Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www. elsevier. com/locate/apjtd

Document heading doi: 10.1016/S2222-1808(12)60067-X

Memory-enhancing activity of *Anacyclus pyrethrum* in albino Wistar rats

K Sujith[1*], C Ronald Darwin[1], Sathish[2], V Suba[3]

[1]*Department of Pharmacology, K. K College of Pharmacy, Chennai, Tamilnadu, India*

[2]*Department of pharmacology, Vels College of pharmacy, Chennai Tamilnadu, India*

[3]*Department of pharmacology, Nationalinstitute of siddha, Chennai, Tamilnadu, India*

ARTICLE INFO

Article history:
Received 23 December 2011
Received in revised form 2 January 2012
Accepted 27 February 2012
Available online 28 August 2012

Keywords:
Anacyclus pyrethrum
Scopolamine
Memory
Piracetam
Cholinesterase

ABSTRACT

Objective: To explore the potential effect of ethanolic extract of *Anacyclus pyrethrum* (*A. pyrethrum*) in memory dysfunction. **Methods:** Memory impairment was produced by administration of scopolamine (1mg/kg *i. p*) in rats. Passive avoidance paradigms, elevated plus maze and social learning task was used to assess learning and memory. **Results:** *A. pyrethrum* extract treated group decreased transfer latency in elevated plus maze model paradigm which is an indicative of cognition improvement. In case of passive avoidance paradigm extract treated group exhibited prounced effect in reversal of scopolamine induced amnesia which was revealed by increase in step down latency. Social learning task also revealed the memory enhancing activity of *A. pyrethrum* extract. **Conclusions**: Ethanolic extract of *A. pyrethrum* has been demonstrated to improve cognitive processes by enhancing memory in different experimental paradigms such as passive avoidance paradigms, elevated plus maze and social learning task when administered orallyBrain cholinesterase level was measured to assess central cholinergic activity. The treatment with drugs, which increase cholinergic neurotransmission, causes an improvement in cognitive deficits. The present study suggest that ethanolic extract of *A. pyrethrum* increased brain cholinesterase level and hence it possess memory enhancing activity in scopolamine induced amnesia model by enhancing central cholinergic neurotransmission.

1. Introduction

Memory is the most important function of the brain. Memory is the process by which experiences are recorded and can be used to adapt their responses to the environment and it is vital for survival[1,2] Central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions[3,4]. The dementing condition that has received the utmost attention in the past decade is Alzheimer disease. Impaired cognitive functions are the major features of Alzheimer disease (AD)[5,6]. Loss of cholinergic neurons in nucleus basalis magnocellularis of cortex is one of the most prominent feature of AD, primarily accounting for memory loss[7,8]. Scopolamine is a centrally acting cholinergic agent which causes impairment in learning[9,10]. The treament with drugs which increase cholinergic neurotransmission causes an improvement in cognitive deficits in AD[11,12]. Herbal medicine emphasizes prevention of disease, rejuvenation of our body systems and it extends the life span and makes life healthy[13,14]. Medicinal herbs are indispensible part of traditional medicine and extensive research is done all over the world due to easy access, low cost, lesser side effects and ancestral experience. Plant extracts may also provide a source of new compound as many synthetic drugs have been originated from herbal sources. *Anacyclus pyrethrum* (*A. pyrethrum*), family Asteraceae is used in traditional system of medicine and it is regarded as a tonic to the nervous system[15,16]. The roots contain anacyclin, pellitorine, hydrocarolin, inulin, traces of volatile oil and seasamin. *A. pyrethrum* is a perennial, procumbent herb, which is found throughout India. The plant roots is reported for antinflammatory[17,18], immunostimulating[19,20] anabolic, aphrodisiac activities[21]. However its antiamnesic

*Corresponding author: K. Sujith, Department of Pharmacology, K. K College of Pharmacy, Chennai, Tamilnadu, India.
Telephone: +91944511082
E-mail: sujipillai76@yahoo.co.in

potential remains to be explored. Therefore present study has been undertaken to investigate the beneficial effects of ethanolic extract of *A. pyrethrum* on cognitive function and cholinesterase activity in scopolamine induced amnesia in rats.

2. Materials and methods

2.1. Plant material

The roots of *A. pyrethrum* were procured from ayurvedic drug store in trivandrum, Kerala and the sample was authenticated for their correct botanical identity by professor Jayaraman, National institute of herbal science, Chennai and voucher specimen (no: KK/P/097) of the plant has been be deposited in the department.

