Journal of Translational Science

Research Article ISSN: 2059-268X

Improved spatial learning and memory performance following Tualang honey treatment during cerebral hypoperfusion-induced neurodegeneration

Anil Kumar Saxena*, Hnin P Phyu, Imad M Al-Ani and Oothuman P

Department of Basic Medical Sciences, Faculty of Medicine, International Islamic University Malaysia, Kuantan, Malaysia

Abstract

Honey has shown potential therapeutic properties in traditional medicine. This study investigated the cognition enhancing potential of Tualang honey in chronic cerebral hypoperfusion- induced neurodegeneration by permanent bilateral common carotid arteries ligation (2 vessels occlusion- 2VO) in rats. Previous studies have shown that rats subjected to 2VO (2 vessel occlusion) experienced cognitive deficits, oxidative stress and neuronal injury in the hippocampus of the brain. We studied the effect of Tualang honey on learning and memory using the Morris water maze (MWM) in rats. Rats were randomly divided into four groups (n=10); Sham control, untreated 2VO (2VO), honey treated 2VO (2VO+H) and honey treated group (H). Each group was again divided into two subgroups (n=5): one for long term memory assessment and another for short term memory and relearning tasks. After 10 weeks of treatment, all rat groups were tested for cognitive assessment by MWM (Morris water maze). 2VO+H rats had better spatial learning and memory performance than untreated 2VO rats in MWM tasks ($p<0.05$). However, there was no significant difference between Sham rats and H group rats ($p>0.05$). This study shows that Malaysian Tualang honey might have therapeutic potential for the treatment of chronic cerebral hypoperfusion related neurodegenerative disorders such as Alzheimer's disease (AD) in which cognitive impairment is profound.

Introduction

Neurodegenerative disorders including Alzheimer's disease (AD) and particularly dementia are associated with aging and reduced cerebral blood flow [1]. Disturbances of the cerebral blood flow (CBF) have been correlated with the cognitive impairment in elderly people, as well as with the development of several types of dementia. Both dementia and cognitive decline are conditions associated with aging [1].

AD (Alzheimer's disease) is an age-associated neurodegenerative disease that is characterized by a progressive impairment of memory and cognitive ability and other neurobehavioral manifestations due to loss of hippocampal and cortical neurons [2]. The hallmark abnormalities of AD are deposition of the abnormal beta-amyloid protein fragments (plaques) and twisted strands of the protein tau (tangles) [1,3,4]. Although a large number of studies had been carried out, the cause of AD is still unknown.

Honey possesses numerous beneficial effects including antimicrobial, antiviral, antiparasitory, anti-inflammatory, antioxidant, antimutagenic, neuroprotective, antitumor, wound healing and cardio protective effects [5-7]. These activities are mainly due to the phenolic compounds such as flavonoid. These antioxidant and radical scavenging effects are seen in all types of honeys. Nevertheless, these effects are in different proportions in different honey depending on the geographical areas, different floral source as well as climate [8]. Tualang honey is used commonly as a medicinal product as well as food in Malaysia.

Permanent, bilateral occlusion of the common carotid arteries (2VO) in rats have been validated as an experimental model to study the effects of chronic cerebral hypoperfusion on cognitive function and neurodegenerative processes [1,9]. Morris Water Maze has been widely used as a measure to investigate spatial learning and memory in laboratory rats, one of the most frequently used methods in behavioral neuroscience [10].

We studied the congnition enhancing potential of Tualang honey *i.e.,* increased memory and learning in honey treated rats comparing with control rats in 2VO rat model.

Methods and materials

Forty male Sprague-Dawley rats weighing 250-300 g were used for the study. The rats were obtained from registered animal supplier in Malaysia. All animal experiments were approved by the ethical committee of the International Islamic University Malaysia IIUM. All rats were housed in cages (2 rats per cage) at a temperature of 26 ± 1°C and 12h light/dark cycle. All animals were treated in accordance with the *Guidelines for The Care and Use of Laboratory Animals of the National Institute of Health.* The standard food pellets and tap water were allowed ad libitum.

Malaysian Tualang honey

Tualang Honey used in this study was kindly supplied by Federal Agricultural Marketing Authority (FAMA), Kedah, Malaysia. The honey has the following composition: total reducing sugar (64.3%) comprising of fructose (34.3%), glucose (26.2%), maltose (3.8%);

Correspondence to: Anil Kumar Saxena, Department of Basic Medical Sciences, Faculty of Medicine, International Islamic University Malaysia, Kuantan, Malaysia, **E-mail:** drak.saxena@gmail.com

Key words: *spatial learning, memory, honey, chronic cerebral hypoperfusion*

Received: June 07, 2016; **Accepted:** June 24, 2016; **Published:** June 27, 2016

fructose/glucose ratio (1.31) and water (22.0%).

Morris water maze

The water maze consisted of a black circular pool (200 cm in diameter, 60 cm in height) filled to a depth of 30 cm with water to cover a black platform. A black platform about 10 cm in diameter was submerged 2 cm below the water surface and was placed at the midpoint of south west (SW) quadrant. The water was maintained at a temperature of about 26 ± 1°C. Various extra-maze cues were positioned on the wall of room around the water maze pool so that the rats could learn the position of the platform. The extra-maze cues were exactly the same throughout the study. The pool was geographically divided into four quadrants and had four points designed as starting positions called north, east, south and west at equal distances on the rim (N, S, W, or E). A video camera was set up in the ceiling above the pool and sessions were recorded using a software (ANY- maze, Stoelting, U.S.A) in separate protocols by a keyboard based behavioral tracking system.

