Journal of Scientific & Innovative Research

Evaluation of Polyherbal Preparation Divya Medha Vati for Nootropic, Anxiolytic and Anticholinesterase Activity

Ashok A Muchandi*, Shashikant C Dhawale

Ashok A Muchandi
Department of Pharmacology, Adarsh College of Pharmacy, Vita, Maharashtra. India

Shashikant C Dhawale
Department of Pharmacology, School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

Abstract

Divya Medha Vati is an Ayurvedic polyherbal preparation extensively used in the treatment of depression, stress, loss of memory both in children and adults. In the present study, aqueous solutions of dose (200 and 400 mg/kg, p.o) of Divya Medha Vati was evaluated for nootropic, antianxiety and anticholinesterase activity by using animal models. The dose dependent significant decrease in radial arm maze and Morris water maze performance was found in Divya Medha Vati treated group as compared to respective control group. Scopolamine and sodium nitrite was used for memory intoxication. The significant (p<0.001) increase in social interaction time was an indication of anxiolytic action in treated group. The dose dependent inhibitory action against haloperidol induced catalepsy and clonidine induced hypothermia was seen in Divya Medha Vati treated group. In addition, the treated group showed significant (p<0.001) decrease in level of anticholinesterase activity as compared with control group. Thus, polyherbal preparation Divya Medha Vati was proved potential remedy for cognition disorders.

Keywords: Alzheimer, Anticholinesterase, Divya Medha Vati, Nootropic.

INTRODUCTION

Memory is a multifaceted function of brain which involves multiple neuronal pathways to record sensory stimuli, events, information, and retain them over short or long periods [1]. Impairments in learning and memory may supplement with cognitive disorders like Schizophrenia, Alzheimer's disease amnesia, and depression. These diseases have huge burden on poor memory, lower retention and slow recall of today's stressful and competitive life [2]. Though, commonly prescribed AChE inhibitors, such as Donepezil®, Rivastigmine, and Galantamine were widely prescribed to treat cognition deficit but clinical appraisal of these drugs has shown the incidence of relapses, side effects, and drug interactions [3]. This has been the rationale to search and development of new nootropics, which includes herbal drugs. In recent years, efforts have been made to find novel nootropic agents for the prevention or treatment of memory disorders which involves; which involves numerous Medhya plants like *Zingiber officinale, Bacopa monniera*, *Ocimum sanctum* Linn., *Vaccinium angustifolium, Glycyrrhiza glabra* [4]. Several multi-herbal formulations such as Mentat [5], Bramhi Ghrita [6], SR-105 [7] and Smrithi [8] have been proved for memory enhancement activity.

Divya Medha Vati is an excellent polyherbal remedy produced by Patanjali Ayurveda, India, containing a blend of herbs and extracts that are beneficial in the treatment of depression, stress, loss of memory both in children and adults. It contains Brahmi, Shankhapushpi, Vach, Ustekhadoos, Gaajwan, Maalkaangni, Jatamansi, Saunth, Ashwagandha, Prawal Pisthi, Jaharmohra Pisthi, Moti Pisthi, and extracts of Brahmi, Jyotismati and Shankhapushpi [9]. Considering the literatures survey and no available data of preclinical experiment; the present study was undertaken to evaluate cognition enhancement, anxiolytic and anticholinesterase activity the Divya Medha Vati tablets in experimental animal models.

MATERIALS AND METHODS

Reagents and Drugs

Dithiobis nitrobenzoic acid (DTNB), Metrifonate (Sigma-Aldrich, USA), Scopolamine (German

Correspondence:
Ashok A Muchandi
Department of Pharmacology, Adarsh College of Pharmacy, Vita, Maharashtra. India

Remedies, India), Piracetam (Uni-UCB, India) Divya Medha Vati Tablets (Patanjali Ayurveda, India). All other reagents and solvents used in the experiment were of standard analytical grade.

Animals

Wistar albino rats of both sex (180-250 g) and Swiss albino mice (25-30 g) were used for cognition enhancement activity. All the experimental animals were maintained under standard husbandry conditions (Temp. 22-28 °C; relative humidity 65 ± 10%) and are given standard food pellet (Hindustan Lever) and water *ad libitum* throughout the experimental period. The experimental protocol received approval from the Institutional Animal Ethical Committee (IAEC Clearance: MCPL/IAEC/12-13/04).

