AMELIORATIVE EFFECT OF DRIED LEAVES EXTRACT OF *CALOTROPIS GIGENTEA* (L) ON SCOPOLAMINE INDUCED MEMORY IMPAIRMENT IN RATS

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Abstract

C. gigentea is frequently utilized ethnopharmacologically for a variety of disease, including CNS disorders. They can counteract reactive oxygen species (ROS) by acting as reducing agents, hydrogen donors and free radical quenchers. The impact of dried extract leaves of *C. gigentea (L)* on Scopolamine hydrobromide (ScHBr) induced memory impairment in rats, cholinesterase activity, and oxidative stress is investigated in this study. *C. gigantea* was chosen for this study because there is insufficient statistical evidence to justify its traditional use in medicine. This plant's extract was tested for various pharmacological activities. Scopolamine induced amnesia in Albino rats of each sex, weighing around 200g to 250g. Nootropic drug used in this trial was Piracetam, which was given at a dosage of 120mg/kg.p.o of body weight. Scopolamine hydrobromide, which tends to enhance AChE activity, is one of the amnesia-causing drugs. The extract dose was given to animals for the Elevated plus Maze (EPM) and Morris Water Maze (MWM) trials. An effort was made to analyze the plant part,namely the leaves of *C. gigentea* (L). The presence of bioactive chemical elements such as alkaloids and flavonoids aids in their identification, determination, and demonstrationof their ability to treat various ailments. Scopolamine hydrobromide (ScHBr) has been used to induce amnesia in rats. Plant extracts have been shown to lessen TBARS & AChE when compared to Scopolamine-induced rats. *C. gigentea* leaves extract has been shown to reduce latency times in maze tests, suggesting that it could be employed to treat cognitive impairment disease in the future

Keywords: Reactive oxygen species (ROS), Scopolamine hydrobromide (ScHBr), Acetyl Cholinesterase (AChE), Thio Barbituric Acid Reactive Substances (TBARS), Transfer latency (TL).

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Introduction

Alzheimer's disease (AD) (named after the German psychiatric Alois Alzheimer) is the most common type of dementia and can be defined as a slowly progressive neurodegenerative disease characterized by neuritic plaques and neurofibrillary tangles as a result of amyloid-beta peptide's (Aβ) accumulation in the most affected area of the brain, the medial temporal lobe and neocortical structures [1]. Alois Alzheimer noticed a presence of amyloid plaques and a massive loss of neurons while examining the brain of his first patient that suffered from memory loss and change of personality before dying and described the condition as a serious disease of the cerebral cortex. Emil Kraepelin named this medical condition Alzheimer's disease for the first time in his 8th edition psychiatry handbook [2,3]. Progressive loss of cognitive functions can be caused by cerebral disorder like Alzheimer's disease (AD) or other factors such as intoxications, infections, abnormality in the pulmonary and circulatory systems, which causes a reduction in the oxygen supply to the brain, nutritional deficiency, vitamin B12 deficiency, tumors, and others [4,5]. At present, there are around 50 million AD patients worldwide and this number is projected to double every 5 years and will increase to reach 152 million by 2050. AD burden affects individuals, their families, and the economy, with estimated global costs of US$1 trillion annually. At present, there is no cure for Alzheimer's disease, although there are available treatments that just improve the symptoms [6,7]. The purpose of this review is to give a brief description about AD diagnosis, pathology, causes, and current treatments, and to highlight the recent development of compounds that could prevent or treat AD by targeting several pathogenic mechanisms, such as Aβ and tau aggregation, and misfolding, inflammation, oxidative damage, and others.

PLANT PROFILE

Calotropis gigantea is a massive shrub that can reach a height of 4 meters (13 feet). It bears clusters of waxy white or lavender flowers. Each flower has five pointed petals and a thin, graceful "crown" that rises from the center to contain the stamens. Calotropis has a valvate aestivation, which means that the sepals or petals in a whorl only meet at the margins, without overlapping. Oval, light green leaves and a milky stem characterize this herb. *Calotropis gigantea* latex is composed of cardiac glycosides, fatty acids, and calcium oxalate. It contains cardiac glycosides, β-sitosterol, madrine, saponins, alkaloids, tannins, trisaccharide's, and flavanols, according to reports. According to a preliminary analysis, the leaves extracts contain a high concentration of bioactive secondary molecules such as alkaloids, tannins, saponin, flavonoids, and glycosides. The presence of these components indicates that this species may have some medicinal value.

MATERIAL AND METHOD

Collection of Plant

The leaves of *Calotropis gigantea (L)* were collected in December 2022 from a dry coastal area in Kaushambi, Uttar Pradesh, India. The leaves were dried under normal environmental condition and authenticated by **Dr. O.N. Murya,** Principal Scientist, Botanical Survey of India Central Regional Center, 10 Chatham Lines, Allahabad-211002.

EXTRACTION OF LEAVES:

Extraction of leaves 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 [8].

EXTRACTION OF SUCCESSIVE SOLVENTS:

The type of solvent used in the extraction process is fundamental to the effective determination of biologically dynamic compounds from plant material. A strong solvent in plant extractions has properties such as ease of evaporation at low heat, low toxicity, fast physiologic absorption of the extract, preservative activity, and the inability to allow the extract to complex or dissociate. The following solvents are used in the extraction procedures: ethanol, chloroform, methanol, and water.

DIRECT METHANOLIC EXTRACTION:

The leaves of *Calotropis gigantea (L)* were shade dried at room temperature. The shade dried plant material was coarsely ground and extracted with petroleum ether in a soxhlet apparatus. The extraction was preceded until the sample had been defatted. The marc obtained after petroleum ether extraction was extracted with methanol in a soxhlet apparatus. To ensure full extraction, the extraction was extended for 72 hours. The extract was condensed and dried to a constant weight using a rotary evaporator [9].

Calotropis gigantea (L): Quantitative Pharmacognostic analysis gave moisture content 18.7%, total ash value 6.5%, acid insoluble ash

4.0% and water-soluble ash 0.08%, water insoluble ash 2.5%. Different extractive values are: In Petroleum ether 4.16%, Chloroform 1.2%, Methanol 4.52% and water-soluble extractive value 10.12%. Water soluble extractives are more than alcohol soluble extractives show more water-soluble constituents in the roots [10].

PHYTOCHEMICAL SCREENING

The leaves were gathered, dried in the shade, and ground into a coarse powder. In a Soxhlet apparatus, the powdered leaves were removed with Petroleum ether, Chloroform, Alcohol, Methanol, and Distilled water. The extracts were filtered, and the solvent was separated using low-pressure distillation. The percentage yields were determined, and the extracts were then tested for Alkaloids, Glycosides, Flavonoids, Carbohydrates, and Tannins using phytochemical tests [11].
Phytoconstituents found in extracts obtained from successive solvent extractions were determined using a variety of chemical [12].

QUANTITATIVE CHEMICAL EVALUATION:

DETECTION OF ALKALOID

Dragendroff's Test:
Take 1ml of extract + add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An **orange-red** precipitate indicates the presence of alkaloids.

Mayer's Test:
Take 1ml of extract + add 1 ml of Mayer's reagent (Potassium mercuric iodide solution).
Whitish **yellow** or **cream**-colored precipitate indicates the presence of alkaloids.

Hager's Test:
Take 1 ml of extract + add 3ml of Hager's reagent (Saturated aqueous solution of picric acid).
Yellow colored precipitate indicates the presence of alkaloids.

Wagner's Test:
Take 1ml of extract + add 2 ml of Wagner's reagent (Iodine in Potassium Iodide). Formation of reddish-brown precipitate indicates the presence of alkaloids [13].

DETECTION OF GLYCOSIDE

Legal Test:
Dissolve the extract in (1ml) pyridine + (add) 1ml sodium nitroprusside solution to make it alkaline. The formation of **pink red** to **red** color shows the presence of steroidal glycosides.

Baljet Test:
The test extracts (1 ml) + adds 1ml of sodium picrate solution. The **yellow** to **orange** color reveals the presence of steroidal glycosides.

Brontrager's Test:
Add a few ml of dilute sulphuric acid + the extract solution (1ml) boiled. Filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of **red** color of the ammonical layer shows the presence of anthraquinone glycosides [13].

