Original Article

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Cassia tora A Potential Cognition Enhancer in Rats with Experimentally Induced Amnesia

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ABSTRACT

Objective: Present study investigates the effect of ethanolic *C. tora* leaves extract on cognitive function in scopolamine and electroshock induced amnesia in rats. **Methods:** To explore the potential effect, *C. tora* extract was screened by administrating three doses (100, 200 and 400 mg/kg) for 14 days using scopolamine-induced amnesia model. The dose having maximum activity was selected (400 mg/kg) and evaluated by scopolamine and electroshock induced amnesia model, cognition parameters assessed using elevated plus maze, passive avoidance and Morris water maze. The biochemical parameters such as AChE and β amyloid$_{1-42}$ level from brain homogenate were estimated on 15th day. *In vitro* acetylcholinesterase microplate assay of *C. tora* extract at 25, 50, 100, 250 and 500 µg/ml concentration was performed. **Results:** *C. tora* extract (400 mg/kg) showed increase in escape latency ($p<0.001$) and time spent in target quadrant ($p<0.05$) while decrease in transfer latency ($p<0.001$) was observed in scopolamine induced amnesia. Further, pre-treatment with *C. tora* (400 mg/kg) significantly reversed the behavioral impairment in scopolamine and electroconvulsive shock induced amnesia also AChE activity ($p<0.001$) and β amyloid1-42 level ($p<0.001$) were significantly decreased and IC$_{50}$ of *C. tora* extract was found out to be 198.59 µg/ml. **Conclusion:** Above finding concludes that ethanolic *C. tora* leaves extract possesses cognition enhancing property in scopolamine and electroshock induced amnesia models.

Key words: Acetylcholinesterase, Alzheimer's disease, IC$_{50}$, β Amyloid$_{1-42}$, Memory and learning.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by cognitive decline, neuronal inflammation, and extracellular aggregation of β amyloid. Cognitive function involves memory, attention, perception, language and psychomotor functions.[1] Nootropics are drugs commonly used to treat cognitive defect in AD patient.[2] Currently, FDA approved drugs for AD include Donepezil, Rivastigmine, Galantamine, and Memantine which slows the progression of disease but has greater side effects, hence for safer approach traditional herbal medicines need to be explored. Several natural products claim to possess cognitive effect in traditional system of medicine, but lack scientific evidence. Also, evaluation of natural products may be useful in identification of a potential new drug that can be used to treat cognitive impairment.[3]

Cassia tora (*Senna tora* L.) belongs to family *Leguminosae*, is a small shrub which is found throughout the tropical parts of Asian and African countries. *C. tora* is a well-known

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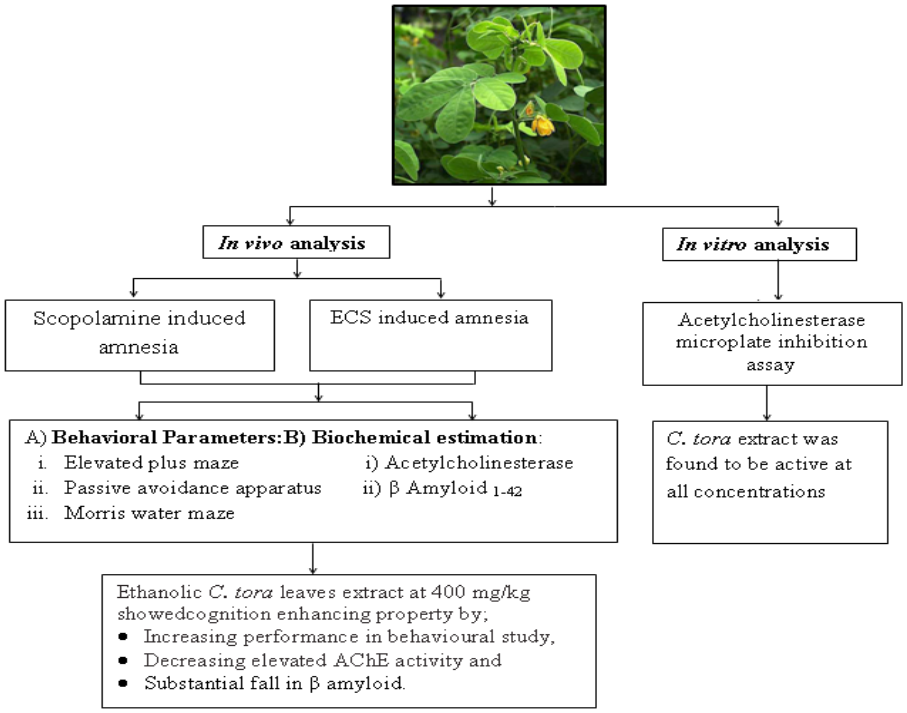

