Metabolic Brain Disease (2019) 34:87–92
https://doi.org/10.1007/s11011-018-0324-1

ORIGINAL ARTICLE

Effect of *Nelumbo nucifera* fruit on scopolamine induced memory deficits and motor coordination

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

Received: 1 May 2018 / Accepted: 24 September 2018 / Published online: 1 October 2018

Abstract

From prehistoric time till today herbal medications are supposed to have neuroprotective effects both by inhibiting acetyl cholinesterase enzyme or antioxidant ability and are also affordable. Thus extensive studies are necessary to investigate the pharmacological effects of herbal plants. The goal of the present study was to ascertain the outcome of *Nelumbo nucifera* fruit (NNF) on scopolamine induced amnestic rats along with motor coordination in mice in order to explore its pharmacological use in disorders like Alzheimer's disease (AD). The effect of NNF on learning and memory was assessed by Morris water maze test using 35 Wister rats weighing 200–230 g evenly divided in to five groups. While motor coordination was assessed using Rot rod test, 35 male locally bred albino mice weighing 20–25 g were equally divided in to five groups. Group I was kept as control (10 ml/kg gum tragacanth). Group II, III and IV were labeled as treated groups (NNF 50, 100 and 200 mg/kg). Group V served as reference group (piracetam 200 mg/kg). All drugs were given by oral route as a single dose for 15 days in both experiments to rats and mice, however scopolamine (1 mg/kg IP) was used in Morris water maze test 40 min after the administration of drugs to rats for the induction of amnesia. In Morris water maze test, *N. nucifera* fruit caused highly significant and significant decrease in escape latency in the amnestic rats at 200 and 100 mg/kg as compared to control. In Rota rod test, *N. nucifera* fruit did not exhibit any notable changes in the riding time at any dose as compared to control. *N. nucifera* fruit have demonstrated ameliorating effects on memory without affecting muscle coordination. Hence NNF seems to have great potential for therapeutic application in memory disorders, such as AD which may be due to its ability to enhance cholinergic neurotransmission and exerting antioxidant effect and thus encourage more preclinical and clinical trials in this field.

Keywords *Nelumbo nucifera* · Alzheimer's disease · Morris water maze · Rota rod test

Introduction

Learning is the aptitude of an individual to gain new information, where memory is defined as the process by which brain encodes and stores the acquired knowledge and reproduces the same whenever needed (Sherwood 2015). Prevention and delay in the onset of memory disorders have a great impact on society by reducing the disease burden and finances (Oliveira et al. 2009). Alzheimer's disease (AD) is a progressive, multifactorial dementing condition with rising prevalence and incidence especially in elderly (Iriti et al. 2010). The drugs that increase cholinergic neurotransmission improve cognitive presentation in AD and other dementing illnesses (Pattewar et al. 2011; Abhinav et al. 2010). Similarly scopolamine, a cholinolytic agent diminishes learning and memory in rodents and humans through blockade of central muscarinic (M) receptors (Kwon et al. 2009).

From prehistoric time till today herbal medications are supposed have neuroprotective effects either by inhibiting acetyl cholinesterase enzyme or antioxidant ability (Rabiei et al. 2014) and are also affordable (Leonardo et al. 2000). Thus extensive studies are necessary to investigate the pharmacological effects of herbal plants, which would enable discovery of novel drugs from herbal source permitting their use to benefit mankind. (Rajput et al. 2017).

✉ Rafeeq Alam Khan
rkhan1959@gmail.com

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

[2] Department of Pharmacology, Multan Medical & Dental College, Multan, Pakistan

[3] Karachi Institute of Medical Sciences, CMH Malir, Karachi, Pakistan

Nelumbo nucifera represents *Nymphaeaceae*, family of plants, is extensively grown in the humid areas of India, China, Thailand, Australia and Pakistan (Mukherjee et al. 2009). The fruit is comprised of seeds which are embedded in the pod. The pod is green in color, in contrast seed is black in color, oblong, ovoid or roundish in shape, approximately 1.5 cm wide and 1.0 cm elongated and is consolidated in whorls (Sridhar and Bhat 2007). The whole fruit is eatable especially the seeds that needs to be skinned individually before they are consumed (Moro et al. 2013).

