

An Anthocyanins-Rich Corn Derived Herbal Tea Improves Memory, Oxidative Stress Status and the Functions of Cholinergic and Monoaminergic Systems in Hippocampus

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Background: The anthocyanins-rich herbal tea has been developed from corn cob based on the memory enhancing effect of anthocyanins. The authors hypothesized that the product should improve memory function. However, no supported evidence concerning the health benefit and possible underlying effect of the developed product is available.

Objective: To determine the effect of an anthocyanins-rich corn derived herbal tea on spatial memory and to explore the possible underlying mechanisms by focusing on the roles of oxidative stress status and the functions of cholinergic and monoaminergic systems in hippocampus.

Materials and Methods: Male Wistar rats, weighing 250 to 300 g, were orally given herbal tea from cob of purple corn (*Zea mays*) at doses of 14, 28 and 56 mg/kg BW once daily for 28 days. All rats were determined spatial memory after the single administration and every 7 days throughout an experimental period. The oxidative stress status indices including MDA level and the activities of SOD, CAT and GSH-Px in hippocampus were assessed at the end of study. The activities of AChE and MAO in the mentioned area were also determined.

Results: The medium and low doses of the developed tea improved spatial memory and suppressed AChE and MAO activities in hippocampus. The decreased MDA level was also observed in rats treated with the medium dose of corn cob herbal tea. However, no significant changes of SOD, CAT and GSH-Px activities were observed.

Conclusion: Corn cob herbal tea shows a memory enhancing effect. The mechanism occurs partly via the suppression of AChE and MAO. Therefore, it shows the potential to serve as cognitive enhancing tea. However, the clinical trial study and subchronic toxicity test are essential to confirm the benefit and consumption safety.

Keywords: *Zea mays*, Anthocyanins, Memory, Oxidative stress, Cholinergic, Monoaminergic

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Herbs have been long-term used for treating various ailments in Asian countries. Traditionally, the water extract of herbs have been used as decoction and infusion tea. Due to the increasing health concern, herbal medicine has gained much attention. To date, various valuable parts of herbs have been prepared as infusion tea and claimed for health promotion. Unfortunately, less scientific evidence for safety consumption and the beneficial effects is available.

Recently, polyphenolic compounds such as flavonoids including anthocyanins have been reported to

provide numerous health benefits such as anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties^(1,2). It has been clearly demonstrated that anthocyanins and its metabolites can pass the blood brain barrier (BBB). In addition, other flavonoids and their metabolites can also pass the BBB⁽³⁾. Recent findings have shown that anthocyanins can attenuate the neurodegeneration in cellular model of Parkinson's disease⁽⁴⁾. Based on its ability to pass BBB and the neuroprotection mentioned earlier, the cognitive enhancing effect of substances which are rich in anthocyanins and other flavonoids have gained attention.

Purple corn or *Zea mays* L. (purple color) are widely consumed in Thailand. It has been reported that the main ingredient of water extract is anthocyanins whereas the main ingredients in non-polar solvent are phenolic acids such as p-coumaric, vanillic acid, protocatechuic acids, flavonoids such as quercetin and a hesperidin derivatives⁽⁵⁾. Purple corn extract has been reported to improve many oxidative stress

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related disorders⁽⁶⁾ such as diabetic cataract⁽⁷⁾. In addition, consumption of anthocyanins rich foods also improves memory⁽⁸⁾. Interestingly, the cob of purple corn, a waste product derived from corn agricultural industry, also contains abundant anthocyanins⁽⁹⁾. This raises the hypothesis that the purple corn cob may possibly improve memory performance and oxidative status. Currently, most researches concerning functional food have not been carried out by testing the pharmacological activity of the product directly. Therefore, in the view of consumers, they still can't strongly assure the effect of product. In order to serve the realistic approach in the view of the consumer, we aimed to determine the effect of an anthocyanin-rich corn derived herbal tea on spatial memory, oxidative stress status and the functions of cholinergic and monoaminergic systems in hippocampus.

Materials and Methods

Plant material and preparation of the herbal infusion

Purple waxy corn (open pollinated cultivar) was harvested during January to February 2012 and kindly provided by Assoc. Prof. Dr. Kamol Lertrat, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. The dried cobs of *Z. mays* were pulverized and roasted for 3 minutes. 3.6 grams of the roasted *Z. mays* were packed into tea bags. Infusion of *Z. mays* cob was prepared by adding 250 ml of boiling water (equivalent to a tea cup) to the bags (each bag contained 3.6 g of *Z. mays* corn cob powder). The infusions were brewed for 6 minutes and after the bag was withdrawn, the resulting solutions were cooled and kept at -20°C for further experiments. *Z. mays* cob tea used in this study contained the total phenolic compound and anthocyanin at the concentration of 10.57±0.12 mg GAE and 46.05±0.06 mg/L cyanidin-3-glucoside equivalents.

