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ORIGINAL ARTICLE

Effect of *Trigonella foenum-graecum* Linn. seeds methanol extract on learning and memory

Tahira Assad[1] · Rafeeq Alam Khan[2] · Muhammad Ali Rajput[3]

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Abstract

Prevention and delay in the onset of memory disorders will have a great impact on society by reducing the disease burden and finances. Drugs available for the treatment of learning and memory disorders are few. There is need to develop a better drug, several studies have shown the therapeutic effectiveness of herbal extracts for the learning and memory disorders because of their neuroprotective effects, hence herbs should be evaluated scientifically to form a basis for the future discovery of newer drugs. In this study, effect of *Trigonella-foenum graecum L.* seeds methanol extract (TFGS-ME) was evaluated in mice on learning and memory process by both exteroceptive and interoceptive behavioral models at three different doses. Elevated plus maze test was employed to assess the effect on learning and memory as an exteroceptive behavioral test. Scopolamine-induced amnesia was performed to assess effect on learning and memory as interoceptive behavior test. In both tests, it was found that animals received extract at 200 mg/kg exhibited a highly noteworthy decline in transfer latency on both acquisition and retention days in contrast to control animals, suggestive of improved learning and memory process. Results were equivalent to the standard drug piracetam at similar dose indicating that TFGS-ME improves learning and memory process and has significant potential as an antiamnesic agent. Hence there is need to separate the dietary components which may play a vibrant role in the future invention of novel drugs.

Keywords Learning · Memory · *Trigonella-foenum graecum L.* seeds · Elevated plus maze test · Scopolamine induced amnesia

Introduction

Learning is the ability of an individual to gain new information, whereas memory is defined as the process by which brain encodes and stores the acquired knowledge and recalls the same whenever needed (Sherwood 2015).

Memory can be affected by various pathologies such as neurodegenerative diseases, stroke, tumor, anxiety, head injury, lack of oxygen, heart surgery, undernourishment, attention deficit disorder, depression, harmful effects of drugs, and old age (Joshi et al. 2007; Budson and Price 2005). Prevention and delay in the onset of memory disorders will have a great impact on society by reducing the disease burden and finances (Oliveira et al. 2009).

The process of learning and memory involve long-lasting modifications of the brain, various neurotransmitters are involved in this process such as acetylcholine, dopamine, and serotonin. These neurotransmitters activate the receptor linked enzymes which produce intercellular messengers. Numerous studies have revealed the contribution of the cholinergic system in learning and memory process of the brain (Maurer and Williams 2017; Peter et al. 2016). It has been proved that Alzheimer's disease is associated with damage to cholinergic neurons in the cerebral cortex and hippocampus areas of the brain and decreased the action of the enzyme choline acetyl transferase. Based on this finding, acetyl cholinesterase (AChE) inhibitors were developed to treat dementia associated with Alzheimer's disease (Kim et al. 2007).

The Ayurveda system of traditional medicines has gained a lot of popularity in this respect (Oh et al. 2005). Numerous plants, characterized as the "Medhya" are reported to improve

✉ Rafeeq Alam Khan
rkhan1959@gmail.com

[1] CMH, Karachi Institute of Medical Sciences, Malir Cantonment, Karachi, Pakistan

[2] Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi 75270, Pakistan

[3] Department of Pharmacology, Multan Medical College, Multan, Pakistan

the process of cognition (Joshi and Parle 2006). Adams assembled facts on more than 150 plant species for many age-related cognitive disorders. Various phenolic compounds, such as resveratrol obtained from grapes and red wine, curcumin isolated from turmeric and epigallocatechin acquired from green tea revealed neuroprotective effects (Frank and Gupta 2005; Sun et al. 2008). Antiamnesic effects of various plants extracts are well documented in the literature (Hsieh et al. 2010).

Dementia which is a gradual loss of memory is widespread among elderly people. Expenditure estimates go beyond a billion Euros in Europe only. Alzheimer's disease is responsible for many cases of dementia, followed by vascular, Lewy body type and dementia induced by Parkinson's disease. The pathogenesis of the disease is of diverse type and indicate various abnormalities such as β-amyloidosis, ischemic damages, α-synucleinopathy, neurotransmitter abnormalities including abnormalities in cholinergic, glutamatergic and serotonergic systems, abnormal tau, inflammation, oxidative stress, cell death and neuroplasticity (Ballard et al. 2007; Muñoz-Torrero 2008).

