



## Nootropic activity of *Calotropis gigantea* (Linn) root against scopolamine induce amnesia in albino rats

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### Abstract

**Objective:** The current study investigated that defensive effect of methanolic extract of *Calotropis gigantea* (Linn.) roots on learning and memory functions in scopolamine induced memory deficits rats.

**Methods:** In scopolamine (SCO) induced cognitive deficit rat model Wistar albino rats weighing 150-200 g were divided into 8 groups (6 animals per group). After seven days of treatment animals were at once scarified, the estimation of markers of oxidative stress in the brain was measured. The protective and cognitive enhancing effects of MCGE on cognitive shortfall rats induced by scopolamine were investigated by assessing the elevated plus maze, the passive avoidance test and the Morris water maze test. In order to prove the underlying mechanisms of memory enhancing effects of MCGE, activities of AChE, oxidative stress markers such as GSH and MDA were measured.

**Results:** MCGE at a dose 200 mg/kg may be useful for the cognitive improvement via regulation of cholinergic marker enzyme activities and the antioxidant defense system in SCO induced cognitive deficit rats. MCGE at a dose 200 mg/kg may be protective for the brain via antioxidant defense system in rats.

**Conclusion:** These conclusions suggest the possible neuroprotective role for *C. gigantea*; therefore it seems that *C. gigantea* may show to be an anti Alzheimer mediator in view of its memory enhancing property observed in the present study.

**Keywords:** *Calotropis gigantea* (Linn.), Cognitive Functions, Scopolamine, TBARS, Memory

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### Introduction

Natural world has exceptional us with many herbs having supernatural exhausted properties that are used widely in number of ailments. The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Today, as much as 80% of the world's population depends on traditional medicine as primary health care needs. Ayurveda is an intricate system of healing that originated in India thousands of years ago. Herbs are an excellent alternative to antibiotics in the treatment of infectious diseases, with wider antibacterial effects as well as various antifungal and antiviral actions. Some herbal formulations serve as detoxification agents, antioxidants, and anti-cancer therapies.

The main objective of this work was to investigate indigenous plants used in protection against cognitive dysfunction in India. We hereby reported our findings related to cognitive dysfunction activities of plant *Calotropis gigantea* from some in vitro model studies and Sedative and anxiolytic effects of methanolic extract of *Calotropis gigantea* root [1].

### Materials and methods

Plant materials

The root of *Calotropis gigantea* (Linn.) collected from botanical garden of Shambhunath Institute of Pharmacy, U.P. India. The roots were dried under normal environmental conditions and were authenticated (TR No. 097099) Botanical Survey of India Central Regional Center, Allahabad, U.P. before of commencement of the study.

#### Preparation of extract

Extraction of roots was done by successive solvent extraction. Soxhlet apparatus was used for the successive solvent extraction. The solvents for extraction were selected on the basis of their polarity. The roots of *Calotropis gigantea* coarse and air dried drug material were washed and allowed to dry in shade for a week and then grounded into fine powder in mixer grinder. 100 g of dried powder of roots was subjected to Soxhlet extraction with 500 ml of solvents for 4 h starting from petroleum ether (60-80 °C). The extraction was continued till the defeating of the material had taken place. The marc obtained after petroleum ether was subjected to methanol extraction in Soxhlet apparatus. Extraction was concentrated by distilling off the solvent and then evaporated to dryness on water bath. Hence the present study used

methanolic *Calotropis gigantea* extract (MCGE).

#### Animals

All the animals and experiments were approved and conducted as per the guidelines of Institutional Animal Ethical Committee (IAEC) of SIP (SIT-IAEC/016/09/16) ethical norms were strictly followed during all experiment procedures. Used Wistar albino strains of young healthy adult rats 200-250 g, procured from NIN (National Institute of Nutrition's) Hyderabad. All animals housed in cage kept at the temperature (22-25 °C) natural light-dark cycle. They had free access to slandered pellet diet and tap water.

#### Drugs and administrations

All animals were divided in to eight groups and each group consisting of six animals. Each animals receiving different treatment orally for seven days. GP I- served as normal control (NC), GP II-2 received SCO (2 mg/kg i.p.), GP III- served as per se group received piracetam (PR) (200 mg/kg i.p.), GP IV & GP V-serve as MCGE (100 mg/kg p.o.), MCGE (200 mg/kg p.o.), GP VI-received piracetam (PR) (200 mg/kg i.p.) for 7days respectively followed by SCO (2 mg/kg i.p.), GP VII & VIII-serve as CGME (100 mg/kg p.o.) and MCGE (200 mg/kg p.o.) for 7 days respectively followed by SCO (2 mg/kg i.p.).

