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Research Article

Cognition improving potential of aqueous extract of roots of *Asparagus adscendens* Roxb. in mice

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Abstract

The present study involves investigations on the nootropic activity of aqueous extract of dried roots of *Asparagus adscendens* Roxb. (AAR) in mice. Elevated plus maze and water maze were the models used for testing learning and memory. Scopolamine (0.4 mg/kg, i.p.) and natural aging induced amnesia were the interocpetive behavioral models. Piracetam (200 mg/kg, ip) was used as standard drug. AAR (50 and 100 mg/kg, p.o.) significantly attenuated amnesic deficits induced by scopolamine and natural aging. AAR found to increase whole brain acetyl cholinesterase inhibition activity in mice. Aqueous extract of *A. adscendens* can be useful in treatment of preliminary symptoms of dementia in elderly.

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

Introduction

Increasing life expectancy has produced a dramatic rise in the number of cases of age-associated diseases, including dementia. Alzheimer's disease (AD) is the most frequent cause of dementia, accounting for 60–80% of all cases[1] and epidemiological studies indicate that AD will become even more incident by midcentury, constituting a major personal and societal tragedy. AD is primarily a condition of late life, roughly doubling in prevalence every 5 years after age 65 and affects some 47 million people worldwide [2]. This number is predicted to increase in the next two decades [3]. The total cost of dementia was estimated around $818 billion in 2010 and has been projected to hit $1 trillion by 2018 worldwide. This becomes even more dramatic because nearly 60% of people affected by dementia live in low- and middle-income countries [4].

From time immemorial, plants and plant products are being used for management of cognitive impairment in India. Ayurveda, the Indian system of medicine describes the use of medhyarasayana (intellect promoting) drugs in the treatment 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 [5].

Asparagusadscendens is a flowering perennial, spring plant species in the genus Asparagus [6]; 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 [7]. *Asparagus adscendens*Roxb.is known by various common names i.e. Shatawari, Safedmusli, 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 [8]. 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 [9]. It was initially grown in thick forest in natural form, and is a customary medicinal plant [11].

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. Interoceptivebehavioural 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[11]

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. Dried and cleaned root powder (1000 g) was soaked in purified water in the ratio of 1:20 (w/v) and subjected to extraction by maceration. The extract was filtered, concentrated and dried in freeze drier 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) was diluted in normal saline and administered intra peritoneally. Volume of administration was 1 ml/ 100 g. 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, Govt. of India.

Acute toxicity studies

Organization for Economic Co-operation and Development (OECD) guideline no. 423 was adopted for conducting acute toxicity tests. Aadscendens aqueous extract (AAR) 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. AAR was administered at the same time on each day. 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 [12].

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 [13-14]. 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.

Interoceptive behavioral model

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 [15-17].

Estimation of brain acetyl cholinesterase (AChE) activity

The animals were sacrificed by cervical decapitation under light anesthesia on the 8th day, 90 min 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 spectroscopically using the Ellman method [18].

Morris water maze (MWM)

The MWM test was employed to assess learning and memory of the animals. MWM is a swimming model where the animals learn to escape on to a hidden platform. In the present study the target quadrant was Q4. The mice were subjected to 4 consecutive trials every day with a gap of 5 minutes for 4 e days continuously, during which they were permitted to escape to the hidden platform and to be remained for 20 sec. If the animal was not able to find the hidden platform within 120 seconds, the mouse was gently pushed and guided to the platform and permitted to stay on the platform for further 20 seconds. Escape latency time to identify the hidden platform in Morris water maze was the index of acquisition (learning). The starting point on every day to conduct 4 acquisition trials was changed as described below and Q4 was maintained as the target quadrant in all the acquisition trials. The starting point for dropping the mice into water maze on day 1 for four consecutive acquisition trials was in the sequence Q1, Q2, Q3 Q4 and so on. The sequence change in starting point was as follows. Day 1: Q1, Q2, Q3, Q4 Day 2: Q2, Q3, Q4, Q1 Day 3: Q3, Q4, Q1, Q2 Day 4: Q4, Q1, Q2, Q3. Mean escape latency time (ELT) was calculated for each day of the trial. On the 5th day the hidden platform was removed, each mouse was permitted to explore the pool for 120 seconds. The animal was made to take 4 such trials with 5-minute interval time and every trial had a different starting point covering all the 4 quadrants. The mean of time spent by the animal in all 4 quadrants was recorded. The TSTQ (time spent in target quadrant) in Q4 as compared to time spent in other quadrants in locating missing platform was considered as an index of retrieval (memory). Utmost care was employed to ensure that relative location of water maze with respect to any other objects in the laboratory serving as visual clues was not disturbed during the total duration of the study. All the trials were completed between 09:00 and 17:00 hours [19-20].

