Original Article

Evaluation of the Neuroprotective and Cognitive Enhancing Effects of *Cucurbita moschata*

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Background: To date, the requirement of the neuroprotectant and cognitive enhancer is increasing. However, most of the available substances in the market still have the limitation efficacy and expensive. Therefore the novel substance which is effective, cheap and easy to approach is required. Based on the neuroprotection and memory enhancing effects of substances possessing antioxidant activity, aforementioned effects of Pumpkin or *Cucurbita moschata* (*C. moschata*), a plant containing high contents of antioxidant, has been considered.

Objective: To determine effect of *C. moschata* on spatial memory neuron density, oxidative stress and AChE activity in hippocampus.

Materials and Methods: Male Wistar rats, weighing 180 to 200 g, were orally given the aqueous extract of *C. moschata* at doses of 50, 150 and 450 mg.kg^{-1} BW for 28 days. The animals were assessed spatial memory using Morris water maze test every 7 days throughout the study period. At the end of study, the determination of neuron density, oxidative stress and AChE activity in hippocampus of the experimental were performed.

Results: All doses of extract enhanced memory but decreased MDA level in hippocampus. The decreased AChE activity in hippocampus was observed only in rats treated with the low and medium doses of extract whereas the elevation of SOD was observed only in the low dose of extract treated group. No significant change in neuron density was observed in rats treated with the plant extract at all dosage range used in this study. Our data suggested that the memory enhancing effect of *C. moschata* extract might be attributed partly to the decreased oxidative stress and the enhanced cholinergic function.

Conclusion: *C. moschata* extract is the potential functional food to enhance memory which is cheap and easy to approach. However, further researches about the underlying mechanism and possible active ingredients are essential.

Keywords: *Cucurbita moschata*, Memory, Oxidative stress, Acetylcholinesterase

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The prevalence of age-related neurodegeneration and cognitive impairment are continually increasing due to the increased human life expectancy[1]. Therefore, it has been recognized as the important health problem in this decade[2]. Despite the increasing importance, the therapeutic efficacy is still very limited. Most therapeutic agents used nowadays can only slow down the progress of neurodegeneration and provide a temporary abatement of symptomatology but no long-term solutions toward a cure have been established[2]. In addition, the most commonly used drug still targets only at the inactivation process of acetylcholine (ACh) via the suppression of acetylcholinesterase (AChE) enzyme. However, many side effects are observed and the price of the drug is expensive. Therefore, the effective prevention strategy which is cheap and easy to approach is required.

Food has been long term used as medicine in traditional folklore of many countries. Based on the shift of health paradigm which emphasizes on the prevention strategy nowadays, the applications of functional foods as neuroprotectant and cognitive enhancer have gained much attention. Recently, it has been well established that plant-based foods abound with natural biologically active compounds such as polyphenols, vitamin C or α-carotene which produce a great benefit on brain health[3-6]. In addition, our previous study has demonstrated that a polyphenol and α-carotene rich plant such as *Mangifera indica* fruit can improve both neurodegeneration and cognitive impairment[7]. However, this fruit is available only some period of the year and the price is expensive. Therefore, the brain health benefit of a substance which is available all year round and easy to approach has been considered.

Pumpkin or *Cucurbita moschata* Decne, a plant in a family of Cucurbitaceae, is a vegetable which is widely consumed in Asian countries including in Thailand. In Thailand,

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many parts of pumpkin have been consumed both as food and as medicine. Lave-buds and young leaves are widely consumed as vegetable. It has been shown that the leaves of pumpkin contained high flavonoids and carotenoids contents together with the antioxidant activity[8]. This raised the hypothesis that aqueous extract of leave-buds and young leaves of *C. moschata* might protect against neurodegeneration and cognitive impairment. Therefore, this study was carried out to determine the effect of *C. moschata* on spatial memory and neuron density in hippocampus in health rats. In addition, the effects of the plant extract on the oxidative stress markers and AChE were further investigated to determine the possible underlying mechanisms.

Materials and Methods

Chemicals

Donepezil hydrochloride (Aricept 5 mg/tablet) (Pfizer Pharmaceuticals Inc) and vitamin C (500 mg/tablet) (Government Pharmaceutical Organization) were used as positive control in this study. All chemical substances used in this study were of analytical grade.