Graphical Abstract

medicinal plant, consumed by Indians in the form of vegetable, traditionally used as laxative and in treatment of leprosy and various other skin disorders.[4] According to Ayurveda, leaves are useful as anthelmintic, liver tonic, cardiotonic, expectorant, dyspepsia and proved beneficial in constipation, bronchitis and also have effective anti-oxidant property.[5,6] *C. tora* leaves principally contains polyphenols, myricyl alcohol, crysophenol, anthraquinone glycosides, β-sitosterol, flavonoids, emodin, stigmasterol, quercetin, iso-quercetin, palmitic, stearic, succinic and d-tartaric acid.[7]

Hence present study investigates the effect of ethanolic *C. tora* leaves extract on cognitive function. Acetylcholinesterase activity and β amyloid$_{1-42}$ level in rat brain using scopolamine and Electroconvulsive shock (ECS) induced amnesia.

MATERIALS AND METHODS

Plant collection and extraction

Fresh *C. tora* leaves were collected in the month of August. Plant material was taxonomically identified and authenticated by botanist from Regional Medical Research Centre, Belagavi, India and herbarium with voucher number M-93 was deposited at Regional Medical Research Centre, Belagavi, India for future reference.

Leaves were shade dried, powdered and extracted with 90% ethanol at room temperature for 48 h by repeated shaking. The extract was then filtered and concentrated by using rotary evaporator.

Animals

Male Wistar rats (150–250 g) were selected for study. Rats were kept under observation, at controlled room temperature, 12 h light–dark cycle and were allowed free access to food and water. Approval was obtained (KLECOP/IAEC/Res. 18) from the Institutional Animal Ethics Committee for conducting this study.

Experimental Design

In vitro acetylcholinesterase microplate inhibition assay

AChE activity was measured using a modified 96-well microplate assay[8] based on Ellman's method.[9] Acetylthiocholine iodide was used as substrate and Ellman's Reagent (DTNB) was used for measurement of acetylcholinesterase activity. AChE used in the assay was from electric eel (type VI-S lyophilized powder, 149 U/mg solid, 241 U/mg of protein). In 96 well plate, 140 µL sodium phosphate buffer, 10 µL (100 mM, pH 8.0) DTNB, test compound solution 25, 50, 100, 250 and 500 µg/ml (20 µL) and acetylcholinesterase (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 i.e. 5-thio-2-nitrobenzoate anion as the result

of the reaction of DTNB with thio-choline, released by the enzymatic hydrolysis of acetylthiocholine at a wavelength of 412 nm. The rate of hydrolysis of AChE was measured after 5 min over 20 min using a 96-well microplate reader (Thermoscientific, MultiskanGO). The IC_{50} values were calculated using a software program.