Acute toxicity test

The LD50 study of Divya Medha Vati was determined according to OECD guidelines No. 425 by using albino mice of either sex (20–25 g). There are no toxic signs and mortality during 48 h observation period and LD50 was more than of 2000 mg/kg. Thus, effective oral dose 200 and 400 mg/kg in aqueous solution was exposed to animals for pharmacological activity.

Radial arm maze model

It serves as exteroceptive behavioral model, consisting of 36 cm diameter octagonal central hub with eight radial arms. Each arm (45 x 15 cm and 12 cm sides), had small plastic food container mounted at 30 cm from the central hub; elevated 50 cm above the floor [10].

Experimental screening procedure

Average daily food intake for animals was assessed and 85% of this quantity was provided to generate food-motivated performance during the experiment. Before administration of drugs, the rats were trained to choose the arm freely to get the food with upper cut off limit of 300 sec. The training session was considered to be complete when animal visited all eight arms. The time taken by each rat to find the food along with number of reentries was considered to assess radial maze task performance. After successful completion of training, the animals were divided into five groups containing 6 animals each.

- Group-I: Control received normal saline (10 ml/kg)
- Group-II: Normal saline against Scopolamine (1 mg/kg, i.p.)
- Group-III: Standard drug Piracetam (400 mg kg, p.o) against scopolamine (1 mg/kg, i.p.)
- Group-IV: Aqueous solution of DMV (200 mg/kg, p.o) against scopolamine (1 mg/kg, i.p.)
- Group-V: Aqueous solution DMV (400 mg kg, p.o) against scopolamine (1 mg/kg, i.p.)

Animals were treated with standard drug Piracetam and aqueous solutions of DMV (200 and 400 mg/kg) for 10 successive days; after 24 h of last dose (11 day), the amnesia was induced by injecting scopolamine (1mg/kp, i.p). The individual animals of group were exposed to radial arm after 60 min administration of scopolamine. The number of days required for making the mice learned and the latency to find the food along with number of initial correct entries of learned rat was recorded as the effect of the drug on learning and memory process [10, 11].

Morris water maze

The apparatus used consist of a circular water tank (100 cm in diameter)

filled to a depth of 30 cm with water (25 ± 2 °C). The tank was divided into four equal quadrants and a small platform (9 cm width) was concealed in the center of one of the quadrants 2 cm below the water level. The platform remained in the fix position during the training session; and subsequently randomly to evaluate the effect of the drugs on spatial working cognition. The mice were individually released into the water and allowed 90 s to find the platform. Animals exposed to trials until the performance was stable [12].

Evaluation for Learning Facilitation Activity

The preselected mice were divided into five groups containing 6 animals each are treated.

- Group I: Vehicle control, received normal saline (10 ml/kg, p.o), for seven days
- Group II: Served as amnesia control, injected sodium nitrite (95 mg/kg, s.c) after 24 hrs (8th day) of consecutive 7 days of vehicle administration
- Group III: Received standard drug Piracetam (400 mg kg, p.o) for seven successive days and sodium nitrite (95 mg/kg, s.c) after 24 h of last dose.
- Group IV: Received aqueous solution of DMV (200 mg/kg, p.o) for seven successive days and sodium nitrite (95 mg/kg, s.c) after 24 h of last dose.
- Group V: Received aqueous solution of DMV (400 mg kg, p.o) for seven successive days and sodium nitrite (95 mg/kg, s.c) after 24 h of last dose.

After 60 min of sodium nitrite intoxication on 8th day, mice were released into the tank individually and allowed to find the hidden platform. Distance travelled and times taken to find the hidden platform (escape latency) at a fixed location (working memory) or changing location (short term spatial memory) was recorded [13].

Social interaction test

The social interaction arena was an open topped wooden box (60 × 60 × 35 cm) with solid floor, placed in dimly lit room. Rats were placed individually in the test arena for 5 min familiarization session on two consecutive days. On day 3rd, different groups of rat were treated with, normal saline (10 ml/kg. p.o), diazepam (1 mg/kg, i.p) and two doses of DMV 200 and 400 mg/kg, p.o., after 60 min rats were paired on weight and sex basis and placed in the box for 5 min. Social interactions (sniffing, following, kicking, boxing, biting and grooming over the partner) were recorded for each pair of rats [14, 15].

