ORIGINAL ARTICLE

Memory-enhancing activity of *Rose alba* in mice

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Alzheimer's disease (AD) is a neurodegenerative disorder currently without an effective treatment. Impairment of memory is the initial and most significant symptom of AD. Memantine is the first novel class of AD medications acting on the glutaminergic system and produces symptomatic improvement in learning. Nootropic agents such as piracetam, aniracetam, and choline esterase inhibitors like donepezil are being used to improve memory, mood, and behavior, but the resulting side-effects associated with these agents have made their use limited. The present study was undertaken to investigate the effects of *Rose alba* (RA) on learning and memory in mice. Male Swiss albino mice (3 months old) weighing around 25 g were employed in the present investigation. Elevated plus-maze and passive-avoidance apparatus served as the exteroceptive behavioral models, and diazepam-induced amnesia served as the interoceptive behavioral models. RA (100 and 200 mg/kg p.o.) was administered for eight successive days to the mice. Piracetam (200 mg/kg i.p.) was used as a standard nootropic agent. RA improved learning and memory of mice as indicated by decreased transfer-latency and increased step-down latency. RA significantly reversed the amnesia induced by diazepam (1 mg/kg, i.p.). The results indicate that the aqueous extract of calyces of RA might prove to be a useful memory restorative agent in the treatment of cognitive disorders.

Key words: *Rose alba,* nootropic, Alzheimer 's disease

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes, and ultimately death.[1] It is a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity, and language.[2] Memantine is the first novel class of AD medications acting on the glutaminergic system.[3] It regulates glutamate which is involved in the information process, storage and retrieval.[4] It is the only agent currently approved for treatment of AD.[5] It produces symptomatic improvement in learning under the conditions of tonic NMDA receptor activation.[6] Nootropic agents such as piracetam,[7] pramiracetam, aniracetam,[8] and choline esterase inhibitors like donepezil are being primarily used to improve memory. However, the resulting adverse effects associated with these agents have limited their use[9,10] and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. The central cholinergic pathways play a prominent role in learning and memory processes.[11] Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals and human beings.[12] In the recent years, there has been a rise in the interest of scientific community and pharmaceutical laboratories to explore the pharmacological actions of herbs. Several plants have been reported to possess nootropic activity. The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence.[13] Plants like *Bacopa monniera,*[14] *Azadirachta indica,*[15] *Withania somnifera,*[16] as well as *Ocimum sanctum,*[17] have been investigated for their effect on cognitive functions. The present study was undertaken to investigate the effects of *Rose alba* (RA) on learning and memory in mice by using elevated plus-maze and passive-avoidance test. RA *(*family: Rosaceae) flower is bitter, acrid, pungent, with flavours; cooling astringent to the bowels, aphrodisiac; cures, 'tridosha' stomatitis, leprosy, biliousness, burning sensation; purifies the blood; improves the complexion, the taste, and the appetite. The flower smells sweet; enriches the blood; carminative, laxative; lessens inflammation; useful in cold and catarrh of the nose, headache, toothache, bronchitis, disease of the lungs, ophthalmia, rheumatism; applied to piles; and its perfume is a tonic for the brain and the heart.[18]

MATERIALS AND METHODS

Animals

Male Swiss albino mice (3 months old), weighing around 25 g, were used in the present study. They had free access to food and water and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 hours each. The animals were acclimatized for at least 5 days before behavioral experiments. Experiments were

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Received: 20-03-2008; **Accepted:** 05-11-2008; **DOI:** 10.4103/0973-8258.56283

carried out between 09:00 and 16:00 hours. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee (IAEC) regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals.[19] IAEC approved the experimental protocol, and care of animals was taken as per the guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Preparation of Extract

The flowers were extracted by the maceration process. The mixture was filtered and evaporated to dryness. The dark brownish semisolid mass obtained was stored in a well-closed airtight light-resistant container.

Drugs

Durgs such as piracetam (UCB India Pvt. Ltd) and diazepam (Ranbaxy, India) were used in the present study.

Laboratory Models for Testing Learning and Memory

(i) Elevated plus-maze.
(ii) Passive shock avoidance paradigm.
(iii) Diazepam-induced amnesia

Experimental Protocol

In the present investigation, the mice were divided into different groups (Control, diazepam treated, piracetam treated, RA extract and RA + diazepam treated groups) comprising six animals each for investigations using various interoceptive (stimulus lies within the body) as well as exteroceptive (stimulus lies outside the body) memory models. RA (100 and 200 mg/kg, i.p.) was administered to mice of different groups. These mice were exposed to the training session using elevated plus-maze or passive avoidance apparatus on eighth day after 90 min of the last dose. Retention (memory) of the learned task was recorded after 24 hours i.e., on ninth day. Amnesia was induced in separate groups (interoceptive model) by diazepam (1 mg/kg, i.p.) on eighth day after 90 minutes of the last dose. Piracetam (200 mg/kg, i.p.), an established nootropic agent was injected for 8 days to the positive control group of animals.

Exteroceptive Behavior Models

Elevated plus-maze

Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in mice. The procedure, technique and end point for testing memory were followed as per the parameters described by the investigators working in the area of psychopharmacology.[20, 21] The elevated plus-maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) extended from a central platform (5 cm × 5 cm), and the maze was elevated to a height of 25 cm from the floor.[22] On the first day (i.e., eighth day of drug treatment), each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24 hours after the first day trial (i.e., ninth day, 24 hours after last dose). Significant reduction in the TL value of retention indicated improvement in memory.

