บทความวิจัย (Research Article)

Continuous supplementation of perilla seed oil is safe and facilitates the learning and memory in adult rats

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Abstract

The perilla plant (*Perilla frutescens L. Britton*) is a member of emblements that has been used as ingredients for local food in many Asian countries. Its seed oil contains abundant of valuable omega-3 fatty acid including α-linoleic acid (ALA). Recent studies showed many advantage roles of omega-3 fatty acid such as anti-inflammation, anti-depression, as well as enhancement of learning and memory. However, the safety has not been reported in dietary perilla seed oil consumption and its potential to alter learning and memory in normal subjects. The present study aimed to investigate the safety dose of perilla seed oil consumption. Forty individuals of eight-week male Wistar rats were divided into 5 groups of vehicles (soybean oil; negative control), fish oil 500 mg (positive control), perilla seed oil at the dosage of 50, 100 and 200 mg/kg, respectively. Daily oral administered of each substance, were continuously performed for 8 weeks. The results showed that all dosages did not alter any blood profiles of the rats compared to soybean and fish oil. Moreover, perilla oil did not affect the weight gain or vital organ weights. The effect of perilla seed oil consumption on learning and memory was investigated by novel objective recognition (NOR) test and Morris water maze (MWM) test on week 0, 4 and 8. The results showed that after administered of 50 mg/kg perilla seed oil for 8 weeks significantly augmented NOR test, but not for MWM test. The study found that the sub-chronic of 50 mg/kg of perilla oil supplement is not only safe but also, facilitates the memory of objects recognition in young adult rats.

Keywords: Perilla seed oil, safety dose, learning and memory, omega-3 fatty acids, fish oil

Introduction

Perilla (*Perilla frutescens L. Britton*) is a member of emblements in Lamiaceae family. The plant is wildly grown and being used as ingredients of local food, also as herb in Asian countries including Thailand, Laos, Myanmar, Vietnam, China, India, Japan and Korea. [1] The perilla seeds are globular (0.5 to 2 mm in diameter). The colors are various from white, gray, brown and dark brown. [2, 3] It has been known that perilla seed oil abundantly contain essential fatty acids, α-linolenic acid (ALA, 76.33% w/w), linoleic acid (LA, 12.90% w/w) and vitamin E. [4, 5] ALA serves as a precursor for synthesis of long chain omega - 3 fatty acids including eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA).[6] It has been reported that EPA and DHA exhibit many physiological roles in mammals such as maintaining cell membrane fluidity, inhibiting inflammatory process, [7] decreasing depressive signs, [8] improving memory abilities in non-demented elderly. [9] EPA and DHA are also concentrated in synaptic membrane in the brain and retina. [10]

The required ALA level for human varies between 1.1 g/day in female and 1.6 g/day in male depending on the age, thus providing approximately 0.3 to 0.45 g EPA and DHA per day. [6] Although the health benefits of ALA have been propagandized, the safety dose of daily perilla seed oil consumption has not been reported yet. The study was designed to investigate the safety dose of continuous daily perilla seed oil supplementation in adult healthy rats. Moreover, the effect of perilla seed oil on alteration of learning and memory in two models of cognitive function test was examined.

Material and Method

Perilla seed oil preparation

The perilla oil used in the study was provided by the former study project. [5] It was cool-pressed and then was kept at 4°C until used.

Animal preparation

The eight-week male Wistar rats were purchased from the national laboratory animal research center. They were acclimatized for two weeks at the animal room at laboratory animal research center, University of Phayao prior to the beginning of the experiment. The animals were housed in polysulfone cages and had free accessed to food and water under controlled condition of 24 ± 1°C, humidity of 50 to 70%, and 12-h light-dark cycle. The experimental protocols were approved by the institutional animal research ethical committee (code: 5701040016).

The animals were randomly divided into an eight-rat per group for 5 groups including vehicle (soybean oil; negative control), commercial fish oil 500 mg/kg BW (positive control), perilla seed oil 50, 100 and 200 mg/kg BW, respectively. Each rat was daily measured its weight in the morning to calculate the consumption amount. Each substance was orally administered once a day in the morning for 8 weeks.