Haloperidol induced catalepsy

Rats were divided into four groups. The control group received vehicle (normal saline, 10 ml/kg p.o) whereas the other group received DMV (200 and 400 mg/kg) for two consecutive days. On the 3rd day, animals received haloperidol (1 mg/kg i.p); 60 min after the vehicle and DMV treatment. Control group received only haloperidol (1 mg/kg i.p). After the treatment, the forepaws of the rat were placed on rod of 1.2 cm diameter upraised 7 cm from the floor. The stages of catatonia induced with haloperidol were studied at 30, 60, 90 and 120 minute [16].

Clonidine induced hypothermia

Clonidine (0.1 mg/kg i.p) was administered to normal (saline treated), standard drug piracetam (400), DMV 200 and 400 mg/kg treated group animals after two consecutive days pretreatment. The control group administered Clonidine (0.1 mg/kg i.p) only. The rectal temperature was

recorded using digital telethermometer (Dolphin, India) after 30 min of clonidine injection upto 120 min [17].

Estimation of brain acetyl cholinesterase (AChE) level

The different groups of animals were treated vehicle (normal saline), standard drug Metrifonate (50 mg/kg, p.o) and test drug DMV 200 and 400 mg/kg for seven consecutive days. Scopolamine (1.4 mg/kg, i.p) was injected after 60 min of last dose treatment. The animals were sacrificed by cervical decapitation under light anesthesia on the 8th day; whole brain was isolated, weighed and homogenized by adding 10 volumes of normal saline. The homogenate was centrifuged at 3000 rpm for 10 min and resultant supernatant liquid was used for estimation of cholinesterase.

Brain cholinesterase activity was measured by the method of Ellman *et al*. (1961) [18] with slight modification. Briefly 0.5 ml of the supernatant of brain homogenate was pipette out in to 25 ml volumetric flask and dilution was made with the help of freshly prepared DTNB (10 mg in 100 of Sorenson phosphate buffer, pH 8.0) solution. From the volumetric flask, 4 ml of solution was transferred to two test tubes. Into one of the test tube, 2 drops of serine solution was added, used to adjust zero. 1ml of substrate solution (75 mg of acetyl choline iodide per 50 ml of distilled water) was added to both test tubes and incubated for 10 min at 30ºC. The resulting yellow color was measured by using spectrophotometer at 420 nm [19].

Statistical Analysis

The data were statically analyzed by student's t-test all the value were expressed as Mean ± SEM, followed by one way ANOVA and Dunnet's t-test. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Cognitive deficit is the condition associated with memory impairment and if the symptoms are left untreated it result in an ominous threat like Alzheimer disease. Various animal models and tasks were used to evaluate memory enhancement, anxiolytic activity of herbal preparation.

Effect on radial arm maze performance

This model is widely used to study spatial reference and spatial working memory in animals [20]. The significant increased radial arm maze performance in scopolamine (1 mg/kg i.p) induced amnesia rats was found to be 194.12 sec as compared to vehicle control group (137.85 sec); indicating impairment in cognition functions. DMV 200 and 400 mg/kg treated group significantly reversed the effects of scopolamine in dose dependent manner. The Piracetam (400 mg/kg) showed significant ($p < 0.001$) decreased performance of radial arm maze task as compared to scopolamine treated group. The percentage memory retention in scopolamine treated group was markedly decreased (36.32%) as compared to that of normal vehicle group (94.95%). The percentage of memory retention in standard drug Piracetam, DMV 200 and DMV 400 mg/kg treated group were exhibited 84.75, 61.96 and 72.22% respectively. Thus, decrease in radial arm performance and increased percentage memory retention of DMV against scopolamine induced dementia is correspondence of improvement of spatial reference and spatial working memory (Table 1).

