed extract on brain lipid peroxidation in scopolamine-treated mice and found that the administration of neither scopolamine nor longan seed extract exerted effect on malondialdehyde level of the brain. In conclusion, the present study has demonstrated the beneficial effects of longan seed extract for learning and memory deficit induced by scopolamine. Further investigation is needed to explore whether longan seed extract could be beneficial for memory impairment in Alzheimer’s disease in which cholinergic deficit is one of the hallmarks.

Keywords: Euphoria longana; Learning and memory; Longan; Morris water maze; Scopolamine
Introduction

The increase in life expectancy during the 20\textsuperscript{th} century had concomitantly increased the number of people suffering of age related diseases. Dementia, a clinical syndrome characterized by the development of multiple cognitive deficits that are severe enough to interfere with daily functioning, has become a major public health issue \cite{1,2}. It is imposing a tremendous economic impact on both affected individuals and the entire society \cite{3}. The most common form of dementia among older people is Alzheimer’s disease (AD) \cite{4}. The pathophysiology of AD is complex and involves several different biochemical pathways. The key symptoms of AD are primarily caused by cholinergic dysfunction. It is known that acetylcholine (ACh) is an important neurotransmitter related to memory and learning \cite{5}. Based on a cholinergic hypothesis, many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity via acetylcholinesterase inhibitors (AChEI) \cite{6}. However, AChEI presents some limitations as its short half-life and excessive side effects caused by activation of peripheral cholinergic systems \cite{5} and currently there are no effective treatments available for dementia or the progression of AD \cite{7}.

\textit{Euphoria longana} (Sapindaceae), commonly known as longan, is natively grown in tropical countries. Dried pulp of \textit{E. longana} has been used traditionally to treat symptoms such as deficiency of spleen and heart, palpitations, insomnia, and poor memory \cite{8}. Studies have shown that \textit{E. longana} contains many phenolic constituents such as gallic acid, ellagic acid and corilagin. Ethanolic extract from the seeds of \textit{E. longana} has been shown to possess antioxidant effect which could be beneficial for memory deficit \cite{9}. Similarities in the memory impairments between Alzheimer patients and scopolamine-treated animals have been reported, and it has been proposed that scopolamine, a muscarinic cholinergic receptor antagonist, could serve as a useful pharmacological tool to produce a partial model of the disorder \cite{10}. For such reason, we primarily evaluated the effect of ethanolic extracts of longan seed on mice with memory impairment induced by scopolamine, using the Morris water maze \cite{11} and the step-down avoidance test \cite{12}.

Materials and Methods

\subsection*{Animals}

Male ICR mice, weighing 25-30 g, were obtained from National Laboratory Animal Center, Mahidol University, Salaya, Nakompathom, Thailand. Prior to testing, animals were housed 8 mice per cage for one week in the Animal House of the Faculty of Pharmaceutical Sciences, Chulalongkorn University. They were allowed free access to water and food \textit{ad libitum}, and maintained in a constant temperature (25 ± 1 °C) environment under a 12-h light/dark cycle. Animal treatment and maintenance were carried out with an approval of the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

\subsection*{Chemicals}

Scopolamine hydrobromide, sodium hydrogen phosphate-2-hydrate, sodium dihydrogen phosphate-2-hydrate, acetic acid, sodium dodecyl sulfate, thiobarbituric acid, N-butanol, pyridine, 1, 1, 3, 3-Tetraethoxy-propane (Malondialdehyde) were purchased from Sigma Chemical Co., USA. Absolute ethanol was obtained from GPO, Thailand and Normal saline solution was purchased from Thai Nakorn Patana Co., Ltd., Thailand.

\subsection*{Preparation of longan seed extract}

Dried longan seeds were obtained from the local supplier in Chiangmai province, Thailand. They were dried, ground and 500 grams of dried longan seed powder was extracted with 1 liter of absolute ethanol in soxhlet apparatus for 1 week. The pooled extracts were concentrated and then evaporated under vacuum and further evaporated by heating on 80 °C water bath to dryness. This process yielded 80 grams of crude longan seed extract, which is stored at room temperature in a closed container.

\subsection*{Preparation and administration of the test compounds}

In the present study, longan seed extract was suspended in vehicle (0.5% carboxymethylcellulose (CMC) in water) and given orally to mice once daily at doses of 300 and 1000 mg/kg body weight (B.W.).
Scopolamine was dissolved in physiological saline. Scopolamine was intraperitoneally injected at a dose of 1 mg/kg B.W. The control animals were orally administered with vehicle (0.5% CMC) and were intraperitoneally injected with saline.

**Induction of memory deficits by scopolamine**

To study the effects of longan seed extract on impairment of learning and memory induced by scopolamine. Four groups of animals were used: 2 groups of mice (n = 6 per group) were administered orally with vehicle, another 2 with the extracts of longan seed (300 or 1,000 mg/kg B.W.) Followed 30 minutes later, an intraperitoneal injection of normal saline (0.3 ml) was administrated to control group (vehicle) and scopolamine (1 mg/kg B.W.) to other groups. Behavioral tests were carried out at 30 minutes after the injection of scopolamine.

