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นิพนธ์ต้นฉบับ

Memory Enhancing Effects of Virgin Rice Bran Oil Derived from Thai Brown Rice in Mice

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Abstract

Rationale and Objective: Oil extracted from rice bran has been found to possess antioxidant activity; and as it is also rich in γ-oryzanol, vitamin E and phytosterols, it has many health benefits. This study aimed to determine the effects of virgin rice bran oil derived from Thai brown rice by using cold-press technology on learning and memory in mice.

Methodology: Male ICR mice were force-fed either with distilled water, corn oil or virgin rice bran oil (VRBO) at the dose of 5 ml/kg body weight, once daily for 21 days. Learning and memory were assessed by a fear-aggravated test and a passive avoidance test (PAT) on day 18 (training day) and day 19 (test day), and a spatial learning test and the Morris water maze test (MWM) on days 19-21. Latency time in PAT, escape latency time, and time in right quadrant in the MWM were determined. On each test day, 30 min after treatment, mice were intraperitoneally injected with either distilled water or 1 mg/kg scopolamine and 15 min later were subjected to the tests.

Results: VBRO was found to improve learning and memory of mice in both fear-aggravated and spatial learning tests, compared to the water or corn oil-treated groups. However, at the dose of 5 ml/kg/day, VBRO could not antagonize the memory deficit induced by 1 mg/kg scopolamine.

Discussion and Conclusion: The results suggest that VBRO has a cognitive enhancing effect. VBRO, derived from Thai brown rice by cold-press technology, is generally rich in essential fatty acids, phytosterols, and compounds with antioxidative activities. Thus, VBRO might be one of the nutraceuticals for brain health, especially for learning and memory enhancement.

Key words: **Virgin rice bran oil, Thai brown rice, learning and memory, mice**

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Rationale and Objectives

Dementia is a chronic or persistent disorder of the mental processes caused by brain disease or injury and marked by learning and memory disorders, personality changes, and impaired reasoning. The impairments of cognitive function are commonly accompanied and occasionally preceded by deterioration in emotional control, social behavior, or motivation.[1] The World Health Organization estimated that the number of patients suffering from dementias will be approximately double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050[2] and the burdens of dementias are enormous.[3] Recent estimates for total cost per dementia patient in Thailand for the year 2015 were $1,810, resulting in a total Asia Pacific cost of $184,868 million.[4] From above, dementia seems to be a global burden and an important problem in public health. Therefore, it is challenging to look for novel substances to enhance the memory and prevent patients from dementia.

Rice (*Oryza sativa* L.) is a major source of nourishment for the world's population, especially in Asia. World production of rice is estimated at around 680 million tons, equivalent to that of wheat.[5] Rice bran is a by product from the rice milling process and a good source of vitamins, minerals, and other bioactive compounds.[6] Oil extracted from rice bran is well known as containing many high potency antioxidant substances such as γ-oryzanol, phytosterols, tocopherols, tocotrienols, ferulate esters, β-sitosterol, and campesterol.[7] Previous reports showed that rice bran oil plays an important role in preventing heart attack, reducing cholesterol level (LDL-C), increasing HDL in the blood,[8-10] modulates the immune system by enhancing B-lymphocyte proliferation,[11] and improve memory function.[12]

By a cold pressed technology, virgin rice bran oil (VRBO) derived from Thai brown rice is one of the special essential oils which, as a whole food, provides greater nutrition and is a source of more complex micronutrients especially natural antioxidants phytosterols and gamma oryzanol.[13-15] In this study, the effects of VRBO on learning and memory in mice were investigated.

Methodology

Materials and chemicals

VRBO used in this study was obtained from the Manufacturer in Chaiyaphum Province, Thailand. Corn oil and scopolamine hydrochloride (Sigma-Aldrich, UK) were used.

Experimental animals

Male ICR mice 9 weeks of age, weighing 30-35 g, were obtained from the National

Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand. Animals were housed in groups under standard conditions (ambient temperature 25±2°C, humidity 60-70%, 12-h light/dark cycle (lights on at 7:00 h) and allowed free access to both food pellets and water. They were allowed to acclimatize to these housing conditions for 1 week prior to the experiments. All behavioral experiments were carried out between 8:00 and 16:00 h. The experimental protocol was approved by the Animal Ethics Committee of Khon Kaen University based on the Ethics of Animal Experimentation of the National Research Council of Thailand. Record No. AEKKU 39/2557 and reference No. 0514.1.12.2/46.

