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# AMELIORATION OF COGNITIVE DEFICIT BY ETHANOLIC LEAF EXTRACT OF *ELAEOCARPUS TECTORIUS LOUR.* IN SCOPOLAMINE INDUCED DEMENTIA IN WISTAR RAT MODEL

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## ABSTRACT

### PROBLEM

Dementia is a chronic and progressive neurodegenerative disorder characterized by a gradual decline in memory, learning ability, and cognitive functions, ultimately affecting daily activities and quality of life. Alzheimer's disease represents the most common form of dementia and is associated with neuronal loss, oxidative stress, cholinergic dysfunction, and neuroinflammation. Currently available drugs, such as acetylcholinesterase inhibitors, provide only temporary symptomatic relief and do not effectively prevent disease progression. In addition, long-term use of these drugs may be associated with adverse effects. Therefore, there is a growing need to explore safer and more effective therapeutic alternatives, particularly from natural sources, that can target multiple pathological pathways involved in dementia.

### APPROACH

The present study investigated the neuroprotective potential of the ethanolic leaf extract of *Elaeocarpus tectorius* (ELEET) using a scopolamine-induced dementia model in Wistar rats. Phytochemical screening and GC–MS analysis were performed to identify the bioactive constituents present in the extract. The antioxidant activity of ELEET was evaluated in vitro to assess its ability to neutralize free radicals. Cognitive and behavioural functions were assessed using well-established tests, including the Morris water maze, Y-maze, novel object recognition, passive avoidance, and open field tests. Biochemical analyses were carried out to measure neurotransmitter levels, acetylcholinesterase activity, antioxidant enzyme status, and lipid peroxidation. Histopathological examination of brain tissues was conducted to evaluate neuronal integrity and structural changes.

### FINDINGS

Phytochemical and GC–MS analyses revealed that ELEET contains flavonoids, terpenoids, and phenolic compounds, which are known for their antioxidant and neuroprotective properties. The extract exhibited strong antioxidant activity, with an $IC_{50}$ value of 28.8 µg/mL, indicating its potential to reduce oxidative stress. Behavioural studies showed that scopolamine administration caused significant cognitive impairment, while ELEET treatment markedly improved learning, memory, and exploratory behaviour. The higher dose of ELEET (400 mg/kg) demonstrated the most significant effects and was comparable to the standard anti-dementia drug donepezil. Biochemical results indicated significant inhibition of acetylcholinesterase activity, leading to improved cholinergic transmission. Additionally, ELEET enhanced endogenous antioxidant defenses by increasing levels of superoxide dismutase, glutathione peroxidase, and reduced glutathione, while significantly reducing lipid peroxidation. Histopathological findings supported these results by showing reduced neuronal damage and preservation of normal brain architecture in ELEET-treated groups.

## CONCLUSION

The findings of this study demonstrate that *Elaeocarpus tectorius* ethanoic leaf extract exerts significant neuroprotective and memory-enhancing effects against scopolamine-induced dementia. Its beneficial effects are mediated through multiple mechanisms, including antioxidant activity, cholinergic modulation, and protection against neuronal damage. These results suggest that *E. tectorius* has strong potential as a natural, multi-target therapeutic agent for the management and prevention of dementia and may contribute to the development of safer alternative treatments in the future.

**Keywords**: Dementia, Alzheimer's diseases, Scopolamine, *Oxidative stress,* Antioxidant activity, Acetylcholinesterase inhibition, Neuroprotection.

## INTRODUCTION

Alzheimer's disease (AD) accounts for 70% of dementia cases, a debilitating illness that is prevalent in elderly populations. AD is characterized by neuropathological features such as neuronal death, intracellular protein called tau deposits, amyloid beta peptide (Ab) accumulation in extracellular plaques, and, more recently, a discernible loss of synapses. [1]

Cholinergic hypofunction is one of the traits of AD. Cholinergic neuronal and axonal abnormalities, a significant loss of cholinergic neurons, and a reduction in the number of post-synaptic neurons susceptible to acetylcholine (Ach) have all been associated with it. There is evidence linking Aβ and tau diseases to cholinergic hypofunction.[2] Currently, rather than concentrating on a long-term cure, dementia treatment aims to reduce its symptoms. Enhancing cholinergic transmission is the key method which is used in therapy. Galantamine, donepezil, and rivastigmine are examples of cholinesterase inhibitors that are widely used to treat mild to severe disease stages. By effectively halting the depletion of acetylcholine, a neurotransmitter crucial for memory and cognitive processes, these medications enhance cholinergic activity in the brain.[3]

Scopolamine (Scop), a non-selective, antagonistic post-synaptic muscarinic receptor inhibitor, can cause cognitive impairment in both humans and animals. A popular pharmacological method for examining memory impairments, especially those that resemble Alzheimer's disease, is the scopolamine-induced dementia paradigm in rats. Scopolamine blocks muscarinic acetylcholine receptors when it is injected intraperitoneally, which causes cholinergic neurotransmission to be disturbed and therefore results in cognitive impairments and increased oxidative stress in the brain, as evidenced by higher concentrations of reactive oxygen species and lipid peroxidation products, coincides with this disturbance. Memory problems are made worse by these metabolic changes, which also lead to neuronal death. The model is a valuable tool for preclinical screening of possible therapeutic compounds aimed at improving cognitive dysfunctions associated with dementia because of its repeatability and applicability to human neurodegenerative disorders.[4]

Elaeocarpus tectorius (Lour.) Poir., pertaining to the Elaeocarpaceae family, is a tropical evergreen tree indigenous to the Indian Subcontinent and Indo-China. This species thrives in wet tropical environments and is recognized for its distinctive blue to purple drupe-like fruits. Ethnobotanical applications of E. tectorius encompass the treatment of various ailments, including diabetes, hypertension, and inflammatory conditions. Phytochemical analyses have identified a diverse array of bioactive compounds within the plant, such as alkaloids, flavonoids, glycosides, tannins, triterpenes, and fatty acids. These constituents contribute to its pharmacological activities, which include antioxidant, anti-inflammatory, antidiabetic, and antimicrobial effects. Its potential to manage diabetic complications and damage caused by oxidative stress is also being recognized by recent investigations. Elaeocarpus tectorius has promising as a natural treatment for a number of illnesses due to its diverse medicinal qualities.[5]

## GUT BRAIN AXIS VS SCOPOLAMINE

The brain's central nervous system (CNS) and the gastrointestinal system are connected by the intricate, two-way gut-brain axis (GBA). This complex system facilitates dynamic signal exchanges between the gut and the brain by including several routes, such as the vagus nerve, which is part of the enteric nervous system, which is part of the immune system, and the system of endocrines. Comprising over 80% afferent fibres, the vagus

nerve is the main route via which gut-derived signals are sent to the brainstem, which subsequently forwards the information to higher cognitive centres.[6,7]

Furthermore, the gut microbiota generates a variety of metabolites, including biological amines (e.g., dopamine, serotonin, and GABA), short-chain fatty acids (SCFAs), and other neuroactive substances that can affect brain function by modifying immunological responses, blood-brain barrier permeability, and neurotransmitter systems. The pathophysiology of a number of neurological conditions, such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease, has been linked to changes in the composition of the gut microbiota, or dysbiosis. This underscores the vital role that gut health plays in preserving mental health and cognitive function. Developing therapeutic approaches that target the gut microbiota in order to manage or prevent neurodegenerative illnesses and other cognitive deficits requires an understanding of the mechanisms behind the GBA. The relevance of the GBA in controlling glial activities and affecting the onset and course of neurodegenerative illnesses has been highlighted by recent research that has revealed the two-way interaction between the gut microbiota and the central nervous system. Additionally, the gut microbiota can affect behaviour and brain development by directly secreting neuromodulators and neurotransmitters that affect the body or control their expression. It appears that the GBA is involved in both physiological and pathological situations since proinflammatory mediators and other immune system components generated by the gut microbiota can also impact brain function. [8,9]

In summary, the gut-brain axis is a complex communication network in which the gut microbiota significantly affects behaviour and brain function. Maintaining a healthy gut microbiota is crucial for overall wellbeing since disruptions in this axis, including those brought on by dysbiosis, can have serious effects on neurological disorders and mental health.

