

# Antiamnesic potentiality of *Argyreia speciosa* (Burm.f) Boj. in mice

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Several '*rasayana*' herbs that are enlisted in Indian system of medicine have been in use for the treatment of age-related neurodegenerative disorders including Alzheimer's disease (AD). Roots of *Argyreia speciosa* are used in several Ayurvedic preparations as brain tonic and nervine tonic. The present work was undertaken to justify the traditional claim of the plant as nootropic and antiamnesic agent in mice. The ethyl acetate and ethanolic fractions (EtAS) of roots were selected for the study. Exteroceptive behavioural models such as elevated plus maze and Water maze were used to assess the short-term memory, whereas, scopolamine and natural ageing-induced amnesia served as interoceptive models. The whole brain acetyl cholinesterase activity was measured to assess the effect of *A. speciosa* on the central cholinergic system. Scopolamine (0.4 mg/kg, i.p.) increased the transfer latency significantly ( $P < 0.01$ ) in young mice on the first and second day as compared to control indicating the impairment of memory. Pretreatment with EAAS (100 and 200 mg/kg, p.o.) significantly ( $P < 0.01$ ) attenuated scopolamine and ageing-induced amnesia. Escape latency time was recorded in the water maze model as an index of acquisition, and trials were conducted for 4 days. The mean time spent in target quadrant (TSTQ) during retrieval trial on fifth day was taken as the index of retrieval (memory). EAAS (100 and 200 mg/kg, p.o.) administered before the training trial (from day 1 to day 4), significantly ( $P < 0.01$ ) attenuated scopolamine and ageing-induced decrease in TSTQ during the retrieval test on the fifth day. EAAS (100 and 200 mg/kg, p.o.) significantly produced reduction in whole brain acetyl cholinesterase (AChE) activity of both young and aged mice thus exhibiting anti-AChE activity in whole brain homogenate compared to Piracetam, scopolamine and control groups of mice. The results indicate that *A. speciosa* has significant nootropic and antiamnesic activity, justifying its traditional use in Ayurveda.

**Key words:** Antiamnesic, *Argyreia speciosa*, ayurveda, nootropic, *rasayana*

## INTRODUCTION

Stress can be defined as the sum total of all reactions of the body, which disturb the normal physiological condition and result in a state of threatened homeostasis. Stress and free radicals have been implicated in the loss of memory, concentration and also AD.<sup>[1,2]</sup> AD, the most common form of dementia in the elderly population, is characterized by an insidious onset with memory impairment and an inexorable progression of cognitive decline. In addition to the cognitive impairment of functions such as memory and language, AD patients frequently show abnormal behaviour such as delusion agitation and wandering.<sup>[3-5]</sup> The personality distortions interfere with the patient's professional life, social activities and relationships.<sup>[6]</sup> Nootropic agents such as Piracetam, aniracetum and cholinesterase inhibitors like donepezil are being used for improving memory, mood and behaviour,<sup>[7]</sup> but the resulting side effects associated with these agents have made their applicability limited. Several '*rasayana*' herbs that are enlisted in Indian system of medicine have been in use for the treatment of age-related neurodegenerative disorders.

*Argyreia speciosa* (Burm.f) Boj. (Convolvulaceae) is commonly known as *Vrudhadaruka* in Indian system of medicine. Roots of *A. speciosa* are used in Ayurveda as aphrodisiac, rejuvenating, intellect promoting, brain tonic, in the treatment of infected wounds, bronchitis, syphilis and pulmonary tuberculosis.<sup>[8-10]</sup> The plant has been screened for anti-inflammatory,<sup>[11]</sup> immunomodulatory<sup>[12]</sup> and hepatoprotective activities.<sup>[13]</sup> Flavonoid sulphates such as Kaempferol 7-O methyl 3-sulphate, Quercetin 3'7 di-O methyl 3-sulphate<sup>[14]</sup> and Stigmasteryl *p*-hydroxycinnamate<sup>[15]</sup> have been reported from the roots. Two constituents such as hexadecanyl *p*-hydroxycinnamate and scopoletin were found to inhibit the growth of fungi isolated from diseased parts of some medicinal and aromatic plants.<sup>[16]</sup> In the present study, the antiamnesic activity of ethyl acetate (EAAS) and ethanol fractions (EtAS) of *A. speciosa* was investigated by employing both exteroceptive and interoceptive models. Elevated plus maze and Morris water maze are used to assess the short-term memory. Interoceptive behavioural models such as scopolamine and natural ageing-induced amnesia are widely used models to stimulate dementia in AD.

