Research Article

Improved blood oxidative status is not associated with better memory performance in postmenopausal women receiving Tualang honey supplementation

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Abstract: We examined association between the changes in blood oxidative stress level/activity and the changes in memory performance in postmenopausal women receiving Tualang honey (TH). The verbal learning and memory performances of thirty nine postmenopausal women were assessed using the Malay version of the Auditory Verbal Learning Test (MVAVLT) and their oxidative stress levels/activities were determined using commercially available kits before and 16 weeks after TH supplementation. The plasma glutathione peroxidase (GPx) and catalase (CAT) activities were considerably increased but the 4-hydroxynonenal (4-HNE) level was notably decreased after 16 weeks of TH supplementation. There were positive correlations between the changes in plasma GPx and the changes in trial A6 scores ($r = 0.48$, $P < 0.05$) and between the changes in plasma CAT and the changes in recognition score ($r = 0.32$, $P < 0.05$). TH supplementation for 16 weeks reduced blood oxidative stress but most of the changes in blood oxidative stress level/activity were not significantly correlated with the changes in memory performance.

Keywords: Tualang honey; postmenopausal women; oxidative stress; memory performance

Introduction

Central nervous system (CNS) is vulnerable to oxidative stress [1, 2] and inflammation [3] especially during aging. Both inflammatory and oxidative stress processes potentially play a role in the development of various neurodegenerative disorders such as Alzheimer or Parkinson disease in human as well as in animal models. A previous study showed that late menopause women perform significantly worse at cognitive function tests (planning and mental flexibility) than their counterparts in early menopause [4]. More recent evidence indicates that cognition declines progressively over the menopause, with significant reduction in processing speed and working memory [5, 6]. The treatment of choice is hormone replacement therapy (HRT) but, despite its effectiveness for symptomatic relief, there has been a marked and global decline in the HRT use because of concerns over possible side effects [7].

Herbal and dietary therapies are among the most common alternatives to HRT. Dietary intake can influence the onset and incidence of neurodegenerative diseases by protecting neuronal vulnerability, enhancing existing neuronal function and/or stimulating neuronal regeneration. Studies in humans and animals have highlighted the potential of dietary antioxidant supplementation to influence cognition and learning [8-14]. Youdim and colleagues [15] reported that flavonoids supplementation was able to give

Table 1. Characteristics of the participants

Age (years)	55.44 (0.52)
Menarche age (years)	13.28 (0.25)
Menopausal age (years)	50.08 (0.46)
Duration of menopause (years)	5.33 (0.53)
Educational status, n (%)	
Primary	6 (15.4)
Secondary	18 (46.1)
Tertiary	15 (38.5)

Values are expressed as mean (SEM) for age, menarche, menopausal age and duration of menopause and n (%) for educational status.

direct and indirect effect on learning/memory-induced signaling cascades. The antioxidant properties of flavonoids were able to scavenge reactive oxygen species or exert influence on the intracellular redox status [16].

In addition, studies on the effects of soy isoflavones supplementation especially in postmenopausal women showed positive association [17-19] and favourable effect on cognitive function [20] particularly in verbal memory of postmenopausal women [21]. Six and twelve-week of isoflavones supplementation demonstrated a positive effect on the frontal lobe function [22]. Furthermore, animal studies have also indicated that isoflavones are capable of improving cognitive function [23-26]. However there are still conflicting data on the beneficial effects of soy isoflavones as some trials reported that it does not lead to cognitive improvements. A six-month trial indicates that 80 mg soy-derived isoflavones supplementation did not improve cognitive performance in generally healthy Chinese postmenopausal women [19]. Another double-blind randomized trial using soy protein containing isoflavones also reported negative finding [27]. These contradictory findings could be explained by the differences in evaluation of functional status, utilization of various cognitive tools and age of participants.

Apart from isoflavones, studies using vitamins E and C supplementation on memory function in menopausal women [28, 29] and menopausal animal model [30] also revealed mixed results. Women who received vitamin E and C supplementation had significantly better memory performance than women who had never received vitamin E or C [28]. In that study there was a positive correlation trend between mean memory performance scores and duration of supplementation. Animal study also revealed that cognitive impairment in ovariectomized rats could be prevented by pretreatment with vitamins E and C for 30 days [30]. Kang et al. [29] however reported that long-term vitamin E supplementation did not provide cognitive benefits in generally healthy older women. Similarly Engelhart et al [31] reported antioxidants were not related to cognitive decline in nondemented subjects and there was no association between plasma levels of vitamin A and E, and Alzheimer disease or cognitive decline.