The seeds of *N. nucifera* contain high contents of amino acid, fat, asparagines, unsaturated fatty acids, protein, starch, tannins and saponins. The principal secondary metabolites contained in the seeds are alkaloids, predominantly lotusine, isoliensinine, liensinine, dauricine, pronuciferine, nuciferine, roemerine, procyanidin, armepavine and neferine along with considerable amounts of Gallic acid, isoquininolinol and carbohydrates (Mukherjee et al. 2009). Moreover remarkable amounts of numerous minerals such as potassium, magnesium, calcium, sodium, chromium, iron, manganese, copper and zinc are also present (Indrayan et al. 2005). Recent study has revealed the presence of alkaloids, flavonoids, saponins, terpenoids and tannins (Rajput and Khan 2017). Procyanidin, an alkaloid was likewise identified and extracted from the pods of fruit (Mukherjee et al. 2009).

Customarily these fruits are utilized as a healthy food in Asia and as a remedy for several disorders including inflammation, fever, palpitation, hypertension, arrhythmia, sleep problems, skin diseases and leprosy (Slavin and Lloyd 2012; Mukherjee et al. 2009; Varshney and Rzóska 1976; Chopra et al. 1956).

Recently study conducted on the acute toxicity of the NNF extract in mice revealed its LD50 value to be greater than 5000 mg/kg and also confirmed its anxiolytic, antidepressant and antiepileptic effects (Rajput and Khan 2017; Rajput et al. 2017) but very limited work has been done regarding its effects on memory and motor coordination, thus existing study was focused to assess the effects of NNF extract on memory deficits in order to explore its pharmacological use as a memory boosting agent in disorders like AD. Moreover its effect on motor coordination was also identified.

Materials and methods

Animals and grouping

The effect of NNF on memory and motor coordination was assessed on the population of 35 male Wister rats weighing 200–230 g and locally bred albino mice weighing 20–25 g which were evenly distributed in 5 groups ($n = 7$). The research committee representing Department of Pharmacology legitimizes the usage of animals for experiments in accordance with the approach provided by NACLAR (National Advisory Committee for Laboratory Animal Research 2004) and NIH (The National Institute of Health Guide for the Care and Use of Laboratory Animals 2010).

Animal housing

Animals were retained in plastic cages with conservation of room temperature at 22 ± 3 °C. The dampness was kept at 50 to 60% in a switching 12-h light/dark sequence. All animals were given normal diet prepared in laboratory plus water as desired. The rats were transferred to the laboratory almost 60 min before the commencement of experiments. The experiments were executed all through day time. Before the dose administration, general health of animals was evaluated all through the adaptation phase for a week mainly observing edema, diarrhea, lack of activity and ulceration.

Plant authentication and grounding of extract

The fruits of *N. nucifera* were acquired from the native fruit shop of Qasimabad, Hyderabad, Pakistan in June 2015. The fruits were recognized and validated by the Department of Pharmacognosy, Karachi University and receipt sample no NNF-03 was issued.

Grounding of crude extract was completed utilizing cold extraction technique (Hossain et al. 2010). Six kg fruits were cleaned with tap water and then the seeds were manually peeled off from the pods, after they were chopped and then dried out under shade for 6 days and later was ground to fine powder. Similarly, the pods were first dried up under shade for 3 days and then ground to coarse powder. The seed powder along with the seed pod powder was soaked in 10 liters of 98% ethanol for a month with occasional shaking and stirring until the color of the solvent turn into black.

The solvent so obtained was filtered utilizing filter paper Whatman No. 1. Subsequently the material was evaporated under reduced pressure in a rotary evaporator at 40 °C to 45 °C followed by freeze drying at −30 °C. The solid Lyophilized material so attained was stored at −20 °C until further use in doses of 50, 100 and 200 mg/kg by oral route. The quantity finally obtained was 400 g of dried fruit extract.