2VO procedure

Permanent bilateral occlusion of the common carotid arteries (2VO) was done to induce chronic cerebral hypoperfusion in rats. Rats were anaesthetized with ketamine 90 mg/kg and xylazine 20 mg/kg (intraperitoneally) and were allowed to breath spontaneously throughout the surgical procedure. The common carotid arteries were exposed via a ventral cervical incision, and separated from the carotid sheaths and vagus nerves; care was taken to avoid the injury of vagus nerves. Both arteries were doubly ligated with silk sutures. The same procedure was performed on the Sham group except that the common carotid arteries were not ligated. After the operation, all animals were returned to their cages with free access to food and water.

Experimental study

After acclimatization for 1 week, animals were randomly divided into 4 groups (10 rats per group) *i.e.,* (A) Sham operated group; (B) untreated 2VO group (2VO); (C) honey-treated plus 2VO (2VO+H) group; and (D) honey-treated group (H). Rats in honey-treated group and honey-treated plus 2VO group received freshly prepared honey orally by gavage every morning for 10 weeks. The honey at a dose of 1.2 g/kg body weight was freshly diluted with distilled water each time before it was administered. This dose was calculated relative to the local human consumption of honey which was based on the body surface area normalization method [11].

Morris water maze task

Various Morris water maze (MWM) behavioral tasks were conducted in the present study. It involved several components that consisted of attention, concept of rules of task, reference memory, working memory and relearning tasks for short and long term memory assessment. It also included cued version phase to detect sensorimotor dysfunction and visual impairment in animal models after 2VO surgery. The MWM protocols vary between study groups but the basic principles of the test is the same [12].

For MWM (Morris water maze) test, the animals from group A, B, C and D were again divided into 2 subgroups; Memory Group (n=5) and Learning Group (n=5). The memory group animals were accessed for the long term memory whilst the learning group animals were tested for short term memory and relearning. The animals in learning group were not exposed to MWM before 2VO. In contrast, the memory group animals were familiarized with MWM. The objectives of Morris water maze experiment are to access the cognitive function of rats before and after 2VO in experimental animals and the effect of Tualang honey on memory and learning in rats between Group A and D.

Long term memory protocol (Figure 1)

This protocol consisted of 4 phases *i.e.,* Habituation phase, Acquisition phase, Retention probe test (probe trial), and Cued version phase.

Short term memory and relearning protocol (Figure 2)

This protocol consisted of 5 phases *i.e.,* Habituation phase, Acquisition phase, Retention probe test, Re-learning phase and Cued version phase. All the tests were conducted after 2VO surgery. The rats were gently placed with the tail end lower, into the water, supporting them in the hand. They were put facing the side walls of the water maze, so the head did not go under water, from one of the four starting position. Care has been taken not to drop the head first as this will give stress to the animals and impair their learning abilities.

Habituation phase: All the animals underwent this pre-training phase. In this phase, the escape platform (EP) was visible 2 cm above the water and situated in the center of water maze pool. The rats were allowed 120 seconds (s) to reach the EP. If any rat failed to find the EP within 120s, it was gently guided to reach EP. When the rat reached EP, it was allowed to stay on it for 30s to learn the location of the platform and orientate to the extra-maze visual cues. All rats had four trials per day for 2 days with different starting points of MWM pool (N, S, W, E). The duration for inter-trial interval was 2 minutes. Rats were dried with a towel and returned to their cages after finishing all trials.

Acquisition phase: The acquisition phase includes trials in which the rats must learn the location of a hidden platform submerged under the water surface in a circular swimming pool. The animals were trained to locate the hidden EP, which was located in a fixed location (*i.e.,* SW zone) throughout this phase. Rats were subjected to 4 trials per day for 4 days as described in habituation phase.

Each rat was placed on EP for 30s to get orientation of the new location of the hidden EP before starting first trial. The trial began when the rat was placed in the water at one of the four starting positions which was chosen at random. The escape latency time (in seconds) to reach the hidden platform and the swimming distance (in meter) travelled by the rat until its entry to EP zone were recorded by computerized

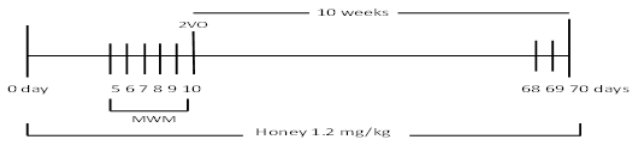


Figure 1. Experimental study for long term memory groups.

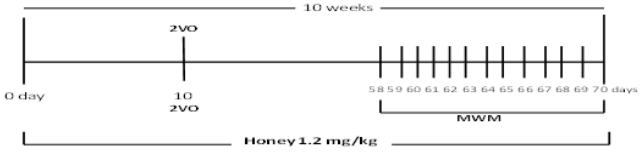


Figure 2. Experimental study for short term memory and re-learning groups.

video tracking system in a separate ANY-maze protocol. If the rat failed to find the platform within 120s, the training was terminated and a maximum score of 120s was assigned. The mean latency in seconds and mean distance travelled in meters for each training day were recorded.

Retention memory test (probe trial): The probe test was conducted one day after acquisition phase. It consisted of a single trial, as described before, with the removal of EP from the pool. The rats were released into the water from the quadrant opposite where the platform had been located. The rats were allowed to swim for 60s in the pool before they were removed from water by hand.

The time that rats spent in the SW zone previously containing the EP was taken as indicative of retention capacity. The parameters recorded in this phase were the frequency of crossings over the EP zone and the time spent by each rat in the target zone (SW zone).

Re-learning phase (working memory): The aim of this phase was to assess the effect of 2VO on the working memory of animals; whether honey treatments used prior to surgery was able to preserve the learning capability. The trial also compared the ability of groups A and D whether honey treated rats could perform better than SHAM controls. This study phase consisted of four trials per day on 3 consecutive days with the location of escape platform being changed daily (SE, NE and NW zones). Each trial was conducted similar to the reference memory phase, with a two minute inter-trial interval. Latency to find the platform was measured in each trial and the mean latency for daily trials performed in the three consecutive days were calculated. The rats were allowed to locate the novel platform position each day.