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 AAR 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 transfer latency using elevated plus-maze

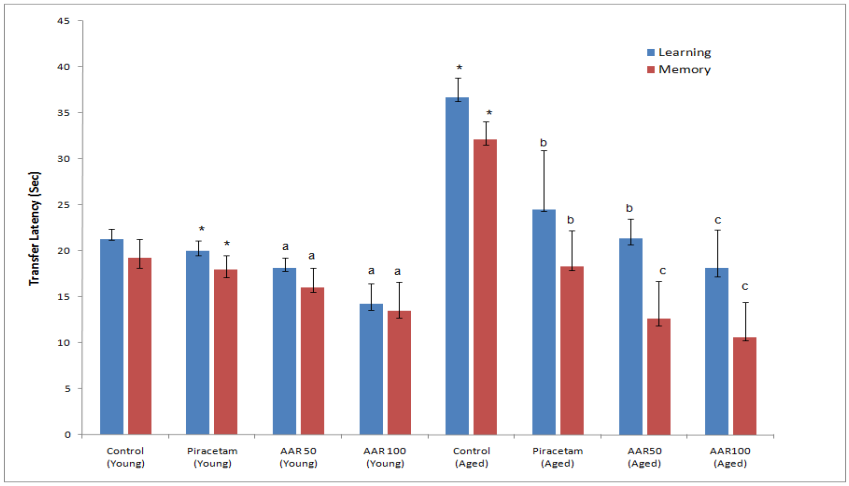
Transfer Latency (TL) of second day (day 9th of drug treatment) reflected retention of learned task or memory. The young animals treated with AAA (50 and 100 mg/kg, p.o.) showed reduction in TL of 9th day, thus enhanced memory, when compared with control group. AR (50 and 100 mg/kg, p.o.) also exhibited significant improvement in memory of aged mice (Fig. 1). Scopolamine (0.4 mg/kg, i.p.) and injected before training significantly increased ($P< 0.01$) the TL of 9th day indicating amnesia. AR (50 and 100 mg/kg, p.o.) reversed successfully the amnesia induced by scopolamine (Fig. 2). Piracetam (200mg/kg, i.p.) was the standard drug and it significantly improved memory ($P < 0.01$) of both young and aged mice.

Discussion

Selective loss of cholinergic neurons and decrease

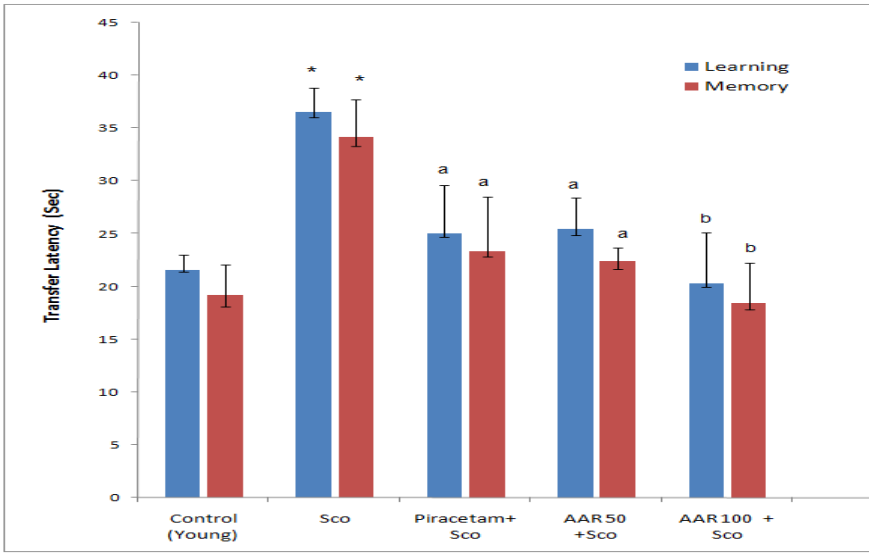
in cholinacetyltransferase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type [21-22]. Blocking cholinesterase induced hydrolysis of Ach and the subsequent increase in ACh concentration in the central synapses and the enhancement of cholinergic function provides the symptomatic improvements observed in patients with probable AD who are treated with cholinesterase inhibitors [23-24].

