Evaluation of Nootropic Potential of *Leucaena leucocephala* on Wister Rats

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Abstract Introduction: *Leucaena leucocephala* is a commonly used plant in the Ayurveda system of medicine to alleviate several ailments. Different parts of *Leucaena leucocephala* are used for the treatment of ailments such as diabetes, ascariasis, intestinal parasitism and trichinosis. It is also used as an emmenagogue, emollient and as nutritious forage for cattle. **Objective:** This study was conducted to evaluate the plant extract's effect on memory and learning using *in-vivo* models and *ex-vivo* models. **Methods:** The plant extract was prepared by using the Soxhlet extraction procedure. The effect of the extract on nootropic activity was assessed against scopolamine-induced amnesia using different exteroceptive models such as Elevated plus maze, Y maze and Hebb William's maze. Acetylcholinesterase activity of the rat brain homogenate was estimated using Ellman's method. The activity of the extract was compared with the standard drug piracetam. **Results:** On pre-treatment with ethanolic extract of *L. leucocephala*, *in-vivo* studies showed significantly improved spatial learning and memory against scopolamine-induced amnesia in Wistar rat models in a dose-dependent manner. *Ex-vivo* studies of rat whole brain homogenate showed a significant decrease in acetylcholinesterase activity in extract-treated animals. **Conclusion:** The ethanolic extract of the leaves of *L. leucocephala* showed significant nootropic potential against animals induced by amnesia using scopolamine.

Keywords Anticholinesterase, Cognition, Elevated Plus Maze, Hebb Williams Maze, Nootropic Potential, Y Maze

1. Introduction

In the treatment of memory disorders like Alzheimer's disease, the structural diversity of the phytoconstituents present in the plants makes them a potential lead compound for the quest of drugs for the treatment [1]. Cognition is the ability of the brain to comprehend, store and retrieve the sequences of information experienced through the sensory organs [2,3]. Large numbers of plants are being screened for their nootropic, memory enhancing activity. Plants such as *bacopa monnieri*, *acorus calamus*, *withania somnifera*, *evolvulus alsinoides*, *emblica officinalis*, *centella asiatica*, *zinziber officinale*, *celastrus panniculatus*, *vitis vinifera*, *thespesia populnea*, *Panax ginseng*, *Prunus amygdalus*, *Clitoria ternatea* L., *Hypericum perforatum*, *Cornus officinalis*, *Glycyrrhiza glabra* and *Pueraria tuberosa* have showed significant memory enhancing activity [3]. *L. leucocephala* has its origin in Central America where it has been recognised for its fodder values over 400 years ago. Now it is widely spread over Southern Asia and neighbouring islands [4].

Flavonoids like Isorhamnetin, Caffeic acid, Chrysoeriol, isorhamnetin 3-o-galactoside, Kaempferol-3-o-rubinoside, Quercetin-3-o-rhamnoside, Luteolin-7-o-glucoside) [5] Polyphenols like Ficaprenol, Terpenoids like Squalene, Lupeol, Steroid like *B*-sitostenone [6], β -sitosterol, stig-mastenone [7], Tannins like Gallic acid, Flavone [8], Chlorophylls like aristophyll-c⁵ are present in the *L. leucocephala*. Antidiabetic [9], Antiproliferative [10], Anthelmintic [11], Antimicrobial [12], Nutritive [13], anti-piles [14], Hypoglycemic and Hypolipidemic [15] activities of *L. leucocephala* have been reported in the literature. A limited study has been carried out on the nootropic activity using *L. leucocephala*. Dzoyem JP et al. reported the anti-inflammatory, antioxidant activity and Acetylcholine esterase inhibitor properties of the plant. Since antioxidants offer a neuroprotective effect, they can be potential molecules for investigation [10]. With this hypothesis, the present study was carried out to assess the nootropic effect of *L. leucocephala* leaves' extract based of antioxidant and acetylcholine esterase inhibitory

activity of the plant extract reported earlier.

2. Materials and Methods

The systematic approach of the study procedure is illustrated in the Figure 1.

2.1. Materials

The chemicals used in the study were of analytical grade. Scopalamine, piracetam, and acetylcholine iodine were procured from Sigma Aldrich, St. Louis, USA. Chemical including anhydrous calcium chloride, sodium carboxy methyl cellulose, potassium dihydrogen citrate was procured from Loba Chemie Pvt. Ltd., Mumbai, hydroxymethyl hydrochloride (Tris HCL) and Ellman's reagent from Himedia, sodium hydroxide and ethanol from Merck Pvt. Ltd., Mumbai and Methanol was procured from Nice chemicals Pvt. Ltd. Kochi, Kerala.