Honey had a variety of phytochemicals which serves as sources of dietary antioxidants [32]. Antioxidant capacity of honey appeared to be due to its non enzymatic antioxidants such as ascorbate, α- tocopherol and β carotene which may influence the total antioxidant activity [32, 33] as well as its capacity to increase the plasma antioxidant enzymes [34]. Honey had been shown to reduce cognitive impairment in human as well as in animal model. Al-Himyari et al. [35] found that only 95 subjects who received honey compared to 394 who received placebo developed dementia in a 5-year study involving 2893 subjects aged 65 and older. They concluded that honey acts as natural preventive therapy for both cognitive decline and dementia. A previous animal study reported that honey-fed rats performed significantly better than those fed with sucrose or a sugar-free diet in the Y maze task [36]. Recently, our research team has reported that Tualang honey (TH) was able to improve immediate memory in postmenopausal women comparable with that of estrogen progestin therapy [37] and improve both short-term and long-term memory in stressed ovariectomized (OVX) rats compared with that of untreated stressed OVX rats [38].

Based on our recent findings, the present study aims to examine the association between the changes in oxidative stress level/activity and the changes in memory performance in postmenopausal women receiving TH supplementation.

Materials and methods

Procedures and design

The study protocol was approved by the Research and Ethics Committee of Universiti Sains Malaysia (USM). This study involved 39 postmenopausal women aged between 45 and 60 years. The participants were recruited from the Family Medicine and the Obstetrics and Gynecology Clinic, Hospital USM. Participants were literate and were not taking any form of medication that could alter their memory function, or any other herbal or hormone replacement 3 months prior to the study. Subjects were not chosen if they smoked or had a history of drug or alcohol abuse or a history of serious medical, surgical, mental or gynecological diseases. Participants underwent physical examinations including a pelvic ultrasonography to exclude endometrial hyperplasia.

Table 2. MVAVLT performance of the participants at pre- and post-intervention.

Trial	Pre-intervention	Post-intervention
A1	5.7(0.3)	7.9(0.3)[a]
A2	8.2(0.4)	9.3(0.3)[a]
A3	9.5(0.3)	10.2(0.3)[a]
A4	10.1(0.4)	11.3(0.3)[a]
A5	11.4(0.4)	11.7(0.3)
A1-A5	45.2(1.4)	51.5(1.1)[a]
B	5.8(0.3)	5.9(0.3)
A6	8.4(0.5)	10.4(0.4)[a]
A7	9.7(0.4)	10.7(0.4)[a]
Recognition	13.5(0.3)	14.6(0.1)[a]

MVAVLT scores are expressed as mean (SEM). Pre- and post-intervention scores were compared using paired t-test. [a]$P<0.05$ was considered statistically significant.

Postmenopausal women who had fulfilled the criteria were briefed concerning the nature of the study and their consent to participate was taken. During the initial visit, participants' verbal learning and memory performance was assessed and blood was obtained for oxidative stress levels/activities. The participants received 20 gm TH sachet for 16 weeks. Their health status and compliance were monitored at 8 weeks. After 16 weeks, their verbal learning and memory performance and oxidative stress status were reassessed.

Determination of memory performance

The participants' verbal learning and memory performance were assessed using the Malay version of the Auditory Verbal Learning Test (MVAVLT) as previously described [37, 39]. The test consists of two different lists (A and B) of 15 unrelated words. The list A was read over five different trials and the participants were asked to repeat it (A1 to A5). Another list of 15 unrelated words (List B) was read and the participants were asked to repeat it (B). Immediately after that the participants were asked to recall words in list A (A6) without prior presentation of list A. After 20 minutes of rest, the participants were asked to recall the words in list A. Finally, the participants were asked to recognize the words from list A interspersed among semantically or phonetically related words in the third list that comprised 30 words. The score from A1 to A5 trials represents immediate auditory-verbal memory. Total learning score (A1 + A2 + A3 + A4 + A5) represents the memory acquisition. Scores from B, A6 and A7 trials represent the interference, immediate memory after interference and delayed memory, respectively. The recognition score reflects the ability of the participants to

Table 3. Blood oxidative stress level/activity of the participants at pre- and post-intervention.