2.2. Preparation of extracts

The roots of *A. pyrethrum* was powdered (500g) and ethanolic extract was prepared by simple maceration process using 2L of ethanol. The ethanolic extract was evaporated under reduced pressure using rotavapor evaporator. The yield of the extract was 0. 93% w/w. A suspension was prepared using 2% v/v tween 80 and administered orally.

2.3. Animals

Albino wistar rats of either sex approximately same age group having weight 150-200g were used after being acclimatized for a week at laboratory conditions. They were provided standard rodent pellet diet (Lipton India) and water ad libitum. The animals had free access to food and water and maintained under 12:12 h light and dark cycle. All experiments were carried out during day time from 09. 00 to 17. 00 h. The institutional animal ethical committee approved the protocol (no:290/04/V/CPCSEA/IAEC/PHA-24-29).

2.4. Elevated plus maze test

Rats were divided into six groups consisting of 6 animals per group. Groups are treated with control vehicle (2%v/v tween 80), scopolamine, extract at a dose of 50, 100, 200 mg/kg and other group treated with standard drug piracetam (200 mg/kg, *p.o*). All the animals were treated for 14 days and at the end of treatment period all the extract treated animals were subjected to scopolamine (1 mg/kg *i.p*)[22] 60 minutes after administration of extract, except the first group which served as vehicle control. The elevated plus maze was described as tool for testing memory by the investigator working in the field of psychopharmacology[23]. Elevated plus maze served as extroceptive behavioral model to evaluate learning and memory in rats[24]. The elevated plus maze consisted of two open arms and two closed arms (50cmx10cmx40cm) with an open roof arranged so that the two arms are opposite to each other. The maze was elevated to a height of 50 cm. On the 14th day respectively each rat was placed at end of the open arm, facing away from the central platform. Transfer latency was time taken by the rats to move in to the covered arm with all its four paws, transfer latency was recorded. If the animals did not enter in to one of the covered arms with in 90 s, it was gently pushed in to one of the two covered arms and transfer latency was assigned as 90s. The rat was allowed to explore the maze for 10s and returned to the home cage. Twenty four hours later *i.e.* on 15th day transfer latency was recorded again. The measurement of transfer latency on the day 14 served as parameter for acquisition and those on day 15 served as parameter for retention of memory

2.5. Passive avoidance paradigm

Rats were divided into six groups consisting of 6 per group. Groups are treated with control vehicle (2%v/v tween 80), scopolamine, extract at a dose of 50, 100, 200mg/kg, other group treated with standard drug piracetam (200 mg/kg, *p.o*). At the end of treatment period all the animals were subjected to scopolamine (1 mg/kg *i. p*) 60 min after administration, except the first group which served as vehicle control. Passive Avoidance Behavior based on negative reinforcement was used to examine the long-term memory[25]. The apparatus consisted of a box (27 cm x 27 cm x 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (made up of 3mmstainless-steel rods set 8mm apart), with a wooden platform (10cmx7cmx1. 7cm) in the center of the grid floor. The box was illuminated with a 15W bulb during the experimental period. Electric shock was delivered to the grid floor. The rats were initially trained and was gently placed on the wooden platform set in the center of the grid floor. When the rat stepped down and placed all its paws on the grid floor, shocks (50hz:1.5mA;1s) were delivered for 15 seconds and the step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the rat to step down from the wooden platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2-15 seconds during the training session were used for the acquisition and the retention test. The acquisition task was carried out 90 min after the training session. During the acquisition test, animals was removed from the shock free zone if they did not step down for a period of 60 seconds Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 seconds[26]. Significant increase in SDL value indicated improvement in memory. the animals were euthanized by cervical decapitation and the brains were isolated to evaluate the anticholinesterase activity of *A. pyrethrum*.

2.6. Social recognition task

Rats were divided into five groups consisting of 6 animals per group. Groups received control vehicle 2% v/v tween 80, extracts at a dose of 50, 100, 200 mg/kg, and standard drug piracetam (200 mg/kg, *p.o*) The social learning test was performed according to method of Monndadori C[27]. Male Wistar rats (350-450 g) were used for the experiments and juvenile males (90-110 g) were used as social stimuli. The first day of the experiment, a juvenile rat was introduced in to the adult males cage and the time spent in social investigatory behavior by the adult male within a 5 min fixed interval was recorded and after 24 h, either the same juvenile or an unfamiliar one was placed again into the mature males cage and social investigatory behavior was recorded in a 5 minutes interval.