Animal treatment

Male ICR mice were divided into five groups with 6-8 animals in each group. Animals were forced-fed either with distilled water (as the control), corn oil (as the oil supplemented diet control[16]), or VRBO at the dose of 5 ml/kg body weight (BW) once daily for 21 days. Mice were subjected to learning and memory tests by using a fear-aggravated test and passive avoidance test (PAT) on day 18 (training day) and day 19 (test day) and spatial learning test and the Morris water maze test (MWM) on day 19-21. Latency time in PAT, escape latency time, and time in right quadrant in MWM were determined. On each test day, 30 min after treatment, mice were intraperitoneally injected either with distilled water or 1 mg/kg BW scopolamine and 15 min later were subjected to the tests.

Passive avoidance test[17]

The step-through inhibitory avoidance apparatus box (50 cm x 25 cm x 25 cm) comprised of two equal compartments was installed on the floor of the dark compartment to produce foot shock. Intermittent electric shocks (50 Hz, 3 s, and 1 mA intensity) were conveyed to the grid floor of the dark compartment by an insulated stimulator. On the training day, each animal was gently placed in the light compartment of the apparatus. Thirty sec later, the guillotine door was opened allowing the animal to enter the dark compartment. Latency time (time taken for a mouse to enter the dark compartment after door opening) was recorded as training latency time. Once the animal entered the dark compartment, the door was closed and an electrical foot shock was delivered through the stainless steel rods. Twenty-four hours after the training on test day, the animal was placed in the light compartment for 30 sec. Then the door was opened and latency time (test latency time) was recorded. The cut-off time of 300 sec was applied for those animals which still remained in the light compart-

ment. During these sessions, no electric shock was applied. Data were expressed as mean ± SEM of percentage change of latency time [(test latency time - training latency time) x 100/training latency time].

Morris water maze test[18]

The water maze consisted of a plastic pool (70 cm in diameter x 23 cm tall) filled with milky water (25 ^{o}C, 6 cm deep) divided into 4 quadrants by strings placed over the top of the pool crossing the center and four visual cues, different in shape and color, were put on the edge of the pool. In the center of one quadrant (target quadrant), a removable escape platform was placed 1 cm below the water level. The placement of the water tank and platform were the same in all acquisition trials. From the beginning, the mouse was placed on the platform for 1 min then placed into the water facing the edge of the pool at opposite quadrant and allowed a maximal time of 1 min for each mouse to locate the platform. In the case where the mouse could not find the platform, it was manually guided to the platform and left on the platform for 15 sec. The time that the animal was placed into the water until climbing onto the hidden platform was recorded as escape latency. Total of 3 trials of the test were performed with 10-min intervals. In Probe trial, 1 hour after the 3rd trial, the platform was removed and time of mouse swimming in target quadrant in 60 seconds was then recorded as time in right quadrant.

Statistical analysis

All data were analyzed using Sigma stat, version 3.5. They were expressed as mean± SEM, analyzed by analysis of variance (ANOVA) followed by the Tukey test and Independent *t*-test. *p*-value <0.05 was considered statistically significant.

Results

Effects of VRBO on fear-aggravated memory

The effect of VRBO on fear-aggravated memory was shown in **Figure 1**. Mice received distilled water or corn oil (5 ml/kg BW) had a percentage change of latency time of -0.09±1.11 and -2.91±2.12, respectively. No difference among the two groups could be seen. Mice treated with VRBO showed a significant increase in percentage change of latency time (26.82±8.55), represented by the increase in learning and memory, when compared to distilled water-treated group. Although no significant change in percentage change of latency time could be seen in mice receiving scopolamine (1 mg/kg BW) when compared to the distilled water-treated group, VRBO could not antagonize the effect of scopola-