	Pre- intervention	Post-intervention
Blood GSH: GSSG ratio	25.2 (7.6)	39.7 (11.5)
Plasma SOD (U/ml)	0.068 (0.005)	0.060 (0.005)
Plasma CAT (nmol/min/ml)	4.1(0.5)	5.6 (0.5)[a]
Plasma GPx (nmol/min/ml)	75.3 (5.5)	90.1 (6.5)[a]
Plasma 4-HNE (μg/ml)	3.6 (0.23)	3.1(0.23)[a]

Values are expressed as mean (SEM). *GSH:GSSG*, ratio of reduced to oxidized glutathione; glutathione peroxidase (GPx); 4-hydroxynonenal (4-HNE); catalase (CAT) and superoxide dismutase (SOD). Statistical comparisons were made between pre- and post-intervention using paired *t* test. [a]$P< 0.05$ was considered statistically significant.

recognize words from list A.

Determination of blood oxidative stress level/activity

Blood samples for the determination of oxidative stress level/activity were collected into appropriate EDTA bottles. A total of 150 µl whole blood was used for the determination of reduced glutathione to oxidized glutathione (GSH:GSSG) ratio. The remaining blood was centrifuged at 1000g for 10 minutes at 4°C to obtain plasma, aliquoted and stored at -80°C until assayed.

The blood GSH:GSSG ratio, plasma catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined using a commercially available kit (Calbiochem, Darmstadt, Germany). Plasma 4-hydroxynonenal (4-HNE) was ascertained using the Oxiselect HNE-His adduct Elisa kit (Cell Biolab Biolabs, U.S.A.). Procedures for the verification of antioxidant enzymes and lipid oxidative damage were in accordance with the manufacturer's protocol.

Statistical analysis

The results were analyzed using PASW Statistics version 20 software. A paired *t* test was used to examine the differences in MVAVLT scores and blood oxidative stress values before and 16 weeks after TH supplementation. Pearson correlation coefficient was used to examine the association between the changes in blood oxidative stress level/activity and the changes in memory performance. A probability values < 0.05 was considered statistically significant.

Results

A total number of 39 postmenopausal women participated in this study and the participants' characteristics are summarized in Table 1. To ensure that participant's blood glucose levels did not confound the memory performance, we have measured the fasting blood glucose (FBS) at pre- and post-interventions. The results showed no

Table 4. Correlation between the changes in blood oxidative stress levels/activity and the changes in memory performance.

	Δ GSH:GSSG	Δ SOD	Δ CAT	Δ GPx	Δ 4-HNE
Δ A1	-0.10	0.65	0.72	0.75	0.19
Δ A2	0.22	0.13	0.65	0.87	0.06
Δ A3	0.26	0.11	0.90	0.50	0.94
Δ A4	-0.02	0.12	0.42	0.33	0.39
Δ A5	0.10	0.80	0.82	0.86	0.37
Δ A1-A5	0.12	0.11	0.71	0.06	0.89
Δ B1	0.14	0.40	0.30	-0.04	0.53
Δ A6	0.13	0.94	0.17	0.41[a]	0.73
Δ A7	0.07	0.20	0.08	0.22	0.25
Δ Recognition	-0.04	0.80	0.32[a]	0.19	0.71

Values are expressed as correlation coefficients (r).
Δ *GSH:GSSG*, changes in the ratio of reduced to oxidized glutathione; Δ GPx, changes in the activity of glutathione peroxidase; Δ 4-HNE, changes in the level of 4-hydroxynonenal; Δ CAT, changes in the activity of catalase; Δ SOD, changes in the level of superoxide dismutase. [a]$P < 0.05$ was considered statistically significant.

significant difference in the FBS at pre-and post-intervention. (5.40 ± 0.23 mmol/L and 5.63 ± 0.20 mmol/L, respectively).

The mean MVAVLT scores before and 16 weeks after TH supplementation were compared for each trial using a paired *t* test as shown in Table 2. The mean scores for trials A1, A2, A3 and A4 (immediate memory) were significantly increased when comparing before and 16 weeks after TH supplementation. The mean scores for total learning were significantly increased after TH supplementation when compared to its baseline value. The mean scores for trials A6 (immediate memory after interference with list B), A7 (delayed memory) and recognition memory were also significantly increased when contrasting before and after TH.