Preparation of drugs

Gum tragacanth powder obtained from Merck was used up to prepare suspension for the control animals and 3 doses of test group i.e. NNF extracts 50, 100 & 200 mg/kg. 2% gum tragacanth was administered to control group as placebo in the dose of 10 ml/kg orally. 100 ml of warm distilled water was added in 2 g gum tragacanth powder to form 2% suspension. Every time fresh suspension was made for dosing (Madhu et al. 2009; Rajput et al. 2013).

Piracetam 800 mg tablets were procured from one of the renowned medical stores in Karachi and were crushed to powder and diluted in distilled water and administered to animals as reference drug in the dose of 200 mg/kg by mouth (Aslam and Sultana 2015).

Scopolamine hydrobromide trihydrate powder was obtained from Merck and diluted in 0.9% normal saline. It was administered through intraperitoneal (IP) route at a dose of 1 mg/kg for the induction of amnesia in rats (Peng et al. 1997).

Morris water maze task

Richard Morris invented Morris water maze (MWM) test as the most powerful test of hippocampal function that is even valid today to assess learning and memory. It is more widely used than its precursors (radial-arm maze, passive avoidance, T mazes and their variations) because the effects on MWM test post treatment have been more extensively reproduced than the effects observed with any other learning task and is also comparatively easy to set (Morris 1981; Vorhees and Williams 2006).

To assess the effect of NNF extract on memory a population of 35 male Wister rats weighing 200–230 g were used and were evenly separated in to five groups, $n = 7$. Group I was labeled as control and given gum tragacanth. Group II, III, IV were designated as treated groups and were administered 50, 100 and 200 mg/kg of NNF extract. Group V served as reference group and was given piracetam 200 mg/kg. All drugs were given by mouth through orogastric tube once a day for fifteen consecutive days. On the day of experiment i.e. 15th day scopolamine hydrobromide trihydrate was administered by intraperitoneal route in the dose of 1 mg/kg, 40 min after the administration of drugs to animals of all groups for the induction of amnesia then MWM task was performed. NNF extract was initially tested in a dose of 20 mg/kg for 15 days but no significant effects were observed.

Procedure

MWM comprised of a round pool of water tank (60 cm in diameter, 25 cm in height) occupied up to 20 cm with water at 26 °C ± 1. The water tank was made cloudy with safe milk. The tank was then divided into four equal parts with the help of two strings, located at the right angle to one another on the edge of the pool. An immersed stage with top painted in white was put inside the target part (Q4) of the pool with its top apparent 1 cm above the surface of water during the training session. The position of the stage was not changed throughout the training session. During the training session every rat was exposed to four continuous trials every day with a gap of 5 min, during which it was allowed to go on the hidden platform and stay there for 20 s. During the test session, the rats were quietly put in the water between quadrants, facing the wall of pool with drop area changing for every trial and allowed 120 s to find immersed platform (top painted surface immersed 1 cm beneath the water). Rats which could not detect the platform in 120 s were directed carefully to the platform and were allowed to stay there for 20 s. The scored parameter was escape latency (EL) i.e. the time taken by the animal (rat or mouse) to move from the start quadrant in order to explore the hidden platform in the target quadrant and climb on to it (Dhingra and Kumar 2012).

Rota rod test

The Rota rod test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The test estimates riding time in seconds. It can evaluate balance, grip strength and motor coordination of the animal mainly after traumatic brain injury or to test the effect of experimental drugs (Perez et al. 1998; Dunham and Miya 1957). Locally bred albino mice of either sex weighing 20–25 g were used for the assessment of the effect of NNF extract on motor coordination, animals were allocated in to five groups, each having seven animals. Group I was kept as control and given gum tragacanth. Group II, III, IV served as treated and were administered 50, 100 and 200 mg/kg of NNF extract. Group V served as reference and was given piracetam 200 mg/kg. All drugs were given by mouth through orogastric tube once a day for fifteen consecutive days. NNF extract was initially tested in a dose of 20 mg/kg for 15 days but no significant effects were observed.