Medicines available for the treatment of dementia are few and include the acetyl cholinesterase inhibitors, butyrylcholinesterase inhibitors (in few cases) and memantine (a glutamate NMDA receptor antagonist). Additional drugs often advised to some patients include antipsychotics, sedatives, and antidepressants. Cholinesterase inhibitor drugs increase the level of the neurotransmitter acetylcholine in the brain by inactivating the enzyme acetylcholinesterase. Acetylcholinesterase inhibitors include rivastigmine, donepezil, galantamine and tacrine (Orhan et al. 2009).

Scopolamine, a cholinergic antagonist drug can induce impairment of learning and memory in experimental animals (Misane and Ögren 2003; Ballard et al. 2005). It is a widely accepted method to evaluate antiamnesic effects of herbal extracts (Bejar et al. 1999).

Several studies on various plants all over the world have shown the therapeutic effectiveness of herbal medicines on memory because of their neuroprotective effects either by inhibiting acetyl cholinesterase enzyme or antioxidant ability (Rabiei et al. 2014). Plants are more efficacious have less harmful effects and are more cost-effective. Hence studies on plant extracts may offer a basis for the development of novel compounds (Katiyar et al. 2012).

Trigonella foenum-graecum Linn. (Fenugreek) is a famous ancient medicinal plant pertaining to the family Fabaceae, various parts of which are consumed as food component such as leaves and seeds (Nathiya et al. 2014).

This study was accomplished using seeds of *T. foenum graecum L.* Seeds are small (3–5 mm long, 2 mm thick) having a firm consistency ranging from yellowish brown to brown in color. They are flattened and have a distinctive rhomboid shape. They have a typical peppery odor, bitter and mucilaginous taste (Nasroallah et al. 2013).

Seeds are traditionally used as a spice in food. Ancient people used it customarily for various medicinal uses. It is a rich source of various bioactive constituents such as saponins (fenugreekine, diosgenin), alkaloids (trigonelline, gentianine, carpaine), amino acids (4-Hydroxy isoleucin, arginine) polyphenols and flavonoids (Wani and Kumar 2016; He et al. 2015; Dande and Patil 2012; Mandegary et al. 2012). Literature review showed various scientifically appraised activities such as hypoglycemic (Raju et al. 2001), hypolipidemic (Ribes et al. 1987), antioxidant (Rababah et al. 2004), anticancer (Shabbeer et al. 2009) and antiulcer activities (Kaur et al. 2011). Transfer latency is the parameter used to measure acquisition and retention process of memory (Pellow et al. 1985).

The present study was specially designed to evaluate the effect of *T. foenum graecum L.* seeds methanol extract (TFGSME) on learning and memory process in mice.

Materials and methods

Formulation of extract

The cold extraction method was executed to formulate crude extract of methanol (Hossain et al. 2010). Seeds (5 kg) purchased from the local herbal store were purified from all scums manually and crushed to obtain a fine powder, which was immersed in 80% 1000 ml methanol for 10 days in air-tight jars with their caps tightly closed. Jars were shaken frequently every 2 days until the solvent turn out to be medium brown in color. The acquired solvent was sieved first through cotton and then by What-Mann No.1 filter paper. Subsequently, the obtained filtrate was vaporized under reduced pressure by means of a rotary evaporator at 45°C, followed by freeze- drying at −30 °C of temperature. The crude extract of methanol so developed was preserved in Petri dishes and kept at −20°C temperature for further use. The yield of extract obtained was 1120 mg of dry weight.

Animals

This study was conducted at Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, the University of Karachi after the approval of Board of Advanced Study and Research (BASR) of University of Karachi. The albino mice of either gender weighing 18–24 g were procured from Hussain Ebrahim Jamal (HEJ) Research Institute of Chemistry, University of Karachi. All the animals were retained in plastic cages placed at the animal house of Department of Pharmacology, University of Karachi, under strict conditions of temperature (22 ± 2°C) and humidity

(50–60%) in an alternating 12-h light/dark cycle. Animals were fed with standard food and water regularly. Guidelines of National Institute of Health (NIH) were followed for handling and experimentation on animals (National Research Council 1996).