By investigating the possibility of antioxidant activity and behavioural recovery following treatment of the ethanolic extract of E. tectorius leaf, the current study seeks to assess scopolamine-induced dementia in rats. Additionally, the relationship between the behavioural scores and the amounts of the main indicators of oxidative stress from the hippocampus regions of rats administered scopolamine as a result of ethanolic extract administration was investigated.[10]

## RESOURCES AND METHODS

### 2.1.CHEMICALS

Scopolamine Hydrobromide (Sigma Aldrich Chemicals Pvt. Ltd. India), Ellman's Reagent [5,5-dithio-bis-2-nitrobenzoic acid (Sigma Aldrich Chemicals Pvt. Ltd. India), Trichloroacetic acid (Merch Chemicals Pvt. Ltd. India), Phosphoric acid (Merch Chemicals Pvt. Ltd. India), Thio barbituric acids (Merch Chemicals Pvt. Ltd. India), Donepezil hydrochloric acid (Aricept), and EDTA (Merch Chemicals Pvt. Ltd. India).

### 2.2. PLANT COLLECTION AND AUTHORIZATION

Elaeocarpus tectorius leaves were gathered from the Nilgiris Hills' surrounding regions in Tamil Nadu, India. Dr. S.S. Hameed, a botanist from the Botanical Survey of India, Southern Regional Centre, TNAU Campus, Coimbatore, Tamil Nadu, verified the plant specimen. (BSI/SRC/5/23/2024-25/Tech./182) is the reference number.

### 2.3.PLANT MATERIAL AND PREPARATION OF ETHANOLIC EXTRACT

To get rid of any dust, each leaf that was collected was thoroughly cleansed and rinsed with distilled water. The scrubbed and dried leaves have been crushed into a coarse powder using a hand grinder. First, weigh 200 g of finely crushed leaves and put them in a Soxhlet thimble made of cellulose or filter paper to remove chlorophyll and accomplish defatting of plant material using Soxhlet extraction. Because it efficiently recovers lipophilic compounds while eliminating chlorophyll, choose petroleum ether (75%) as the solvent.[11,12] Attach the condenser and thimble holder to complete the Soxhlet apparatus, making sure that all of the connections are leak-proof. To maintain a steady boiling rate of one to two cycles per hour, add 500 mL of the petroleum ether to a round-bottom flask and heat it gently. The solvent will percolate through the thimble, carry the extracted compounds into the flask, evaporate, condense in the condenser, and drip onto the sample. The solvent in the siphon tube should turn colourless after 8 hours of extraction, signifying full defatting and pigment removal. Spread the defatted plant material on a sanitized tray after removing the thimble. Before

moving further with other extractions, like using ethanol or methanol to isolate other desirable chemicals, let it air-dry to eliminate any remaining solvent. [13,14]

### 2.4.FOLLOWED BY COLD MACERATION:

For biological research, the E. tectorius marc was once more immersed in alcohol (ethanol) for 15 days, then filtered and evaporated under lower pressure using a heating mantle at 45°C. After that, it was stored at 4°C in a hermetic glass container.[15]

## PHARMACOGNOSTICAL STUDIES

### 3.1.ELAEOCARPUS TECTORIUS EXTRACT SOLUBILIZATION: SOLUBILITY TEST

To attain a 10 mg/mL concentration, weigh around 100 mg of dried Elaeocarpus tectorius extract (ethanolic leaf extract) and then add 10 mL of the distilled water to a sterile glass container. Stir vigorously with a magnetic stirrer for 30 to 60 minutes at ambient temperature (25 to 30 °C). Allow the mixture to stand for a little while after mixing so that it may be seen. [16]

### 3.2.PRELIMINARY PHYTOCHEMICAL ANALYSIS

A preliminary screening of phytochemicals was carried out in order to identify the different phytochemicals included in the extracts. The leaf extract was tested for alkaloids using Meyer's test, phenolics and tannins using the ferric chloride test, flavonoids using the Shinoda test, saponins using the foam test, and steroids and terpenoids using the Liebermann-Burchard test. [17,18]

## *IN VITRO* ANTIOXIDANT STUDIES

### 4.1.DPPH Free Radical Scavenging Assay

The Blois technique was used to conduct the assay. A new stock solution containing 0.3 mM DPPH in ethanol was made. Different concentrations of test samples (10, 20, 40, 60, 80, and 100 µg/ml) and reference standards (5, 10, 15, 20, 25, and 30 µg/ml) were added to 1 millilitre of this DPPH solution. Following a thorough shake, the mixtures were left to stand at room temperature for 30 minutes in the absence of light. The absorbance of each reaction mixture at the wavelength of 517 nm was measured after incubation and compared to a blank. The reference antioxidant for comparison was quercetin. The test sample was not used in the control reaction. Each experiment was conducted in triplicate to ensure accuracy and reproducibility.[19,20]

### 4.2.GC-MS CHARACTERIZATION OF ELAEOCARPUS TECTORIUS LEAF EXTRACT

The gas chromatography-mass spectrometry (GC-MS) method was adjusted with specific parameters to ensure reliable analysis. By setting the injector port temperature to 250°C, the sample was successfully vaporized. The source and interface temperatures were set at 200°C and 280°C, respectively, to optimize ionization conditions. The oven temperature regimen, which began at 70°C for a period of three minutes, stepped up to 150°C at 5°C/min, and then increased to 300°C at 8°C/min, allowed for a complete separation of the compounds. A split-less injection mode was employed with a DB- 35 MS non-Polar columns (0.25 mm OD × 0.25 µm ID × 30 m length, Agilent Co., USA) with a 1:50 split ratio. Helium was the carrier gas, and its flow rate was 1 mL/min. The mass spectrometer was set up to scan between 50 and 650 Da, the source was maintained at 200°C, and the vacuum pressure was kept below 40 mTorr. Using electron collision at 70 eV, ionization was accomplished. The device has a built-in pre-filter to cut down on neutral particles. Two built-in libraries, NIST4 and WILEY9, each with approximately five million references, were used to analyze the data. If a compound's spectral fit value was 700 or higher, it was deemed positively identified. [21-23]

### EXPERIMENTAL ANIMALS

Wistar albino male rats, weighing between 150 and 200 grams. The animals were kept in controlled environments with a 12-hour light/dark cycle and a temperature range of 20 to 250 degrees Celsius. Throughout the trial, all of the rats were fed a commercial pelletized chow meal and had unlimited access to fresh water; weight growth was recorded at 14-day intervals. Every animal procedure was carried out with the ethical committee's consent and in compliance with guidelines for the responsible handling and care of lab

animals. The Institutional Animal Ethical Committee (IAEC) KMCRET/ReRc/MPharm/110/2024 for Animal Care approved the study's protocols, which were in compliance with the Government of India's Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines. The methods used in the study were also conducted in compliance with the approved guidelines. The animals (Reg. No:971/bc/06-CCSEA) were purchased from the Biogen Laboratory Animal Facility in Bangalore. (Table 1)

**Table 1**. Animals approved by IAEC

| | |
|---|---|
| **Species** | Wistar albino rat |
| **Sex** | Male |
| **No. of animals** | 30 |
| **Duration of study** | 28 days |

## 5.1.PRETREATMENT WITH ETHANOLIC EXTRACT OF *ELAEOCARPUS TECTORIUS*

The Wistar albino rats were divided into 5 groups, each consisting of six rats. For this investigation, a total of 30 rats were required, and they were split equally among 5 groups. The entire course of treatment was given over a 28-day period. (Table-2)

