Research Article

Ameliorative Effects of Roots of *Asparagus adscendens* Roxb. on Cognitive Impairments and Brain Aging Induced By Scopolamine and Diazepam in Animal Models Relevant to Alzheimer's Disease

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ABSTRACT

Memory loss in dementia is often the most disabling feature of many disorders, impairing the normal daily activities of the patients and profoundly affecting their families. Treatment of cognitive disorders like dementia and Alzheimer's disease has been challenging since no potential drug is available at present with proved efficacy. In the present study, nootropic activity of methanolic extract of roots of *Asparagus adscendens* Roxb.(AR) was studied in mice. Elevated plus maze and passive avoidance paradigm were employed to evaluate learning and memory. Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) were used to induce amnesia in mice. AR (50 and 100 mg/kg, p.o.) significantly attenuated amnesic deficits induced by scopolamine, diazepam and natural aging. Furthermore, it also reversed aging induced amnesia due to natural aging of mice. AR profoundly increased whole brain acetyl cholinesterase inhibition activity. Hence, AR might prove to be a useful memory restorative agent in the treatment of dementia seen in the elderly. The underlying mechanism of its action may be attributed to its antioxidant and acetyl cholinesterase inhibition properties.

Keywords: *Acetyl cholinesterase, Asparagus adscendens, Dementia, Memory, Scopolamine*.

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INTRODUCTION

Memory function is vulnerable to a variety of pathologic processes including neurodegenerative diseases, strokes, tumors, head trauma, hypoxia, cardiac surgery, malnutrition, attention deficit disorder, depression, anxiety, the side effects of medication, and normal ageing[1]. Alzheimer's disease is a progressive neurodegenerative brain disorder characterized by impairment of new information storage or retrieval[2]. 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[3]. With the increase of life expectancy and the consequent increase in the number of patients suffering from brain degenerative disorders, the search for products which reduce or minimize cognitive deficits associated with aging has become very crucial. It is estimated that within the next 50 years, approximately 30% of the world population will be aged 65 years or aged[4]; more importantly by the year 2025, 70% of world's aged population will be living in developing countries[5]. The National Institute of Health predicts, if the current trend continues, there will be more than 8.5 million AD patients by the year 2030 in USA alone[6-7]. Nootropic agents such as piracetam[8], pramiracetam,

aniracetam[9] and choline esterase inhibitors like Donepezil® are presently used for improving memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use[10] and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders.

For thousands of years, plants have been used for cognitive impairment in India. Ayurveda, the Indian system of medicine describes the use of medhya rasayana (rejuvenating and intellect promoting) drugs in the management of nervous disorders. The Ayurvedic concept of rasayana consists of specialized class of drugs which prevent ageing, increase longevity, impart immunity, improve mental functions and add vigor and vitality of the body[11].

Asparagus adscendens is a flowering perennial, spring plant species in the genus Asparagus[12]; which is a large genus of herbs and under shrubs with stout, tuberous roots and erect or climbing stems. It was once classified in the lily family, like its Allium cousins, onions and garlic, but the Liliaceae has been split and the onion-like plants are now in the family Amaryllidaceae and asparagus in the Asparagaceae family[13]. *Asparagus adscendens* Roxb. is known by various common names i.e. Shatawari, Safed musli, Shatavar, Shatamuli, Sahasrapal, Sainsarbuti. It was initially grown in thick forest in natural form, and is a customary medicinal plant; is an herb with sub-erect lanceolate leaves and tuberous root system. The plant form of *Asparagusadscendens* is a shrub of struggling nature much branched, spines with woody stem, It can grow up to an utmost height of 1.5 feet. Cladodes are 0.6-1.2 cm long linear in shape but stout, straight, bear spines. Flowers are small, white, 3-4 cm across, solitary or fascicled with copious racemes. Fruits are 0.8 cm in diameter, globes, and 3 lobed berries with only one seed[14]. Tubers can grow up to a depth of 10 inch. Asparagus is a sub-erect prickly shrub with white tuberous root that grows well in tropical and sub-tropical climates with heights up to 1,500 meters. *Asparagus adscendens* is usually found throughout India and Himalayan Mountain ranges. Naturally occurs in forests of western Himalaya, Gujarat, Madhya Pradesh, Karnataka and Maharashtra States that are listed in the endangered species of India[15]. It was initially grown in thick forest in natural form, and is a customary medicinal plant.

