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In vivo Investigation of antiamnesic effect of *Asparagus racemosus* root extract in scopolamine induced amnesic mice

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Abstract
Asparagus racemosus, mentioned as Medhya Rasayanas in ancient Ayurveda which improve brain function, memory, and intelligence. The present study was focused to investigate its effects on learning and memory. Study was carried out on young adult male mice (25 gm ± 5 wt.). Elevated plus maze and Passive avoidance paradigm were used for the study. Methanolic root extract (100 mg/ kg wt. p. o.) was given orally. Experimental animals were given Scopolamine hydrobromide (1 mg/ kg wt. i. p.) was administered for 7 successive days to adult mice before starting the AR treatment. Intra peritoneal injection of scopolamine increased the TL in exteroceptive behavioral model Elevated plus maze and remarkable decrease in step through transfer latency in Passive avoidance paradigm. Pretreatment for 7 successive days of asparagus racemosus reversed these effects of scopolamine in mice. Thus *Asparagus racemosus* root extract possesses memory enhancing potential and validating its utility as a curing drug for treatment of dementia.

Keywords: *Asparagus racemosus*, elevated plus maze, passive avoidance test, step through latency, scopolamine.

Introduction
The neurodegeneration in specific area of central nervous system plays an important role in development of various neuronal disorders. Dementia is one condition which is associated with decline in function, cognition, behavior, and other thinking skills that affects a person's ability to perform everyday activities, caused by damage to nerve cells in the brain. Alzheimer's disease (AD) is the most common form of dementia among aged people characterized by a progressive loss of memory, impairment of cognitive function, synaptic dysfunction and diffuse neuronal loss coupled with classic histopathology changes.[1,2]
Phytoestrogens are plant -derived non-steroidal Xenoestrogens with endogenous estrogen like functions in mammals. Phytoestrogens are structurally and functionally similar to mammalian endogenous estrogen found in seeds, fruits, and vegetable. Phytoestrogens are often considered as a natural and safer alternative to the synthetic conjugated used in hormone therapy. They have stable structure with low molecular weight which makes them to cross cell membrane and interact to intracellular enzymes and intracellular messengers. [3]There are strong evidences that phytoestrogens can increase spine density and synapse formation in the hippocampus of adult brain. [4]
The estrogenic plant compounds are also present in food, including herbs and seasonings (garlic, parsley), grains (soybeans, wheat, and rice), vegetables (beans, carrots, and potatoes), fruits (date, pomegranates, cherries, apples) and drinks (coffee). [5] Phytoestrogen have many subtypes including isoflavones, coumestans, lignanschalcones, flavones and prenylflavonoids. [6] The two most studied phytoestrogen groups are lignans and isoflavones. Lignans are products of intestinal microbial breakdown of compounds found in whole grains, fibers, flax seeds and many fruits and vegetables. [7] *Asparagus racemosus* or Shatavari is a well- known therapeutic agent in Ayurveda reffered as "rasayana" and well accepted as female tonic due its phytoestogenic properties. [8] The roots and leaves of *Asparagus racemosus* pussesestogenic properties. [9, 10] Roots in medicinal oils can be used for nervous and rheumatic pain. In Ayurveda, it is commonly used for cardiac abnormality and intelligence as well as memory modulator. Thus in the present study we evaluated the role *Asparagus racemosus* for treatment of amnesic behavior.
Scopolamine induced amnesic rodent model was used in this study as it is one of the well-established animal model for memory dysfunction. [11] Scopolamine is a non-selective receptor

Antagonist to muscarine receptors and produce impairment of learning acquisition as well as short-term memory lossby reducing acetylcholine level in hippocampus. [12] Similarities have been observed between patient with Alzheimer's disease and scopolamine treated animals. [13]

Estrogen plays an important role in various brain functions; it has been proved as an effective neuroprotectant and improves cognitive impairments caused by neurodegeneration. Clinical studies have shown that estrogen therapy at postmenopausal women play an important role in cognitive impairment as well as learning and memory. [14] Many studies indicates that estrogen promote neuronal survival, growth and reduce neuronal apoptosis. [15] Estrogen depletion at menopause causes the onset of the development of AD in women.

