

**Evaluation of effect on *Cascabela thevetia* leaf extract on spatial memory in rodent**Pawan Kushwah^{1*}, Bharat Kumar Tyagi¹¹*Institute of Professional Studies, College of Pharmacy, Gwalior, Madhya Pradesh, India***Abstract**

The present study aimed to evaluate the effect of hydroalcoholic leaf extract of *Cascabela thevetia* on spatial memory in scopolamine-induced memory-impaired rodents. The leaves were extracted using a hydroalcoholic solvent system and subjected to phytochemical screening, total phenolic content, total flavonoid content, and antioxidant activity assessment by DPPH radical scavenging assay. The extract exhibited the presence of flavonoids, phenolics, and sterols, with total phenolic and flavonoid contents of 91.84 mg GAE/g and 113.22 mg QE/g, respectively. Antioxidant evaluation demonstrated concentration-dependent DPPH radical scavenging activity, reaching $58.9 \pm 1.10\%$ inhibition at 250 $\mu\text{g/mL}$. Nootropic activity was assessed using the Morris Water Maze model in scopolamine-treated rodents. Scopolamine significantly impaired spatial learning and memory, as evidenced by increased escape latency. Treatment with *Cascabela thevetia* extract at doses of 200 and 400 mg/kg significantly reduced the time required to locate the hidden platform, with the 400 mg/kg dose producing the greatest improvement (18.00 ± 0.894 s on Day 4) compared with the scopolamine control group (52.00 ± 2.577 s). The findings suggest that *Cascabela thevetia* leaf extract possesses significant antioxidant and memory-enhancing activities, possibly mediated through its high phenolic and flavonoid content. Thus, the plant may serve as a promising natural therapeutic candidate for the management of cognitive dysfunction and memory impairment.

Keywords: *Cascabela thevetia*, Spatial Memory, Morris Water Maze, Nootropic Activity, Scopolamine

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Introduction

Dementia is a predominant condition, with changing lifestyle. Conditions like Alzheimer's disease and Parkinson's disease are accompanied with progressive dementia. The WHO statistics indicate that currently more than 55 million people throughout the world are suffering from dementia. This number is expected to reach 78 million by 2030 and 139 million till 2050 [1].

Literature studies reveal that several medicinal plants have been studied for their memory enhancing effects in test animal. Most of these effects are presumed to be modulated through the presence of phenolics and flavonoids in the extracts of various parts of these plants.

Cascabela thevetia is a flowering plant of family Apocynaceae and its flowers and leaves have been explored for a wide array of pharmacological actions [2-8]. Several flavonoids and phenolic acids have also been isolated from the plant [9]. This prompted us to investigate the effect on *Cascabela thevetia* leaf extract on its ability to induce improvement in spatial learning in rodent.

The objective of the present work is to assess the effect of *Cascabela thevetia* leaf extract on scopolamine- induced learning and memory impairment in rodents.

Material and Methods

Collection, identification and preparation of the plant material

The plants of *Cascabela thevetia* were purchased from Shubham Nursery, Bhopal, Madhya Pradesh and the preliminary identification of the plant was done at RB Science, Bhopal. The leaves of the plant were washed with distilled water and dried in shade (preventing from direct sunlight). The dried leaf has been powdered using slow speed blender and is kept in closed airtight container.

Extraction of leaves

The extraction was carried out using ethanol as the solvent by hot continuous extraction method using a soxhlet apparatus. Briefly, 90 g of powder was packed in a thimble and the thimble was placed in the extractor of the soxhlet apparatus. 450 mL of hydroalcoholic solution (ethanol-water, 80-20) was flown down the extractor in to the round bottom flask. The flask was heated at 50°C to carry out the extraction process. The extraction was carried out for 7 hours (complete extraction of contents was confirmed by the clear solution in the siphon tube of the extractor). The extract was filtered hot through Whatman filter paper in order to remove any impurity. The extract was then concentrated on boiling water bath to obtain the oleo-resinous residue. The oleo-resinous extract was collected and placed in desiccators to remove the excessive moisture. The dried extract was weighed and stored in desiccator for further analysis [10-13].