Figure 1. Schematic presentation of study procedure

2.2. Preparation of Extract

The leaves of *Leucaena leucocephala* were collected from Deralakatte, Mangalore, Karnataka, India. The leaves were collected during the month of July 2016. Plant was authenticated by Dr. K.V. Nagalakshamma, Associate Professor, HoD, Department of Botany, St. Aloysius College, (Autonomous) Mangalore-575003. Voucher specimen was kept in the herbarium of the institute. The leaves were washed and dried under shade at room temperature. The dried leaves crushed into a coarse powder. Seven hundred grams of coarse powder was extracted with ethanol by Soxhlet extractor for five cycles. The extract was concentrated using a rotary flash evaporator under reduced pressure. However, we could not get pure dry powder using the rotary flash evaporator. The greenish brown viscous extract obtained was stored in a desiccator free from moisture and humidity. The percentage yield of the viscous extract was 12.

2.3. Preliminary Qualitative Phytochemical Investigation

Qualitative phytochemical examination of the ethanolic extract of leaves of *L. leucocephala* was carried out using standard methods for alkaloids (Dragendorff's test, Hager's test, Wagner's test, Mayer's test), reducing sugar (Molisch test, Benedict's test, Fehling's test, Tollen's test), flavonoids (Shinoda test), saponins, tannins, steroids (Liebermann-Burchard's test, Salkowski reaction), proteins (Biuret test, Millon's test) and triterpenoids (Liebermann-Burchard's test) [16,17].

2.4. Animals

Albino Wistar rats of either sex, weighing 150-200 g, were obtained from the committee for the purpose of control and supervision of experiments on animals (CPCSEA) approved animal house of Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences (Ref.1781/PO/EReBi/S/2014/CPCSEA), Mangalore, India. After being divided into different groups, all the animals were kept in separate cages, with free access to food (standard dry pellet diet) and water *ad libitum*. These animals were kept at a temperature of 23-27 °C with light and dark cycle of 12 hrs following the standard laboratory conditions. The bedding used for the animals was a paddy husk, which was changed at least twice a week. After the completion of experiment the animals were anaesthetized and the animals were sacrificed by cervical dislocation.

2.5. Selection of Dose

Three dose levels were selected namely 200 mg/kg, 400 mg/kg and 800 mg/kg body weight based on acute toxicity study result. The extract was found to be nontoxic and safe when tested at a dose level of 2000 mg/kg. The test

was carried out as per organization for economic co-operation and development (OECD) guideline 425, up and down method (Acute oral toxicity test). As we predicted low toxicity of the extract, a single dose of 2000 mg/kg body weight was administered orally using a gavage needle to a female rat weighing 180 g. The animal was observed for 4 hours continuously and later on daily basis for any toxic CNS, CVS, and autonomic symptoms. No morbidity or mortality was observed during the observation period.

2.6. Treatment Schedule and Evaluation of Nootropic Activity

Forty-eight albino, Wistar rats of either sex, weighing about 150-200 g were divided into eight groups of six animals each (i.e., n=6) for the study. Group I served as normal control and received the formulation vehicle (0.6% w/v sodium CMC, 10ml/kg, once daily, oral). Group II received scopolamine (1 mg/kg, i.p.) only on the 8th day of the study. Piracetam (200 mg/kg, oral, once a day for 8 days) and *L. leucocephala* extract (800 mg/kg, oral, once a day for 8 days) were administered to groups III and IV. Animals of groups V, VI, VII and VIII were administered with standard piracetam and test compounds for eight days and on the 8th day; amnesia was induced with one dose of scopolamine (1 mg/kg). After 45 minutes of administration of the test drug, the nootropic activity was assessed using three maze-models: Elevated plus maze, Y-maze, and Hebb-William's maze. The trials were carried out again on the next day (9th day) 24 hrs. Since the first exposure and the retention index was recorded, this served as the scale for determining the nootropic potential of the drug.

