

The Memory Enhancing Effects of the Extract from the Fruit Hull of Mangosteen (*Garcinia mangostana* L.) in Healthy Adult Male Rats

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Abstract. The extract from the fruit hull of mangosteen (GME) possesses a wide variety of biological activities; however, its effects on learning and memory remain to be elucidated. Thus, the aim of the present study was to investigate the effects of GME on spatial learning and memory of adult male Wistar rats. Rats received vehicle (10% tween80, 1 ml/kg), vitamin E (40 mg/ml/kg), GME (500, 1000 or 2000 mg/ml/kg) orally once daily for 30 days. Spatial memory and learning of rats were tested by Morris water maze test. The protocol consisted of 21 training trials (3 times per day for 7 days on day 24-30) and probe trial on day 30. In all groups, time to find platform on day 7 of training trial was significantly decreased from day 1. Time spent in target quadrant was significantly increased in vitamin E-treated group and 500 mg/ml/kg GME-treated group when compared to vehicle treated group. No significant difference was found in the number of entry into the target quadrant between groups. Thus, GME appears to improve learning and memory in adult rats. GME may promote neuroprotective effects; however, the underlying mechanisms are still not fully understood.

Keywords: Mangosteen, learning, memory, morris water maze, rat

1. Introduction

The fruit hull of mangosteen, a rich source of polyphenols (xanthone, tannin, flavonoid and anthocyanins) has been used in Southeast Asia traditional medicine for the treatment of skin infections, wound, and diarrhea [1], [2]. These polyphenolic compounds are of plant secondary metabolites that have been reported to possess a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, anti-diabetic and acetylcholinesterase inhibitory activities and to be efficient scavenger of reactive oxygen species (ROS), and to possess neuroprotective properties [3]-[5]. The benefit of polyphenols on protection against Alzheimer's disease and other memory problems suggested the potential role of polyphenols found in the fruit hull of mangosteen in the prevention and treatment of memory impairment. It is hope that natural antioxidant found in the fruit hull of mangosteen could also enhance memory. Therefore, the effects of the crude extract from the fruit hull of mangosteen (GME) on memory in healthy adult rats were investigated.

2. Materials and Methods

2.1. Plant Material

Mangosteen (*Garcinia mangostana* Linn.) fruits were purchased from local market in Nakhon Ratchasima province during May-October 2007.

2.2. Preparation of Plant Extract

The fruit hull was washed with copious amounts of water. The fruit hull was then cut into small thin pieces and allowed to air dry at room temperature. The dried hull was powdered using an electric mill with a

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1.0 mm mesh. The dried powder will be extracted in 85% aqueous ethanol using maceration method for 7 days in the dark at room temperature. The extract was filtered through Whatman filter paper No. 1. After filtration, the extract was evaporated (Rotavapor model R-205, Buchi, Switzerland) under vacuum to absolute dryness the aqueous extract was lyophilized (Labconco Corporation Ltd., Missouri, USA) and kept at -20 °C until further used.

2.3. Animals

Male Wistar rats (8 weeks old) were obtained from the Animal Care and Use Committee Guidelines of Suranaree University of Technology. They were maintained under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 20 ± 1 °C) with free access to food and water.

Rats were randomly assigned to five groups of 6 rats each as follows: group 1: Control (10% tween80, 1 ml/kg), group 2: vitamin E (40 mg/ml/kg), group 3: GME500 (the crude extract from the fruit hull of mangosteen (GME), 500 mg/ml/kg), group 4: GME1000 (GME, 1000 mg/ml/kg) and group5: GME2000 (GME, 2000 mg/ml/kg). All rats were tested for their spatial memory using Morris water maze test.

2.4. Morris Water Maze Test

The Morris water maze test is widely used to study spatial memory and learning in rodent. Morris water maze test was developed by Richard Morris at the University of St Andrews in Scotland [6].

The apparatus consists of large circular pool (2.3 m in diameter and 63 cm in height) which contains no internal cues, stimuli, markings on objects, but is surrounded by stable, salient extra-maze cues. The pool was filled to a depth of 21.5 cm with water at temperature of 25 °C and made opaque with white non-toxic water paint (TOA, Co., Ltd, Thailand). The pool was divided into four quadrants of equal areas (Q1, Q2, Q3 and Q4 (with platform)) and surrounded by extra maze distal visual cues of different shape. A white platform (19.5 cm in diameter and 28.5 cm in height) was submerged 2 cm below the water surface and placed in the center of Q4 and it was located in the same position on every trial. A video camera is placed above the centre of the pool to capture images of the swimming animal.

