**Calotropis procera**: A potential cognition enhancer in scopolamine and electroconvulsive shock-induced amnesia in rats

Rohit Malabade, Ashok D. Taranalli

**ABSTRACT**

**Objectives**: Present study evaluates the effect of *Calotropis procera* (Apocynaceae) dry latex on cognitive function in rats using scopolamine and electroconvulsive shock (ECS) induced amnesia model.

**Materials and Methods**: Male Wistar rats were pretreated with 200, 400 and 800 mg/kg of *C. procera* dry latex in scopolamine-induced amnesia model. Dose showing maximum effect in cognitive performance was selected and further evaluated using scopolamine and ECS-induced amnesia model for its effect on neurochemical enzymes and cognitive performance. Acetylcholinesterase (AChE) activity, β amyloid$_{1-42}$ and dopamine level were analyzed, while the cognitive performance was assessed by elevated plus maze, step-through passive avoidance test, and Morris water maze. Simultaneously, *C. procera* dry latex (25, 50, 100, 250, 500, and 1000 μg/mL) was screened for in vitro AChE inhibition assay.

**Results**: Pretreatment with (200, 400 and 800 mg/kg) *C. procera* dry latex shows dose-dependent increase in cognitive performance in scopolamine-induced amnesia. Further, pretreatment with the selected dose (800 mg/kg) showed significant improvement in transfer latency ($P < 0.001$, $P < 0.01$), escape latency ($P < 0.05$), time spent in target quadrant ($P < 0.001$) also significant decrease in AChE activity ($P < 0.05$), β amyloid$_{1-42}$ level ($P < 0.001$), and increase in dopamine level ($P < 0.01$) in rat brain homogenate when compared with scopolamine and ECS disease control groups. IC$_{50}$ for *C. procera* dry latex was found to be <1000 μg/mL.

**Conclusions**: Pretreatment with *C. procera* dry latex (800 mg/kg) produced significant cognition enhancement by improving cognitive performance and decreasing the marker neurochemical enzyme activity in scopolamine and ECS-induced amnesia model.

**KEY WORDS**: Acetylcholinesterase, Alzheimer’s disease, Dopamine, β amyloid$_{1-42}$

---

### Introduction

Cognitive impairment is one of the most common condition and a major health problem in the geriatric population. Progressive loss of cognitive ability is a primary characteristic of Alzheimer’s disease, which particularly affects daily activities such as memory, learning, and problem solving. Also, decreased cholinergic transmission and rise in oxidative stress, neuroinflammatory reactions in brain is a reason for cognitive impairment.

In the present study, scopolamine and electroconvulsive shock (ECS) induced amnesia was used as a pharmacological model. Scopolamine a muscarinic receptor antagonist interferes...
with central cholinergic functions and process of memory and learning which lead to cognitive decline in animals. ECS is an animal model of seizure induction that typically induces amnesia. ECS produces both anterograde and retrograde amnesia. ECS affects different region of brain, it is likely to have different molecular pathways which all together contribute to cognitive impairment in rats.

The pathophysiology of cognition impairment is complex and involves several different biochemical pathways. In recent years, herbal remedies are reported to contain several active compounds which made them a popular choice to enhance cognitive function and to alleviate other functions associated with cognitive disorders. Calotropis procera (Asclepiadaceae) is an important medicinal plant, which was used as a drug of choice for various ailments in ancient time. Pharmacological properties of C. procera are associated with its latex, which is rich in biologically active compounds like cardenolides, triterpenoids, resins, proteolytic enzymes, flavonoids, tannins, sterol, and terpenes. Pharmacologically, latex has proved to possess potent anti-inflammatory, analgesic activity. The free radical scavenging and antioxidant property is comparable to standard antioxidant (Vitamin C) and also root extract of C. procera possess anticonvulsant activity.

Potential of C. procera latex has been proved scientifically for the treatment of various diseases including central nervous system disorders, but the cognition-enhancing property of C. procera latex on rats is not well understood. Therefore, the study was undertaken to investigate the effect of C. procera dry latex on cognitive function in rats using scopolamine and ECS-induced amnesia model.