Plant preparation

Leave-bud and young leaves of *C. moschata* were collected from organic farm at Amphoe Srithat, Udornthani province. They were thoroughly washed with tap water, wet sortation, cut, dried and ground into powder and extract with water by maceration technique. The solution was then filtered through Whatman filter paper number 1. The filtrate was evaporated by using rotator evaporator. The percentage yield of the plant extract was 13.7. The phenolic contents of the extract determined via Folin-Ciocalteu method was 488.100±0.003 mg of GAE/100 g of plant.

Animals

Adult male Wistar rats weighing 180 to 200 g were obtained from National Laboratory Animal Center, Salaya, Nakhon Pathom. They were housed in group of 5 per cage in standard metal cages at 22±2°C and light environment was controlled at 12: 12 h light-dark cycle. All animals were given access to food and water ad libitum. The experiments were performed to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC).

The experimental protocols were approved by the Institutional Animal Care and Use Committee, Khon Kaen University, Thailand.

Experimental design

After the acclimatization, the animals were divided into 6 groups containing 16 animals each.

Group I) Naive intact which receive no treatment.

Group II) Vehicle treated group; rats in this group received distilled water which used as vehicle in the present study.

Group III) Donepezil treated group; animals in this group were orally treated with donepezil, a cholinesterase inhibitor which was used as standard drug for cognitive impairment.

Group IV to VI) Pumpkin extract treated groups; rats in these groups were orally given the water extract of leave-buds and young leaves of pumpkin at doses of 50, 150 and 450 mg/kg BW respectively.

All rats in Group II to Group IV were treated with the assigned substances for 28 days. The determination of memory was performed by using Morris water maze test after single administration and every 7 days throughout the 28-day experimental period. At the end of study, rats were sacrificed, hippocampi were isolated and determined the neuron density in CA1, CA2, CA3 and dentate gyrus of hippocampus. In addition, the oxidative stress markers including the level of malondialdehyde (MDA) and the activities of main scavenger enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in hippocampus were also investigated.

Morris Water Maze Test

Spatial memory of each rat was assessed via Morris water maze test. According to this test, the animal must learn and memorize the location of the immersed platform in the water bath tub with opaque surface by forming the relationship between its location and the location of immersed platform via the external cue of the room environment. In brief, each rat was exposed to the metal pool (170 cm in diameter x58 cm tall) which was divided into 4 quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the center of the pool. Then, it was filled with tap water (25°C, 40 cm deep) and covered the surface with a nontoxic milk powder. The removable platform was immersed below the water level at the center of one quadrant and remained there throughout training. The rats had to memorize the platform location in relation to various environmental cues because there was nothing that directly showed the location of the platform in and outside the pool. Therefore, the placement of the water tank and platform was the same in all acquisition trials. Each rat was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool and the animal was allowed to swim until it found and climbed onto the platform. During training session, the rat was gently placed on the platform by experimenter when it could not reach the platform in 60 s. In either case, the subject was left on the platform for 15 s and removed from the pool. The time for animals to climb onto the hidden platform was recorded as escape latency. In order to determine the capability of the animals to retrieve and retain information, the platform was removed 24 hr later and the rat was released into the quadrant diagonally opposite to that which contained the platform. Time spent in the region that previously contained the platform was recorded as retention time. Prior to Morris water maze testing, all rats were habituated to swimming and they were trained with 4-trial shaping procedures with a 20 min inter-trial interval for 3 days. In each trial, the animal was quickly

dried with towel before being returned to the cage[9]. All tests were carried out within 45 minutes after the administration of vehicle or plant extract or donepezil, a cholinesterase inhibitor, which served as positive control.

Histology study

After anesthesia with sodium pentobarbital (60 mg/kg BW), the brain fixation was performed via transcardial perfusion with fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3. After the perfusion, the brains were removed and stored in a fixative solution containing 30% sucrose solution at 4°C until the brains were saturated and sank to the bottom of brain jars. The specimens were frozen rapidly and 30 µM thick sections were cut on cryostat. They were rinsed in the phosphate buffer and picked up on slides coated with 0.01% of aqueous solution of a high molecular weight poly L-lysine. Then, the duplicate coronal sections of brains were stained with 0.75% cresyl violet, dehydrated through graded alcohols (70%, 95%, 100% 2x), placed in xylene and coverslips using DPX mountant.