Thus in present study we used scopolamine induced amnesic mice model to study the memory enhancing effects of the *Asparagus racemosus*.

2. Materials and methods

2.1 Animals

Adult healthy Swiss albino male mice weighing approximately 25 gm ±5 were used in this study. The mice were maintained in polyutherine cages separately for one week to acclimatize the laboratory environment. They had free access to commercial standard food pellets and water. The mice were maintained on 12:12 hour light and dark cycle. All the experiments were carried out between 0900 and 1500h. Handling and usage of animals agreed strictly with the regulation and guideline set by international norms. All protocols were made to reduce the number of animals used. Protocols was approved by Institutional animal ethical committee (IAEC Log.No.973/ac/06/CPCSEA).

2.3 Preparation of plant extract

Asparagus racemosus roots were purchased from the local supplier. The roots were dried under shade, pulverized by a mechanical grinder and stored in airtight container for further use. The air-dried and powdered material [500 gm.] was extracted with 100% methanol in a Soxhlet extractor for 35 hours. The solvent was evaporated at low temperature under reduced pressure in a Rota evaporator; to obtain dry mass completely free from the solvent. The extract gave positive tests for polyphenols, flavonoids, tannins, saponins and glycosides. The semisolid mass was kept in refrigerator and dissolved in distilled water just before use.

2.4 Drugs and treatment

All chemical used were purchased from commercial supplier and were analytical grade. *Asparagus racemosus* methanolic root extract was dissolved in distilled water and administered per orally. Scopolamine hydro bromide was dissolved in saline and administered intra peritoneal after one hour of *Asparagus racemosus* dose.

2.5 Treatment protocol

Adult Swiss albino male mice were used for experimental study. Animal were grouped in six separately groups (N=6). Dose was selected on the basis of a pilot study and reports available in the literature**.** All the drugs were administered in the morning session i.e. 8.00-900 AM each day.

Table 1: Animal groups and treatment Table

Group		Treatment	Duration
Group I N=06	Control	Vehicle	7 Days
Group II N=06	AR	Scopolamine (1mg/kg I.P.)	7 Days
Group III N=06	SCO	Asparagus racemosus (100mg/kg P.O.) + Scopolamine (1mg/kg I.P.)	7 Days
Group IV N=06	SCO +AR	Asparagus racemosus (100mg/kg P.O.)	7 Days

Table 2: Schematic illustration for Behavioral study (Elevated plus mazes EPM)

Treatment	Dose	Dose	Dose	Dose	Dose	Dose	Dose	No Dose
Day	1	2	3	4	5	6	7	8
Treatment	No treatment	No treatment	No treatment	No treatment	No treatment	No treatment	Training EPM	Retention Memory Test EPM

Table 3: Schematic illustration for Behavioral study (Passive avoidance test PAT)

Treatment	Dose	Dose	Dose	Dose	Dose	Dose	Dose	No Dose
Day	1	2	3	4	5	6	7	8
Treatment	No treatment	No treatment	No treatment	No treatment	No treatment	No treatment	Training PAT	Retention Memory Test PAT

2.6 Elevated plus maze (EPM)

The elevated plus maze is the most commonly used for the study of exteroceptive behavioral (when the stimulus existed outside the box) to evaluate learning and memory in mice. [16] The maze consists of two open arms (16 cm x 5 cm) and two covered arms (16 cm x 5 cm x 12 cm) with central platform (5 cm x 5 cm). The arms extended from central platform and maze is elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 sec, it is gently pushed into one of the two covered arms and the TL was assigned as 90 seconds. Memory retention was examined on the second day, 24 hours after the first day's trial. [16, 17]