Experimental animals

Healthy male Wistar rats (250 to 300 g) were obtained from National Laboratory Animal Center, Salaya, Nakhon Pathom. They were randomly housed 5 per cage and maintained in 12: 12 light:dark cycle and given access to food and water ad libitum. The experiments were strictly performed in accordance with the internationally accepted principles for laboratory use and care of European Community (EEC directive of 1986; 86/609/EEC). The experiment protocols were approved by the Institutional Animal Care and Unit Committee Khon Kaen University, Thailand (AEKKU AA/2556).

Experimental protocols

After 14 days of acclimatization, the rats were divided into 7 groups, each containing 5 rats.

Group I: Naive control; rats in this group received no treatment.

Group II: Vehicle treated group; rats were orally given distilled water and served as a control group.

Group III: Vitamin C treated group; all animals in this group were orally given ascorbic acid at dose of 250 mg/kg BW and served as a positive control based on its cognitive enhancing effect⁽¹⁰⁾.

Group IV: Donepezil treated group; all rats in this group were treated with donepezil, an acetylcholinesterase inhibitor (AChEI), at the dose 1 mg/kg BW and also served as positive control.

Group V to VII: ZM treated groups, rats in these groups were orally treated with infusion of corn derived herbal tea at doses of 14, 28 and 56 mg/kg BW respectively, once daily. Each substance was administered at the volume of 1.5 ml. All animals except that in group I were treated with the assigned substances for 28 days. The spatial memory was determined using Morris water maze test after the single administration and every 7 days throughout the study period. The possible underlying mechanisms including oxidative stress markers and activities of acetylcholinesterase (AChE) and monoamine oxidase (MAO), an indirect indicator to reflect the activities of cholinergic and monoaminergic systems were determined at the end of the experiment.

Morris water maze test

Spatial memory was assessed by using Morris water maze test⁽¹¹⁾. Briefly, rats were trained for 5 days to remember the location of a platform which was immersed in the center of 1 quadrant. Since the surface of the water was covered with non-toxic milk, the location of the immersed platform was invisible. The rats must learn to identify the location of the platform by using the relationship of its location and the platform location with the aid of environmental cues. On the test day, each rat was gently placed into quadrant which was opposite to the quadrant with the platform and time which the rat spent to climb onto the platform was recorded as escape latency. The evaluation of retention memory was performed 24 hours later. The animal was exposed to the same situation except that the immersed platform was removed. Time which each rat spent swimming in the quadrant which previously immersed the platform was recorded and regarded as retention time. All assessments of Morris water maze test were carried out within 30 minutes after the administration of the assigned substances. Either the decreased escape latency or the enhanced retention time indicated enhanced memory.

Preparation of brain homogenate and protein determination

After the anesthetization with 60 mg/kg BW of Sodium thiopental, rats were transcardially perfused with cold normal saline solution to remove blood from brain tissues. The brain of each rat was quickly removed and hippocampus, a crucial area of spatial memory, was isolated and prepared as brain homogenate with a 50 volume of phosphate buffer saline (PBS) and used for the determination of protein, oxidative markers, AChE and MAO. The protein concentration was determined in brain homogenate volume

by the method of Lowry and colleagues⁽¹²⁾ using bovine serum albumin as standard.

Determination of oxidative status in hippocampus

Malondialdehyde (MDA), a lipid peroxidation process product, was determined by using Thiobarbituric acid (TBA) reaction⁽¹³⁾. In brief, the pinkish red chromogen with the absorbance at 532 nm which occurred as the result of the reaction between the secondary end product of the oxidation of polyunsaturated fatty acids and TBA was measured. The measured MDA level was normalized by its own protein concentration and expressed as nmol/mg protein.

The activity of superoxide dismutase (SOD) was determined via the inhibition of cytochrome C reduction mediated via superoxide anions generated by xanthine-xanthine oxidase and monitored at 550 nm⁽¹⁴⁾. In this study, catalase (CAT) activity was measured by determining the decomposition rate of H₂O₂ via the titrimetric method. According to this method, the activity of enzyme was measured indirectly by measuring the residual H₂O₂ which was titrated by potassium permanganate at 515 nm⁽¹⁵⁾. Glutathione peroxidase (GSH-Px) activity was determined by measuring the rate of decreased of nicotinamide adeninedinucleotide phosphate (NADPH) at 340 nm which was directly proportional the activity of GSH-Px⁽¹⁶⁾. The activities of SOD, CAT and GSH-Px were expressed as units per milligram protein.