2.7. Maze Models

2.7.1. Elevated plus-maze

This model is an incentive based exteroceptive model which is used for the screening of nootropic agents and anxiolytics. The apparatus has two closed and two open arms with a length of 50 cm and a width of 10 cm and are perpendicular to each other. The maze has an elevated position, which is 50 cm from the ground. For taking the trial, the animal was placed in one of the open arms of the maze such that the animal faced away from the central platform. The animal was observed and the time taken by the rat to reach the closed arm from the open arm was recorded as the initial transfer latency (ITL). For all the trials, the cut-off time was 90 seconds. A similar trial was carried after 24hr of the ITL and that was recorded as the retention transfer latency (RTL). These both recorded parameters were designated as acquisition (learning/ITL) and retention (memory/RTL), respectively [18,19]. A decline in the transfer latency on repeated maze exposures will be graded as successful memory retention.

From the above observations, inflexion ration (IR) was calculated:

$$IR = L_0 - L_1 / L_1$$

IR = Inflexion ratio

L_0 = Initial transfer latency (In sec)

L_1 = Retention transfer latency (In sec)

2.7.2. Y-maze

The spatial working memory in rodents was determined by using Y-Maze. This model is based on the constant altering behaviour of animals. In Y-maze, there are three horizontal arms that are aligned at an angle of 120°. The maze arms are of length 40 cm, 3 cm in width, and 12 cm high walls. The three arms may be labeled as start arm (A), a reward arm having food (B), and a random arm (C). The maze is made of dark polyvinyl plastic, which is opaque. With food as a reward, the animals are allowed to perform a suitable search operation. This index is used in temporal measurement and error scoring, which are the intricate parameters to evaluate the retention ability in a given drug known to possess nootropic activity. All the animals should properly be trained before subjecting them to test [20].

For the trial, the animals were placed on the start arm and were allowed to move all through the maze for 8 mins, and every arm entry was recorded. The alternating behaviour of animals was expressed when the animals know which arm it has already visited. The arm re-entry was tabulated in the form of a triad, which were ABC or BCA or CAB or ACB, and so on. The triad was only considered if the animal shows alternating arm entries and not repeated entry, i.e., ABA or BCB or CBC, and so on. Short-term memory retention was assessed using this alteration. From the recorded arm entries and the alterations, the % alterations were calculated using the formula.

$$\% \text{ Alternation} = [(\text{Number of alternations}) / (\text{Total arm entries} - 2)] \times 100$$

2.7.3. Hebb-William's maze

Spatial memory, as well as working memory, can be determined using the Hebb-Williams maze, which is an exteroceptive model having three chambers that are used to assess animal behaviour. With the help of a wooden partition, chambers are detached from each other. The animal is kept in the first chamber, which is called the start box. The second chamber consists of a twisted corridor that separates the start box and the reward area [21]. This area is called an exploratory area. The animal fasts overnight. For taking a trial, the animal was placed in the start box. The time taken for the animal to get to the reward chamber was noted. A zero-watt red colour bulb was used to prevent any nocturnal interruptions during the test. ITL was noted on the first day of the test (8th day of

drug treatment). For an additional 3 minutes, the animals were allowed to explore all over the maze. The animals were then exposed to food for 1 hr to bring about stimulation. RTL was determined on the next day. Both the values were taken, and the IR was calculated [22].

2.8. Ex-vivo Studies

2.8.1. Collection of brain samples

Rats were anesthetized and sacrificed humanely after the experiments. The skull was cut open, and the whole brain was removed carefully, weighed and washed using cold saline in a cold bath and homogenized. The brain was homogenized using Potter-Elvehjem homogenizer (20 mg of brain tissue in one ml of 0.1M phosphate buffer). Acetylcholine esterase activity was estimated by using the supernatant liquid separated from the homogenate after centrifuging it for 10 minutes at a speed of 3000 rotation per minute [23].

2.8.2. Estimation of acetylcholinesterase activity by Ellman's method

Acetylcholine esterase is an enzyme, whose main function is hydrolysis of acetylcholine which results in the termination of transmission of cholinergic synapses. So, in cognitive dysfunctions like Alzheimer's diseases, AChE is considered to cause extensive loss of cholinergic neurons in the brain [24]. Ellman's method is used to determine the AChE activity. In this method AChE acts on Acetylthiocholine (ATC), which is an artificial substrate and results in the release of thiocholine and acetic acid. Further this thiocholine is made to react with -SH reagent 5,5-dithio-bis-(2, nitro benzoic acid) (DTNB), resulting in the conversion of thiocholine to thionitro benzoic acid, a yellow-coloured anion with absorption maxima 412 nm. 1.36×10^{-4} molar/cm is the molar extinction coefficient of thionitrobenzoic acid. By using UV spectrophotometer, the concentration of thionitro benzoic acid is determined which is directly proportional to the AChE activity [25].