2.7 Passive avoidance apparatus (PAT)

The passive avoidance test is simple and rapid test method for memory assessment.[18]The passive avoidance test apparatus consisted of two adjoining compartments, a dark chamber (20cm×20cm×20 cm) with black walls and a light chamber (20×10×10 cm) with transparent walls. Both compartment equipped with a stainless steel grid floor spacing of 1mm and 3 mm diameter located at 2 cm above the base of chamber. The dark chamber is connected with a light chamber via a guillotine door with dimensions of 5×7 cm. The light chamber illuminated by a stimulus light (60 W light bulb) located overhead. The dark compartment contained steel rods capable of delivering an electric shock to the animal's feet. The electroshock apparatus capable of producing a current of 1.5 mA for any desired time was connected to a grid for delivering foot shocks. On the acquisition trial each mice was placed in the light compartment. After the period of 120 second of habituation, guillotine door opened, and the initial latency to enter the dark compartment with all four paws was recorded then mice immediately removed and returned to the home cage. The mice were placed in the light compartment facing away from the dark compartment. After 30 min mice again place in the light compartment and when the mice moved to dark compartment the door closed and they received an electric shock (0.25mA) for duration of 3 second then mice returned to their home cage. On the test day (24 hours after training), the placed in the light compartment, facing away from the dark compartment and the latency period to enter the dark compartment was recorded and described as step through latency. The retention trial period were set at limit of 300 second cut off time. The step though latency is a basic indicator of memory formation; the shorter the latency, the weaker the memory.

2.8 Statistical Analysis

All data were analyzed by SPSS software designed for statistical analysis. The all data were expressed as mean ± SEM. Statistical analysis done using one-way ANOVA followed by Turkey's test. In all test, the criterion for statistical significance was $p<0.05$.

3. Results

3.1 Elevated plus maze test

On the 7th day of experimental protocol schedule, acquisition latency was measured. Retention was observed at Transfer latency (TL) on 8th day to evaluate learning and memory in mice using elevated plus maze. On the 7th day and 8th day scopolamine administration mice showed significant difference (P <.0001) and remarkable increase (82.00 ± 2.53 and 64.50 ± 7.43) in TL, when compared to control (21.17 ± 1.07 and 13.33 ± 1.28) and AR treated mice (16.17 ±1.72 and 11.17±1.19). During experiment, AR administration did not reveal any change (P<0.0001) when compared to control in TL. Administration of AR followed by scopolamine exhibit significant decrease (P<0.0001) (52.33± 3.70 and 38.17 ± 4.26) in TL. When compared to scopolamine treated mice indicating improved retention memory.

Table 4: Transfer latency in seconds in the model of Elevated plus maze test

Groups	1st Day TL Mean ±SEM	2nd Day TL Mean ±SEM
Control	21.17± 1.08	13.33±1.28
AR	16.17±1.72	11.17±1.19
SCO	82.00±2.53	64.50±7.34
SCO +AR	52.33±3.70	38.17±4.26

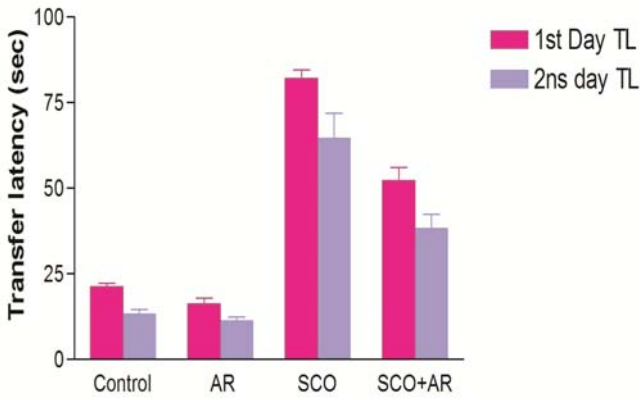

Fig1: Effect of asparagus racemosus Elevated plus maze (Transfer Latency)