DETECTION OF TANNINS

Take the little quantity of test extract + mixed with basic lead acetate solution Formation of **white precipitates** indicates the presence of tannins.
Take 1ml of the extract + add ferric chloride solution. Formation of a **dark blue** or **greenish black** colour product shows the presence of tannins.
Take little quantity of test extract + treated with potassium ferric cyanide and ammonia solution. Give a **deep red** colour indicates the presence of tannins.[13].

DETECTION OF SAPONINS

Take small quantity of extract separately + add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm **layer of foam** indicates the presence of Saponins.

DETECTION OF FLAVONOIDS

Shinoda Test:
Take the test solution + add few magnesium turnings + add concentrate hydrochloric acid drop wise. Pink scarlet **crimson red** or **occasionally green** to **blue** color appears after few minutes [14].

DETECTION OF VITAMIN C

Dilute 1 ml of (2% w/v) solution of drug with 5 ml of water + add 1 drop of freshly prepared (5% w/v) solution of sodium nitroprusside + 2 ml of dilute NaOH solution then add 0.6 ml of HCL drop wise and stir. Show yellow color turns blue [15].

TEST FOR STEROIDS AND STEROLS

Libermann-Burchard Test:
1gm of the test extract + dissolved in a few drops of chloroform + 3 ml of acetic anhydride + 3 ml of glacial acetic acid was added then heat and cooled under the tap and drops of concentrated sulphuric acid was added along the sides of the test tube. Bluish-green coloured appearance shows, the presence of sterol.

Salkowski Test:
Dissolve the extract in chloroform + add equal volume of conc. H_2SO_4. Formation of **bluish red** to **cherry** colour in chloroform layer and green fluorescence in the acid layer represents the

steroidal components in the tested [13].

DETECTION OF CARBOHYDRATES AND SUGARS

Molisch's Test:

Take 2 ml of the extract + add 1ml of α-napthol solution + add concentrated sulphuric acid through the side of the test tube. **Purple** or **reddish violet** colour at the junction of the two liquids reveals the presence of carbohydrates.

Fehling's Test:

Take 1 ml of the extract + add equal quantities of Fehling solution A and B, upon heating formation of a **brick red precipitate** indicates the presence of reducing sugars.

Benedict's test:

Take 5 ml of Benedict's reagent + add 1ml of extract solution and boil for 2 minutes and cool. Formation of **red precipitate** shows the presence of sugars [13].

Experimental Animal

In vivo study was performed on Albino wistar rat (100-150gm) in the animal Shambhunath Institute of Pharmacy, Prayagraj with the prior approval from Institutional Animal Ethical Committee (IAEC) bearing approval no IAEC/010/10/19. From study performed according to CPCSEA. Healthy adult albino wistar rat (100-150gm) of either sex was brought from Saha -Enterprise Kolkata, West Bengal (Reg. No: - 1828/PO/Bt/S/15/CPCSEA). The animal was housed under standard condition as prescribed and had a proper approach to water and feed with the exclusion of food deprivation during the period of blood sampling throughout the experiment.

Experimental Protocol

For the scopolamine induced cognitive dysfunction the animals were grouped in eight group having six animals in each group as following:

i. **Group I** served as Normal control.
ii. **Group II** served as standard (positive control) treated with 200 mg/kg b.w. of Piracetam i.p.
iii. **Group III** served as Neurotoxin (negative control) group treated with 2 mg/kg b.w. scopolamine hydrobromide i.p.
iv. **Group IV** served as test group treated with 100 mg/kg b.w. *Calotropis gigantea* (L.) methanolic leaves extract orally.
v. **Group V** served as test group treated with 200 mg/kg b.w. *Calotropis gigantea* (L.) methanolic leaves extract orally.
vi. **Group VI** served as test group treated with 100 mg/kg b.w. *Calotropis gigantea* (L.) methanolic leaves extract orally + Scopolamine hydrobromide 2 mg/kg b.w., i.p.
vii. **Group VII** served as test group treated with 200 mg/kg b.w. *Calotropis gigantea* (L.) methanolic leaves extract orally + Scopolamine hydrobromide 2 mg/kg b.w., i.p.
viii. **Group VIII** served as Piracetam + Scopolamine group treated with 200 mg/kg b.w. of Piracetam i.p. + Scopolamine hydrobromide 2 mg/kg b.w., i.p.

ACUTE ORAL TOXICITY

The extracts will be subjected to an acute toxicity trial to determine a safe dosage using the Organization for Economic Cooperation and Development's (423) acute oral toxic class process (OECD). The acute oral toxicity review was carried out in accordance with OECD guidelines-423. Animals were examined separately for behavioral profile (alertness, restlessness, irritability, and fearfulness) as well as neurological profile (spontaneous movement, reactivity, contact response, and pain response) and autonomic profile (defecation and urination) after oral administration of methanol extracts of C. gigantea leaves [16].

TEST DOSE SELECTION

There was one control group, one standard (positive control), one negative control and two treatment groups. The 200 and 400 mg/kg doses of *C. gigantea* Alcoholic leaves extract were given to the treatment classes. The methanol extract of *C. gigantea* leaves did not cause lethality or toxic reactions in mice until the end of the study period. According to the toxicity scale, the methanol extract is "unclassified."

TEST DOSE PREPARATION

The use of an aqueous solution/suspension of the test dosage, as suggested by OECD recommendations, should be considered first. As previously mentioned, the methanol extract of *C. gigantea* leaves was mainly soluble in 1 percent tween 80 suspension and 0.5 percent CMC in regular saline solution [17]. As a result, its solubility was tested first, and an aqueous suspension was prepared. Just before administration, the doses were freshly packed.

ROUTE OF ADMINISTRATION

The test dose was administered in a single dose orally.

EXPERIMENTAL DESIGN

PROCEDURE:

The experimental design was chosen in such a way that the effect of *Calotropis Gigantea* at various

doses against Scopolamine-induced amnesia could be tested after 7 days. The extracts' neuroprotective and nootropic behavior was investigated in eight groups of six rats each. The classes are as follows:

Group I: was the management group. For seven consecutive days, the vehicle was delivered orally. On day seven, the animals were allowed to swim 90 minutes after vehicle administration, and an escape latency of 24 hours was observed (i.e., on eighth day).

Group II: This was the control group for the amnesic patients. For seven consecutive days, vehicle was administered and Scopolamine (2 mg/kg) was injected i.p. every 90 minutes. On day seven, the animal was allowed to swim 60 minutes after administration, and 24 hours later, escape latency was observed (i.e., on eighth day).

Group III: On seven consecutive days, piracetam (200 mg/kg, i.p.) was injected. On the seventh day, 60 minutes after the piracetam injection, scopolamine (2 mg/kg), i.p. was administered. On day seven, the animal was allowed to swim 90 minutes after extract administration, and an escape latency of 24 hours was observed (i.e., on eighth day).

Group IV: A methanolic extract of the root of *Calotropis gigantea* (100 mg/kg, p.o.) was administered orally for seven consecutive days, followed by an i.p. injection of Scopolamine (2 mg/kg) 90 minutes after the extract was administered on day seven. 45 minutes after injection and 24 hours later, an escape lag was observed (i.e., on eighth day)

Group V: A methanolic extract of the root of *Calotropis gigantea* (200 mg/kg, p.o.) was administered orally for seven consecutive days, followed by an i.p. injection of Scopolamine (2 mg/kg) 90 minutes after the extract was administered on day seven. 45 minutes after injection and 24 hours later, an escape lag was observed (i.e., on eighth day)

Group VI: On seven consecutive days, Piracetam (200 mg/kg, i.p.) was injected. On the seventh day, 60 minutes after the Piracetam injection, scopolamine (2 mg/kg), i.p. was administered. On day seven, the animal was allowed to swim 90 minutes after extract administration, and an escape latency of 24 hours was observed (i.e., on eighth day).

Group VII: On seven consecutive days, a methanolic extract of the root of *C. gigantea* (100 mg/kg, p.o.) was administered orally. Scopolamine (2 mg/kg) was injected intravenously. On day seven, the animal was allowed to swim 90 minutes after extract administration, and an escape latency of 24 hours was observed (i.e., on eighth day).

