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Short Communication

Effects of Vitexin on Scopolamine-Induced Memory Impairment in Rats

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Abstract

Various synthetic derivatives of natural flavonoids are known to have neuroactive properties. The present study aimed to investigate the effects of vitexin (5, 7, 4-trihydroxyflavone-8-glucoside), a flavonoid found in such plants as tartary buckwheat sprouts, wheat leaves phenolome, Mimosa pudica Linn and Passiflora spp, on scopolamine-induced memory impairment in rats. To achieve this goal, we assessed the effects of vitexin on memory retrieval in the presence or absence of scopolamine using a step-through passive avoidance trial. In the first part of the study, vitexin (25, 50, and 100 μM) was administered intracerebroventricularly (i.c.v.) before acquisition trials. In the second part, vitexin, at the same doses, was administered before scopolamine (10 μg, i.c.v.) and before the acquisition trials. During retention tests, vitexin (100 μM) in the absence of scopolamine significantly increased the step-through latencies compared to scopolamine. In addition, vitexin (100 μM) significantly reversed the shorter step-through latencies induced by scopolamine ($P < 0.05$). These results indicate that vitexin has a potential role in enhancing memory retrieval. A possible mechanism is modulation of cholinergic receptors; however, other mechanisms may be involved in its effects in acute exposure.

Key Words: flavonoid, memory retrieval, passive avoidance, scopolamine, vitexin

Introduction

Flavonoids are a group of structurally related phenyl benzopyrones. They are subdivided into the following main classes: flavones, flavanones, flavonols, isoflavones, flavans and flavan-3-ols. Because they are found in all plants, they are constituents of the human diet (13). Flavonoid derivatives have been considered useful in treatment for neurodegenerative disorders such as Alzheimer disease (AD) (45).

In recent years, several mechanisms have been examined for signalling pathways that underlie improved cognitive function; flavonoids activate the extracellular signal-regulated protein kinase cAMP-response element-binding protein (ERK-CREB) pathway and the phosphoinositide 3-kinase-mTOR (PI3-kinase-mTOR) cascade, leading to changes in synaptic plasticity. They are also capable of influencing neurogenesis through the activation of PI3-kinase-AkteNOS (44). Moreover, flavonoids have been reported to block oxidative-induced neuronal damage by preventing the activation of caspase-3, thus, providing evidence in support of their potent anti-apoptotic action (37).

In a brain-imaging study of humans, the consumption of flavanol-rich cocoa was shown to enhance cortical blood flow (8). This result is important for increasing cerebrovascular function, especially in the hippocampus, a brain region that is important for memory and that may facilitate adult neurogenesis

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(10). New hippocampal cells are clustered near blood vessels that proliferate in response to vascular growth factors and may influence memory (29).

Several lines of evidence suggest that flavonoids have beneficial effects on memory and learning (39). The effects of flavonoid-rich foods and pure flavonoids on neurocognitive ability have been shown in rodents (14, 16, 18, 47). Rodent models have been used as models of human declarative memory to predict the potential effects of flavonoids on human cognitive performance (34). Previous reports have also established a role for flavonoids in preventing human dementia (5). It has shown that flavonoids have acetylcholinesterase inhibitory activities (20, 42).

Scopolamine is used as a standard drug for inducing cognitive deficits in healthy humans and animals (21). Scopolamine is a muscarinic receptor antagonist with amnestic properties and is a widely used model for characterizing potential cognition-enhancing drugs (8). Blockade of central muscarinic receptors may induce a pattern of cognitive impairment in AD patients (4).

Vitexin (5, 7, 4-trihydroxyflavone-8-glucoside) is a c-glycosilated flavone often found in plants such as tartary buckwheat sprouts, wheat leaves phenolome, Mimosa pudica Linn and Passiflora spp (25, 31, 46, 48). It has been reported that vitexin has several pharmacological properties, including antinociceptive, antispasmodic, antimyeloperoxidase and α-glucosidase inhibitory activities (11, 12, 23, 32, 33, 38). In addition, vitexin has been shown to have free radical scavenging activity in ultraviolet B (UVB)-irradiated cultured human dermal fibroblasts (19). Recently, we have shown that vitexin has anticonvulsant effects in rats treated with pentylenetetrazole (PTZ) (1).