Determination of acetylcholinesterase (AChE) and monoamine oxidase (MAO) activities

AChE activity in brain homogenate was measured with the modified method of Ellman et al⁽¹⁷⁾ by using acetylthiocholine iodide (ATCI) as a substrate and measured the yellow color formation at 415 nm. AChE activity was expressed as $\mu\text{mol/min/g}$ protein. In this study, the activity of MAO was determined by using Tyramine as substrate and measured the color formation

spectrophotometrically at 490 nm⁽¹⁸⁾.

Statistical analysis

Data are presented as mean \pm standard error of mean (SEM). The statistical analysis of the experiment was carried out using IBM SPSS Statistic (version 21). Data were analyzed using one-way analysis of variance (ANOVA), following by Tukey post hoc test. A probability levels less than 0.05 were accepted as significance.

Results

Effect of herbal tea from the cob of *Z. mays* (purple color) on spatial memory

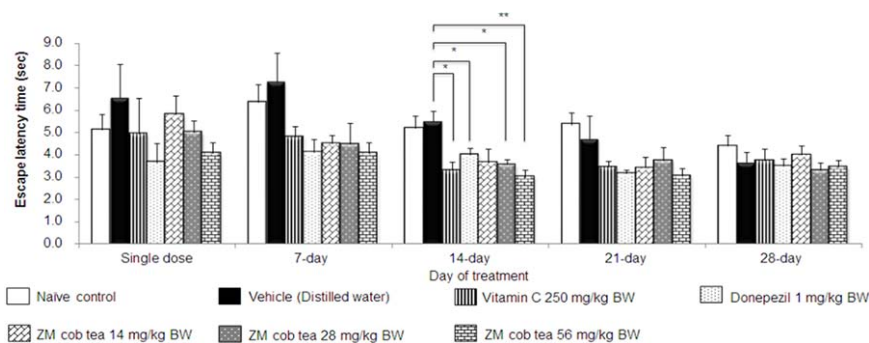
In Morris water maze test nepezil and the medium and high doses of herbal tea from cob of *Z. mays* (purple color) also significantly enhanced retention time at 7 days of treatment (p -value <0.05 all; compared to vehicle treated group). No other significant changes were observed as shown in Figure 2.

Effect of herbal tea from the cob of *Z. mays* (purple color) on oxidative status in hippocampus

Figure 3 showed the effect of herbal tea from the cob of *Z. mays* (purple color) on MDA level and on the activities of SOD, CAT and GSH-Px in hippocampus. It was found that vehicle produced no changes in all parameters just mentioned. Rats which subjected to either Donepezil or medium dose of herbal tea from cob of *Z. mays* (purple color) treatment showed the decreased MDA level in hippocampus (p -value <0.05 all; compared to vehicle treated group). Unfortunately, no significant changes of SOD, CAT and GSH-Px activities were observed.

Effect of herbal tea from the cob of *Z. mays* (purple color) on cholinergic and monoaminergic functions

The effect of herbal tea from the cob of *Z. mays* (purple color) on cholinergic function in hippocampus was



*** p -value <0.05 and 0.01 respectively; compared to vehicle treated group
ZM = *Z. mays*

Figure 1. Effect of herbal tea from cob of *Z. mays* (purple color) on escape latency in Morris water test ($n = 5/$ group). Data were expressed as mean \pm SEM.

