

Research Article



Memory Enhancing Activity of Ethanolic Extract of *Delonix regia* Leaves against Scopolamine induced Amnesia in Swiss Albino Mice

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ABSTRACT

In traditional system of medicine, various parts of *Delonix regia* have been used for many ailments. The objective of the present study was to evaluate the memory enhancing activity of Ethanolic Extract of *Delonix regia* leaves (EEDRL) against scopolamine induced amnesia by using Elevated Plus Maze, Y Maze and Morris Water Maze Models. Ethanolic Extract of *Delonix regia* was prepared then subjected to phytochemical analysis revealed the presence of flavonoids, alkaloids, carbohydrates, tannins, steroids, terpenoids, phenols and saponins. Acute oral toxicity was performed as per OECD guidelines 423. Based on this, two dose levels of EEDRL were chosen as 200 mg/kg and 400 mg/kg for pharmacological screening. Swiss albino mice were divided into five groups of six animals each. EEDRL at a dose levels 200 mg/kg & 400 mg/kg showed increase in inflexion ratio in Elevated Plus Maze, increase in Percentage alterations in Y Maze & decrease in Escape latency in Morris Water Maze Model compared to disease control in dose dependent manner which indicates that the EEDRL reverses the scopolamine induced amnesia in mice. The memory enhancing activity in mice might be due to facilitation of cholinergic transmission. Hence it can be concluded that *Delonix regia* appears to be a promising candidate for improving memory, and it would be worthwhile to explore the potential of this plant in the management of Alzheimer patient.

Keywords: *Delonix regia*, Scopolamine induced amnesia, Alzheimer's disease.

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INTRODUCTION

Memory is the ability of an individual to record the event, information and retains them over short or long periods of time. The different conditions such as age, stress, and emotion may lead to memory loss, amnesia, anxiety, high blood pressure, dementia to more threat like schizophrenia and Alzheimer's disease^{1,2}.

Alzheimer's disease (AD) is the most common neurodegenerative disease that can be characterized through a progressive loss of episodic memory and cognitive function, with later deficiency of language and visuospatial abilities. Such changes are often accompanied by behavioural disorders such as apathy, aggressiveness and depression. Anatomopathologically, AD is characterized by senile plaques, composed of a nucleus of β -amyloid protein accumulation ($A\beta_{42}$), as extracellular lesions and neurofibrillary tangles composed of phosphorylated tau protein and which are intraneuronal findings. Loss of cholinergic cells particularly in the basal forebrain is accompanied by loss of the neurotransmitter acetylcholine³.

Scopolamine is a centrally acting anti-cholinergic agent which causes impairment in learning. The treatment with drugs which increase cholinergic neurotransmission causes an improvement in cognitive deficits in AD⁴. Therefore, Scopolamine-induced amnesic animal models are used to screen for agents that are claimed to have cognition-enhancing activity through stimulation of the cholinergic system, thus making them candidates for the treatment of AD. AChE inhibitors from general chemical classes such as physostigmine, tacrine, galantamine, and heptylphysostigmine have been tested for the symptomatic treatment of AD. Medicinal plants are playing a significant role in the management of AD and memory deficit⁵.

Delonix regia also known as the Royal Poinciana or Flamboyant, is a species of flowering plants in the pea family, Fabaceae and subfamily Caesalponioideae. In some countries, *D. regia* has folkloric used as a medicinal agent to treat some disorders, such as constipation, inflammation, rheumatoid arthritis, diabetes, pneumonia, and malaria. Many biological activity substances in the extracts of *D. regia* were reported to have anti-inflammatory, antioxidant, antimicrobial, anti-diarrhoeal, antidiabetic, hepatoprotective, wound healing, and gastroprotective activity⁶. However, its anti-amnesic potential yet to be proved. Therefore, present study was aimed to investigate the beneficial effects of ethanolic extract of *Delonix regia* Leaves (EEDRL) on cognitive function in scopolamine induced amnesia in mice.