Materials and Methods

Collection and Preparation of Plant Material

The C. procera plant was identified in Belagavi District, Karnataka, India and was authenticated by Dr. Harsha Hegde, Scientist F, Regional Medical Research Centre, Belagavi, Karnataka, India, whereas herbarium is preserved for future reference. The latex was collected from the aerial parts of the plant. It was dried under shade at ambient temperature. The dry latex was triturated with water and aqueous suspension thus obtained was administered orally to rats.

Experimental Animals

The study was carried out in Male Wistar rats (150–200 g). Animals were kept in plastic cages in a room with a controlled temperature of 25 ± 2°C, 12/12 h light/dark cycle, food, and water ad libitum. The rats were acclimatized for a period of 1-week before starting the experiments. All the experimental procedures were performed between 9 am and 4 pm. Before commencement of the experiment, animal ethical approval was obtained from the Institutional Animal Ethical Committee.

In vitro Acetylcholinesterase Microplate Inhibition Assay

Acetylcholinesterase (AChE) activity was measured using a modified 96-well microplate assay based on Ellman’s method. The test compound solution having concentration 25, 50, 100, 250, 500, and 1000 μg/mL (20 μL) and AChE (20 μL) were mixed and incubated for 15 min (25°C). The reaction was then initiated by the addition of acetylthiocholine (10 μL). The hydrolysis of acetylthiocholine was monitored by the formation of yellow color, that is, 5-thio-2-nitrobenzoate anion at 412 nm for every 5 min over 20 min using a 96-well microplate reader (Thermo Scientific, Multiskan GO). The IC_{50} values were calculated using a software program.

Experimental Design

Part I

C. procera dry latex was screened using scopolamine-induced amnesia model, by administrating three doses, that is, 200, 400, and 800 mg/kg. For this rats were divided into six groups (n = 6 animal/group) Group 1 – normal control, Group 2 – disease control (scopolamine, 3 mg/kg, i.p.), Group 3 – standard control (Mentat - 100 mg/kg, p.o. for 14 days), and Groups 4–6 – received 200, 400, and 800 mg/kg of C. procera dry latex p.o. for 14 days. The acquisition trial for elevated plus maze, step-through passive avoidance, and Morris water maze was carried on 14th day and retention was tested on 15th day. Dose showing maximum activity in scopolamine-induced amnesia model was selected.

Part II

Selected dose was further evaluated by scopolamine and ECS-induced amnesia model for its effect on AChE activity, β amyloid_{42}, dopamine concentration in rat brain homogenate and for its cognitive performance in elevated plus maze, step-through passive avoidance, and Morris water maze. For this study, rats were divided into seven groups; Group 1 – normal control, Group 2 – scopolamine, 3 mg/kg, i.p., Group 3 – scopolamine + Mentat (100 mg/kg, p.o.), Group 4 – scopolamine + C. procera dry latex (800 mg/kg, p.o.), Group 5 – received ECS (10 mA current for 0.2 s), Group 6 – ECS + Mentat (100 mg/kg), Group 7 – ECS + C. procera dry latex (800 mg/kg). Mentat and C. procera dry latex was administered orally for 14 days, while scopolamine and ECS was administered after acquisition trial (14th day) which provoked cognitive impairment in rats.

Electroconvulsive Shock

An electric current (60 Hz, 2 s, and 20 mA) was delivered to the restrained rat by applying the corneal electrodes of the ECS apparatus. The shock was delivered immediately after the acquisition trial in disease control and treatment groups.

Elevated Plus Maze

The apparatus consists of a wooden structure, raised to a height of 40 cm from the floor. This apparatus is composed of four arms, two closed and two open arms. In acquisition trial, each rat was placed at the end of an open arm facing away from the center. The time taken to enter any one of the closed arms was recorded as transfer latency. All four legs inside the closed arm are counted as an entry. Cut off time allotted for each rat was 180 s. Retention trial was conducted 24 h after the first trial and transfer latency was recorded. Shortened transfer latency was considered as an index of improvement of memory.