Grouping and dosing

35 Swiss albino mice were randomly distributed into 5 different groups, each comprised of 7 animals. One group was tagged as the control group and received distilled water 1 ml/kg orally once a day. The second group was labeled as the standard drug group and was administered Piracetam (GlaxoSmithKline) at 200 mg/kg body weight through intraperitoneal route. Three test groups received TFGS-ME in three varying dosages (50, 100 and 200 mg/kg body weight respectively) orally once a day. All the treatments were continued for 8 days. Transfer latency (TL) was measured 90 min after the last dose on acquisition day (Day 8). Memory was noted on retention day (Day 9) 24 h after the first day's test.

Testing

Elevated plus maze test was employed to assess the effect on learning and memory as an exteroceptive behavioral test. Scopolamine-induced amnesia was performed to assess the effect on learning and memory as interoceptive behavior test.

Elevated plus maze

The elevated plus maze functioned as the exteroceptive behavioral test (in which provocation is present exterior to the body) to assess learning and cognition function (Joshi and Parle 2006). The tool is comprised of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm), spreading from a platform (5 cm × 5 cm) positioned in the center. The maze is situated 5 cm above the floor. On day 1, each mouse was positioned at the termination corner of an open arm, located away from the central platform and transfer latency was recorded. Transfer latency is defined as the time spent by the mouse to reach into one of the covered arms by using all four legs. If the mouse could not reach into one of the covered arms within 90 s, it is transferred into one of the two covered arms and the transfer latency was marked as 90 s. The mouse was permitted to search the maze for further 10 s and then sent back to its residing cage. Cognition was tested on the day 2, 24 h after the first day's test. Significant reduction in transfer latency signifies improved learning and memory (Dhingra et al. 2004; Parle et al. 2004).

Scopolamine-induced amnesia

Scopolamine hydrobromide (Sigma-Aldrich Inc., St. Louis, MO, USA) was used to induce amnesia in experimental animals. Scopolamine hydrobromide was administered to animals of all groups at 0.4 mg/kg body weight through intraperitoneal route 90 min after the last treatment on acquisition day (Day 8) except to the standard drug group which received the injection 60 min after the last treatment, after 8 continuous days of treatment. Transfer latency was measured 45 min after the administration of scopolamine hydrobromide. Memory was observed on retention day (Day 9), 24 h after the first day's test (Saini et al. 2011).

Statistics

Data analysis was done utilizing Superior Performance Statistical Software (SPSS)-23 and is presented as mean ± SEM. Anova followed by post hoc Tukey test was performed for comparisons of values with control. Values of $P < 0.05$ were considered as significant and $P < 0.01$ as highly significant.

Results

Table 1 demonstrates the effect of TFGS-ME and piracetam on transfer latency in mice in Elevated plus maze test after 8 days of uninterrupted administration. Animals received TFGS-ME at doses of 50 and 100 mg/kg body weight showed a decrease in transfer latency on both acquisition and retention day but the decrease was statistically inconsequential as compared to control animals. However, animals received TFGS-ME extract at 200 mg/kg body weight showed a highly noteworthy decrease in transfer latency

Table 1 Effect of TFGS-ME and piracetam on transfer latency in elevated plus maze

Group/Treatment	Transfer latency (Sec)	
	Day 8 (Acquisition Day)	Day 9 (Retention Day)
Distilled Water 1 ml/kg	28.13 ± 1.4	26.14 ± 1.3
Piracetam 200 mg/kg	16.31 ± 1.1**	8.20 ± 0.2**
TFGS- ME I 50 mg/kg	25.23 ± 2.4	22.31 ± 1.5
TFGS- ME II 100 mg/kg	20.31 ± 3.1	18.56 ± 1.2
TFGS- ME III 200 mg/kg	15.34 ± 1.7 **	9.67 ± 1.3**

TFGS-ME Trigonella Foenum Graecum Seeds-Methanol Extract

$n = 7$, Figures are presented as mean ± standard error of mean

*P ≤ 0.05 noteworthy in contrast to control group

**$P \leq 0.001$ highly noteworthy in contrast to control group

on both acquisition and retention day i.e. 15.34 ± 1.7 s and 9.67 ± 1.3 s in contrast to control animals. These effects were similar to animals which received standard drug piracetam at 200 mg/kg body weight.