Group VIII: For seven consecutive days, methanolic extract of *C. gigantea* leaves (200 mg/kg, p.o.) was administered orally; scopolamine (2 mg/kg, i.p.) was administered. On day seven, the animal was allowed to swim 90 minutes after extract administration, and an escape latency of 24 hours was observed (i.e., on eighth day).

The extracts' neuroprotective and nootropic behavior was investigated in eight groups of six rats each. Identified a procedure for inducing cognitive dysfunction. For eight days, oral doses of leaves extract (100 and 200 mg/kg) were administered. Scopolamine hydrobromide (2 mg/kg, i.p.) was used to cause cognitive dysfunction in the extract-treated groups 90 minutes after the final dose was administered (on the eighth day). The vehicle control group received regular saline. On the final day, the neurotoxin (negative control) group received scopolamine hydrobromide (on 8th day). For eight days, groups IV to VII received an oral methanolic extract of leaves (100 and 200 mg/kg). In the positive control group, rats received Piracetam (200 mg/kg, i.p.) for eight consecutive days. Scopolamine 2 mg/kg, i.p. was given 60 minutes after the Piracetam injection on the eighth day. Piracetam (200 mg/kg, i.p.) was administered to rats in Group VIII for eight consecutive days. Scopolamine 2 mg/kg, i.p. was given 60 minutes after the Piracetam injection on the eighth day. The neuroprotective and nootropic effects were quantified using time in transit latency measurements (TL). Transfer latency was determined 45 minutes after Scopolamine hydrobromide injection on the seventh day and again 24 hours later (i.e., on the eighth day) [18].

Morris water maze:

Construction of Morris water maze:

The Morris water maze consists of large circular tank made of black opaque PVC or hard board coated with fiber glass and resin and then surface painted white (1.8 – 2.0m in diameter and 0.4 – 0.6m height). The pool is filled with water (20 - 22°c) to a depth of 0.3 – 0.4m, and rendered opaque by addition of small quantity of milk or non-toxic white color. The pool is fixed with filling and draining facilities and mounted on a frame so that the water is at waist level. The floor of circular tank is marked off in to four equal quadrants arbitrarily designed north, south, east or west. And escape platform is made of Plexiglas with a13 cm square platform attached to a 34cm long clear Plexiglas cylindrical pedestal (3cm diameter) mounted on a 1 sq. m (5mm thick) Plexiglas base. The top of the platform is covered with a coarse material that provides a good grip for the rat when climbing on

a platform. For the hidden platform task, water is added to circular tank to a level 2cm above the top of the platform. Water maze represents a versatile tool in which a number of distinct tasks can be measured. The simplest measure of performance is the Latency to escape from the water on to the hidden platform.

ELEVATED PLUS MAZE

The elevated plus maze was used to test learning and memory in rats using an exteroceptive behavioural paradigm (in which the stimulus was external to the body). Two exposed arms (16 cm x5 cm) and two covered arms (16 cm x 5 cm x 12cm) were used in the apparatus. The arms extended from a central platform (5cm x 5cm), and the labyrinth was raised to a height of 25cm from the floor. Each rat was mounted at the end of an open arm faced away from the central platform on the first day. The time required for the rat to enter each of the covered arms with all four legs was described as transfer latency (TL). On the first day, each animal's TL was determined. The rat was given another two minutes to navigate the maze before being returned to its cage. 24 hours after the first day experiment, retention of this studied challenge was tested [18].

BIOCHEMICAL ESTIMATIONS:

PREPARATION OF TISSUES:

On the eighth day, immediately after cervical dislocation, test animals from both classes were slaughtered and the brains of each animal were carefully separated, mounted on Petridish, over frost, and measured. Rinse the whole brain with ice-cold regular saline. A 10% homogenate of brain extracts was processed using a homogenizer in 0.1 M chilled phosphate buffer (pH 7.4). To isolate the radioactive debris from the homogenates, they were centrifuged at 800 g for 5 minutes at 4 0C. To extract the supernatant, the supernatant was centrifuged at 10,500 g for 20 minutes at 4 ^{o} C. The supernatant was obtained from each animal in each group and subjected to biochemical analysis [19].

PROTEIN ESTIMATION:

Protein levels were determined in all brain samples to determine GSH, MDA, and Ache function. Protein concentrations were determined using Lowry's 1951 process.

Method of Examination

0.1ml supernatant was taken, combined with 0.9ml DDW and 5ml alkaline working reagent, thoroughly mixed, and incubated at room temperature for 10 minutes. 0.5 ml Folin-phenol reagent was added and incubated for an additional 30 minutes at room temperature. At 750 nm, absorbance was determined in comparison to a reagent blank. As a standard, bovine serum albumin (BSA) (1 mg/ml) was estimated in the range of 50-1000 g and the protein content of the sample was determined as mg/ml [20].

Measurement of GSH:

GSH was calculated by reacting it with 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) to produce a yellow chromophore that was spectrophotometrically calibrated. Glutathione was quantified using the Ellman equation. To isolate the proteins, an equivalent volume of homogenate was combined with 10% trichloroacetic acid (500l each) and centrifuged at 2000 g for 10 minutes at 4 0C. The supernatant was used to determine the GSH concentration. 2 ml 0.1M phosphate buffer (pH 7.4), 0.5 ml DTNB, and 0.4 ml double distilled water is added to 0.1 ml of this supernatant. Within 15 minutes, the mixture was vigorously shook on a vortex and the absorbance at 412 nm was measured. The concentration of GSH was determined using a glutathione standard curve and expressed as g/mg protein [21].

Measurement of lipid peroxidation (MDA):

Thiobarbituric acid reactive species (TBARS), a marker of lipid peroxidation, were determined (We quantified malondialdehyde (MDA) to look at some short-term improvements in lipid peroxidation. Specifically, 1 ml of supernatant was extracted from the 10% tissue homogenate. To this, 0.5 ml of 30% TCA (Trichloroacetic acid) was added, followed by 0.5 ml of 0.8 percent TBA (Thiobarbituric acid) reagent dissolved in 95% ethanol. Aluminum foil was used to protect the tubes and they were kept in a shaking water bath for 30 minutes at 900C. After 30 minutes, tubes were removed and placed in ice-cold water for another 30 minutes. This were then centrifuged for 15 minutes at 3000 rpm. The supernatant was isolated and its absorbance at 540 nm at room temperature was measured against a suitable void. 1 ml distilled water, 0.5 ml 30% TCA, and 0.5 ml 0.8 percent TBA constitute the blank. The TBARS values were calculated using the following formula: n moles MDA/mg protein [22].

Measurement of Acetyl cholinesterase (AChE):

Acetyl cholinesterase (AChE) assay:

Acetyl cholinesterase activation is a marker of prolonged cholinergic system degeneration in the brain. The amounts of acetyl cholinesterase in the brain were quantified using Ellman's tool. 0.05 ml supernatant, 3 ml 0.01 M sodium phosphate buffer (pH 8), 0.1 ml acetylthiocholine iodide, and 0.1 ml

DTNB were added to the assay mixture (Ellman reagent). The shift in absorbance at 412 nm was determined immediately using a spectrophotometer. The results were determined using the chromophore's molar extinction coefficient (1.36 × 104 $M^{-1}cm^{-1}$) and are expressed in moles of acetylcholine hydrolyzed/min/mg protein [23,24].

$$R = \frac{\delta OD x\ Volume\ of\ Assay}{Ex\ mg\ Protein}$$

Where, **R** is the rate of enzyme activity in "n" mole of acetylcholine iodide hydrolized per minute per mg of protein.
δOD is the change in absorbance per minute, and **E** is the extinction coefficient (1.36 x 104 M – 1 cm- 1)

STATISITICAL 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.