Morphological analysis

Five coronal sections of each rat in each group were studied quantitatively. Neuronal counts in hippocampus were performed by eye using a 40x magnification with final field 255 μm^2 according to the following stereotaxic coordinates: AP -4.8 mm, lateral $\pm$2.4 to 6 mm, and depth 3 to 8 mm. The observer was blind to the treatment at the time of analysis. Viable-stained neurons were identified on the basis of a stained soma with at least two visible processes. Counts were made in five adjacent fields and the mean number extrapolated to give total number of neurons per 255 μm^2. All data are represented as number of neurons per 255 μm^2.

Determination of oxidative stress markers

Hippocampi were isolated and homogenized with a buffer consisting of 10 mM sucrose, 10 mM Tris-HCl, and 0.1 mM EDTA (pH 7.4). Then the brain homogenates were centrifuged at 3,000 g for 15 min at 4°C. The supernatant was used for the determination of MDA level, and the activities of SOD, CAT and GSH-Px.

Lipid peroxidation determination

The level of MDA, a product of lipid peroxidation process, was determined via thiobarbituric reaction method with a slightly modified method of Garcia[10]. In brief, 0.75 mL thiobarbituric acid was mixed with the solution containing 100 µL of supernatant and boiled in water bath at 95°C for 1 hour. Absorbance of the color was determined at $\geq$532 nm. Concentration of MDA was calculated using a series of standard solution of 1, 1, 3, 3-tetramethoxypropane (TMP) and expressed as nM/mg protein.

Scavenger enzymes assays

SOD assay was carried out based on the reaction of this enzyme in a xanthine/xanthine oxidase system. The rate of reduction of cytochrome c was assessed at 550 nm via spectrometer (UV-1601, Shimadzu). SOD activity was expressed as units per milligram of protein (U/ mg protein)[11]. One unit of enzyme activity was defined as the quantity of SOD required to inhibit the rate of reduction of cytochrome c by 50%.

Catalase activity was measured by assessing the rate of H_2O_2 decomposition at absorbance at 240 nm according to the slightly modified method of Aebi[12]. In brief, 10 µL of sample was mixed with 150 µL of 50 mM potassium phosphate, pH 7.0, 25 µL 0.05 M KMnO4, 25 µL of 5N H_2SO_4 and 50 µL 0.1M of H_2O_2 at room temperature under dark condition. The activity of catalase was expressed as U/ mg protein.

GSH-Px was assessed by using t-butylhydroperoxide as a substrate. The optical density was spectrophotometrically recorded at 340 nm. One unit of the enzyme was defined as micromoles (µmol) of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute[13]. The GSH-Px activity was expressed as U/mg protein.

Determination of AChE

AChE activity was evaluated using amount of nitrobenzoate liberated via the reaction of thiocholine and dithiobis-nitrobenzoic acid as index. The determination of nitrobenzoate was performed at 405nm[14]. The experiment was run in triplicate.

Statistical analysis

Data were presented as mean $\pm$ standard error of mean (SEM). Statistical significance was analyzed by One-way analysis of variance (ANOVA), followed by Tukey-HSD post hoc test. A probability levels less than 0.05 were accepted as significance.

Results

Effect of C. moschata on spatial memory

In the present study, vehicle treatment failed to produce significant changes in escape latency throughout 28-day study period. Donepezil decreased escape latency throughout the study period. However, the significant reduction of escape latency was observed only after the single administration and at 7, 14 and 21 days of treatment (p-value <0.001, 0.01, 0.001 and 0.01 respectively, compared to vehicle treated group). After single dose of treatment, the water extract of buds and young leaves of *C. moschata*, all rats which received the extract at doses of 50, 150 and 450 mg/kg BW significantly decreased escape latency (p-value <0.01, 0.01 and 0.05 respectively; compared to vehicle treated group). Rats which received the extract at doses of 150 and 450 mg/kg BW also showed the significant decreased escape latency at 7 (p-value <0.01 all; compared to vehicle treated group), 14 (p-value <0.01 all; compared to vehicle treated group), 21 (p-value <0.001 and 0.01 respectively; compared to vehicle treated group) and 28 days of treatment (p-value <0.01 and 0.05 respectively; compared to vehicle treated group). However, the significant reduction of escape

latencies in rats which received the extract at dose of 50 mg/kg BW were observed only at 7, 14, and 21 days of treatment (p-value <0.01, 0.05 and 0.01 respectively; compared to vehicle treated group) as shown in Figure 1.

