

The Effects of Dark Purple Glutinous Rice Variety Leum Phua on Scopolamine-Induced Memory Deficits in Mice

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Background and Objective: Leum Phua is a special variety of glutinous rice which has been reported to have very high antioxidant property. Oxidative stress is widely accepted as an important factor in destroying many biological systems including nervous system. The aim of this experiment is to *investigate the effects of* Leum Phua rice on brain function, especially learning and memory, in male mice.

Methods: Morris water maze and passive avoidance tests were used to test the effects of Leum Phua rice aqueous extract (LP) on normal and scopolamine-induced memory deficit in young adult mice. LP was given to mice at the dose of 90 or 180 mg/kg by gavages once daily. On day 15, mice were trained and, on day 16 were tested in passive avoidance apparatus. Another group of animals, on day 15-17, animals were tested in Morris water maze. The latency time for entering the dark chamber (in pas-

sive avoidance) and step on platform (in Morris water maze) were determined. On each test day, certain groups of animals were received intraperitoneal injection of 1 mg/kg scopolamine 15 min before the test.

Results: The results showed that LP at either 90 or 180 mg/kg could improve learning and memory in mice in both passive avoidance and Morris water maze tests. Learning and memory of the animals were impaired by scopolamine treatment and the effects could be antagonized by LP, especially at 180 mg/kg.

Conclusion: The results suggested that LP has learning and memory enhancing effects. The effect of LP might be due to high concentration of antioxidants in Luem Phua rice.

Key words: dark purple glutinous rice var. Leum Phua, learning and memory, scopolamine-induced memory deficit

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Introduction

Memory impairment associated with stress and aging is an important problem of human health around the world and is often one of the early signs of dementia. In addition to memory loss, symptoms of dementia can include difficulties with language, judgment, and insight, failure to recognize people, disorientation, mood changes, hallucinations, delusions, and the gradual loss

of ability to perform all tasks of daily living¹. As a number of older people are increasing, an increasing in demented population is noted, therefore, it is challenging to look for novel substances to enhance the memory and prevent patients from dementia.

Leum Phua glutinous rice was developed by Phitsanulok Rice Research Center. The rice has dark-purple pericarp and contains high levels of certain



nutrients such as vitamin E, omega6, iron (Fe), calcium (Ca) and anthocyanins. Additionally, Leum Phua rice has been reported to have antioxidant activity higher than other black rice². Several recent reports showed that antioxidant agents could prevent and improve many diseases such as cancer, atherosclerosis³ and Alzheimer's disease⁴. Therefore, with high antioxidant property, Leum Phua rice could be one of the nutraceuticals for the brain.

Objective

The aim of this experiment is to *investigate the effect of LP on learning and memory in male mice.*

Methods

LP used in this study was received from Department of Chemistry, Faculty of Science, Chiang Mai University. Fear memory was test using passive avoidance test. Mice were orally administered with distilled water (as a control), 90 mg/kg LP or 180 mg/kg LP once daily for 8 days. Passive avoidance was done on day 15 (train day) and day 16 (test day). At 30 min after first treatment (oral), mice received second treatment (intraperitoneal injection,i.p.) (Table 1). The test was performed 15 min

after the second treatment. Mouse was placed in the light compartment of the passive avoidance apparatus. The time taken for a mouse to enter the dark compartment after door opening was defined as latency time. Latency time was recorded up to 300 s. Spatial memory was evaluated by Morris water maze. Mice were orally administered with distilled water (as a control), 90 mg/kg LP or 180 mg/kg LP once daily for 17 days. On day 15-17, specified as test day 1, 2 and 3. Thirty minutes after first treatment (oral), mice received second treatment (i.p.) (Table 1). The test was performed 15 min after the second treatment. The Morris water maze was consisted of milky water filled plastic pool in which a circular platform submerged below the water surface was placed in the center of one quadrant (the target quadrant). The pool was divided into four quadrants of equal areas. From the beginning, mouse was placed on the platform for 1 min then it was placed into the water facing the edge of the pool at opposite quadrant, and allowed the maximal time of 1 min to locate the platform. The time animal used to locate the platform was expressed as escape latency. If the animal failed to find the platform in 60 s, it was guided to the platform and placed on the platform for 15 s. The test was done to 3 trials/ day with an inter-trial interval of

Table1 Treatment conditions in passive avoidance and Morris water maze tests.

Group	First treatment (oral)	Second treatment (i.p.)
1. Control	Distilled water	Distilled water
2. LPL	LP 90 mg/kg	Distilled water
3. LPH	LP 180 mg/kg	Distilled water
4. Sco	Distilled water	Scopolamine 1 mg/kg
5. LPL+Sco	LP 90 mg/kg	Scopolamine 1 mg/kg
6. LPH+Sco	LP 180 mg/kg	Scopolamine 1 mg/kg

15 min on each day of test⁵.

Data were assessed by one-way analysis of variance (ANOVA) and all pairwise multiple comparison procedures (Fisher's LSD method). P-value less than 0.05 was considered as statistically significant.

Result

Passive avoidance test

The result of this study, as shown in Fig1, a significant increase in learning and memory ability in mice treated with LP 90 mg/kg and 180 mg/kg could be observed as seen by a significant increase of

percentage change of latency time on test day when compared with the control group. The percentage change of latency time of scopolamine-treated mice was significantly lower than the control group. Interestingly, LP 180 mg/kg, but not 90 mg/kg, could reverse the effect of scopolamine on memory.