In the present study, two sets of experiments were conducted. We first investigate the dose-response of vitexin alone, followed by the effects of vitexin with scopolamine interactions.

Materials and Methods

Animals

Male Wistar rats (200-250 *g*) were obtained from the Razi Institute (Karaj, Iran) and housed in groups of four per cage under standard laboratory conditions. They were kept at constant room temperature (21 ± 2°C) under a normal 12 h light/12 h dark (12L:12D) regime with free access to food and water. All the animal experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) to minimize the number of animals and their suffering.

Drugs

Vitexin was purchased from Fluka (Buches, Swetzerland). The other drugs used in this study were ketamine (Rotexmedica, GmbH, Germany) and xylazine (Loughrea Co, Galway, Ireland). Scopolamine was purchased in an injectable form from Daru Pakhsh (Tehran, Iran). Vitexin was dissolved in saline. Vitexin, scopolamine and saline were administered intracerebroventricularly (i.c.v.) in a volume of 5 µl.

Surgery and Experimental Procedures

All the rats were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). The skull surface was exposed, and the head was oriented such that the bregma and lambda skull sutures were at the same vertical levels. Stereotaxic coordinates for the left lateral ventricle were: anterior-posterior (AP), -0.92 mm from bregma; medial-lateral (ML), 1.6 mm from the midline; and dorsal-ventral (DV), 3.5 mm from the skull surface (30). For chronic i.c.v. cannulation, a stainless steel guide cannula (21-gauge) was implanted to terminate 1 mm above the left lateral ventricle (2.4 mm ventral from the skull surface). This cannula was attached to the skull with a stainless steel screw and dental acrylic cement. Housed individually, the rats were allowed to recover from surgery for at least a week. On the day of the experiment, the rats were transferred to the experimental room 1 h prior to the experiment. For intracerebroventricular drug administration, the rats were gently hand restrained and drug infusions were made using an injection needle (24-gauge) inserted into the guide cannula connected through a polyethylene tube to a 5 µl Hamilton syringe (28).

Experimental Procedure

The rats were divided into eight groups of 10 animals each. The first group was administered saline (5 µl, i.c.v) 30 min before acquisition trials. The second group received scopolamine (10 µg, i.c.v.) 30 min before the acquisition trials. The next three groups received vitexin (25, 50 or 100 µM, i.c.v.) 30 min before the acquisition trials. In the remaining three groups, vitexin (25, 50 or 100 µM, i.c.v.) was administered 15 min before scopolamine (10 µg, i.c.v.) and 45 min before the acquisition trials.

Passive Avoidance Apparatus

The passive avoidance apparatus comprised of

a learning box consisting of a light (white) and a dark (black) compartment, 20 × 20 × 30 cm each. A guillotine door opening (6 × 6 cm) was made on the floor in the center of the partition between the two compartments. Stainless steel grids (5 mm in diameter) were placed at 1 cm intervals (distance between the centers of grids) on the floor of the dark compartment to produce a foot shock (Borj Sanat, Tehran, Iran).

All the rats were allowed to habituate to the experimental room for at 30 min prior to the experiments. This standard was applied across experiments both in the vitexin dose response and the vitexin with scopolamine treatment. Then, each rat was gently placed in the light compartment of the apparatus. After 5 sec, the guillotine door was opened and the rat was allowed to enter the dark compartment. The latency with which each rat crossed into the dark compartment was recorded. The rats that waited more than 100 sec to cross into the dark compartment were eliminated from the experiments. Once the rat crossed with all four paws into the next compartment, the guillotine door was closed and the rat was returned to its home cage. The acquisition trial was performed 30 min after the habituation trial. The rat was placed in the light compartment, and the guillotine door was opened 5 sec later. As soon as the rat crossed into the dark compartment, the door was closed and a foot shock (0.5 mA intensity, 3 sec) was immediately delivered to the grid floor of the dark room by an insulated stimulator (26). One day after training, retention tests were performed to evaluate memory performance. Each rat was placed in the light compartment for 20 sec, the door was opened, and the step-through latency for entering into the dark compartment was measured. The test session ended when the rat entered the dark compartment or remained in the light compartment for 300 sec. No electric shock was applied during these sessions (27).