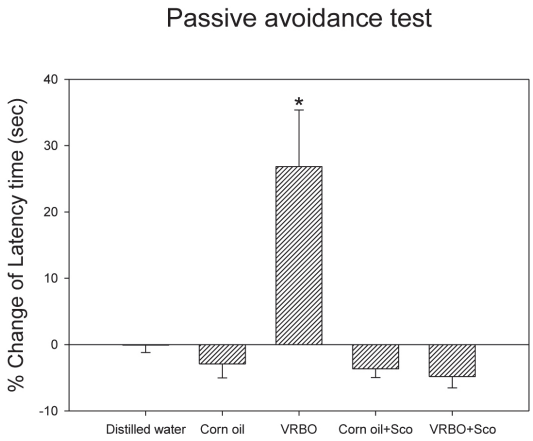

Figure 1. Effect of VRBO on fear aggravated memory. Mice were forced-fed either with distilled water, corn oil or VRBO at the dose of 5 ml/kg BW, once daily. On day 18 and day 19, mice were intraperitoneally injected either with distilled water or 1 mg/kg body weight of scopolamine (Sco) at 30 min after oral treatment. Mice were subjected to passive avoidance test at 15 min after injection. *represented a significant difference when compared to distilled water-treated group.

mine on latency time.

Effects of VRBO on spatial memory

Effects of VRBO (5 ml/kg BW) on spatial memory tested by Morris water maze test were shown in **Figures 2** and **3**. Mice treated with distilled water showed a significantly lower ability to learn, as the escape latency in trial 1 of days 20 and 21 were shorter than that in day 19. Mice treated with corn oil had escape latency time in day 19, 20 and 21 comparable to the distilled water-treated group. The animals treated with VRBO showed a significant decrease in escape latency time when compared to the distilled water-treated group on the same test day. The effects of VRBO in increasing learning and memory ability could be seen in all test days.

On days 20 and 21, mice pretreated either with corn oil or VRBO and received scopolamine injection, had a comparable escape latency time but significantly longer than distilled water- and corn oil-treated groups **(Figure 2)**. The results of trials 2 and 3 were in the same pattern as trial 1 (data not shown). Following probe trial sessions, VRBO signifi-

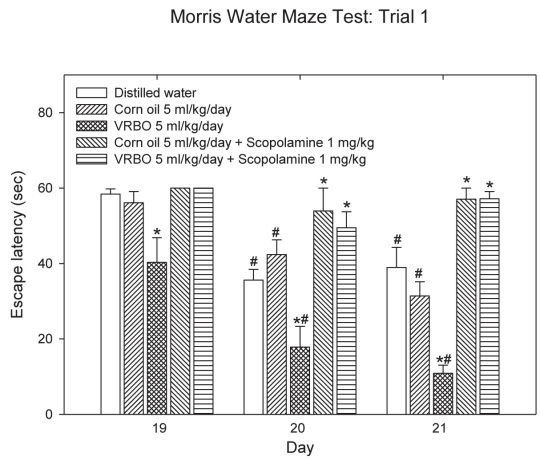

Figure 2. Effect of VRBO on spatial memory (trial 1). Mice were forced-fed either with distilled water, corn oil or VRBO at the dose of 5 ml/kg BW, once daily for 21 days. On day 19-21, mice were intraperitoneally injected either with distilled water or 1 mg/kg body weight of scopolamine at 30 min after oral treatment. Mice were subjected to the Morris water maze test at 15 min after injection. *represented a significant difference when compared to distilled water-treated group on the same test day, #represented a significant difference when compared to the same treatment group on day 1.