Morris water maze test

The effects of LP on escape latency of trial 1 were shown in Fig 2. On test day 1, only mice treated with LP 180 mg/kg showed a significant increase in learning and memory ability as seen by a significant decrease of escape latency when compared to the control group. On

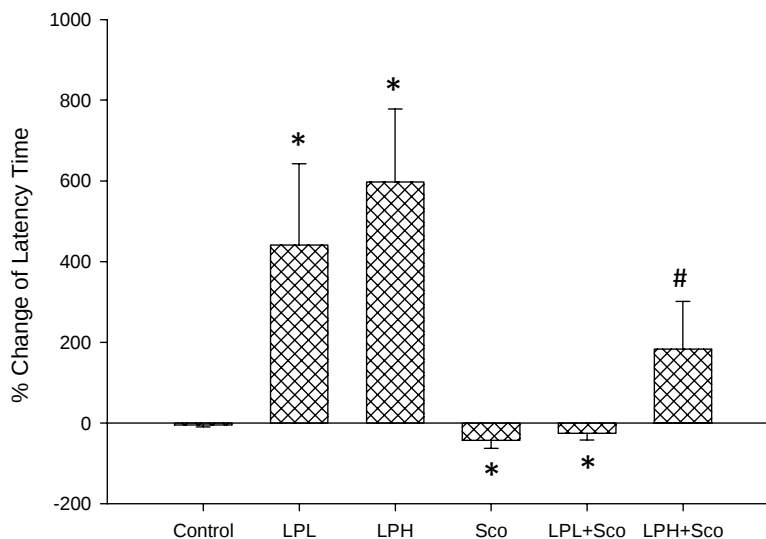


Figure 1 The effect of LP on latency time in the passive avoidance test in mice. Data were represent as percentage change and presented as mean $\pm$ SD. * significant different when compared to the control group; # significant different when compared to scopolamine-treated group.

test day 2 and 3, every groups of animal except scopolamine-treated group, showed an increase in learning and memory when compared to the same treatment on day 1. Interestingly, on test day 3, LP 90 mg/kg, LP 180 mg/kg and scopolamine + LP 180 mg/kg showed significant lower escape latency time when compared to the control on the same day. The results suggest the antagonist effects of LP against scopolamine. .

The results of trials 2 and 3 were in the same pattern as trial 1 (data not shown).

Conclusion

The results in this study suggested that LP has the cognitive enhancing effect in both fear memory test (passive avoidance test) and spatial memory test (Morris water maze test). In addition, LP could also antagonize the sco-

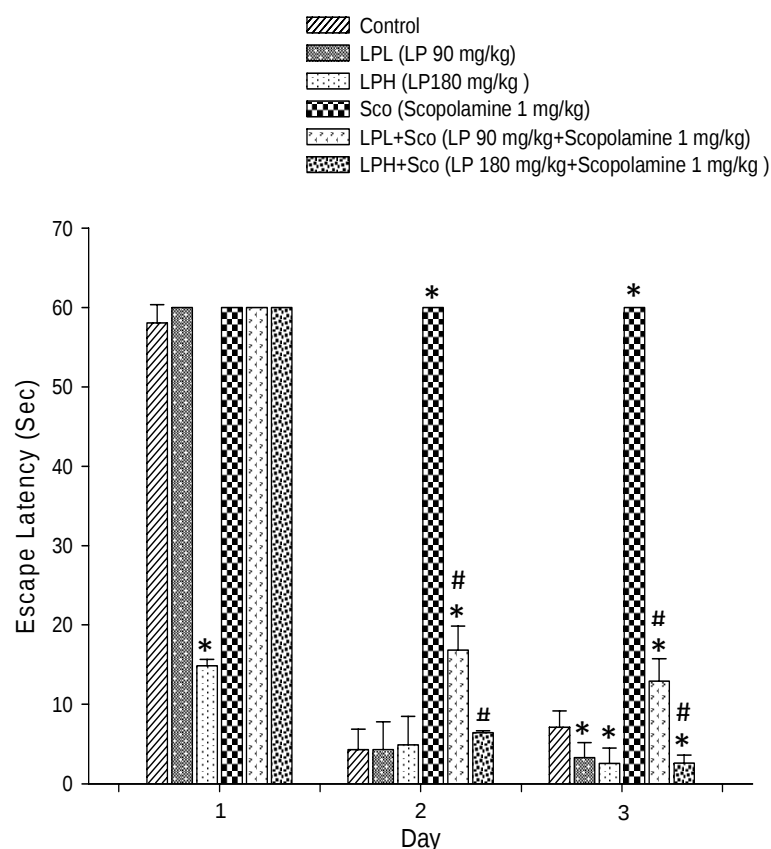


Figure 2 The effects of LP treatment on escape latency in Morris water maze test in mice. Data were presented as mean $\pm$ SD. * significant different when compared to the control group in the same test day; # significant different when compared to scopolamine-treated group in the same test day.

polamine-induced memory deficit effect, especially at the dose of 180 mg/kg. It has been shown that Leum Phua rice contains very high concentration of anthocyanins which reported to have learning and memory enhancing effects. Thus, the effect of LP on learning and memory might be the results of the high antioxidant property of anthocyanins in the rice. This study then suggests that Leum Phua rice might be one of the nutraceutical for brain health, especially for learning and memory.

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