**Morris water maze (MWM)**

The Morris water maze protocol was adapted from D’Hooge R. and De Deyn P.P. [11]. It consisted of a circular pool in a room with geometric shapes on the wall serving as spatial cues. The pool, which was 70 cm in diameter and painted with black color, filled with 13 cm depth of 25 ± 1°C water. A platform (6 cm diameter) was placed into one quadrant of the pool and submerged 1 cm below the water surface. For the training, all mice were trained to locate the submerged platform in a constant location. The training day consisted of four trials. During training, a mouse was started at one of four starting points and allowed to swim until it located the platform or until 60 seconds had elapsed. The mouse remained on the platform for 15 seconds before being dried off and transferred to a holding cage. If the mouse did not reach the platform within 60 seconds, it was gently guided there by the experimenter. The mouse was continuously trained for 4 times at 4 starting points. Acquisition trial was started 24 hours after the final training trial and continued daily for 5 days. The latency of escaping onto the platform was recorded.

**Step-down test**

A step-down passive avoidance was examined using apparatus consisted of plexiglass chamber. The inside dimensions of the activity cage are, length 35 cm; width 23 cm; and height 20 cm. The cage floor is made of evenly spaced stainless steel bars (3 mm diameter) that are spaced 11 mm apart, and a plastic platform (5 cm diameter, 4 cm height) set on the grid in one corner. Electric stimulation was given through the grid connected with a scrambled shock generator (1 Hz, 1 ms, 36 V dc). Mouse was allowed to get adapted to environment in the cage for 3 minutes without electric shock. Then it was placed on the platform, electric shocks were delivered to the grid when the mouse stepped down from the platform. The electric shocks were still delivered for 5 minutes. After 24 hours of training, mouse was placed on the platform for retention test. The electric shocks were still delivered for 5 minutes. Step-down latency, the time that elapsed until the mouse stepped down from the platform, and number of errors was recorded. If the mouse did not step down from the platform within 300 seconds, the retention test was terminated and the maximal step-down latency of 300 seconds was recorded. An error was counted whenever the mouse stepped down from the platform and the number of errors made in 5 minutes was recorded.

**Spontaneous locomotor activity test**

A spontaneous locomotor activity test was carried out by using apparatus consisted of activity cage and counting unit. The inside dimensions of the activity cage are, length 35 cm; width 23 cm; and height 20 cm. The cage floor is made of evenly spaced stainless steel bars (3 mm diameter) that are spaced 11 mm apart, connected to the circuit of counting unit, UGO BASILE model 7430 (7431+7432). The registered numbers or counts of movements were recorded at 5 minutes intervals. The apparatus was placed in light and sound attenuated, and ventilated testing room [13].

**Lipid peroxidation assay**

Following the behavioral testing, the animals were decapitated and the brains were quickly removed.
cleaned with ice-cold saline and stored at -80°C. Brain tissue samples were thawed and homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates from mice brain were separated and used to determine the marker of oxidative stress (malondialdehyde, MDA) by the method previously described by Ohkawa [14]. The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulphate (8.1%) were added to 0.1 ml of processed tissue samples, then heated at 100°C for 60 minutes. The mixture was cooled with tap water and 5 ml of n-butanol/pyridine (15:1). 1 ml of distilled water were added. The mixture was vortexed vigorously. After centrifugation at 2,500 rpm for 20 minutes, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. The concentration of MDA was expressed as µmol/g tissue.

Data analysis

All data are expressed as the mean value for the group ± standard error of the mean (SEM). Statistical analyses were performed by one-way ANOVA and Duncan post-hoc test for planned comparisons between a control versus different treatment groups. A significance value of $P < 0.05$ was considered as statistically significant.

Results

Effects of longan seed extract on Morris water maze test

The effect of longan seed extract (300 and 1,000 mg/kg, p.o.) on memory and learning was evaluated using the Morris water maze test. As shown in Figure 1, the scopolamine-treated group significantly exhibited longer escape latencies throughout the whole trial schedule than did the control group ($P < 0.05$). On day 5, the escape latencies of scopolamine-treated mice and normal saline-treated mice were 30.86 ± 7.09 and 12.65 ± 2.11 seconds respectively. Longan seed extract (300 mg/kg) had no significant effect on scopolamine induced memory deficit in mice. However, longan seed extract (1,000 mg/kg) significantly shortened the escape latencies prolonged by scopolamine treatment on day 3 and 5 ($P < 0.05$). On day 5, the escape latencies of longan seed extract-treated mice and scopolamine-treated mice were 16.14 ± 3.69 and 30.86 ± 7.09 seconds, respectively (Figure 1).