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## MATERIALS AND METHODS

### Preparation of Extracts

Roots of *A. speciosa* were collected from hilly areas surrounding Dharwad, Karnataka province, India, and authenticated by qualified taxonomist, Department of Botany, Karnataka University, Dharwad. A herbarium specimen was kept in Dept. of Pharmacognosy (SETCPD/Ph.cog/herb/33/2006). The roots were dried under shade and powdered. The dried powder was exhaustively extracted successively using EAAS and ethanol (95%), respectively. Both the extracts were concentrated by rotary flash evaporator followed by freeze drying. The percentage yield of dry extracts was found to be 1.76% and 0.82% for EtAS and EAAS, respectively. Suspensions of the extracts were prepared in Tween-80 and distilled water (2:8) and used to assess anti-amnesic activity.

### Acute Toxicity Studies

Acute toxicity study was carried out using Swiss albino mice (25-30 g) by up and down/staircase method as per CPCSEA guidelines. Both the extracts were orally administered to different groups of young and aged mice at doses of 50, 300, 1000 and 2000 mg/kg body weight, respectively. Animals were observed for 48 h to study the general behaviour of animals, signs of discomfort and nervous manifestations.<sup>[17]</sup>

### Drugs

Scopolamine hydrobromide (Sigma Aldrich, USA), piracetam (Nootropil, UCB India Pvt. Ltd, Vapi, Gujarat), diazepam (Calmose, Ranbaxy, India) phenytoin (Dialantin suspension, Parke Davis) were diluted in normal saline. Volume of oral and i.p. administration was 1 ml/100 g of mouse.

### Experimental Animals

Swiss mice of either sex weighing around 18 g (young ones, aged 8 weeks) and 25 g (older ones, aged 28 weeks) were used in the present study. Animals were procured from disease-free animal house of BLDEA's medical college and Research centre, Bijapur, Karnataka, India. They were acclimatized to the laboratory conditions for 5 days before behavioural studies. The animals had free access to food and water and were maintained under 12:12 h light and dark cycles. All the readings were taken during the same time of the day i.e. between 6 and 8 PM. Institutional Animals Ethics Committee (IAEC) had approved the experimental protocol, and care of animals was taken as per the CPCSEA guidelines, Animal welfare division, Ministry of Environment and forests, Govt. of India.

### Experimental Design

#### Elevated plus maze

The elevated plus maze served as the exteroceptive behavioural model to evaluate learning and memory

in mice. The apparatus consisted of two open arms (16 cm×16 cm) and two covered arms (16 cm×5 cm×12 cm). The arms extended from a central platform (5 cm×5 cm), and maze was developed 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 one of the covered arms within the 90 s, it was gently pushed into one of the two covered arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for 10 s and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day.<sup>[18,19]</sup>

Group I and Group XV: Represented control groups for young and aged mice (n=6). 10 ml/kg distilled water, p.o., was administered for 8 days. TL was noted after 45 min of administration on eighth day and after 24 h (ninth day).

Group II and Group III: Piracetam, 200 mg/kg, i.p., was injected to both young and aged mice, respectively. TL was noted after 45 min of injection and on the ninth day.

Group IV: Scopolamine hydrobromide (0.4 mg/kg, i.p) was administered to young mice and TL was noted after 45 min of injection on eighth day and after 24 h (ninth day).

Group V and Group VI: EtAS extract, 100 mg and 200 mg/kg, was administered orally to young mice for 8 days. The last dose was given 45 min before subjecting the animals to the elevated plus maze test. TL was noted on eighth day and ninth day.

Group VII and Group VIII: EAAS extract, 100 mg and 200 mg/kg, was administered orally to young mice for 8 days. The last dose was given 45 min before subjecting the animals to the elevated plus maze test. TL was noted on eighth day and ninth day.

Group IX and Group X: EtAS extract, 100 mg and 200 mg/kg, was administered orally to aged mice for 8 days. The last dose was given 45 min before subjecting the animals to the elevated plus maze test. TL was noted on eighth day and ninth day.

