

ผลของสารสกัดเกสรบัวหลวงต่อความจำบกพร่องในหนูแรทที่ถูกเหนี่ยวนำด้วยแอมิลอยด์บีต้า

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Effect of *Nelumbo nucifera* Stamen Extract on Memory Deficits in A β -induced Rat

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Received: 16 December 2019

Accepted: 20 February 2020

หลักการและวัตถุประสงค์: เกสรบัวหลวงเป็นพืชสำคัญทางการแพทย์แผนไทย มีฤทธิ์ต้านเบาหวาน ด้านอนุมูลอิสระ และยับยั้งเอนไซม์อะซิติลโคลีนเอสเทอเรส นิยมบริโภคเป็นอาหารสุขภาพ การศึกษานี้เป็นการประเมินผลของสารสกัดเกสรบัวหลวงต่อภาวะความจำบกพร่องในหนูแรทที่ถูกเหนี่ยวนำด้วยแอมิลอยด์บีต้า

วิธีการศึกษา: หนูแรท เพศผู้ พันธุ์ Sprague Dawley จำนวน 40 ตัว แบ่งเป็น 5 กลุ่ม กลุ่มละ 8 ตัว ดังนี้ กลุ่มที่ 1 กลุ่มควบคุมปกติ กลุ่มที่ 2 กลุ่ม V+A β ได้รับ 0.5% sodium carboxymethyl cellulose ทางปาก และถูกฉีดด้วยแอมิลอยด์บีต้าเข้า lateral ventricles กลุ่มที่ 3 กลุ่ม Vit C+A β ได้รับวิตามินซีขนาด 200 มก./กก. ทางปาก และถูกฉีดด้วยแอมิลอยด์บีต้าเข้า lateral ventricles กลุ่มที่ 4 และ 5 กลุ่ม NNSE250+A β , NNSE500+A β ได้รับสารสกัดเกสรบัวหลวงขนาด 250 และ 500 มก./กก. ทางปาก ตามลำดับ และถูกฉีดด้วยแอมิลอยด์บีต้าเข้า lateral ventricles ทุกกลุ่มได้รับสารตามกลุ่มต่อเนื่องทุกวันเป็นเวลา 33 วัน ในวันที่ 21 ของการรักษา ทำการฉีดแอมิลอยด์บีต้าเข้าโพรงสมองทั้ง 2 ข้างของหนูแรท หลังจากนั้น 10 วัน ประเมินการเคลื่อนไหวและทดสอบ

Background and Objectives: *Nelumbo nucifera* (lotus) is a key plant in Thai traditional medicine that has anti-diabetic and anti-oxidant activities, and inhibits acetylcholinesterase enzyme. It is widely consumed as functional food. This study investigated the effect of *N. nucifera* stamen extract (NNSE) on memory deficit in amyloid beta (A β)-induced rat.

Methods: Forty adult male Sprague Dawley rats were divided into 5 groups, each group consisting of 8 rats as follows: Group 1, the normal control group. Group 2, the vehicle plus A β group (V+A β), received 0.5% sodium carboxymethyl cellulose, orally and was injected with amyloid- β via the lateral ventricles. Group 3, the vitamin C plus A β group (Vit C+A β), received vitamin C orally at 200 mg/kgBW., and was injected with A β via the lateral ventricles. Group 4, and 5, the NNSE plus A β groups (NNSE250+A β , NNSE500+A β), received NNSE orally at doses of 250, and 500 mg/kgBW, respectively, and were injected

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ความจำชนิดรู้จำด้วยอุปกรณ์ทดสอบ open field และ novel object recognition ตามลำดับ