In the present study, the nootropic effects of *A. adscendens* were investigated by employing both exteroceptive and interoceptive models. The stimulus lies outside the body in exteroceptive behavioral models, whereas, it lies within the body in case of interoceptive behavioral models. Elevated plus maze is a neutral exteroceptive model used to assess short-term memory whereas, passive avoidance apparatus is a punishment based exteroceptive model used to test long-term memory. Interoceptive behavioural models such as scopolamine, diazepam and natural aging induced amnesia are widely cited as models simulating human dementia in general and Alzheimer's disease in particular.[16]

MATERIAL AND METHODS

Plant material

The roots of *A. adscendens* were collected from Biligiri Ranga Hills of Chamarajanagar district. The plant material was identified and authenticated by the first author and a voucher specimen (SVCP-214-AA01) was deposited in the department. The shade-dried roots were powdered and passed through 100-mesh sieve. Root powder (1000 g) was soaked in methanol in the ratio of 1:20 (w/v) and subjected to extraction using soxhlet apparatus. The extract was filtered, concentrated using rotavapourator apparatus, and dried in freeze drier (Freezone®, Labonco, USA) with high vacuum. A suspension was prepared using distilled water containing 1% (w/v) tween 20 and was administered orally.

Drugs and reagents

Scopolamine hydrobromide (Sigma Aldrich, USA), diazepam (Calmpose, Ranbaxy, India) and piracetam (Nootropil, UCB India Pvt. Ltd., India) were diluted in normal saline and administered intra peritoneally. Volume of administration was 1 ml/ 100 g. All the drugs were administered in the morning session i.e. 8 AM- 9 AM on each day. 5, 5'-dithiobis nitrobenzoic acid (DTNB, Ellman's reagent, Sigma, USA) and acetyl thiocholine (Sigma, USA) were used.

Animals

Swiss mice of either sex weighing around 18- 20 g (younger ones, aged 3-4 months) and more than 30 g (aged ones, aged 12-15 months) were used in the present study. Animals were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and were maintained under 12:12 h light and dark cycles. All experiments were carried out during day time from 0900 to 1400 h. Institutional Animals Ethics Committee (IAEC) had approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Acute toxicity studies

Organization for Economic Co-operation and Development (OECD) guideline no. 423 was adopted for conducting acute toxicity tests. *A. adscendens* methanolic extract (AR) at different doses (10-2000 mg/kg) was administered orally to mice with the help of a specially designed oral needle connected to a polythene tube. AR was administered at the same time on each day (i.e. 8 AM- 9 AM). During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any, for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia and mortality were observed. The doses selected were 50 and 100 mg/kg/day.[17]

Elevated plus-maze

Elevated plus-maze served as the exteroceptive behavioral model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as reported earlier[18-19]. 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. On the first day, 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 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 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 was examined 24 h after the first day trial.

Passive shock avoidance paradigm

Passive avoidance behavior based on negative reinforcement was recorded to examine the long-term memory. The apparatus consisted of a box [27 X 27 X 27 cm] having three walls of wood and one wall of Plexiglas, featuring a grid floor [3 mm stainless steel rods set 8 mm apart], with a wooden platform [10 X 7 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 [20V AC] was delivered to the grid floor. Training 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 and placed all its paws on the grid floor, shocks were delivered for 15 sec and the step-down latency [SDL] was recorded. SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range [2-15 sec] during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 sec, electric shocks were delivered for 15 sec. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 sec. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec.[20,21,22]

Interoceptive behavioral models

Scopolamine induced amnesia: Amnesia was induced by administration of scopolamine hydrobromide (0.4 mg/kg, ip) on 8th day and the TL recorded. Retention was recorded after 24 hr. AR (50 and 100 mg/kg, po) and piracetam (200 mg/kg) were administered for 8 days successively. On 8th day, after 45 min of administration of doses, scopolamine was

administered and TL was noted after 45 min. SDL was recorded on 9th day.[23]

Diazepam induced amnesia: Diazepam, 1mg/kg, i.p was administered to young mice and TL was noted after 45 min of injection on 8th day and after 24 hr. AR (50 and 100 mg/kg, p.o.) and piracetam (200 mg/kg, i.p.) were administered for 8 successive days. After 60 min of administration of the last dose on 8th day, diazepam (1 mg/kg, ip) was administered. TL was noted after 45 min of administration of diazepam and after 24 hr. SDL was recorded on 9th day.[24]

Collection of brain samples

The animals were sacrificed by cervical decapitation under light anesthesia on the 8th day, 90 mins after administration of the last dose of. Immediately after decapitation whole brain was carefully removed from the skull. For preparation of brain 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% w/v sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of brain acetylcholinesterase activity.