Cued version phase: In order to assess the animal's sensory, motor coordination and motivation function especially in 2VO groups, the capability of rats to escape to a visible platform was tested in this study. The platform was raised 2 cm above the water surface, and a red flag in 10 cm height was placed on the platform. A single trial was subjected to each rat with visible platform in the center of MWM pool in all cued trials. The objective of this part of the test was to assure that the results of the initial phases of water maze test were not due to unspecific sensorimotor or visual impairment.

Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) version 16.0 and presented as mean ± S.E.M. One way ANOVA was used to assess the differences among groups. If the results were statistically significant, the differences between groups were assessed by post-hoc Tukey's test. A value of $p<0.05$ was considered as statistically significant at 95% confidence interval.

Results

Long term memory assessment

Reference memory performance: The spatial memory and learning assessment is demonstrated through Morris water maze performance. Chronic cerebral hypoperfusion animal model induced by permanent bilateral common carotid arteries occlusion demonstrated impaired spatial memory and learning on Morris water maze. Figures 3A and 3B show the results of the escape latencies and swimming distances travelled to find the hidden platform of all rats in memory group during reference memory test phase before and after 2VO.

Escape latency time: Before 2VO procedure, all animal groups quickly learned to locate the hidden platform in day 1, 2 and 3. The escape latency time to locate the hidden platform for all groups decreased day by day in 3 days before 2VO. The ANOVA revealed no significant effect on escape latencies and swimming distance within the groups ($p>0.05$) in all 3 days. It suggested that all rats improved their spatial learning effectively across the 3 days training period.

On 68th post-operative day, all the rats were tested to find the hidden platform for assessment of their long term memory. The spatial learning memory curves obtained with MWM test on Day 68 demonstrated that 10 weeks of experimental cerebral hypoperfusion induced significant impairment of reference memory in untreated 2VO rats on water maze. This indicates that chronic cerebral hypoperfusion affected the performance of the rats in water maze.

ANOVA performed on the escape latency time on Day 68 in acquisition trials showed a significant group difference ($p<0.005$). The Tukey's post hoc test showed that 2VO group had longer escape latency

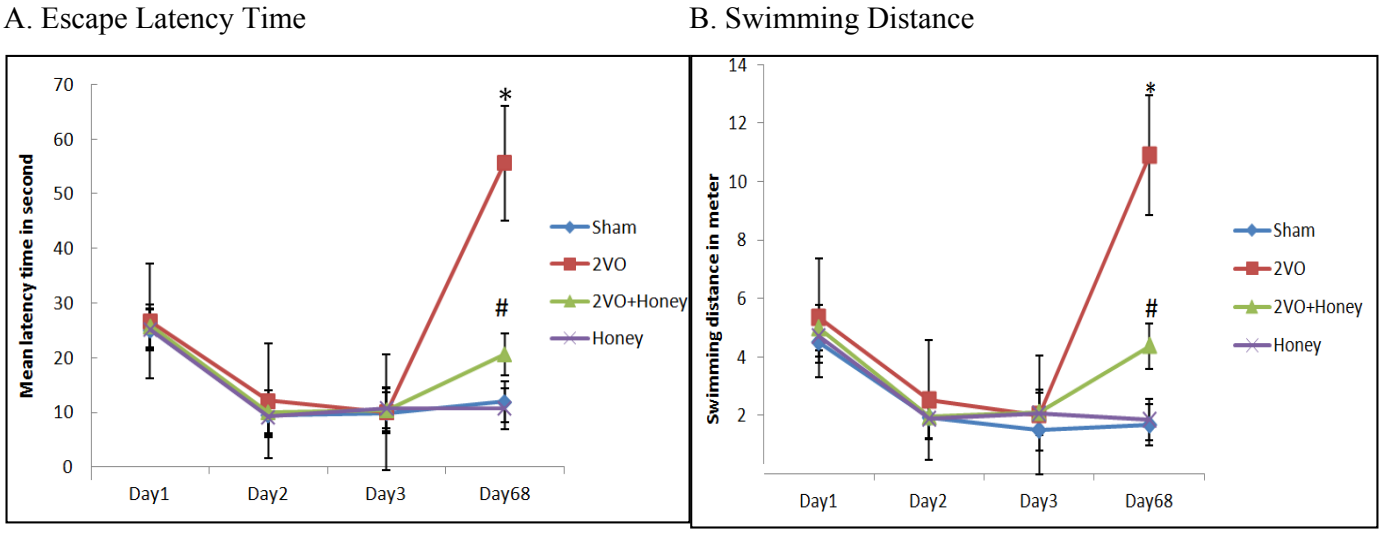


Figure 3. A. Escape latency time for LTM on 68th post-operative day with MWM test in honey treated rats compared to sham and 2VO groups; **B.** Swimming distance for LTM on 68th post-operative day with MWM test in honey treated rats compared to sham and 2VO groups. Significance with Tukey's test following one-way ANOVA is indicated as *$p<0.01$ (Sham *vs.* 2VO) and #$p<0.01$ (2VO+H *vs.* 2VO).

time as compared with Sham rats ($p<0.01$). It indicated that 2VO resulted significant cognitive impairment. Sham group performance was not significantly different with that of 2VO+H group ($p>0.05$) on Day 68. The 2VO+H group had significantly reduced escape latency time compared to untreated 2VO group ($p<0.05$), demonstrating that honey is effective in improving spatial learning in cerebral hypoperfused rats. On further analysis, there was no significant difference between Sham and 2VO+H ($p=0.836$). The swimming duration of honey treated group and Sham group were not significantly different too ($p=0.999$) on Day 68 (Figure 3A).

Swimming distance: ANOVA performed on the swimming distance on Day68 in acquisition phase demonstrated a significant difference between groups. Sham group had a relatively short distance to find the hidden platform as compared with 2VO group on Day 68 after 2VO indicating a good retention of spatial memory; $p<0.01$, post hoc test, (Fig 3B). The Turkey's post-hoc test showed that honey treated 2VO group revealed reduced significant swimming distance compared to that of untreated 2VO group ($p<0.01$). On further analysis, there was no significant difference between Sham and honey treated 2VO; $p=0.656$). The swimming distance to reach the hidden platform on Day68 between honey treated group and Sham group were also not significant ($p=0.999$) (Figure 3B).