ผลการศึกษา: สารสกัดเกสรบัวหลวงขนาด 500 มก./กก. สามารถเพิ่มความเร็วและระยะทางการเคลื่อนที่อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ในหนูที่ถูกเหนี่ยวนำด้วยสารแอมิลอยด์บีต้า มากไปกว่านั้นสารสกัดเกสรบัวหลวงทั้งขนาด 250 และ 500 มก./กก. ยังช่วยฟื้นฟูความจำชนิดรู้จำระยะสั้นในหนูที่มีความบกพร่องทางสติปัญญาอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$ และ $p < 0.01$) และสารสกัดเกสรบัวหลวงทั้งสองขนาดยังมีแนวโน้มช่วยฟื้นฟูความจำชนิดรู้จำระยะยาวในหนูที่มีความจำบกพร่อง

สรุป: สารสกัดเกสรบัวหลวงสามารถฟื้นฟูความจำบกพร่องที่ถูกเหนี่ยวนำด้วยแอมิลอยด์บีต้าโดยเฉพาะความจำระยะสั้น นอกจากนี้สารสกัดเกสรบัวหลวงปริมาณสูงยังกระตุ้นการทำงานของกล้ามเนื้อด้วยการเพิ่มความเร็วและระยะทางการเคลื่อนที่

คำสำคัญ: แอมิลอยด์บีต้า เกสรบัวหลวง การเรียนรู้และความจำ ภาวะเครียดออกซิเดชัน

with A β via the lateral ventricles. All treatments were performed for 33 consecutive days. At day 21, A β (1-42) was injected via both sides of lateral ventricles. Ten days after A β 1-42 injection, the rats were tested for locomotor activity and recognition memory by using the open field and novel object recognition tasks, respectively.

Results: The NNSE at dose of 500 mg/kg BW significantly increase the velocity and moving distance in locomotor activity in rats after induced with A β . Moreover, NNSE at doses of 250 and 500 mg/kgBW significantly improve short-term recognition in cognitively impaired rats. These doses of NNSE also tend to improve long-term recognition in memory impaired rats.

Conclusions: NNSE improve memory impairments induced by A β , especially short-term memory. Furthermore, the high doses of NNSE stimulates muscle function by increasing speed and movement distance.

Keywords: amyloid- β , *Nelumbo nucifera* stamen, novel object recognition, oxidative stress

ศรีนครินทร์เวชสาร 2562; 35(3): 266-271. • Srinagarind Med J 2019; 35(3): 266-271.

Introduction

Memory deficiency can occur in many diseases, a major one of them is Alzheimer's disease (AD), which causes neuronal damage. The number of AD patients have been projected to increase up to 75 million by 2030 and 131 million by 2050¹. It is still unclear on which factors cause AD but it is believed that the accumulation of amyloid beta (A β) plaque is one of the factors associated with AD onset. There are many Alzheimer's patients that have high level of free radicals and oxidative stress in the brain. Many studies suggested that oxidative stress may be one of the earliest alterations that occur during the initiation and development of AD^{2,3}. There are many medications used for AD treatment, each of them might have some side effects. Many herbs were studied and used to treat and reduce an occurrence of AD such as *Piper nigrum*⁴, *Phyllanthus acidus*⁵, and *Nelumbo nucifera*⁶. Several studies have been carried out toward the isolation of natural products those are applicable for development of new anti-AD drugs, including *N. nucifera* stamen. Several phytochemical compounds such as flavonoids, triterpenoids,

carotenoids and phenolics have been isolated from *N. nucifera* stamens⁷⁻⁹. These compounds possessed antioxidant and anti-inflammatory effects¹⁰. *N. nucifera* stamen extract also improved memory deficit in rat treated with scopolamine⁶. Therefore, this study aimed to investigate the effect of *N. nucifera* stamen extract on the improvement of memory deficit in rat induced by A β .

Materials and Methods

Plant preparation

The *N. nucifera* stamen extract (NNSE) was obtained from the department of Pharmaceutical Chemistry, faculty of Pharmacy, Khon Kaen university, Thailand. Briefly, the *N. nucifera* stamen extract was prepared by macerating 3 kilograms of dried *N. nucifera* stamen in 46 L of 95% ethyl alcohol. Then it is incubated at room temperature for 3 days before being evaporated with the rotary evaporator at 40°C and then freeze-dried.