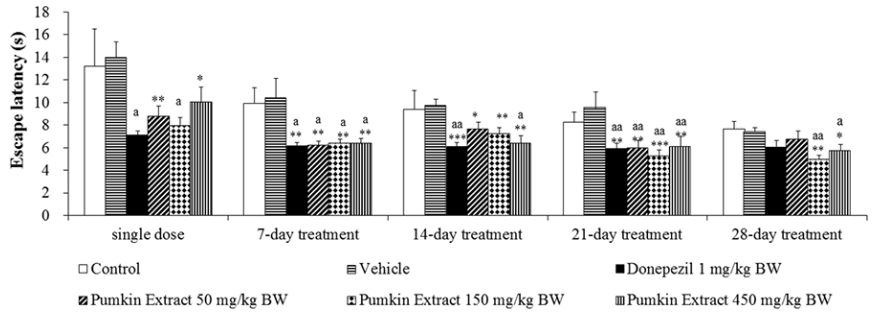
The effect of *C. moschata* extract on retention time was also investigated and results are shown in Figure 2. Vehicle treatment also failed to show the significant changes of retention time throughout the experimental period. Both Donepezil treated rats and the low dose treated rats showed the significant increase in retention time only at 28 days of treatments (p-value <0.01 and 0.001 respectively; compared to vehicle treated group). Rats which treated with *C. moschata* at dose of 150 mg/kg BW significantly enhanced retention time after single administration and at 14, 21 and 28 days after treatment (p-value <0.01, 0.001, 0.01 and 0.001 respectively; compared to vehicle treated group). The high dose treatment group showed the significant enhanced retention time after single administration and at 14 and 28 days of treatment (p-value <0.01, 0.001 and 0.001 respectively; compared to vehicle treated group).

Effect of C. moschata on AChE activity

On the basis of crucial roles of cholinergic system and hippocampus on memory, the authors also investigated the effect of *C. moschata* extract on AChE activity in hippocampus and results were shown in Figure 3. Vehicle showed no effect on AChE activity. Donepezil treated rats and rats which received the extract at doses of 50 and 150 mg/kg BW showed the significant reduction of AChE activity in hippocampus whereas no significant change was observed in rats treated with the extract at dose of 450 mg/kg BW.

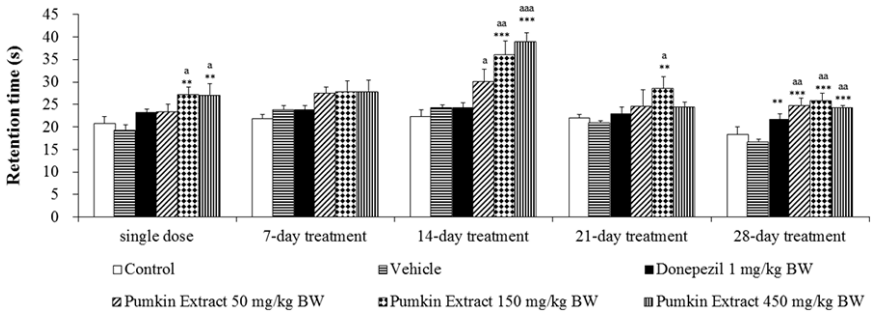
Effect of C.moschata on oxidative stress markers

Figure 4 to 7 showed the effect of *C. moschata* extract on MDA, SOD, GSH-Px and CAT activities in hippocampus. It was found that no significant changes of all parameters mentioned earlier in vehicle treated rats when