Table 1: Effect of Divya Medha Vati on radial arm maze performance

Treatment (mg/kg)	Radial arm maze performance			Percentage memory retention
	Before scopolamine		After scopolamine	
	Average time taken (sec)	Average days taken to learn	Average time taken (sec) (11 day)	
Vehicle control (normal saline)	131.21 ± 1.24	6.9	137.85 ± 1.14	94.95%
Scopolamine (1)	118.45 ± 1.17	7.6	194.12 ± 1.35###	36.32%
Piracetam (400) + Scopolamine (1)	37.81 ± 1.08	4.4	43.67 ± 2.01***	84.75%
DMV (200) + Scopolamine (1)	93.65 ± 1.21	6.3	129.28 ± 1.99**	61.96%
DMV (400) + Scopolamine (1)	87.53 ± 1.32	6.0	111.85 ± 1.41***	72.22%

Values represent Mean ± SEM (n=6), one way ANOVA followed by Dunnet's t-test. ###$p < 0.001$ as compared to vehicle control, **$p < 0.01$ and ***$p < 0.001$ as compared to scopolamine group.

Effect on Morris water maze task

Morris (1981) introduced the water maze task, has been used widely to study the neurobiological mechanisms of spatial localization or navigation task for the nootropic agents [21]. The results of escape latency for finding the hidden platform are depicted in Table 2. The sodium nitrite injected group showed marked increase in escape latency and distance travelled as compared to normal group. The DMV 200 and 400 mg/kg treated group exhibited significant decrease in both transfer latency and distance travelled when compared with sodium nitrite intoxication group; which suggest that aqueous solution of DMV improved facilitating learning and memory processes. However, reduced distance travelled thought to be via improved cognition activity. Piracetam, an existing nootropic agent significantly ($p < 0.001$) reverses sodium nitrite induced memory impairments, as expected.

**Table 2:** Effect of Divya Medha Vati on Morris water maze performance

Treatment (mg/kg)	Morris water maze performance			
	Escape latency (sec)		Distance travelled (cm)	
	Working memory	Preferential memory	Working memory	Preferential memory
Vehicle control (Normal saline)	36.4 ± 1.8	34.1 ± 1.1	217.8 ± 5.4	219.4 ± 3.1
Sodium nitrite (95)	$87.6 \pm 1.4^{\#\#\#}$	$81.2 \pm 1.5^{\#\#\#}$	$385.4 \pm 4.2^{\#\#\#}$	$373.9 \pm 4.6^{\#\#\#}$
Piracetam (400) + Sodium nitrite (95)	$43.8 \pm 1.2^{***}$	$42.6 \pm 1.4^{***}$	$188.7 \pm 4.5^{***}$	$182.5 \pm 5.1^{***}$
DMV (200) + Sodium nitrite (95)	$67.7 \pm 1.3^{**}$	$63.8 \pm 1.2^{**}$	$223.6 \pm 3.8^{***}$	$219.7 \pm 4.7^{***}$
DMV (400) + Sodium nitrite (95)	$51.4 \pm 1.7^{***}$	$49.5 \pm 1.7^{***}$	$198.5 \pm 4.6^{***}$	$201.4 \pm 3.9^{***}$

Values represent Mean $\pm$ SEM (n=6), one way ANOVA followed by Dunnet's t-test. $^{\#\#\#}p<0.001$ as compared to vehicle control, $^{**}p<0.01$ and $^{***}p<0.001$ as compared to scopolamine group.

Effect on social interaction behavior

Generally an increase in social interaction is revealing of an anxiolytic effect, whereas a specific decrease in social interaction indicates an anxiogenic effect. This performance suggest use a natural form of behavior as the dependent measure and the dependent variable is the time spent by pairs of rats in social interaction with the partner. [22, 23] In the present study, sodium nitrite treated group showed significant (p<0.001) decrease in social interaction time as compared with normal vehicle treated group. The rats treated with standard drug diazepam and test solutions DMV 200 and 400 mg/kg showed significant increase in social interaction time as compared to sodium nitrite induced intoxication animals; is an indication of anxiolytic action (Figure 1).
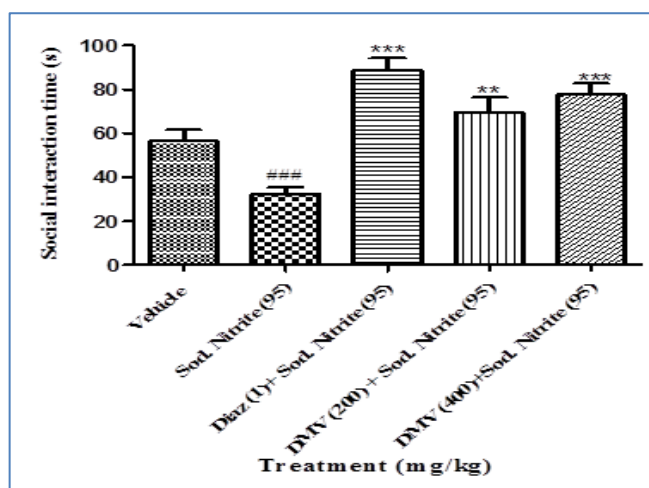
Figure 1: Effect of Divya Medha Vati on social interaction time. Values represent Mean $\pm$ SEM (n=6), one way ANOVA followed by Dunnet's t-test. $^{\#\#\#}p<0.001$ as compared to vehicle control, $^{***}p<0.001$ as compared to sodium nitrite group.