In vivo pharmacological studies

Screening of C. tora extract using scopolamine-induced amnesia

Dose showing maximum activity was selected by screening *C. tora* extract at three different doses (100, 200 and 400 mg/kg)[10] using scopolamine-induced amnesia model. The rats were divided into six groups (n=6 animal per group) Group 1–Control, Group 2–Negative control (scopolamine, 3 mg/kg, i.p.), Group 3–Standard treatment (scopolamine + mentat for 14 days) and Group 4-6–received 100, 200 and 400 mg/kg of *C. tora* extract for 14 days. Scopolamine was administered on 14th day to all groups except the normal control group. 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.

Evaluation of C. tora (400 mg/kg) using scopolamine and ECS induced amnesia

The dose showing maximum activity was selected and further evaluated for its effect on biochemical and cognitive parameter using scopolamine and Electroconvulsive shock (ECS) amnesia rat model. For this study rats were divided into 7 groups (n=6 animal per group) Group 1–Normal control, Group 2–Disease control (scopolamine, 3 mg/kg, i.p.), Group 3–Standard control (scopolamine + mentat for 14 days), Group 4-Scopolamine + *C. tora* extract, Group 5 – received ECS (10 mA current for 0.2 s), Group 6–ECS + Mentat, Group 7–ECS + *C. tora* extract. Mentat and *C. tora* extract was administered for 14 days, while scopolamine and ECS was administered after acquisition trial (14th day) which provoked cognitive impairment. Cognition improvement was assessed with three behavioral parameters, while biochemical parameters assessed by estimating acetylcholinesterase activity and β amyloid$_{1-42}$ content from rat brain homogenate.

Elevated plus maze

The apparatus consists of two open arms (50×10 cm) and two closed arms (50×10×40 cm) extended from a central platform (10×10 cm). The maze is elevated to a height of 40 cm from the floor. During *acquisition trial*, each rat was placed at the end of an open arm facing away from the centre. 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 sec. *Retention trial* was conducted 24 h after the first trial and transfer latency was recorded in a similar manner as mentioned before. Shortened transfer latency was considered as an index of improvement of memory.[11]

Step through passive avoidance apparatus

It consists of two compartment, an illuminated compartment and dark compartment connected by a guillotine door. The floors of the two compartments were constructed of stainless steel rods (3 mm in diameter, 10 mm apart) through which foot-shock could be delivered from a constant current source. *Acquisition trial:* We placed a rat in the illuminated compartment and 10 sec later the guillotine door was raised. Upon entering the dark compartment the door was closed and a 50 Hz, 1 mA constant current shock was applied for 2 sec immediately after the animal had entered the dark compartment. In all experiments the rat was re-trained in the apparatus and received a foot shock each time if it re-entered the dark compartment in 120 sec. Acquisition trial was terminated when the rat remained in the light compartment for 120 consecutive seconds. All the groups were trained and the escape latencies were assessed. *Retention trial*: This was evaluated on 15th day, by placing the animal into the light compartment and recording their latency to enter the dark compartment (four paws in). If the animal had not entered to the dark compartment within 300 sec, it was returned to its cage and a maximum latency of 300 sec was recorded. This escape latency served as a measure of retention performance of the step-through avoidance response.[12]

Morris water maze

In Morris water maze each animal was subjected to four acquisition trials per day for four consecutive days and their memory was tested on the 5th day. A plastic circular swimming pool (117 cm in diameter, 60 cm high) was filled to a depth of 25 cm with water. Pool was divided into four quadrants (Q1, Q2, Q3 and Q4). An 10×10 cm plexiglass platform, onto which the rat could escape, was positioned in the centre of one of the quadrant (Q4), 2 cm below the water surface. One day before the test, each rat was placed in the pool for 60 sec; this free swim enabled the rat to become habituated to the training environment. If the rat did not find the platform in 120 sec, it was manually placed on the platform for a 30 sec rest. Whereas on day 5, time spent in target quadrant (Q4) served as an index of retrieval or memory.[13]

Electroconvulsive shock

An electric current (60 Hz, 2 sec 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.[14]