Passive avoidance paradigm

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory.[23,24] The apparatus consisted of a box (27 cm × 27 cm × 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 × 7 cm × 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 (20 V, AC) was delivered to the grid floor.[25] Training (i.e., eighth day of drug treatment) was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped-down placing all its paws on the grid floor, shocks were delivered for 15 seconds and the step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to the grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2–15 seconds during the first test were used for the second session and the retention test. The second session was carried out 90 minutes after the first test. During the second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from the shock-free zone, if they did not step down for a period of 60 seconds and were subjected to the retention test. Retention (memory) was tested after 24 hours (i.e., ninth day, 24 hours after last dose) 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.

RESULTS

Effect on Transfer Latency using Elevated Plus-maze

TL of second day (day 9 of drug treatment) reflected retention of learned task or memory. The mice treated with RA (100 and 200 mg/kg, i.p.) showed dose-dependent reduction in TL of ninth day, indicating significant improvement in memory, when compared with the control group. Diazepam

Table 1: Effect of *Rose alba* extract on transfer latency of mice using elevated plus-maze paradigm

Treatment	Dose (mg/ kg)	TL on first day	TL after 24 hours
Control (distilled water)	10	24.85 ± 3.79	26.85 ± 2.86
Diazepam	1	49.7 ± 7.03*	29.31 ± 3.05*
Piracetam	200	20.04 ± 3.79*	12.0 ± 2.236*
RA	100	22.64 ± 1.463*	20 ± 2.29*
RA	200	21.07 ± 4.15*	19.38 ± 2.324*
Piracetam + diazepam	200 1	27.34 ± 1.92#	17.43 ± 3.0#
RA + diazepam	200 1	24.32 ± 3.07#	21.85 ± 2.82#

TL - Transfer latency, Each group consists of six animals. Values are expressed as mean ± S.E.M. ANOVA followed by the unpaired '*t*'-test. **P* <0.05 as compared to the control group; #*P* <0.05 as compared to diazepam alone.

(1 ml/kg, i.p.) injected before training significantly increased ($P < 0.05$) the TL of ninth day indicating impairment in memory (amnesia). The mice treated with RA (100 and 200 mg/ kg, i.p.) for nine successive days reversed successfully the amnesia-induced diazepam. Piracetam (used as the positive control) at the dose of 200 mg/kg, i.p. improved memory ($P < 0.05$) of mice and reversed the amnesia induced by diazepam as expected [Table 1].

Effect on Step-down Latency using Passive Avoidance Paradigm

SDL of second day (day 9 of drug treatment) reflected the long-term memory of animals. Various concentrations of RA (100 and 200 mg/kg, i.p.) administered to mice for 8 days showed dose-dependent increase in SDL values as compared to respective control groups. RA (100 and 200 mg/kg, i.p.) administered for 8 days reversed diazepam-nduced amnesia. The groups of mice, which were treated with piracetam (200 mg/kg, i.p.) for eight successive days showed improvement in memory of mice [Table 2].

Table 2: Effect of *Rose alba* extract on step-down latency of mice using passive shock avoidance apparatus

Treatment	Dose (mg/kg)	SDL after 24 hours
Control (distilled water)	10	118 ± 3.42
Diazepam	1	49.7 ± 7.03*
Piracetam	200	300 ± 1.87*
RA	100	282.1 ± 4.27*
RA	200	300 ± 6.38*
Piracetam + diazepam	200 1	28.2 ± 2.19#
RA + diazepam	200 0.4	245 ± 3.29#

SDL - Step-down latency, Each group consists of six animals. Values are expressed in mean ± S.E.M. ANOVA followed by the unpaired '*t*'-test. **P* <0.05 as compared to the control group; #*P* <0.05 as compared to diazepam alone

DISCUSSION

AD is a neurodegenerative disorder currently without an effective treatment. Impairment of memory is the initial and most significant symptom of AD. AD is associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy, and psychosis that impair daily living.[1] The most common cause of dementia in the elderly is probably AD.[27] Despite the severity and high prevalence of this disease, the allopathic system of medicine is yet to provide a satisfactory antidote. The central cholinergic system plays an important role in learning and memory.[28] In the present study, RA extract (100 and 200 mg/kg) administered orally for 8 days improved learning and memory of mice significantly in both the exteroceptive behavioral models employed. Furthermore, pretreatment with RA (100 and 200 mg/kg) for 8 days also protected the animals from learning and memory impairment produced by interoceptive stimuli (diazepam) when assessed on passive avoidance paradigm. Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals.[29] Passive avoidance behavior is based on negative reinforcement and is used to examine long-term memory.[30] Both piracetam and RA extract meet major criteria for nootropic activity, namely improvement of memory in the absence of cognitive deficit. However, further investigations using more experimental paradigms are required for further confirmation of nootropic potential of RA in the treatment of various cognitive disorders.

CONCLUSION

In the present investigation, RA has shown promise as a memory-enhancing agent in mice in the laboratory models employed.

ACKNOWLEDGMENT

Authors are grateful to UCB India, Ltd, Vapi (Gujarat, India), for the generous supply of piracetam as a gift sample.

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Source of Support: Nil, **Conflict of Interest:** None declared.