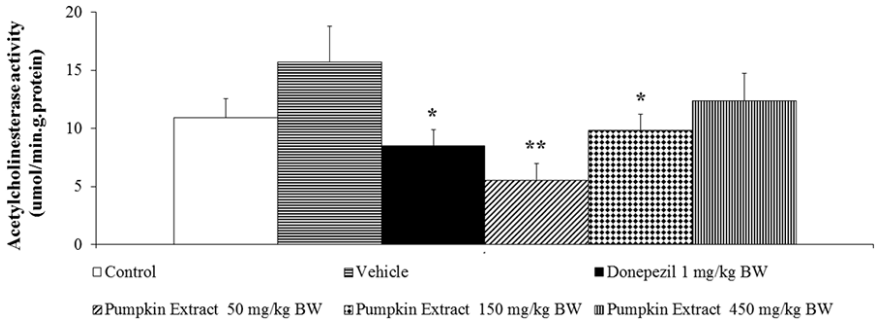
a, aa p-value <0.01 and 0.05 compare with control, *, ** p-value <0.001, 0.01 and 0.05 compare with vehicle treated group

Figure 1. The effect of *C. moschata* extract on escape latencies in Morris water maze test. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. They were determined the escape latency or the time to reach the platform in Morris water maze test at 1, 7, 14, 21 and 28 days. Data are presented as mean ± SEM, n = 8/group.



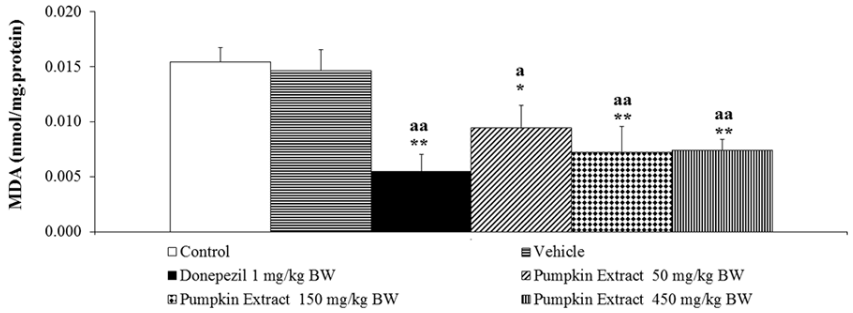
a, aa, aaa p-value <0.001, 0.01 and 0.05 compare with control, *, **, *** p-value <0.001, 0.01 and 0.05 compare with vehicle treated group

Figure 2. The effect of *C. moschata* extract on retention time in Morris water maze test. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. They were determined the escape latency or the time to reach the platform in Morris water maze test at 1, 7, 14, 21 and 28 days. Data are presented as mean ± SEM, n = 8/group.



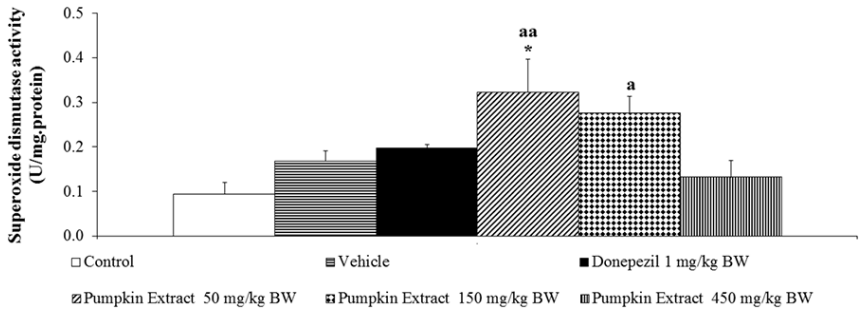
*, ** *p*-value <0.01 and 0.05 compare with vehicle treated group

Figure 3. The effect of *C. moschata* extract on AChE activity in hippocampus. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. Data are presented as mean ± SEM, n = 8/group.



*, **p-value <0.01 and 0.05 compare with vehicle treated group.