2.8.3. Estimation of Acetylcholinesterase by microplate assay

Acetylcholinesterase activity was measured using 96-well microplate reader based on Ellman's method. In the 96-well plates, 25 µl of 15 mM ACTI in water, 125 µl of samples of 3mM DTNB in buffer C (50 mM Tris-hydrochloric acid, pH 8, containing 0.1 M NaCl and 0.02 M magnesium chloride), 50 µl of buffer B (50mM Tris-HCl, pH 8, comprising 0.1% bovine serum Albumin), 25 µl of samples (10mg/ml, in methyl alcohol, diluted ten times with buffer A) were added. The absorbance was measured at 405 nm every 13 seconds for 5 times [26,27].

2.9. Statistical Analysis

The recorded data were expressed in terms of Mean $\pm$

SEM and analyzed in statistical package for the social science (SPSS version 20.0) using one way analysis of variance (ANOVA) and post hoc Scheffe's test. The findings were interpreted for possible activity. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Preliminary Phytochemical Analysis of an Ethanolic Extract of *L. Leucocephala* Leaves

The extract was analyzed for the presence of alkaloids, reducing sugars, flavonoids, tannins, saponins, steroids, proteins and triterpenoids using standard methods stated earlier. The extract showed the presence of all the phytoconstituents stated above. The results are given in Table 1. However, we have not carried out quantitative estimation of these compounds in the present study.

Table 1. Phytochemical evaluation of ethanolic extract of *L. leucocephala* leaves

Phytoconstituents	Test	Result
Alkaloids	Dragendroff's test	+
	Hager's test	+
	Wagner's test	+
	Mayer's test	+
Reducing sugars	Molisch's test	+
	Benedict's test	+
	Fehling's test	+
	Tollen's test	+
Flavonoids	Shinoda test	+
Tannins		+
Saponins		+
Steroids	Libermann-Burchard's test	+
	Salkowski test	+
Proteins	Biuret test	+
	Million's test	+
Triterpenoids		+

3.2. Acute Toxicity Studies

Acute toxicity study was carried out using female

albino rats. The ethanolic extract of the leaves of *L. leucocephala* was found to be safe at a dose level of 2000 mg/kg body weight when administered by oral route. The animals were observed for 24 hours for any sign of toxicity following oral administration of the extract suspension (single dose). Even after 24 hours animals were found well tolerated. There were no signs of toxicity, morbidity or mortality. The observations are given in Table 2.

Table 2. Acute toxicity study observations

signs	observation	signs	observation
Motor activity	Normal	Ataxia	Absent
Tremors	Absent	Lighting reflex	Normal
Clonic convulsion	Absent	Sedation	Absent
Straub reaction	Absent	Muscle relaxation	Absent
Pilo-erection	Absent	Arching and rolling	Absent
Muscle spasm	Absent	Ptosis	Absent
catatonia	Absent	Lacrimation	Normal
Exophthalmos	Absent	Writhing	Absent
Diarrhoea	Absent	Reparation: Depression Stimulation Failure	Normal
Salivation: Viscid Watery	Normal	Skin colour: Blanching Cyanosis Vasodilatation	Normal

3.3. Elevated Plus-Maze

Evaluation of the nootropic potential of the ethanolic extract of *L. leucocephala* was carried out using elevated plus-maze. The result showed that those groups of animals in which amnesia was induced by scopolamine (1 mg/kg b.w. i.p.) after pre-treatment with *L. leucocephala* (200 mg/kg, 400 mg/kg and 800 mg/kg) for eight days showed significant decrease in ITL, when compared to normal group as well as scopolamine challenged group. The results are shown in Table 3.