Step through Passive Avoidance Test

The apparatus consisted of a light and dark compartment connected by an opaque guillotine door. The floors of both compartments were made of stainless steel rods (3 mm diameter) spaced 1 cm apart. Acquisition trial: The rats were placed in the light compartment and 5 s later the
guillotine door was raised. Rats have natural preference for the dark environment. The latency to enter the dark compartment (escape latency) was recorded when the animal had placed all four paws in the dark compartment. After the animal had spontaneously entered the dark compartment, the guillotine door was lowered, and a mild electrical shock (0.5 mA) was applied for 2 s. After 30 s, the rat was removed from the dark compartment and returned to its home cage. Retention test: Escape latency was recorded during the retention trial and if the rat did not enter the dark compartment within 300 s, the retention test was terminated and a ceiling score of 300 s was assigned.\[15\]

**Morris Water Maze Test**

It consists of circular pool filled with water, a platform (10 cm × 10 cm) was submerged 2 cm below the water surface. Each rat was given four trials on each testing day for 4 days and retention of memory was tested on 5th day. The starting position was randomized over each testing day but remained same for all the rats in each trial. During each trial, the rat was placed in the water with its head pointed toward the side wall and allowed 90 s to search for the hidden platform. One day before the test, each rat was placed in the pool for 60 s; this free swim enabled the rat to become habituated to the training environment. If the rat did not find the platform in 120 s, it was manually placed on the platform for a 30 s rest. Whereas on day 5, time spent in target quadrant (Q4) served as an index of retrieval or memory.\[16\]

**Assay of Acetylcholinesterase Activity**

Rats were sacrificed by cervical dislocation and whole brain was dissected out, 20 mg of brain tissue/mL of phosphate buffer (0.1 M; pH 8) was homogenized and 0.4 mL aliquot of brain homogenate was added to a cuvette containing 2.6 mL of 0.1 M phosphate buffer. 100 μL of 5.5’-dithiobis-2-nitrobenzoic acid reagent. The substrate acetylthiocholine iodide 20 μL was added and change in optical absorbance was measured every 2 min for 10 min at 412 nm to provide a measure of enzyme activity.\[12\]

**Estimation of Dopamine**

The content of dopamine in the brain tissue was determined as per the method described by Schlumpf et al.\[17\]

**Estimation of β Amyloid**\[14,2\]

The content of β amyloid\[14,2\] in the brain tissue was determined according to the manual of sandwich ELISA kit for rat β amyloid\[14,2\] (YH Bioresearch Laboratory, Shanghai, China).

**Statistical Analysis**

The results were expressed as mean ± standard error of mean. The statistical significance was determined by one-way analysis of variance followed by Dunnett’s test. P < 0.05 was considered to be statistically significant.

**Results**

**In vitro Acetylcholinesterase Microplate Inhibition Assay**

*C. procera* dry latex was found to be active at all concentrations (25, 50, 100, 250, 500, and 1000 μg/mL) in *in vitro* AChE microplate inhibition assay and IC\[50\] was found out to be <1000 μg/mL.

**Part I: Screening of Calotropis procera using Scopolamine-induced Amnesia**

Three different doses (200, 400, and 800 mg/kg) of *C. procera* dry latex was administered in scopolamine-induced amnesia model. At a dose of 800 mg/kg, transfer latency was significantly decreased (*P < 0.001*) in elevated plus maze, also significant increase (*P < 0.01*) was observed in step-through latency and time spent in Q4 was significantly increased in Morris water maze when compared with disease control group as indicated in Table 1.

**Part II: Evaluation of Calotropis procera (800 mg/kg) using Scopolamine and Electroconvulsive Shock-Induced Amnesia**

**Elevated plus maze**

Retention trial showed a significant increase in transfer latency in scopolamine (*P < 0.01*) and ECS (*P < 0.5*) disease control groups indicating cognitive impairment in rats. Pretreatment with *C. procera* dry latex significantly decreased transfer latency in Sco + *C. procera* (800 mg/kg) (*P < 0.001*) and ECS + *C. procera* (800 mg/kg) (*P < 0.01*) group when compared with respective disease control groups [Figure 1].

**Step through passive avoidance test**

Administration of scopolamine (*P < 0.01*) and ECS (*P < 0.05*) decreased step down latency, while pretreatment with *C. procera* dry latex (800 mg/kg) markedly increased (*P < 0.05*) escape latency and reversed scopolamine-induced cognitive impairment. Furthermore, rats treated with Mentat also showed increase (*P < 0.001*) in escape latency [Figure 2].