As recommended according to OECD guidelines the use of an aqueous solution/suspension of the test dose should be considered first. As reported the mostly solubility of *C. gigantea* roots methanol extract was in 1% suspension of tween 80 and in 0.5% CMC in normal saline solution [2].

#### Induction of amnesia

On the 7th day scopolamine 2 mg/kg, i.p. was administered which induces amnesia. On 8<sup>th</sup> day after 24 hrs, the animals were studies behavioral activities like, transfer latency, scape latency and step down latency. On 8<sup>th</sup> day immediately after the test animals from all groups was sacrificed by cervical dislocation and brain of individual animal was carefully isolated, placed on Petridis, over ice, and weighed. Then homogenate was prepared from the brain slices and biochemical estimations were carried out.

#### Behavioral test

##### Passive shock avoidance

Passive avoidance, based on negative reinforcement, was recorded to examine the long-term memory. Electric shock was delivered to the grid floor. When the rat was placed in the chamber and placed its paw on the grid floor, shock (foot shock: 50 Hz; 1.5 mA; 1 s) was delivered and transfer latency time (TLT) was recorded. TLT is defined as the time taken by the rat to step down and place all four paws on the grid floor. Rats showing SDL in the range of 2-15 s during the training session were taken for the acquisition and the retention task [3].

##### Elevated plus maze

The elevated plus maze served as the exteroceptive

behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in rats. Transfer latency (TL) was taken as the time taken by the rat to move into any one of the covered arms with all its four legs. TL was recorded on the first day for the each animal. The rat was allowed to explore the maze for another 2 min. and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial [3].

##### Morris water maze test

The water maze test is a widely accepted method for memory testing. A trial began by placing the animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape on the platform within 120 sec, it was gently placed on platform and allowed to stay for 15 s. and then returned to the home cage. The escape latencies (sec) to reach the platform were recorded [4].

##### Biochemical estimations

A 10% homogenate of brain samples were prepared by homogenizing in 0.1 M chilled phosphate buffer (pH 7.4) using homogenizer. The homogenates were centrifuged at 800 × g for 5 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10,500 × g for 20 min at 4 °C to get the supernatant. Supernatant was collected from individual animal from all groups and subjected to the biochemical estimations [5]. Protein was measured in all brain samples for GSH, MDA and AChE activity measurement. Protein was measured according to the method of Lowry 1951.

##### Tissue preparations

On the 8<sup>th</sup> day immediately after the test animals from all groups were sacrificed by cervical dislocation and brain of individual animal was carefully isolated, placed on Petridis, over ice, and weighed. Whole brain sample were rinsed with ice-cold normal saline. A 10% homogenate of brain samples were prepared by homogenizing in 0.1 M chilled phosphate buffer (pH 7.4) using homogenizer. The homogenates were centrifuged at 800 × g for 5 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10,500 × g for 20 min at 4 °C to get the supernatant. Supernatant was collected from individual animal from all groups and subjected to the biochemical estimations determined according to Otari et al. (2012) [5].

##### Estimation of malondialdehyde (MDA)

Thiobarbituric acid reactive species (TBARS), a measure of lipid peroxidation was measured as described by Ohkawa et al. Malondialdehyde (MDA) was quantified to detect any short-term changes in lipid peroxidation. Briefly, 1 ml of supernatant was taken from the 10% tissue homogenate. 0.5 ml of 30% TCA (Trichloroacetic acid) was added to it, followed by 0.5 ml of 0.8% TBA (Thiobarbituric acid) reagent dissolved in 95% ethanol. The tubes were covered with aluminum foil and kept in shaking water bath for 30 min at 90

°C. After 30 min, tubes were taken out and kept in ice-cold water for 30 min. Then, these were centrifuged at 3000 rpm for 15 min. The supernatant was separated and the absorbance of supernatant was read at 532 nm at room temperature against appropriate blank. Blank consist of 1 ml distilled water, 0.5 ml 30% TCA, and 0.5 ml 0.8% TBA. TBARS values were expressed as n moles MDA/mg protein [6].

#### Estimation of glutathione (GSH)

GSH was determined by its reaction with 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) to yield a yellow chromophore which was measured spectrophotometrically. Glutathione was measured according to the method of Ellman. The equal quantity of homogenate was mixed with 10% trichloroacetic acid (500 µl each) and centrifuged at 2000 × g for 10 min at 4 °C to separate the proteins. The supernatant was used for GSH estimation. To 0.1 ml of this supernatant, 2 ml of 0.1M phosphate buffer (pH 7.4), 0.5 ml of DTNB and 0.4 ml of double distilled water were added. The mixture was shaken vigorously on vortex and the absorbance was read at 412 nm within 15 min. GSH concentration was calculated by using standard curve prepared with reduced glutathione and expressed as µg/mg protein [7].

