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PLUMBAGO ZEYLANICA ROOTS: A POTENTIAL SOURCE FOR IMPROVEMENT OF LEARNING AND MEMORY

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ABSTRACT

The present study is to investigate the effect of *Plumbago zeylanica* roots on learning and memory of mice. The exteroceptive behaviour model (Elevated plus maze and Passive avoidance paradigm) and interoceptive behaviour model i.e. scopolamine induced amnesia were employed to evaluate the effect of *Plumbago zeylanica* roots on learning and memory of mice. The Chloroform extract of *Plumbago zeylanica* (100, 200 and 400 mg/kg. p.o.) was administered for 10 successive days in separate group of animals. The *Plumbago zeylanica* at dose 200mg/kg. has shown promising memory enhancing effect in mice. Furthermore, the extract significantly reversed the amnesia induced by scopolamine (0.4mg/kg i.p.). The reversal of scopolamine induced amnesia may be due to facilitation of cholinergic transmission in mice brain. Antioxidant, hypolipidaemic and anti-atherosclerotic properties of *P. zeylanica* may be contributing favourably to memory enhancing effect.

KEY WORDS

Plumbago zeylanica, learning, memory, amnesia

INTRODUCTION

Plumbago zeylanica Linn (Plumbaginaceae) is a perennial shrub found wild in South India and West Bengal. It is also cultivated in gardens through out India. The roots of *P. zeylanica* (popularly known as 'Chitrak') is reported to possess great pharmacological importance in traditional system of medicine and employed clinically for their antifertility, germicidal, antileprotic and anti-inflammatory activities[1-2]. The plant is also reported to possess central nervous system stimulatory, hepatoprotective, antioxidant, hypolipidaemic and anti-atherosclerotic properties[3-6]. Scientist are undertaking research constantly to identify a moiety to improve learning and memory since there is lack of satisfactory drugs in allopathic system of medicine. In the present paper we have made an effort in this direction by selecting *P. zeylanica*, a potential medicinal shrub.

MATERIAL AND METHODS

Plant material

The roots of *P. zeylanica* purchased in July 2005 from Khari bawri market, Delhi. The roots were taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum division, National Institute of Science Communication and Informational Resources (NISCAIR), New Delhi, India. A voucher specimen is preserved in the department for the further reference.

Preparation of P. zeylanica root extract (PZE)

The roots (500 gm) were sliced into small pieces and pulverized using a mechanical grinder. The powdered roots were subjected to soxhlet extraction with chloroform for 72 hours. After exhaustive extraction the extract was filtered and concentrated by

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vacuum evaporator so as to yield dark yellow extract (6.96 gm; yield 1.39%)

Drugs

Scopolamine hydrobromide (Sigma chemicals Co., St.Louis, USA), Tween 80 (Loba Chemie, Mumbai, India) were used in the present study.

Vehicle

The emulsion was prepared with 100, 200 and 400 mg extract triturated with 1ml of Tween 80 adding small volume of water and then make up the volume upto 10 ml. which was ready for oral administration. Scopolamine hydrobromide was dissolved in normal saline and injected intraperitoneally.

Experimental animals

Young Swiss albino mice weighing around 20-30 gm are used for the present study. Animals are procured from disease free small animal house of CCS Haryana Agriculture University, Hisar. The animals had free access to food, clean water and were housed in a natural light-dark cycle (12hour each). The study animals were acclimatized for at least 7-days to laboratory conditions prior to undertaking behaviour studies. Experimental protocol was approved by the Institutional Animal Ethics Committee and care of laboratory animals was taken as per guidance of Ministry of Forest and Environment, Government of India (registration no.0436)

Laboratory models employed to evaluate learning and memory

Passive avoidance behaviour based on negative reinforcement was used to examine the long term memory.

Interoceptive behaviour model

Scopolamine induced amnesia

Exteroceptive behaviour model

(i) *Elevated plus maze*: Elevated plus-maze serves as the exteroceptive behavior model to evaluate learning and memory in mice and procedure for evaluation of learning and memory was followed as per the parameters described by investigators[7-8]. The elevated plus maze apparatus for mice consists 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 is elevated to a height of 25 cm from the floor. On the first day, each mice is placed at the end of an open arm, facing away from the central platform. Transfer Latency **(TL)** is defined as the time taken by the animal to move into one of the enclosed arms with all its four legs. **TL** is recorded on the first day for each animal. The mice is allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task is examined 24 h after the first day trial.