Effect on haloperidol induced catalepsy

Nucleus accumbens and corpus striatum are major brain regions associated with antipsychotics induced catalepsy which seems as a result of blockade of dopaminergic transmissions. The neuroleptic induced catalepsy was suppressed by the drugs which increases the dopaminergic neurotransmission in the brain region [24]. Haloperidol is a non-selective D2 receptor blocker, induces catalepsy by preventing dopaminergic flow in in the striatum. In this study, DMV 200 and 400 mg/kg dose significantly antagonized haloperidol induced catalepsy score after 60 min of administration (Table 3). Thus, ability of the DMV to potentiate or inhibit the haloperidol-induced catalepsy in rats is a suggestive of a reduction or augmentation of dopaminergic neurotransmission in corpus striatum of brain region.

Table 3: Effect of Divya Medha Vati on Haloperidol induced catalepsy score

Treatment (mg/kg)	Cataleptic scores at different time period (min)			
	30	60	90	120
Vehicle control (Normal saline)	17.21 ± 0.51	18.61 ± 0.65	19.21 ± 0.54	17.96 ± 0.41
Haloperidol (1)	15.32 ± 1.45	$27.51 \pm 1.28^{\#\#}$	$34.85 \pm 1.65^{\#\#\#}$	$42.23 \pm 1.27^{\#\#\#}$
DMV (200) + Haloperidol (1)	15.07 ± 0.91	$13.87 \pm 0.54^{**}$	$12.27 \pm 0.47^{***}$	$11.86 \pm 0.82^{***}$
DMV (400) + Haloperidol (1)	14.01 ± 0.63	$9.13 \pm 1.12^{***}$	$8.63 \pm 1.09^{***}$	$8.21 \pm 1.27^{***}$

Values represent Mean $\pm$ SEM (n=6), one way ANOVA followed by Dunnet's t-test. $^{\#\#\#}p<0.001$ as compared to vehicle control, $^{**}p<0.01$ and $^{***}p<0.001$ as compared to Haloperidol group.

Effect on clonidine induced hypothermia

Clonidine induced hypothermia was widely used to evaluate the effect of drug influencing nor-adrenaline mediated behavior in rodents. The clonidine is α- receptor agonist which facilitates secretion of noradrenaline (NA) in the brain which activates cold sensitive neurons in hypothalamus, results in hypothermia [25]. Administration of clonidine produced a fall in rectal temperature in control animals as compared to vehicle treated rats. The rats treated with standard drug piracetam showed increase in rectal temperature. Administration of DMV 200 and 400 mg/kg inhibited clonidine induced hypothermia in dose dependent manner (Table 4). This suggested that, nootropic activity of DMV is linked with the ability to decrease the transmission of NA in hypothalamus.

Effect on brain acetyl cholinesterase (AChE) level

Review of literature has shown that, acetylcholine plays a significant role in memory functions both in humans and rodents. Moreover, scopolamine is a muscarinic antagonist, causes excess phosphorylation of tau proteins leading to the formation of β-amyloid by interfering with muscarinic activity that induce memory impairment [26]. Cholinergic or muscarinic agonists known to be prevent the formation of β-amyloids acts through the GSK-3 enzyme pathway and improve memory [27]. Thus, AChE inhibitors serves as the rationale for the symptomatic treatment of AD, made a new array of therapy in the early stages. The present findings showed that, scopolamine treated group significantly (p<0.01) increased level of AChE as compared with vehicle control group. Metrifonate (50 mg/kg) effectively antagonized the effect of scopolamine. DMV 200 and 400 mg/kg exhibited dose dependent inhibition of level of AChE as compared with scopolamine treated group. Thus, nootropic potential of DMV may contribute with its anticholinesterase activity (Figure 2).