Biochemical Study

Assay of Acetylcholinesterase activity

The estimation of acetylcholinesterase activity was based on Ellman's method. Post-treatment animals were sacrificed by cervical dislocation and brain was isolated and weighed. Whole brain was rinsed with ice cold saline and homogenized by making 20 mg of the tissue per ml in chilled phosphate buffer (pH 7.4). Homogenate were centrifuged at 800 g for 5 min. The supernatant thus obtained was used for AChE estimation. A total of 0.4 ml supernatant was added to a cuvette containing 2.6 ml phosphate buffer (0.1 mol/L, pH 8) and 100 µL of 5,5'-dithiobis-(2-nitrobenzoic acid). The content of the cuvette were mixed thoroughly and absorbance was measured at 412 nm by a UV spectrophotometer. When absorbance reaches a stable value, it was recorded as the basal reading. The substrate of 20 µL of acetylthiocholine iodide was added and the change in absorbance was recorded for a period of 10 min at intervals of 2 min. Change in the absorbance/min was thus determined. The mean change in absorbance was considered for calculation using following formula and acetylcholinesterase activity was measured as moles/min/g of tissue.[9]

$$R=\Delta A \times 5.74 \times 10^{-4} / Co$$

Where, R=Rate, in moles substrate hydrolysed per min/g of tissue.

ΔA=Change in absorbance per min. Co=Original concentration of tissue.

Estimation of β amyloid$_{1-42}$

The measurement of rat β amyloid$_{1-42}$ levels was conducted according to manual of sandwich ELISA kit for rat β amyloid$_{1-42}$ (YH Bioresearch Laboratory, Shanghai, China). Rat brain was homogenized in phosphate buffer (7.4) and centrifuged at 5000 g and supernatant was used for detection of β amyloid$_{1-42}$. Standard curve analysis was run in parallel to test samples. The absorbance was measured in the multi scan spectrum spectrophotometer (Thermo scientific, Multiskan GO) at OD 450 nm. All the readings were performed in triplicate.

Statistical analysis

All the results were expressed as mean ± standard error of mean (SEM). Data were analysed using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.P<0.05 was considered to be statistically significant.

RESULTS

In vitro acetylcholinesterase microplate inhibition assay

The ethanolic *C. tora* leaves at 25, 50, 100, 250 and 500 µg/ml concentrations was found to be effective in *in vitro* acetylcholinesterase microplate inhibition assay. IC_{50} was found to be 198.59 *µg/ml*.

In vivo Pharmacological studies

Screening of C. tora extract using scopolamine-induced amnesia

Transfer latency in elevated plus maze is time taken by rat to move from open arm to closed arm with all its four legs and escape latency is the time taken by rat to enter from light compartment to dark compartment. Among three doses of *C. tora* extract (100, 200 and 400 mg/kg) administered in scopolamine-induced amnesia model, 400 mg/kg dose showed significant reduction (p<0.01) in transfer latency also significant increase (p<0.01) in escape latency and time spent in target quadrant was increased (p<0.05) in Morris water maze. All the treatment groups are compared with disease control group, as shown in Table 1.

Table 1: Effect of *C. tora* (CT) on–100, 200 and 400 mg/kg dose on transfer latency, escape latency and time spent in target quadrant using scopolamine-induced amnesia model in rats

Groupings	Transfer latency (sec)	Escape latency (sec)	Time spent in target quadrant (sec)
Normal control	19.0±1.265	20.67±1.145	4.6±0.5578
Disease control (Scopolamine)	27.0±1.125***	13.83±0.8433**	6.1±0.6009**
Standard control (Mentat)	13.0±0.8944***	16.40±0.9189**	8.5±0.4282**
CT–100 mg/kg	20.50±2.377*	14.67±0.4216	4.6±0.3651
CT–200 mg/kg	19.33±1.726	16.0±1.291**	5±0.5578
CT–400 mg/kg	22.83±1.621**	15.33±1.358**	7.5±0.4282*