Table 2 demonstrates the effect of TFGS-ME and piracetam on transfer latency in mice on scopolamine-induced amnesia after 8 days of uninterrupted administration.

Animals received TFGS-ME at doses of 50 and 100 mg/kg body weight showed decrease in transfer latency on both acquisition and retention day but the decrease was statistically inconsequential as compared to control animals, however animals received TFGS-ME extract at 200 mg/kg body weight showed highly noteworthy decrease in transfer latency at both acquisition and retention day i.e. 20.03 ± 1.2 s, 13.12 ± 1.6 s respectively in contrast to control animals. These effects were comparable to the effects of animals received standard drug piracetam at 200 mg/kg body weight.

Discussion

In this study, the effect of TFGS-ME was also evaluated on learning and memory by both exteroceptive and interoceptive behavioral models. In both tests, it was found that animals receiving extract at 200 mg/kg of dose exhibited a highly noteworthy decline in transfer latency on both acquisition and retention day in contrast to control animals. Results were equivalent to the standard drug Piracetam at a similar dose.

Diet and lifestyle perform a crucial role in slowing the onset as well as the development of neurodegenerative ailments and considerably enhancing memory function (Spencer 2008; Nurk et al. 2009). It has been found that diet rich in polyphenols have revealed favorable influences on learning and memory process and are linked with a decreased chance of dementia (Beking and Vieira 2010). Polyphenols and their metabolites have modulatory actions on protein and lipid kinase signaling pathways (Williams et al. 2004). These may even protect the neurons against the harms induced by inflammation and oxidative stress (Calabrese et al. 2012). These have the ability to reverse the age-related neuronal changes (Chan et al. 2006; Ashok et al. 2010). *T. foenum graecum L.* seeds are a rich source of polyphenols (Rayyan et al. 2010). Thus learning and memory enhancing activities are might be due to the presence of these polyphenols.

It has been found that the flavonol rutin increased the levels of noradrenaline and dopamine neurotransmitters in the brain, thus improving spatial memory in aged rats (Pyrzanowska et al. 2012). Improvement in learning and memory can be due to flavonol rutin present in *T. foenum graecum L.* seeds (Rayyan et al. 2010).

Alkaloids have also shown antiamnesic effect in scopolamine induced memory impairment in mice (Chuong et al. 2014). Trigonelline, an important alkaloid of *T. foenum graecum L.* seeds has shown neuroprotective and memory boosting effect in animals (Zhou et al. 2012). Saponins also have favorable effect on learning and memory (Jiang et al. 2007). Diosgenin, an important saponin content of *T. foenum graecum L.* seeds considerably upgraded learning and memory in mice, it was suggested that memory boosting effects of diosgenin may be facilitated by increasing antioxidant enzymatic activities (Chiu et al. 2011). Improvement in learning and memory can be due to the presence of alkaloids and saponins content of *T. foenum graecum L.* seeds.

Present study indicates that *T. foenum graecum L.* improves learning and memory and has significant potential

Table 2 Effect of TFGS-ME and piracetam on transfer latency in scopolamine-induced amnesia

Group	Dosage	Transfer latency (Sec)	
		Day 8 (Acquisition Day)	Day 9 (Retention Day)
D/Water + Scopolamine Hydrobromide	1 ml/kg + 0.4 mg/kg	26.11 ± 2.0	41.32 ± 2.4
Piracetam + Scopolamine Hydrobromide	200 mg/kg + 0.4 mg/kg	15.10 ± 1.7**	8.32 ± 1.3**
TFGS- ME I + Scopolamine Hydrobromide	50 mg/kg + 0.4 mg/kg	21.13 ± 1.2	19.44 ± 0.9
TFGS- ME II + Scopolamine Hydrobromide	100 mg/kg + 0.4 mg/kg	20.14 ± 1.3	17.31 ± 0.4
TFGS- ME III + Scopolamine Hydrobromide	200 mg/kg + 0.4 mg/kg	20.03 ± 1.2*	13.12 ± 1.6**

TFGS-ME Trigonella Foenum Graecum Seeds-Methanol Extract

$n = 7$, Data is presented as mean ± standard error of mean

*$P \leq 0.05$ noteworthy in contrast to control group

**$P \leq 0.001$ highly noteworthy in contrast to control group

as antiamnesic agent. Hence there is extreme need to isolate the dietary constituents which may play a dynamic role in future invention of novel drugs.