**Table 2**. Treatment and route of administration

| S. No | Groups | Quantity of Samples | Sex | Group Description | Intervention |
|---|---|---|---|---|---|
| 1. | Group I | 6 | Male | Vehicle control | Normal saline *(p.o)* (0.9%W/V) |
| 2. | Group II | | | Negative control | Only scopolamine *(i.p)* (2mg/kg) |
| 3. | Group III | | | Positive control | Scopolamine *(i.p)* (2mg/kg) +Donepezil *(p.o)* (2.5mg/kg) |
| 4. | Group IV | | | Test 1 | Scopolamine *(i.p)* (2mg/kg) + EEET *(p.o)* (low dose -200mg/kg) |
| 5. | Group V | | | Test 2 | Scopolamine *(i.p)* (2mg/kg) + EEET *(p.o)* (high dose-400mg/kg) |

## 5.6.PREPARATION OF ETHANOLIC EXTRACT FROM ELAEOCARPUS TECTORIUS LEAVES

Prior to administration of medicine, each animal was weighed separately. The appropriate dosage for every animal was determined based on its body weight. Distilled water serves as a solvent in the formulation of 200 and 400 mg/kg of EEET.The appropriate dosage for every animal was determined based on its body weight. To eliminate air bubbles, the EEET (200 and 400 mg/kg) was sonicated after being completely dispersed in distilled water without any aggregates.[15]

## 5.7.ADMINISTRATION OF ELAEOCARPUS TECTORIUS ETHANOLIC LEAF EXTRACT

Prior to administering EEET, each animal should be weighed in order to remove any potential inaccuracies in dose calculation. EEET was diluted in distilled water and given orally (*p.o.)* to groups (G3–G5) for 14 days as a pretreatment (200 mg/kg for a low dosage and 400 mg/kg for a high dose), except for Group I and II. (fig 1,2)

**Fig1:** Ethanolic extract of *Elaeocarpus tectorius*

**Fig 2:** Administration of ET extract (*p.o.*)

## 5.8.INDUCTION OF DEMENTIA

Scopolamine was administered in order to produce Dementia. Rats in various groups were administered scopolamine via the peritoneum (*i.p.*) at a concentration of 0.1 ml per 100 grams of rat weight, following its dissolution in distilled water. Since 2mg/kg of scopolamine had been shown to cause cognitive deterioration in rats, this dose was given to them. Dementia was induced to all the animals (G2 – G5) by intraperitoneal injection of scopolamine(2mg/kg) except Group 1. For G3-G4, animals were treated with the extract for a period of 14days and then dementia was induced at the15$^{th}$ day and continues till the end of 28 days after 14 days to evaluate the drug's pretreatment impact. Animals were observed for mortality, morbidity and signs of toxicity once daily. [24,25] (fig 3)

**Fig 3:** Intraperitoneal injection of scopolamine

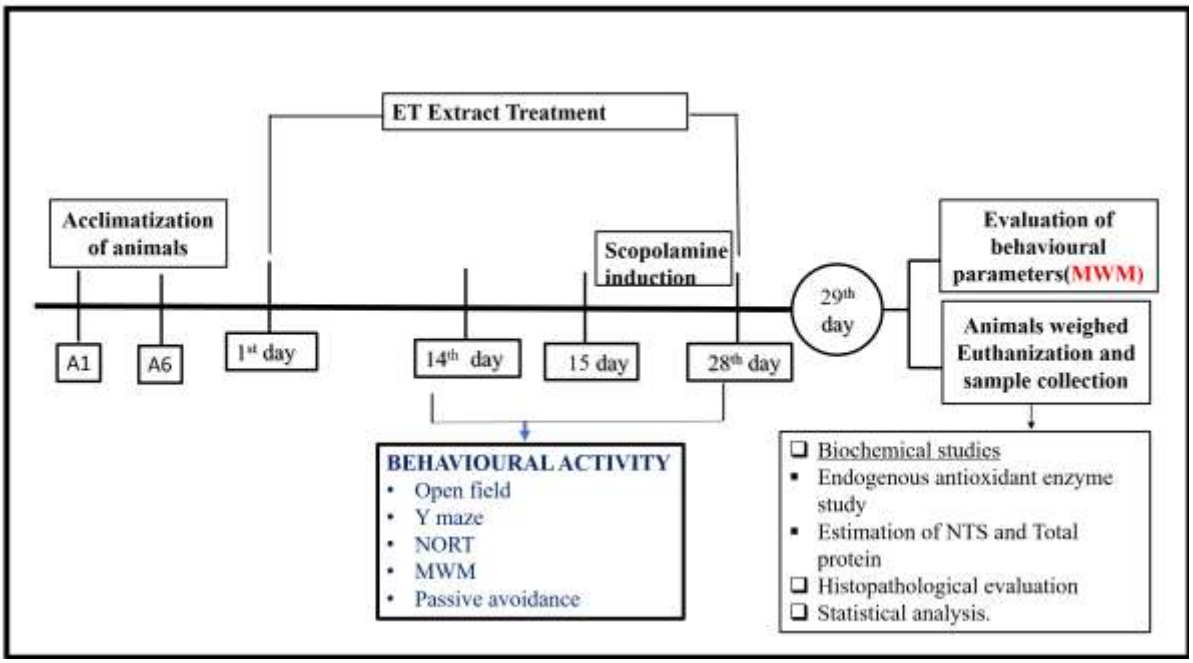



**Fig 4:** Diagrammatic representation of the experimental protocol. Thirty rats were equally split up into five groups. Group 1 was the control group; Group 2 was only induction with scopolamine (2 mg/kg); Group 3 was donepezil (2.5 mg/kg) plus scopolamine; Group IV was EEET (200 mg/kg) plus scopolamine; and Group V was EEET (400 mg/kg) plus scopolamine. After 14 days of pretreatment with EEET and donepezil, the rats received 14 days of scopolamine throughout a 28-day period. Water maze, Y maze, passive avoidance, and open field were evaluated as memory evaluation parameters. All of the rats were killed after the behavioural tests, and their brains were collected for additional biochemical analysis.

## EXPERIMENTAL DESIGN FOR PREVENTION ORIENTED STUDY

### 6.1.BEHAVIOURAL STUDIES

On Days 14 and 28 were used for the behavioral testing, which included the open field test (OFT), Y-maze test (YMT), novel object recognition test (NORT), passive avoidance and water maze. The protocol and experimental design are shown in fig:4

### 6.1.1.OPEN FIELD TEST: EXPLORATORY RESPONSE

Rats' exploratory behaviour was frequently assessed using the open field test (OFT). The apparatus was a clear plexiglass box with a bottom that was precisely 40 cm long by 40 cm broad and divided into 16 equal squares. Each rat was placed in the centre of the field and allowed to move freely about the box. The number of squares that all four paws successfully traversed was used to determine the number of crossings.[26]

### 6.1.2.Y MAZE TEST: SPONTANEOUS ALTERNATION BEHAVIOUR

spontaneous alternation behaviour was evaluated. using the Y-maze test to assess short-term working memory. Rats' exploratory behaviour and capacity to investigate a new location were assessed using spontaneous alternation behaviour (SAB). Rats usually investigate an arm of the maze that differs from the one they have already explored. The rats were placed in the centre of the maze and allowed to roam freely among its three arms for eight minutes. By counting the number of arm entries and triads, the percent of spontaneous alternation behaviour was determined. An arm entry occurred when all four paws of the rat were within the arm.[27]

The following formula was used to calculate the percentage of spontaneous alternation behaviour:

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

### 6.1.3.NOVEL OBJECT RECOGNITION TEST: RECOGNITION MEMORY

To evaluate cognitive function, NORT was utilized. The test was carried out in a 40 cm length, 40 cm wide, and 40 cm high clear plexiglass box with a constant illumination level of 40 lux. The NORT was divided into three phases: habituation, training, and testing. On the initial day of the habituation phase, the animals were placed back in their normal cage after exploring the empty box for five minutes. During the training phase, the animals explored two familiar things in the box for five minutes. On the day of evaluation, which was 24 hours after the training, the animals were given two distinct objects: object A, to which they had been accustomed, and object B, to which they had not. The rats pointed their noses at the objects and then scented them from a distance of less than two centimeters. The box and the objects were cleaned with a solution of 70% ethanol after each rat had inspected them.[28]