RESULT
EXTRACTIVE VALUES:
The organoleptic investigation's findings, such as the extraction yield of *Calotropis gigantea (L.)* laeves extract, are shown in Table 1. In the hot extraction process, the extraction yields of various solvents may be categorised as follows: aqueous extracts > alcohol > chloroform> petroleum ether. When the solvents, temperature, and sample extraction ratios are raised, the percentage of extraction yield rises (Spela Stangler Herodez). The crude extracts of *Calotropis gigantea (L.)* showed a wide spectrum of colours. Methanol and aqueous extracts are brown and light yellow in appearance, but petroleum ether and chloroform extracts are colourless and pale green. Furthermore, petroleum ether and chloroform extracts have strong bitter flavours, whereas methanol extract has a harsh taste [25].

Table 1: The Extractive values of *Calotropis gigantea (L.)* leaves powder by hot extraction method:

S.N.	Nature of Extract	Values (% w/w) by hot extraction
1	Petroleum ether	3.75
2	Chloroform	2.42
3	Alcohol	1.75
4	Methanol	12.25
5	Aqueous	2.87

ASH VALUE:
The ash content of the medication remained after incineration, which simply indicates inorganic salts naturally exist in the drug, clinging to it, or are intentionally added to it as a type of adulteration. Ash values, especially in powdered form, are useful in assessing the quality and purity of a crude medication. The goal of washing vegetable medicines is to get rid of any organic debris that could interfere with an analytical result [26]. *Calotropis gigantea (L.)* leaves has a total ash concentration of 12.46 percent, with water soluble ash being higher than acid insoluble ash at 1.6 percent and 0.97 percent, respectively (Table 2).

Table 2: Ash value of *Calotropis gigantea (L.)* leaves powder:

S.N.	Physical Contents	Values (% w/w)
1	Moisture contents	87.2
2	Total ash value	12.46
3	Acid insoluble ash	0.97
4	Water soluble ash	1.6

FLUORESCENCE STUDIES OF POWDERED DRUGS:
The fluorescence analysis is sensitive enough to provide precise and accurate assessment across a wide concentration range. Each compound's fluorescence hue is distinct. When a non-fluorescent chemical is combined with fluorescent impurities, it can fluoresce. Both conventional and UV light were used to observe the colour of the extracts from organic and inorganic solvents. *Calotropis gigantea* (L.) leaves fluorescence analysis is tabulated using several chemical reagents (Table 3).

Table 3: Fluorescent studies of powder drug of *Calotropis gigantea* (L.) sleaves:

S.N.	Solvent Treatment	Visible light	Short UV (252 nm)	Long UV (366 nm)
1.	Drug as such	Yellowish white	Light yellow	Alice blue
2.	Drug and 1M.HCL	Wheat	Khaki	Dark khaki
3.	Drug and picric acid	Yellow	Greenish	Black
4.	Drug and petroleum ether	Light cream	Yellowish green	Cream
5.	Drug and Chloroform	Light yellowish	White	Blue violet
6.	Drug and methanol	Light yellow	Yellowish green	Khaki

PHYTOCHEMICAL SCREENING:

The presence of the following chemical components was discovered in a phytochemical analysis of *Calotropis gigantea* (L.) leaves extract.

Table 4: Phytochemical Screening of Methanolic extracts of *Calotropis gigantea* (L.) leaves:

S.N.	Constituents	Methanolic extract	Aqueous extract
1	Alkaloid	Present	Easily seen/noticed
2	Glycoside	Absent	Easily seen/noticed
3	Tannins	Present	Present
4	Saponins	Easily seen/noticed	Easily seen
5	Flavonoids	Easily seen	Easily seen
6	Terpenoids	Absent	Absent

PHARMACOLOGICAL EVALUATION

Determination of Oral Acute Toxicity Study:

At dosages of 2000 mg/kg (Orally), *Calotropis gigantea (L)* (leaves) Alcoholic extract had no influence on the mice's behavioural responses, and no mortality was detected during a 72-hour period. These findings suggest that Alcoholic leaves extract does not have a hazardous profile.

Exteroceptive behavioural models:

Evaluations of in scopolamine-induced amnesia in rats, the effects of an alcoholic extract of *Calotropis gigentia linn* leaves on transfer latency (elevated plus maze paradigm). The effects of an alcoholic extract of *Calotropis gigentia linn* leaves on transfer latency (elevated pus maze paradigm) in rats with scopolamine-induced amnesia (Mean ±SD).

Table 5: Alcoholic extract of *Calotropis gigentia (L)* transfer latency (EPMP) in scopolamine-induced amnesia in rats

S.no	Treatment groups	TL on acquisition day (sec) 7 day	TL on retention day (sec) 8 day
1	Control group	44.50 ± 15	41.25±8.70
2	Inducing agent (ScHbr) 1	87.60± 17.2*	84.17±15.72*
3	Plant part extract (250) + Inducing agent (Schbr) 1	51.5±5.5[a]	48.00±4.2[b]
4	Plant part extract (500) + Inducing agent (Schbr)1	47.5±6.7[a]	42.02±3[b]
5	Piracetam (120) +Inducing agent (Schbr)1	41.2±10.92[a]	39.27±1.64[b]

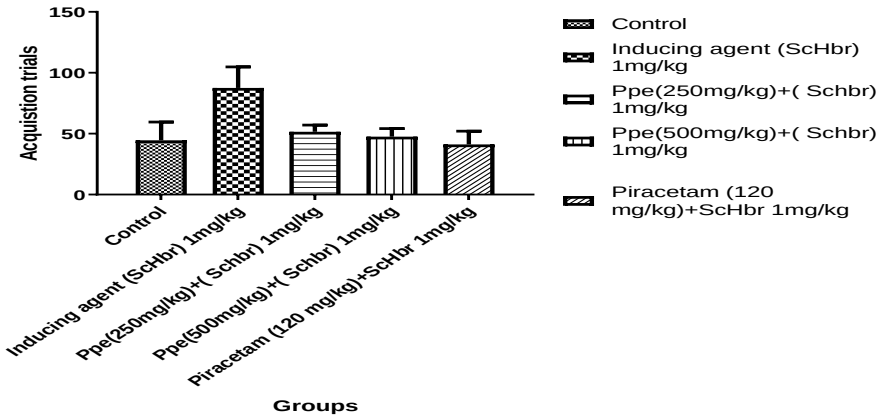


Figure 1 Effects of a *Calotropis gigentia (L)* alcoholic extract on acquisition trials transfer latency (elevated plus maze paradigm) in ScHbr-induced amnesia in rats (Mean ± SD).

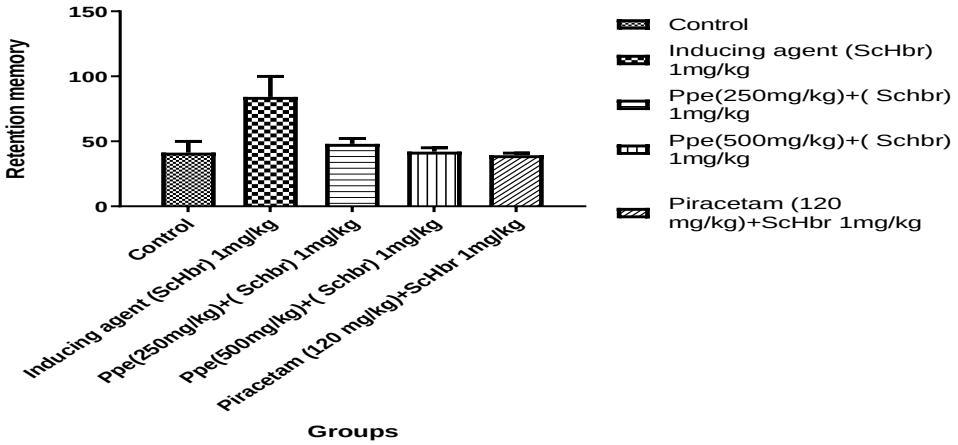


Figure 2: Effects of Alcoholic extract of *Calotropis gigentea (L) leaves* on retention trial transfer latency (elevated plus maze paradigms) on ScHbr in rats (Mean ± SD)