Procedure

Rota rod apparatus used in current study was composed of a base stage and a horizontal iron rod having 3 cm diameter and 30 cm length, with a non-slippery surface. Animals were tested for their capacity to hold the rod at the speed of 16 rpm for 5 min. The animals were preselected by a learning period of 24 h before the test on their capacity to persist on the rod at16 rpm for 2 min. Forty minutes after the treatment with extract and drug, all the test animals were exclusively allowed to stay on to the rod at the speed of 16 rpm and were observed for a period of 30, 60 and 90 min after dosing. Time interval between the mounting of the animal on the rotating rod and falling off were recorded as the performance time. Time spent in the apparatus was witnessed for a duration of 5 min by means of a stop watch (Perez et al. 1998).

Statistical analysis

The analysis of data was accomplished by taking average and standard error to the average utilizing two sample student T- test and values of $P < 0.05$ were considered as notable and $P < 0.005$ as highly notable. All statistical

procedures were performed utilizing SPSS software version 20 (Walpole 1982).

Results

Effect of *N. nucifera* fruit on scopolamine induced memory deficits in rats

Table 1 exhibits the effect of NNF extract and Piracetam on Escape Latency (the time in seconds taken by the animals to get to the hidden platform) of amnestic rats in Morris water maze test. NNF extract at 200 mg/kg caused highly significant decrease in the escape latency of amnestic rats as compared to control whereas at 100 mg/kg there was significant decrease in the escape latency as compared to control, however at 50 mg/kg the effects of NNF were comparable to control.

Effect of NNF on motor coordination in mice

Table 2 reveals the effect of NNF extract and Piracetam on motor coordination in mice utilizing Rota rod test. The effects of NNF extract at all three doses i.e. 50, 100 and 200 mg/kg were almost comparable to control in riding time. However Piracetam caused highly significant increase in the riding time after 30 and 60 min, but it remained significantly increased after 90 min as compared to control.

Discussion

Current study reveals the effect of NNF extract on escape latency in amnestic rats induced by scopolamine in Morris water maze test. NNF extract showed highly significant decrease in the escape latency in amnestic rats at 200 mg/kg as compared to control whereas at 100 mg/kg the fruit extract showed a significant decrease in the escape latency as compared to control.

Table 1 Effect of NNF and Piracetam on Escape Latency of amnestic rats in Morris water maze test

Groups/ Dose (mg/kg)	Escape Latency sec
Control 2% Gum tragacanth	109 ± 4.6
Test group I NNF 50	100.6 ± 2.3
Test group II NNF 100	94.9 ± 3.2*
Test group III NNF 200	79.2 ± 4.2**
Reference group Piracetam 200	72.5 ± 3.4**

n = 7. Values are Mean ± S.E.M

*$p < 0.05$ significant in comparison to control

**$p < 0.005$ highly significant in comparison to control

Table 2 Effect of NNF and Piracetam on motor coordination in Rota rod test

Groups/Dose (mg/kg)	Riding time sec		
	30 min	60 min	90 min
Control 2% Gum tragacanth	116.6 ± 7.4	111.2 ± 0.7	143.3 ± 3.6
Test group I NNF 50	114.6 ± 7.8	107.6 ± 1.5	140.0 ± 3.6
Test group II NNF 100	117.3 ± 8.9	111.65 ± 1.4	136.4 ± 3.9
Test group III NNF 200	120.2 ± 5.4	113.65 ± 1.7	144.2 ± 1.9
Reference group Piracetam 200	149.3 ± 3.4**	159.4 ± 3.2**	153 ± 0.82*

n = 7. Values are Mean ± S.E.M

*$p < 0.05$ significant in comparison to control

**$p < 0.005$ highly significant in comparison to control

Current study also exhibited the effect of NNF extract on motor coordination in mice by recording riding time on Rota rod. NNF extract at any dose i.e. 50, 100 and 200 mg/kg did not exhibited any substantial change in the riding time as compared to control. Hence muscle coordination was not affected significantly at any dose of the fruit extract.