Retention probe task

***Time spent in SW Zone*:** On Day 69, all groups of rats underwent retention probe trial. The performance of all rat groups was plotted by analyzing the time spent in the target zone (*i.e.,* SW zone) where the hidden platform had been previously located. Memory was evaluated by measuring the swimming time in the target quadrant without the platform.

One way ANOVA showed a significant effect between all groups ($p<0.01$). The post hoc Tukey's also showed that Sham rats spent more time in SW zone *i.e.,* target zone than that of untreated 2VO rats ($p<0.01$). The post-hoc test on retention performance also revealed that 2VO+H group spent significantly longer time in SW zone than untreated 2VO group ($p<0.05$). There was no significant difference effect ($p=0.153$) between Sham and 2VO+H group. Time spent in SW zone of Sham and H groups were also not different ($p=0.999$). (Figure 4A).

Number of EP crossing: In Probe retention trial for long term memory assessment, the number of EP crossing was also noted together with time spent in SW zone. Sham rats crossed EP 3 times (60%), 4 times (20%) and 5 times (20%) respectively. H group rats crossed EP 5 times (60%), 4 times (20%) and 1 time (20%). 2VO rats did not cross EP at all was 60% while 2VO+H rats crossed EP at least 2 times was 80% and 20% for 1 time. (Figure 4B)

Short term memory assessment

Reference memory performance: For short term memory assessment (STM) on MWM, animals were trained to find escape platform (EP) in SW zone for 4 days and their performance in terms of escape latency time and swimming distance to reach the hidden platform in each day were analyzed.

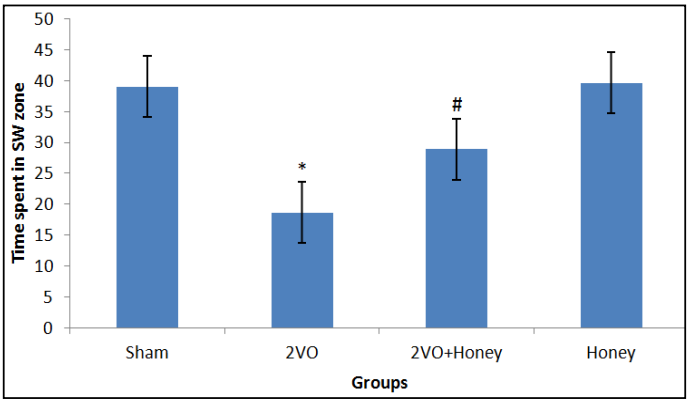
Escape latency time: All animals were given training 4 trials per day for 4 days for reference memory phase after finishing habituation phase. MWM training was started on Day 60 after 2VO. Spatial learning curves obtained in Morris water maze test demonstrated that experimental cerebral hypoperfusion in 2VO rats induced a marked decrease in learning performance. Moreover, the untreated 2VO group did not show day-to-day improvement over the 4 days training period in reference memory task.

The untreated 2VO rats showed a significant impairment of reference memory performance in STM task as compared with Sham rats. One way ANOVA revealed significant effect of group difference in escape latency time in all 4 days. Tukey's test showed that 2VO group showed significantly increased escape latency time, compared with those of Sham group ($p<0.05$ on Day 1, $p<0.01$ on Day 2 and Day 3 and $p<0.001$ on Day 4 respectively) Figure 5A.

On further analysis, 2VO+H group showed significantly reduced escape latency time ($p<0.05$ on Day 3 and $p<0.01$ on Day 4) as compared with untreated 2VO group. Although there were no significant effect between Sham *vs.* 2VO+H; Sham vs. Honey treated group ($p>0.05$ in all 4 days).

Swimming distance: ANOVA performed on the swimming distance of acquisition trials showed also significant effect of group difference. Tukey's test revealed that Sham group showed significantly reduced swimming distance than untreated 2VO group ($p<0.05$ on Day

A. Time Spent in SW Zone



B. Number of EP Crossing

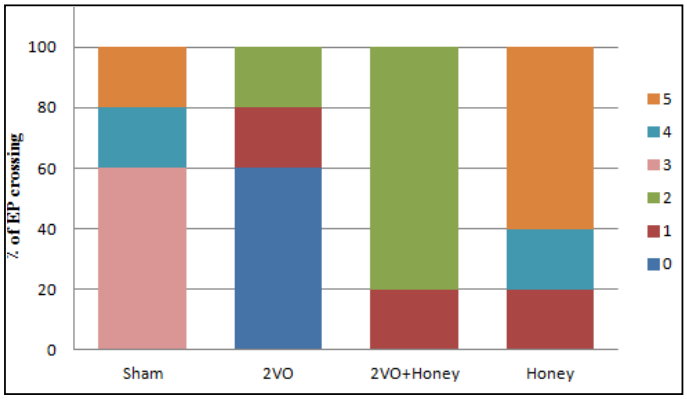


Figure 4. A. Time spent in the target zone in seconds on 69th post-operative day in retention probe test with MWM test in honey treated rats compared to sham and 2VO groups; **B.** % of EP crossing on 69th post-operative day in retention probe test with MWM test in honey treated rats compared to sham and 2VO groups. ANOVA showed significant differences between all groups. Significance with Tukey's test indicated as *$p<0.01$ (Sham *vs.* 2VO) and #$p<0.05$ (2VO *vs.* 2VO+H).