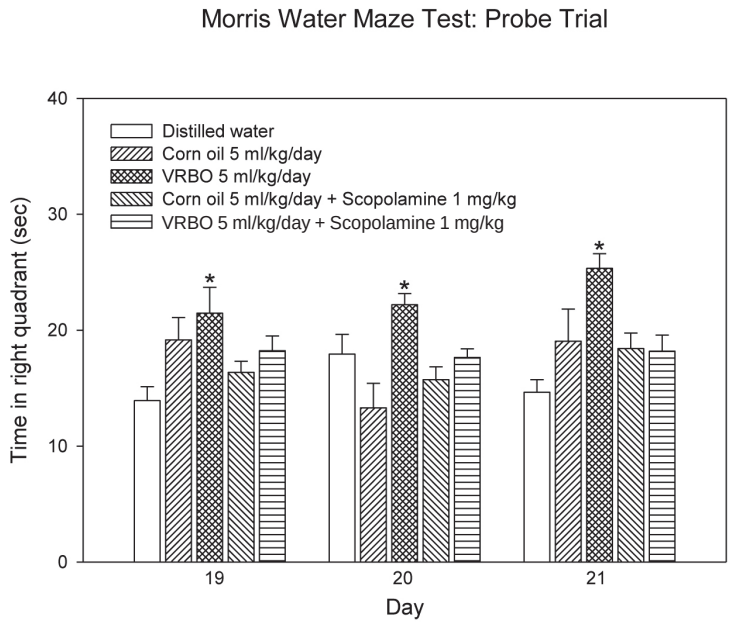

Figure 3. Effect of VRBO on time in right quadrant in Morris water maze test. Mice were forced-fed either with distilled water, corn oil or VRBO at the dose of 5 ml/kg BW, once daily for 21 days. On day 19-21, mice were intraperitoneally injected either with distilled water or 1 mg/kg body weight of scopolamine at 30 min after oral treatment. Mice were subjected to Morris water maze test at 15 min after injection. *represented a significant difference when compared to distilled water-treated group on the same test day.

cantly increased time in right quadrant when compared to the distilled water- or corn oil-treated groups (**Figure 3**) but could not antagonize the effect of scopolamine.

Discussion

In the present study, we observed the effects of VRBO on Learning and Memory in passive avoidance test and Morris water maze test in mice. The results from this study show that VRBO could enhance learning and memory in mice. The increase in learning and memory was clearly seen in both fear-aggravated memory (passive avoidance test) and spatial memory (Morris water maze test). However, VRBO could not antagonize the amnesic effect of scopolamine in both memory test models. Spatial memory has been suggested to involve hippocampus[19] whereas fear memory involves amydala.[20] It might be the case that VRBO could have effect on both hippocampus and amydala in a manner that could increase in learning and memory.

Many neurotransmitters including acetylcholine (ACh), glutamate (Glu), gamma amino butyric acid (GABA) and amines are believed to have roles in learning and memory. Although the effects of ACh on memory have

to be regarded separately for the acquisition, consolidation, and recall phase for different memory systems[21], it remains a fact that ACh acts on cholinergic receptors that are widely distributed throughout in the brain. Scopolamine and muscarinic cholinergic receptor antagonist has been shown to impair cognitive performances[22] especially spatial learning and memory.[23] It exerts amnesic effect equally in various behavioral models of memory including Morris water maze.[17] In this study, VRBO at the dose 5 ml/kg BW could not antagonize the amnesic effect of scopolamine. Stigmasterol (10 mg/kg BW), one of the phytosterols found in rice bran oil[24], was shown to ameliorate scopolamine-induced memory dysfunction in mice.[21] Nevertheless, concerning the content of stigmasterol in rice bran oil (14.00-19.28 %)[25], the dose that we used in this study was much lower than was used in the study by Park et al.[25] It was suggested that the cognitive ameliorative effects against scopolamine of stigmasterol were mediated by the enhancement of cholinergic neurotransmission system via the activation of estrogen or NMDA receptors.[25] Activation of NMDA receptor also suggested to involve the long term potentiation and brain neuroplasticity which contribute to learning and memory formation.[26]

Oxidative stress has been implicated in progression of a number of dementia as one of the risk factors in the development of cognitive disorders. Many studies have shown that rice bran oil has antioxidant properties.[27] Tocopherols and γ-oryzanols are the main antioxidants present in the rice bran. Antioxidant activity of γ-oryzanols is almost 10 times higher than that of tocopherols, while tocotrienols have 40-60 times greater antioxidant power than those of tocopherols in different biological systems.[28] Hence, as a whole grain essential oil, VRBO that contain many active antioxidant components, might increase antioxidant activity in the brain and improve memory function.[29]

Conclusion

The results in this study suggested that VRBO has the cognitive enhancing effect in both a fear-aggravated test, passive avoidance test (PAT), spatial learning test, and the Morris water maze test (MWM). However, at the dose tested, it could not antagonize the scopolamine-induced memory deficit effect in both PAT and MWM. VRBO might be one of the nutraceutical for brain health, especially for learning and memory.