The safety dosage of Perilla seed oil used

At the end of the experiment, all rats were pushed to sleep in a of carbon dioxide box. The heart blood was collected by 24 G needle. The blood glucose, cholesterol profile (total cholesterol, high density lipoprotein – HDL, low density lipoprotein – LDL, triglyceride - TG), liver profile (total protein, aspartate aminotransferase - AST, alanine aminotransferase - ALT), and renal profile (blood urea nitrogen – BUN, creatinine), were investigated. In addition, the weight of vital organs was also measured.

Effects of perilla seed oil on learning and memory

Two behavioral tests were performed to determine the effects of daily perilla oil administration learning and memory **(Figure 1)** NOR test represented the recognition the cognitive functions of the brain. [11-14] The MWM test represented the spatial memory. [15, 16]

The study NOR protocol was modified from Antunes and Biala. [11] One day before the first orally administered of such substance, each rat was trained in a box contained 2 duplicated objects (A, A´). Each rat was allowed to explore the objects for 10 minutes then was returned to the home cage. The time that the rat approached each object was recorded. Next 24-hours, the rats were fed by the testing materials of the group. Thirty minutes later, each rat was put into the box for 5 minutes to acclimatize then one of the previous object and a novel object that had different shape were put into the box (A, B). The time spent with each object was recorded for 5 minutes. The recognition index (RI) was calculated to determine the discrimination ability. The RI equals the time spent with B divided by total time spent with both objects (A+B).

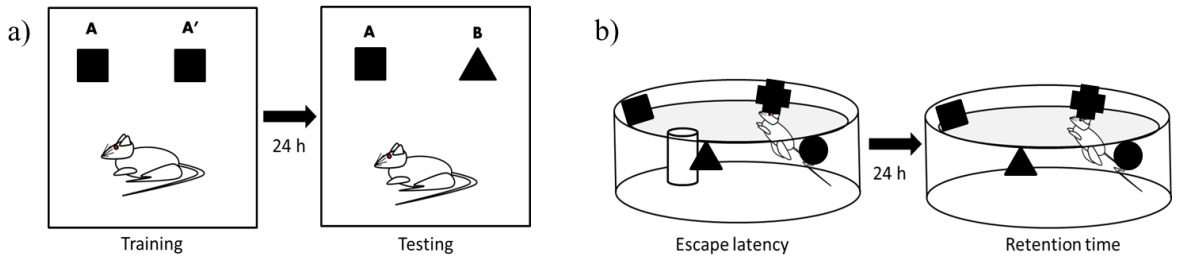

Figure 1 The behavioral model of learning and memory: a) the novel object recognition test; b) the Morris water maze test

The protocol of MWM testing was modified from Vorhees et al. [15] and Alvin V. [16] After the NOR test was performed, the rat was introduced to the MWM test. The MWM test was performed 4 hours after the first administration of the testing materials to determine the escape latency. Then 24 hours later, the test was performed again to measure the retention time.

The experiment apparatus consists of a black plastic pool filled with water (22 ± 1°C) and equally divided into four quadrants. Water was made opaque by the addition of nontoxic talcum powder to disassemble the platform. A platform was placed in the midpoint of one quadrant submerged 1 cm beneath the surface of the water. Visual cues were placed above the wall of each quadrant for navigation. In the training session (1 day prior to the test session), each rat was placed into the pool and was allowed to swim (max 1 min) to find the hidden platform for 4 trials. When the rat found the platform, it was allowed to stay on the platform for 10 seconds. If it failed to reach the hidden platform within 1 min, it was guided to the platform then was allowed to stay on the platform for 10 seconds to help it remember the platform location. Escape latency (time to find the platform) was recorded. Next 24-hour, each rat was put into the pool without the hidden platform. It was allowed to swim freely for 1 min. Retention time (time stayed in the previous target quadrant) was recorded.

Statistical analysis

The data were expressed as mean (standard deviation – SD), and analyzed using analysis of variance (ANOVA) followed by the least significant difference (LSD) post-hoc test. The *p*-value <0.05 was considered to be statistically significant.