The training Trial: on the day before training trials, the animal was placed in the pool and allowed to swim for 60 s in the absent of the escape platform. During the seven training days, the animal was given three trials sessions each day with an inter-trial interval of 1 min. The trial began when animal were randomly placed in the water at one of the locations (Q1, Q2, and Q3) with its head facing the wall of the pool and allowed 60 s to swim, search and climb up for the platform. Once the animal found the platform, rat was allowed to remain on the platform for 60 s. The escape latency to the platform (the time taken to find the platform) was recorded. If the animal did not locate the platform within 60 s and the animal was guided to the platform by experimenter and let the animal sit on platform for 15 s. If animal jumped off, guide it back. Once the animal has completed all three trials, dry off with a towel. The three trial training process was repeated for all the animals consecutively.

Probe Trails: On day 7 after training was completed, the experimenter conducted a probe trial in which the escape platform was removed from the pool. The animals were released from the quadrant opposite where the platform had been location and allowed to swim for 60 s, after which the rat was taken out of the pool. Generally, a well-trained rat swam to the target quadrant of the pool and repeatedly acrossed the former location of the platform until starting to search elsewhere. The time spent in target quadrant and the number of entry into the target quadrant was recorded.

Rats in group 1 were daily orally administered with 10% tween 80 (1 ml/kg), rats in group 2 were daily orally administered with positive control vitamin E (40 mg/ml/kg, [7]), rats in group 3, 4 and 5 were daily orally administered with the crude extract from the fruit hull of mangosteen at a dose of 500, 1000, and 2000 mg/ml/kg, respectively, for 30 days. The protocol consisted of 21 training trials (3 times per day for 7 days) and probe trial on the last day (day 30). Twenty one training trials were performed 1 hour after each treatment on day 24 to day 30. On day 30, one hour after the last training trial, all rats were tested for their spatial memory using the Morris Water Maze test (probe trial). The time spent in target quadrant and the number of entry into the target quadrant was recorded.

2.5. Statistical Analysis

Results were expressed as mean \pm standard error of mean (SEM). Data were analyzed by One-way ANOVA followed by Student-Newman-Keuls using the software SigmaStat (version 3.5, Systat Software Inc., USA.). *P*-values less than 0.05 ($P < 0.05$) were considered statistically significant.

3. Results

The results of Morris water maze test are presented in Fig. 1, 2 and 3. The escape latency to the platform (the time taken to find the platform) on training days (day 1 to day 7) of all groups on day 7 was significantly lower than on day 1 (Fig. 1). There was no significant difference in the escape latency between groups on any training days. In probe trial, the time spent in target quadrant was significantly enhanced by vitamin E (40 mg/kg) and the crude extract from the fruit hull of mangosteen at a dose of 500 mg/ml/kg (GME500) for 30 days, compared to control group (10% tween80) (Fig. 2). The increase in time spend in target quadrant by the crude extract from the fruit hull of mangosteen was not dose-dependent. Receiving the crude extract from the fruit hull of mangosteen at doses of 1000 and 2000 mg/ml/kg (GME1000 and GME2000) for 30 days did not show significant difference from control group (Fig. 2). The number of entry into the target quadrant of all groups did not demonstrate any significant difference between groups. Oral administration of vitamin E (40 mg/kg) and the crude extract from the fruit hull of mangosteen at a dose of 2000 mg/ml/kg (GME2000) for 30 days tended to increase the number of entry into the target quadrant.

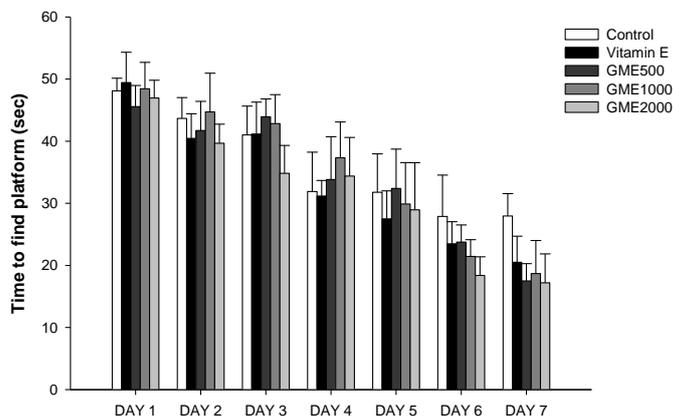


Fig. 1: Effects of vitamin E and the crude extract from the fruit hull of mangosteen on time to find platform on training days (day 1 to day 7) of adult rats in Morris water maze test. Values are expressed as mean \pm SEM; n=6 per group.