Histology

At the end of the experiments, the rats were injected with 2 μl of crystal violet and deeply anesthetized. Their brains were removed and fixed in 10% formaldehyde solution. For histological examination of the cannula and needle placement in the lateral ventricle region, 100 μm-thick sections were taken and the cannula track was examined in each rat. Only those rats whose cannulae were exactly placed in the left ventricle were used for further analysis (28). Thus, only behavioral results for animals with correct cannula placement were considered.

Data Analysis

The data were analyzed three times as follows: [1] control versus scopolamine treatment analyzed by Student's *t*-test, [2] vitexin dose response comparing with the control *vs.* 25, 50 or 100 μM vitexin analyzed by one-way ANOVA, [3] vitexin with scopolamine treatment analyzed by one-way ANOVA. A probability $P < 0.05$ was considered statistically significant.

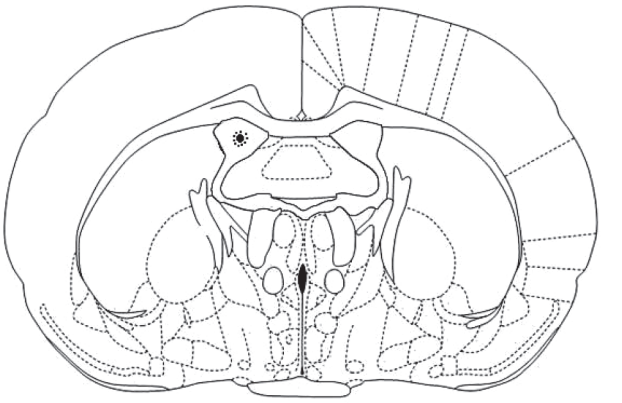

Fig. 1. Schematic representation of cannula sites aimed unilaterally at the intracerebroventricular (icv) region using the stereotaxic atlas of Paxinos and Watson (30). Correct placement of cannula tips of ten animals were shown by filled circles.

Results

Histological evaluation of the brains of control and drug treated rats confirmed that in all rats in each group, the cannula were in correct position (Fig. 1).

The step-through latency times were not significantly different between the experimental and control groups during the acquisition test (Fig. 2A). During the retention test, the step-through latency of the scopolamine-treated group was significantly lower than that of the control group ($P < 0.05$) (*t*-test) (Fig. 2B).

The step-through latencies of the groups treated with vitexin alone (25, 50 or 100 μM) were not significant at the studied doses compared to the control (one-way ANOVA, Tukey-Kramer test).

However, vitexin alone at a dosage of 100 μM significantly increased the step-through latency compared to scopolamine treatment (one-way ANOVA, Tukey-Kramer test) (Fig. 2B).

The step-through latencies of the groups treated with vitexin (25, 50 or 100 μM) with scopolamine were not significant at the studied doses compared to the control (one-way ANOVA, Tukey-Kramer test).

Furthermore, in the analysis of the groups treated with vitexin (25, 50 or 100 μM) with scopolamine by