2.7. Biochemical estimations

For preparation of homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0. 9% sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of cholinesterase level.

2.8. Estimation of cholinergic status in rat brain

The cholinergic marker, cholinesterase was estimated in the whole brain according to Ellman method[28]. The end point was formation of yellow color due to reaction of thiocholine from acetylcholine iodide in presence of dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using spectrophotometer. The sample was first treated with 5, 5′-dinitrobenzoic acid (DTNB) and the optical density (OD) of yellow color compound formed during the reaction at 412 nm every minute was measured.

3. Results

3.1. Elevated plus maze

The effect of vehicle, scopolamine control, *A. pyrethrum* (50 mg/kg, 100 mg/kg, 200 mg/kg) and piracetam were evaluated at end of day 14. Transfer latency on 14th day of drug treatment reflected learning behavior of animals, where as transfer latency of next day reflected retention of information or memory. Scopolamine hydro bromide (1 mg/kg *i. p*) group showed a significant increase in transfer latency values on acquisition as wells as on the retention days as compared with vehicle control rats, indicating impairment in learning and memory. The *A. pyrethrum* at dose level of 50, 100, 200 mg/kg orally demonstrated decrease in transfer latency in a dose dependent manner on transfer latency on 14th day and 15th day in elevated plus maze test as compared to scopolamine control and successfully reversed memory deficit induced by scopolamine (P<0.001). Piracetam used as positive control at a dose of 200 mg/kg also improved learning and memory in rats and reversed the amnesia induced by scopolamine. (table 1). the results obtained was statistically significant (P<0.001).

Table 1
Effect of *Anacyclus pyrethrum* (AP) ethanolic extract on transfer latency (s) (elevated plus maze paradigm) in scopolamine induced amnesia in rats.

Treatment groups	Transfer latency	
	acquisition day 14	retention day 15
Control (vehicle, *p.o*)	34. 66±2. 53	27. 33±1. 40
Scopolamine hydro bromide	135. 33±3. 58	133. 16±4. 85
AP (50mg/kg*p.o*) +Scopolamine	31. 53±2. 63*	16. 83±1. 51*
AP (100mg/kg*p.o*) +Scopolamine	14. 50±0. 88*	9. 00±1. 46*
AP (200mg/kg*p.o*) +Scopolamine	15. 33±1. 054*	6. 16±1. 13*
Piracetam (200mg/kg) +Scopolamine	26. 50±2. 43*	7. 20±1. 18*

Statistical significance test was done by ANOVA followed by Dunnet's *t* test (n=6), Values are mean ± SEM of 6 animals per group.
*P < 0. 001 vs. Scopolamine- treated group

3.2. Passive avoidance paradigm

Step down latency of the second day, 15th day of drug treatment reflected long term memory of animals. Scopolamine (1 mg/kg *i. p*) decreased step down latency in acquisition and retention test indicating impairment in memory. *A. pyrethrum* at a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract orally administered for 14 days markedly (P<0.001) increased step down latency and reversed scopolamine induced amnesia. The group of rats which were treated with piracetam (200 mg/kg *p.o*) for 14 days showed improvement (P<0.001) in memory and reversed amnesia induced by scopolamine. Results were shown in Table 2.

Table 2
Effect of *Anacyclus pyrethrum* (AP) ethanolic extract on step down latency (passive avoidance paradigm) in scopolamine induced amnesia in rats.

Treatment groups	Step down latency	
	acquisition day 14	retention day 15
Control (vehicle, *p.o*)	3. 33±0. 49	3. 16±0. 47
Scopolamine hydro bromide	1. 18±0. 20	1. 38±0. 15
AP (50mg/kg*p.o*) +Scopolamine	9. 63±0. 76*	12. 83±1. 07*
AP (100mg/kg*p.o*) +Scopolamine	13. 83±1. 13*	18. 50±0. 562*
AP (200mg/kg*p.o*) +Scopolamine	23. 16±0. 79*	28. 16±0. 94*
Piracetam (200mg/kg) +Scopolamine	20. 38±0. 94*	34. 36±1. 13*

Statistical significance test was done by ANOVA followed by Dunnet's *t* test (n=6), values are mean ± SEM of 6 animals per group
*P < 0. 001 Vs scopolamine- treated group.

3.3. Social learning test

In the social learning test, results demonstrate that extract of *A. pyrethrum* decreases the investigation time of the same juvenile rat in the forgetting procedure, indicating that the extract enhances short-term social memory in rats and duration of exploration of the familiar partner in the second session of the test was similar to the standard drug piracetam. The facilitation of social memory, demonstrates that the extract displays memory-enhancing properties even when administered orally. Results were shown in Table 3.