Scopolamine-induced amnesia in test subjects is a generally cited model that simulates human dementia as a rule and AD in specific (Joshi and Megeri 2008). The administration of scopolamine results in a transient memory loss when given shortly before the experiment. The effectiveness of various cholinomimetic medications to reverse the amnestic effects of scopolamine is currently well described in animals and humans (Kanwal et al. 2010). Recent studies have revealed the M1 Muscarinic acetylcholine receptor binding activities of several flavonoids and also proposed their usefulness in the treatment of AD (Swaminathan et al. 2014).

Several studies have suggested an inverse association between flavonoids intake and various disorders. Previous studies submitted that the administration of flavonoid as dietary supplements improves learning and memory by enhancing blood flow to the brain (Shang et al. 2005; Cambay et al. 2011). Recent study revolves around the synaptic change affecting learning and memory, since flavonoids have direct role in signaling pathways modulating cAMP, CREB and PKC (Wilcox et al. 1999). Another possible mechanism through which flavonoids enhance memory is binding to regulatory proteins e.g. cAMP response element-binding protein (CREB), responsible for the expression of important genes linked to memory. Flavonoids may also enhance neuronal

protein synthesis that may form more synapses and neurotransmitters thereby increasing the strength of communication between neurons and flow of information (Spencer 2010).

Another study suggested that flavonoids possibly promote CREB activation and enhances the levels of brain-derived neurotrophic factor in the hippocampus. Since CREB is required for the assembly of neutrophins-protein, that may lead to neuronal survival, differentiation and function (Hernandez and Abel 2008). Recently Cho et al. 2013 suggested that antioxidant action of flavonoids affect memory and learning by modulating antioxidant enzyme activity or by interrupting signaling cascade and plays important role in blocking oxidative neuronal injury. Hence all of these suggested mechanisms may influence considerably in the memory boosting effects of flavonoids.

In another study saponins extracted from dried leaves of *Albizzia lebbeck* exhibited noteworthy effect on nootropic activity without affecting muscle coordination in albino mice (Une et al. 2001). Neferine, an alkaloid present in the seeds of *N. nucifera* fruit has exhibited antianxiety effects in the elevated plus maze test without affecting muscle coordination as demonstrated in the Rota rod test whereas in the same study diazepam showed antianxiety and muscle relaxant effects as well (Sugimoto et al. 2008). Moreover, neferine has also exhibited antiamnesic effect due to its AChE inhibitory action. Previous studies have shown that *N. nucifera* semen and seedpod inhibited AChE activity and improved memory by inducing choline acetyl transferase (CHAT) expression (Kim et al. 2014). Procyanidins isolated from the *Nelumbo nucifera* seedpod ameliorate scopolamine induced memory impairment by inhibiting AChE activity (Zhang et al. 2009).

Hence *N. nucifera* fruit is a rich source of all of these secondary metabolites i.e. flavonoids, saponins and alkaloids (neferine & procyanidins) which have shown memory boosting effects in various studies, hence it may be suggested that *N. nucifera* fruit has exhibited memory ameliorating effects in scopolamine induced amnesia in rodents through synergistic action of these constituents.

Conclusion

NNF extract have demonstrated ameliorating effects on memory which may be due to its ability to enhance cholinergic neurotransmission and exerting antioxidant effect. Hence NNF extract seems to have great potential for therapeutic applications in the management of memory disorders, such as AD however further studies on biochemical markers of AD such acetylcholinesterase activity, BDNF are required to further reveal the exact role of this fruit on memory deficits.

Acknowledgements The authors are grateful to Dr. Mohtesheem, Associate Professor and Chairman Department of Pharmacognosy for identification and authentication of NNF. The authors are also thankful to Prof. Dr. Iqbal Chaudhary, Director, HEJ, Research Institute of Chemistry for granting permission to use Institute laboratory and workshop facilities. The current research project was performed following endorsement by the Board of Advance Studies & Research (BASR), University of Karachi, Karachi.

