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Leaf extract of ethnomedicinally important Bharangi (*Clerodendrum serratum*) may improve neuromodulatory activity in mice model

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Ancient civilization to present-day cultural and technological development, human over the world has been using plants as a source of natural medicine for their basic needs. *Clerodendrum serratum* (Linn.) Moonis one such traditionally well-accepted plant used vigorously in Indian ayurvedic therapeutic purposes. Despite its ethnomedicinal significance, the neuroprotective prospects and relevance of *Clerodendrum serratum* (CSL) remain unaddressed. Therefore, in the present study, we explored several *in-vivo* antioxidant enzymatic assays followed by acetylcholinesterase enzyme (AChE) inhibitory activity against neurodegenerative disorders (NDs) in mice model and explore the beneficial impact of the plant extract. Furthermore, the *in-silico* molecular docking approach was employed to evaluate the inhibitory potential of the plant-derived bioactive compounds against the imperative proteins associated with neurodegenerative disorders. The plant extract showed promising results in all *in-vivo* enzymatic assays indicating its positive role over several neurodegenerative disseases namely, Alzheimer's and Parkinson's disease. The histological and *in-silico* investigation also reveled this plant extract is promising in elevating neurodegenerative disorders. Therefore, it seems likely that this plant can be used as an anti-cognitive agent for future drug development.

Keywords: Acetylcholinesterase, *Clerodendrum serratum*, Ethnomedicinal, Molecular docking, Neurodegeneration.

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Every culture all over the world has been using plants for natural medicine and depends on nature for their existence since ancient times. The therapeutic relevance of medicinal plants in India has led to extensive exploration of their medicinal attributes[1-3]. Several phytocompounds including tannins, alkaloids, steroids, phenols and flavanoids find widespread applications in treating various ailments[4]. The phytocompounds nullify the oxidative stress in the human body rendering immunity against chronic diseases. Different neurodegenerative disorders (NDs) like dementia, Alzheimer's disease (AD) and Parkinson's disease (PD)[5,6] are caused by reactive oxygen and nitrogen species as consequences of oxidative stress. It has been reported that neurodegenerative disorders can be balanced by the antioxidative defense system[7].

Clerodendrum serratum (Linn.) Moon, a semi-woody shrub, locally known as '*Bharangi'* belonging to Lamiaceae family, is found in some parts of Asia and Africa. Ethnomedicinally, *C. serratum* is chiefly used for respiratory diseases viz. colds, bronchitis, bronchial asthma and tuberculosis[8]. Water decoction of *C. serratum* is used as a remedy to high blood pressure in Malayasia[9]. In the Indian traditional system of therapeutics, the plant was mentioned as an important ingredient of several Ayurvedic preparations like *Dashmoolarishtha* (a health tonic, prepared from the roots of 10 medicinal species), *Chavanprasha* (used to treat fever, asthma, anorexia and stomachache) and *Panchatikta ghrita guggul* (prescribed for a neurological disorder, herpes, inflammation and arthritis) to treat regular ailments[10]. Reviews on *Clerodendrum serratum* reported preliminary antioxidant, antibacterial and anti-inflammatory activities[11,12].

Quite surprisingly, no major step has still been carried out to validate the neuroprotective relevance of *Clerodendrum serratum* (CSL). In our previous reports, we have successfully been justified the ethnomedicinal claims of *Hippophae salicifolia*[6]. Therefore, an initiative was undertaken to analyze a

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detailed neuroprotective effect of CSL in mice models. In this regard, the neurotherapeutic activity of *Clerodendrum serratum* leaf (CSL) was executed in the present study using different *in-vivo* antioxidant methods as well as *in-silico* molecular docking analysis justifying its beneficial effects over oxidative stress.

Materials and Methods

Plant material

C. serratum (CSL) leaves were collected during April and May 2017, from Azra, Guwahati, Assam (26.1445° N, 91.7362° E).

Preparation of plant extracts

A mechanical grinder was used to pulverize air-dried (3 weeks) disease-free fresh leaves of *C. serratum* into a fine powder. The crushed leaves of CSL (10 g) were extracted in 70% methanol (v/v) for 6-7 hours using soxhlation procedure. Rotary evaporator was used to concentrate the extract. Finally, the extract was lyophilized to obtain dry powder for further use.