***p<0.001, **p<0.01, *p<0.05 vs disease control.

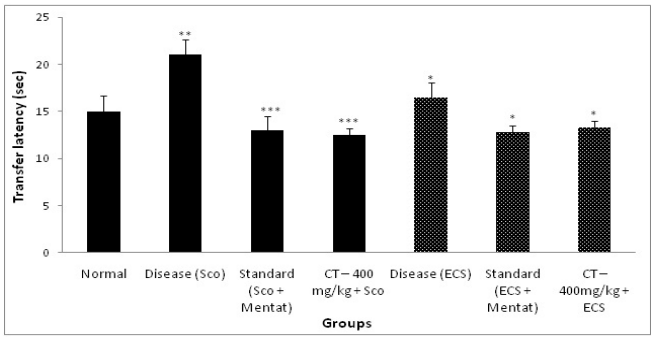

Figure 1: Effect of *C. tora* (CT)–400 mg/kg extract on transfer latency in Scopolamine and ECS induced amnesia

All values represent mean ± S.E.M. ***p<0.001, **p<0.01, *p<0.05 vs disease control.

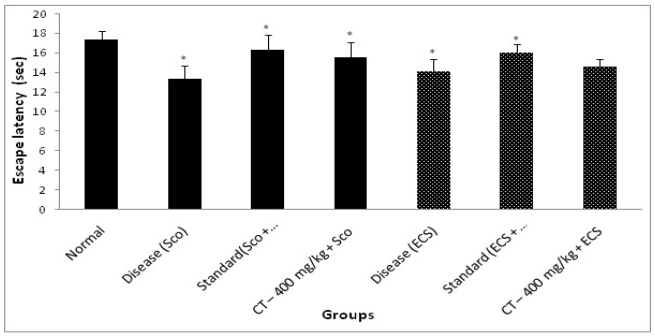

Figure 2: Effect of *C. tora* (CT)–400 mg/kg on escape latency in Scopolamine and ECS induced amnesia

All values represent mean ± S.E.M. *p<0.05 vs disease control.

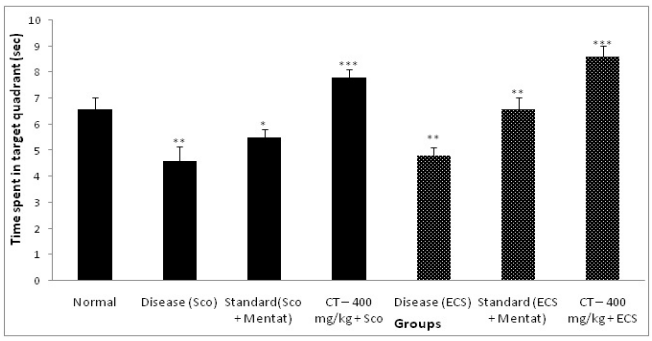

Figure 3: Effect of *C. tora* (CT)-400 mg/kg on time spent in target quadrant in Scopolamine and ECS induced amnesia

All values represent mean ± S.E.M. ***p<0.001, **p<0.01, *p<0.05 vs disease control.

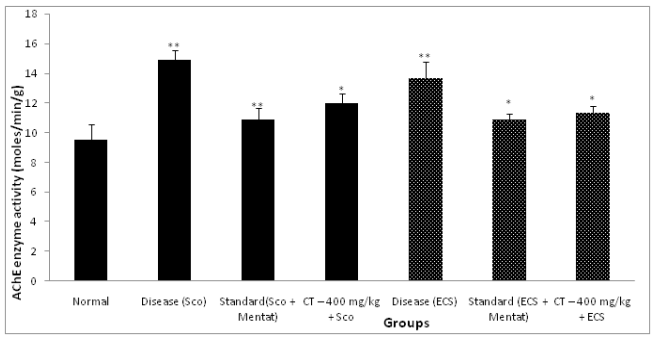

Figure 4: Effect of *C. tora* (CT)–400 mg/kg on AChE enzyme activity in Scopolamine and ECS induced amnesia

All values represent mean ± S.E.M. **p<0.01, *p<0.05 vs disease control.