#### Estimation of acetyl cholinesterase

Acetyl cholinesterase activity is a marker of extended loss of the cholinergic system in the brain. The quantitative measurement of acetyl cholinesterase levels in brain was

performed according to the method of Ellman. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured immediately at 412 nm using spectrophotometer. Results were calculated using molar extinction coefficient of chromophore ( $1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ ) and expressed as n moles of acetylcholine hydrolyzed/ min/ mg protein [8, 9].

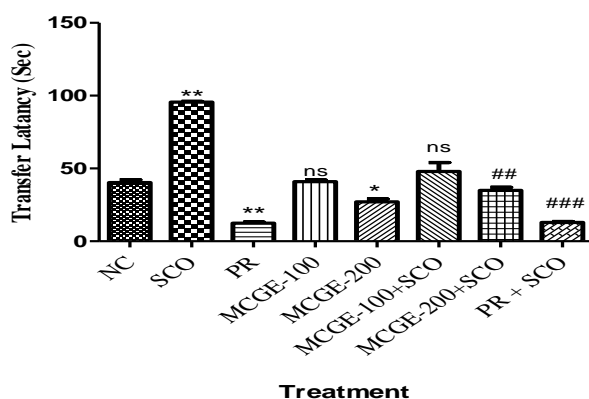
#### Statistical analysis

The values were expressed as mean±SEM from 6 animals. The results were subjected to statistical analysis by using one-way ANOVA followed by Dennett's test to calculate the significant difference if any among the groups.  $P < 0.05$  was considered as significant.

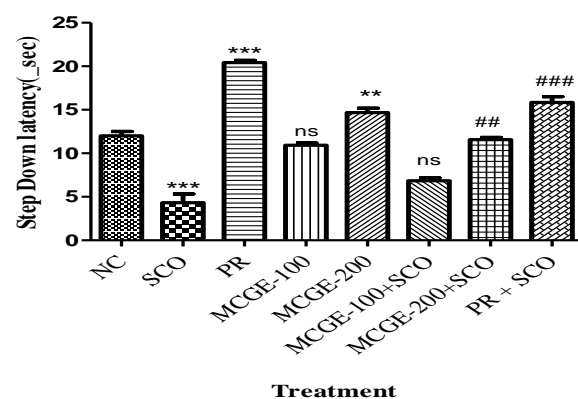
### Results and discussion

#### Elevated plus maze

Transfer Latency (TL) of first day (on 7th day of drug treatment) reflected acquisition of learning behavior of animals. The MCGE at a dose 200 mg/kg, p.o. for 7 successive days, successfully reversed memory deficits (neuronal damage) induced by scopolamine ( $p < 0.05$ ). Piracetam (used as the positive control) at a dose of 200 mg/kg, i.p. also improved learning and memory in rats and reversed the neuronal deficits induced by scopolamine as expected (Figure1).



**Figure 1: Elevated plus maze;** Each group consists 6 animals (n=6). Values are expressed as Mean ± SEM, One-way ANOVA followed by Dunnett's test. \* $p < 0.05$  vs day 7th TL in normal control, #  $p < 0.05$  vs day 7th TL in scopolamine, \*\* $p < 0.05$  vs day 8th TL in normal control, ###  $p < 0.05$  vs day 8th TL in scopolamine.



**Figure 2: Step-down latency;** Each group consists of 6 animals (n=6). Values are expressed as Mean ± SEM, One-way ANOVA followed by Dunnett's test. \* $p < 0.05$  vs day 8th TL in normal control, ###  $p < 0.05$  vs day 8th TL in scopolamine.

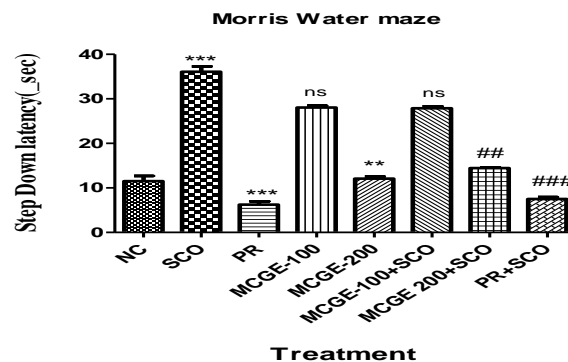
#### Step-down latency

Electric shock was delivered to the grid floor. When the rat was placed in the chamber and placed its paw on the grid floor, shock (foot shock: 50 Hz; 1.5 mA; 1 s) was delivered

and Step down latency time (Sec) was recorded. The MCGE at a dose 200 mg/kg, p.o. for 7 successive days, successfully reversed memory deficits (neuronal damage) induced by scopolamine ( $p < 0.05$ ). Piracetam (used as the positive

control) at a dose of 200 mg/kg, i.p. also improved learning and memory and treated rats showed significant increase step down latency time when reversed the neuronal deficits induced by scopolamine as expected (Fig.2).Morris water maze