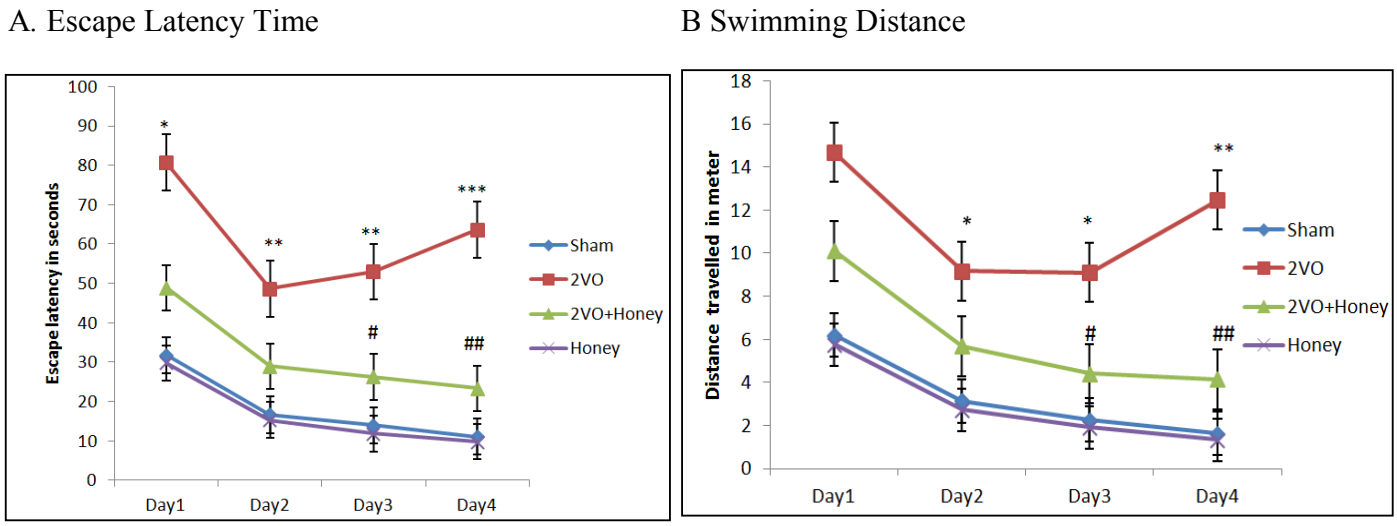


Figure 5. A. Escape latency time to reach EP for STM on MWM test in honey treated rats compared to sham and 2VO groups; **B.** Swimming distance to reach the EP for STM on MWM test in honey treated rats compared to sham and 2VO groups.
Significance with Tukey's test following ANOVA is indicated as *p<0.05, **p<0.01, ***p<0.001 (Sham *vs.* 2VO); #p<0.05, ##p<0.01 (2VO *vs.* 2VO+H).

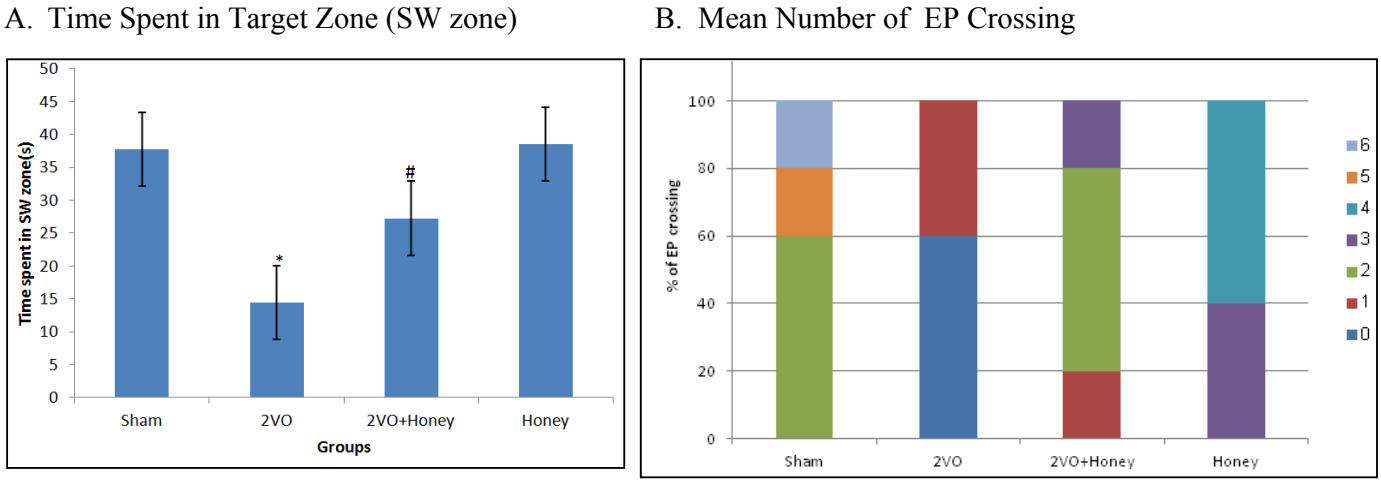


Figure 6. A. Time spent in the target zone on MWM test for STM in honey treated rats compared to sham and 2VO groups; **B.** % of EP crossing (*i.e.,* SW zone) in seconds in probe retention trial on MWM test for STM in honey treated rats compared to sham and 2VO groups. Significance with Tukey's test following ANOVA is indicated as *p<0.05 (Sham *vs.* 2VO), #p<0.05 (2VO *vs.* 2VO+H).

2 and Day 3, p<0.01 on Day 4). Furthermore, 2VO+H group performed much better than untreated 2VO group (p<0.05 on Day 3 and p<0.01 on Day 4 respectively). On further analysis, there was no significant difference between Sham *vs.* 2VO+H in all 4 days (p>0.05). There was no significantly better performance of Honey treated group than that of Sham group in all 4 days of experiment (p>0.05) (Figure 5B).

Retention probe trial

Time spent in target zone (SW zone): On Day 5, retention probe trial also showed that untreated 2VO rats spent less time in target zone than Sham rats (p<0.05) and 2VO+H (p<0.05). There was no significant difference between Sham and Honey treated group (p=0.999): Sham *vs.* 2VO+H (p=0.386) (Figure 6A).