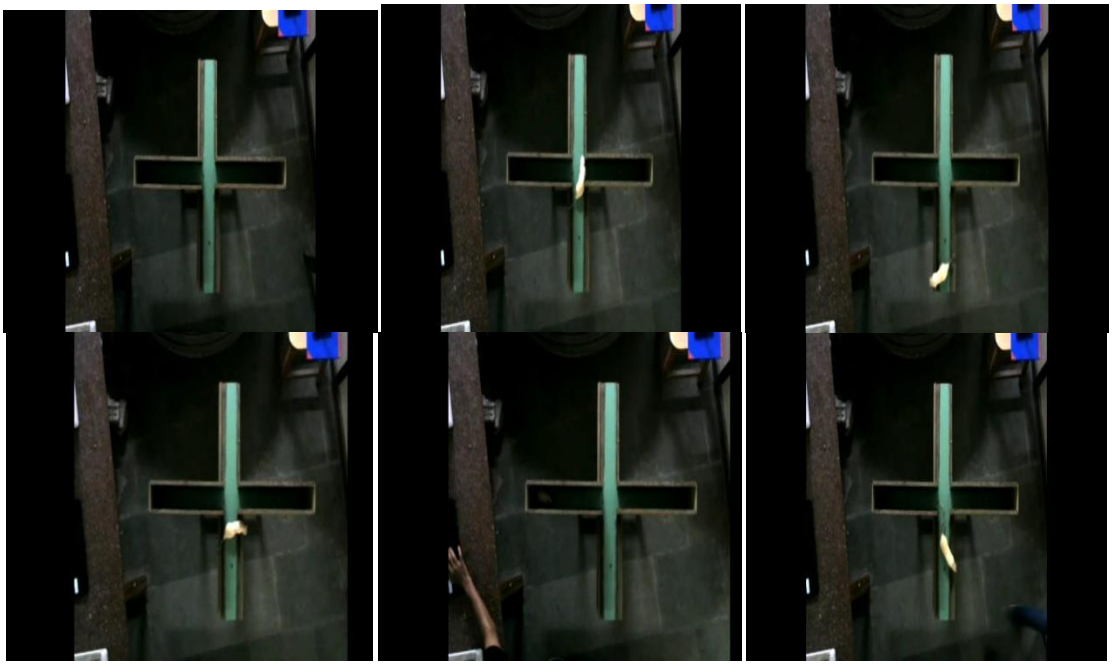


Figure 3: Analysis of experimental animals movement on elevated plus maze paradigms.

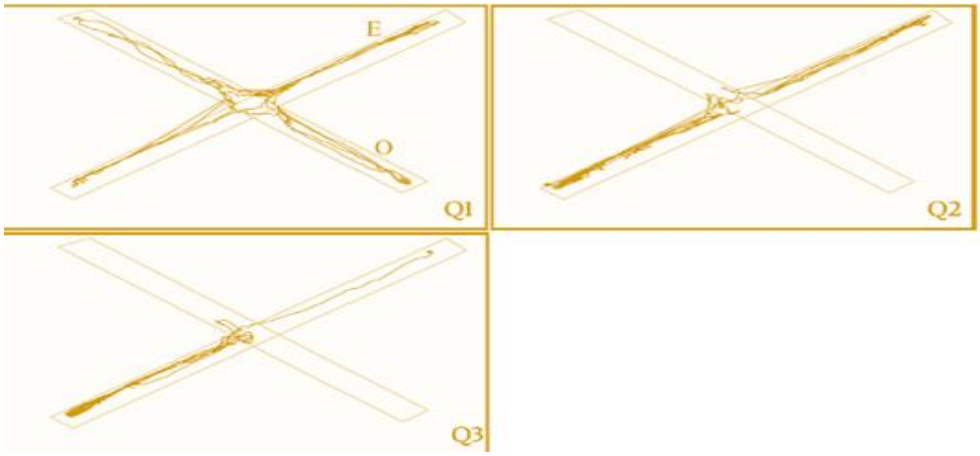


Figure 4: Motion-tracking software analysis of rat movement in elevated plus maze scenarios. Q1 denotes the control group, while Q2 denotes the experimental group. Q2= Plant extract (500 mg/kg), Q3= Piracetam (120 mg/kg).

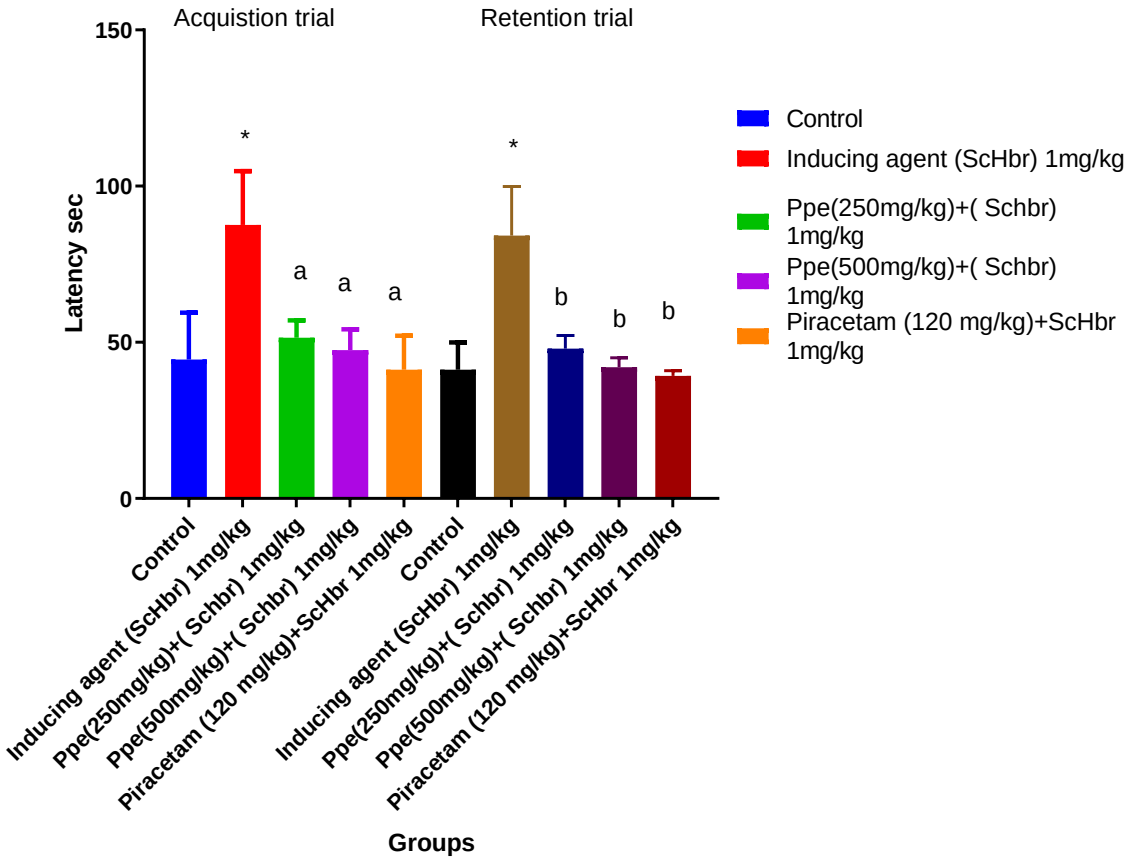


Figure 5: Effect of alcoholic extract of plant extract materials on TL and RL on EPM. N=6, and values are expressed as mean+ SEM. One way. ANOVA followed by Dunnet's test, $^{*}p < 0.05$, as comparing with control, ^{a} P < 0.05 compared with ScHBr treated group, ^{b}P< 0.05 compared with control group.

Spatial reference learning and memory

In scopolamine-induced amnesia in rats, the effects of an alcoholic extract of *Calotropis gigentia (L)* leaves on transfer latency (Morri's water maze paradigm) were assessed (Mean ± SD). In scopolamine-induced amnesia in rats, the effects of an alcoholic extract of *Calotropis gigentia (L)* leaves on transfer latency (Morri's water maze paradigm) are shown in Table 4.6 (Mean ±SD).

Table 6: Effects of alcoholic extract of *Calotropis gigentia (L)* leaves on transfer latency (MWMP) in scopolamine-induced amnesia in rats.