Estimation of brain acetyl cholinesterase (AChE) activity

The time frame of cholinesterase activity estimation was similar to behavioral tests i.e. 8 AM- 11 AM on each day. On the 9th day the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured spectroscopically using the Ellman method.[25]

Locomotor function:

Locomotor activity of control and drug treated animals was measured using a photoactometer (INCO, Ambala, India).

Statistical Analysis

All the results were expressed as mean Standard error. The data was analyzed using ANOVA followed by Tukey-Kramer test.

RESULTS

Acute toxicity study

No mortality was observed following oral administration of AR even with the highest dose (2000 mg/kg). However, AR at doses more than 800 mg/kg, produced profuse watery stools in animals. Both the doses of AR did not exert any toxic effect on the normal behavior of the mice.

Effect on locomotor activity

In the present study, AR (50 and 100 mg/kg) did not show any significant change in the locomotor function of animals (score: 222.6±8 and 228.1±7) when tested on photoactometer as compared to control group (score 216.4±12).

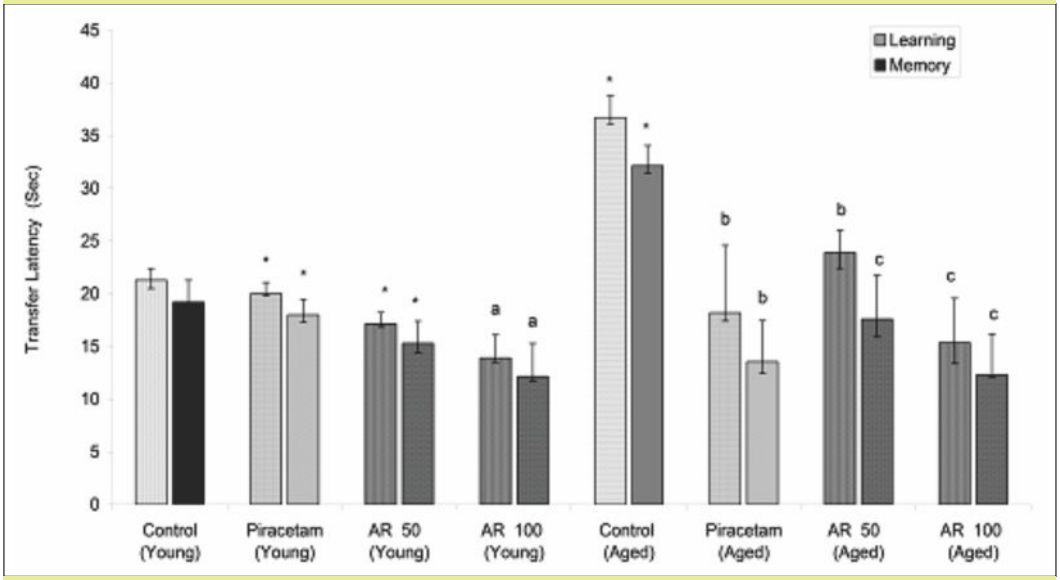
Effect on transfer latency using elevated plus-maze

Transfer Latency (TL) of second day (day 9th of drug treatment) reflected retention of learned task or memory. The young animals treated with AR (50 and 100 mg/kg, p.o.) showed dose- dependent reduction in TL of 9th day, indicating significant improvement in memory, when compared with control group. These doses of AR (50 and 100 mg/kg, p.o.) also produced significant improvement in memory of aged mice (Fig. 1). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) injected before training significantly increased ($P< 0.01$) the TL of 9th day indicating impairment in memory (amnesia). The mice treated with AR (50 and 100 mg/kg, p.o.) for 9 successive days reversed successfully the amnesia induced by both scopolamine and diazepam (Fig. 2). Piracetam (used as the positive control) at the dose of 200 mg/kg, i.p. improved memory ($P < 0.01$) of both young and aged mice and reversed the amnesia induced by scopolamine and diazepam as expected.