The following formula was used to calculate the discrimination ratio (DR):

DR = (Exploration time of Object B − Exploration time of Object A) / (Exploration time of Object B + Exploration time of Object A)

### 6.1.4.MORRIS WATER MAZE TEST: SPATIAL MEMORY

Memory and spatial learning were evaluated using the Morris water maze. The maze consisted of a large circular pool of opaque water and four quadrants. During the training phase, which lasted from the first to the fifth day, the animals were placed in the water at various starting places and allowed free space for swimming around looking for of the hidden platform. To assist them in locating the platform, visual cues were provided. The time it took to locate the platform, or escape latency (EL), was recorded.After a successful search, rats were taken back to their home cage after spending 20 seconds on the platform. When the rats were unable to arrive at the platform in a duration of 120 seconds, the researcher gently led them there and held them there for 20 seconds. A probing experiment was used to see whether the rat could recall the platform's prior placement on the fourteenth day following its removal. The spatial location test, which evaluated the rat's capacity to use memory to find the missing platform, was followed by this one.[29]

### 6.1.5.PASSIVE AVOIDANCE PARADIGM

This test evaluates a small laboratory animal's short-term or long-term memory. Participants in this test learn to avoid circumstances in which a painful stimulus, such a foot shock, has already been given. The rats were placed in a light-filled shuttle box compartment and given the passive avoidance test. A guillotine door separated the light area from the dark compartment. After 30 seconds of acclimatization, the rat entered the dark chamber and the door to the guillotine was opened and closed. The patient received a low-intensity plantar shock (0.5 mA; 10 s) in the dark compartment.

The animal's transfer latency time (TLT) was recorded in seconds while it was relocated from one location to another. A trial lasted for 270 seconds. A second trial was conducted 24 hours following the first one to evaluate retention, while the initial evaluation was for acquisition. To prevent reacquisition, the shock was not administered during the retention trials. When comparing retention trials to acquisition trials, the learning criteria was defined as an increase in the TLT.[30]

### 6.2.BODY WEIGHT ANALYSIS

Following the proper therapy and induction, the animals' body weight was measured on days 1, 14, and 28.

### 6.3.SAMPLE COLLECTION

The animals were slaughtered after the last behavioural assessment, and their brains were immediately removed and placed in designated containers. These were then kept at 4°C to maintain tissue integrity for further examinations.

## BIOCHEMICAL ANALYSIS

### 7.1.ESTIMATION OF ENDOGENOUS ENZYMATIC AND NON-ENZYMATIC ANTI OXIDANT LEVELS IN RAT BRAIN

Rats were sacrificed after treatment, and their brains were separated, cleaned with regular saline, and kept for

12 hours to conduct in vivo antioxidant research. The separated brain was homogenized with 0.1M Tris-HCl buffer (pH 7.4) using a powered Teflon-coated homogenizer to produce a 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 5˚C. Supernatant was collected for in vivo studies.

### 7.2. CONCENTRATION OF TOTAL PROTEIN

The supernatant of the right hippocampal homogenate prepared with lysis buffer solution was converted into a whole-cell protein lysate. The lysate's total protein content was measured using a commercial protein assay kit and the colorimetric Bradford method (Bio-Rad, USA), with bovine serum albumin (BSA) acting as a reference control. [31]

### 7.3.MEASUREMENT OF ANTIOXIDANT ACTIVITY

#### 7.3.1.SOD

After mixing, agitating, and centrifuging 0.25 ml cooled ethanol, 0.15 ml chilled chloroform, 0.5 ml brain homogenate, and 0.5 ml distilled water at 2000 rpm. 0.5 ml of the supernatant was combined with 1.5 ml of buffer and 0.4 ml of epinephrine to start the reaction. The intensity of the optical density change was measured at 480 nm per minute using a UV-VIS spectrophotometer. SOD activity was measured in U/mg. An enzyme unit is the change in optical density per minute when the enzyme blocks 50% of the adrenochrome transition.[32]

#### 7.3.2.GPX

0.4 ml of buffer, 0.1 ml of enzyme (brain homogenate), 0.2 ml of EDTA, a solution of sodium azide, reduced glutathione, and H2O2 were added, and the entire mixture was incubated for 10 minutes at 37°C. The tubes underwent centrifugation with the addition of 0.5 ml of TCA to halt the process. After adding 0.5 ml of supernatant to 3 ml of sodium hydrogen phosphate and 1 ml of DTNB solution, the colour produced was quickly quantified at 412 nm using a double beam UV-VIS spectrophotometer. The activity of serum glutathione peroxidase is expressed in µg/mg .[33,34]

#### 7.3.3.GSH

After mixing 250 µL of tissue homogenate with 1 mL of 5% TCA, the mixture was centrifuged at 3000g for 10 minutes at room temperature. 250 µL of the supernatant was mixed with 1.5 mL of phosphate buffer solution.After adding 250µL of 0.6mM DTNB, absorbance was measured within 10 minutes at 412 nm. A standard curve made with reduced glutathione (1 mg/mL) and expressed as µg/mg protein was used to calculate the GSH concentration.[35]

#### 7.3.4.ESTIMATION OF LIPID PEROXIDATION(TBARS)

A volume of 1 mL of reaction mixture was prepared for the test. It contained 0.02 mL of 100 mM ferric chloride, 0.58 mL of 0.1M a solution of phosphate buffer (pH 7.4), 0.2 mL of 100 mM ascorbic acid, and 0.2 mL of brain homogenate supernatant. The resultant mixture was incubated for one hour at 37°C in a shaking water bath. To stop the reaction, one millilitre of a 10% trichloroacetic acid solution was added. After that, the tubes were centrifuged for ten minutes at 2500 RPM. The amount of lipid peroxidation (TBARS) that had formed in each sample was determined by measuring the absorbance of the supernatant at 535 nm using a spectrophotometer. The resulting TBARS molecular coefficient of extinction was 1.56 × 105 M 1 cm-1, or Nm/TBARS/min/mg of tissue at 37°C.[36]

## ESTIMATION OF BRAIN NEUROTRANSMITTERS

### 8.1.DOPAMINE

0.5 mL of the iodine mixture (0.1 M in ethanol) was added for oxidation after 0.25 mL of 0.4 M HCl and 0.5 mL of the sodium acetate buffer (pH 6.9) were added to 1 mL of aqueous phase. To halt the reaction, 0.5 mL of Na2SO3 solution was added after two minutes. 0.5 mL of acetic acid was added after 1.5 minutes. The solution was then heated to 100°C for six minutes.The spectrofluorometer's both emission and excitation spectra were obtained once the sample had come to room temperature. Dopamine and noradrenaline values were obtained between 330 and 375 nm. The oxidation step's reagents were added to blanks for the experiment

in the opposite order (iodine comes after sodium sulfite).The standard was a different concentration of noradrenaline (1 mg/mL) and dopamine.[37]

### 8.2.SEROTONIN

OPT reagent (1.75 mL) was added to 1.4 mL of aqueous extract. For ten minutes, the fluorophore was produced by heating to 100°C. When the materials were at room temperature, measurements were made in the spectrofluorometer between 360 and 470 nm. OPT-free concentrated HCI was used as a blank. The standard was serotonin (1 mg/mL) at various concentrations.[38]

### 8.3.ESTIMATION OF BRAIN GABA AND GLUTAMATE CONTENT

A solution of 0.14 M ninhydrin and 0.1 mL of tissue homogenate were combined in 0.5 M bicarbonate-carbonate buffer (pH 9.95) in a water bath that was set at 60°C for 30 minutes. The copper tartrate reagent (5 mL) was added after cooling. A spectrofluorometer was used to measure the fluorescence at 377/455 nm after ten minutes. As requirements, glutamate (1 mg/ml) and GABA in different quantities were used.[39]