Table 3

Effect of *A. pyrethrum* (AP) ethanolic extract on social recognition task

Treatment	Investigation time (secs)	
	First presentation	Second presentation
Control (vehicle, *p.o*)	59±6. 99	54. 33±4. 25
AP (50 mg/kg, *p.o*)	56. 33±1. 22	24. 66±0. 88*
AP (100 mg/kg, *p.o*)	57. 66±3. 70	19. 33±1. 56*
AP (200 mg/kg, *p.o*)	57. 33±3. 35	9. 00±0. 73*
Piracetam (200 mg/kg)	59±3. 27	8. 66±0. 49*

Statistical significance test was done by ANOVA followed by Dunnet's *t* test (n=6), Values are mean ± SEM of 6 animals per group.
*$P < 0.001$ Vs scopolamine- treated group.

3.4. Effect on brain cholinesterase activity

A. pyrethrum at dose of 50 mg/kg, 100 mg/kg and 200 mg/kg *p.o* significantly ($P<0.001$) reduced the levels of cholinesterase as compared to scopolamine treated group by Ellman's kinetic calorimetric method, which is considered as indicator of inhibition of cholinesterase activity in rat brain after 14 days of treatment. Piracetam (20 mg/kg *p.o*) significantly ($P<0.001$) reduced the levels of cholinesterase and indicated in Table 4.

Table 4

Estimation of the Acetylcholinestrease activity in the brain homogenate in scopolamine induced amnesia in rats

Treatment groups	Acetylcholinesterase level (m moles)
Control (vehicle, *p.o.*)	47. 44±0. 72
Scopolamine hydrobromide	68. 03±2. 08
AP (50 mg/kg *p.o.*) +Scopolamine	40. 58±0. 56*
AP (100 mg/kg *p.o.*) +Scopolamine	36. 38±1. 20*
AP (200 mg/kg *p.o.*) +Scopolamine	29. 25±1. 92*
Piracetam (200 mg/kg) +Scopolamine	28. 81±2. 52*

Statistical significance test was done by ANOVA followed by Dunnet's *t* test (n=6), Values are mean ± SEM of 6 animals per group.
*$P < 0.001$ vs scopolamine- treated group.

4. Discussion

Alzheimer disease is a neurodegenerative disorder without an effective treatment. Alzheimer disease is associated with decline in cognitive abilities, patient also have non cognitive symptoms such as depression, apathy and psychosis that impair learning[29,30]. Progressive memory loss, dementia, cognitive deficit are curently seen as medical and social problems of disastrous dimension[31-33]. The administration of antimuscarnic agent scopolamine produces transient memory deficit. Scopolamine amnesia test is widely used as primary screening test for antialzheimer drug[34,35]. In the present study suggest that ethanolic extract of *A. pyrethrum* possess memory enhancing activity in scopolamine induced amnesia model. *A. pyrethrum* extract treated rats showed decrease in transfer latency in elevated plus maze model paradigm which is an indicative of cognition improvement. In case of passive avoidance paradigm administration of *A. pyrethrum* extract for 14 days exhibited prounced effect in reversal of scopolamine induced amnesia which was revealed by increase in step down latency. *A. pyrethrum* has been demonstrated to ameliorate cognitive processes, not only preventing amnesia induced by pharmacological treatments in elevated plus maze and passive avoidance test, but also by producing facilitation of social memory in a social learning task which demonstrates that the extract displays memory-enhancing properties even when administered orally. Acetylcholine is considered as the most important neurotransmitter involved the regulation of cognitive functions. Cholinergic neurons plays important role in cognitive deficit associated with Alzheimer disease and neurodegenerative disease[36,37]. It has been demonstrated that impairment in learning, memory and behavior observed in the patients with dementia are caused at least by changes within cholinergic system[38,39]. Facilitation of central cholinergic activity improves memory[40,41]. In the present study *A. pyrethrum* inhibited acetylcholinestrease enzyme, there by elevating acetylcholine concentration in the brain homogenate and ultimately improved memory in rats. These findings suggest the possible neuroprotective role for *A. pyrethrum*, therefore it seem that *A. pyrethrum* may prove to be useful anti Alzheimer agent in view of its memory enhancing property observed in the present study. However further investigation is warranted to explore the possible involvement of other neurotransmitters responsible for memory improving property of *A. pyrethrum*.