Table 4: Effect of Divya Medha Vati on Clonidine induced hypothermia

Treatment (mg/kg)	Rectal Temperature ºC			
	30 min	60 min	90 min	120 min
Vehicle (normal saline)	35.89 ± 0.14	35.85 ± 0.13	35.01 ± 0.15	35.37 ± 0.17
Clonidine (0.1)	34.89 ± 0.01	34.02 ± 0.11	33.37 ± 0.18	33.35 ± 0.17
Piracetam (400) + Clonidine (0.1)	34.99 ± 0.12	35.15 ± 0.16	35.79 ± 0.10	35.98 ± 0.13
DMV (200) + Clonidine (0.1)	34.90 ± 0.19	34.67 ± 0.19	33.96 ± 0.15	34.01 ± 0.08
DMV (400) + Clonidine (0.1)	34.97 ± 0.13	35.13 ± 0.18	35.71 ± 0.19	35.83 ± 0.07

Values represent Mean ± SEM (n=6).
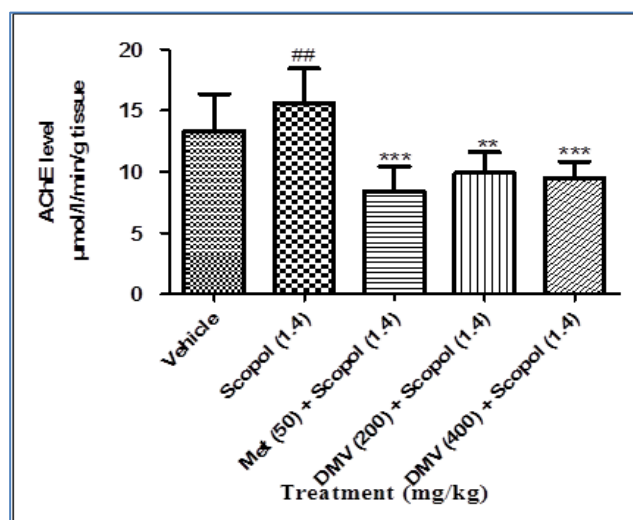
Figure 2: Effect of Divya Medha Vati on brain AChE level. Values represent Mean ± SEM (n=6), one way ANOVA followed by Dunnet's t-test. ##p<0.01 as compared to vehicle control, **p<0.01 and ***p<0.001 as compared to scopolamine group

CONCLUSION

In the present study, we observed that, aqueous solution of Divya Medha Vati significantly improved learning and memory. It also exhibited potential anxiolytic action and significantly reduced the level of AChE. Thus, Diva Medha Vati was found to be effective in the treatment of cognition deficient and psychobehavioural disorders.

REFERECES

1. Reddy DS. Assessment of nootropic and amnestic activity of centrally acting agents. Ind. J. Phamacol. 1997; 29:208-221.
2. Stephenie Schnorr. Course cognitive neuroscience. LACDR/medical pharmacology, Leiden University, 2009.
3. Kim DH, Yoon BH, Kim YW, Lee S, Bum YS, Jung JW. The Seed Extract of *Cassia obtusifolia* ameliorates learning and memory impairments induced by scopolamine or transient cerebral hypoperfusion in mice. Journal of Pharmacol. Sci. 2007; 105:82-93.
4. Pattewar AV, Katedeshmukh RG, Vyawahare NS, Kagathara VG. Phytomedicines and Cognition. Int. J of Pharmaceut. Sci. and Res. 2011; 2(4):778-791.
5. Bhattacharya SK. Nootropic Effect of BR-16A (Mentat), a Psychotropic Herbal Formulation, on Cognitive Deficits-induced by Prenatal Undernutrition, Postnatal Environmental Impoverishment and Hypoxia in Rats. Ind. J of Exp. Biol. 1994; 32(1):31-39.
6. Achliya G, Barabde U, Wadodkar S, Dorle A. Effect of Bramhi Ghrita, an polyherbal formulation on learning and memory paradigms in experimental animals. Ind. J of Pharmacol. 2004; 36(3):159-162.
7. Ladde S, Gouda S, Venkat RN. Evaluation of memory enhancing activity of SR-105 in experimental animals. Int. J of Res. in Ayur & Pharm. 2011; 2(3):973-977.