Group XI and XII: EAAS extract, 100 mg and 200 mg/kg, was administered orally to aged mice for 8 days. The last dose was given 45 min before subjecting the animals to the elevated plus maze test. TL was noted on eighth day and ninth day.

Group XIII: EtAS 200mg/kg, p.o. was administered to young mice for 8 days. After 45 min of administration of

the last dose on eighth day, scopolamine hydrobromide (0.4 mg/kg, i.p) was administered. TL was noted after 45 min of administration of scopolamine and on the ninth day.

Group XIV: EAAS 200 mg/kg, p.o. was administered to young mice for 8 days. After 45 min of administration of the last dose on eighth day, scopolamine hydrobromide (0.4 mg/kg, i.p) was administered. TL was noted after 45 min of administration of scopolamine and on the ninth day.

### Morris water maze

It consisted of a circular water tank (150 cm diameter, 45 cm height), filled with water maintained at 25°C. The water was made opaque with a white coloured non-toxic dye. The tank is divided into four quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm<sup>2</sup>) of 29 cm height was located in the center of one of these four quadrants. The position of platform was kept unaltered throughout the training sessions. In the present study, the target quadrant was Q4. Each animal was subjected to four consecutive trails on each day with a gap of 5 min for 4 consecutive days, during which they are allowed to escape on to the hidden platform and to remain there for 20 s. In case the animal was unable to locate the hidden platform within 120 s, it was gently guided to the platform and allowed to remain on the platform for 20 s. Escape latency time (ELT) to locate the hidden platform in water maze was taken as an index of acquisition or learning. Starting position on each day to conduct four 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 sequenced Q1, Q2, Q3 Q4 and so on. Sequence change of the 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 ELT was calculated for each day of the trial. On fifth day, the platform was removed and each mouse was placed in water for 120 s. The animal was subjected to four such trials and each trial had a different starting point covering all the four quadrants. The mean time spent by an animal in all four quadrants was recorded. The time spent in the target quadrants Q4 as compared to time spent in other quadrants in search of the missing platform was taken as an index of retrieval. Care was taken that the relative location of water maze with respect to other objects in laboratory serving as visual clues was not disturbed during the total duration of the study.<sup>[20]</sup>

### Estimation of Brain Acetyl Cholinesterase Activity

The whole brain acetyl cholinesterase (AChE) activity was measured using the Ellman method.<sup>[21]</sup> Animals were euthanized on the ninth day by cervical dislocation and brain tissue was removed carefully to avoid any injuries. Tissue was homogenized in normal saline and centrifuged. The supernatant was used to estimate the AChE activity. The end point was the formation of yellow colour due to the reaction of thiocholine from acetylcholine iodide in the 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 the yellow colour compound formed during the reaction at 412 nm every minute was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula.

$$R = \frac{\$ O.D. \times \text{volume of assay (5ml)}}{E \times \text{mg of protein}}$$

where R=rate of enzyme activity in 'n' mole of acetyl choline iodide hydrolysed/min/mg protein. \$O.D.=change in absorbance/min, E=extinction coefficient – 13,600/M/cm.

Group I: Control group treated with normal saline

Group II: Served as Phenytoin (12 mg/kg, p.o.) treated group

Group III: and IV: EtAS 100 mg and 200 mg treated group

Group V: and VI: EAAS, 100 and 200 mg treated group.

Group VII: Treated with piracetam (200 mg/kg, p.o.)

### Statistical Analysis

The data were expressed as mean±SEM. The data were analysed using one-way ANOVA followed by Tukey–kramer test.  $P < 0.01$  was considered significant.

## RESULTS

### Effect on Transfer Latency using Elevated Plus Maze

Aged mice showed higher TL values on first day and second day as compared to young mice, indicating impairment in learning (ageing-induced amnesia). Scopolamine (0.4 mg/kg, i.p.) increased the transfer latency significantly ( $P < 0.01$ ) in young mice on first and second day as compared to control indicating impairment of memory. Treatment with piracetam (200 mg/kg, i.p.) for 8 days decreased TL as compared to the control group, indicating improvement in both learning and memory. Pretreatment with EAAS (100 and 200 mg/kg, p.o.) decreased the TL on eighth day and ninth day in young and aged mice ( $P < 0.01$ ) when compared to control groups. Higher dose of EAAS (200 mg/kg, p.o.) significantly enhanced anti-amnesic property

in aged animals rather than young mice as reflected by marked decrease in TL on eighth day and ninth day when subjected to EPM tests. EAAS (200 mg/kg, p.o.) exerted profound enhancement of memory in young mice and protected them from against scopolamine ( $P<0.01$ ) and ageing-induced amnesia. The results are summarized in Figure 1. The activity of mice on Elevated plus maze is shown in Figure 2.