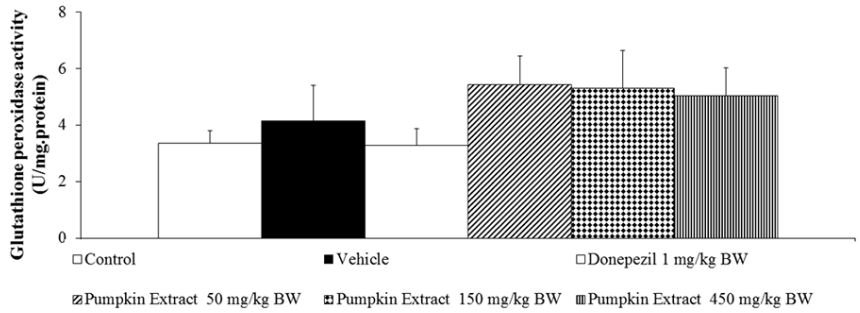
Figure 4. The effect of *C. moschata* extract on MDA level in hippocampus. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. Data are presented as mean ± SEM, n = 8/group.



*,** p-value <0.01 and 0.05 compare with vehicle treated group

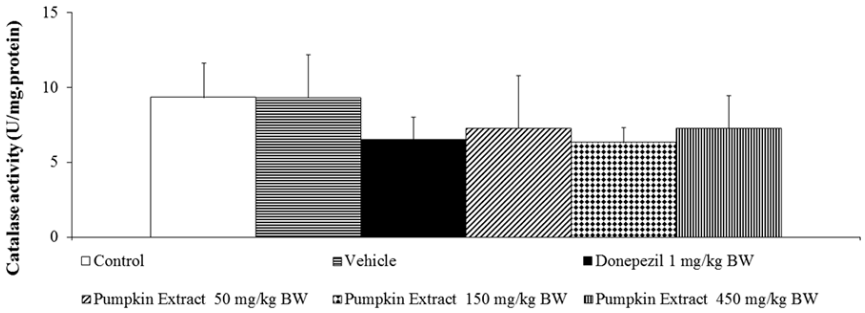
Figure 5. The effect of *C. moschata* extract on SOD activities in hippocampus. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. Data are presented as mean ± SEM, n = 8/group.

compared to control group. The reduction of MDA level in hippocampus was observed in Donepezil treated rats and in rats which received the extract at doses of 50, 150 and 450 mg/kg BW (*p*-value <0.01, 0.05, 0.01 and 0.01 respectively; compared to vehicle treated group). However, the scavenger enzymes which showed significant change was observed only



*,** *p*-value <0.01 and 0.05 compare with vehicle treated group

Figure 6. Showed the effect of *C. moschata* extract on GSH-Px activities in hippocampus. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. Data are presented as mean ± SEM, n = 8/group.



*,** p-value <0.01 and 0.05 compare with vehicle treated group

Figure 7. The effect of *C. moschata* extract on CAT activities in hippocampus. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. Data are presented as mean ± SEM, n = 8/group.

in rats treated with low dose extract (*p*-value <0.05; compared to vehicle treated group). No other significant changes were observed in any treatments.

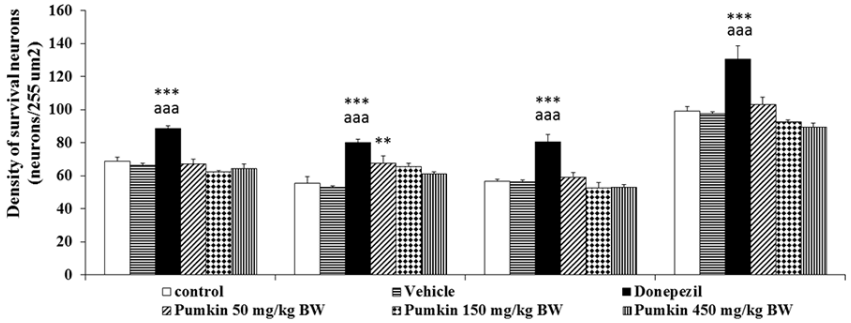
Effect of C. moschata on neuron density in hippocampus

Figure 8 showed the effect of *C. moschata* on neuron density in various subregions of hippocampus including CA1, CA2, CA3 and dentate gyrus. Our results showed that vehicle produced no significant changes of neuron in all investigated areas. It was found that rats which received Donepezil showed the enhanced neuron density in all areas mentioned earlier (*p*-value <0.001 all; compared to vehicle treated group) whereas no significant changes in neuron density were observed in rats treated with the extract at all doses used in the present study.