(ii) *Pasive Avoidance Paradigm*: Passive avoidance behaviour, based on negative reinforcement is used to examine the long-term memory[9]. The apparatus consists of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of Plexiglass, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box is illuminated with a 15 W bulb during the experimental period. Electric shock (20 V. A.C.) is delivered to the grid floor. Training is carried out in two similar sessions. Each mouse is gently placed on the wooden platform set in the center of the grid floor. When the mice stepped-down placing all its paws on the grid floor, shocks are delivered for 15 seconds and the step-down latency (**SDL**) is recorded. **SDL** is defined as the time taken by the mice to step down from the wooden platform to grid floor with all its paws on the grid floor. Animals showing **SDL** in the range of 2-15 seconds during the first test are used for the second session and the retention test. The second-session is carried out 90

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minutes after the first test. When the animals step down before 60 seconds, electric shocks are delivered for 15 seconds. During the second test animals are removed from shock free zone, if they do not step down for a period of 60 seconds. Retention is tested after 24 h in a similar manner, except that the electric shocks are not applied to the grid floor observing an upper cut-off time of 300 seconds.

Drug Protocol

In the present investigation the mice were divided into 12 different groups for employing various interoceptive and exteroceptive memory models. Each group was comprised of a minimum five animals. The Chloroform extract (**PZE** ,100, 200 and 400 mg/kg. p.o.) was administered for ten successive days. After 90 minutes of administration of last dose of extract on 10th day, mice were exposed to an training session using elevated plus maze and passive avoidance paradigm and transfer latency and step down latency were recorded. Amnesia was induced in separate group by scopolamine (0.4mg/kg; i.p.) before training. **TL** and **SDL** was noted down after 45 minutes of scopolamine injection. Retention (memory) was recorded after 24 hours (on eleventh day). All control group receive vehicle for 10 days.

Statistical Analysis

All results were expressed as mean ± standard error mean (SEM). Data was analyzed using one way ANOVA followed by Dunnett's t-test and Student's unpaired t-test. P-values<0.05 were considered as statistically significant.

RESULTS

In the present study, **PZE** administered orally for ten successive days improves the learning and memory in mice as reflected by the lowering of **TL** (Table-1 & Fig.I).

Table 1.

Effect of PZE on TL of mice using Elevated Plus-Maze

Sr. no.	Extract/Drug	Dose(kg^{-1})	TL (Sec.)	TL after 24 hrs.
1.	Control	10 ml.	32.2±7.24	18.6±3.93
2.	PZE for 10 days	100 mg.	26.4±5.54	12.6±3.70
3.	PZE for 10 days	200 mg.	12.2±2.87*	6.8±0.86*
4	PZE for 10 days	400 mg.	19.8±3.71*	8.9±1.96*
5.	Scopolamine	0.4 mg.	57.6±6.65*	32.8±6.42
6.	PZE(10 days)+Scopolamine	100mg./0.4mg	41.2±5.04	22.6±3.12
7.	PZE(10 days)+Scopolamine	200mg./0.4mg	26.6±6.16**	16.2±3.59**
8.	PZE(10 days)+Scopolamine	400mg./0.4mg	36.8±5.95*	19.9±3.35*

Values are expressed as mean ±SEM.
n= 5; $^{*}P < 0.05$ as compared to control group.
$^{**}P < 0.05$ as compared to scopolamine alone.
Effect of extract on TL in seconds

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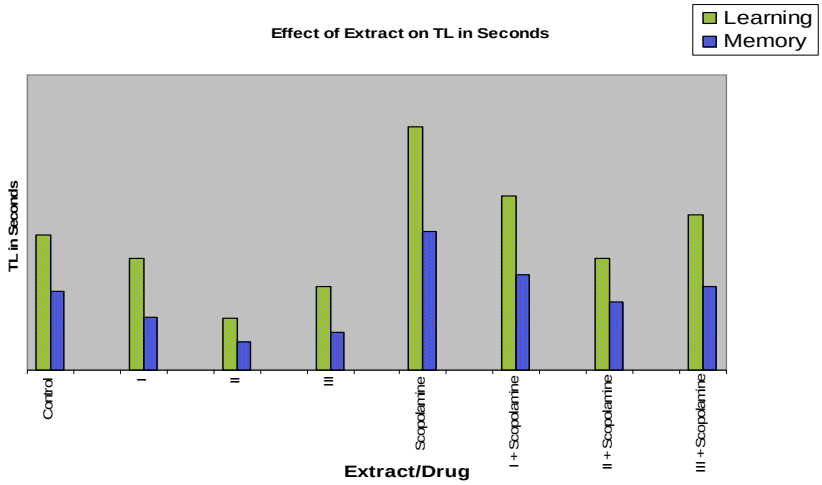

Figure-I

The extract also enhances the Step down latency **SDL** (Table-2 & Fig.II). Further the pretreatment with **PZE** also protected the animal from memory impairment produced by interoceptive stimuli i.e. scopolamine.