Acknowledgements

Authors are thankful to the management of vels university, Chennai for providing necessary research facility to carry out this research project.

References

[1] Kaur H, Singh D, Singh B, Goel RK. Anti-amnesic effect of Ficus religiosa in scopolamine-induced anterograde and retrograde amnesia. *Pharmaceutical Biology* 2010;**1**:1-8.
[2] Shaji KS, Roy KG, Jacob KS. Behavioral symptoms and caregiver burden in dementia. *Indian J Psychiatry* 2009; **51**(1): 45-49.
[3] Persson CM, Wallin AK, Levander S, Minthon L. Changes in cognitive domains during three years in patients with Alzheimer's disease treated with donepezil. *BMC Neurol*. 2009; **9**: 1-7.
[4] Rahul A, Ethika T, Gunjan S, and Chandishwar N. Cholinergic influence on memory stages: A study on scopolamine amnesic

mice. *Indian J Pharmacol* 2009; **41**(4): 192-196.
[5] Iriti M, Vitalini S, Fico G, Faoro F. Neuroprotective herbs and foods from different traditional medicines and diets. *Molecules* 2010; **15**(5): 3517-55.
[6] Espiritu DA, Rashid H, Mast BT, Fitzgerald J, Steinberg J, Lichtenberg PA. Depression, cognitive impairment and function in Alzheimer's disease. *Int J Geriatr Psychiatry* 2001; **16**(11): 1098-1103.
[7] Prajapati CG, Galani VJ, Patel JS. Review on learning and memory. Inventi Rapid: *Molecular pharmacology* 2011; **2**: 1-8.
[8] Deepika S, Munish P, Ashok K, Nirmal S, Amteshwar S J. Antiamnesic effect of stevioside in scopolamine-treated rats. *Indian J Pharmacol* 2010; **42**: 164-167.
[9] Habbu PV, Mahadevan KM, Shastry RA, Chilakwad SR. Antiamnesic potentiality of Argyreia speciosa (Burm. f) Boj. in mice. *Int Journal Green pharm* 2010; **4**(2): 83-89.
[10] Brian MB, James NR, Glen TP, Robert MD, Robert JS ,Gregory RJ. Cognitive deficits in rats after forebrain cholinergic depletion are reversed by a novel NO mimetic nitrate ester. *Neuropsychopharmacol* 2007; **32**: 505-513.
[11] Pattewar RG, Katedeshmukh, Vyawahare NS, KagatharaVG. *Phytomedicines and cognition Int Journal pharm sci res* 2011; **2**(4): 778-791.
[12] Abhinav K, Jogender M, Madhusudana K, Vegi GMN, Yogendra KG, Ramakrishna S. Anti-amnesic activity of *Vitex negundo* in scopolamine induced amnesia in rats. *Pharmacol Pharm* 2010; **1**: 1-8.
[13] Singh AK, Gupta A, Mishra AK, Gupta V, Bansal P, Kumar S. Medicinal Plant for Curing Alzheimer's Disease. *Int Journal Pharm Biol Arch* 2010; **1**(2): 108- 114.
[14] Guru Prasad BR, Hegde SN. Use of *Drosophila* as a model organism in medicine. *J Med Med Sci* 2010: **1**(12): 589-593.
[15] Nadkarni AK. Indian Materia Medica (popular prakashan), Mumbai. 1989; **1**: 97-98.
[16] Khare CP. *Indian medicinal plants an illustrated dictionary*. Berlin, Germany: Springer-Verlag; 2007, p. 749-755.
[17] Victor R, Esther R, Salvador C, Joseplglesias. Antinflammatory activity of some extracts from plants used in the traditional medicine of north Africa countries. *Farmacognesia* 1995; **10**: 150-153.
[18] Nadkarni KM. Indian plants and drugs with their medicinal properties and uses. Vol. I. New Delhi: Srishti Book Distributors; 2005, p. 97-98.
[19] Bendjeddou D, Lalaoui K, Satta D. Immunostimulating activity of the hot water-soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galanga* and *Citrullus colocynthis*. *J Ethnopharmacol* 2003; **88**: 155-160.
[20] Bendjeddou D, Lalaoui K, Satta D. Immunologically active polysaccharides Isolated from *Anacyclus pyrethrum*. *Libyan Agric Res Cen J Int* 2010; **1**(3): 128-133.
[21] Vikas S, Mayank T, Nagendra SC, Vinod KD. Evaluated the Anabolic, Aphrodisiac and Reproductive Activity of *Anacyclus pyrethrum* DC in Male Rats. *Sci Pharm* 2009; **77**: 97-110.
