



Evaluation of *Anacyclus pyrethrum* root extract on Learning and Memory-enhancing activity in albino Wistar rats

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Abstract

To explore the potential effect of ethanolic extract of *Anacyclus pyrethrum* (*A. pyrethrum*) in memory dysfunction. 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. *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 pronounced 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. 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 orally brain cholinesterase level was measured to assess central cholinergic activity. 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.

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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 (Kaur *et al.*, 2010 and shaji *et al.*, 2009). Central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions (Persson *et al.*, 2009 and Rahul *et al.*, 2009). 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) (Iriti *et al.*, 2010 and Espiritu *et al.*, 2001). Loss of cholinergic neurons in nucleus basalis magnocellularis of cortex is one of the most prominent features of AD, primarily accounting for memory loss (Prajapati *et al.*, 2011 and Deepika *et al.*, 2010). Scopolamine is a centrally acting cholinergic agent which causes impairment in learning (Habhu *et al.*, 2010 and Brain *et al.*, 2007). The treatment with drugs which increase cholinergic neurotransmission causes an improvement in cognitive deficits in AD (Pattewar *et al.*, 2011 and Abhinav *et al.*, 2010). Herbal medicine emphasizes prevention of disease, rejuvenation of our body systems and it extends the life span and makes life healthy (Singh *et al.*, 2010 and Guru *et al.*, 2010). Medicinal herbs are indispensable 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 compounds as many synthetic drugs have been originated from herbal sources. *Anacyclus pyrethrum*, family Asteraceae is used in traditional system of medicine and it is regarded as a tonic to the nervous system (Nadkarni, 1989 and Khare, 2007). The roots contain anacyclin, pellitorine, hydrocarolin, inulin, traces of volatile oil and sesamin. *A. pyrethrum* is a perennial, procumbent herb, which is found throughout India. The plant roots are reported for anti-inflammatory (Victor *et al.*, 1995 and Nadkarni, 2005) immunostimulating, (Bendjeddou *et al.*, 2003 & 2010), anabolic, aphrodisiac activities (Vikas *et al.*, 2009). However its anti-amnesic 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.

Materials and methods

Plant material

The roots of the plant *A. pyrethrum* were collected from Rajshahi University campus, Bangladesh. It was identified and authenticated in the Department of Botany, Rajshahi University, Bangladesh.

Preparation of extracts

Fresh roots of the plant were washed under running tap water and air dried for about 4 weeks and then homogenized to fine powder and stored in airtight bottles. The powder of roots (500g) was extracted with 2L of ethanol using conical flask in a shaking incubator at 28°C for two days. The extract was filtered and evaporated until dryness. The extract was stored at 4°C. The yield of the extract was 0.93% w/w. A suspension was prepared using 2% v/v tween 80 and administered orally.

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 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.

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 100, 200, 400 mg/kg and other group treated with standard drug piracetam (400 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.*) (Vaisaman and Pelled, 2009) 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 (Kirti *et al.*, 2010). Elevated plus maze served as exteroceptive behavioral model to evaluate learning and memory in rats (Raghavendra *et al.*, 2009). The elevated plus maze consisted of two open arms and two closed arms (50 cm x 10 cm x 40 cm) 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 into one of the covered arms within 90 s, it was gently pushed in to one of the two covered arms and transfer latency was assigned as 90 s. The rat was allowed to explore the maze for 10 s 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

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 100, 200, 400 mg/kg, other group treated with standard drug piracetam (400 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 (Nade *et al.*, 2011). 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 3 mm stainless-steel rods set 8 mm apart), with a wooden platform (10 cm x 7 cm x 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock was delivered to the grid floor. The rats were initially

trained and were 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 (50 Hz: 1.5 mA; 1 s) 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 were 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 (Nilufar *et al.*, 2009). 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*.

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 100, 200, 400 mg/kg, and standard drug piracetam (400 mg/kg, *p.o*). The social learning test was performed according to method of Monndadori (1992). 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.

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.

Estimation of cholinergic status in rat brain

The cholinergic marker, cholinesterase was estimated in the whole brain according to Ellman method (1961).

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.

Results

Elevated plus maze

The effect of vehicle, scopolamine control, *A. pyrethrum* (100 mg/kg, 200 mg/kg, 400 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 well as on the retention days as compared with vehicle control rats, indicating impairment in learning and memory.