Table 2.

Effect of PZE on SDL of mice using Passive avoidance paradigm

Sr.no	Extract/Drug	Dose(kg^{-1})	SDL after 24 hours of training(seconds)
1.	Control	10 ml.	38.4±7.22
2.	PZE for 10 days	100 mg.	80.4±10.47*
3.	PZE for 10 days	200 mg.	187.6±15.41*
4.	PZE for 10 days	400 mg.	142.1±12.85*
5.	Scopolamine	0.4 mg.	21.6±3.32
6.	PZE(10 days)+Scopolamine	100mg./0.4mg	40.6±8.18
7.	PZE(10 days)+Scopolamine	200mg./0.4mg	62.8±7.66**
8.	PZE(10 days)+Scopolamine	400mg./0.4mg	44.51±7.92*

Values are expressed as mean ± SEM.
*n= 5; * P < 0.05 as compared to control group.*
*** P < 0.05 as compared to scopolamine alone.*

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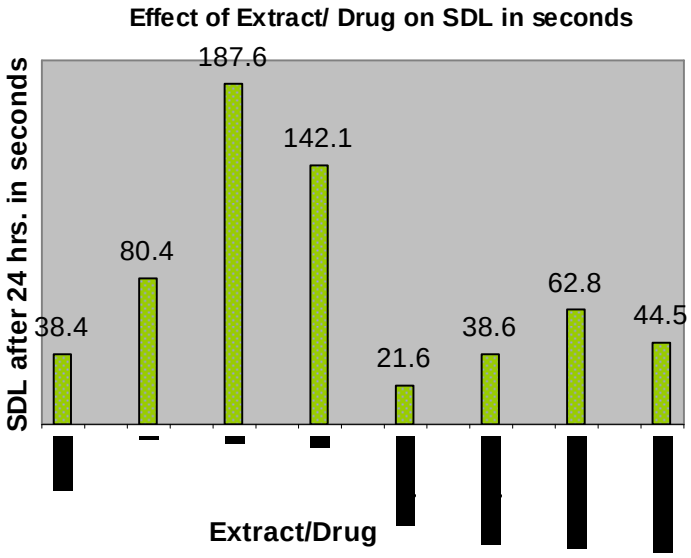

Figure-II

DISCUSSION

Memory forms one of the most complex function of brain and ultimately involves multiple neuronal pathways and neurotransmitter pathway. Cognition is that operation of mind by means of which we become aware of our surroundings objects and thoughts. Alzheimer's disease (AD) represents one of the most common cause of dementia (loss of memory) caused due to degeneration of the cerebral neurons[10]. Immuno histochemical studies suggested that the inflammation of certain regions in brain exists in Alzheimer's disease patinents. This hypothesis supported by the observation that there was reduction in symptom of AD upon chronic use of anti-inflammatory drugs[11]. The main histological feature of AD includes the extracellular deposition of the β-amyloid plaques[12]. Enhanced accumulation of of cholestrol levels in blood increases the β-amyloid deposition in cells[13]. Investigations revealed that high levels of cholestrol contributes to the pathogenesis of the dementia disorder[14-15]. Anti-atherosclerotic and hypolipidaemic effect of plumbagin from *P. zeylanica*[5] might be contributing to the observed improved memory in mice.

Oxygen free radicals have been shown to be neurotoxic[12] and furthermore antioxidant rich diet improved the cerebellar physiology and motor learning in aged rats[17]. Antioxidant property of *P. zeylanica*[6] might be responsible for the scavenging of free radicals in brain and reduce the oxidative stress of brain cells resulting in improved neuronal function and reduce damage to brain cells and there by improves the memory. The present investigation thus establishes that *Plumbago zeylanica* roots are potential source for the improvement of learning and memory in mice.

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providing the necessary raw material for the research work.

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