### 8.4.ESTIMATION OF BRAIN AchE LEVEL

The approach previously revealed by Ellman et al. was used to perform the anti-cholinesterase assay. This test involved mixing 0.05 ml of the supernatant with 3 ml of 0.1M phosphate buffer solution (pH 7), 0.1 ml of AchE iodide, and 0.1 ml of the Ellman reagent. The resultant combination was measured spectrophotometrically, and the absorbance at 412 nm was calculated. The enzyme activity was expressed as µmol/min/mg tissue after measurement.[40]

## HISTOPATHALOGICAL STUDIES

The brains of the rats were removed after they were sacrificed and preserved in a 10% neutral buffered formalin solution. After that, the preserved brain tissues were kept at 48°C in a 10% neutral buffer. The brains were then fixed in paraffin according to conventional protocols. The brain slices fixed in paraffin underwent haematoxylin and eosin staining. Under a microscope, the hippocampus lesions were examined at 40X and 10X.[41]

## STASTICAL ANALYSIS

The findings were displayed as mean ± SEM. For statistical analysis, Dunnett's test and a one-way analysis of variance (ANOVA) were employed. P-values were considered statistically significant if they were less than 0.05.[42]

## RESULTS:

### 1.YIELD PERCENTAGE AND EXTRACT PROPERTIES

The table displayed the Elaeocarpus tectorius extract yield as a percentage. Every extract was kept at 4°C in a tightly sealed container.

**Table 3.** Percentage yield of extract of *Elaeocarpus tectorius*

| Extract | Plant material utilized in grams | Amount of extract obtained (g) | Percentage yield (%w/w) |
|---|---|---|---|
| Solvent 90% Ethanol extract | 200 | 22 | 11 |

## 2.EEET SOLUBILIZATION:

### SOLUBILITY TEST

Approximately 100 mg of the *Elaeocarpus tectorius* leaf extract was added to 10 mL of distilled water at room temperature (~25 °C). The mixture was stirred manually and then left undisturbed for 15 minutes. The solution remained clear with no signs of precipitation, indicating good aqueous solubility.

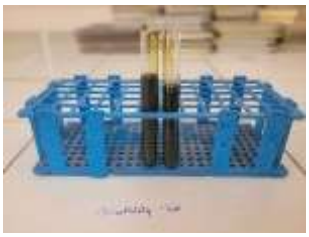


**Fig 5.** Solubility of Elaeocarpus tectorius ethanolic extract

### 3.Preliminary phytochemical screening

The ethanolic leaf extracts of Elaeocarpus tectorius contained a range of phytochemicals, including alkaloids, phenol compounds, flavonoids, tannins, terpenoids, saponins, and steroids, based on first phytochemical screening.

**Table 4.** Phytochemical screening of leaves of *Elaeocarpus tectorius*

| S.NO | PHYTOCONSTITUENT | ETHANOLIC LEAF EXTRACT OF E*laeocarpus tectorius* (ELEET) |
|---|---|---|
| 1. | Phenols | +++ |
| 2. | Alkaloids | ++ |
| 3. | Flavonoids | +++ |
| 4. | Steroids, Triterpenoids | + |
| 5. | Carbohydrates | + |
| 6. | Saponins | + |
| 7. | Glycosides | + |
| 8. | Proteins and amino acids | + |

### 4.Gc-Ms Characterization of Ethanolic Leaf Extract of *Elaeocarpus tectorius*

**Table 5.** GC-MS-identified bioactive components

| S.NO | NAME OF THE COMPOUND | MOLICULAR FORMULA | MW | PEAK AREA % |
|---|---|---|---|---|
| 1. | Phenylethyl alcohol | $C_8H_{10}O$ | 122 | 0.85 |
| 2. | 5-Methyl-1,3-benzenediol | $C_7H_8O_2$ | 124 | 0.64 |
| 3. | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl | $C_6H_8O_4$ | 144 | 11.23 |
| 4. | Benzyl alcohol | $C_7H_8O$ | 108 | 0.95 |
| 5. | 2-Methoxy-4-vinylphenol | $C_9H_{10}O_2$ | 150 | 6.15 |
| 6. | 4-hydroxy-3- methoxy-benzaldehyde | $C_8H_8O_3$ | 152 | 4.85 |
| 7. | α- Terpineol | $C_{10}H_{18}O$ | 154 | 0.92 |
| 8. | 9,12- Octadecadienoic acid (Z,Z)- (Linoleic acid) | $C_{18}H_{32}O_2$ | 280 | 5.59 |
| 9. | Oleic Acid | $C_{18}H_{34}O_2$ | 282 | 0.61 |
| 10. | n-Hexadecanoic acid (Palmitic acid) | $C_{16}H_{32}O_2$ | 256 | 0.82 |
| 11. | Heptadecane | $C_{17}H_{36}$ | 240 | 0.72 |
| 12. | Tetradecanedioic acid (Myristic acid) | $C_{14}H_{26}O_4$ | 258 | 6.05 |
| 13. | Cyclotetracosane | $C_{24}H_{48}$ | 336 | 4.89 |
| 14. | 1-Nonadecene | $C_{19}H_{38}$ | 266 | 1.36 |
| 15. | Linoelaidic acid | $C_{18}H_{32}O_2$ | 280 | 0.34 |
| 16. | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 1.42 |
| 17. | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 1.25 |
| 18. | Phytol | $C_{20}H_{40}O$ | 296 | 1.12 |
| 19. | Benzene propanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, ethyl ester | $C_{19}H_{30}O_3$ | 306 | 10.53 |
| 20. | Ellagic acid | $C_{14}H_6O_8$ | 302 | 1.05 |
| 21 | Cyclopropane octanoic acid, 2-[(2-pentylcyclopropyl) methyl]-, methyl ester, trans, | $C_{22}H_{38}O_2$ | 334 | 14.23 |
| 22 | (E)-9-Octadecenoic acid ethyl ester | $C_{20}H_{38}O_2$ | 310 | 0.04 |
| 23 | Quercetin 7,3',4'-trimethoxy | $C_{18}H_{16}O_7$ | 344 | 0.64 |

| | | | | |
|---|---|---|---|---|
| 24 | Flurandrenolide | $C_{24}H_{33}FO_6$ | 436 | 0.48 |
| 25 | L-Ascorbic acid, 6-octadecanoate | $C_{24}H_{42}O_7$ | 442 | 0.45 |
| 26 | 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z) | $C_{23}H_{34}O_2$ | 342 | 0.55 |
| 27 | Pentacosane | $C_{25}H_{52}$ | 353 | 0.89 |
| 28 | Benzenedicarboxylic acid, bis(2-ethylhexyl) ester | $C_{24}H_{38}O_4$ | 390 | 8.12 |
| 29 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 3.59 |
| 30 | Cholest-2-ene-2-methanol, (5à) | $C_{30}H_{50}OS$ | 458 | 5.13 |

The National Institute of Standards and Technology (NIST4) and WILEY9 databases were used to interpret the GC-MS mass spectrum. The known component's spectrum and the known components' spectrums kept in the built-in library were compared.