The oxidative stress levels/activities measured at before and after TH in postmenopausal women are summarized in Table 3. The plasma CAT and GPx activities were significantly increased after TH supplementation. The blood GSH:GSSG ratio, an indicator of oxidative stress, was increased but was not statistically significant. There appeared almost no change in the plasma SOD activity after TH supplementation. The plasma 4-HNE levels, however, showed considerable reduction after 16 weeks after TH supplementation.

The Pearson correlation coefficient revealed a significantly positive association between the changes in trial A6 scores (immediate memory after interference) and the changes in plasma GPx and between the changes in recognition and the changes in plasma CAT. No associations were found between the changes in other oxidative stress levels/activities and the changes in scores of other trials (Table 4).

Discussion

The present study found improvement in learning, immediate, delayed and recognition memory after 16 weeks of TH supplementation. The learning and memory improvement may be explained by increased neuronal proliferation in the specific hippocampal regions that is associated with learning and memory in ovariectomised rats treated with TH [38]. The neuronal proliferation could be attributed to the antioxidant properties of TH.

In a study on nine different Malaysian honey from different origins using Manuka honey as a gold standard, TH has been shown to have more free radical scavenging and antioxidant activity than other honeys [40, 41]. TH has total phenolic content of 251.7 ± 7.9 mg gallic acid/kg honey, total antioxidant activity of 322.1 ± 9.7 μM Fe(II)) and antiradical activity of 41.30 ± 0.78 (% inhibition) [42]. The main groups of phenolic compounds present in honey are in the forms of flavonoids such as catechin, kaempferol, naringenin, luteolin and apigenin [41].

Flavonoids if taken at high concentrations are able to regulate estrogen receptor activity by selectively modulating the membrane and nuclear actions without interfering with estrogen receptor activities in other tissues or organs [44, 45]. Studies by Youdim et al. indicated that certain flavonoids were able to penetrate the blood brain barrier (BBB) *in vitro* and *in situ* models [46-48]. Flavonoids also have the ability to interact with neuronal signaling pathways [49, 50] and have potential to inhibit neuroinflammatory processes in the brain [51, 52].

Based on the above data, there is a possible association between oxidative status and memory performance. Thus the present study aimed to examine the association between the changes in blood oxidative stress level/activity and the changes in memory performance in postmenopausal women receiving TH.

The present study revealed no significant correlation between the changes in blood oxidative stress level/activity and the changes in memory performance except for the changes in the plasma GPx and the changes in trial A6 scores and between the changes in plasma CAT and the changes in recognition scores. Our findings are supported by a recent study which reported no tight association between the increased cholinergic neurons density in hippocampus that is associated with learning memory and the decreased MDA level in hippocampus [53].

In the present study, it is possible that the learning and memory enhancing effect of TH occurred partly via the increased plasma GPx and CAT activities which in turn gave rise to the decreased plasma HNE level. However, we could not exclude other beneficial effects of TH such as improvement in brain cholinergic system (unpublished), increased BDNF levels [54] and enhanced cerebral blood flow, and all these may contribute to the learning and memory improvement.

Our suggestions on the effects of oxidative stress on cognitive function are supported by findings from previous studies [53, 55, 56]. Palumbo et al. [55] reported that chronic psychosocial stress-induced reactive oxygen species (ROS) in the hippocampus caused morphological changes in the hippocampal CA3 region and a decline in cognitive function. Thus, chronic stress-induced ROS and the consequent oxidative damage may contribute to impairment in cognitive function. Another previous study by Kuhad and colleagues [56] reported that oxidative stress in the diabetic rat brain caused cognitive dysfunction and antioxidant treatment improved the cognitive dysfunction. Wattanathorn et al. [53] suggested that the cognitive enhancing effect of *M. indica* extract occurred partly via the increased SOD and GSH-Px activities which in turn gave rise to the decreased MDA level in hippocampus. The decreased oxidative stress in turn induced the increased cholinergic neurons density and resulted in the improved spatial memory. However, they could not exclude other factors which may play a role in learning memory including the enhanced cerebral blood flow and the alteration of other neurochemical systems [53].