S.no	Treatment groups	TL on acquisition day (sec) 10th day	TL on retention day (sec) 11 day
1	Control group	54.2±5.33	50.2±8.18
2	Inducing agent (ScHbr) 1	108.4± 6.52*	103.5±5.78*
3	Plant part extract (250) +Inducing agent (Schbr) 1	60.8 ± 4.78^{a}	56±1.93^{b}
4	Plant part extract (500) + Inducing agent (Schbr)1	58.5±5.76^{a}	55±4.34^{b}
5	Piracetam (120) +Inducing agent (Schbr)1	56.2±4.79^{a}	51±3.24^{b}

Figure 6: Effects of. Alcoholic extract of *Calotropis gigentia (L)* leaves on acquisition trials transfer latency (morris water maze paradigm) in ScHbr- induced amnesia in rats (Mean ± SD).

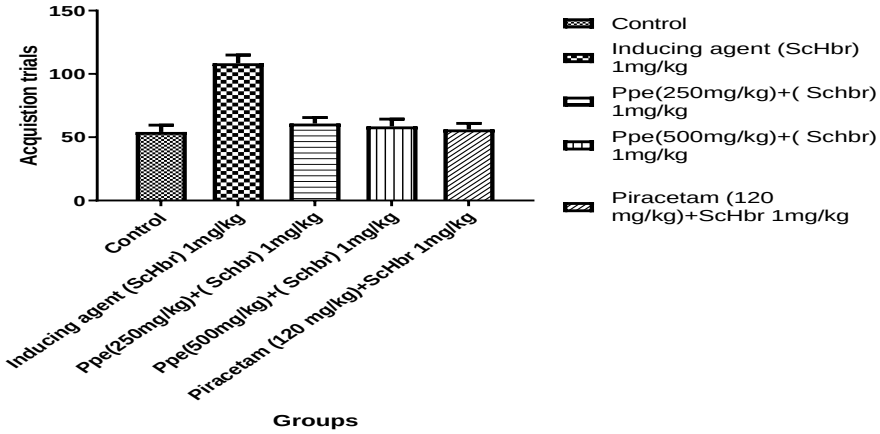


Figure 7: Analysis of rat movement in Morri's water maze paradigms showing various quadrants and positions of platform.

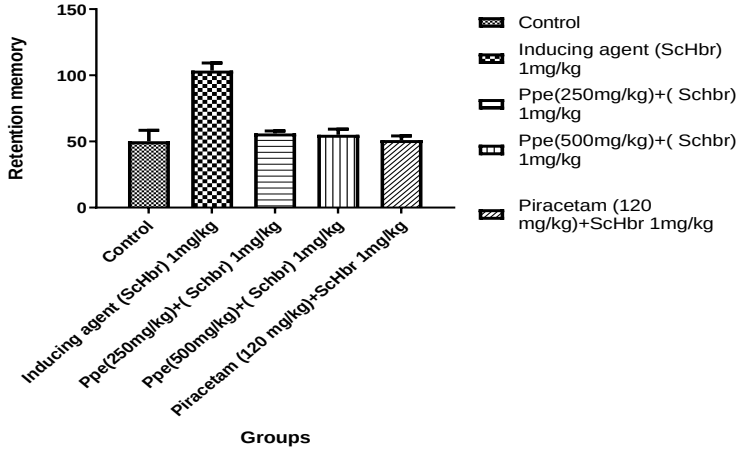


Figure 8: Effects of. Alcoholic extract of *Calotropis gigentia (L)* leaves on retentions trials transfer latency (Morris water maze paradigm) in ScHbr- induced amnesia in rats (Mean ± SD).

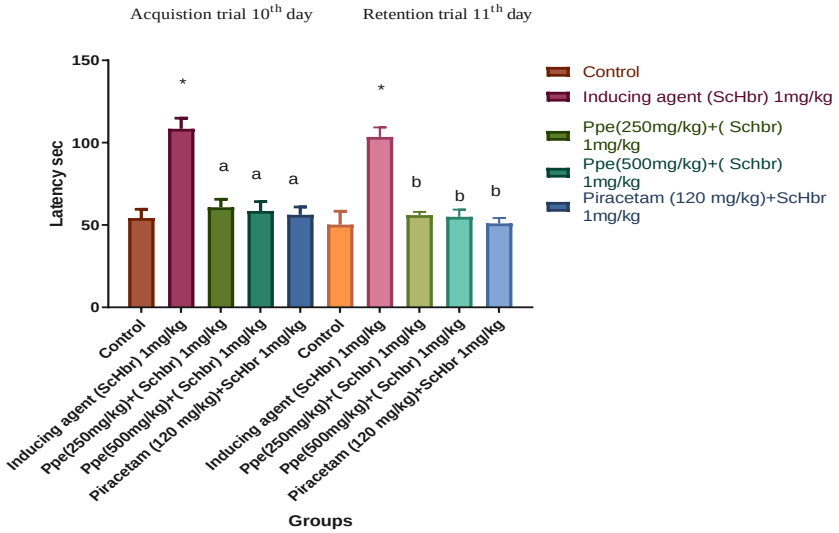


Figure 9: Effect of alcoholic extract of plant part on TL and RL 0n MWM. N=6, and values are expressed as mean+ SEM. One way ANOVA foll0wed by Dunnet's test, $^{*}p < 0.05$, as comparing with control, ^{a} $P < 0.05$ compared with ScHBr treated gr0up, $^{b}P < 0.05$ compared with control group.

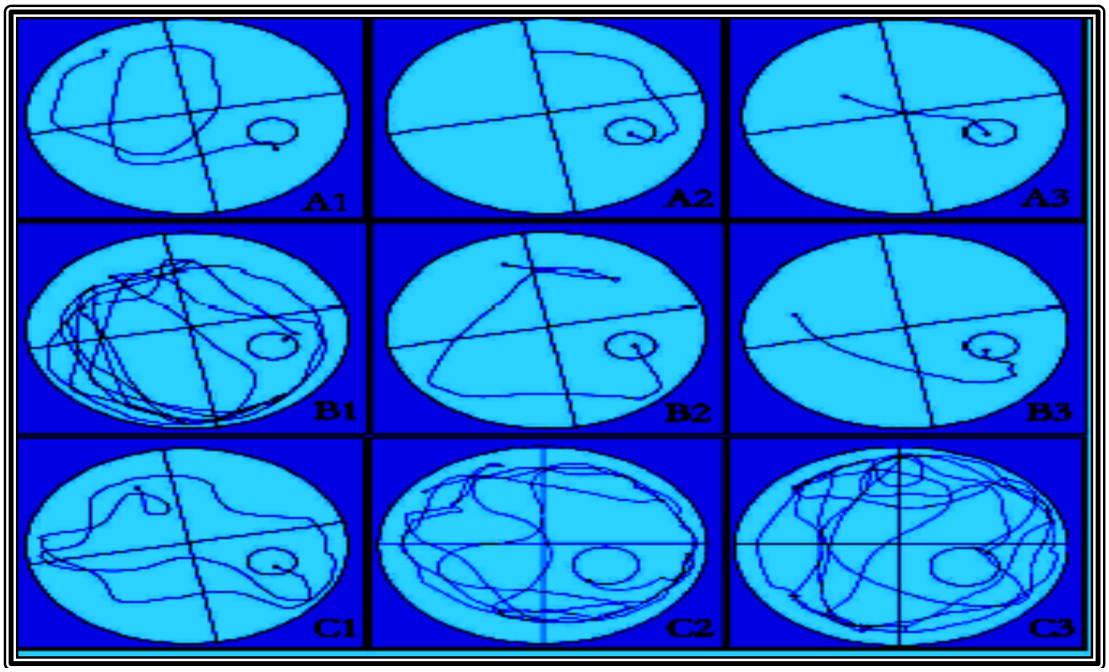


Figure 9: Standard medicine of Piracetam treated rats, dose of extract of seed of plant part extract at 500mg/kg body weight, and Control treated groups are shown in series A, B, and C.

Estimation of AChE activity

In scopolamine-induced amnesia in rats, the effects of an alcoholic extract of Calotropis gigentia (L) leaves on AChE activity were measured (Mean ±SD).

Table 7: Effects of a *Calotropis gigentia* (L) leaf alcoholic extract on AChE activity in rats with scopolamine-induced amnesia (Mean ±SD).