[22] Vaisaman N, Pelled D. N-3phosphatidylserine attenuated scopolamine induced amnesia in middle aged rats. *Prog Neuro Pyscho pharmacol Biol Pyschiatry* 2009; **33**: 952-959.
[23] Kirti, Kulkarni S, Kasture SB, Mengi SA. Efficacy study of Prunus amygdalus (almond) nuts in scopolamine-induced amnesia in rats. *Indian J Pharmacol* 2010; **42**(3): 168-173.
[24] Raghavendra M, Maiti R, Shafalika K, Acharya SB. Role of Ocimum sanctum in the experimental model of Alzheimer's disease in rats. *Int Journal Green pharm* 2009; **3**(1): 6-15.
[25] Nade VS, Kanhere SV, Kawale LA, Yadav AV. Cognitive enhancing and antioxidant activity of ethyl acetate soluble fraction of the methanol extract of Hibiscus rosa sinensis in scopolamine-induced amnesia. *Indian J Pharmacol*. 2011; **43**(2):137-42.
[26] Nilofar S N, Sommath NM, Rahul SA, Chandrakanth SM. Memory enhancing activity of Rosa Alba in mice. *Int Journal Green pharm* 2009; **3**: 239-242.
[27] Monndadori C, Preiswerk G, Jaekel. Treatment with a GABA B receptor blocker improves the cognitive performance of mice, rats and rhesus monkeys. *J pharmacol commun* 1992; **2**: 93.
[28] Ellman GL, Courtney DK, Andres V, Featherstone RM. A new and rapid calometeric determination of acetylcholinestrease activity. *Biochemical Pharmacology* 1961; **2**: 88-95.
[29] Fratiglioni, Winblad B. Prevention of Alzheimer's disease and dementia. Major findings from the Kungsholmen *Project. Physiol. Behav* 2007; **92**(1-2): 98-104.
[30] Zhang H, Han T, Zhang L, Yu CH, Wan DG, Rahman K et al: Effects of tenuifolin extracted from radix polygalae on learning and memory: A behavioral and biochemical study on aged and amnesic mice. *Phytome* 2008; **15**: 587-594.
[31] Joshi H, Milind P. Evaluation of the antiamnesic effects of *Phyllanthus amarus* in mice. *Colombia Médica* 2007; **38**: 132-113.
[32] Adiga S, Trivedi P, Ravichandra V, Deb D, Mehta F. Effect of *Punica granatum* peel extract on learning and memory in rats. *Asian Pac J Trop Med* 2010; **3**(9): 687-690.
[33] Otari KV, Bichewar OG, Shete RV, Upasani CD. Effect of hydroalcoholic extract of *Vitex negundo* Linn. leaves on learning and memory in normal and cognitive deficit mice. *Asian Pac J Trop Med* 2010; **3**(9): S104-S111.
[34] Joshi H, Navneet K, Jyotibala C. Evaluation of nootropic effect of Argyreia speciosa in mice. *J Health Sci* 2007; **53**(4): 382-388.
[35] Han CK, Park YH, Jin DQ, Hwang YK, Oh KB, Han JS. SK-PC-B70M from *Pulsatilla koreana* improves scopolamine-induced impairments of memory consolidation and spatial working memory. Brain Research 2007; **1184**: 251-1259.
[36] Jagdeep SD, Prasad DN, Rajiv G, Avinash CT. Role of traditional medicine in neuropsychopharmacology. *Asian J Pharm Clin Res* 2009; **2**(2): 72-76.
[37] Kumar S, Maheshwari KK, Singh V. Effects of *Mangifera indica* fruit extract on cognitive deficits in mice. *J Environ Biol* 2009; **30**(4): 563-566.
[38] Bihaqi SW, Sharma M, Singh AP, Tiwari M. Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. *J Ethnopharmacol* 2009; **124**(3): 409-415.
[39] Ahmed T, Gilani AH. 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* 2009; **91**: 554-559.
[40] Heo H, Shin Y, Cho W, Choi Y, Kim H, Kwon YK. Memory improvement in ibotenic acid induced model rats by extracts of *Scutellaria baicalensis*. *J Ethnopharmacol* 2009; **122**(1): 20-27.
[41] Apryani E, Hidayat TM, Moklas MAA, Fakurazi S, Idayu NF. Effects of mitragynine from *Mitragyna speciosa* Korth leaves on working memory. *J Ethnopharmacol* 2010; **129**: 357-360.