MATERIALS AND METHODS

Plant material

The plant material was collected from Trichy district, Tamil Nadu (India), in the month of May 2020. The plant material was identified and authenticated by Dr. V. Nandagopalan, Controller of Examinations, Associate Professor in Botany, National College (Autonomous), Tiruchirappalli.

Preparation of Plant extract

The dried coarsely powdered sample of *Delonix regia* leaves was first extracted with Petroleum ether (60-80°C) in Soxhlet apparatus and then using ethanol as a solvent at 60 - 70°C. Then the extract was concentrated with the help of hot plate. The solvent was removed by distillation under reduced pressure⁷.

Phytochemical Screening

The preliminary phytochemical screening was carried to identify the phytoconstituents. The result showed that the presence of flavonoids, alkaloids, carbohydrates, tannins, steroids, terpenoids, phenols and saponins⁷.

Animal Experimentation

The experimental protocols for the memory enhancing activities have been approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Indian National Sciences Academy for the use and care of experimental animals. IAEC approved this proposal with approval number PCP/IAEC/004/2020.

Toxicity studies⁸

Acute Toxic Class method: Guideline number- 423

According to Organization for Economic Cooperation Development (OECD) guideline 423, Ethanolic Extract *Delonix regia* Leaves (EEDRL) at a dose level of 5, 50, 300 and 2000 mg/kg [orally (p.o.)] was used for acute oral toxicity study. EEDR administered at a dose of 2000 mg/kg body weight did not produce any behavioural abnormalities in the animals. As all tested animals survived, the oral LD₅₀ of EEDRL in mice was found to be 200 mg/kg body weight.

Selection of doses

For the assessment of antialzheimer's activity by animal models, two dose levels of EEDR were chosen in such a way that low dose was approximately one-tenth of the maximum dose during acute toxicity studies, and a high dose, which was twice that of one-tenth dose (200 mg/kg and 400 mg/kg).

Memory enhancing activity of Ethanolic Extract of *Delonix regia* Leaves (EEDRL)

Experimental Animals

Healthy Swiss albino mice of either sex weighing between 20 and 25 g were taken for the evaluating nootropic activity. They were housed in polypropylene cages containing bedding material as husk and maintained under

controlled conditions of temperature (25±2°C), humidity (55±5%) and 12 hrs light and 12 hrs dark cycles. They were fed with standard pellet diet with water ad libitum.

Scopolamine induced amnesia

Scopolamine is a muscarinic Ach receptor antagonist that can cause learning and memory deficits by disrupting cholinergic neurotransmission; this compound has been used to induce amnesia in experimental animal

Experimental Design⁹

Adult male Swiss albino mice of either sex were divided into five groups. Each group containing 6 animals. The following were the groups:

Group I served as Normal control received Normal saline *p.o.*, daily for 15 days.

Group II served as Negative control received Normal saline *p.o.*, daily for 15 days

Group III served as Positive control received Piracetam 200 mg/kg *i.p.*, for 15 days.

Group IV and **Group V** served as Treatment groups received EEDRL 200 mg/kg and 400 mg/kg *p.o.* respectively daily for 15 days.

After 45 min of administration of the last dose on 15th day, amnesia was induced by administration of scopolamine (1mg/ kg *i.p.*) to animals in all groups except group I. After 45 min of administration of scopolamine, initial transfer latency was recorded on Elevated Plus Maze and Retention Transfer Latency was recorded after 24 h of the first exposure on Elevated Plus Maze and also the trials were taken on Y Maze Model and Morris water maze.

Behavioral Model^{9,10}

Elevated Plus Maze test (EPM)