Discussion

The current study has clearly demonstrated that *C. moschata* enhances spatial memory. All doses of *C. moschata* improves oxidative status by decreasing MDA level but only the extract at doses of 50 and 150 mg.kg^{-1} can suppress AChE activity in hippocampus of normal rats.

During the last decade, most studies about the memory enhancing effect focus only on the improvement of memory deficit. The enhanced brain tonic effect has gained less attention. However, in the view of consumer, smart nutrients which can enhance memory even in normal condition are required. Basically, the smart nutrients exert their effects to enhance memory by the mechanisms as describe following: to minimize the brain damage and the natural deterioration of one's brain functions, to repair some of the damage and to enhance brain functions above usual levels. Both age-related changes and life under stressful situation nowadays can enhance oxidative stress which in turn destroys brain cells resulting in the decreased brain function capacity. In this study, it was found rats which received the extract at all doses used in this study showed the increased memory performance and the decreased MDA level which reflected the decreased oxidative status without the significant change of neuron density. Based on the previous finding the oxidative stress could also disturb the function of cerebral endothelial cells which in turn decreased the oxygen and



[aaa] *p*-value <0.001 compare with control, **,*** *p*-value <0.001 and 0.01 compare with vehicle treated group

Figure 8. The effect of *C. moschata* on neuron density in various subregions of hippocampus including CA1, CA2, CA3 and dentate gyrus. Rats were treated with vehicle or donepezil or *C. moschata* extract at various doses ranging from 50, 150, 450 mg/kg BW via oral route for 28 days. Data are presented as mean ± SEM, n = 8/group.

nutrients supply[15]. Therefore, the authors did suggest that *C. moschata* enhanced memory partly via the decreased oxidative stress which in turn improved the function of cerebral endothelial cells resulting in the enhanced oxygen and nutrients supply leading to the improved memory[16].

Although the decreased oxidative stress was observed, no tightly association between the alterations of MDA and the activities of scavenger enzymes was observed. Only low dose of extract showed the enhanced SOD activity and no other changes of scavenger enzymes were observed whereas the reduction of MDA was observed in the rats which received the extract treatment at all doses used in the present study. These suggested that other factors such as the increased oxidative stress buffering system via the non-enzymatic scavenger system such as high flavonoids and carotenoids induced by *C. moschata* extract[8]. In addition, the extract might possibly decrease the oxidative stress formation. However, these required further investigation.

Our data also clearly demonstrated that the extract at doses of 50 and 150 mg.kg^{-1} could suppress the activity of AChE in hippocampus while no change of AChE was observed in the mentioned area of the high dose treatment group. These data suggested that the extract at medium and low dose also enhance memory partly via the enhanced cholinergic function by suppressing AChE giving rise to the elevation of available ACh which in turn enhanced the spatial memory.

The underlying mechanism of the extract appeared to depend on dose of the extract. The principal mechanism which was responsible for the memory enhancing effect of the low dose effect appeared to be associated with the enhanced cholinergic function. However, the decreased oxidative stress was also contributed the role. The memory enhancing effect of the medium dose of extract also occurred via both the suppression of AChE and the decreased oxidative stress. Based on the changes of both parameters mentioned earlier in this group, the principal mechanism appeared to occur via the decreased oxidative stress. It was found that the underlying mechanism for memory enhancing effect of the high dose of extract appeared to occur only via the decreased oxidative stress.

Conclusion

The present study has clearly revealed that consumption of *C. moschata* at a period of 28 days can effectively enhance memory. Since the effective dose of this plant extract is low and no report concerning toxicity at this dose level is observed, it may be used as a functional food to enhance brain functions especially memory above usual levels. However, further researches to identify the precise underlying mechanism and possible active ingredients are very much essential.

What is already known on this topic?

Acetylcholinesterase inhibitor, therapeutic agents for cognitive impairment have many side effects and the price of the drug is expensive. The applications of functional foods as neuroprotectant and cognitive enhancer from plant-based foods flourish with natural biologically active compounds produce a great benefit on brain health. Pumpkin or *Cucurbita moschata* Decne, have been consumed both as food and as medicine in ASEAN countries. The leaves of pumpkin contained high flavonoids and carotenoids contents together with the antioxidant activity.