Animals

Forty adult male Sprague-Dawley rats of age 6 - 8

weeks weighed 200-400 grams were obtained from Nomura Siam International Co., Ltd., Bangkok, Thailand. They were raised at the Northeast Laboratory Animal Center, Khon Kaen University. They were housed at 4 rats per cage (37.5 x 48 x 21cm) in controlled room under constant temperature at 23 ± 2 °C and lighting control (12-h light/12-h dark cycle). The rats were acclimatized for 7 days before starting the experiment. All experiments were approved by the Ethics Committee of Khon Kaen University, Thailand (AEKKU 25/61).

Experimental design

The rats were divided into 5 groups (n=8): Group 1 normal control, Group 2 received 0.5% sodium carboxymethyl cellulose (NaCMC), Group 3 received vitamin C at a dose of 200 mg/kg/BW, p.o., Group 4 and 5 received NNSE at a dose of 250 and 500 mg/kg/BW, p.o. daily, respectively. All rats of group 2 to 5 were injected with Aβ₁₋₄₂ into both sides of lateral ventricle by determining the location with stereotaxic atlas of Paxinos and Watson¹¹. After the rat recovery from the injection, they were administered with the extracts daily until the day 33rd of the experiment. Then, the locomotor activity of all rats was assessed by open field test. The recognition memory of all rats was also assessed by novel object recognition task (NOR) (Figure 1). All behaviors of the rats were tracked, observed and recorded using a video camera.

Open field test

The open filed task was used to assess locomotor activity of the rats. The task consists of a video camera and an opaque black square box (depth 50x width 50x high 40 cm)¹², of which its floor is divided into 16 small compartments. The experiment contains two sessions, habituation and test sessions. Habitua-

tion is the process of adapting to the environment in the box which placed the rat in the center and letting the rat move freely for 5 minutes. The test session is similar to the habituation. The movement behavior of the rats was analyzed by Noldus EthoVision XT program to determine the rat velocity and total moving distance.

NOR test

The recognition memory of the rats was assessed by NOR test. Briefly, the equipment consists of an opaque black square box (depth 50x width 50x high 40 cm)¹² and cylindrical, triangular, and square shape objects. NOR test consists of two phases, namely familiarization phase and test phase. To begin the experiment, the rats were let to freely explore two identical objects for 5 minutes in the familiarization phase. After finished, one familiar object was removed and changed to a novel object, and then a test phase was performed two time, at 5 minutes and 24 hours¹². The rats were then allowed to explore both objects for 5 minutes to assess short-term memory¹³. For long-term memory, the rats were allowed to explore another set of familiar and novel objects for five minutes, at 24 hours after the familiarization phase¹⁴. The movement and the time used to explore each object of each rat were determined from video footages recorded during the whole experiment. The rats were considered exploring an object when the nose of a rat inhales and touches the object less than or equal to two centimeters. Exploration time of familiar and novel objects were used to calculate Discrimination index (DI) by using a following formula¹⁵. All equipment used in this behavior assessment were wiped clean with 70% alcohol between sessions.

Discrimination index (DI) = (TN-TF)/(TN+TF)
 where, TN = Time spent in explore novel object
 and TF = Time spent in explore familiar object