Phytochemical Screening of the extract

The extract was tested for the presence of various phytochemicals using the reported methods. Alkaloids (Mayer, Wagner, Dragendorff test), saponin (Froth test), glycosides (Bontrager, Keller-kiliani test), triterpenoids (Salkowski test), tannins and phenolics (Gelatin, ferric chloride, vanillin-hydrochloride, lead acetate, alkaline reagent test), flavonoids (Shinoda, zinc-hydrochloride reduction, alkaline reagent test), protein (Ninhydrin, Millon's test), and carbohydrates (Molisch's test) were tested qualitatively [11-14].

Total Phenolic Content

The total phenolic content in the leaves extract would be determined quantitatively using Folin-Ciocalteu reagent method, using gallic acid as the reference standard. For total phenolic content determination, 200 μ L of sample was mixed with 1.4 mL purified water and 100 μ L of Folin-Ciocalteu reagent. After incubating at room temperature for 15 min, 300 μ L of 20% Na_2CO_3 aqueous solution was added and the mixture was allowed to incubate at room temperature for 2 h. The absorbance of the solution was measured at 760 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the analytical curve. The control solution contained 200 μ L of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample [15].

Total Flavonoid Content Determination

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150 μ g/ml were prepared in methanol. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 5% sodium nitrite was added to 1 mL of extract and after 10 min, 1 mL of 2% AlCl_3 methanolic solution was added to it and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm [16].

DPPH Radical Scavenging Assay

DPPH solution was prepared by taking 8 mg of DPPH using a chemical balance dissolving with 100 ml 99.5% ethanol and finally kept in dark for 2 hrs. 1mm solution of DPPH of extract solution (50-250 μ g/ml) were prepared in ethanol. 1.5 ml of the extract solution was added to 1.5 ml of

DPPH Solution. The absorbance was measured at 517 nm against the corresponding blank solution. The control sample used was 3 ml of DPPH.

Pharmacological Evaluation of the extracts

Animal

The male/female Wistar mice of amid 1 to 2 months of age weighing between 25-35 g were used. The rodents let liberated and fed with pallet diet (Lipton India Ltd, Mumbai, Ind.) with water *ad libitum*. All the laboratory conditions and animals were maintained as per CCSEA guidelines throughout the experiments.

Acute Toxicity study

The short-term toxic effects of the extract was performed within prescribed guideline set by OECD guideline no. 423. The albino rats of 150-200 Gms were employed for study and kept in 12hour day-night cycle with ad libitum. The principle was based on use of minimal animals. The extracts were suspended in 1% Tween 80, prepared in distilled water. Animals were fasted for 12 hours with ad libitum and extracts were administered orally with increasing doses to 2000 mg/kg considering as maximum weight. The crucial observation made in first 4 hours to check for any bizarre behaviors like changes in skin and fur, eye, hyperactivity, grooming, convulsions, sedation, hypothermia, salivation, tremor, coma, lethargy, body weight, and mortality. From the observations and study, 1/10th and 1/5th of the LD used as therapeutic dose & cut-off values were chosen as 200 & 400 mg/kg to evaluate dose dependent action for the assessment of nootropic activity [17].

Grouping of animal for treatment

The animals were divided in 10 groups with 6 in every cluster. Group 1 control vehicle (0.9% NaCl); Group 2 was injected with scopolamine (SCOP) 2mg/kg intraperitoneally for 21 days; Group 3 and 4 were administered with hydroalcoholic extract of *Cascabela thevetia* (CTHE) at dose of 200 and 400 mg/kg/p.o respectively and injected with SCOP (2 mg/kg) for 21 days.