Effect on step-down latency using passive avoidance paradigm

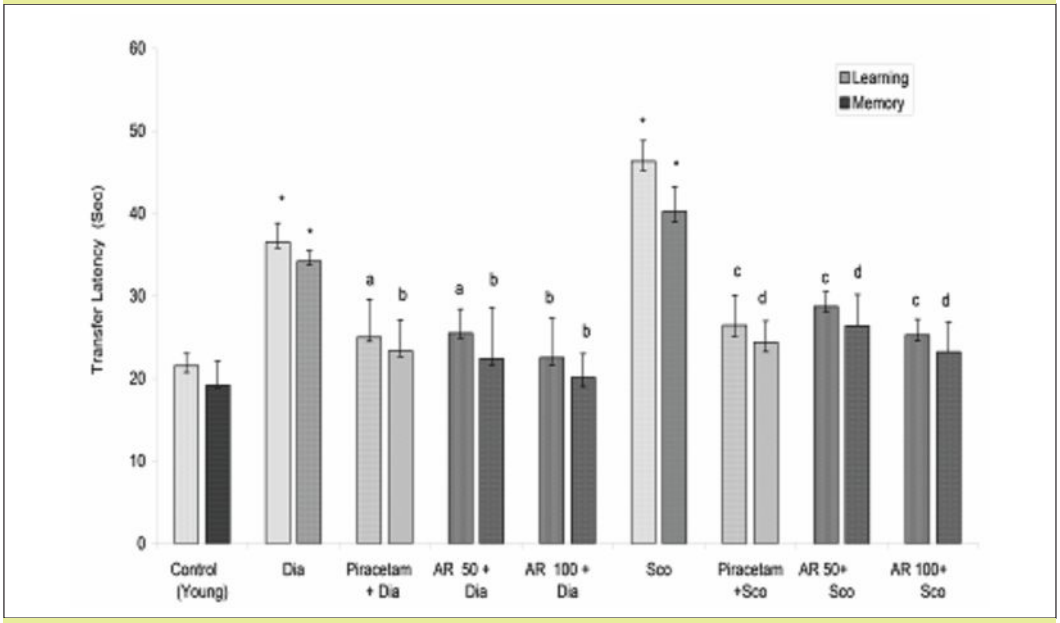
Step down Latency (SDL) of second day (9th day of drug treatment) reflected the long-term memory of animals. Various doses of AR (50 and 100 mg/kg, p.o.) administered to young and aged mice for 8 days, showed dose-dependent increase in SDL values as

Fig. 1: Effect of *A. adscendens* (AR, 50 and 100 mg/kg, p.o.) on transfer latency of young and aged mice using elevated plus maze.



Values are mean ±S.E.M. (n=6).
** indicates P< 0.01 as compared to control group of young mice, **a** indicates P< 0.001 as compared to control group of young mice.*
***b** indicates P< 0.01 as compared to control group of aged mice, **c** indicates P< 0.001 as compared to control group of aged mice.*
(One way ANOVA followed by Tukey-Kramer multiple comparison tests)

Fig. 2: Effect of *A. adscendens* (AR, 50 and 100 mg/kg, p.o.) on diazepam (Dia, 1 mg/kg, i.p.) and scopolamine (Sco, 0.4 mg/kg, i.p.) induced amnesia in young mice using elevated plus maze.



Values are mean ±S.E.M. (n=6).
** indicates P< 0.01 as compared to control group of young mice, **a** indicates P< 0.01 as compared to diazepam (Dia) group alone.*
***b** indicates P< 0.001 as compared to diazepam (Dia) group alone, **c** indicates P< 0.01 as compared to scopolamine (Sco) group alone.*
***d** indicates P< 0.001 as compared to scopolamine (Sco) group alone*
(One way ANOVA followed by Tukey-Kramer multiple comparison tests)

compared to respective control groups (Fig. 3). AR (50 and 100 mg/kg, p.o.) administered for 9 days reversed memory deficits due to ageing induced amnesia. Scopolamine and diazepam significantly ($P< 0.01$) decreased SDL values as compared to control group of young mice, indicating impairment of memory (amnesia). AR administered for 9 days reversed memory deficits induced by both scopolamine and diazepam (Fig. 4). The groups of mice, which were treated with piracetam (200 mg/kg, i.p.) for 8 successive days showed improvement in memory of young as well as aged mice.