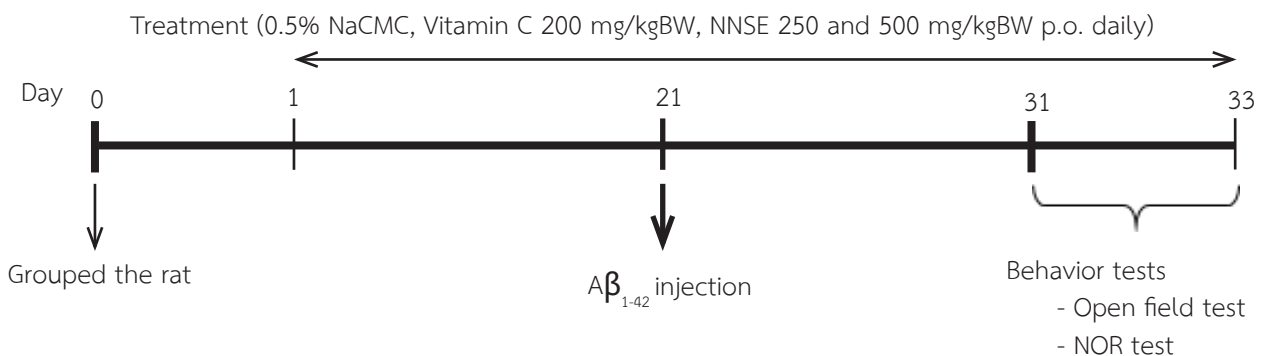


Figure 1 Timeline of the experimental process (Aβ₁₋₄₂: amyloid-β (1-42), NaCMC: sodium carboxymethyl cellulose, NNSE: *N. nucifera* stamen extract, NOR: novel object recognition

Statistical analysis

Means of moving distance, velocity, and DI were calculated and expressed as Mean ± SEM (Standard error of the mean). One-way Analysis of Variance (ANOVA) was used to determine the differences in these variables among treatment groups. All statistical analyses were performed at the 95% confidence level.

Results

Effect of NNSE on locomotor activity

The velocity and moving distance are two indexes used for assessing the locomotor activity of the rats. The results showed that muscle function of the Aβ-induced rats does not impair in comparison to control rats. Whereas the NNSE at the high dose showed the significantly increased the velocity and moving distance of the rats when compare to V+Aβ group (p < 0.05) (Figure 2A and 2B).

Effect of NNSE on recognition memory

NOR test is used to assess the object recognition memory which was reported as the DI. The results showed that injection of Aβ causes memory impairment in rats. For short-term memory, rats

received NNSE 250 and 500 mg/kg BW showed the significant higher DI (p<0.05 and p<0.01) than those of the Aβvehicle (V+Aβ) rats (Figure 3B). For long-term memory, vitamin C treated rats showed significantly higher DI compared to V+Aβ group. Rats received two

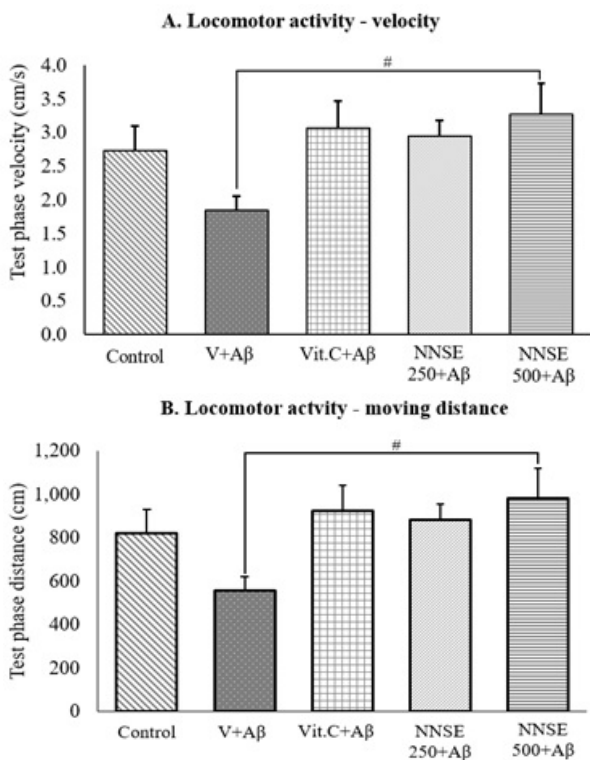


Figure 2 Locomotor activity of the rats during 5 min test phase shows velocity (A.) and moving distance (B.) Data are presented as mean ± S.E.M. (n = 8/group), # : significant differences from vehicle+Aβ group at p < 0.05