Table 3. Effect of *L. leucocephala* extract on transfer latency using elevated plus maze

Groups	Treatment (n=6)	ITL (secs)	RTL (secs)	Inflexion Ratio
Normal control	Normal (Vehicle)	47.50 ± 0.56	43.35 ± .88	0.109 ± .01
Disease control	Scopolamine (1 mg/kg)	56.83 ± 6.90	51 ± 4.03	0.098 ± .004
Piracetam	Piracetam (200 mg/kg)	20.16 ± 2.16	15 ± 2.74	0.575 ± .271
Extract	<i>L. leucocephala</i> (800 mg/kg)	23.33 ± 3.21	17.16 ± 2.18	0.357 ± .062
Standard	Piracetam (200 mg/kg) + Scopolamine (1 mg/kg)	25 ± 2.80	18.35 ± 2.13	0.394 ± .176
T-1	Extract (200 mg/kg) + Scopolamine (1 mg/kg)	40 ± 4.44	34.33 ± 3.83 ^b	0.153 ± .034 ^b
T-2	Extract (400 mg/kg) + Scopolamine (1 mg/kg)	33 ± 5.47 ^a	26.83 ± 4.19 ^b	0.224 ± .035 ^b
T-3	Extract (800 mg/kg) + Scopolamine (1 mg/kg)	26.16 ± 2.18 ^a	19.83 ± 1.55 ^b	0.318 ± .045 ^a

a = p<0.05, b = p<0.05 when compared to disease control group of ITL and RTL respectively.

Table 4. Effect of *L. leucocephala* extract on transfer latency using Hebb-Williams Maze

Groups	Treatment (n=6)	ITL (secs)	RTL (secs)
Normal control	Normal (Vehicle)	129.5 ± 10.99	108.8 ± 8.79
Disease control	Scopolamine (1 mg/kg)	149.67 ± 10.69	132.8 ± 9.05
Piracetam	Piracetam (200 mg/kg)	35.83 ± 3.09	26 ± 3.87 ^b
Extract	<i>L. leucocephala</i> (800 mg/kg)	40.66 ± 1.78	32.66 ± 1.56
Standard	Piracetam (200 mg/kg) + Scopolamine (1 mg/kg)	37.83 ± 1.95	28.33 ± 1.76
T-1	Extract (200 mg/kg) + Scopolamine (1 mg/kg)	50.16 ± 4.20 ^a	44.50 ± 3.93 ^b
T-2	Extract (400 mg/kg) + Scopolamine (1 mg/kg)	43.66 ± 4.50 ^a	35.50 ± 3.50 ^b
T-3	Extract (800 mg/kg) + Scopolamine (1 mg/kg)	41.66 ± 5.12 ^a	33 ± 4.35 ^b

a = p<0.05, b = p<0.05 when compared to disease control group

Table 5. Effect of *L. leucocephala* extract on the number of arm entries using Y maze

Groups	Treatment (n=6)	No. of arm entries on day 8 (Mean ± SEM)	No. of arm entries on day 9 (Mean ± SEM)
Normal control	Normal (Vehicle)	27.5 ± .76	22.16 ± .47
Disease control	Scopolamine (1 mg/kg)	32.3 ± 2.57	26.16 ± 1.77
Piracetam	Piracetam (200 mg/kg)	14.5 ± 2.30	10.83 ± 1.62
Extract	<i>L. leucocephala</i> (800 mg/kg)	15 ± 1.34	11.16 ± 1.13
Standard	Piracetam (200 mg/kg) + Scopolamine (1 mg/kg)	16.5 ± 3.32	11.50 ± 1.8
T-1	Extract (200 mg/kg) + Scopolamine (1 mg/kg)	20.16 ± 2.30 ^a	14.83 ± 1.7 ^b
T-2	Extract (400 mg/kg) + Scopolamine (1 mg/kg)	18 ± 2.26 ^a	14.16 ± 1.19 ^b
T-3	Extract (800 mg/kg) + Scopolamine (1 mg/kg)	17.3 ± 1.20 ^a	13 ± 1 ^b

a = p<0.05, b = p<0.05 when compared to disease control group

3.4. Hebb-William's Maze

The result suggested that those groups of animals in which amnesia was induced by scopolamine (1 mg/kg b.w. i.p.) after pre-treatment with *L. leucocephala* (200 mg/kg, 400 mg/kg and 800 mg/kg) for successive eight days showed a significant decrease in ITL, when compared to normal group as well as scopolamine challenged group.

Data are illustrated in Table 4.

3.5. Y Maze

3.5.1. Effect on the number of arm entries

Groups in which the amnesia was induced by scopolamine (1 mg/kg i.p) after pre-treatment with the

extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) for eight days successively showed a significant decrease in the number of arm entries when compared to the normal group and the scopolamine challenged group. The results are shown in Table 5.