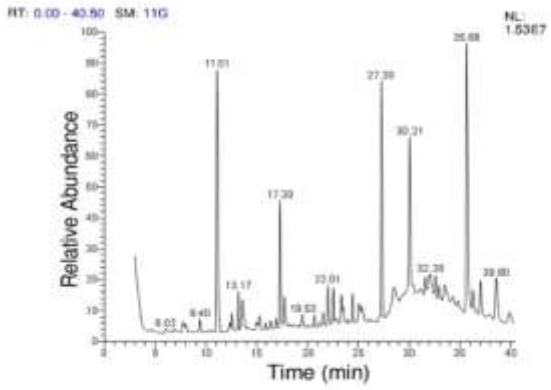



**Fig 6.** GC-MS TIC Chromatogram

## *5.INVITRO* ANTIOXIDANT STUDY

### DPPH Free Radical Scavenging Assay

**Table 6.** Ascorbic acids and ET -DPPH radical scavenging assay percentage inhibition and IC50 values

| CONCENTRATION | % INHIBITION OF STANDARD | % INHIBITION OF SAMPLE |
|---|---|---|
| 10 | 35.00 | 6.6 |
| 20 | 48.75 | 25 |
| 40 | 61.25 | 48 |
| 60 | 72.50 | 66.6 |
| 80 | 82.50 | 80 |
| 100 | 90 | 85 |

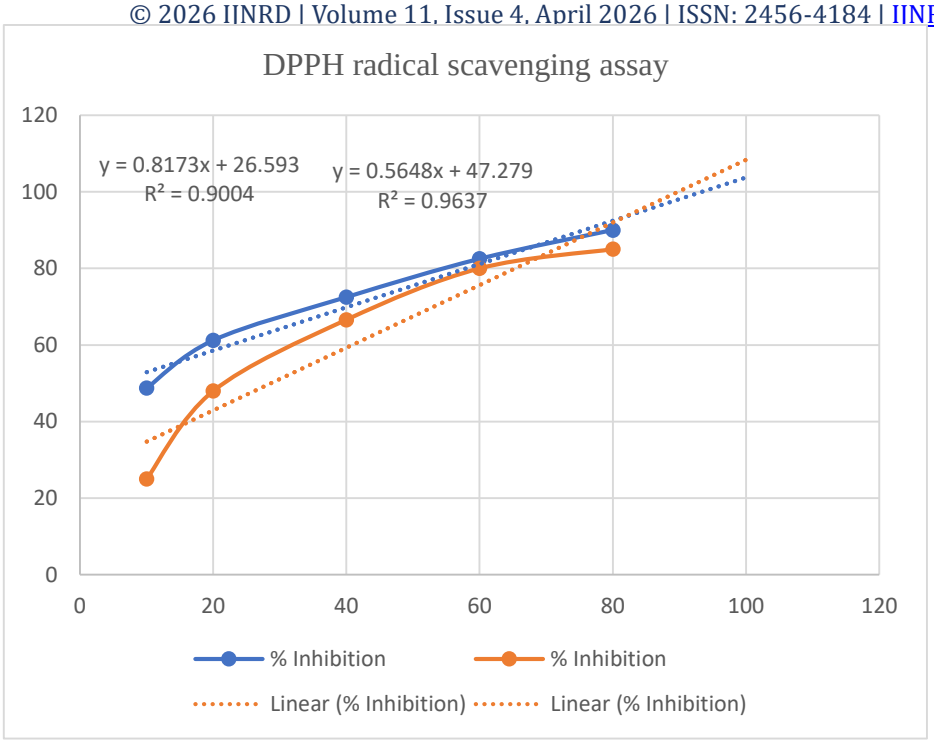



**Fig 6.** DPPH radical Scavenging assay of ascorbic acid

As a reference standard, ascorbic acid has a concentration-dependent DPPH radical scavenging activity between 10 and 100 µg/ml. The IC50 value of the sample (ET extract) was **28.8µg/ml.**

## 6.PHARMACOLOGICAL EVALUATION

### EFFECT OF VARIOUS DOSES OF EEET ON BODY WEIGHT ANALYSIS

Over the course of the experiment, the control animals' body weight increased normally and steadily, indicating healthy growth. The scopolamine-only group, on the other hand, demonstrated a significant decrease in body weight by day 28 ($p < 0.001$ in comparison to control), demonstrating the detrimental systemic effects of scopolamine. When scopolamine was combined with the standard drug (donepezil), the decline in body weight was prevented, and values remained stable, showing a significant improvement over scopolamine treatment alone ($p < 0.01$). Body weight was substantially higher than that of the scopolamine group ($p < 0.05$), indicating a modest protective effect from the administration of 200 mg/kg of EEET. With body weight values that were almost identical to those of the control group ($p < 0.001$ vs. scopolamine; non-significant vs. control), the higher dose of EEET (400 mg/kg) provided the strongest protection. Overall, the data indicate that 400 mg/kg of EEET produced outcomes similar to those of the conventional treatment, suggesting that it has a dose-dependent protective action against scopolamine-induced weight loss.

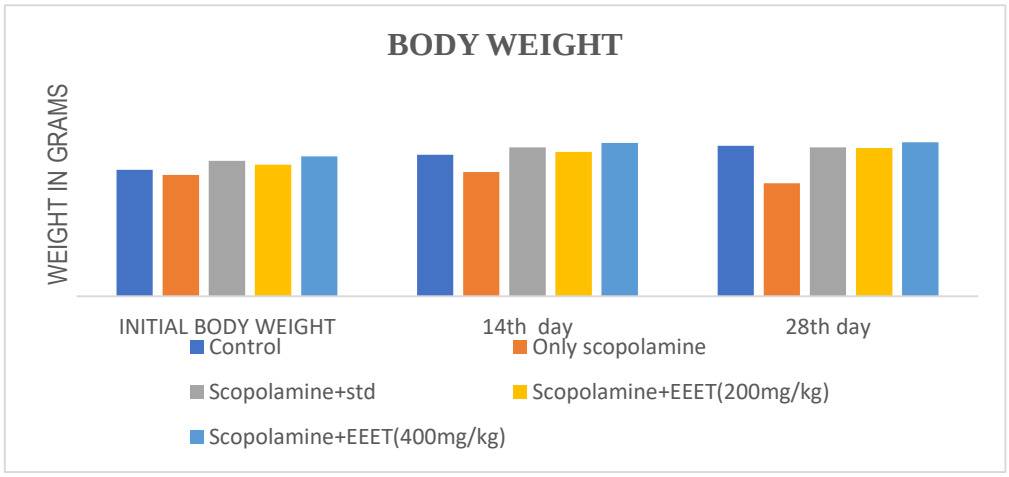



**Fig 7.** Impact of different dosages of EEET (200mg/kg,400mg/kg) on initial, 14, 28$^{th}$ day body weight of control and treated groups.

## EFFECT OF VARIOUS DOSES OF EEET ON ORGAN WEIGHT ANALYSIS

When scopolamine is administered, the weight of the brain, liver, and stomach is significantly decreased in comparison to control animals. However, as the "ns" (non-significant) notation indicates, administration of 200 and 400 mg/kg doses of Elaeocarpus extract (EEET) did not significantly reverse these losses. On the other hand, donepezil treatment at a standard dose of 1.88±0.115 for brain weight, 8.17±0.185 for liver weight, and 15±1.03 for gut weight shown notable improvements, indicating that it was effective in reducing the weight loss caused by scopolamine in these organs.