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Compliance with ethical standards

Conflict of interest Authors have no conflict of interest to declare.

References

Ashok P, Koti BC, Thippeswamy AHM, Tikare VP, Dabadi P, Viswanathaswamy AHM (2010) Evaluation of anti-inflammatory activity of *Centratherum anthelminticum* (L) Kuntze seed. Indian J Pharm Sci 72(6):697–703. https://doi.org/10.4103/0250-474X.84577

Ballard CG, Greig NH, Guillozet-Bongaarts AL, Enz A, Darvesh S (2005) Cholinesterases: roles in the brain during health and disease. Curr Alzheimer Res 2(3):307–318

Ballard CG, Chalmers KA, Todd C, McKeith IG, O'Brien JT, Wilcock G, Love S, Perry EK (2007) Cholinesterase inhibitors reduce cortical Aβ in dementia with Lewy bodies. Neurology 68(20):1726–1729. https://doi.org/10.1212/01.wnl.0000261920.03297.64

Bejar C, Wang RH, Weinstock M (1999) Effect of rivastigmine on scopolamine-induced memory impairment in rats. Eur J Pharmacol 383(3):231–240

Beking K, Vieira A (2010) Flavonoid intake and disability-adjusted life years due to Alzheimer's and related dementias: a population based study involving twenty three developed countries. *Public Health Nutr* 13(9):1403–1409. https://doi.org/10.1017/S1368980009992990

Budson AE, Price BH (2005) Memory dysfunction. N Engl J Med 352(7):692–699

Calabrese V, Cornelius C, Dinkova-Kostova AT, Iavicoli I, Di Paola R, Koverech A, Cuzzorcrea S, Rizzarelli E, Calabrese EJ (2012) Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. Biochim Biophys Acta 1822(5):753–783. https://doi.org/10.1016/j.bbadis.2011.11.002

Chan YC, Hosoda K, Tsai CJ, Yamamoto S, Wang MF (2006) Favorable effects of tea on reducing the cognitive deficits and brain morphological changes in senescence-accelerated mice. J Nutr Sci Vitaminol 52(4):266–273

Chiu CS, Chiu YJ, Wu LY, Lu TC, Huang TH, Hsieh MT, Lu CY, Peng WH (2011) Diosgenin ameliorates cognition deficit and attenuates oxidative damage in senescent mice induced by D-galactose. Am J Chin Med 39(03):551–563. https://doi.org/10.1142/S0192415X11009020

Chuong NN, Trung BH, Luan TC, Hung TM, Dang NH, Dat NT (2014) Anti-amnesic effect of alkaloid fraction from *Lycopodiella cernua* (L.) Pic. Serm. on scopolamine-induced memory impairment in mice. Neurosci Lett 575:42–46. https://doi.org/10.1016/j.neulet.2014.05.031

Dande P, Patil S (2012) Evaluation of Saponins from *Trigonella foenum graecum Seeds* for its Antifertility Activity. Asian J Pharm Clin Res 5(3):154–157

Dhingra D, Parle M, Kulkarni SK (2004) Memory enhancing activity of *Glycyrrhiza glabra* in mice. J Ethnopharmacol 91(2–3):361–365. https://doi.org/10.1016/j.jep.2004.01.016

Frank B, Gupta S (2005) A review of antioxidants and Alzheimer's disease. Ann Clin Psychiatry 17(4):269–286

He Y, Ding C, Wang X, Wang H, Suo Y (2015) Using response surface methodology to optimize countercurrent chromatographic separation of polyphenol compounds from fenugreek (*Trigonella foenum-graecum* L.) Seeds. J Liq Chromatogr, R T 38(1):29–35. https://doi.org/10.1080/10826076.2013.864981