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บทคัดย่อ

ผลเพิ่มความจำของผลิตภัณฑ์น้ำมันรำข้าวบริสุทธิ์จากข้าวกล้องไทย ในหนูเม้าส์

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หลักการและวัตถุประสงค์: น้ำมันรำข้าวเป็นน้ำมันพืชที่มีฤทธิ์ต้านอนุมูลอิสระ และประกอบด้วยสารสำคัญที่มีปริมาณสูง เช่น แกมมาออไรซานอล (γ-oryzanol), วิตามินอี และไฟโตสเตอรอล (Phytosterols) ซึ่งมีประโยชน์ต่อสุขภาพ เพื่อศึกษาผลของน้ำมันรำข้าวบริสุทธิ์สกัดด้วยวิธีสกัดเย็นจากข้าวกล้องไทย ต่อการเรียนรู้และความจำในหนูเม้าส์

ระเบียบวิธีศึกษา: หนูเม้าส์เพศผู้ สายพันธุ์ไอซีอาร์ ถูกป้อนด้วย น้ำกลั่น, น้ำมันข้าวโพด หรือ น้ำมันรำข้าวบริสุทธิ์ ในขนาด 5 มิลลิลิตรต่อกิโลกรัมน้ำหนักตัว วันละครั้ง ติดต่อกันเป็นเวลา 21 วัน หนูเม้าส์ได้รับการทดสอบการเรียนรู้และความจำโดยทดสอบที่ใช้ความกลัวเป็นตัวกระตุ้น ด้วย Passive avoidance test (PAT) ในวันที่ 18 (วันที่ฝึกฝน) ต่อเนื่องด้วย วันที่ 19 (วันที่ทดสอบ) และ ทดสอบความจำเกี่ยวกับสถานที่และตำแหน่งวัตถุ ด้วย Morris water maze test (MWM) ในวันที่ 19-21 ผลจากการทดสอบถูกวัดออกมาเป็น Latency time ใน PAT และ Escape latency time และ Time in right quadrant ใน MWM ในวันที่ถูกทดสอบหนูเม้าส์ถูกเหนี่ยวนำให้เกิดสภาวะความจำบกพร่องด้วยการฉีด scopolamine (1 มิลลิกรัมต่อกิโลกรัม) หรือ ฉีดน้ำกลั่น (สำหรับกลุ่มควบคุม) เข้าช่องท้องที่เวลา 30 นาทีหลังป้อนสาร หลังจากนั้น 15 นาทีหนูเม้าส์ถูกนำไปทดสอบ

ผลการศึกษา: ผลการศึกษาแสดงว่า น้ำมันรำข้าวบริสุทธิ์ สามารถเพิ่มความจำและการเรียนรู้ในหนูเม้าส์ได้ทั้งใน PAT และ MWM เมื่อเปรียบเทียบกับกลุ่มที่ได้รับน้ำ หรือน้ำมันข้าวโพด อย่างไรก็ตาม น้ำมันรำข้าวบริสุทธิ์ ในปริมาณ 5 มิลลิลิตรต่อกิโลกรัมน้ำหนักตัวต่อวัน ไม่สามารถต้านฤทธิ์ scopolamine ปริมาณ 1 มิลลิกรัมต่อกิโลกรัมที่ทำให้ความจำบกพร่อง

อภิปรายและสรุปผล: น้ำมันรำข้าวบริสุทธิ์ มีผลเสริมสร้างการเรียนรู้และความจำ น้ำมันรำข้าวบริสุทธิ์เป็นน้ำมันข้าวกล้องบริสุทธิ์สกัดด้วยวิธีธรรมชาติจากข้าวไทยที่ไม่ผ่านการแปรรูป ซึ่งเป็นผลิตภัณฑ์ที่มีสารสำคัญมากมาย เช่น กรดไขมันที่จำเป็น ไฟโตสเตอรอล (Phytosterols) และสารต้านอนุมูลอิสระ อาจมีส่วนในการเสริมสร้างการทำงานของสมองและพัฒนาความจำ ดังนั้น น้ำมันรำข้าวบริสุทธิ์ จึงน่าจะเป็นหนึ่งในผลิตภัณฑ์เสริมอาหารสำหรับบำรุงสมอง

คำสำคัญ : **น้ำมันรำข้าวบริสุทธิ์, ข้าวกล้องไทย, การเรียนรู้และความจำ, หนูเม้าส์**