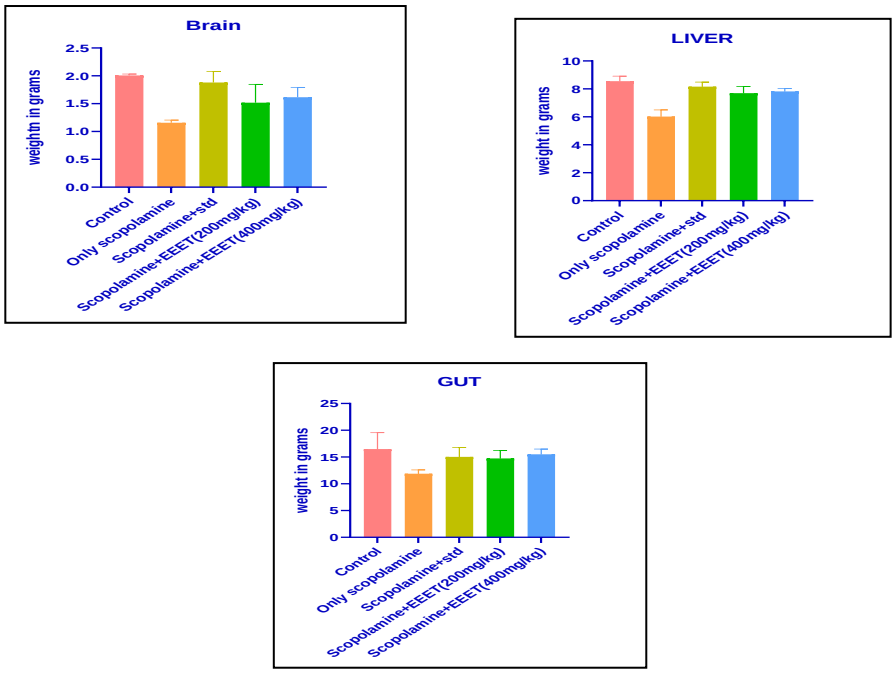



**Fig 8.** Effect of various doses of EEET on brain, gut and liver weight of control and treated groups.

## 7.EVALUATION OF BEHAVIORAL PARAMETERS

### OPEN FIELD TEST

The number of crossings was used to evaluate the rats' memory and exploratory behaviour. The control group exhibited conventional behaviour and maintained constant activity levels, with a slight increase in crossings from 10.3 prior to injection to 11.3 following treatment. As crossings dropped from 11 to 5, the scopolamine-treated group's exploration activity declined, indicating memory impairment and a decreased desire to explore. When donepezil (a common medication) was administered, exploratory behaviour improved; crossings increased from 11 to 13 ($P < 0.01$), indicating that memory function had been restored. Crossings increased from 10 to 9.3 ($P < 0.05$) in the 200 mg/kg-treated group of E. tectorius leaf extract (EEET), indicating a modest improvement; crossings improved from 10.5 to 11.7 ($P < 0.01$) in the 400 mg/kg treated group of EEET. These findings suggest that while donepezil and EEET, particularly at larger dosages, aid in enhancing cognitive abilities and activity in rats, scopolamine markedly reduces memory and exploratory behaviour.

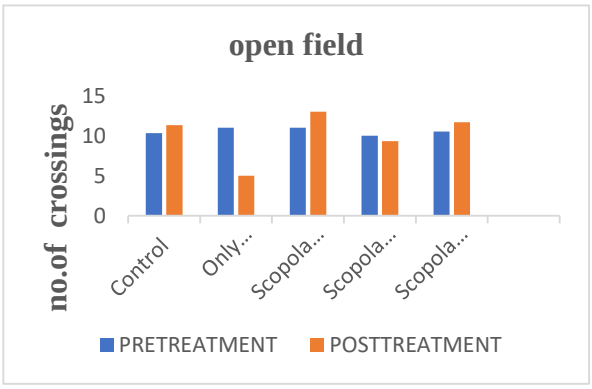
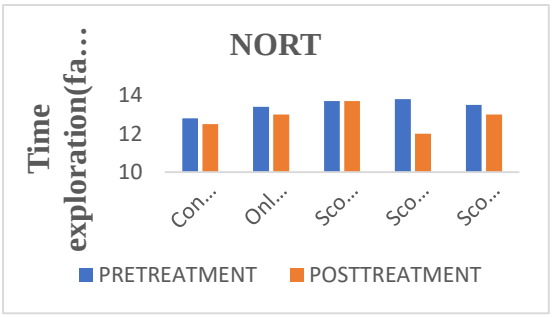



**Fig 9.** Effect of various doses of EEET (200&400mg/kg) on open field test of control and treated groups. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

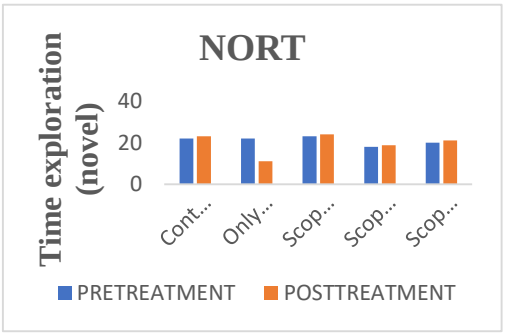
### NOVEL OBJECT RECOGNITION TEST

Rats' recognition memory was evaluated utilizing the Discrimination Index (DI), which contrasted their exploration of known and unfamiliar objects. DI scores for the control group increased marginally from 0.27 prior to treatment to 0.29 following treatment, indicating steady memory performance. DI dropped from 0.24 to -0.08, showing a substantial impairment in recognizing ability, in the scopolamine-treated group. Memory improved after taking the conventional medication donepezil; the DI increased from 0.26 to 0.27 ($P < 0.01$), indicating a protective effect. Rats given 200 mg/kg of E. tectorius leaf extract (EEET) showed a moderate improvement in DI, going from 0.13 to 0.21 ($P < 0.05$), but rats given 400 mg/kg showed a higher increase, going from 0.19 to 0.23 ($P < 0.05$). These findings demonstrate that while donepezil and EEET, especially at larger dosages, aid in restoring memory function, scopolamine inhibits recognition memory.

**(A).**




**(B).**



**(C)**

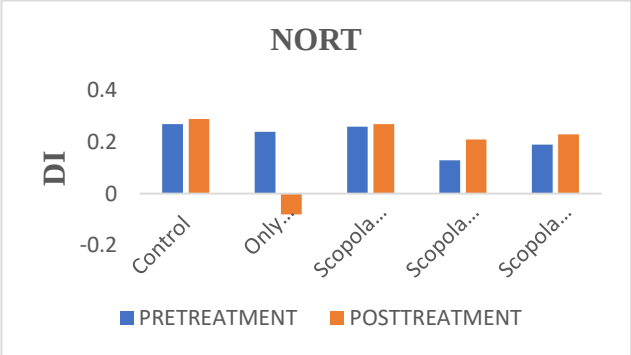



**Fig 10.** Effect of various doses of EEET (200&400mg/kg) on NORT of control and treated groups. (A). Time spent in exploring a familiar object. (B). Time spent in exploring a novel objection. (C). discrimination index

## Y MAZE

Rats' spontaneous alternation behaviours (SAB%) were assessed using the Y-maze test to gauge their short-term memory. The SAB% of the control group increased from 82% prior to treatment to 110% following treatment, indicating a typical improvement in memory ability. SAB% dropped from 75% to 27% in the group that received scopolamine alone, indicating a significant deterioration in memory. SAB% increased from 71% to 134% ($P < 0.01$) in rats given with the conventional medication donepezil, showing a remarkable recovery in memory and a strong restoration of scopolamine-induced impairment. The group that received 200 mg/kg of E. tectorius leaf extract (EEET) improved from 83% to 88% ($P < 0.05$), but the 400 mg/kg group that was given of EEET improved from 84% to 115% ($P < 0.05$). These results show that scopolamine impairs memory, but donepezil and EEET both successfully enhance memory function in rats, particularly at larger dosages.

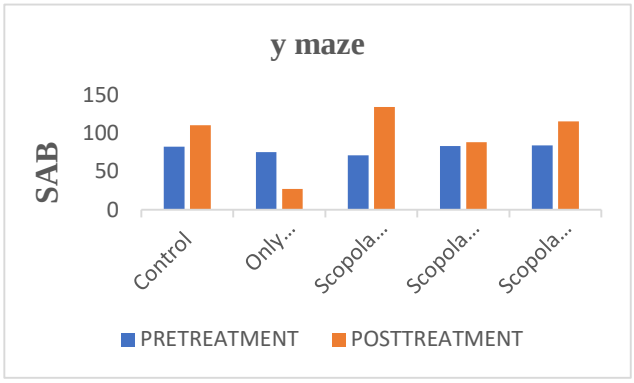



**Fig 11.** Effect of various doses of EEET (200&400mg/kg) on Y Maze of control and treated groups. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M.(n = 6 per group) is the outcome.