Results

The safety dosage of perilla seed oil used

After 8-week administration of the testing materials, the results showed that all groups of perilla seed oil consumption did not increase body weight gain of the rats compared to the controls. **(Table 1)** At the end of the experiment, the vital organs' weight of each experimental group such as liver, kidneys, spleen, heart, and testes were measured. In comparison among the groups, the values were not significantly changed. The data indicated that daily consumption for 2 months has no bad effects on the growth of the animals.

Table 1 Effect of perilla seed oil on the change of average body weight and vital organs

Group	Body weight gained (g)	Weight of vital organs (g)				
		Liver	Kidneys (both)	Spleen	Heart	Testes (both)
Vehicle (negative control)	92.49 (18.761)	15.12 (1.534)	2.59 (0.316)	0.99 (0.190)	1.44 (0.179)	3.95 (0.167)
Fish oil 500 mg (positive control)	102.54 (16.102)	15.86 (1.861)	2.67 (0.232)	1.04 (0.867)	1.46 (0.171)	3.90 (0.278)
Perilla seed oil 50 mg	93.82 (23.184)	15.33 (0.962)	2.65 (0.233)	1.00 (0.095)	1.43 (0.109)	3.94 (0.158)
Perilla seed oil 100 mg	94.85 (12.554)	15.05 (1.343)	2.57 (0.249)	1.04 (0.096)	1.41 (0.161)	3.85 (0.268)
Perilla seed oil 200 mg	94.97 (12.124)	15.43 (1.578)	2.81 (0.561)	0.89 (0.304)	1.50 (0.155)	4.06 (0.259)

Note: Data shown as mean (SD)

The dosage of perilla seed oil consumption had no significant change in blood glucose and cholesterol profiles, compared to the neither negative nor positive controls. **(Table 2)** The results reflected the safety of perilla seed oil. Moreover, considering the liver and kidney functions, the data did not show any change after perilla seed oil consumption for 8 weeks. These data might indicate that perilla seed oil is safe to consume.

Table 2 Effect of perilla seed oil on average blood glucose, cholesterol profile, liver and kidney functions

Group	Blood chemistry (mg/dL)					Liver function			Kidney function	
	Blood glucose	Total cholesterol	HDL	LDL	TG	Total Protein (g/dL)	AST (IU/L)	ALT (IU/L)	BUN (mg/dL)	Creatinine (mg/dL)
Vehicle (negative control)	168.33 (44.843)	104.33 (12.727)	19.11 (2.571)	58.00 (10.356)	136.00 (25.174)	5.50 (0.307)	92.77 (28.752)	46.55 (14.098)	25.25 (2.549)	0.98 (0.253)
Fish oil 500 mg (positive control)	167.10 (25.760)	101.78 (18.130)	19.56 (3.609)	54.444 (16.455)	139.00 (21.800)	5.58 (0.603)	90.00 (28.053)	48.56 (22.908)	26.67 (2.291)	1.12 (0.327)
Perilla seed oil 50 mg	164.05 (28.181)	100.90 (9.170)	19.30 (1.636)	54.20 (8.929)	137.60 (17.933)	5.16 (1.77)	94.00 (36.325)	47.20 (20.799)	25.40 (3.169)	1.21 (0.395)
Perilla seed oil 100 mg	162.00 (40.222)	102.20 (15.346)	19.70 (3.36)	55.30 (16.680)	136.80 (21.159)	5.29 (0.128)	98.90 (38.939)	49.60 (16.674)	27.60 (2.836)	0.90 (0.200)
Perilla seed oil 200 mg	152.19 (29.626)	104.30 (10.893)	19.40 (1.505)	59.40 (11.692)	127.30 (23.730)	4.88 (0.388)	85.20 (46.300)	38.50 (13.368)	28.60 (3.339)	1.14 (0.271)

Note: Data shown as mean (SD)

Effects of perilla seed oil on learning and emory

After 8-week continuous administration of perilla seed oil, it was observed that the dose of 50 mg/kg significantly increased RI from the beginning (p=0.034). Moreover, it significantly augmented the RI, in comparison with the negative (p=0.039) and positive control (p=0.001), respectively **(Figure 2)**. While the doses of 100 and 200 mg/kg showed no difference compared with the controls.