Animals and care

In this experiment, inbreed male Swiss albino mice (8-9 weeks age and 34±2gms body weight) were kept in polypropylene cage (6 male mice/group, n=6) in the animal house with food and water. Throughout the experiment a constant temperature (25 ±2 °C) and humidity (55±5 %) was maintained.

Acute toxicity analysis

The acute toxicity of CSL extract was assessed following OECD guidelines. Animals were divided into five groups (n=6). The plant extract was fed orally with increasing concentrations (250, 500, 1000, and 2000 mg/kg body weight) to the mice. Subsequently, all the groups were methodically monitored to note the development of toxicological symptoms at different time interval.

Experimental setup and administration of the dose

The animals (30 male mice) were divided into five groups and this treatment was carried out daily for 7 days:

Group I: Control, received normal saline water; Group II: Scopolamine group received Scopolamine (0.5 mg/kg/day BW) intraperitonially; Group III: Donepezil group received scopolamine (0.5mg/kg/day BW) and donepezil (10 mg/kg/day BW); Group IV: Mice received scopolamine (0.5 mg/kg/day BW) and low dose extract of CSL at 100 mg/kg/day BW; Group V: Mice received scopolamine (0.5 mg/kg/day BW) and high dose extract of CSL at 400 mg/kg/day BW respectively. On the 8th day, plant extracts/standard drug/normal saline was administrated orally after 90 min. of scopolamine treatment followed by behavioral tests performed after 45 min of injection. At 9th day of the last injection the behavioral test was performed to measure the retention time. All the trial and examining sessions were carried out at night, preferably between 19:00 and 23:00 h due to the restless movement of the mice at night.

Passive avoidance task

The methods of Reddy[13] was employed to study the passive avoidance task.

Preparation of brain tissue samples

After behavioral evaluations, the animals were sacrificed (2% mild ether) by cervical dislocation. The brain of the sacrificed mice of each group were removed quickly from the skull and washed consciously in ice cold normal saline. Subsequently, homogenization was performed using phosphate buffer (50mM; pH 7.4), potassium chloride (KCl; 120mM), and finally BHT (0.004%, w/v) was added to prevent auto-oxidation of the samples. Centrifugation was performed at 3000 rpm for 10 min at 4ºC.

In-vivo brain enzymatic assays

Acetylcholinesterase inhibiting activity (AChE), DPPH, Catalase (CAT), Reduced Glutathione (GSH), Superoxide dismutase (SOD) activity and MDA quantification were measured according to the standard methods[14].

Histopathological examination

Mice brains were removed and were fixed in 10% formaldehyde solution. The method opted by Knodell *et al.*[15] was used to prepare, stain and cut the brain sections.

GC-MS analysis

GC-MS analysis was conducted as perthe standard protocol with slight modifications.

Molecular Docking

The major compounds present in CSL were used for the purpose of molecular docking analysis against dopamine receptor D3 protein (PDB ID- 3PBL). The protein structure was retrived from the Protein Data Bank. The AutoDockVina[16] software was employed to

perform the molecular docking as per the scheme opted by Dutta *et al.*[14].

Statistical analysis

Dunnett's test implemented in KyPlot software (version 5.0) was employed to perform one-way analysis of variance (ANOVA) statistical test with a significance level at $P< 0.05$.

Results and Discussion

Acute toxicity study

At high dose of CSL extract (2000 mg/kg), no death was observed in the mice. Therefore, in the present study $1/20^{th}$ (100 mg/kg) and $1/5^{th}$ (400 mg/kg) were considered safe for *in-vivo* studies.

Body weight changes

Table 1 depicts the mice body weight of the different groups and no significant weight gain was observed during this experiment.