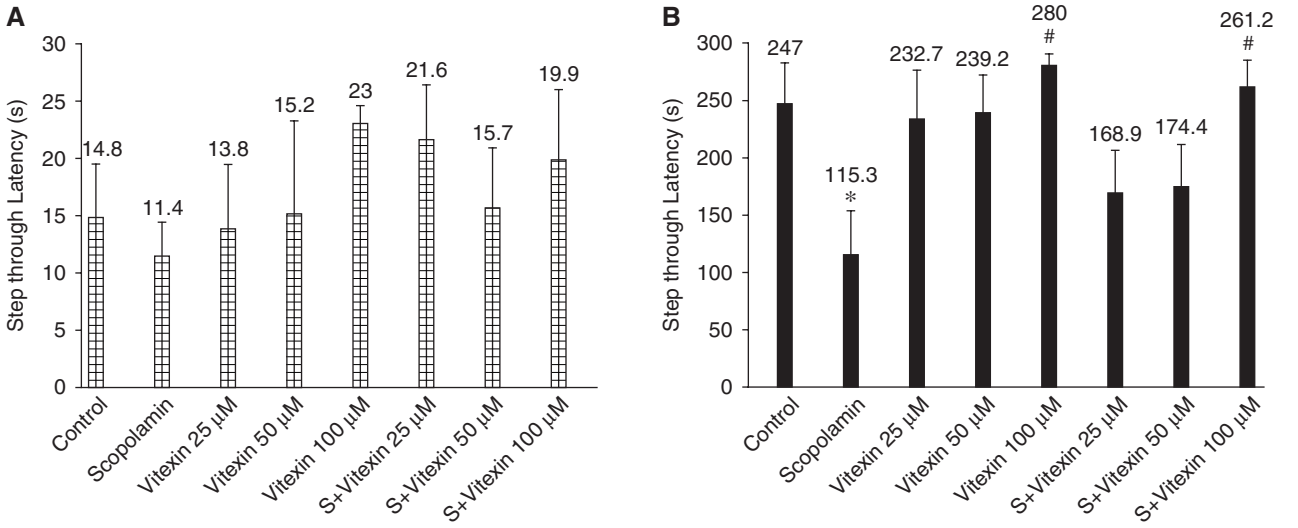

Fig. 2. Effects of vitexin and scopolamine on the step-through latencies in acquisition trials (A) and retention trials (B) in rats. In the first group, saline (5 µl, i.c.v.) was given 30 min before the acquisition trial. Memory impairment was induced by scopolamine treatment (10 µg, i.c.v.), and acquisition trial was carried out 30 min after its treatment. Rats were injected vitexin (25, 50 or 100 µM, i.c.v.) 30 min before the acquisition trials. For the study on the effects of vitexin on the scopolamine-induced memory deficits model, rats were administered vitexin (25, 50 or 100 µM, i.c.v.) 15 min before scopolamine (10 µg, i.c.v.) and 45 min before the acquisition trials (A). At 24 h after the acquisition trials, retention trials were done (B). Values are expressed as means ± SEM (n = 10). $^{*}P < 0.05$, compared to control (*t*-test); $^{\#}P < 0.05$, compared to scopolamine-treated group, Tukey-Kramer test.

one-way ANOVA, vitexin (100 µM) significantly reversed the shorter step-through latencies of scopolamine ($P < 0.05$) (one-way ANOVA, Tukey-Kramer test) (Fig. 2B).

Discussion

Data of the present study show that the step-through latency of the scopolamine-treated group was significantly lower than that of the control group in the retention test. Vitexin alone at 100 µM significantly increased the step-through latency compared to scopolamine.

Vitexin administration prior to scopolamine in the acquisition trials is followed by attenuated impairment retention of the avoidance test in rats. Vitexin at a dose of 100 µM significantly reversed scopolamine-induced memory impairment.

Despite some limitations that it fails to replicate the pathological aspects and the progressive degenerative nature of Alzheimer's disease (43), scopolamine-induced memory impairment, particularly when coupled with a version of the inhibitory avoidance task, provides a relatively rapid phenotypic screening tool for drug discovery in the field of cognition enhancement (7). Similar results have been obtained for other related compounds such as rutin, quercetin, resveratrol and curcuminoids, all of which showed potential protective effects against scopolamine-induced memory impairment (3, 9, 35).

One of the receptors proposed to act as a flavonoid-binding site in neurons is the $GABA_A$ receptor (2, 15). Muscarinic receptor subtypes are localized in pyramidal and nonpyramidal cells in the hippocampus (22). It has been shown that muscarinic receptors can enhance GABAergic synaptic transmission through a presynaptic and postsynaptic mechanism (49). The rapid release of GABA in response to acetylcholine is mediated by a different subtype of muscarinic receptors (24). Activation of cholinergic receptors seems to cause direct excitation of GABAergic interneurons (17). Moreover, GABAergic inhibition is known to control the flow of information in a cortical circuit that is important for performance of certain forms of memory (6). These findings suggest that vitexin possibly acts *via* modulation of the GABAergic system to attenuate scopolamine-induced memory impairment. For this reason, the effects of vitexin on memory were more significant in the presence of scopolamine.

As an active component of *Urtica circularis*, vitexin has shown potent antinociceptive effects in a chemical model of nociception in mice. The possible role of cholinergic activation has been discussed for antinociceptive effects of the extract (13). Furthermore, as the role of acetylcholinesterase inhibitory activity of flavonoids, vitexin could have cholinergic properties (20, 42). As shown by our results, it is pos-

sible that vitexin improves cognition by modulating cholinergic neurotransmission.