8. Jyothi V, Satyavati D, Pradeep Kumar C, Maheshwara R. Evaluation of nootropic activity of smrithi: a polyherbal formulation. The Pharma Innovation – Journal. 2014; 3(3):33-41.

9. Jyothi Shenoy. Ayur Times. Divya Medha Vati, 2016. Available from: < https://www.ayurtimes.com/divya-medha-vati/ > (accessed 11.04.2017)

10. Reddy DS. Assessment of Nootropic and amnesic activity of centrally acting drugs. Ind. J. of Pharmacol. 1997; 29:208-221.

11. Oltan DS, Walker JA, Wolf WA. A disconnection analysis of Hippocampal function. Brain Res. 1982; 233:241-53.

12. Achliya G, Barbade U, Wadodkar S, Dorle A. Effect of Brahmi Ghrita, a Polyherbal Formulation on Learning and Memory Paradigms in Experimental Animals. Ind. J. of Pharmacol. 2004; 36(3):159-162.

13. Dong-mei W, Jun Lu, Yuan-lin Z, Zhong Z, Qun S. Purple sweet potato color repairs D-galactose-induced spatial learning and memory impairment by regulating the expression of synaptic proteins. Neurobiol. of Learning and Memory. 2008; 90:19-27.

14. File SE. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Methods. 1996; 2:219-38.

15. Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, *et al*. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes Brain Behav. 2004; 3:287-302.

16. Dikshit SK, Tewari PV, Dixit SP. Anticonvulsant activity of Canscora decussata Roem & Schult. Indian Journal of Physiology and Pharmacology. 1972; 16(1):81-83.

17. Farooq SM, Alla TR, Venkat Rao N, Prasad K, Shalam, Nandakumar K, *et al*. A study on CNS effects of Milk extract of nuts of Semi-carpusana cardium (Anacardaceae). Pharma-cologyonline. 2007; 1:49-63.

18. Ellman GL, Courtney DK, Andres V, Feathestone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961; 7:88-95.

19. Vasudevan M, Parle M. Pharmacological evidence for the potential of Daucus carota in the management of cognitive dysfunction. Bio. Pharm. Bull. 2006; 29:1154-1161.

20. Sunil N Kshirsagar. Nootropic Activity of dried Seed Kernels of *Caesalpinia crista* Linn against Scopolamine induced Amnesia in Mice. International Journal of PharmTech Research. Int. J. Pharm Tech Res. 2011, 3(1).

21. Kulkarni SK. Hand book of experimental pharmacology. 3rd ed. Delhi: Vallabh Prakashan, 1999.

22. Kumar D, Bhat ZA, Kumar V, Khan NA, Chashoo IA, Zargar MI. Effects of Stachys tibetica essential oil in anxiety. Eur J Integr Med. 2012; 4:e169-e176.

23. File SE, Seth P. A review of 25 years of the social interaction test. Eur J Pharmacol. 2003; 463:35-53.

24. Rang HP, Ritter J, Flower RJ, Henderson G. Rang & Dale's Pharmacology. Amsterdam: Elsevier, 2016.

25. Saleem Ahmed, Sridhar KA, Syed Aamir, Salahuddin MD, Monowar Hussain. An experimental study of ethanolic seed extract of vigna mungo linn. for nootropic activity on albino rats. Journal of Pharmaceutical Research. 2016; 15(3):91.

26. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: A review of progress. J Neurol Neurosurg Psychiatr. 1999; 66:137-147.

27. Forlenza OV, Spink JM, Dayanandan R, Anderton BH, Olesen oF, Lovestone S. Muscarinic agonists reduce tau phosphorylation in non-neuronal cells via GSK-3beta inhibition and in neurons. J Neural Transm. 2000; 107:1201-1212.