Evaluation of C. tora (400 mg/kg) using scopolamine and ECS induced amnesia

Elevated plus maze

Transfer latency was significantly increased in both disease control groups (p<0.01, p<0.05), which indicates cognitive impairment in rats. While pre-treated *C. tora* (400 mg/kg) extract groups shows significant decreased in transfer latency in *C. tora* (400 mg/kg) + Sco (p<0.001) and *C. tora* (400 mg/kg) + ECS (p<0.05) induced group when compared with disease control group (Figure 1).

Step through passive avoidance

Pre-treatment of *C. tora* (400 mg/kg) extract increases escape latency in *C. tora* (400 mg/kg) + Sco induced group, while no significant difference in *C. tora* (400 mg/kg) + ECS induced group. While escape latency was found to be decreased in disease control groups i.e. scopolamine and ECS induced group (Figure 2).

Morris water maze

Time spent in target quadrant was significantly increased in both *C. tora* (400 mg/kg) pre-treated groups (p<0.001) when compared with disease control. While administration of scopolamine and ECS in disease control group showed decreased (p<0.01) time spent in target quadrant (Figure 3).

Biochemical estimation

Assay of acetylcholinesterase activity

Exposure of scopolamine and ECS significantly elevate (p<0.01) AChE activity, while pre-treatment with *C. tora* (400 mg/kg) decreased the elevated AChE activity to normal in *C. tora* (400 mg/kg) + Sco (p<0.05) and no significant change was observed in *C. tora* (400 mg/kg) + ECS, when compared to disease control groups. (Figure 4)

Estimation of β amyloid$_{1-42}$

β amyloid$_{1-42}$ levels were significantly increased (p<0.001) in Scopolamine and ECS administered group when compared with normal control, while substantial fall in (p<0.001) βamyloid$_{1-42}$ level was observed in pre-treated groups when compared with disease control groups (Figure 5).

DISCUSSION

Present study investigated the effect of ethanolic *C. tora* leaves extract on scopolamine and ECS induced cognitive

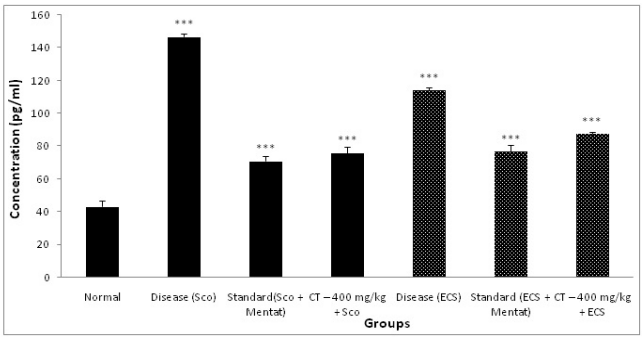

Figure 5: Effect of *C. tora* (CT)–400 mg/kg on concentration of β amyloid1-42 in Scopolamine and ECS induced amnesia

All values represent mean ± S.E.M. ***p<0.001 vs disease control.

impairment in rats using behavioral and biochemical paradigm. Scopolamine, a non-selective centrally acting muscarinic receptor antagonist, impairs learning and memory in rodents which is associated with reduced cerebral blood flow, increased oxidative stress and acetylcholinesterase activity in rat brain. Administration of scopolamine a day before retention trial induced cognitive impairment as tested by behavioral and biochemical paradigm.[15] ECS induces both anterograde and retrograde amnesia, stimulates neuro plasticity and causes changes in structures of hippocampus, amygdala, and prefrontal cortex. ECS induces amnesia in animals by facilitating serotonergic transmission.[16]

Dose dependant increase in activity of ethanolic *C. tora* leaves extract was observed in scopolamine induced amnesia model. At 400 mg/kg dose, significant improvement in transfer latency, escape latency and time spent in target quadrant was observed. Hence, 400 mg/kg dose showing maximum activity was selected and further evaluated using scopolamine and ECS induced amnesia model.