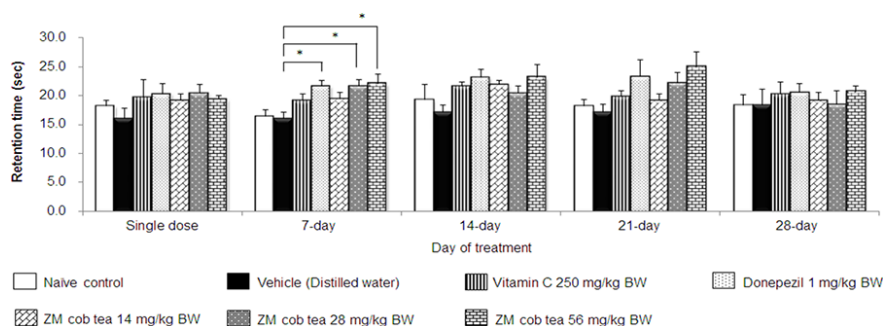
also investigated by using the activity of AChE as indirect indicator to indicate cholinergic function and the results were shown in Figure 4. Our data clearly demonstrated that both Vitamin C and Donepezil treated rats significantly decreased AChE activity in hippocampus (p -value <0.01 all; compared to vehicle treated group). Rats which received herbal tea from the cob of *Z. mays* (purple color) at doses of 14, 28 and 56 mg/kg BW also significantly decreased the activity of AChE in the mentioned area (p -value <0.05 , 0.001 and 0.01 respectively; compared to vehicle treated group). The authors also assessed the effect of herbal tea from the cob of *Z. mays* (purple color) on monoaminergic function in hippocampus and the activity of monoamine oxidase (MAO) was used as an indicator to reflect the monoaminergic function. The results were shown in Figure 5. Vehicle treated rats showed no significant different MAO activity when compared to control. Donepezil treated rats showed the significant decrease in MAO activity in hippocampus (p -value <0.001 ; compared to vehicle treated group) while no significant change was observed in Vitamin C treated rats. Interestingly, rats treated with herbal tea from the cob of *Z. mays* (purple color) at doses of 14, 28 and 56 mg/kg BW showed the significant reduction of MAO activity in hippocampus (p -value <0.001 ,

0.001 and 0.01 respectively; compared to vehicle treated group).

Discussion

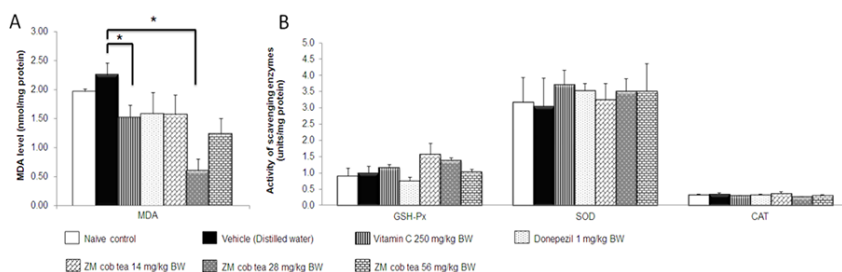
The current study has revealed for the first time that herbal tea from cob of *Z. mays* (purple color) at doses of 28 and 56 mg/kg BW show the positive modulation on learning and memory in rats. The suppression of AChE and MAO activities in hippocampus were also observed in both groups. However, the decreased MDA was observed only in rats which received herbal tea from cob of *Z. mays* (purple color) at dose of 24 mg/kg BW and no significant changes of scavenger enzymes such as SOD, CAT and GSH-Px were observed.

Spatial memory is defined as a memory of topographical layout of environments, and enables goal directed navigation on the basis of cognitive map⁽¹⁹⁾. This type of memory is associated with the function of hippocampus^(20,21). It has been reported that spatial memory is under the influence of many factors such as cholinergic⁽²²⁾ and monoaminergic⁽²³⁾ functions. In addition to the factors just mentioned, oxidative stress also plays the important role on spatial memory⁽²⁴⁾. Therefore, the modulation of these



* p -value <0.05 and 0.01 respectively; compared to vehicle treated group)

Figure 2. Effect of herbal tea from cob of *Z. mays* (purple color) on retention time in Morris water maze test ($n = 5$ /group). Data were expressed as mean \pm SEM.



* p -value <0.05 ; compared to vehicle treated group

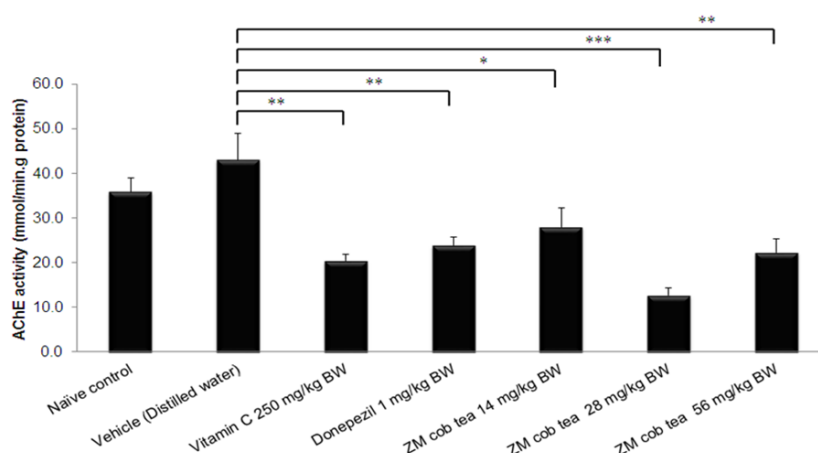
Figure 3. Effect of herbal tea from cob of *Z. mays* (purple color) on oxidative status (A) malondialdehyde (MDA), (B) Scavenging enzymes activities including superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH-Px) in hippocampus ($n = 5$ /group). Data were expressed as mean \pm SEM.

factors may in turn modify spatial memory.