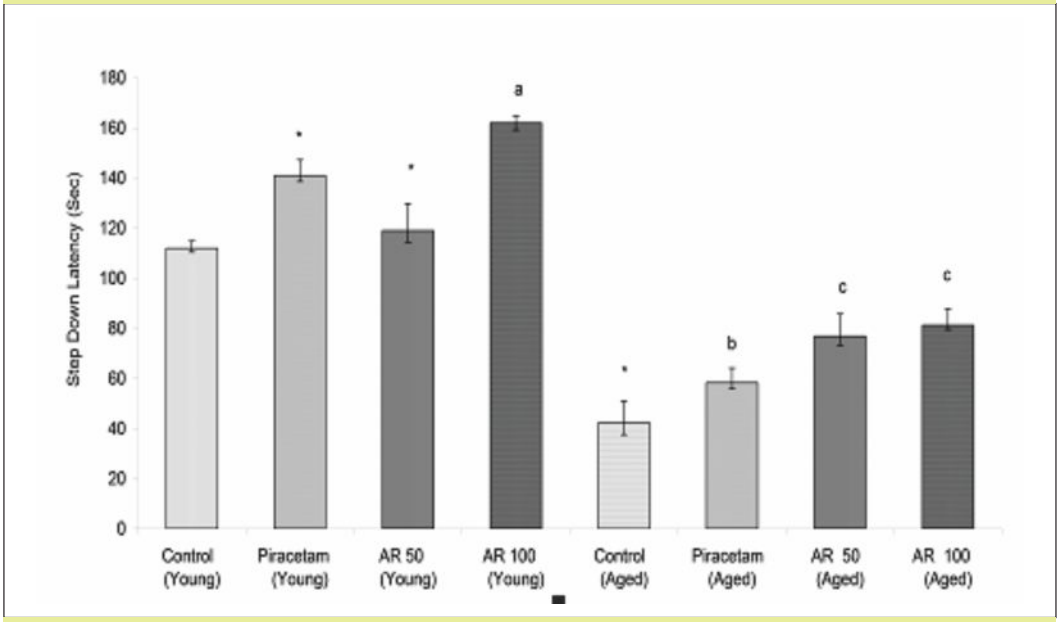
Effect on brain cholinesterase activity

AR (50 and 100 mg/kg, p.o.) showed a remarkable reduction in brain cholinesterase activity in young and aged mice, AR (50 and 100 mg/kg, p.o.) reduced cholinesterase activity in young and aged mice.

DISCUSSION

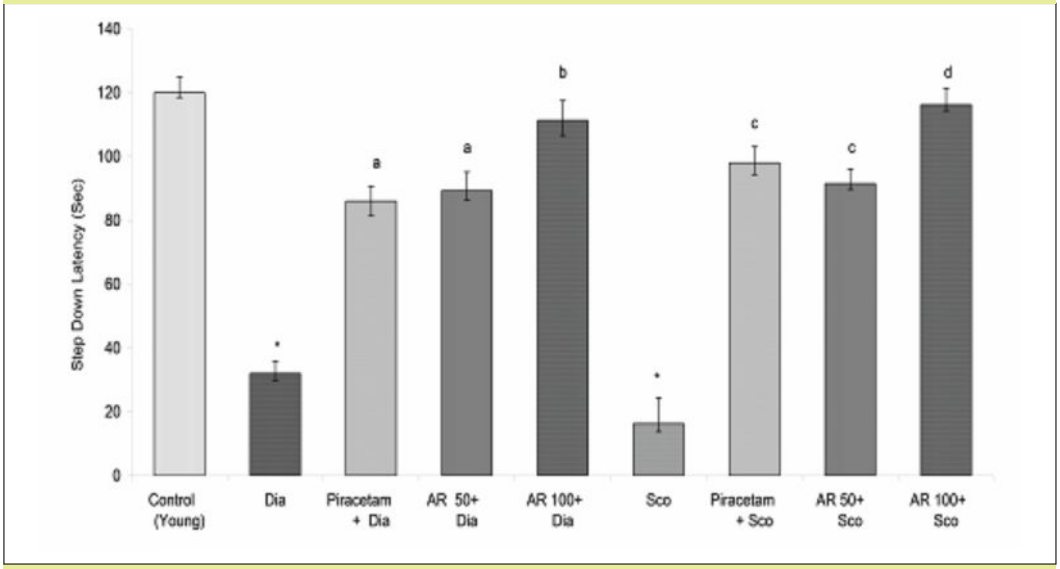
The symptoms of dementia are related to impaired neurotransmission and degeneration of neuronal circuits in the affected areas of brain[26]. Cognitive deterioration occurring in patients with probable AD is associated with a progressive loss of cholinergic neurons and a consequent decline in levels of acetylcholine (ACh) in the brain, particularly in the temporal and parietal neocortex and hippocampus[27]. These suggest that impairment of cholinergic function contributes to the symptoms of dementia and that the patients with dementia could potentially benefit from cholinergic replacement therapy. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. There is extensive evidence linking the central cholinergic system to memory[28]. Cognitive dysfunction has been shown to be associated with reduced cholinergic transmission and the facilitation of central cholinergic transmission with improved memory[29]. Selective loss of cholinergic neurons and decrease in cholinacetyltransferase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type[30]. Blocking