In conclusion, 16 weeks of TH supplementation increased blood antioxidant capacity and reduced lipid

oxidative damage. Most of the changes in blood oxidative stress levels/activities, however, were not significantly correlated with the changes in memory performance of postmenopausal women.

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Conflict of interests

The authors declare no conflict of interest.

References

[1] Joseph JA, Denisova N, Fisher D, Shukitt-Hale B, Bickford P, Prior R, et al. Membrane and receptor modifications of oxidative stress vulnerability in aging: nutritional considerations. Ann NY Acad Sci 1998; 854: 268-276.

[2] Joseph JA, Denisova N, Fisher D, Bickford P, Prior R, Cao G. Age-related neurodegeneration and oxidative stress: putative nutritional intervention. Neurol Clin 1998; 16: 747-755.

[3] Chang HN, Wang SR, Chiang SC, Teng WJ, Chen ML, Tsai JJ, et al. The relationship of aging to endotoxin shock and to production of TNF-alpha. J Gerontol A 1996; 51: M220-M222.

[4] Elsabagh S, Hartley DE, File SE. Cognitive function in late versus early postmenopausal stage. Maturitas 2007; 56: 84-93.

[5] Greendale GA, Wight RG, Huang M-H, Avis N, Gold EB, Joffe H, et al. Menopause-associated symptoms and cognitive performance: results from the study of women's health across the nation. Am J Epidemiol 2010; 171(11): 1214-24.

[6] Schaafsma M, Homewood J, Taylor A. Subjective cognitive complaints at menopause associated with declines in performance of verbal memory and attentional processes. Climacteric 2010; 13(1): 84-98.

[7] Magyar Z, Berkes E, Csapo Z, Papp Z. Effect of hormone replacement therapy on postmenopausal endometrial bleeding. Pathol Oncol Res 2007; 13: 351-359.

[8] Galli RL, Shukitt-Hale B, Youdim KA, Joseph JA. Fruit polyphenolics and brain aging: nutritional interventions targeting age-related neuronal and behavioral deficits. Ann N Y Acad Sci 2002; 959: 128-132.

[9] Haque AM, Hashimoto M, Katakura M, Tanabe Y, Hara Y, Shido O. Long-term administration of green tea catechins improves spatial cognition learning ability in rats. J Nutr 2006; 136: 1043-1047.

[10] Kuriyama S, Hozawa A, Ohmori K, Shimazu T, Matsui T, Ebihara S, et al. Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. Am J Clin Nutr 2006; 83: 355-361.

[11] Unno K, Takabayashi F, Kishido T, Oku N. Suppressive effect of green tea catechins on morphologic and functional regression of the brain in aged mice with accelerated senescence (SAMP10). Exp Gerontol 2004; 39: 1027-1034.

[12] Wang Y, Wang L, Wu J, Cai J. The in vivo synaptic plasticity mechanism of EGb 761-induced enhancement of spatial learning and memory in aged rats. Br J Pharmacol 2006; 148: 147-153.

[13] Youdim KA, Joseph JA. A possible emerging role ofphytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. Free Radic Biol Med 2001; 30: 583-594.

[14] Youdim KA, Spencer JP, Schroeter H, Rice-Evans C. Dietary flavonoids as potential neuroprotectants. Biol Chem 2002; 383: 503-519.

[15] Youdim K A, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an in situ model of the blood–brain barrier. Free Radical Biology and Medicine 2004; 36(5): 592-604.

[16] Rice-Evans C, Miller N. Antioxidant activities of flavonoids as bioactive components of food. Biochemical Society Transactions 1996; 24(3): 790-794.

[17] File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H. Eating soya improves human memory. Psychopharmacology (Berl) 2001; 157: 430-436.

[18] Kim H, Xia H, Li L, Gewin J. Attenuation of neurodegeneration-relevant modifications of brain proteins by dietary soy. Biofactors 2000; 12: 243-250.

[19] Ho SC, Chan AS, Ho YP, So EK, Sham A, Zee B, Woo JL. Effects of soy isoflavone supplementation on cognitive function in Chinese postmenopausal women: a double-blind, randomized, controlled trial. Menopause 2007; 14: 489-499.