Mean number of EP crossing: For short term memory assessment, the frequency of EP crossing was also measured during Probe retention task, all rats from Sham, H and 2VO+H groups crossed EP. Sham rats crossed EP 6 times (20%), 5 times (20%) and 2 times (60%). H group rats crossed EP 4 times (60%) and 3 times (40%) respectively. Untreated 2VO rats did not cross EP at all was 60%. 2VO+H rats crossed EP 2 times (60%), 3 times (20%) and 1 time (20%) (Figure 6B).

These results suggested that honey treatment can significantly affect the performance of chronic cerebral hypoperfused rats. It highlighted that all the rats in group A, B, C and D reached the hidden platform in reference memory test was not by chance. They learnt from extra maze cues to search for the hidden platform. As expected the performance of 2VO rats was worst on MWM as they had memory and learning impairment as a result of cerebral hypoperfusion induced by 2VO procedure.

Working memory result (Re-learning)

In this phase, all the animals were given 4 trials per day for 3 days (Day 67, 68 and 69) with the alteration of escape platform in NW, NE and SE respectively. One way ANOVA showed that there were significant differences in escape latency time between groups in all 3 days.

The Tukey's test revealed that untreated 2VO group demonstrated significantly increased swimming time as compared to Sham group ($p<0.01$ on day 1 and $p<0.05$ on days 2 and 3 respectively). The Tukey's test showed that 2VO+H group ($p<0.05$ on days 67 and 69) demonstrated significantly reduced swimming latency time, compared to the untreated 2VO. In addition, there were no significant differences between Sham and 2VO+H ($p>0.05$) along all 3 days. The Tukey's test also revealed no significant differences ($p>0.05$ on all 3 days) between Sham and honey treated groups (Figure 7A).

Cued version result

This test was conducted in all four groups of rats: n=10 (Sham, 2VO, 2VO+H and H) one day after retention probe task. There were no significant differences among all four groups.

All rat groups underwent cued version phase after finishing reference memory phase, probe trials and working memory trial and relearning phase (only for learning group) to exclude the effect of surgery and motor dysfunction due to chronic cerebral hypoperfusion. All rats reached visible escape platform located in the center of pool with no significant escape latency time ($p=0.98$) (Figure 7B).

This means that the animals that underwent 2VO for MWM *i.e.,* group B and C did not have any evidence of motor deficit and visual defect.

Discussion

Honey has been known as food as well as natural remedy for various illnesses. Researchers are looking for more evidence of honey's medicinal benefits. Honey possesses many beneficial properties such as neuroprotective, anti-oxidative, anti-inflammatory, antibacterial, wound healing, anticancer etc. [5,6,13,14]. Based on the current literature search, there has been very little research on the effect of honey on learning and memory on chronic cerebral hypoperfusion induced neurodegeneration in animal models.

The objective of this study was to evaluate the cognition enhancing potential of Malaysian Tualang honey in honey treated rats compared with Sham rats in the 2VO model of chronic cerebral hypoperfusion. In the current study, there were no differences among honey treated group (given honey for 10 weeks) and Sham control on performing MWM tasks. Although, one study has found that long term treatment of honey on rats (given honey for 52 weeks) improved spatial memory on Y maze task [15]. Therefore, the present study may need longer duration of honey treatment to establish the fact that honey could enhance memory.

Previous studies related to neurodegeneration demonstrated mostly short term memory assessment [16-18]. This study demonstrated short term memory, long term memory and also re-learning assessment by analyzing escape latency time, distance travelled to reach escape platform and time spent in target zone on MWM tasks.

Chronic cerebral hypoperfusion induced by permanent bilateral occlusion of the common carotid arteries (2VO) in rats has been reported to be an effective model of neurodegenerative disorders especially Alzheimer's disease (AD) [1,19]. 2VO induced chronic cerebral hypoperfusion enhances the formation of Reactive Oxygen Species in the brain [20]. Oxidative stress due to chronic cerebral hypoperfusion is one of the hypotheses in the pathogenesis of AD. It has been stated that oxidative stress has been linked to the neuronal cell death that is associated with neurodegenerative disorders like AD.

There are studies which proved oxidative damage is related to the impairment in memory and learning [15]. The 2VO rat model produced impaired spatial memory and learning along with apoptotic neuronal damage in the hippocampus [1,12,21-22]. Therefore we have assessed the cognitive capacity of Sprague-Dawley rats at 10th week that underwent 2VO in the present study.

This study proved that chronic cerebral hypoperfusion caused neuronal cell death [23] and cognitive impairment. The hippocampus of brain is very sensitive to ischemia and plays an important role in memory and learning. Similar to previous studies [16,18,24-25]. 2VO rats demonstrated spatial memory and learning impairment on MWM tasks in this study. Animals from 2VO group had the longest escape latency time, distance travelled and the least time spent in target zone in spatial memory and hippocampus dependent tasks of MWM. It may be possible that 2VO rats developed cognitive impairment due to oxidative stress caused by chronic cerebral hypoperfusion. Oxidative stress is widely recognized nowadays as an important feature in the pathogenesis of neuronal damage and reduced antioxidant abilities [16,18,20,26]. Azman, *et al.* [26] reported thatTualang honey protects