Passive avoidance test

The present study accounted for an inclusive report of the neurotherapeutic effect of CSL on memory deficits in a mouse model of amnesia (passive avoidance test) scopolamine treatment. The fear-motivated avoidance test was used to study the animal behavioral changes in presence of an unplesant stimulus (electric foot-shock) as a part of long-term memory. Table 2 revealed that the initial latency time to enter the dark chamber was significantly longer in the mice given only scopolamine as compared to the control group suggesting the amnesic effect of mice. The treatment with CSL extract significantlyattenuated the scopolamine-induced memory deficit in mice to a great extent and also associated with the short-term memory (STL) improvement (Table 2) suggesting the anti-amnesic effect of extracts in the scopolamine-induced rodent model. While considering brain AChE inhibitory activity, CSL extract increased the cholinergic activity to reverse the memory impairment in mice (Fig. 1A). Hence, it can be inferred that CSL could be a potent AChE-inhibitors by hindering the destruction of Ach[17,18].

Table 1 — Effects of *Clerodendrum serratum* on the body weight of the treated mice

	Initial Weight	Final Weight	% body weight change
Control	34.35±1.07	36.45±2.03 ^{NS}	5.67±2.37▲
Scopolamine	36.95±1.34	32.71±1.86**	11.51±1.79▼
Donepezil	35.57±1.26	36.93±1.63^{NS}	3.63±1.99▲
CSL Low	32.78±1.47	35.41±0.90^{NS}	7.43±3.03▲
CSL High	33.68±1.96	35.43±1.67*	4.98±1.16▲

Weight (mean ± SD) in gram, * P<=0.05, ** P<=0.01, ^{NS} = Non significant. Final body weight was compared with the initial body weight of the corresponding group. ▲ Increase weight; ▼ Decrease weight.

Table 2 — Effect of CSL extract on scopolamine-induced memory impairment in the passive avoidance test

Groups	IL (Sec.)	STL (Sec.)
Group I (Control)	18.13±1.99	132.41±8.29
Group II (SCP)	107.23±4.83	80.14±1.33
Group III (SCP + Donepezil)	27.12±2.19	176.79±2.19
Group IV (SCP + CSL low)	57.78±4.34	130.24±0.83
Group V (SCP + CSL high)	35.66±2.39	165.23±1.45

IL- Initial latency; STL- Step-Through Latency; SCP- Scopolamine [Data represented as mean ± SD].

In-vivo antioxidant assays

In this experiment, scopolamine treatment significantly reduced the antioxidant capacity of DPPH, enzymatic catalase and SOD (superoxide dismutase) (Fig. 1B, 1D, 1F) and non-enzymatic reduced glutathione (GSH) system in brain tissues (Fig. 1E). At the same time, CSL treatment significantly increased the percent of inhibition of DPPH, catalase, SOD, and reduced glutathione when compared with scopolaminegroups. Figure 1C illustrates MDA or Lipid peroxidation level in the treated groups. The MDA content increased from 28.26 ± 2.89mM/litre (in control) to 51.89 ± 5.17 mM/litre in the scopolamine group. The increased MDA level was lowered to 32.93 ± 3.98mM/litre after a high dose of CSL administration significantly. Hence, the results suggest the anti-oxidative potential of CSL that contributed to effective neuronal plasticity and memory function. Thus, most importantly, we provide the first evidence for a potent neurotherapeutic role of CSL in the protection from ROSmediated neuronal damage as well as we identified some of the responsible target phytocompounds that could be treated as future CNS drug. It has been well speculated that every cellular organism sustains its own antioxidant stability to protect tissues from oxidative damage at a certain stage. SOD, catalase, GSH, etc. are the fundamental antioxidant enzymes that protect tissues from highly reactive hydroxyl radicals and superoxide anions, linked with NDs[19,20].

Histopathological examination

The normal structure of the cerebral cortex and hippocampus in control mice is shown by