**Morris water maze**

Pretreatment with *C. procera* dry latex (800 mg/kg) showed significant increase (*P < 0.001*) in time spent in Q4 when compared with scopolamine (*P < 0.01*) and ECS (*P < 0.01*) disease control groups [Figure 3].

**Acetylcholinesterase activity**

Pretreatment with *C. procera* dry latex (800 mg/kg) significantly reduced (*P < 0.05*) the AChE activity as compared to disease control groups (*P < 0.01*). Mentat also significantly decreased AChE activity in Sco + Mentat (*P < 0.01*) group and ECS + Mentat (*P < 0.05*) as indicated in Figure 4.

**Dopamine level**

Exposure to scopolamine and ECS significantly reduced (*P < 0.001*) level of dopamine in disease control groups and pretreatment with *C. procera* significantly increased (*P < 0.01*) level of dopamine when compared with disease control groups [Figure 5].

**β amyloid**\[14,2\] level

Scopolamine and ECS administered group showed significant increase (*P < 0.001*) in β amyloid\[14,2\] levels when compared with normal control, while significant fall in (*P < 0.001*) β amyloid\[14,2\] level was observed in pretreated groups when compared with disease control [Figure 6].

**Discussion**

Present study investigated the effect of *C. procera* dry latex on scopolamine and ECS-induced amnesia in rats using elevated plus maze which is used to screen drugs used in learning and memory.
The step-through passive avoidance test generally accepted as an indicator of long-term memory in animals. Morris water maze was used to assess hippocampal-dependent spatial learning ability. Furthermore, AChE activity, β amyloid$_{1-42}$ level, and dopamine concentration were analyzed from rat brain homogenate.

_C. procera_ dry latex was administered at three doses (200, 400, and 800 mg/kg) in scopolamine-induced amnesia model and results revealed a dose dependent effect. The higher dose (800 mg/kg) being able to produce significant results in elevated plus maze, step-through passive avoidance, and Morris water maze, which was comparable with the standard. Further, 800 mg/kg dose was selected to evaluate the effect of _C. procera_ dry latex on cognitive performance and biochemical changes using scopolamine and ECS-induced amnesia model.

Pretreatment with _C. procera_ dry latex (800 mg/kg) in scopolamine and ECS-induced amnesia model demonstrated a significant reduction of transfer latency in elevated plus maze, significant elevation of escape latency in passive avoidance test. Also in Morris water maze the mean time spent in the Q4 was significantly increased during the retention trial. The results show that _C. procera_ dry latex (800 mg/kg) improved the cognitive performance in scopolamine and ECS-induced cognitive impairment in rats.

Increase in cholinergic activity and inhibition of AChE enzyme is a promising therapy to treat a cognitive defect.

### Table 1:

| Groupings                      | Transfer latency (s) | Escape latency (s) | Time spent in target quadrant (s) |
|--------------------------------|----------------------|--------------------|---------------------------------|
| Normal control                 | 19±1.265             | 20.67±1.145        | 5.5±0.5578                      |
| Negative control (scopolamine) | 27±1.125***          | 14.83±0.843***     | 6.1±0.6009**                    |
| Standard control (Mentat)-100 mg/kg | 13±0.894***         | 16.40±0.918**      | 8.5±0.4282***                   |
| CP-200 mg/kg                   | 23±1.065*            | 15.5±0.992**       | 7.1±0.60**                      |
| CP-400 mg/kg                   | 18.16±1.887**        | 13.83±1.138*       | 7±0.365**                       |
| CP-800 mg/kg                   | 16.35±1.308***       | 16.8±1.046**       | 8±0.258***                      |

All values represent mean±SEM. ***P<0.001, **P<0.01, *P<0.05 versus disease control. SEM=Standard error of the mean, CP=Calotropis procera

---

**Figure 1:** Effect of _Calotropis procera_ (Calotropis procera - 800 mg/kg) extract on transfer latency in scopolamine and electroconvulsive shock-induced amnesia. All values represent mean ± standard error of mean. ***P < 0.001, **P < 0.01, *P < 0.05 vs disease control