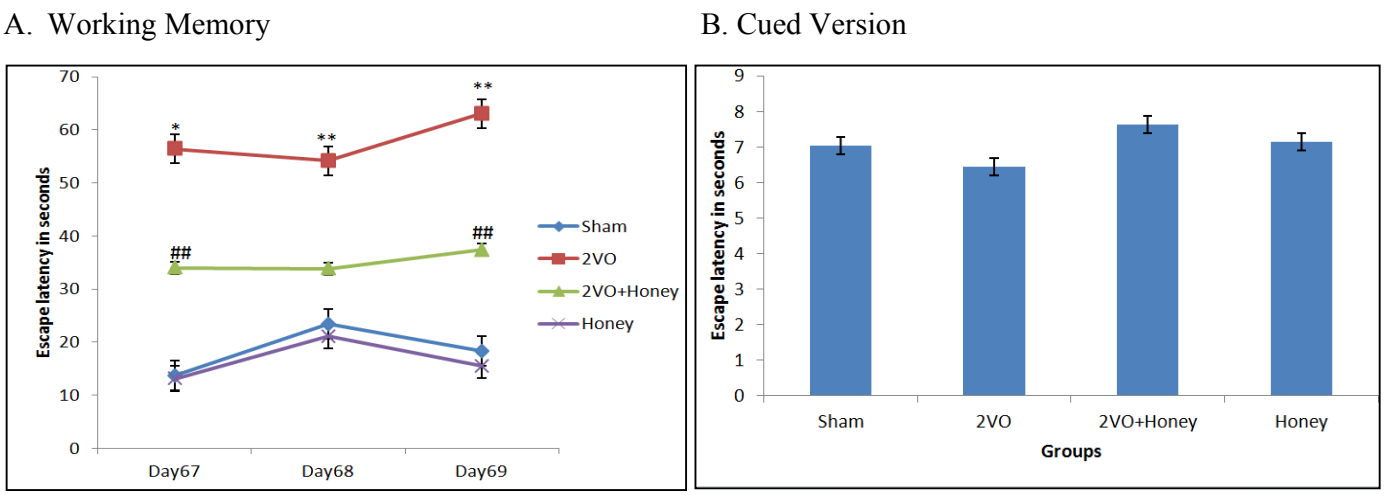


Figure 7. A. Comparison of working memory performance for STM (Re-learning phase) on MWM test in honey treated rats compared to sham and 2VO groups; **B.** Performance of cued version phase on MWM test in honey treated rats compared to sham and 2VO groups. Significance with Tukey's test following one way ANOVA is indicates as *$p<0.01$, **$p<0.05$ (SHAM *vs.* 2VO) #$p<0.05$ (2VO *vs.* 2VO+H).

against memory decline due to noise stress-induced exposure and/or ageing.

Our results showed that supplementation of Malaysian Tualang honey for 10 weeks effectively improved the MWM performance in honey treated 2VO rats compared to untreated 2VO rat. Corresponding with the result of MWM test, chronic cerebral hypoperfusion induced by 2VO demonstrated significant neuronal damage in the hippocampal CA1 region in untreated 2VO rats as compared to Sham controls. However, honey treated 2VO rats had less neuronal cell loss *i.e.,* significant increase in the viable neurons as compared with untreated 2VO rats [23]. Significant correlation was observed between impaired spatial memory and learning and loss of neurons in CA1 region of hippocampus. Therefore, this study showed that supplementation with Tualang honey significantly improved the cognitive behavior and neuronal loss due to chronic cerebral hypoperfusion.

Thus, daily administration of Malaysian Tualang honey attenuated the cognitive impairment caused by chronic cerebral hypoperfusion. This could be due to potent antioxidant free radical scavenging and anti-inflammatory properties of honey. These properties might play an important role in improving cognitive behavior. Antioxidant and anti-inflammatory properties of honey [5] could be the underlying cause for the reduction in damaging effect of oxidative stress induced neurodegeneration and cognitive impairment. These findings need for further research on the mechanism of action of honey on oxidative stress pathways and also to determine the levels of oxidative stress markers in the rat brain to support this hypothesis.

Chepulis *et al.* [15] have reported that consumption of antioxidants can attenuate oxidative damage and improve cognitive performance in animals. In addition, honey can also enhance the body's antioxidants like blood vitamin C concentration by 47%, β-carotene by 3%, uric acid by 12%, and glutathione reductase by 7% [5].

The beneficial effects of honey have also been attributed to the possible polyphenolic contents and some other constituents. It has also been stated that honey may have antioxidant and neuroprotective effects due to these constituents [5,27]. Besides, anti-inflammatory effects of honey may be due to its controlling the formation of free radicals released from the inflamed tissues. Moreover, reduction of inflammation by honey could be due to its direct anti-inflammatory effect which has been supported in animal studies [5].

The pharmacotherapy of neurodegenerative disorder is limited mostly to symptomatic treatments that do not alter the course of the underlying disease. The available treatment for AD is much more limited in effectiveness and there is no definitive cure for Alzheimer's disease at the present time [28].

There are numerous alternative medicines that claim to be beneficial for Alzheimer's disease. Some prominent herbal remedies reported to be effective in Alzheimer's disease are Ginkgo Biloba, Hyuperzine A, Coenzyme Q10 and Phosphatidyl Serine. These products have shown to have positive effects in Alzheimer's treatments [28].

A study has suggested that it will be valuable to investigate the effects of honey in humans in the management of chronic diseases associated with oxidative stress [27].

Further studies are needed to determine the levels of oxidative markers and inflammatory markers to support the hypothesis of honey as antioxidant and anti-inflammatory agent in attenuating cognitive impairment in AD animal model for the improvement in treatment of AD. Further research may also need to explore the exact mechanism how the constituents of honey attenuated ROS produced by oxidative stress in chronic cerebral hypoperfusion.

Acknowledgement

Authors would like to thank IIUM Research Management Centre for sponsoring and supporting this research study (grant # EDW B 11-137-0615).