S. No	Treatment groups	AChE (g/min/mole of tissue)
1	Control group	0.183 ± 0.001
2	Inducing agent (ScHbr) 1	0.250 ± 0.005*
3	C.gigentea (250mg/kg) + Inducing agent (Schbr) 1	0.200 ± 0.004[a]
4	C.gigentea (500mg/kg) + Inducing agent Schbr)1	0.180 ± 0.005[a]
5	Piracetam (120mg/kg) +Inducing agent (Schbr)1	0.185 ± 0.006[a]

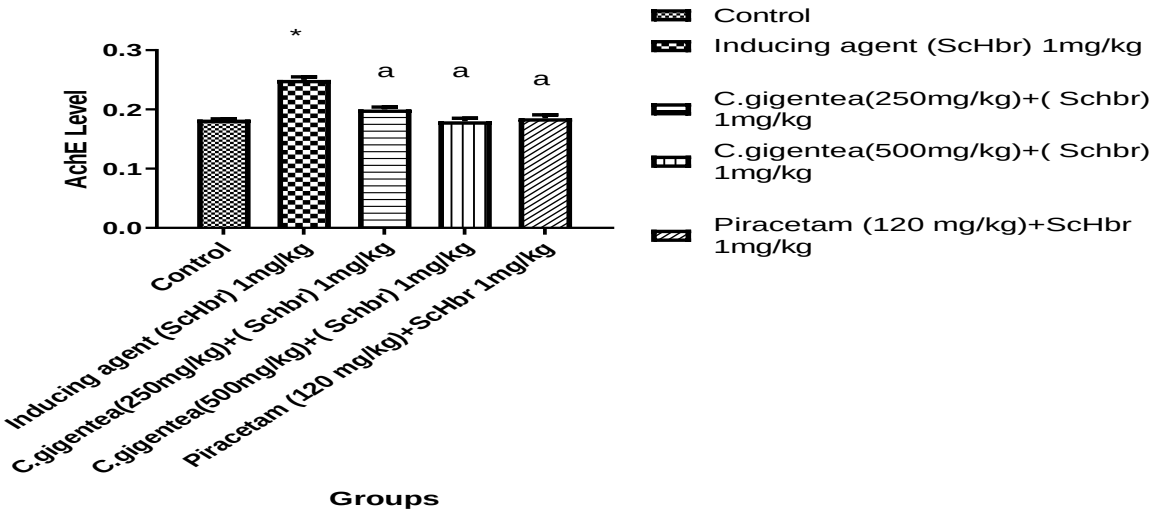


Figure 10: Effects of alcoholic extract of *calotropis gigentea (L)*leaves on AChE level in the brain on scopolamine-induced amnesia in rats (Mean ± SD)

N=6. One way ANOVA followed by Dunnet's test, $^{*}p < 0.05$, as comparing with control ^{a} $P < 0.05$ compared with ScHBr treated group.

Estimation of TBARS levels in the brains

The effects of a calotropis gigentea(L) leaf alcoholic extract on T.B.A.R.S levels in the brain during scopolamine-induced amnesia in rats (Mean ±SD)

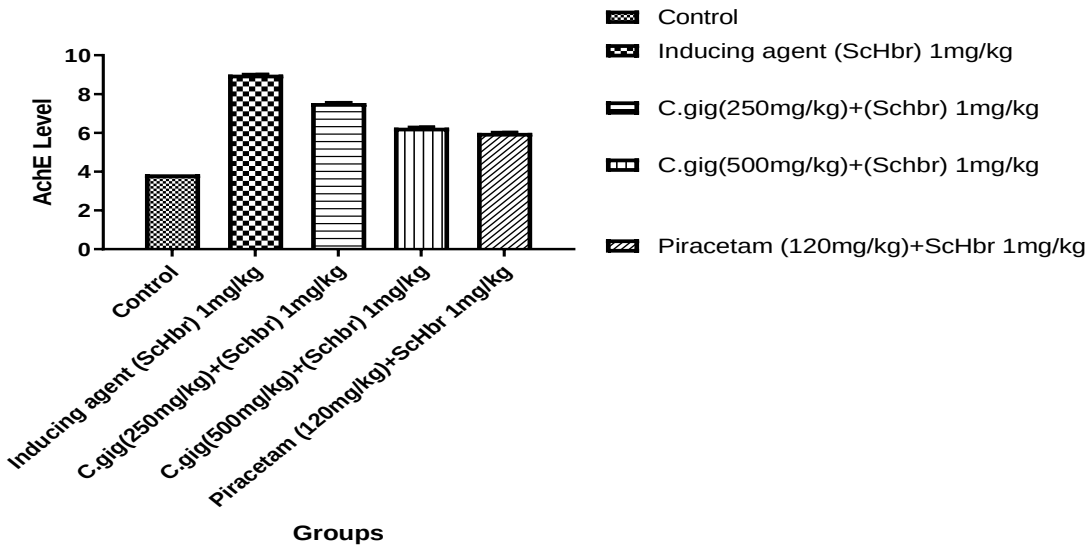


Figure 11: Effects of a Calotropis gigentea (L) leaf alcoholic extract on T.B.A.R.S levels in the brain on scopolamine-induced amnesia in rats (Mean ±SD)

Assessment of Histopathology study

The experimental mice were given extract, which is dried leaf of *C.gigentea* at doses of 250mg/kg and 500mg/kg body weight, orally for a total of 28 days prior to the histopathology study. All of the experimental animals were in good health at this time.

Table 8 On experimental animals, the histopathological effect of dried extract of leaf is shown

Variable	Control	Leaves of plant extract (250mg/kg/day)	Leaves of plant extract (500mg/kg/day)
Creatinine (mg/dl)	0.32 ± 0.02	0.30 ± 0.03	0.31 ± 0.07
Cholesterol(mg/dl)	92.27 ± 0.1	90.15 ± 1.0	89.1 ± 0.01
Bilirubin (mg/dl)	0.19 ± 0.02	0.20 ± 0.01	0.22 ± 0.01
SGPT	6.0 ± 0.02	6.3 ± 0.4	6.2 ± 0.03
Protein	5.27 ± 0.04	5.30 ± 0.03	5.29 ± 0.01
WBC (x 10^3 µ/l)	6.5 ± 0.5	6.8 ± 0.3	7.20 ± 0.5
RBC (x 10^6 µ / l)	7.5 ± 0.6	7.5 ± 0.3	7.7 ± 0.03
Haemoglobin (%)	13.6 ± 0.3	13.7 ± 0.2	13.6 ± 0.4
Platelets (x 10^3)	857 ± 65.2	860 ± 70.6	875 ± 55.2

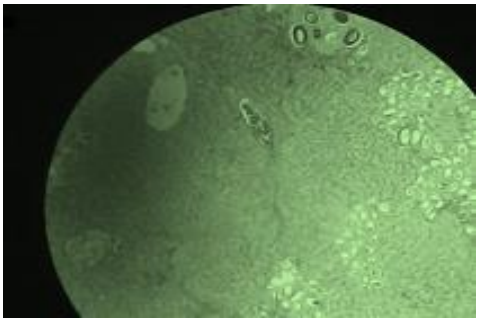
When dried leaves of plant extract were given at doses of 250 mg/kg/day and 500 mg/kg/day, organs such as the heart, kidney, brain, lung, and spleen were tested, and no significant differences were found between the control and treatment groups.