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



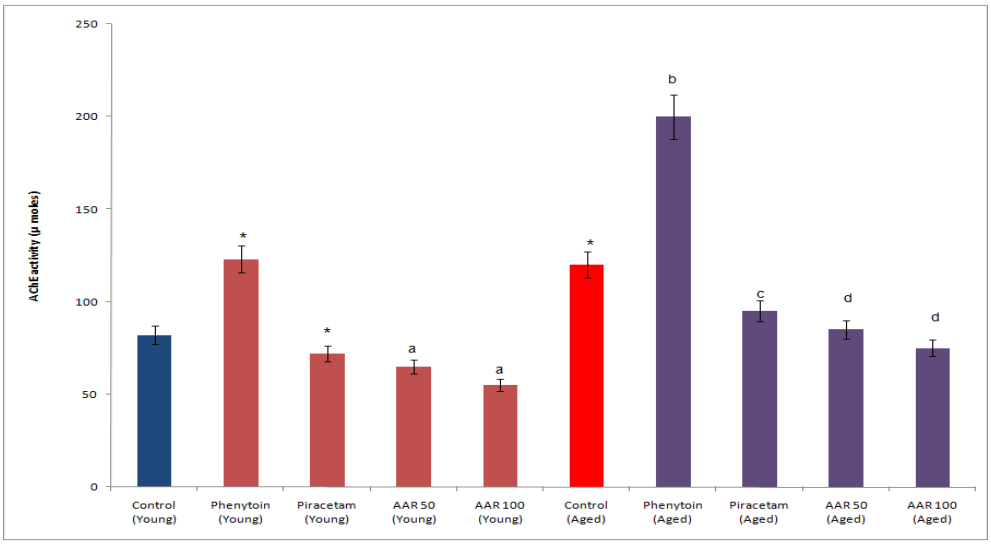
Values are mean ±S.E.M. (n=6). * indicates P< 0.01 as compared to control group of young mice. a indicates P< 0.001 as compared to control group of young mice. b indicates P< 0.01 as compared to control group of aged mice. c indicates P< 0.001 as compared to control group of aged mice. (One way ANOVA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

Fig. 2 Effect of *A. adscendens* (AAR, 50 and 100 mg/kg, p.o.) on scopolamine (Sco, 0.4 mg/kg, i.p.) induced amnesia



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 scopolamine (Sco) group. b indicates P< 0.001 as compared to scopolamine (Sco) group.

Fig. 3 Effect of *A. adscendens* (AAR, 50 and 100 mg/kg, p.o.) on brain cholinesterase (AChE) activity



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.

Table-1: Effect of *A. adscendens* (AAR, 50 and 100 mg/kg, p.o.) on Escape Latency time (ELT) & Time Spent in Target Quadrant (TSTQ)

Treatment	ELT (21st day)	ELT (24th day)	TSTQ (25th day)
Control (10 ml/kg p.o)	95.06±0.49	48.3±0.25	50.05±0.3
Piracetam (200mg/kg i.p.)	75.10±0.7*	39.05±0.4*	70.22+1.1*
AAR (50mg/kg p.o)	80.36±0.1 a	47.08±0.8 a	59.65±0.6a
AAR (100mg/kg p.o)	77.34±0.5a	40.24±0.6a	37.15±0.6a
Scopolamine (0.4mg/kg, i.p.)	130±2.9b	123±3.6b	95±1.4b
AAR (50mg/kg p.o) + Sco	85.1±0.5	82.21±0.7b	99.2±0.62
AAR (100mg/kg p.o) + Sco	80.31±0.96	70.18±0.4	117±0.1

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 scopolamine (Sco) group. b indicates P< 0.001 as compared to scopolamine (Sco) group.

The present study proves that aqueous extract of *A. adscendens* is a anti-cholinesterase agent. It also exhibited neuroprotective activity due to its facilitatory effect on retention of learned task. Piracetam (200 mg/kg, i.p.) and AAR (50 and 100 mg/kg, p.o.), on the other hand significantly (P<0.05) lowered this activity indicating the possible counteracting actions of these drugs on the cholinergic system. AAR also attenuated the scopolamine-induced impairment in learning and memory, when assessed on Morris water maze. Both piracetam and *A. adscendens* improved memory in absence of cognitive deficits. In the present study, *AAR* significantly inhibited the AChE activity in the mice whole brain homogenate, indicating its cognition improving efficacies. Our earlier research findings using *Nardostachys jatamansi* [25], *Foeniculum vulgere* [26], *Asparagus adscendens* [27] and *Zingiber officinale* [28] have displayed a link between memory improving effect and cholinesterase inhibition [28-30]. 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 and aging induced amnesia in mice. However, Further studies 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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