The water maze test is a widely accepted method for memory testing. A trial began by placing the animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape on the platform within 120 sec, it was gently placed on platform and allowed to stay for 15 sec. The escape latencies (sec) to reach the platform were recorded. Memory deficits (neuronal damage) induced by scopolamine ( $p < 0.05$ ). Piracetam (used as the positive control) at a dose of 200 mg/kg, i.p. also improved learning and memory and test rats which receive MCGE 100 and 200 mg/kg p.o. for 7 successive days, successfully reversed, but the higher dose showed significant difference in scape latency time (sec) compared to the lower dose of MCGE and normal control group (Fig.3).



**Figure 3: Morris water maze;** Each group consists of 6 animals (n=6). Values are expressed as Mean  $\pm$  SEM, One-way ANOVA followed by Dunnett's test. \* $p < 0.05$  vs day 8<sup>th</sup> TL in normal control, ##  $p < 0.05$  vs day 8<sup>th</sup> TL in scopolamine

**Table 1: Effect of methanolic extract of *C. gigantea* 100 and 200 mg/kg, comparison of normal control, piracetam and scopolamine on MDA, glutathione, and AchE levels in brains of normal rats on 8<sup>th</sup> day**

Treatment	MDA (nmol/g tissue)	Glutathione (g/g tissue)	AchE (n moles)
Normal control	1.45 $\pm$ 0.1318	2.413 $\pm$ 0.1699	5.532 $\pm$ 0.3361
Scopolamine	3.362*** $\pm$ 0.1558	0.645** $\pm$ 0.05476	21.55** $\pm$ 0.4643
Piracetam	0.4048*** $\pm$ 0.09529	2.065** $\pm$ 0.04137	10.35*** $\pm$ 0.6637
MCGE 100	1.395 $\pm$ 0.1219	0.685 $\pm$ 0.1091	5.532 $\pm$ 0.3361
MCGE 200	0.0595** $\pm$ 0.005303	1.547** $\pm$ 0.1659	10.69** $\pm$ 0.7335
MCGE 100+SCO	1.307 $\pm$ 0.1698	0.9867 $\pm$ 0.2494	5.798 $\pm$ 0.5546
MCGE 200+SCO	0.0505### $\pm$ 0.005365	1.565### $\pm$ 0.1311	10.75### $\pm$ 0.3825
PR+SCO	0.3715#### $\pm$ 0.103	2.257#### $\pm$ 0.1478	7.693#### $\pm$ 0.2358

NC–normal control, SCO–Scopolamine, PR–Piracetam, MCGE–Methanolic *Calotropis gigantea* extract (lower and higher dose). Data represented as Mean  $\pm$  SEM (n=6). Significance by One-way ANOVA followed by Dunnett's test. \* $p < 0.05$  vs normal control (NC), ###  $p < 0.05$  vs scopolamine treated group. Effect of MCGE on brain AChE activity in normal rats and scopolamine induced cognitive deficit rats.

Pretreatment of scopolamine memory deficit rats with MCGE 200 mg/kg and piracetam 200 mg/kg resulted in a significant decrease in MDA level as compared to scopolamine group. MCGE 200 mg/kg and piracetam 200 mg/kg significantly increased the level of GSH as compared with scopolamine group. In the present study *C. gigantea* inhibited AChE, thereby elevating acetylcholine concentration in the brain and ultimately improved memory in rats. These results suggest that regular consumption of *Calotropis gigantea* roots might be beneficial on brain health.

#### Conclusion

These conclusions suggest the possible neuroprotective role for *C. gigantea*; therefore it seems that *C. gigantea* may show to be an anti-alzheimer mediator in view of its memory enhancing property observed in the present study. Therefore the result suggests that MCGE 200 mg/kg and piracetam 200

mg/kg defend and enhanced learning and memory in rats treated with scopolamine and MCGE 100 mg/kg did not show significant protection and improvements in learning and memory. The results suggested that flavonoids, phenolic compounds etc. present in the extract might be accountable for nootropic activity. The effects may be due to AChE inhibition, antioxidant effect and/or increase in cholinergic transmission by the extract.

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#### Acknowledgement

I am thankful to the all faculty members of Shambhunath Institute of Pharmacy (SIP), Allahabad for providing chemicals and other infrastructure for doing this research work. The work is dedicated to my senior Mr. Rajeev Varma; also include my father Mr. S.R.Verma who gives me support for my research work, thank you papa.

Conflict of Interest: None declared

Received: 28 July 2017, Revised: 28 August 2017, Accepted: 30 August 2017

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