The efficacy of flavonoids on memory performance is dependent on the period of their administration. It seems that the mechanisms of acute and chronic flavonoid administration are different (40). Flavonoids have been shown to influence peripheral and central blood flow. Based on both *in vitro* and *in vivo* studies, these vascular effects are potentially significant because increased cerebrovascular function is known to facilitate adult neurogenesis in the hippocampus (10, 36). After consumption of flavonol-rich foods, cerebral blood flow rapidly increases in a certain region of the human brain (8). It has been suggested that increased cerebral blood flow exerts impacts on acute cognitive performance (41). Moreover, it has been shown that flavonoids may have the potential to reduce brain amyloid-beta (Aß) levels by improving cerebral blood flow (45). However, it should be considered that increased cerebral blood flow occurs both on acute and chronic exposure.

In the present study, it is possible that vitexin directly interacts with cholinergic or/and GABAergic neurons and attenuates scopolamine-induced memory impairment in the passive avoidance task. Alternatively, vitexin may act indirectly and activate some signaling pathway by increasing cerebral blood flow. However, further investigation is required to clarify this issue.

Our behavioral study shows that vitexin administration can attenuate scopolamine-induced memory impairment in rats. Vitexin administration prior to training trials in passive avoidance tests resulted in improved memory retrieval, which was observed in the retention tests. It is possible that vitexin acts by several mechanisms that play potential roles in memory retrieval in rats. Further studies are necessary to evaluate the effects of vitexin on memory retention and to determine its molecular mechanisms.

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References

1. Abbasi, E., Nassiri-Asl, M., Shafeei, M. and Sheikhi, M. Neuroprotective effects of vitexin, a flavonoid, on pentylenetetrazole-induced seizure in rats. *Chem. Biol. Drug Des*. 80: 274-278, 2012.
2. Adachi, N., Tomonaga, S., Tachibana, T., Denbow, D.M. and Furuse, M. (-)-Epigallocatechin gallate attenuates acute stress responses through GABAergic system in the brain. *Eur J. Pharmacol*. 531: 171-175, 2006.
3. Ahmed, T. and Gilani, A.H. Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. *Pharmacol. Biochem. Behav*. 91: 554-559, 2009.
4. Buccafusco, J.J. The revival of scopolamine reversal for assessment of cognition-enhancing drugs. In: *Methods of Behavior Analysis in Neuroscience* edited by Buccafusco, J.J. 2nd Edition. Boca Raton (FL): CRC Press; pp. 230-329, 2009.
5. Commenges, D., Scotet, V., Renaud, S., Jacqmin-Gadda, H., Barberger-Gateau, P. and Dartigues, J.F. Intake of flavonoids and risk of dementia. *Eur. J. Epidemiol*. 16: 357-363, 2000.