The elevated plus maze served as the exteroceptive behavioral model (wherein stimulus existed outside the body) to evaluate learning and memory in mice. Mice were placed individually at the end of either of open arms facing away from centre and the time taken by the animals to move from open arm to enclosed arm was noted on the first day (Initial transfer latency, ITL). Transfer latency (TL) is used as parameter for estimation of memory enhancing property. If the animal did not enter an enclosed arm within 90 s, it was pushed on the back into one enclosed arm and the transfer latency was given as 90s. Later, the animal was allowed to move freely to explore the apparatus for at least 20 s. After the experiment, the animals were returned to their home cages and transfer latency was recorded again after 24 h of the first exposure (retention transfer latency, RTL). The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was used to minimize odour cues. The transfer latency measured on the first- and second-day trial served as an acquisition (learning) and retention (memory) respectively (Kasture et al. 2007). From these, inflexion ratio (IR) was calculated using the formula



$$IR = (L_0 - L_1) / L_0$$

Where, IR = Inflexion ratio, L_0 = Initial transfer latency in seconds, L_1 = Retention transfer latency in seconds. Decreased IR indicates the induction of amnesia and increased IR indicates improvement in cognition and memory impairment.

Y Maze test

Y Maze analysis has been shown to be reliable, non-invasive test to determine cognitive changes in mice through the measurement of the spontaneous alteration behaviour in the Y Maze task. Each mouse was initially placed at the end of arm A, allowed to move freely and the sequence and number of arm entries were recorded manually over 8 min period. The time limit in Y-maze test was 8 min, and every session was stopped after 8 min. An arm entry was counted when the hind paws of the mice were completely within the arm. Spontaneous alteration behavior was defined as three consecutive entries in three different arms (i.e., ABC or BCA etc.) Mouse tends to explore the maze systematically, entering each arm in turn. The ability to alternate required that the mice knew which arm they had already visited. The percentage of triads in which all three arms were represented, i.e., ABC, CAB, or BCA but not BAB, was recorded as an 'alternation' to estimate short term memory. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was used to minimize odour cues. The percentage alternation score for each animal was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation:

Percentage alternation = [(Number of alternations) / (Total arm entries - 2)] × 100

Morris water maze model

The apparatus consisted of a circular pool (1.5 m diameter, 35cm deep) which contained water to a depth of 23 cm (at a temperature of approximately 30°C). A circular platform (10 cm×11.5cm, 20cm tall) was placed in one quadrant of the pool, 1cm above the water level during the acquisition phase. A similar platform was placed 1cm below the water level for retention phase. The position of the platform was not changed in any quadrant during assessment of both the phases. Each trial involved placing the mice in the pool, in 1 of the 4 pool quadrants.

Maze acquisition phase (training)

During the learning period, animals were allowed to swim freely until they found the escape platform. The time taken by the mouse to reach the visual platform was taken as the initial acquisition latency (IAL). If a mouse failed to find the platform within the allotted period (300s, initial learning

period; 180s, scopolamine induced amnesia trials), it was placed on the platform by the experimenter for 30s. During the initial learning period, mice were tested sequentially, 4 times per day, for 3 days, during which the escape platform was located in a fixed position in the centre of the pool

Maze retention phase (testing for retention of the learned task)

During the scopolamine trials, mice were tested sequentially, 3 times per day, with each of the three trials being performed between 30 and 45 min following scopolamine injection. Time taken by the mouse to find the hidden platform on day 4th following start of scopolamine administration was recorded, termed as retention latency (RL)

RESULTS AND DISCUSSION

Effect of Ethanolic extract of *Delonix regia* leaves on transfer latency in Elevated Plus Maze.

Transfer Latency of first day (On 15th day of drug treatment) reflected learning behaviour of animals represented as Initial Transfer Latency (L_0), Transfer Latency of second day (24 hours after Initial Transfer Latency) reflected retention of memory represented as Retention Transfer Latency (L_1)

Inflexion ratio was calculated from transfer latencies which is represented as IR. Decreased IR indicates the induction of amnesia and increased IR indicates improvement of memory.

Inflexion ratio is shown in **Tab. No:1 & Fig. No: 1**. It was observed that Group I control animals showed an inflexion ratio of 0.265 ± 0.007 . Disease control animals showed a significant ($P < 0.0001$) decrease in inflexion ratio of -0.147 ± 0.103 . As decreased inflexion ratio indicates the induction of amnesia which showed that scopolamine (1mg/kg) impaired retention of memory significantly in group II animals.