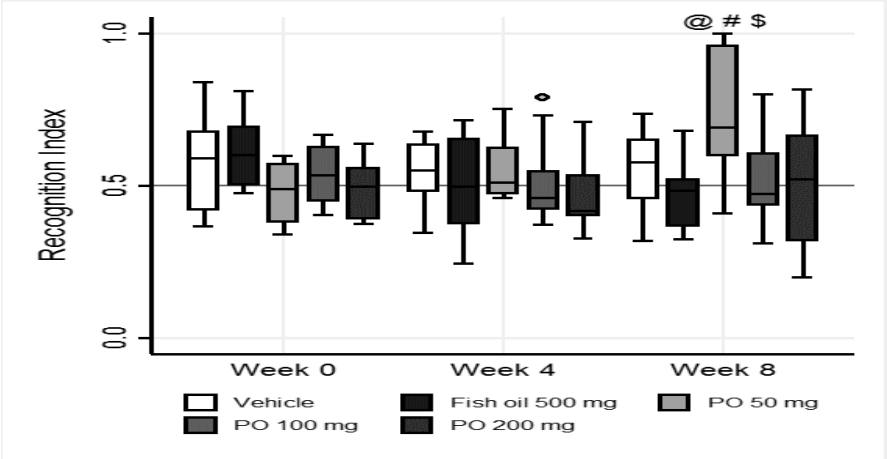

Figure 2 Showing RI values obtained during the object recognition test of rats treated by perilla seed oil in different periods

@ Compared the RI 50 mg/kg perilla oil consumption between week 0 and week 8 (p=0.034)

Compared the RI between vehicle and 50 mg/kg perilla oil consumption at week 8 (p=0.039).

$ Compared the RI between fish oil 500 mg and 50 mg/kg perilla oil consumption at week 8 (p=0.001).

However, the results of the MWM test by all the used doses were inconsistent. Considering the escape latency and retention time of each spot of time series, there was no significant difference among all five groups. **(Figure 3a, 3b)**

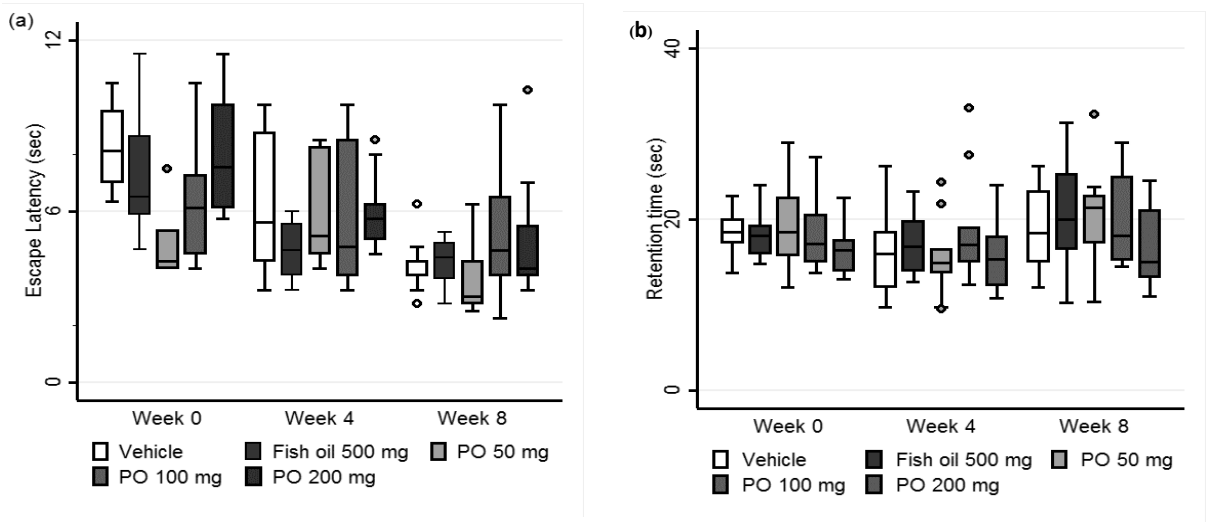

Figure 3 Showing escape latency (a) and retention time (b) obtained during the MWM test of rats treated with perilla seed oil in different periods