References

Abhinav K, Jogender M, Madhusudana K, Vegi GMN, Yogendra KG, Ramakrishna S (2010) Antiamnesic activity of Vitexnegundoin scopolamine induced amnesia in rats. Pharmacol Pharm 1:1–8

Aslam M, Sultana N (2015) Nootropic activity of Vitis vinifera juice in Normal and memory- impaired mice using spatial learning and recognition memory paradigms. Turk J Pharm Sci 12(3):327–336

Cambay Z, Baydas G, Tuzcu M, Bal R (2011) Pomegranate (Punica granatum L.) flower improves learning and memory performance impaired by diabetes mellitus in rats. Acta. Physiol. Ther Hung 98(4):409–420

Cho N, Lee KY, Huh J, Choi JH, Yang H, Jeong EJ, Kim HP, Sung SH (2013) Cognitive-enhancing effects of Rhusverniciflua bark extract and its active flavonoids with neuroprotective and anti-inflammatory activities. Food Chem Toxicol 58:355–361

Chopra RN et al (1956) Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi, p 174

Dhingra D, Kumar V (2012) Memory-enhancing activity of palmatine in mice using elevated plus maze and Morris water maze. Adv Pharmacol Sci 2012:1–7

Dunham NW, Miya TS (1957) A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc 46:208–209

Hernandez P, Abel T (2008) The role of protein synthesis in memory consolidation: Progress amid decades of debate. Neurobiol Learn Mem 89:293–311

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. IJPSR 1:132–138

Indrayan AK et al (2005) Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Curr Sci 89:1252–1255

Iriti M, Vitalini S, Fico G, Faoro F (2010) Neuroprotective herbs and foods from different traditional medicines and diets. Molecules 15: 3517–3555

Joshi H, Megeri K (2008) Antiamnesic evaluation of Clerodendron phlomidis Linn. Bark extract in mice. Braz J Pharm Sci 44:717–725

Kanwal A, Mehla J, Kunchal M, Naidu VGM, Gupta YK, Sistla R (2010) Anti-amnesic activity of Vitex negundo in scopolamine induced amnesia in rats. Pharmacol Pharm 1:1–8 2010

Kim ES, Weon JB, Yun BR, Lee J, Eom MR, Oh KH, Ma CJ (2014) Cognitive enhancing and neuroprotective e ffect of the embryo of the *Nelumbo nucifera* seed. Evid Based Complement Alternat Med 2014(869831):9. https://doi.org/10.1155/2014/869831

Kwon SH, Kim HC, Lee SY, Jang CG (2009) Loganin improves learning and memory impairments induced by scopolamine in mice. Eur J Pharmacol 619:44–49

Leonardo DCL, Franco A, Gustavo ATL, Luciano MA, Lius FMES, Gabriele PDS, Isabela DMA, Jose FNN, Israel F, Karla K (2000) Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. Toxicol Lett 116:189–198

Madhu A, Keerthi PHV, Singh J, Shivalinge GKP (2009) To evaluate the anti-epileptic activity of aqueous root extract of Hemidesmus indicus in rats. Arch Pharm Sci Res 1(1):43–47

Moro CF, Yonekura M, Kouzuma Y, Agrawal GK, Rakwal R (2013) Lotus – a source of food and medicine: current status and future perspectives in context of the seed proteomics. IJLS 7(1):1–5

Morris RGM (1981) Spatial localization does not require the presence of local cues. Learn Motiv 12:239–260

Mukherjee PK, Mukherjee D, Maji AK, Rai S, Mukherjee PK, Saha K, Pal M, Saha BP, Heinrich M (2009) The sacred lotus (Nelumbo nucifera) phytochemical and therapeutic profile. J Pharm Pharmacol 61:407–422

National Advisory Committee for Laboratory Animal Research (2004) Guidelines on the care and use of animals for scientific purposes. P 24