![Figure 1](image-url)
Effects of longan seed extract on step-down passive avoidance test

We assessed whether longan seed extract could improve memory function in passive avoidance learning. As shown in Figure 2, no differences in either initial step-down latency or numbers of error were noted among different treatment groups in acquisition test. However, on the second day, scopolamine (1 mg/kg B.W.) significantly shortened the step-down latency and increased numbers of error when compared to saline-treated group ($P < 0.05$). Retention step-down latencies in saline-treated group and scopolamine-treated group were $266.50 \pm 29.80$ and $61.77 \pm 47.93$ seconds, respectively. Neither the treatment of longan seed extract at the dose of 300 nor 1,000 mg/kg B.W. elicited significant effects on step-down latencies or number of errors when compared to scopolamine-treated group.

Effects of longan seed extract on spontaneous locomotor activity

The spontaneous locomotor activity, measured as movement counting during 5 minutes test period. Scopolamine significantly depressed the spontaneous locomotor activity which was unaltered by the administration of longan seed extract. The total counting of saline-treated group was found to be $257.50 \pm 23.88$ counts whereas it was $101.50 \pm 64.60$ counts in scopolamine treated group. The administration of

![Figure 2](image-url) Effects of scopolamine 1 mg/kg B.W., longan seed extract 300 and 1,000 mg/kg B.W. on learning and memory in step down test, shown as step-down latencies (a) and step-down errors (b) (mean ± SEM, n = 6). # $p < 0.05$ significantly different compared with NSS group
longan seed extract at doses of 300 and 1,000 mg/kg B.W. decreased, but not statistically significant, the locomotor activities to 63.00 ± 37.41 and 8.67 ± 8.27 counts/5 minutes (Figure 3).

**Effects of longan seed extract on brain lipid peroxidation**

As shown in Figure 4, the MDA level of scopolamine treated group was not statistically different from that of saline-treated group. Administration of longan seed extract (both doses) showed no effect on the MDA level. The brain MDA level of saline-treated group, scopolamine-treated group, longan seed extract at the dose of 300 and 1,000 mg/kg B.W. were 792.09 ± 223.06, 583.15 ± 158.46, 635.07 ± 65.23 and 522.72 ± 91.41 µmol/g tissue, respectively.

**Discussion and Conclusion**

In this study, memory deficit was induced by the administration of scopolamine which interferes memory...
and cognitive function in experimental animals by blocking muscarinic receptors [15]. Scopolamine has been extensively used to screen for drugs with potential therapeutic value for dementia [5,16-18]. In line with previous studies, mice treated with scopolamine demonstrated an impairment of learning and memory when assessed by Morris water maze and passive avoidance tests. A longer escape latency in Morris water maze and a shorter step-down latency than those exhibited by mice in control group were noted in scopolamine-treated mice [19,20].

Administration of ethanolic extract of longan seed at the dose of 1,000, but not 300 mg/kg B.W. significantly improved scopolamine-induced memory impairment in the Morris water maze on day 3 and 5 of the treatment. Mice treated with longan seed extract demonstrated shorter escape latency than mice treated with scopolamine. However, such an effect of longan seed was not detected in the step-down avoidance test in which the step-down latency and numbers of error of mice receiving longan seed extract were not significantly different from those receiving scopolamine alone. Lack of ameliorating effect of longan seed extract in step-down passive avoidance paradigm could probably ascribed by difference in sensitivity to interferences with neurochemical system of the behavioral tests in which Morris water maze seemed to be more sensitive than the passive avoidance test [21].

In locomotor activity test, scopolamine has been found to significantly reduce locomotor activity of mice. The same finding has been reported by Kirkby et al., [22] and Ruotsalainen et al. [23]. Based on the results that all doses of longan seed extract given to scopolamine-treated animals had no effect on locomotor activity when compared to scopolamine-treated group, it is suggestive that locomotor behavior had no effect and played no role in an improvement of performance in Morris water maze observed in longan treated mice.

Alzheimer’s disease is one of the most common forms of neurodegenerative disorder characterized by a progressive loss of memory, followed by a complete dementia [24]. Several studies suggest that oxidative stress plays an important role in pathogenesis of neurodegenerative disorder like Alzheimer’s disease. Thus, the progression of neurodegenerative disorder can be inhibited by the use of free radical scavengers and anti-oxidants [25]. MDA is an important marker for lipid peroxidation. In the present study, we found that scopolamine did not increase the lipid peroxidation in comparison to the control group. Also there were no significant differences between longan seed extract-treated groups and scopolamine-treated group, implying that attenuation in learning and memory deficit induced by scopolamine was unlikely to be explained by antioxidant effect. However, the finding that, at the dose of 1000 mg/kg B.W., longan seed extract could attenuate learning and memory deficit induced by scopolamine did suggest the effect of longan seed extract on cholinergic system. Recently, the seed extract of Cassia obtusifolia was reported to ameliorate learning and memory impairment induced by scopolamine via inhibition of acetylcholinesterase [26]. Extensive investigation is needed to identify pharmacologically active component(s) responsible for the anti-amnesic observed as well as its respective mechanism of action.

In conclusion, the present study has demonstrated the beneficial effects of the crude ethanolic extract of longan seed on scopolamine-induced deficit in learning and memory in mice. Further investigation is needed to explore whether longan seed extract could be beneficial for memory impairment in Alzheimer’s disease in which cholinergic deficit is one of the hallmarks.

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