In Morris water maze, scopolamine and ECS administered rats swam for longer time and travelled farther before locating the platform. Pre-treatment with *C. tora* (400 mg/kg) extract significantly improved time spent in target quadrant. There was decrease in transfer latency i.e. rats entered closed arm immediately when placed at open arm in the EPM, which indicates cognitive improvement in *C. tora* (400 mg/kg) + Sco group and *C. tora* (400 mg/kg) + ECS group. In case of the step through passive avoidance paradigm, the escape latency was increased in *C. tora* (400 mg/kg) + Sco and no change was observed in *C. tora* (400 mg/kg) + ECS group, which suggests that animals retained memory of shock, once entered in the dark compartment. Hence, Pre-treatment of *C. tora* (400 mg/kg) extract exhibit pronounced reversal of escape latency in scopolamine induced amnesia while transfer latency and time spent in target quadrant in scopolamine and ECS induced amnesia was reversed.

Pre-treatment with *C. tora* (400 mg/kg) significantly reduced brain acetylcholinesterase activity in scopolamine and ECS induced amnesia, indicating the stimulating actions of *C. tora* extract on the cholinergic system, this was found in close agreement with previous report by Murray *et al.*[17] Hence, in the present study cognitionenhancing effect of *C. tora* extract can be attributed to its acetylcholinesterase inhibitory activityand by probably improving the level of acetylcholine in brain.

Administration of ethanolic *C. tora* (400 mg/kg) extract for 14 days showed marked reduction of β amyloid$_{1-42}$ level in pre-treated groups, these results were in close agreement of previous report by Agnati *et al.*[18] Hence, *C. tora* may be used in delaying the onset and reducing the severity of cognitive impairment.

In vitro acetylcholinesterase microplate assay of ethanolic *C. tora* leaves extract was found to be effective at all concentrations and IC$_{50}$ was found out to be 198.59 µg/ml, which indicate cognitive improvement by *C. tora* extract may be due to its acetylcholinesterase inhibition.

CONCLUSION

Ethanolic *C. tora* leaves extract at 400 mg/kg showed cognition enhancing property in scopolamine and ECS induced amnesia model. Ethanolic *C. tora* leaves extract may be exerting its effect by decreasing AChE activity and β amyloid$_{1-42}$ level in rat brain. Hence, further investigations are required to characterise the active constituents from ethanolic *C. tora* leaves extract responsible for the cognition enhancing activity.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATION

C. tora:	Cassia tora
AD:	Alzheimer's disease
Sco:	Scopolamine
ECS:	Electroconvulsive shock
IC:	Inhibitory concentration

Highlights of Paper

- Hydroalcoholic *Cassia tora* leaves extract at all used concentration inhibits AChE enzyme activity in *in vitro* microplate inhibition assay.
- Pre-treatment with hydroalcoholic *Cassia tora* leaves extract reversed the scopolamine and ECS amnesia induced in rats.
- Also, hydroalcoholic *Cassia tora* leaves extract significantly decreased β amyloid concentration, which can be the probable pathway for its cognition enhancing activity.

Author Profile

- **Mr. Rohit Malabade:** Is a doctoral student at the KLE University, Belagavi, where he graduated in Master of Pharmacy. His doctoral research is focused on *in vivo* and *in vitro* evaluation of selected medicinal plant for cognition enhancing activity.

- **Dr. Ashok Taranalli:** Is currently Dean and Professor in Department of Pharmacology at KLE College of Pharmacy, Belagavi. He has experience in the areas of Pharmacology and Chemistry of Natural Products, his work is focused in Medicinal plants, New Research Drugs and CNS disorders.

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