Recent findings have clearly demonstrated that anthocyanins can suppress AChE⁽²⁵⁾, and monoamine oxidase⁽²⁶⁾ giving rises to the enhanced memory⁽²⁵⁾. In addition, anthocyanins also possessed antioxidant⁽²⁷⁾. It has been reported that antioxidant can also improve memory⁽²⁸⁾. Based on these pieces of information, the authors did suggest that anthocyanins- rich herbal tea from cob of *Z. mays* (purple color) might enhance memory via the multi-target sites. The principal targets appear to be the suppression of AChE and MAO giving rise to the increased available ACh and monoaminergic transmitters in hippocampus resulting in the enhanced attention⁽²⁹⁾ which in turn increased the efficiency of encoding and retrieval of memory⁽³⁰⁾ leading to the enhanced memory. The improved oxidative stress status might also partly play role especially at the medium dose. Although no

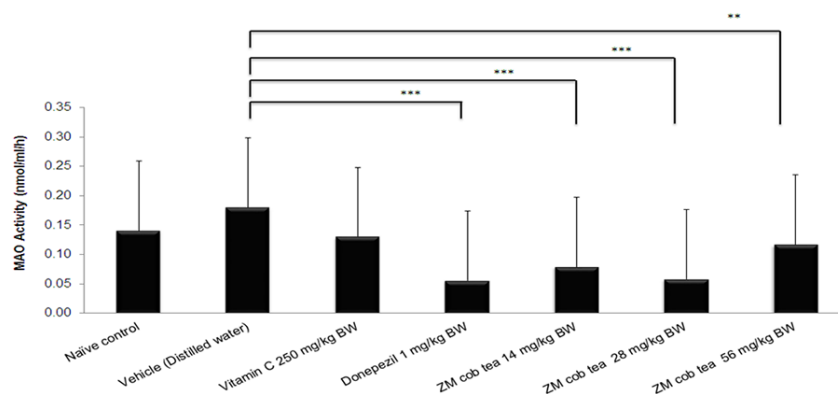
significant changes of the main scavenger enzymes were observed, the level of MDA was decreased. The reduction of MDA level observed in the present study might be due to the decreased oxidative stress and free radicals formation. In addition, the non-enzymatic oxidative stress buffering system induced by herbal tea from cob of *Z. mays* (purple corn) might possibly play role on this phenomenon.

In the present study, it was found that the significant difference both in escape latency and in retention time were loss when the treatment was prolonged. These phenomena might be attributed to the increased learning and memory capacity after the repetitive exposure to the test of the normal healthy rats. In addition, no dose dependent manner was observed. The possible explanation might occur because the relationship between the concentrations of herbal tea from cob of *Z. mays* (purple color) and the memory was



***, ** p-value <0.05, 0.01 and 0.001 respectively; compared to vehicle treated group

Figure 4. Effect of herbal tea from cob of *Z. mays* (purple color) on the activity of acetylcholinesterase (AChE) enzyme in hippocampus (n = 5/group). Data were expressed as mean \pm SEM.



***, ** p-value <0.01 and 0.001 respectively; compared to vehicle treated group

Figure 5. Effect of herbal tea from cob of *Z. mays* (purple color) on the activity of monoamine oxidase (MAO) enzyme in hippocampus (n = 5/group). Data were expressed as mean \pm SEM.

not a simple linear relationship. It has been previously described that spatial memory was under the influence of many factors.

Conclusion

The present study is the first study which clearly demonstrates that cob of *Z. mays* (purple color) can increase its value as herbal tea which provides health benefits. The herbal tea from cob of *Z. mays* (purple color) possesses the memory enhancing effect. This effect can be observed even in healthy condition. The possible underlying mechanism occurs mainly via both the improved cholinergic and monoaminergic functions in the hippocampus leading to increased attention which in turn improves encoding and retrieval processes of memory. The improved oxidative stress status also plays a role at medium concentration. Since the effective doses are very low, it is possible and easy to implement in a practical way. Therefore, herbal tea from cob of *Z. mays* (purple color) is the potential functional food to increase memory capacity. However, further research in clinical trials and subchronic toxicity tests are essential.