Morris water maze test

Spatial learning and memory were assessed using the Morris water maze previously described. Briefly, the testing system was composed of a black circular pool (168 cm in diameter and 50 cm deep) filled with water (temperature 20 ± 2 °C) and surrounded by extra maze distal visual cues of different shape, size and color. The pool was divided in four quadrants. A black circular hidden platform was placed in the northwest (NW) quadrant 2 cm under the water surface so that rat

could escape from swimming. Experimental rats were screened for their swimming ability by recording the latency to reach the visible platform. Rats were trained to exit the water tank onto the platform by using the visual cues. Each rat was placed inside the water tank facing the tank wall, at one of the four randomly selected entry points, once in every block of four trials. The test was performed on four consecutive days (8 trials per day). The starting position was changed randomly for each trial and the animal was allowed to search for 60 s to find the hidden platform. Rat was guided to the platform, if failed to find the platform within 60 s. At the end of the trials, the rat was allowed to remain on the platform for 30 s. Morris water maze training was recorded using a web camera mounted to the ceiling. Recording was performed from 11:00AM to 2:00PM to exclude variations in performance resulting from circadian rhythmicity [18].

Results and Discussion

Extraction Yield and phytochemical screening

The extraction yield of the crude extract obtained using the solvent blend is calculated as the percentage of dry weight of the powdered leaves. The yield of extract was found to be 7.95% w/w. The hydroalcoholic extract of *Cascabela thevetia* contained flavonoids, phenolics and sterols whereas all the other class of constituents were found to be absent in the preliminary phytochemical screening.

Total Phenolic and Flavonoid Content

The total phenolic content in the extract was determined using Folin-Ciocalteu reagent method and are reported as gallic acid equivalent (GAE). The total flavonoid content in the extracts was determined using Aluminum chloride method and are reported as quercetin equivalent (QE). The extract was found to contain 91.84 GAE mg/g of phenolics and 113.22 QE mg/g of flavonoids.

Antioxidant action

The extract was evaluated for its antioxidant potential in terms of DPPH radical scavenging capabilities (Table 1). The DPPH scavenging by the extract was found to dose dependent.

Table 1. DPPH Scavenging potential of *Cascabela thevetia*.

Concentration ($\mu\text{g/mL}$)	DPPH Scavenging %
50	43.1 ± 1.40
100	43.8 ± 1.36
150	48.8 ± 1.13
200	49.4 ± 1.11

250	58.9 ± 1.10
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Values are mean ± SEM of six determinations

Memory assessment in Morris water maze paradigm

MWM (Morris water maze) tests show that average latency to find the hidden platform by experimental rats. Our observations indicate that all experimental groups learned to find hidden platform in four experimental days. This implies that all experimental rats learn to escape swimming by searching hidden platform using visual cues. Results of MWM test in experimental animals confirmed a significant effect of Scopolamine-toxicity, CTHE (200 mg/kg) + SCOP, CTHE (400 mg/kg) + SCOP compared to control on latency to acquire hidden platform. In Two-way ANOVA confirmed a significant interaction between treatment (control vs. SCOP treated) × trial days ($p < 0.001$) and SCOP + CTHE treated × trial days ($p < 0.001$). Non-significant interaction was observed between (control vs. CTHE treated) × trial days ($p > 0.05$) (Table 2).

Water maze tasks were performed to evaluate effect of hydro-alcoholic extract of *Cascabela thevetia* leaf treatment on the spatial memory abilities. Each data point represents the mean (±SEM) latency of the 5 trials for a minimum of six rats performed each day. The mice treated with 400 mg/kg AEPH required only 18.00 ± 0.894 seconds on the 4th day to find the hidden platform as compared to 52.00 ± 3.577 for the scopolamine treated mice (Figure 1).