Hossain MS, Ahmed M, Islam A (2010) Hypolipidemic and hepatoprotective effects of different fractions of ethanolic extract of immature leaves of *Mangifera indica* (Linn.) in alloxan induced diabetic rats. Int J Pharm Sci Res 1(11):132–138

Hsieh MT, Peng WH, Wu CR, Ng KY, Cheng CL, Xu HX (2010) Review on experimental research of herbal medicines with anti-amnesic activity. Planta Med 76(3):203–217. https://doi.org/10.1055/s-0029-1240707

Jiang T, Huang BK, Zhang QY, Han T, Zheng HC, Qin LP (2007) Effect of Liriope platyphylla total saponin on learning, memory and metabolites in aging mice induced by D-galactose. Zhong Xi Yi Jie He Xue Bao 5(6):670–674

Joshi H, Parle M (2006) Zingiber officinale: evaulation of its nootropic effect in mice. AJTCAM 3(1):64–74

Joshi H, Megeri K, Bidchol AM, Kulkarni VH (2007) *Clerodendron phlomidis* Linn improves short term memory of chemically and naturally induced amnesia in mice. Nat Prod Ind J 3(3):166–170

Katiyar C, Gupta A, Kanjilal S, Katiyar S (2012) Drug discovery from plant sources: An integrated approach. Ayu 33(1):10–19. https://doi.org/10.4103/0974-8520.100295

Kaur J, Singh H, Khan MU (2011) Multifarious therapeutic potential of fenugreek: a comprehensive review. IJRPBS 2(3):863–871

Kim DH, Yoon BH, Kim YW, Lee S, Shin BY, Jung JW, Kim HJ, Lee YS, Choi JS, Kim SY, Lee KT, Ryu JH (2007) The seed extract of *Cassia obtusifolia* ameliorates learning and memory impairments induced by scopolamine or transient cerebral hypoperfusion in mice. J Pharmacol Sci 105(1):82–93

Mandegary A, Pournamdari M, Sharififar F, Pournourmohammadi S, Fardiar R, Shooli S (2012) Alkaloid and flavonoid rich fractions of fenugreek seeds (*Trigonella foenum-graecum* L.) with antinociceptive and anti-inflammatory effects. *Food Chem Toxicol* 50(7):2503–2507. https://doi.org/10.1016/j.fct.2012.04.020

Maurer SV, Williams CL (2017) The Cholinergic System Modulates Memory and Hippocampal Plasticity *via* Its Interactions with Non-Neuronal Cells. Front Immunol 8:1489. https://doi.org/10.3389/fimmu.2017.01489

Misane I, Ögren SO (2003) Selective 5-HT1A antagonists WAY 100635 and NAD-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat. Neuropsychopharmacology 28(2):253–264. https://doi.org/10.1038/sj.npp.1300024

Muñoz-Torrero D (2008) Acetylcholinesterase inhibitors as disease-modifying therapies for Alzheimer's disease. Curr Med Chem 15(24):2433–2455

Nasroallah MK, Didarshetaban MB, Pour S, Reza HRS (2013) Fenugreek (*Trigonella foenum-graecum* L.) as a valuable medicinal plant. Inter J Adv Biol Biom Res 1(8):922–931

Nathiya S, Durga M, Devasena T (2014) Therapeutic role of *Trigonella foenum-graecum* [Fenugreek]–a review. *IntJPharmSciRevRes* 27(2):74–80

National Research Council (1996) Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources, Commission on Life Sciences. National Academy of Sciences, Washington, DC

Nurk E, Refsum H, Drevon CA, Tell GS, Nygaard HA, Engedal K, Smith AD (2009) Intake of flavonoid-rich wine, tea, and chocolate by elderly men and women is associated with better cognitive test performance. J Nutr 139(1):120–127. https://doi.org/10.3945/jn.108.095182

Oh MS, Huh Y, Bae H, Ahn DK, Park SK (2005) The multi-herbal formula Guibi-tang enhances memory and increases cell proliferation in the rat hippocampus. Neurosci Lett 379(3):205–208