What is already known on this topic?

Polyphenolic compounds such as flavonoids including anthocyanins have been reported to provide numerous health benefits such as anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties. Purple corn or *Z. mays* L. (purple color) has the main ingredient of water extract as anthocyanins. Purple corn extract has been reported to improve many oxidative stress related disorders such as diabetic cataract and also improves memory.

What this study adds?

The cob of *Z. mays* (purple color) possesses the memory enhancing effect in healthy condition, which resulted from the improvement of both cholinergic and monoaminergic functions in hippocampus. Thus, herbal tea from cob of *Z. mays* (purple color) is the potential functional food to increase memory capacity.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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ชาสมุนไพรจากข้าวโพดที่มีแอนโธไซยานินสูงทำให้ความจำ ความเครียดออกซิเดชันและการทำงานของระบบประสาทโคลิเนอร์จิกและโมโนเอมิเนอร์จิกในฮิปปแคมป์สดีขึ้น

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ภูมิหลัง: ชาสมุนไพรที่มีแอนโธไซยานินสูงถูกพัฒนาขึ้นจากข้าวโพดม่วงเนื่องจากแอนโธไซยานินมีฤทธิ์เพิ่มความจำ ผู้วิจัยตั้งสมมุติฐานว่าผลิตภัณฑ์ที่พัฒนาขึ้นน่าจะเพิ่มความจำดีขึ้น อย่างไรก็ตามปัจจุบันยังไม่มีหลักฐานสนับสนุนเกี่ยวกับประโยชน์ทางสุขภาพและกลไกการออกฤทธิ์ของผลิตภัณฑ์ที่พัฒนาขึ้น

วัตถุประสงค์: เพื่อตรวจสอบฤทธิ์ของชาสมุนไพรที่มีแอนโธไซยานินสูงต่อความจำที่เกี่ยวข้องกับทิศทางและสำรวจกลไกการออกฤทธิ์ที่น่าจะเป็นได้โดยให้ความสนใจต่อสภาวะความเครียดออกซิเดชันและการทำงานของระบบประสาทโคลิเนอร์จิกและโมโนเอมิเนอร์จิกในสมองส่วนฮิปปแคมป์ส

วัสดุและวิธีการ: ได้นำชาสมุนไพรจากข้าวโพดม่วงขนาด 14, 28 และ 56 มิลลิกรัมต่อกิโลกรัมน้ำหนักตัว ให้หนูพันธุ์วิสตาเรพัสน้ำหนัก 250 ถึง 300 กรัม วันละครั้ง 28 วัน และประเมินความจำที่เกี่ยวข้องกับทิศทางหลังได้รับชาสมุนไพรไปครั้งเดียว และทุก 7 วันตลอดระยะเวลาการทดลอง ประเมินดัชนีแสดงสภาวะความเครียดออกซิเดชัน ได้แก่ระดับ MDA และการทำงานของ SOD, CAT และ GSH-Px ในฮิปปแคมป์สเมื่อสิ้นสุดการทดลอง การทำงานของ AChE และ MAO ในบริเวณที่กล่าวข้างต้นเองก็ถูกการประเมินเช่นกัน

ผลการศึกษา: ชาที่พัฒนาขึ้นขนาดกลางและต่ำทำให้ความจำที่เกี่ยวข้องกับทิศทางดีขึ้นและยับยั้งการทำงานของ AChE และ MAO ในฮิปปแคมป์สระดับ MDA ของหนูที่ได้รับชาสมุนไพรจากข้าวโพดขนาดกลางก็ลดลง อย่างไรก็ตามไม่พบการเปลี่ยนแปลงของ SOD, CAT และ GSH-Px

สรุป: ชาสมุนไพรจากข้าวโพดม่วงแสดงฤทธิ์เพิ่มความจำ กลไกส่วนหนึ่งผ่านการยับยั้งการทำงานของ AChE และ MAO ดังนั้นจึงมีศักยภาพในการนำมาใช้เป็นชาเพิ่มความจำ อย่างไรก็ตามยังจำเป็นต้องมีการทดลองทางคลินิกและพิษกึ่งเรื้อรังเพื่อยืนยันประโยชน์และความปลอดภัยในการบริโภค