3.5.2. Effect on % alterations

Group of animals in which the amnesia was induced by giving scopolamine (1 mg/kg i.p) on pre-treatment with extract (200 mg/kg, 400 mg/kg, 800 mg/kg) successively for eight days showed a significant dose-dependent increase in the % alterations when compared to the scopolamine treated group and results were almost comparable with normal groups. Percentage alteration increased in all the extract and in piracetam treated group

when compared to the normal group. The detailed information is referenced in Table 6.

3.6. Biochemical Estimation

3.6.1. Estimation of acetylcholinesterase (AChE) activity

The result of the study showed a significant increase of AChE in the whole brain upon treatment with scopolamine at a dose of 1 mg/kg body weight (i.p.). The amnesia induced group showed a significant dose-dependent decline in AChE when pre-treated with *L. leucocephala* (200 mg/kg, 400 mg/kg and 800 mg/kg) successively for eight days as compared with the scopolamine challenged group. The results are depicted in Table 7.

Table 6. Effect of *L. leucocephala* extract on % Alteration using Y maze

Groups	Treatment (n=6)	% Alteration 8 th day	% Alteration 9 th day
Normal control	Normal (Vehicle)	23.18 ± 1.20	24.66 ± .95
Disease control	Scopolamine (1 mg/kg)	14.72 ± .92	13.87 ± 1.22
Piracetam	Piracetam (200 mg/kg)	27.12 ± 3.39	28.73 ± 1.94
Extract	<i>L. leucocephala</i> (800 mg/kg)	25.83 ± 1.71	27.94 ± 1.58
Standard	Piracetam (200 mg/kg) + Scopolamine(1 mg/kg)	26.84 ± 1.65	29.12 ± 2.69
T-1	Extract (200 mg/kg) + Scopolamine (1 mg/kg)	22.58 ± 1.38	24.65 ± 1.34 ^b
T-2	Extract (400 mg/kg) + Scopolamine (1 mg/kg)	23.84 ± 1.47	25.41 ± 1.51 ^b
T-3	Extract (800 mg/kg) + Scopolamine (1 mg/kg)	25.37 ± .37 ^a	27.42 ± 1.25 ^b

a = p<0.05, b = p<0.05 when compared to disease control group

Table 7. Effect of *L. leucocephala* extract on whole-brain Acetyl cholinesterase

Groups	Treatment	Acetyl cholinesterase enzyme activity (μ moles/min/g tissue)
Normal control	Normal (Vehicle)	1.89 ± 0.089
Disease control	Scopolamine (1 mg/kg)	3.02 ± 0.079
Piracetam	Piracetam (200 mg/kg)	1.65 ± .041
Extract	<i>L. leucocephala</i> (800 mg/kg)	1.75 ± 0.067
Standard	Piracetam (200 mg/kg) + Scopolamine (1 mg/kg)	1.95 ± 0.65
T-1	Extract (200 mg/kg) + Scopolamine (1 mg/kg)	2.40 ± 0.057 ^b
T-2	Extract (400 mg/kg) + Scopolamine (1 mg/kg)	2.32 ± 0.074 ^b
T-3	Extract (800 mg/kg) + Scopolamine (1 mg/kg)	2.20 ± 0.106 ^b

a = p<0.05 when compared to the disease control group

Table 8. Effect of *L. leucocephala* extract on % Inhibition of acetylcholinesterase enzyme