Group III animals pretreated with Piracetam 200mg/kg and Group IV & V were treated with EEDRL 200 mg/kg and EEDRL 400 mg/kg respectively for 15 days before scopolamine administration showed a significant increase ($P < 0.0001$) in inflexion ratio compared to group II scopolamine induced disease control animals. The inflexion ratio of animals treated with Piracetam 200mg/kg was 0.370 ± 0.014 , EEDRL 200mg/kg was 0.452 ± 0.022 and animals treated with EEDRL 400mg/kg was 0.48 ± 0.018 . As the increase in inflexion ratio indicates the improvement in memory.

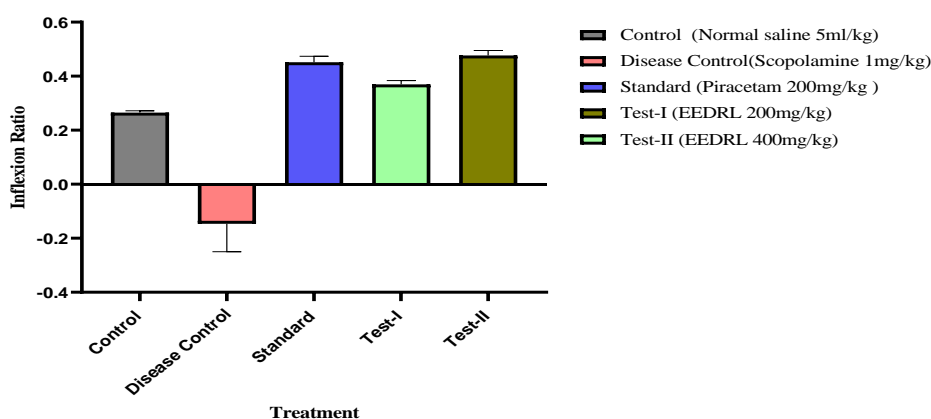
From the results, Treatment with Ethanolic Extract of *Delonix regia* leaves (EEDRL) showed a dose dependent increase in inflexion ratio which indicates the improvement in cognition and memory.



Table 1: Treatment effect on Transfer Latency (By Elevated Plus Maze)

Group	Treatment	Initial Transfer Latency in sec (L ₀)	Retention Transfer Latency in sec (L ₁)	Inflexion Ratio (L ₀ , L ₁)/ L ₀ (IR)
I	Control (Normal Saline 5ml/kg)	53.83±0.44	39.67±0.49	0.265±0.007
II	Disease Control (Scopolamine 1mg/kg)	76.00±0.33####	80.00±0.57####	-0.147±0.103####
III	Standard (piracetam 200 mg/kg)	43.83±0.43****	25.17±0.94****	0.452±0.022****
IV	Test-I (EEDRL 200mg/kg)	46.17±0.55****	29.00±0.36****	0.370±0.014****
V	Test-II (EEDRL 400mg/kg)	39.83±0.31****	20.83±0.60****	0.480±0.018****

Values are mean ± SEM. (n=6). #### indicates P< 0.0001 compared to control, **** indicates P<0.0001 compared to disease control. (one way ANOVA followed by Dunnett's test)

**Figure 1:** Effect of EEDRL on scopolamine induced amnesia in mice using Elevated Plus Maze maze. (Inflexion Ratio)

Effect of Ethanolic extract of *Delonix regia* leaves on Percentage alteration in Y- maze.

Spontaneous alteration refers to the tendency of normal animals to visit the least recently entered or experienced arm of the maze. The percentage alteration was calculated based on number of alterations and total arm entries.

The decrease in percentage alteration indicates the impairment of memory and the increase in percentage alteration indicates the improvement of memory.

Percentage alteration was shown in Table no 2 & Fig. No: 2. It was observed that Group I control animals showed percentage alteration of 33.52±0.75 & the number of alteration and total number of arm entries were 9.0±0.25 & 28.83±0.40 respectively.

Group II Disease control animals administered scopolamine (1mg/kg) on 15th day after receiving normal saline for 15 days showed a significant (P< 0.0001) decrease in percentage alteration 19.42±1.46 when compared to Group I Control animals (33.52±0.75). The number of alterations were significantly decreased 6.5±0.56 and the total arm entries were significantly increased 36.33±0.49 when compared to Group I control animals.