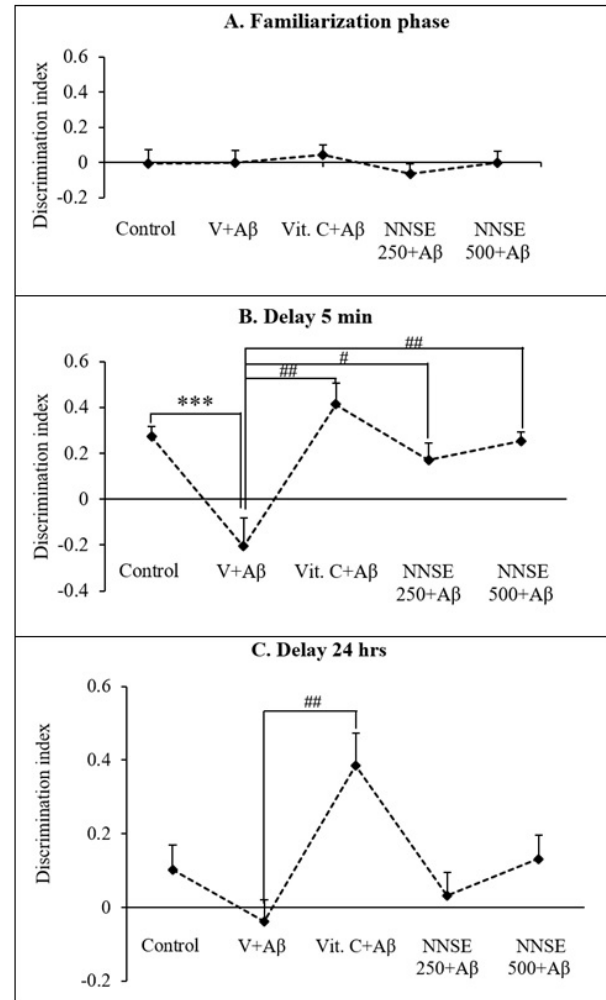


Figure 3 Effects of NNSE on memory performance in Aβ-induced rats as measured by the discrimination index (DI) in the familiarization phase (A.), after a 5 minutes delay (B.) and after 24-hours delay (C.) of NOR task. Data are presented as mean ± S.E.M. (n = 8/group), *, *** : significant differences from control at p < 0.05, and p < 0.001, respectively, #, ## : significant differences from V+Aβ at p < 0.05, and p < 0.01, respectively.

doses of NNSE also showed the higher DI compared to V+Aβ groups (Figure 3C).

Discussion

The findings of this study supported that NNSE extract at the high dose could increase locomotor activity of the rat. Furthermore, the NNSE extracts improved the recognition memory of the rats after inducing impaired memory with Aβ₁₋₄₂. Previous

studies indicated that A β induces oxidative stress and neurotoxicity^{16,17}. Under this situation, the aggregated A β may initiates free radical processes resulting in protein oxidation, lipid peroxidation, reactive oxygen species formation, cellular dysfunction leading to calcium ion accumulation, and subsequent neuronal death¹⁸. Therefore, the use of treatments to reduce A β formation may be helpful in preventing, treating, or delaying the progression of AD. There are various approaches that could be potential candidates to reduce A β levels including the antioxidant compounds in natural products. NNSE extract was reported containing flavonoid, phenolic, and alkaloid that has ability to scavenged free radicals and reduced oxidative stress^{9,19,20}. In this study, memory of NNSE treated rats improvement might be due to the mechanism of the reduction of free radicals and oxidative stress.

Conclusion

The NNSE extract improves memory dysfunction indicating by increased DI. Our finding showed that NNSE extract has potential in neuroprotective effect. These results may be useful in Alzheimer's patients with impaired memory.

Acknowledgement

This study was granted by faculty of Medicine, Khon Kaen university, Thailand (Grant Number IN62344). We are thankful for the NNSE extract used in this study from the Thailand Research Fund, Thailand (Grant umber BDG6080006). We would like to also extend special gratitude for the Northeast laboratory animal center and the faculty of Pharmaceutical Sciences and department of Anatomy, faculty of Medicine, Khon Kaen university, Thailand for their personnel and laboratory technicians involved in this study.

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