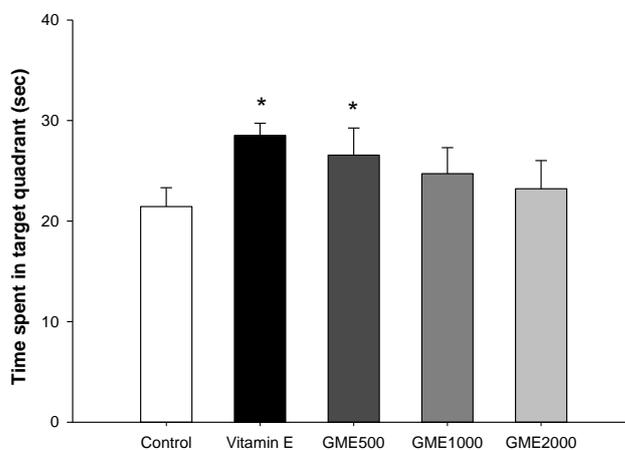


Fig. 2: Effects of vitamin E and the crude extract from the fruit hull of mangosteen on time spent in target quadrant of adult rats in Morris water maze test. Values are expressed as mean \pm SEM; n=6 per group. *Indicates a significant difference ($P < 0.05$) when compared to control group.

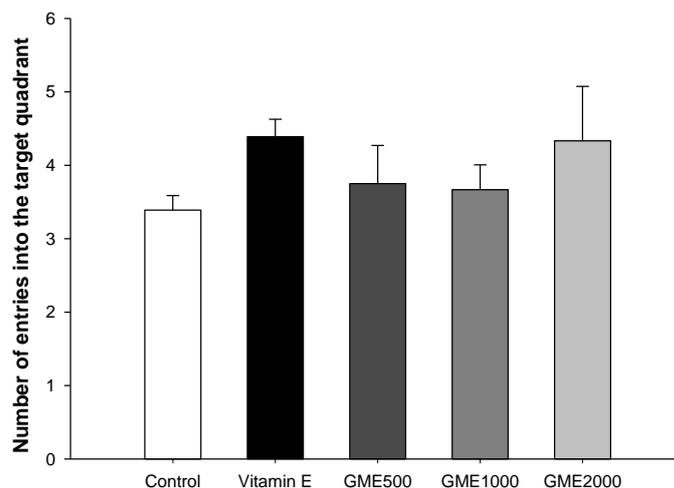


Fig. 3: Effects of the crude extract from the fruit hull of mangosteen on number of entries into the target quadrant of adult rats in Morris water maze. Values are expressed as mean \pm SEM; n=6 per group.

4. Discussion

In adult male rats, significant increases in time to find platform were shown in rats received vitamin E and the crude extract from the fruit hull of mangosteen at a dose of 500 mg/ml/kg for 30 days (Fig. 2). The number of entry into the target quadrant in rats received vitamin E and the crude extract from the fruit hull of mangosteen at a dose of 2000 mg/ml/kg for 30 days were higher, but not significantly different, than control (Fig. 3). These findings indicated that the crude extract from the fruit hull of mangosteen could enhance memory and may be a potent neuroprotectant in adult male rats. The four extracts (water, 50% ethanol, 95% ethanol and ethyl acetate) of the fruit hull of mangosteen showed antioxidant activity estimated by the diphenylpicrylhydrazyl (DPPH) method [8], [9] and neuroprotective activities [9]. Oxidative damage in rat brain tissue exposed to the toxic actions of a free radical generator (ferrous sulfate), an excitotoxic agent (quinolinate), and mitochondrial toxin (3-nitropropionate) could be reduced by α -Mangostin, a xanthone isolated from mangosteen fruit [10]. Bioactive compounds found in the fruit hull of mangosteen such as xanthone, tannin, flavonoid and anthocyanins have positive effects on learning and memory. Flavonoids, proanthocyanidins and terpenoids from *Ginkgo biloba* extracts have been suggested to have a multitude of beneficial effects on central nervous system function, from enhancing cognitive function in dementia to facilitating recovery from acute forms of neural damage such as hypoxia/ischemia [11]. Extracts from plants and fruits containing polyphenol (*Thunbergia laurifolia* Linn, *Centella asiatica*, and *Mangifera indica*) showed significant improvement in learning and memory impairment [12], [13]. The observed improvement in water maze performance may be due to the antioxidant property of polyphenols present in GME. In conclusion, the present findings suggested that GME may be enhancing memory. Based on the results of the Morris water maze tests using adult rats, we found that GME had remarkable cognitive-enhancing effect.

5. Acknowledgements

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6. References

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