As the decrease in percentage alteration in Group II animals administered with scopolamine (1mg/kg) indicates the impairment of memory.

Group III animals pretreated with Piracetam 200mg/kg and Group IV & V were treated with EEDRL 200 mg/kg and EEDRL 400 mg/kg respectively for 15 days before scopolamine administration showed a significant (P< 0.0001) increase in percentage alteration when compared to Disease control animals. The number of alterations were significantly increased and total number of arm entries was significantly decreased after treatment.

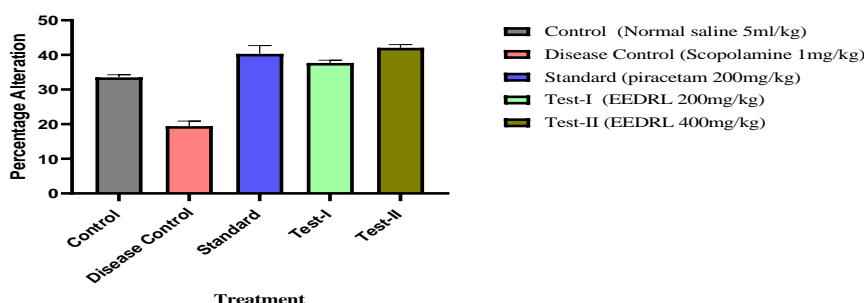
Animals treated with piracetam (200 mg/kg) showed percentage alteration 40.30±2.37, the number of alterations 12.33±0.88 and the total arm entries 32.50±0.56, animals treated with EEDRL 200 mg/kg showed percentage alteration 37.70±0.76, the number of alterations 9.66±0.76 and the total arm entries 27.66±2.04, whereas animals treated with EEDRL 400 mg/kg showed percentage alteration 42.06±0.90, the number of alterations 12.16±0.70 and the total arm entries 30.83±1.13. As the increase in percentage alteration in Group III, IV & V indicates improvement of memory.

From the results, Treatment with Ethanolic Extract of *Delonix regia* leaves (EEDRL) showed a dose dependent increase in Percentage alterations which indicates the improvement in cognition and memory.

Table 2: Treatment effect on Percentage alteration (By Y Maze)

Group	Treatment	Number of Alterations	Total arm entries	Percentage Alteration
I	Control (Normal Saline 5ml/kg)	9.0±0.25	28.83±0.40	33.52±0.75
II	Negative Control (Scopolamine 1mg/kg)	6.5±0.56	36.33±0.49	19.42±1.46####
III	Standard (piracetam 200 mg/kg)	12.33±0.88	32.50±0.56	40.30±2.37****
IV	Test-I (EEDRL 200mg/kg)	9.66±0.76	27.66±2.04	37.70±0.76****
V	Test-II (EEDRL 400mg/kg)	12.16±0.70	30.83±1.13	42.06±0.90****

Values are mean ± SEM. (n=6). #### indicates P< 0.0001 compared to control, **** indicates P<0.0001 compared to disease control. (One way ANOVA followed by Dunnett's test)

**Figure 2:** Effect of EEDRL on scopolamine induced amnesia in mice using Y Maze model. (Percentage alteration)

Effect of Ethanolic extract of *Delonix regia* leaves on Escape Latency in Morris Water Maze Model.

The time taken by the mouse to reach the visual platform was taken as the initial acquisition latency (IAL).

The acquisition latency was observed for three days during the training period (Before scopolamine treatment) which is shown in Table No 3 & Fig. No: 3.

During the scopolamine trials, scopolamine was administered to all groups except Group I control. Time taken by the mouse to find the hidden platform on day 4th following scopolamine administration was recorded, termed as retention latency (RL)

From Table No 3 & Fig. No: 3, it was observed that Group I control animals showed Retention latency 17.17±0.31 whereas, Group II Disease control animals showed significant (P< 0.0001) increase in Retention latency 83.50±0.50 when compared to Group I control animals.