Table 9: Effect on different organs wt/b.wt ratio in experimental animals

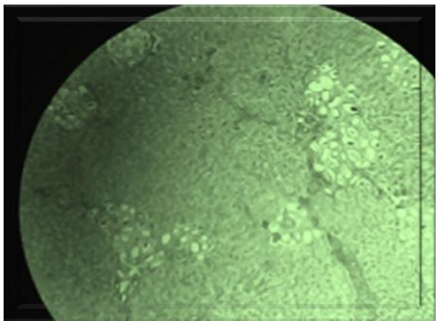
Organs	Control	Leaves of plant extract (250mg/kg/day)	Leaves of plant extract (250mg/kg/day
Liver (x 10^{-3})	32.12 ± 0.05	32.15 ± 0.06	32.06 ± 0.04
Heart (x 10^{-3})	3.71 ± 0.04	3.70 ±0.04	3.71 ± 0.05
Kidney (x 10^{3})	3.28 ± 0.04	3.27 ±0.05	3.27 ± 0.08
Spleen (x 10^{-3})	2.20 ± 0.01	2.16 ± 0.03	2.14 ± 0.06
Lungs (x 10^{-3})	4.85 ± 0.03	4.85 ± 0.04	4.86 ± 0.05
Brain (x 10^{-3})	6.40 ± 0.09	6.41 ± 0.09	6.44 ± 0.08

Data were articulated as mean ± S.E, n=6:

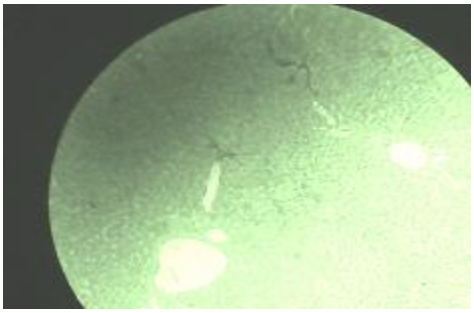
When the dried leaves extract of the plant was given at doses of 250 mg/kg/day and 500 mg/kg/day, and the organs of the heart, kidney, brain, lung, and spleen were inspected under a microscope, there were no significant differences between the control and treated groups.



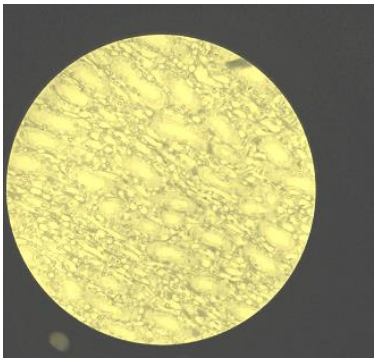
a. Normal kidney of rat kidney



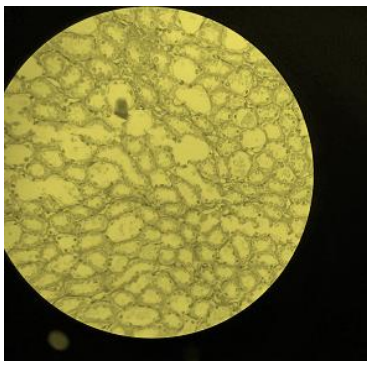
b. Seed of plant extract 250mg/kg b. wt treated



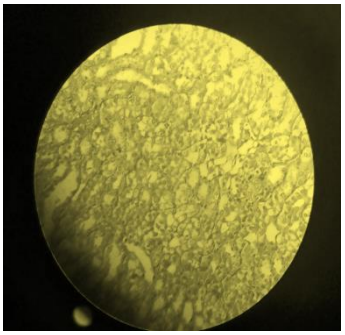
c. Seed of plant extract 500mg/kg b. wt treated extract of kidney



a. Normal liver



b. Seed of plant extract 500mg/kg b. wt treated extract of liver



C. Seed of plant extract at a dose of 250mg/kg b.wt treated

Figure 12: Histopathological slides of various organs.

DISCUSSUION

Products derived from natural sources such as plants, animals, and marine origins are commonly used by homo sapiens to maintain their health. For cognitive impairment diseases like Alzehimer's illness, there is no exact cure. As a result, an effort was made to analyse the plant part, namely the leaves of C*alotropis gigentea (L).* To see how well

it works as a pharmaceutical treatment for Alzheimer's disease. Neuro-apoptotic, neuro-inflammatory, oxidative stress, dementia, amnesia, and a decrease in Acteylcholine levels are all symptoms of cognitive deficits, which play a key role in learning and reasoning. Various parameters have been done for standardisation of alcholic extract of *Calotropis gigentea* (L) leaves, such as the presence of undesired chemicals, as well as determining its various extractive values, physiochemical parameters, and pharmacognistcal parameters. Additionally, look for its numerous indexes, such as the swelling and foaming index. The presence of moisture in the medications is revealed by the loss of drying. The presence of bioactive chemical elements such as alkaloids, glycosides, polyphenolic compounds, flavonoids, carbohydrates, steroids, amino-acids, and amygadlin in plant extracts aids in their identification, determination, and demonstration of their ability to treat various ailments. The important quantitative benchmark was determined by the physicochemical analysis. The amount of moisture in the medication was determined using the loss on drying method. Organic salt, which exists naturally in an extract medicine or is introduced in adulteration form, is represented by ash content. Total ash, acid insoluble ash, and water-soluble ash were all used to determine the ash value. The total ash was used to determine how much residue remained after the fire had been extinguished. Physiological and non-physiological ash are included in the Total Ash value. To ensure that the powder of the crude medication was genuine, fluorescence analysis was performed. Fluorescence analysis of the crude drug revealed distinct colours with different amounts of reagents, confirming the presence of diverse types of components in the medication. Alkaloids, carbohydrates, amino acids, proteins, amygadlin, and flavonoids were found in preliminary phytochemical studies. According to several studies, phenolic compounds, flavonoids, and steroidal glycosides are active elements in the therapy of cognitive impairment. During research, it was shown that the presence of the above bioactive components, as well as their beneficial combined impact, can explain the antioxidant activity of plant extracts of leaves extracts. The quantitative and qualitative estimate of elements contained in the plants extract can be determined via TLC analysis. We also computed plant extract toxicity at various levels (5, 50, 300, 2000 mg/kg), and we found no evidence of toxicity or fatality at any dose, thus we conclude that plant extract leaves materials are safe. Scopolamine – hydrobromide, a frequently proposed model for generating amnesia in experimental rats, has been used. ScHBr possesses anti-muscarinic action, which prevents ACh from attaching to its receptors and promotes anti-cholinesterase activity, allowing it to be degraded. Memory in rats has been tested using two behavioural models: raised plus paradigms and Morris water maze paradigms. On scopolamine-induced rats, doses of alcoholic extract of leaves *Calotropis gigentea (L)* were tested at 250 mg/kg body weight and 500 mg/kg body weight. ScHbr is given at a dose of 1mg/kg body weight to induce amnesia and dementia. According to our experimental protocol, the treatment group of plant extract had a shorter latency period than the ScHBr induction group. This occurred in both models, i.e. EPM and MWM, due to decreased oxidative stress, increased levels of Ach, and decreased anti-cholinesterase enzyme activity. Because it includes a considerable number of phenolic compounds, omega 3 fatty acids, and other antioxidants, *C. gigentea* is a good source of antioxidants. These compounds have the capacity to quench free radicals, which helps to minimise oxidative stress. The concentration of TBARS is used to calculate oxidative stress parameters. Plant extracts have been shown to lessen TBARS levels when compared to those who are given Scopolamine. The amount of TBARS is significantly higher in the scopolamine-treated group. The organ weight/body weight ratio results show that the alcoholic extract of *C. gigentea* leaves extract did not cause organ swelling, atrophy, or hypertrophy, in addition to the hematological and biochemical findings. Furthermore, the absence of significant alterations in the kidney's organ weight/body weight ratio backs up the biochemical findings. Similarly, the liver's organ weight/body weight ratio indicates the absence of any abnormalities in the extract-treated groups' livers.

CONCLUSION

Memory loss, dreamy-state, loss of focus, attention-deficient individuals having an elevated level of activity, and other neuro-related illnesses are the most common symptoms of Alzheimer's and other neuro-related disorders. If an illness like this isn't addressed, it can lead to death. Regrettably, no particular cure for such a condition has yet been established. As a result, efforts have been made to explore and cure such disorders. Poly-phenolic compounds and other chemical ingredients are found in *C.gigentea (L)*. Various animal models have been used to evaluate such illnesses. In both the raised plus maze and the Morris water maze, seed extract has been

demonstrated to reduce latency times. Further biochemical data revealed a drop-in Ache activity and a decrease in TBARS levels, indicating that this medicine could be employed to treat cognitive impairment disease in the future.

Future aspects

Our findings for *Calotropis gigentea (L)* pave the way for future research into molecular mechanisms and the therapeutic effects of isolated plant components in neurological diseases such as Huntington's disease and Parkinson's disease.

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