Oliveira DR, Sanada PF, Saragossa Filho AC, Innocenti LR, Oler G, Cerutti JM, Cerutti SM (2009) Neuromodulatory property of standardized extract *Ginkgo biloba* L.(EGb 761) on memory: behavioral and molecular evidence. Brain Res 1269:68–89. https://doi.org/10.1016/j.brainres.2008.11.105

Orhan G, Orhan I, Subutay-Oztekin N, Ak F, Sener B (2009) Contemporary anticholinesterase pharmaceuticals of natural origin and their synthetic analogues for the treatment of Alzheimer's disease. Recent Pat CNS Drug Discov 4(1):43–51

Parle M, Dhingra D, Kulkarni SK (2004) Improvement of mouse memory by *Myristica fragrans* seeds. J Med Food 7(2):157–161. https://doi.org/10.1089/1096620041224193

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in the rat. J Neurosci Methods 14(3):149–167

Peter J, Lahr J, Minkova L, Lauer E, Grothe MJ, Teipel S, Köstering L, Kaller CP, Heimbach B, Hüll M, Normann C, Nissen C, Reis J, Klöppel S (2016) Contribution of the cholinergic system to verbal memory performance in mild cognitive impairment. J Alzheimers Dis 53(3):991–1001. https://doi.org/10.3233/JAD-160273

Pyrzanowska J, Piechal A, Blecharz-Klin K, Joniec-Maciejak I, Zobel A, Widy-Tyszkiewicz E (2012) Influence of long-term administration of rutin on spatial memory as well as the concentration of brain neurotransmitters in aged rats. Pharmacol Rep 64(4):808–816

Rababah TM, Hettiarachchy NS, Horax R (2004) Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. J Agric Food Chem 52(16):5183–5186. https://doi.org/10.1021/jf049645z

Rabiei Z, Gholami M, Hojjati MR (2014) The *Effects of Cyperus Rotundus* Ethanolic Extract on Motor Coordination in a Rat Model of Alzheimer. J Zanjan Univ Med Sci 22(92):43–54

Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ (2001) *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan *diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes*. Mol Cell Biochem 224(1):45–51

Rayyan S, Fossen T, Andersen OM (2010) Flavone C-glycosides from seeds of fenugreek, *Trigonella foenum-graecum* L. J Agric Food Chem 58(12):7211–7217. https://doi.org/10.1021/jf100848c

Ribes G, Da Costa C, Loubatières-Mariani MM, Sauvaire Y, Baccou JC (1987) Hypocholesterolemic and hypotriglyceridaemic effects of sub-fractions from fenugreek seeds in alloxan diabetic dogs. Phytother Res 1(1):38–43. https://doi.org/10.1002/ptr.2650010109

Saini D, Dhingra AK, Chopra B, Parle M (2011) Psychopharmacological investigation of the nootropic potential of *Trigonella foenum Linn.* in mice. Asian J Pharm Clin Res 4(4):76–84

Shabbeer S, Sobolewski M, Anchoori RK, Kachhap S, Hidalgo M, Jimeno A, Davidson N, Carducci MA, Khan SR (2009) Fenugreek: a naturally occurring edible spice as an anticancer agent. Cancer Biol Ther 8(3):272–278

Sherwood L (2015) Human physiology: from cells to systems. 7the Edition, Cengage learning. pp.157–162.ISBN 978-1-305-44551-2

Spencer JP (2008) Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. Proc Nutr Soc 67(2):238–252. https://doi.org/10.1017/S0029665108007088

Sun AY, Wang Q, Simonyi A, Sun GY (2008) Botanical phenolics and brain health. NeuroMolecular Med 10(4):259–274. https://doi.org/10.1007/s12017-008-8052-z

Wani SA, Kumar P (2016) Fenugreek: A review on its nutraceutical properties and utilization in various food products. J Saudi Soc Agric Sci. https://doi.org/10.1016/j.jssas.2016.01.007

Williams RJ, Spencer JP, Rice-Evans C (2004) Flavonoids: antioxidants or signaling molecules? Free Radic Biol Med 36(7):838–849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Zhou J, Chan L, Zhou S (2012) Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease. Curr Med Chem 19(21):3523–3531