Discussion

To investigate the safety dose of perilla seed oil consumption, the experimental rats were daily fed with perilla seed oil at doses of 50, 100 and 200 mg/kg BW for 8 weeks. The results showed that the rats of all groups gained weight approximately 30%. Our data were consistent with the study reported by Choi et al. [17] The results showed that perilla seed oil and soybean oil consumption for 4 weeks did not alter the gained weight and their vital organs were indifferent. The data suggested that daily consumption of perilla seed oil, 50-200 mg/kg, did not lead to overweight conditions.

The results showed that blood levels of glucose, total cholesterol, HDL, LDL, and TG were not different in each dose. However, blood glucose level in our experiment was slightly higher than that in normal rats. [18-20] It might be caused by the free access to food and water of the animals. Considering the lipid profiles, perilla seed oil supplement did not alter all examined serum cholesterols' concentration compared to neither negative nor positive controls. Compared to Choi and co-workers in 1993 [17] that fed the animals with soybean oil or perilla seed oil mixed diet for 4 weeks. Their results showed that the perilla seed oil mixed diet reduced plasma triglyceride but not altered other lipid parameters. Moreover, Chung and colleagues reported in 2013 [21] that mice fed with perilla seed oil mixed diet for 8 weeks lower plasma LDL concentration compared to olive oil mixed diet, no change in TG, TC, HDL levels. This inconsistency result might cause by different control substance, soybean oil or olive oil. In addition, Kim et.al., 2016 [22] reported the non-diabetic and hypercholesterolemia subjects consumed 1.2 mg/kg/d ALA for 8 weeks showed greater reductions in total- and LDL-cholesterol, ox-LDL, than the placebo. However, our data showed no significant change of lipid profiles after 8 weeks administered of perilla seed oil. The reason might because our rats were healthy.

The liver functions and kidney functions were used as indicators for safety. The liver enzymes, ALT and AST, are biomarkers of liver damage. The high level of those enzymes indicates liver inflammation. On the other hand, a low total protein level in blood stream reflected infection or liver damage. Moreover, BUN and creatinine are markers that indicate renal functions. The increase of both BUN and creatinine indicates azotemia and less function of kidneys.

Our results showed that daily consumption of perilla seed oil did not alter any liver and renal functions. These results were getting along with Zhang et al., 2019 [23] that sub-chronic orally did not produce change in dogs' blood parameters. The results suggested that perilla seed oil is safe to consume daily. The study is based on healthy rats, the only dose of 50 mg/kg (not 100 and 200 mg/kg) had significantly increased the object recognition ability, compared to the controls. It might imply that the neural circuit of the memory had the optimum level of EPA and DHA to improve such ability in healthy young adult rats.

The result seems not to correspond with the varied doses of perilla seed oil due to experimental models. Whereas a study in Korea, the $A\alpha_{25-35}$ induced Alzheimer's disease mouse model which orally received perilla oil in the dose of 100 mg/day for 14 days showed the improvement of cognitive ability by the T maze test, Morris water maze test and novel object recognition. [24, 25] Moreover, Gao 2016 [26] reported that continuously - administered flax seeds for 12 months had a neuroprotective effect on the spatial memory impairment of aged rats.

The limitation of the study is that the subjects were healthy rats, so it may be unspecific design and the significant result may not be demonstrated, or the normal and average level of cognitive ability is ceiling scale. This explanation may be also applied to the inconclusive result of the MWM test for spatial memory. However, even the higher dose than normal requirement the short-term safety is demonstrated in terms of various blood chemistry.

In conclusion, the daily perilla seed oil consumption could be safe, and it might increase the object recognition ability in the rats. Our data suggested further research of food supplements contain of perilla seed oil for facilitating the memory in healthy young adults.

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