[20] Casini ML, Marelli G, Papaleo E, Ferrari A, D'Ambrosio F, Unfer V. Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: a randomized, double-blind, crossover, placebocontrolled study. Fertil Steril 2006; 85: 972-978.

[21] Kritz-Silverstein D, Von Mühlen D, Barrett-Connor E, Bressel MA. Isoflavones and cognitive function in older women: the SOy and Postmenopausal Health In Aging (SOPHIA) Study. Menopause 2003; 10: 196-202.

[22] File SE, Hartley DE, Elsabagh S, Duffy R, Wiseman H. Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. Menopause 2005; 12: 193-201.

[23] Chan YC, Hosoda K, Tsai CJ, Yamamoto S, Wang MF. Favorable effects of tea on reducing the cognitive deficits and brain morphological changes in senescenceaccelerated mice. J Nutr Sci Vitaminol (Tokyo) 2006; 52: 266-273.

[24] Shukitt-Hale B, Carey A, Simon L, Mark DA, Joseph JA. Effects of Concord grape juice on cognitive and motor deficits in aging. Nutrition 2006; 22: 295-302.

[25] Singh A, Naidu PS, Kulkarni SK. Reversal of aging and chronic ethanol-induced cognitive dysfunction by quercetin a bioflavonoid. Free Radic Res 2003; 37: 1245-1252.

[26] Patil CS, Singh VP, Satyanarayan PS, Jain NK, Singh A, Kulkarni SK. Protective effect of flavonoids against aging- and lipopolysaccharide-induced cognitive impairment in mice. Pharmacology 2003; 69: 59-67.

[27] Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, van der Schouw YT. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women. JAMA 2004; 292(1): 65-74.