6. Constantinidis, C., Williams, G.V. and Goldman-Rakic, P.S. A role for inhibition in shaping the temporal flow of information in prefrontal cortex. *Nat. Neurosci*. 5: 175-180, 2002.
7. Ebert, U. and Kirch, W. Scopolamine model of dementia: electroencephalogram findings and cognitive performance. *Eur. J. Clin. Invest*. 28: 944-949, 1998.
8. Francis, S.T., Head, K., Morris, P.G. and Macdonald, I.A. The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J. Cardiovasc. Pharmacol*. 47: S215-S220, 2006.
9. Gacar, N., Mutlu, O., Utkan, T., Celikyurt, I.K., Gocmez, S.S. and Ulak, G. Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and Morris water maze tests in rats. *Pharmacol. Biochem. Behav*. 99: 316-323, 2011.
10. Gage, F.H. Mammalian neural stem cells. *Science* 287: 1433-1438, 2000.
11. Gilani, A.H., Khan, A., Ghayur, M.N., Ali, S.F. and Herzig, J.W. Antispasmodic effects of Rooibos Tea (*Aspalathus linearis*) is mediated predominantly through K^+-channel activation. *Basic Clin. Pharmacol. Toxicol*. 99: 365-373, 2006.
12. Gorzalczany, S., Marrassini, C., Mino, J., Acevedo, C. and Ferraro, G. Antinociceptive activity of ethanolic extract and isolated compounds of *Urtica circularis*. *J. Ethnopharmacol*. 134: 733-738, 2011.
13. Hall, B.J., Chebib, M., Hanrahan, J.R. and Johnston, G.A.R. 6-Methylflavanone, a more efficacious positive allosteric modulator of γ-aminobutyric acid (GABA) action at human recombinant $\alpha_2\beta_2\gamma_{2L}$ than at $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_1\beta_2$ $GABA_A$ receptors expressed in *Xenopus* oocytes. *Eur. J. Pharmacol*. 512: 97-104, 2005.
14. Hartman, R.E., Shah, A., Fagan, A.M., Schwetye, K.E., Parsadanian, M., Schulman, R.N., Finn, M.B. and Holtzman, D.M. Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. *Neurobiol. Dis*. 24: 506-515, 2006.
15. Johnston, G.A. $GABA_A$ receptor channel pharmacology. *Curr. Pharm. Des*. 11: 1867-1885, 2005.
16. Joseph, J.A., Shukitt-Hale, B., Denisova, N.A., Bielinski, D., Martin, A., McEwen, J.J. and Bickford, P.C. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci*. 19: 8114-8121, 1999.
17. Kawaguchi, Y. Selective Cholinergic Modulation of Cortical GABAergic Cell Subtypes. *J. Neurophysiol*. 78: 1743-1747, 1997.
18. Kim, H.K., Kim, M., Kim, S., Kim, M. and Chung, J.H. Effects of green tea polyphenol on cognitive and acetylcholinesterase activities. *Biosci. Biotech. Bioch*. 68: 1977-1979, 2004.
19. Kim, J.H., Lee, B.C., Kim, J.H., Sim, G.S., Lee, D.H., Lee, K.E., Yun, Y.P. and Pyo, H.B. The isolation and antioxidative effects of vitexin from *Acer palmatum*. *Arch. Pharm. Res*. 28: 195-202, 2005.
20. Kim, J.Y., Lee, W.S., Kim, Y.S., Curtis-Long, M.J., Lee, B.W., Ryu, Y.B. and Park, K.H. Isolation of cholinesterase-inhibiting flavonoids from *Morus lhou*. *J. Agric. Food Chem*. 59: 4589-4596, 2011.
21. Klinkenberg, I. and Blokland, A. The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies. *Neurosci. Biolbehav. Rev*. 34: 1307-1350, 2010.