Fig. 3: Effect of *A. adscendens* (AR, 50 and 100 mg/kg, p.o.) on step down latency of young and aged mice using passive avoidance apparatus.



Values are mean ±S.E.M. (n=6).
** indicates P< 0.01 as compared to control group of young mice, **a** indicates P< 0.001 as compared to control group of young mice.*
*__b__ indicates P< 0.01 as compared to control group of aged mice, **c** indicates P< 0.001 as compared to control group of aged mice.*
(One way ANOVA followed by Tukey-Kramer multiple comparison tests)

Fig. 4: Effect of *A. adscendens* (AR, 50 and 100 mg/kg, p.o.) on diazepam (Dia, 1 mg/kg, i.p.) and scopolamine (Sco, 0.4 mg/kg, i.p.) induced amnesia in young mice using passive avoidance apparatus.



Values are mean ±S.E.M. (n=6).
***** indicates P< 0.01 as compared to control group of young mice, **a** indicates P< 0.01 as compared to diazepam (Dia) group alone.*
***b** indicates P< 0.001 as compared to diazepam (Dia) group alone, **c** indicates P< 0.01 as compared to scopolamine (Sco) group alone.*
***d** indicates P< 0.001 as compared to scopolamine (Sco) group alone*
(One way ANOVA followed by Tukey-kramer multiple comparison tests)

cholinesterase induced hydrolysis of Ach and the subsequent increase in ACh concentration in the central synapses and the enhancement of cholinergic function provides the symptomatic improvements observed in patients with probable AD who are treated with cholinesterase inhibitors[31-32].

The present study suggests that *A. adscendens* is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of learned task. Piracetam (200 mg/kg, i.p.) and AR (50 and 100 mg/kg, p.o.), on the other hand significantly (P<0.05) lowered this activity indicating the counteracting actions of these drugs on the cholinergic system. AR also reversed the scopolamine-induced impairment in learning and memory, when assessed on passive avoidance paradigm. Passive avoidance behavior is based on negative reinforcement and is used to examine long-term memory[33]. Both piracetam and *A. adscendens* meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit. In the present study, *A. adscendens* significantly inhibited the AChE activity in the mice whole brain homogenate, indicating its potential in the attenuation of symptoms of cognitive deficits.Our research findings using *Nardostachys jatamansi*[22], *Ocimum sanctum*[24], *Desmodium gangeticum*[28]and *Zingiber officinale*[32]have displayed a link between memory improving effect and cholinesterase inhibition[34]. In the present study, the AR when administered for 8 days to young and aged mice showed significant reduction of brain acetylcholinesterase activity thereby probably facilitating cholinergic transmission and improving memory of animals.

The use of *A. adscendens* in Ayurveda for treatment of various neurodegenerative disorders has been justified by the present study as it showed nootropic potential against scopolamine, diazepam and aging induced amnesia in mice. The memory improving activity of *A. adscendens* may be attributed to its antioxidant, neuroprotective, pro-cholinergic and anti-acetylcholinesterase properties and can be of enormous use in delaying the onset and reducing the

severity of Alzheimer's disease. Further investigations using more experimental paradigms are required for further confirmation of nootropic potential of *A. adscendens* in the treatment of various cognitive disorders.

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