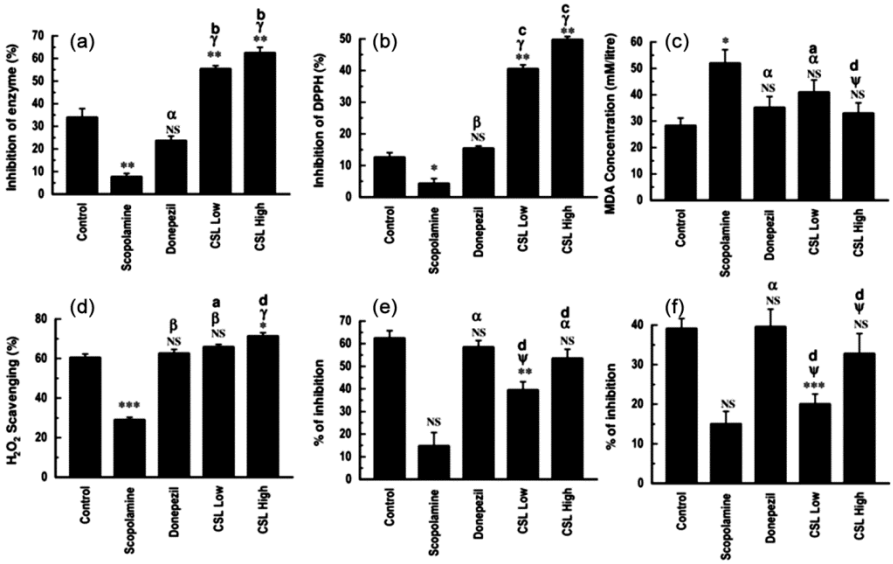

Fig. 1 — **(A)** AChE **(B)** DPPH **(C)** Lipid peroxidation **(D)** Catalase **(E)** GSH and **(F)** SOD activity of CSL extract.

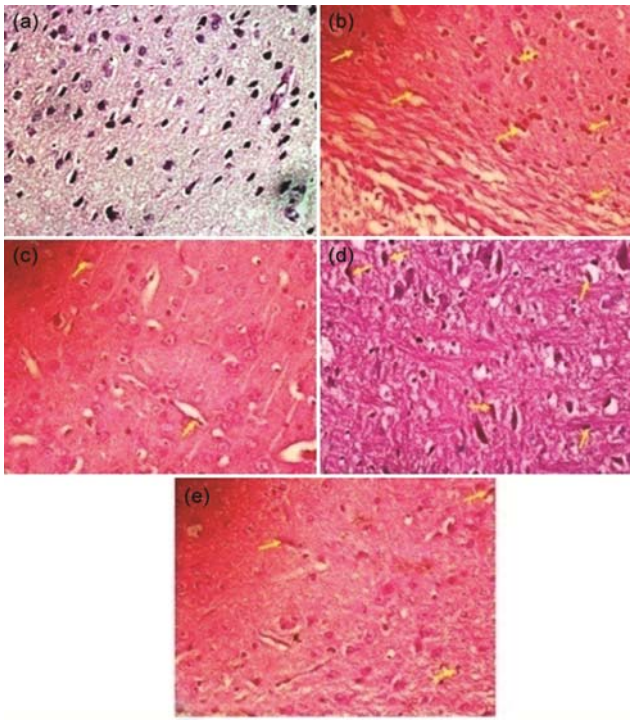

Fig. 2 — Photomicrograph of mice brain (cortex, A-E), 40X. **(A)** Normal neurons, glial cells are shown in control mice. **(B)** Chromatolysis (arrows), Gliosis, and edema in cortex shown in Scopolamine induced mice**. (C)** Donepezil exposure on mice brain showing less necrotic (arrows). **(D)** CSL extract (100mg/kg BW) showing marked gliosis (arrows). **(E)** CSL extract (400mg/kg BW) showing moderate necrotic and degenerative changes.