**Figure 2:** Effect of _Calotropis procera_ (Calotropis procera - 800 mg/kg) on step-through latency in scopolamine and electroconvulsive shock-induced amnesia. All values represent mean ± standard error of mean. ***P < 0.001, **P < 0.01, *P < 0.05 versus disease control

**Figure 3:** Effect of _Calotropis procera_ (Calotropis procera - 800 mg/kg) on time spent in target quadrant in scopolamine and electroconvulsive shock-induced amnesia. All values represent mean ± standard error of mean. ***P < 0.001, **P < 0.01, *P < 0.05 versus disease control

**Figure 4:** Effect of _Calotropis procera_ (Calotropis procera - 800 mg/kg) on Acetylcholinesterase enzyme activity in scopolamine and electroconvulsive shock-induced amnesia. All values represent mean ± standard error of mean. ***P < 0.001, **P < 0.01, *P < 0.05 versus disease control
in Alzheimer’s disease. Administration of *C. procera* dry latex (800 mg/kg) significantly reversed the elevated AChE level in scopolamine and ECS-induced rats. Hence, these results suggest that cognition enhancing the property of *C. procera* dry latex may be due to inhibition of AChE enzyme.

Cognition impairment is characterized by plaque deposition in extracellular spaces by aggregation of β amyloid protein in brain tissue, and β amyloid (1-42) is found predominantly in brain. Extracellular deposition of β amyloid is considered as a biomarker for Alzheimer’s disease. Pretreatment with *C. procera* dry latex (800 mg/kg) demonstrated a significant decrease in the amount of β amyloid (1-42) in rat brain. These results were in co-relation with a previous report by Zhao et al.

In stressful conditions, change in dopamine level is associated with a transient change in behavioral aberrations, memory learning disorders. Administration of *C. procera* dry latex (800 mg/kg) increased dopamine concentration in scopolamine and ECS-induced amnesia model after 14 days of pretreatment. Hence, *C. procera* dry latex may show cognition enhancement by increasing dopamine level.

*C. procera* dry latex inhibit AChE enzyme at 25, 50, 100, 250, 500, and 1000 μg/mL concentration in *in vitro* AChE inhibition microplate assay and IC₅₀ was found to be < 1000 μg/mL. These results provide evidence that *C. procera* dry latex inhibits AChE enzyme activity.

Hence, pretreatment with *C. procera* dry latex (800 mg/kg) demonstrated a significant reversal of cognition impairment in rats by inhibition of AChE enzyme, increasing dopamine level, decreasing β amyloid (1-42) in rat brain, and also by improving cognitive performance in scopolamine and ECS-induced amnesia model.

**Conclusions**

The study provides evidence that *C. procera* dry latex exhibits multiple pathways for cognition enhancement in scopolamine and ECS-induced amnesia model. Beneficial effect of *C. procera* dry latex on cognition may be due to its rich content of biologically active compounds. Further, the study is required to isolate compounds responsible for cognition-enhancing property.
16. Chakravarti K, Avadhani R. Beneficial effect of aqueous extract of *Glycerrizaglabra* on learning and memory using different behavioural models: An experimental study. J Nat Sci Bio Med 2013;4:420-6.

17. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochem Pharmacol 1974;23:2437-46.

18. Gutierres J. Neuroprotective effect of anthocyanins on acetylcholinesterase activity and attenuation of scopolamine induced amnesia in rats. Int J Dev Neurosci 2014;33:88-97.

19. Agrawal R, Tyagi E, Saxena G, Nath C. Cholinergic influence on memory stages: A study on scopolamine amnesic mice. Indian J Pharmacol 2009;41:192-6.

20. Parcha UZ, Qadri I, Hayat K, Hussain T. Biomarkers for Alzheimer’s disease and potential future directions. Aging Neurodegener 2013;1:125-9.

21. Zhao L, Wang JL, Liu R, Li XX, Li JF, Zhang L. Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer’s disease mouse model. Molecules 2013;18:9949-65.

22. Muralidharan P, Balamurugan G, Venu B. Cerebroprotective effect of *Glycyrrhizaglabra* Linn. root extract on hypoxic rats. Bangladesh J Pharmacol 2009;4:60-4.