[28] Grodstein F, Chen J, Willett WC. High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. Am J Clin Nutr 2003; 77(4): 975-984.
[29] Kang JH, Cook N, Manson J, Buring JE, Grodstein F. A randomized trial of vitamin E supplementation and cognitive function in women. Arch Intern Med 2006; 166(22): 2462.
[30] Monteiro SC, Matté C, Bavaresco CS, Netto CA, Wyse AT. Vitamins E and C pretreatment prevents ovariectomy-induced memory deficits in water maze. Neurobiol Learn Mem 2005; 84(3): 192-199.
[31] Engelhart MJ, Ruitenberg A, Meijer J, Kiliaan A, van Swieten JC, Hofman A, et al. Plasma levels of antioxidants are not associated with Alzheimer's disease or cognitive decline. Dement Geriatr Cogn Disord 2005; 19(2-3): 134-139.
[32] Al-Mamary M, AL-Meeri A, Al-Habori M. Antioxidant activities and total phenolics of different types of honey. Nutr Res 2002; 22: 1041-1047.
[33] Aljadi A, Kamaruddin M. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chemistry 2004; 85; 513-518
[34] Shafin N, Zakaria R, Hussain NHN, Othman Z. Tualang Honey Supplementation Reduces Blood Oxidative Stress Levels/Activities in Postmenopausal Women. ISRN Oxidative Medicine Volume 2014, Article ID 364836, 4 pages http://dx.doi.org/10.1155/2014/364836.
[35] Al-Himyari FA. The use of honey as a natural preventive therapy of cognitive decline and dementia in the Middle East. Alzheimer's & Dementia 2009; 5; 247-247.
[36] Chepulis LM, Starkey NJ, Waas JR, Molan PC. The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats. Physiol Behav 2009; 97: 359-368.
[37] Othman Z, Shafin N, Zakaria R, Hussain NHN, Mohammad WMZW. Improvement in immediate memory after 16 weeks of tualang honey (Agro Mas) supplement in healthy postmenopausal women. Menopause 2011; 18(11); 1219-1224.
[38] Al-Rahbi B, Zakaria R, Othman Z, Hassan A, Mohd Ismail ZI, Muthuraju S. Tualang honey supplement improves memory performance and hippocampal morphology in stressed ovariectomized rats. Acta Histochem 2014; 116: 79-88.
[39] Ruzita J, Zahiruddin O, Kamarul I, Muhammad Najib M. Validation of the Malay version of Auditory Verbal Learning Test (MVAVLT) among schizophrenia patients in Hospital Universiti Sains Malaysia (HUSM), Malaysia. ASEAN J Psychiatry 2009; 10(1): 54-74.
[40] Kishore RK, Halim AS, Syazana MS, Sirajudeen KN. Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. Nutr Res 2011; 31(4): 322-325.
[41] Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH. Phenolic Acid Composition and Antioxidant Properties of Malaysian Honeys. J Food Sci 2011; 76(6): C921-C928.
[42] Mohamed M, Sirajudeen K, Swamy M, Yaacob N S, Sulaiman SA. Studies on the antioxidant properties of Tualang honey of Malaysia. African Journal of Traditional, Complementary, and Alternative Medicines 2010; 7(1): 59-63.
[43] Khalil MI, Mahaneem M, Jamalullail SMS, Alam N, Sulaiman SA. Evaluation of Radical Scavenging Activity and Colour Intensity of Nine Malaysian Honeys of Different Origin. J ApiPro ApiMedic Sci 2011; 3(1): 4-11.
[44] Belcher SM. Zsarnovszky A. Estrogenic actions in the brain: estrogen, phytoestrogens, and rapid intracellular signaling mechanisms. J Pharmacol Exp Ther 2001; 299; 408-414.
[45] Galluzzo P, Marino M. Nutritional flavonoids impact on nuclear and extranuclear estrogen receptor activities. Genes Nutr 2006; 1: 161-176.
[46] Youdim KA, Dobbie MS, Kuhnle G, Proteggente AR, Abbott NJ, Rice-Evans C. Interaction between flavonoids and the blood-brain barrier: in vitro studies. J Neurochem 2003; 85: 180-192.
[47] Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an in situ model of the blood-brain barrier. Free Radic Biol Med 2004; 36: 592-604.
[48] Youdim KA, Shukitt-Hale B, Joseph JA. Flavonoids and the brain: interactions at the blood-brain barrier and their physiological effects on the central nervous system. Free Radic Biol Med 2004; 37: 1683-1693.
[49] Schroeter H, Spencer JP, Rice-Evans C, Williams RJ. Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. Biochem J 2001; 358: 547-557.
[50] Smith JV, Burdick AJ, Golik P, Khan I, Wallace D, Luo Y. Anti-apoptoticproperties of Ginkgo biloba extract EGb 761 in differentiated PC12 cells. Cell Mol Biol (Noisy -le-grand) 2002; 48: 699-707.
[51] Chen JC, Ho FM, Pei-Dawn Lee Chao, Chen CP, Jeng KC, Hsu HB, et al. Inhibition of iNOS gene expression by quercetin is mediated by the inhibition of IkappaB kinase, nuclear factor-kappa B and STAT1, and depends on heme oxygenase-1 induction in mouse BV-2 microglia. Eur J Pharmacol 2005; 521: 9-20.
[52] Kim H, Kim YS, Kim SY, Suk K. The plant flavonoid wogonin suppresses death of activated C6 rat glial cells by inhibiting nitric oxide production. Neurosci Lett 2001; 309: 67-71.
[53] Wattanathorn J, Muchimapura S, Thukham-Mee W, Ingkaninan K, Wittaya-Areekul S. Mangifera indica Fruit Extract Improves Memory Impairment, Cholinergic Dysfunction, and Oxidative Stress Damage in Animal Model of Mild Cognitive Impairment. Oxidative Medicine and Cellular Longevity. Volume 2014 (2014), Article ID 132097, 7 pages. http://dx.doi.org/10.1155/2014/132097.
[54] Badriya Al-Rahbi, Rahimah Zakaria, Zahiruddin Othman, Asma' Hassan, Asma Hayati Ahmad. Enhancement of the brain BDNF concentration and restoration of HPA axis reduce depressive-like behaviour in stressed ovariectomised rat treated with Tualang honey. The Scientific World Journal, vol. 2014, Article ID 310821, 8 pages, 2014. doi:10.1155/2014/310821.
[55] Palumbo ML, Fosser NS, Rios H, Zorrilla Zubilete MA, Guelman LR, Cremaschi GA, et al. Loss of hippocampal neuronal nitric oxide synthase contributes to the stress-related deficit in learning and memory. J Neurochem 2007; 102: 261-274.
[56] Kuhad A, Chopra K. Effect of sesamol on diabetes-associated cognitive decline in rats. Exp Brain Res 2008; 185: 411-420.