Group III animals pretreated with Piracetam 200mg/kg and Group IV & V were treated with EEDRL 200 mg/kg and EEDRL 400 mg/kg respectively for 15 days before scopolamine administration showed a significant decrease (P<0.0001) in Retention latency compared to group II scopolamine induced disease control animals. The Retention latency of animals treated with Piracetam 200mg/kg was 14.00±0.36, EEDRL 200mg/kg was 17.17±0.48 and animals treated with EEDRL 400mg/kg was 13.17±0.31. As the decrease in Escape Latency after scopolamine administration (Retention phase) indicates the improvement in memory.

Treatment with Ethanolic Extract of *Delonix regia* leaves (EEDRL) showed a dose dependent decrease in Escape Latency which indicates the improvement in cognition and memory

Table 3: Treatment effect on Escape latency (By Morris Water Maze)

Group	Treatment	Escape Latency Before Scopolamine (Sec) Acquisition Latency			Escape Latency After scopolamine (sec) Retention Latency
		Day 1	Day 2	Day 3	Day 4
I	Control (Normal Saline 5ml/kg)	48.00±0.57	36.83±0.31	28.00±0.36	17.17±0.31
II	Negative Control (Scopolamine 1mg/kg)	49.17±0.47	38.00±0.36	28.50±0.43	83.50±0.50####
III	Standard (piracetam 200 mg/kg)	49.0±0.52****	33.83±0.31****	24.50±0.22****	14.00±0.36****
IV	Test-I (EEDRL 200mg/kg)	48.83±0.48****	35.33±0.33****	25.66±0.21****	17.17±0.48****
V	Test-II (EEDRL 400mg/kg)	47.33±0.33****	33.50±0.22****	23.83±0.31****	13.17±0.31****

Values are mean ± SEM. (n=6). #### indicates P< 0.0001 compared to control, **** indicates P<0.0001 compared to disease control. (Two-way ANOVA followed by Dunnett's test)



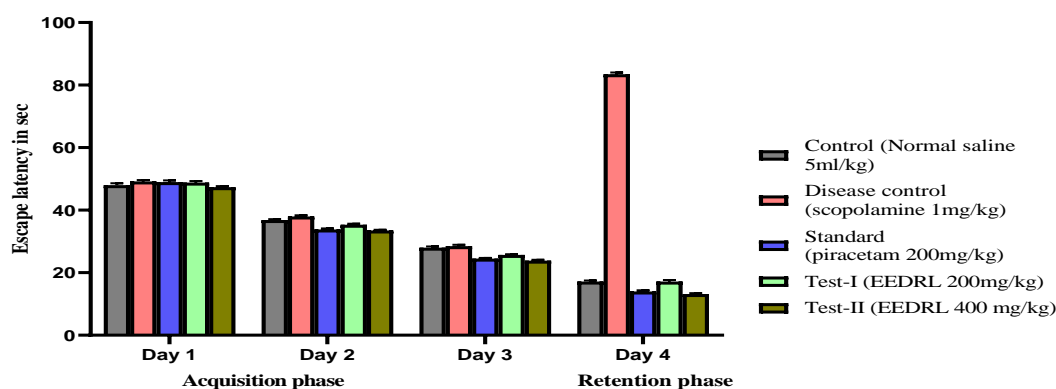


Figure 3: Effect of EEDRL Leaves on learning performance and memory retention against scopolamine induced amnesia by Morris water maze model

CONCLUSION

The results from the present study demonstrated that scopolamine impaired learning & memory process in animals, whereas administration of EEDRL significantly ameliorated scopolamine induced amnesia in elevated plus maze, Y maze & morris water maze model as indicated by significant increase in Inflexion Ratio (IR), increased Percentage alteration & reduction in Escape Latency respectively. The memory enhancing activity in mice may be due to facilitation of cholinergic transmission. Hence it can be concluded that *Delonix regia* leaf Extract appears to be a promising candidate for improving memory, and it would be worthwhile to explore the potential of this plant in the management of Alzheimer patient.

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