National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (2010) Guide for the care and use of laboratory animals. Prepublication draft, 8thedition, vol 6. The National Academies Press, Washington, DC, p 47

Oliveira DR, Sanada PF, Saragossa Filho AC, Innocenti LR, Oler G, Cerutti JM, Cerutti SM (2009) Neuromodulatory property of standardized extract Ginkgo bilobaL.(EGb761) on memory: behavioral and molecular evidence. Brain Res 1269:68–89. https://doi.org/10.1016/j.brainres.2008.11.105

Pattewar RG, Katedeshmukh, Vyawahare NS, Kagathara VG (2011) Phytomedicines and cognition. Int J Pharm Sci Res 2:778–791

Peng WH, Hsieh MT, Wu CR (1997) Effect of long-term administration of berberine on scopolamine-induced amnesia in rats. Jpn J Pharmacol 74:261–266

Perez RM, Perez JA, Garcia LM, Sossa H (1998) Neuro-pharmacological activity of Solanumnigrum fruit. J Ethnopharmacol 62(1):43–48

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

Rajput MA, Khan RA (2017) Phytochemical screening, acute toxicity, anxiolytic and antidepressant activities of *Nelumbo nucifera* fruit. Metab Brain Dis 32(3):743–749. https://doi.org/10.1007/s11011-017-9963-x

Rajput MA, Khan RA, Feroz Z (2013) Evaluation of antiepileptic activity of methanol extract of Trachyspermum Ammi. L. Arch Biol Sci Belgrade 65:815–819. https://doi.org/10.2298/ABS1303815R

Rajput MA, Khan RA, Assad T (2017) Anti-epileptic activity of *Nelumbo nucifera* fruit. Metab Brain Dis 32:1883–1887. https://doi.org/10.1007/s11011-017-0064-7

Shang Y, Cheng J, Qi J, Miao H (2005) Scutellaria flavonoid reduced memory dysfunction and neuronal injury caused by permanent global ischemia in rats. Pharmacol Biochem Behavior 82:67–73

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

Slavin JL, Lloyd B (2012) Health benefits of fruits and vegetables. Adv Nutr 3(4):506–516. https://doi.org/10.3945/an.112.002154

Spencer JP (2010) The impact of fruit flavonoids on memory and cognition. Br J Nutri 104(Suppl 3): 40–47

Sridhar KR, Bhat R (2007) Lotus: a potential nutraceutical source. J Agri Technol 3:143–155

Sugimoto Y, Furutani S, Itoh A, Tanahashi T, Nakajima H, Oshiro H, Sun S, Yamada J (2008) Effects of extracts and neferine from the embryo of *Nelumbo nucifera* seeds on the central nervous system. Phymed 15(12):1117–1124

Swaminathan M, Chee CF, Chin SP, Buckle MJ, Rahman NA, Doughty SW, Chung LY (2014) Flavonoids with M1 muscarinic acetylcholine receptor binding activity. Molecules 19:8933–8948

Une HD, Sarveiya VP, Pal SC, Kasture VS, Kasture SB (2001) Nootropic and anxiolytic activity of saponins of Albizzia lebbeck leaves. Pharmacol Biochem Behav 69(3–4):439–444

Varshney CK, Rzóska J (1976) Aquatic weeds in South East Asia, 1st edn. Springer, New Delhi, p 39

Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 1:848–858

Walpole RE (1982) Introduction of statistics, vol 1982, Third edn. Macmillan Publishing Company, Inc, New York, pp 247–304

Wilcox LJ, Borradaile NM, Huff MW (1999) Antiatherogenic properties of naringenin, a citrus flavonoid. Cardiovasc Drug Rev 17(2):160–178

Zhang L, Xu JQ, Rong S, Xie BJ, Sun ZD, Zhang YJ, Liu LG (2009) The mixture of procyanidins extracted from the lotus seed pod and bilobalide ameliorates scopolamine-induced memory impairment in mice. Neurosci Bull 25(4):203–208. https://doi.org/10.1007/s12264-009-0309-5