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, <i>p.o</i>)	34.66±2.53	27.33±1.40
Scopolamine hydro bromide	135.33±3.58	133.16±4.85
AP(100 mg/kg <i>p.o</i>)+Scopolamine	31.53±2.63*	16.83±1.51*
AP(200 mg/kg <i>p.o</i>)+Scopolamine	14.50±0.88*	9.00±1.46*
AP(400 mg/kg <i>p.o</i>)+Scopolamine	15.33±1.054*	6.16±1.13*
Piracetam(400mg/kg)+Scopolamine	26.50±2.43*	7.20±1.18*

Statistical significance test was done by ANOVA followed by Dunnet's test (n=6), Values are mean ± SEM of 6 animals per group.*P < 0.001 vs. Scopolamine- treated group.

The *A. pyrethrum* at dose level of 100, 200, 400mg/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 400 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).

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 100 mg/kg, 200 mg/kg and 400 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 (400 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.

Social learning test

In the social learning test, results demonstrate the 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.

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

Treatment groups	Transfer latency	
	Acquisition day 14	Retention day 15
Control(vehicle, <i>p.o</i>)	3.33±0.49	3.16±0.47
Scopolamine hydro bromide	1.18±0.20	1.38±0.15
AP(100 mg/kg <i>p.o</i>)+Scopolamine	9.63±0.76*	12.83±1.07*
AP(200 mg/kg <i>p.o</i>)+Scopolamine	13.83±1.13*	18.50±0.562*
AP(400 mg/kg <i>p.o</i>)+Scopolamine	23.16±0.79*	28.16±0.94*
Piracetam(400mg/kg)+Scopolamine	20.38±0.94*	34.36±1.13*

Statistical significance test was done by ANOVA followed by Dunnet'st test (n=6), Values are mean ± SEM of 6 animals per group. *P < 0. 001 vs. Scopolamine- treated group.

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)	
	1st presentation	2nd presentation
Control(vehicle, <i>p.o</i>)	59±6.99	54.33±4.25
AP(100 mg/kg <i>p.o</i>)	56.33±1.22	24.66±0.88*
AP(200 mg/kg <i>p.o</i>)	57.66±3.70	19.33±1.56*
AP(400 mg/kg <i>p.o</i>)	57.33±3.35	9.00±0.73*
Piracetam(400 mg/kg)	58±3.27	8.66±0.49*

Statistical significance test was done by ANOVA followed by Dunnet'st test (n=6), Values are mean ± SEM of 6 animals per group. *P < 0. 001 vs. Scopolamine- treated group.

Effect on brain cholinesterase activity

A. pyrethrum at dose of 100 mg/kg, 200 mg/kg and 400 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 (400mg/kg *p.o*) significantly ($P < 0.001$) reduced the levels of cholinesterase and indicated in Table 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 (Fratiglioni *et al.*, 2007 and Zhang *et al.*, 2008). Progressive memory loss, dementia, cognitive deficit are currently seen as medical and social problems of disastrous dimension (Joshi *et al.*, 2007, Adiga *et*

al., 2010 and Otari *et al.*, 2010). The administration of antimuscarinic agent scopolamine produces transient memory deficit. Scopolamine amnesia test is widely used as primary screening test for anti-Alzheimer drug (Joshi *et al.*, 2007 and Han *et al.*, 2007). 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.

Table 4. Estimation of the Acetylcholinesterase 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(100 mg/kg p.o)+Scopolamine	40.58±0.56*
AP(200 mg/kg p.o)+Scopolamine	36.38±1.20*
AP(400 mg/kg p.o)+Scopolamine	29.25±1.92*
Piracetam(400 mg/kg)+Scopolamine	28.81±2.52*

Statistical significance test was done by ANOVA followed by Dunnet's test (n=6), Values are mean ± SEM of 6 animals per group. *P < 0.001 vs. Scopolamine- treated group.

In case of passive avoidance paradigm administration of *A. pyrethrum* extract for 14 days exhibited pronounced 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 in the regulation of cognitive functions. Cholinergic neurons play an important role in cognitive deficit associated with AD and neurodegenerative disease (Jagdeep *et al.*, 2009 and Kumar *et al.*, 2009).

It has been demonstrated that impairment in learning, memory and behavior observed in the patients with dementia are caused at least by changes within the cholinergic system (Bihaqi *et al.*, 2009 and Ahmed *et al.*, 2009). Facilitation of central cholinergic activity improves memory (Heo *et al.*,

2009 and Apriyani *et al.*, 2010).

Conclusion

In the present study *A. pyrethrum* inhibited acetylcholinesterase enzyme, thereby 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 seems that *A. pyrethrum* may prove to be a useful anti-Alzheimer agent in view of its memory-enhancing properties observed in the present study.

However, further investigation is warranted to explore the possible involvement of other neurotransmitters responsible for memory-improving properties of *A. pyrethrum*.

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