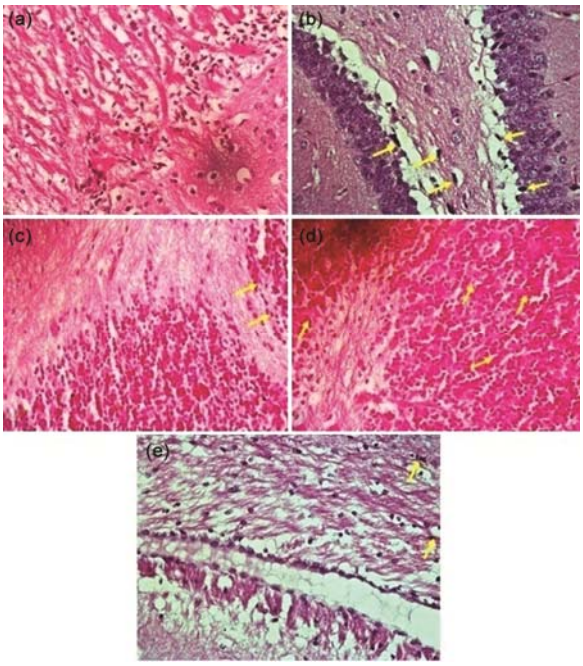

Fig. 3 — Photomicrograph of mice brain (Hippocampus region, A-E), 40X. **(A)** Normal glial cell layer and Purkinje layer shown in control mice. **(B)** Chromatolysis of nuclear material (arrows) and Purkinje neurons are shown in Scopolamine induced mice. **(C)** Donepezil exposed mice shows reduced severity of necrotic (arrows) and degenerative lesions. **(D)** Necrotic degeneration is shown by CSL extracts (1000mg/kg BW) on Purkinje neurons and lysis of glial cells (arrows). **(E)** Significantly less necrotic degeneration is shown by CSL extract (400mg/kg BW) on Purkinje neurons and lysis of glial cells (arrows).

histopathological sections (Fig. 2A and 3A). Mice with scopolamine exposure showed severe gliosis, edema,and chromatolysis (arrows) in the cortex (Fig. 2B), whereas, the hippocampus revealed severe necrotic Purkinje neurons and chromatolysis of nuclear material (Fig. 3B). Mice brain exposed to donepezil showed less necrotic, degenerative changes with normal neurons and glial cells in both the cortex and hippocampus region (Fig. 2C and 3C). In the present study, the injury was down-regulated by a

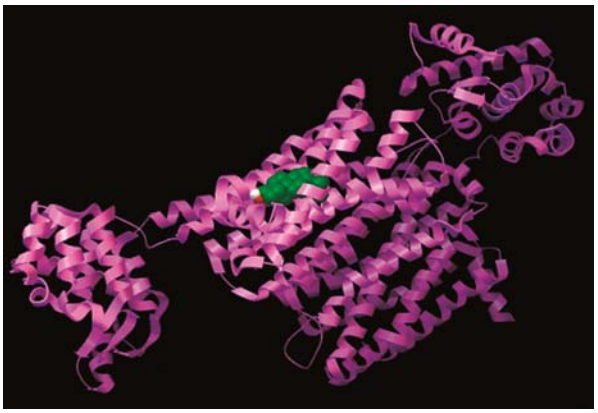

Fig 4 — Molecular Docking view if the secondary structure of dopamine receptor D3 protein (PDB ID- 3pbl) with Stigmasterol.

high dose of CSL extract (400 mg/kg BW) compared to the standard donepezil (Fig. 2E and 3E).

GC-MS analyses

The phytocompounds hexadecanoic acid, phytol, linoleic acid, oleic acid, squalene and stigmasterol were identified in CSL[21] among which linoleic acid, oleic acid, squalene and stigmasterol were noted to be the main bioactive compounds.

Molecular Docking

The phytochemicals hexadecanoic acid, phytol, linoleic acid, oleic acid, squalene and stigmasterol revealed binding energy scores of -3.6, -4.4, -2.9, -3.3, -5.7 and -6.1 kcal/mol respectively. It was interesting to note that stigmasterol displayed decent binding inhibition potential with the dopamine receptor D3 protein (Fig. 4).

Conclusion

The present study is the first ever detailed neuroprotective evaluation of ethnomedicinal plant *Clerodendrum serratum*. Despite of having massive ethnomedicinal utilization, proper clinical study of CSL was not yet executed. Hence, we intended to explore its different therapeutic applications in the present study. Results reflected that CSL was found to be a potent neuroprotective agent with non-toxic nature. In addition, CSL was first time assigned as AChEI over neurodegeneration as per the authors' best knowledge. Therefore, it seems likely that this plantcan be used as an anti-cognitive agent for future drug development in AD and PD.

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Ethical statement

Animal Ethical Committee Permit No.: 840/ac/04/CPCSEA.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Contribution Statement

AS and PK conceived the idea. PK, AKC, SD and AS carried out the research, collected the plant materials and analyzed data. PK, MB and SD carried the rat related experiments. AKC contributed in *in-silico* analysis. All authors wrote the MS and approved it.

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