3.2 Passive avoidance test

On the 7th day of experimental protocol schedule, acquisition latency was measured. Retention was observed at Transfer latency (TL) on 8th day to evaluate learning and memory in mice using passive avoidance test. On the 7th day and 8th day scopolamine administration mice showed significant difference (P <.0001) and remarkable decrease in step through transfer latency (35.83 ± 2.27 and 70.7 ± 2.90), when compared to control (39.67 ± 2.27 and 265.2 ± 2.76) and AR treated mice (28.83 ± 1.85 and 248 ± 3.18). During experiment, AR administration did not reveal any change (P<0.0001) when compared to control in TL. Administration of AR followed by scopolamine exhibit significant decrease (P<0.0001) (31.17± 2.15 and 237 ± 3.29) in TL. When compared to scopolamine treated mice indicating improved retention memory.

Table 5: Transfer latency in seconds in the model of passive avoidance test

Group	1st Day TL Mean ±SEM	2nd Day TL Mean ±SEM
Control	39.67±2.27	265.2±2.76
AR	28.83±1.85	248.3±3.18
SCO	35.83±1.24	70.7±2.90
SCO +AR	31.17±2.15	237.7±3.29

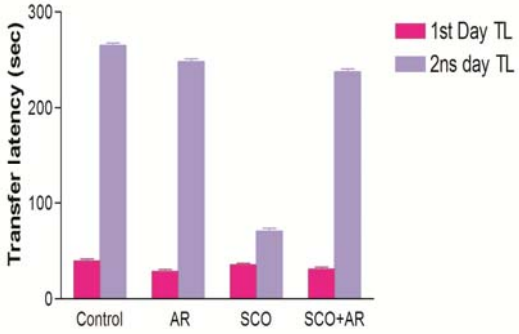

Fig 2: Effect of *Asparagus racemosus* Passive avoidance test

4. Discussion

In both the test-performed animals treated with Scopolamine exhibited a sharp decline in memory retention and treatment with *Asparagus racemosus* improved the condition and was comparable to control animals. Acquisition latency and retention was observed at Transfer latency (TL) was significantly improved after treatment with *Asparagus racemosus* after Elevated plus maze test and Passive avoidance test. In the present study, the effect of improving memory

deficit *Asparagus racemosus* (AR) was evaluated, using the scopolamine induced AD type amnesia in mice. Scopolamine induced amnesic rodent model is one of the well-established animal model for memory dysfunction. [19, 20] Scopolamine, a nonselective muscarinic antagonist block cholinergic neurotransmission and produce impairment in learning and memory processing. [21, 22] Both the long term and short term memory deficit produced by scopolamine. [23, 24]Similarities have been observed between patient with Alzheimer's disease and scopolamine treated animals. Thus scopolamine can provide a use full pharmacological tool to generate a partial model of the disorder. [13]

The cognitive enhancing activity of methanolic root extract of *Asparagus racemosus* on scopolamine induced memory impairment in mice was investigated by using behavioral parameters Elevated plus maze resulted, decrease in retention latency indicated improvement of memory and vice versa. In EPM, it was observed that long-term administration of scopolamine also drastically increase in TL, demonstrating that central cholinergic system plays an important role in learning acquisition. Methanolic root extract of *Asparagus racemosus* decreased TL prolongation induced by scopolamine. Alzheimer's disease (AD) refers to clinical neuronal disorders that occurs in elderly and characterized at least two clinical abnormalities an insidious degradation of memory and neurobehavioral disturbance.[25] The severity and prevalence of AD are not yet under control because the exact pathogenesis of this disease is not known. Therefore attention has been focused on development of alternative and complementary medicines including herbal formulation and phytochemical extract for effective management of AD. [26, 27, 28] The current therapeutic strategies are effective for prohibits that mechanism by which neurons come in to apoptotic or necrotic processes. These therapies include use of natural antioxidants that may be beneficial in aging and neurodegenerative disease. [29 30]

5. Conclusions

In conclusion, the findings of this research work signify the use of herbal remedies with proven efficacy for improving learning and memory. Our finding indicates that *Asparagus racemosus* (Shatavari) has a potential phytoestrogenic source andcould beuseful for management of dementia and Alzheimer's disease.

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