Table 2. Time to reach hidden platform in morris water maze test

Treatment	Dose (mg/kg)	Time to reach platform (sec)			
		Day 1	Day 2	Day 3	Day 4
Vehicle	0.5 ml/kg, i.p.	39.66 ± 2.320***	34.16 ± 2.544***	31.66 ± 2.312***	28.16 ± 1.786***
SCOP	2 mg/kg, i.p.	63.16 ± 3.792	59.33 ± 3.546	56.00 ± 3.816	52.00 ± 2.577
CTHE	200 mg/kg, p.o.	36.83 ± 1.857***	32.00 ± 1.529***	30.16 ± 1.640***	25.83 ± 1.534
	400 mg/kg, p.o.	26.83 ± 2.970***	22.5 ± 2.449***	21.16 ± 1.483***	18.00 ± 0.894***

Values represent means ± SD ($n = 6$), ***($p < 0.0001$)

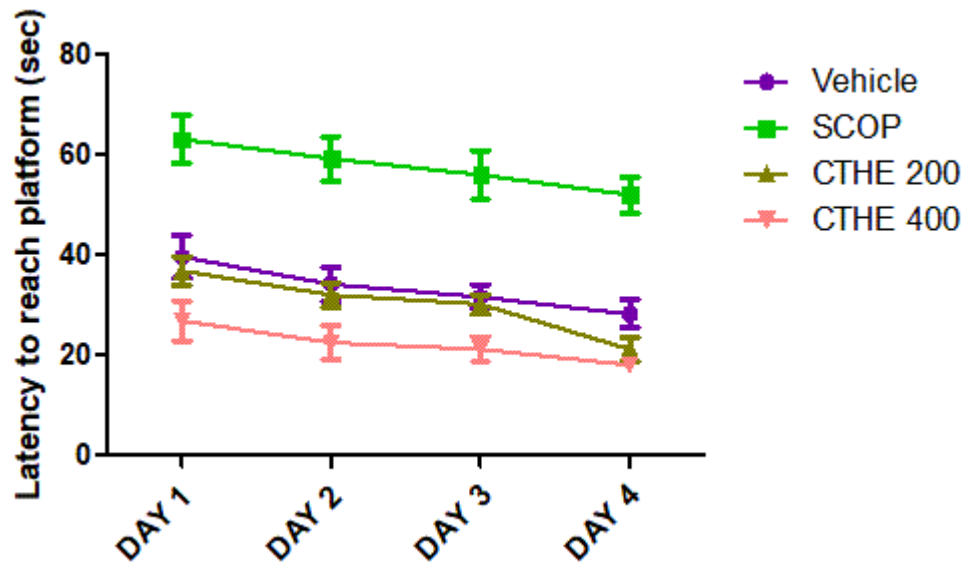


Figure 1. Latency to search platform

Conclusion

The present study demonstrated that the hydroalcoholic leaf extract of *Cascabela thevetia* possesses significant antioxidant and memory-enhancing potential. Phytochemical investigation revealed the presence of phenolic compounds and flavonoids, which were quantified at appreciable levels and may contribute to the observed biological activities. The extract exhibited concentration-dependent free radical scavenging activity in the DPPH assay, confirming its antioxidant capacity.

In the Morris Water Maze model, scopolamine administration produced marked impairment in spatial learning and memory, whereas treatment with *Cascabela thevetia* extract significantly improved cognitive performance by reducing escape latency in a dose-dependent manner. The higher dose (400 mg/kg) showed superior efficacy and markedly reversed scopolamine-induced memory deficits. These findings indicate that the extract has a protective effect against cognitive dysfunction and may improve learning and memory processes.

Overall, the study suggests that *Cascabela thevetia* leaf extract is a promising natural source of neuroprotective and nootropic constituents. The memory-enhancing activity observed may be associated with its antioxidant properties and rich phenolic-flavonoid content. Further studies involving isolation of active phytoconstituents, elucidation of molecular mechanisms, and long-term safety evaluation are warranted to establish its therapeutic potential for the management of memory-related neurological disorders.

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