22. Levey, A.I., Edmunds, S.M., Koliatsos, V., Wiley, R.G. and Helman, C.J. Expression of m1-m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. *J. Neurosci*. 15: 4077-4092, 1995.
23. Li, Y.L., Ma, S.C., Yang, Y.T., Ye, S.M. and But, P.P.H. Antiviral activities of flavonoids and organic acid from *Trollius chinensis* Bunge. *J. Ethnopharmacol*. 79: 365-368, 2002.
24. McCormick, D.A. and Prince, D.A. Two types of muscarinic response to acetylcholine in mammalian cortical neurons. *Proc. Natl. Acad. Sci. USA* 82: 6344-6348, 1985.
25. Moheb, A., Ibrahim, R.K., Roy, R. and Sarhan, F. Changes in wheat leaf phenolome in response to cold acclimation. *Phytochemistry* 72: 2294-2307, 2011.
26. Mutlu, O., Ulak, G., Çelikyurt, I.K., Akar, F.Y. and Erdenm, F. Effects of citalopram on cognitive performance in passive avoidance, elevated plus-maze and three-panel runway tasks in naïve rats. *Chinese J. Physiol*. 54: 36-46, 2011.
27. Nassiri-Asl, M., Sarookhani, M.R., Abbasi E., Zangivand, A.A., Shakiba, E., Sedighi, A. and Rahbari, M. The effects of pretreatment with vitamin B6 on memory retrieval in rats. *Food Chem*. 132: 1046-1048, 2012.
28. Nassiri-Asl, M., Shariati-Rad, S. and Zamansoltani, F. Anticonvulsive effects of intracerebroventricular administration of rutin in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32: 989-993, 2008.
29. Palmer, T.D., Willhoite, A.R. and Gage, F.H. Vascular niche for adult hippocampal neurogenesis. *J. Comp. Neurol*. 425: 479-494, 2000.
30. Paxinos, G. and Watson, C. The Rat Brain in Stereotaxic Coordinates. New York: Academic Press, Inc, 1998.
31. Pereira, C.A., Yariwake, J.H. and McCullagh, M. Distinction of the C-glycosylflavone isomer pairs orientin/isoorientin and vitexin/isovitexin using HPLC-MS exact mass measurement and in-source CID. *Phytochem. Anal*. 16: 295-301, 2005.
32. Quílez, A., Berenguer, B., Gilardoni, G., Souccar, C., de Mendonc, S., Oliveira, L.F.S. and Martín-Calero, M.J. Anti-secretory, anti-inflammatory and anti-*Helicobacter pylori* activities of several fractions isolated from *Piper carpunya Ruiz* & Pav. *J. Ethnopharmacol*. 128: 583-589, 2010.
33. Ragone, M.I., Sella, M., Conforti, P., Volonte, M.G. and Consolini, A.E. The spasmolytic effect of *Aloysia citriodora*, Palau (South American cedron) is partially due to its vitexin but not isovitexin on rat duodenums. *J. Ethnopharmacol*. 113: 258-266, 2007.
34. Rendeiro, C., Spencer, J.P.E., Vauzour, D., Butler, L.T., Ellis, J.A. and Williams, C.M. The impact of flavonoids on spatial memory in rodents: from behaviour to underlying hippocampal mechanisms. *Genes Nutr*. 4: 251-270, 2009.
35. Richetti, S.K., Blank, M., Capiotti, K.M., Piato, A.L., Bogo, M.R., Vianna, M.R. and Bonan, C.D. Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behav. Brain Res*. 217: 10-15, 2011.
36. Schroeter, H., Heiss, C., Balzer, J., Kleinbongard, P., Keen, C.L., Hollenberg, N.K., Sies, H., Kwik-Uribe, C., Schmitz, H.H. and Kelm, M. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc. Natl. Acad. Sci. USA* 103: 1024-1029, 2006.
37. Schroeter, H., Spencer, J.P.E., Rice-Evans, C. and Williams, R.J. Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. *Biochem. J*. 358: 547-557, 2001.
38. Shibano, M., Kakutani, K., Taniguchi, M., Yasuda, M. and Baba, K. Antioxidant constituents in the dayflower (*Commelina communis* L.) and their α-glucosidase-inhibitory activity. *J. Nat. Med*. 62: 349-353, 2008.
39. Spencer, J.P.E. The interactions of flavonoids within neuronal signalling pathways. *Genes Nutr*. 2: 257-273, 2007.
40. Spencer, J.P.E. The impact of fruit flavonoids on memory and cognition. *Brit. J. Nutr*. 104: S40-S47, 2010.
41. Spencer, J.P.E., Vauzour, D. and Rendeiro, C. Flavonoids and cognition: The molecular mechanisms underlying their behavioural effects. *Arch. Biochem. Biophysics* 492: 1-9, 2009.
42. Uriarte-Pueyo, I. and Calvo, M.I. Flavonoids as acetylcholinesterase inhibitors. *Curr. Med. Chem*. 18: 5289-5302, 2011.
43. Van Dam, D. and De Deyn, P.P. Drug discovery in dementia: The role of rodent models. *Nat. Rev. Drug Discovery*. 5: 956-970, 2006.
44. Vauzour, D., Vafeiadou, K., Rodriguez–Mateos, A., Rendeiro C. and Spencer, J.P. The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr*. 3: 115-126, 2008.
45. Williams, R.J. and Spencer, J.P.E. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radic. Biol. Med*. 52: 35-45, 2012.
46. Xu, N., Yang, H., Cui, M., Wan, C. and Liu, S. High-performance liquid chromatography-electrospray ionization-mass spectrometry ligand fishing assay: a method for screening triplex DNA binders from natural plant extracts. *Anal. Chem*. 84: 2562-2568, 2012.
47. Yamamoto, Y., Adachi, Y., Fujii, Y. and Kamei, C. *Ginkgo biloba* extract improves spatial memory in rats mainly but not exclusively *via* a histaminergic mechanism. *Brain Res*. 1129: 161-165, 2007.
48. Zhang, J., Yuan, K., Zhou, W.L., Zhou, J. and Yang, P. Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn. from southern China. *Pharmacogn. Mag*. 7: 35-39, 2011.
49. Zhong, P., Gu, Z., Wang, X., Jiang, H., Feng, J. and Yan, Z. Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. *J. Biol. Chem*. 278: 26888-26896, 2003.