References

1. Farkas E, Luiten PGM, Bari F (2007) Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* 54: 162-180. [Crossref]

2. Minghetti L (2005) Role of inflammation in neurodegenerative diseases. *Curr Opin Neurol* 18: 315-321. [Crossref]

3. Bombi L, Eu-Jung C, Eun-Jung L, Seung-Moo H, Dae-Hyun H, et al. (2011) The neuroprotective effect of methanol extract of gagamjungjihwan and fructus euodiae on ischemia-induced neuronal and cognitive impairment in the rat. *Evid Based Complement Alternat Med* 2011: 685254. [Crossref]

4. Ohta H, Nishikawa H, Kimura H, Anayama H, Miyamoto M (1997) Chronic cerebral hypoperfusion by permanent internal carotid ligation produces learning impairment without brain damage in rats. *Neuroscience* 79: 1039-1050. [Crossref]

5. Bogdanov S, Jurendic T, Sieber R, Gallmann P (2008) Honey for nutrition and health: a review. *J Am Coll Nutr* 27: 677-689. [Crossref]

6. Nik Man NM, Hassan R, Ang CY, Abdullah AD, Mohd Radzi MA, et al. (2015) Antileukemic Effect of Tualang Honey on Acute and Chronic Leukemia Cell Lines. *Biomed Res Int* 2015: 307094. [Crossref]

7. Khalil MI, Tanvir EM, Afroz R, Sulaiman SA, Gan SH (2015) Cardioprotective Effects of Tualang Honey: Amelioration of Cholesterol and Cardiac Enzymes Levels. *Biomed Res Int* 2015: 286051. [Crossref]

8. Ghashm AA, Othman NH, Khattak MN, Ismail NM, Saini R (2010) Antiproliferative effect of Tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines. *BMC Complement Altern Med* 10: 49. [Crossref]

9. Cechetti F, Worm PV, Pereira LO, Siqueira IR, A Netto C (2010) The modified 2VO ischemia protocol causes cognitive impairment similar to that induced by the standard method, but with a better survival rate. *Braz J Med Biol Res* 43: 1178-1183. [Crossref]

10. Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11: 47-60. [Crossref]

11. Mahaneem M, Sulaiman S, Jaafar H, Sirajudeen K, Ismail Z, et al. (2011) Effect of honey on testicular functions in rats exposed to cigarette smoke. *J ApiProd ApiMed Sci* 3: 12-17. [Crossref]

12. Farkas E, Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* 64: 575-611. [Crossref]

13. Mohamed M, Sirajudeen K, Swamy M, Yaacob NS, Sulaiman SA (2009) Studies on the antioxidant properties of Tualang honey of Malaysia. *Afr J Tradit Complement Altern Med* 7: 59-63. [Crossref]

14. Aznan MI, Khan OH, Unar AO, Tuan Sharif SE, Khan AH, et al. (2016) Effect of Tualang honey on the anastomotic wound healing in large bowel anastomosis in rats-A randomized controlled trial. *BMC Complement Altern Med* 16: 28. [Crossref]

15. Chepulis LM, Starkey NJ, Waas JR, Molan PC (2009) The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats. *Physiol Behav* 97: 359-368. [Crossref]

16. Ji HJ, Hu JF, Wang YH, Chen XY, Zhou R, et al. (2010) Osthole improves chronic cerebral hypoperfusion induced cognitive deficits and neuronal damage in hippocampus. *Eur J Pharmacol* 636: 96-101. [Crossref]

17. Onem G, Aral E, Enli Y, Oguz EO, Coskun E, et al. (2006) Neuroprotective effects of L-carnitine and vitamin E alone or in combination against ischemia-reperfusion injury in rats. *J Surg Res* 131: 124-130. [Crossref]

18. Xu Y, Zhang J, Xiong L, Zhang L, Sun D, et al. (2010) Green tea polyphenols inhibit cognitive impairment induced by chronic cerebral hypoperfusion via modulating oxidative stress. *J Nutr Biochem* 21: 741-748. [Crossref]

19. Zakharova EI, Storozheva ZI, Dudchenko AM, Kubatiev AA (2010) Chronic cerebral ischaemia forms new cholinergic mechanisms of learning and memory. *Int J Alzheimers Dis* 2010: 954589. [Crossref]

20. Saxena AK, Abdulmajeed SS, Oothuman P, Mustafa M (2011) Lipid peroxidation in chronic cerebral hypoperfusion-induced neurodegeneration in rats. *The international Medical Journal Malaysia* 10: 3-6.

21. De Jong GI, Farkas E, Stienstra CM, Plass JR, Keijser JN, et al. (1999) Cerebral hypoperfusion yields capillary damage in the hippocampal CA1 area that correlates with spatial memory impairment. *Neuroscience* 91: 203-210. [Crossref]

22. Farkas E, Institóris A, Domoki F, Mihály A, Bari F (2006) The effect of pre- and posttreatment with diazoxide on the early phase of chronic cerebral hypoperfusion in the rat. *Brain Res* 1087: 168-174. [Crossref]

23. Saxena AK, Hnin P Phyu, Imad M Ai-Ani, Norlelawati A Talib (2014) Potential protective effect of honey against chronic cerebral hypoperfusion-induced neurodegeneration in rats. *Journal of the Anatomical Society of India* 63: 151-155.

24. Farkas E, Institóris Á, Domoki F, Mihály A, Luiten PGM, et al. (2004) Diazoxide and dimethyl sulphoxide prevent cerebral hypoperfusion-related learning dysfunction and brain damage after carotid artery occlusion. *Brain Res* 1008: 252-260. [Crossref]

25. He XL, Wang YH, Gao M, Li XX, Zhang TT, et al. (2009) Baicalein protects rat brain mitochondria against chronic cerebral hypoperfusion-induced oxidative damage. *Brain Res* 1249: 212-221. [Crossref]

26. Azman KF, Zakaria R, Abdul Aziz CB, Othman Z (2016) Tualang Honey Attenuates Noise Stress-Induced Memory Deficits in Aged Rats. *Oxid Med Cell Longev* 2016: 1549158. [Crossref]

27. Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012) Honey: a novel antioxidant. *Molecules* 17: 4400-4423. [Crossref]

28. Kelley BJ, Knopman DS (2008) Alternative medicine and Alzheimer disease. *Neurologist* 14: 299-306. [Crossref]