**Effect on Brain Acetyl Cholinesterase Activity**

EAAS (100 and 200 mg/kg, p.o.) significantly produced reduction in whole brain AChE activity of both young and aged mice as compared to the respective control group. The brain AChE activity with phenytion (12 mg/kg, i.p.) exhibited significant elevation that was considered as negative control. Piracetam (200 mg/kg, i.p.) profoundly reduced AChE activity as compared to the control group [Figure 3].

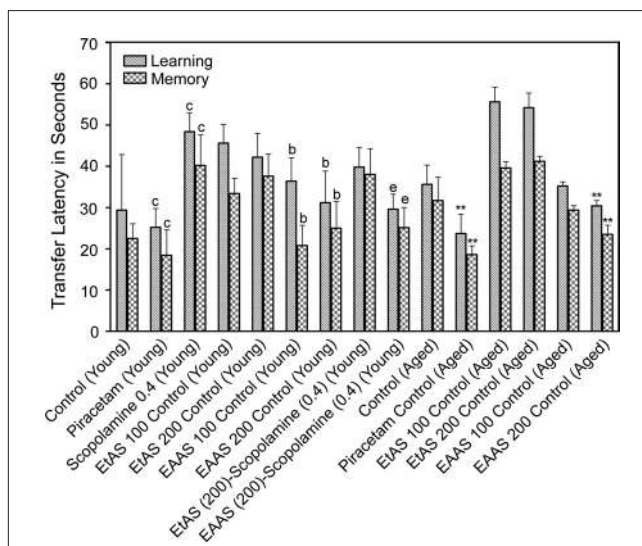
**Effect of *A. speciosa* on Scopolamine-induced Enhancement on ELT in Mice using Water Maze**

A significant decrease ( $P<0.01$ ) in the ELT was observed in the control group mice in their 4 days trial. Scopolamine produced impairment of acquisition and increased the ELT during successive training trials. The action of scopolamine was reversed by pretreatment with EAAS (100 and 200 mg/kg, p.o.) as reflected by a significant decrease ( $P<0.01$ ) in ELT of mice. The results are summarized in Figure 4.

**Effect of *A. speciosa* on Scopolamine and Ageing-induced Alterations in the Time Spent Target Quadrant During Retrieval Trials on Water Maze**

The time spent by young control mice in the target quadrant was more as compared to time spent on other quadrants during retrieval trial on the fifth day. Further scopolamine (0.4 mg/kg, i.p) administered before retrieval trial produced a significant decrease ( $P<0.01$ ) in mean time spent in target quadrant (TSTQ) in search of the missing platform as compared to control (young). Aged mice also decreased TSTQ significantly compared to control (young). The results are shown in Table 1. These observations indicate that scopolamine and natural ageing produced anterograde and retrograde amnesia. Mice treated with piracetam (200 mg/kg, i.p.) produced better effects only in aged mice by decreasing TSTQ as compared to control (aged) mice. EAAS (100 and 200 mg/kg, p.o.) administered before training trial (from day 1 to day 4) significantly ( $P<0.01$ ) attenuated scopolamine and ageing-induced decrease in TSTQ during retrieval test on the fifth day. The results are shown in Table 2. The activity of mice on water maze is shown in Figure 5.