What this study adds?

Consumption of *C. moschata* for 28 days can effectively enhance memory, the underlying mechanism via the decreased oxidative stress. There has no toxicity report and the effective dose is low. Thus, it may be used as a functional food to enhance brain functions especially memory.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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การประเมินฤทธิ์ปกป้องระบบประสาทและฤทธิ์ต่อกระบวนการทางพุทธิปัญญาของฟักทอง

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ภูมิหลัง: ปัจจุบันความต้องการสารที่ปกป้องระบบประสาทและเพิ่มการทำงานของกระบวนการทางพุทธิปัญญาเพิ่มมากขึ้น อย่างไรก็ตามสารที่มีอยู่ในตลาดยังมีประสิทธิภาพจำกัดและมีราคาแพง ดังนั้นสารตัวใหม่ที่มีประสิทธิภาพ ราคาถูก เข้าถึงง่ายจึงเป็นที่ต้องการ สืบเนื่องจากฤทธิ์ปกป้องประสาทและเพิ่มความจำของสารที่มีฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ดังกล่าวข้างต้นของฟักทอง ซึ่งมีสารต้านอนุมูลอิสระสูงจึงได้รับความสนใจ

วัตถุประสงค์: เพื่อตรวจสอบฤทธิ์ของฟักทองต่อความจำที่เกี่ยวข้องกับทิศทาง ความหนาแน่นของเซลล์ประสาท ความเครียดออกซิเดชัน และการทำงานของเอนไซม์อะเซติลโคลีนเอสเตอเรส (AChE) ในฮิบโปแคมปัส

วัสดุและวิธีการ: ได้ทำการป้อนสารสกัดด้วยน้ำของฟักทองขนาด 50, 150 และ 450 มิลลิกรัมต่อกิโลกรัมน้ำหนักตัวให้หนูขาววิสตาร์เพศผู้ที่มีน้ำหนัก 180 ถึง 200 กรัม เป็นเวลา 28 วัน ประเมินความจำที่เกี่ยวข้องกับทิศทางของสัตว์ทดลองด้วย Morris water maze test ทุก 7 วันตลอดระยะเวลาการศึกษา เมื่อสิ้นสุดการศึกษาได้วัดความหนาแน่นของเซลล์ประสาท ความเครียดออกซิเดชันและการทำงานของเอนไซม์อะเซติลโคลีนเอสเตอเรสในฮิบโปแคมปัสของสัตว์ทดลอง

ผลการศึกษา: สารสกัดทุกขนาดที่ใช้ในการศึกษาครั้งนี้เพิ่มความจำแต่ลดระดับมาลอนไดอัลดีไฮด์ในฮิบโปแคมปัส การลดการทำงานของเอนไซม์อะเซติลโคลีนเอสเตอเรสในฮิปโปแคมปัสนั้นพบเฉพาะในหนูที่ได้รับสารสกัดขนาดต่ำและขนาดกลาง ในขณะที่การเพิ่มการทำงานของ SOD พบในกลุ่มที่ได้รับสารสกัดขนาดต่ำ ไม่พบการเปลี่ยนแปลงความหนาแน่นของเซลล์ประสาทอย่างมีนัยสำคัญในหนูที่ได้รับสารสกัดจากฟักทองในทุกช่วงปริมาณขนาดที่ใช้ในการศึกษานี้ ข้อมูลจากการศึกษาของเราชี้นำว่าฤทธิ์เพิ่มความจำของฟักทองน่าจะเป็นผลจากการลดความเครียดออกซิเดชันและเพิ่มการทำงานของระบบประสาทโคลิเนอร์จิค

สรุป: สารสกัดฟักทองเป็นอาหารเสริมสุขภาพที่มีศักยภาพในการเพิ่มความจำที่มีราคาถูกและง่ายต่อการเข้าถึง อย่างไรก็ตามการวิจัยเกี่ยวกับกลไกพื้นฐานและสารที่น่าจะเป็นสารออกฤทธิ์มีความจำเป็น