Groups	Treatment (n=6)	% Inhibition				
		0 (s)	13 (s)	26 (s)	39 (s)	52 (s)
Normal control	Normal (Vehicle)	31.27±0.44	21.7±2.9	19.23±2.07	12.9±1.55	9.4±0.8
Disease control	Scopolamine (1 mg/kg)	25.95 ±0.6	17.25±1.7	15.3±85	4.3±1.3	7.5±1.37
Piracetam	Piracetam (200 mg/kg)	40.42 ±1.0 ^{a,b}	37.21±2.7 ^{a,b}	31.21±1.8 ^{a,b}	23.1±2.67 ^{a,b}	17.3±1.9 ^{a,b}
Extract	<i>L. leucocephala</i> (800 mg/kg)	36.83 ±0.9 ^{a,b}	34.31±0.9 ^{a,b}	28.8±1.9 ^b	19.7±1.46 ^b	14.7±1.3
Standard	Piracetam (200 mg/kg) + Scopolamine (1 mg/kg)	36.5 ±0.49 ^{a,b}	34.12±1.4 ^{a,b}	27.6±2.0 ^b	18.9±1.71 ^b	14.2±1.6
T-1	Extract (200 mg/kg) + Scopolamine (1 mg/kg)	32.84 ±0.7 ^b	25.91±0.7 ^c	19.83±1.84 ^c	13.1±1.65 ^c	9.9±1.64
T-2	Extract (400 mg/kg) + Scopolamine (1 mg/kg)	33.34 ±0.6 ^b	28.18±1.0 ^b	21.3±1.33 ^c	14.7±1.56 ^b	10.3±1.6
T-3	Extract (800 mg/kg) + Scopolamine (1 mg/kg)	34.7 ±0.63 ^b	30.8±1.87 ^b	23.46±1.5 ^{a,b,c}	16.3±1.75 ^b	11.6±0.96

a = p<0.05 when compared to Vehicle (Normal) group, b = p<0.05 when compared to Scopolamine (Treated) group, c = p<0.05 when compared to Group II

3.6.2. Estimation of Acetylcholinesterase by microplate assay

The results obtained from the estimation of % inhibition of acetylcholinesterase enzyme by using microplate reader showed that those animals challenged by scopolamine after pre-treatment with extract at different doses showed a significant increase in % inhibition when compared to the normal and the scopolamine treated group as shown in Table 8.

4. Discussion

L. leucocephala is a common plant which is used as animal fodder and a rich source of protein. Our assumption that the leaf extract will be nontoxic and safe was validated by the acute oral toxicity study. Absence of CNS, autonomic or any other adverse effects enabled us to select three doses, the highest one being 800 mg/kg.

The qualitative estimation of the phytoconstituents showed the presence of alkaloids, carbohydrates, saponins, tannins, steroids, triterpenoids, flavonoids, and proteins. However, we have not carried out quantitative study of these components. Neither the activity of isolated compounds were studied which is one of the limitations.

The study results show that the extract of *L. leucocephala* leaves could improve the learning and memory in experimental rats. The ability of the plant extract to overcome the amnesia produced by scopolamine was assessed by determining the transfer latency, inflexion ratio, % alteration, and several arm entries using established maze models using rats. Rats are quick learners and can be trained more effectively.

Induction of amnesia and cognition deficit using scopolamine is an established and validated procedure. The compounds which can reverse the effect of

scopolamine certainly could be promising drugs for overcoming memory impairment. In this regard, the action of *L. leucocephala* extract at different dose levels is comparable to that of memory enhancing drug piracetam.

Loss of memory and cognitive functions in diseases such as Alzheimer's is attributed partially to the impairment of cholinergic system. Depletion of acetylcholine (ACh) level in brain and its enhanced metabolism by acetyl cholinesterase enzyme (AChE) is observed in such patients. As a consequence, any drug which can prevent the metabolic degradation of ACh by AChE could be a potential nootropic agent. Hence, we studied the level of AChE in rat brain after treating with different doses of the extract in normal and scopolamine challenged rats. The results reveal that the extract significantly reduces the activity of AChE.

As stated in the introduction, many plants show nootropic activity, can improve memory and perhaps retard loosing memory as age advances. *L. leucocephala* can also be a promising candidate. The study was limited by the time and funding resources as it was an academic project. The study lacks quantitative data of the phytoconstituents. Isolating the compounds and testing them may give us an active compound. The histopathological data of rats' brain could be added.

5. Conclusions

Based on the obtained results, it can be concluded that the ethanolic extract of the leaves of *L. leucocephala* showed significant nootropic potential in scopolamine-induced amnesic rats. The nootropic activity was found in both in-vivo and in-vitro models. Further studies can be carried out to establish the exact mechanism involved in the nootropic activity of the plant.

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Author's Contribution

JM and **CSS** designed the study, **JM** collected the data, **JM** and **SC** carried out the analysis of data, **CSS** approved and verified the procedure and the obtained data, **JM** and **SC** prepared the draft manuscript, **CSS** corrected and approved the final copy.

Competing Interest

None of the authors have any competing interest in publishing the findings of this study.

Availability of Data and Material

The additional data will be provided by the corresponding authors on request.

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