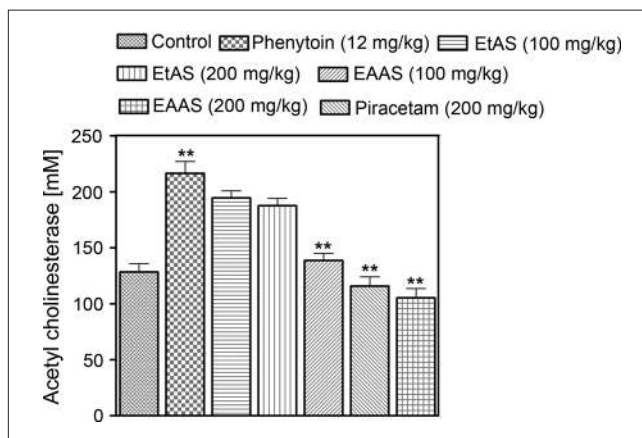
**DISCUSSION**



**Figure 1:** Effect of ethanol and ethyl acetate extracts of *Argyrea speciosa* on transfer latencies of young and aged mice in elevated plus maze model. Values are mean±SEM (n=6). bp<0.01; cp<0.001 compared to control (Young); ep<0.01 compared to scopolamine treated group; \*\*P<0.01 compared to control (Aged)



**Figure 2:** Photograph showing the activity of mice on elevated plus maze. Open arms (16 cm x 16 cm) and two covered arms (16 cm x 5 cm x 12 cm). The arms extended from a central platform (5 cm x 5 cm), and maze was developed to a height of 25 cm from the floor



**Figure 3:** Effect of ethanol and ethyl acetate extracts of *argyrea speciosa* on acetylcholinesterase activity in aged mice. Values are mean±SEM., \*\*P<0.01 compared to control

AD has been identified as a protein misfolding disease due to the accumulation of the abnormally folded amyloid beta protein in the brains of AD patients.<sup>[22]</sup> This neuropathological disorder is increasingly diagnosed in all countries where the number of patients rises exponentially with life expectancy. It has been estimated that about 5% of the population aged more than 65 years are affected by AD. Acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine are the only FDA-approved drugs currently used for the treatment of mild or moderate cases of dementia. Recent reports established an important role for soluble Aβ in the development of AD.<sup>[23]</sup> Earlier studies have demonstrated that the exposure of cells to soluble Aβ could lead to neuronal apoptosis following oxidative stress, pro-inflammatory signals and cytoskeleton perturbations.<sup>[24-27]</sup> Due to its fusogenic properties, the amphiphilic non-aggregated Aβ oligomers could be the proximate effectors of the neuronal degeneration and death occurring in early stages of AD.<sup>[28-30]</sup> Therefore, there is a need for the development of novel therapeutic strategies that target or even better prevent the molecular mechanisms

leading to dementia. Subgroups of Ayurvedic *rasayanas*, known as *medhyarasayanas*, are used to promote intellect and memory. The cognitive promoting effect of *medhyarasayanas* is best seen in children with memory deficits, or when memory is compromised following head injury, prolonged illness or in old age.<sup>[31]</sup> The present study indicates that EAAS fraction of *A. speciosa* is a potential anti-amnesic agent. It also possesses nootropic activity in view of its facilitatory effect on retention and acquired learning. EAAS (100 and 200 mg/kg, p.o.) decreased transfer latencies in both young but more profoundly in aged mice in a dose-dependent manner as compared to respective controls. The central cholinergic system plays an important role in learning and memory. Phenytoin is known to reduce the hippocampal AChE concentration<sup>[32,33]</sup> and causes cognitive impairment. In our study, phenytoin *per se* (12 mg/kg i.p.) significantly elevated brain AChE activity. Piracetam (200 mg/kg, i.p.) and EAAS (100 and 200 mg/kg, p.o.) on the other hand significantly ( $P < 0.01$ ) lowered this activity indicating the counteracting action of the drugs on the cholinergic system. EAAS elicited profound neuroprotective effect in scopolamine treated and older mice compared to control groups and piracetam-treated mice. It significantly inhibited AChE activity in the whole brain homogenate in mice

**Table 1: Effect of *A. speciosa* on the mean time spent in the target quadrants Q4 in young mice using Morris water maze**

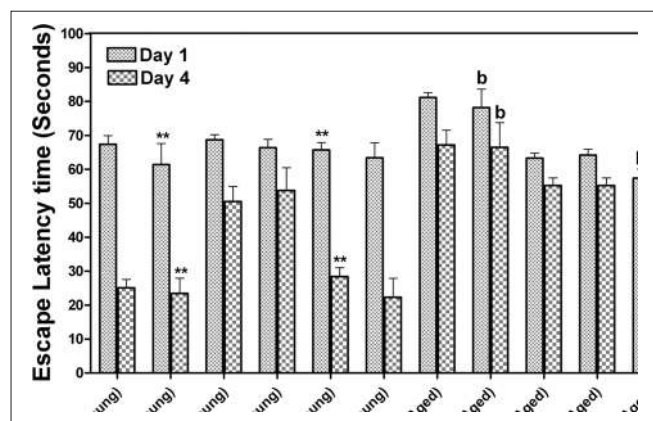
Group	Treatment	Dose (mg/kg, i.p./p.o.)	TSTQ (s)
I	Control	10	68.7 ± 1.23
III	Piracetam	200	54.2 ± 2.13*
V	Scopolamine	0.4	30.2 ± 1.55
VI	EtAS	100	33.2 ± 2.54
VII	EtAS	200	37.4 ± 3.53
VIII	EAAS	100	58.4 ± 2.45 <sup>†</sup>
IX	EAAS	200	62.6 ± 7.23 <sup>††</sup>
X	EtAS+scopolamine	200	36.3 ± 1.24
XI	EAAS+scopolamine	200	56.6 ± 2.74 <sup>†††</sup>

Each value represents mean ± SEM. \* $P < 0.01$  as compared to control, <sup>†</sup> $P < 0.001$  as compared to control, <sup>††</sup> $P < 0.001$  as compared to control, <sup>†††</sup> $P < 0.01$  as compared to scopolamine-treated mice

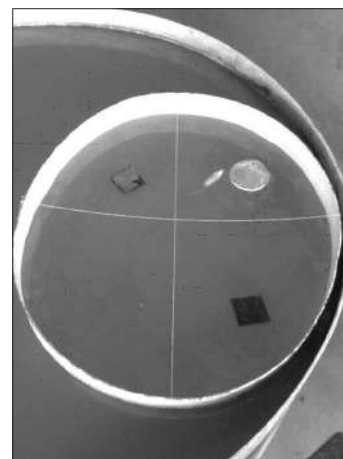
**Table 2: Effect of *A. speciosa* on the mean time spent in the target quadrants Q4 in aged mice using Morris water maze**

Group	Treatment	Dose (mg/kg, i.p./p.o.)	TSTQ (s)
II	Control (aged)	10	29.4 ± 2.44
IV	Piracetam	200	52.4 ± 1.75*
XII	EtAS	100	36.4 ± 3.44
XIII	EtAS	200	38.2 ± 4.34
XIV	EAAS	100	55.2 ± 4.43 <sup>†</sup>
XV	EAAS	200	58.4 ± 2.33 <sup>††</sup>

Each value represents mean ± SEM, \* $P < 0.001$  as compared to control, <sup>†</sup> $P < 0.001$  as compared to control, <sup>††</sup> $P < 0.001$  as compared to control



**Figure 4:** Effect of ethanol and ethyl acetate extracts of *Argyrea speciosa* on escape latency time of young and aged mice using morris water maze. Each value represents ± SEM (n=6), \*\* $P < 0.01$  as compared to control mice (young), <sup>†</sup> $P < 0.01$  as compared to control mice (Aged)



**Figure 5:** Photograph showing the activity of mice on morris water maze (150 cm diameter, 45 cm height). A platform (10 cm<sup>2</sup>) of 29 cm height was located in the centre of one of the four quadrants

indicating its potential in the attenuation of learning and memory deficits especially in aged mice.

In the water maze model, a marked decrease in ELT, during subsequent trials as compared to the first exposure, denotes normal learning ability. The enhancement in the time spent by the animal in the target quadrant reflects successful retention of learned task (or memory).

Amnesia is inability to remember past experiences or loss of memory. Anterograde amnesia is the impairment of memory for events occurring after accident/drug treatment. In such case, new memories are not formed. Retrograde amnesia is the impairment of memory of events which have occurred before the accident or drug treatment. In such case new memories can be formed, but old memories are lost. In the present study, scopolamine (0.4 mg/kg, i.p.) showed anterograde amnesia as indicated by significant decrease in more TSTQ on fifth day in the Morris water maze model. Our observation suggested that EAAS (100 and 200 mg/kg, p.o) reversed the scopolamine and ageing- induced amnesia.

The study justify the traditionally claimed intellect promoting and brain tonic potentiality of roots of *A. speciosa* and further investigations are warranted to explore the possible phytoconstituents from the potent fraction(s) responsible for the management of AD and other cognitive disorders.

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