## MORRIS WATER MAZE

Rats' learning and memory were assessed using the Morris Water Maze test, which measures escape latency time. The control group displayed normal learning ability, as the latency increased from **33.3 seconds before treatment** to **110 seconds after treatment**. Rats treated with scopolamine showed impaired memory, with latency decreasing from **31.4 seconds** to **27 seconds**, indicating difficulty in retaining spatial information. Memory was significantly improved in the group treated with the conventional medication donepezil; delay increased from 22 seconds to 134 seconds ($P < 0.01$). Likewise, rats with 200 mg/kg of E. tectorius leaf extract exhibited a moderate improvement in latency, increasing from 18.3 seconds to 88 seconds ($P < 0.05$). Even more benefits were obtained with the higher dose of 400 mg/kg extract, which increased delay from 26.7 seconds to 115 seconds ($P < 0.05$), nearly matching the performance of the control group. According to these findings, scopolamine impairs memory, but donepezil and EEET, particularly at higher dosages, successfully improve memory and spatial learning.

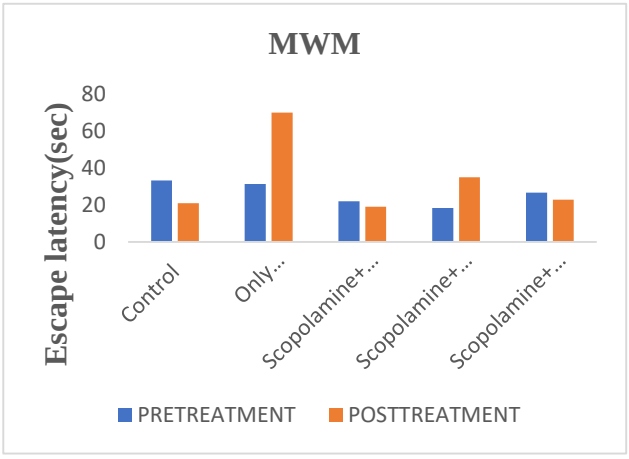
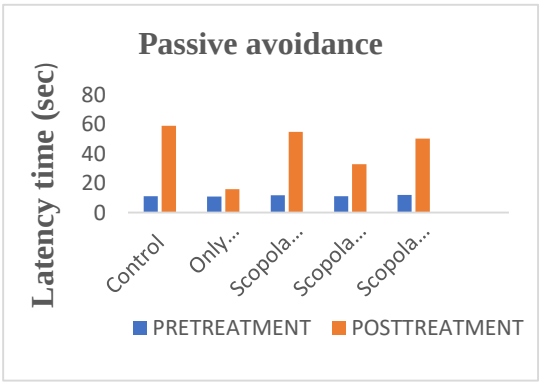
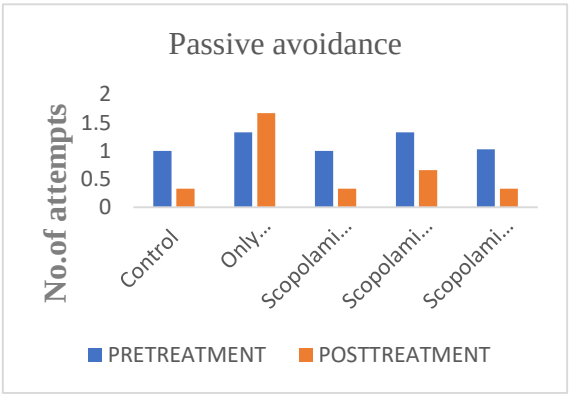



**Fig 12.** Effect of various doses of EEET (200&400mg/kg) on Morri's water Maze of control and treated groups. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M.(n = 6 per group) is the outcome.

**PASSIVE AVOIDANCE**

The number of trials and lag time were used to assess memory function in the passive avoidance test. With latency time rising from 11.2 seconds (pretreatment) to 59.0 seconds (posttreatment) and attempts falling from 1.00 to 0.33, the control group demonstrated significant memory retention. The scopolamine-treated group, on the other hand, showed memory impairment, as tries climbed from 1.33 to 1.67 and latency only increased from 10.9 to 15.9 seconds. Memory was considerably enhanced by donepezil (a common medication); latency increased from 11.8 to 55.0 seconds, and attempts decreased from 1.00 to 0.33 ($P < 0.01$). In a similar vein, E. tectorius leaf extract (EEET) at 200 mg/kg enhanced memory, increasing latency from 11.2 to 33.0 seconds and lowering attempts from 1.33 to 0.66 ($P < 0.05$). In contrast, the 400 mg/kg dose demonstrated higher efficacy, increasing latency from 12.1 to 50.3 seconds and lowering attempts from 1.03 to 0.33 ($P < 0.05$). These findings show that while donepezil and EEET, particularly at larger doses, successfully improve memory performance in rats, scopolamine causes notable memory impairments.




**B.** **(a)**

**(b)**

**Fig 13.** Effect of various doses of EEET (200&400mg/kg) on Passive avoidance test of control and treated groups. (a). latency time, (B). No. of attempts. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

**Impact of Leaf Extract from E. tectorius on Total Protein**

Scopolamine dramatically reduced total protein levels, which fell from 1.25 ± 0.0564 mg/dL in controls to 0.36 ± 0.0109 mg/dL ($P < 0.01$). Protein levels were restored to 1.03 ± 0.192 mg/dL after donepezil treatment, but they were improved to 1.09 ± 0.0423 mg/dL and 1.12 ± 0.056 mg/dL, respectively, by E. tectorius leaf

extract (EEET) at 200 as well as 400 mg/kg (P> 0.05 compared to control), indicating a defence against protein decrease brought on by scopolamine

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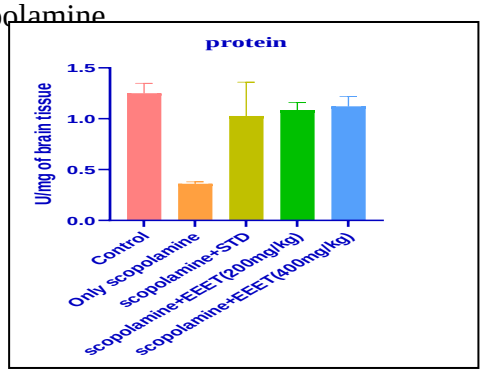
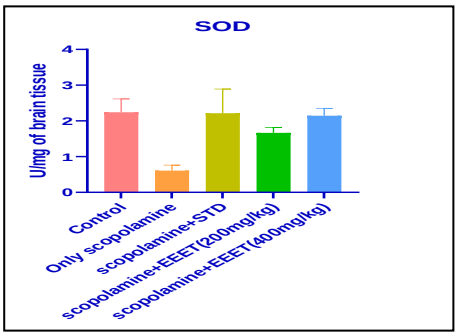
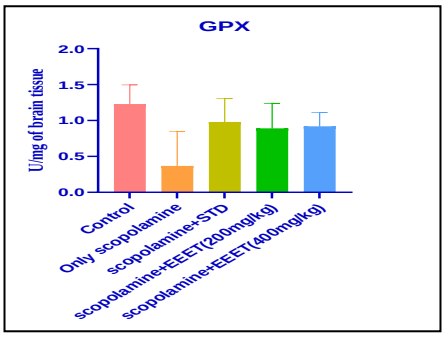



**Fig 14.** Effect of E. tectorius leaf extract (200&400 mg/kg) on Total protein. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

**Impact of Leaf Extract from E. tectorius on brain antioxidant levels**

Rats treated with scopolamine had significantly lower levels of glutathione and antioxidant enzymes. The activity of superoxide dismutase (SOD) dropped from 2.24 ± 0.216 unit/min/mg protein in the control group to 0.61 ± 0.0882 (P < 0.01) in the scopolamine group, indicating oxidative stress. Treatment with donepezil restored SOD to 2.21 ± 0.392 (non- significant), and E. tectorius leaf extract (EEET) at both 200 and 400 mg/kg brought it to 1.67 ± 0.0867 (non-significant) and 2.15 ± 0.119 (non-significant), respectively. Glutathione peroxidase (GPX) activity also declined from 1.23 ± 0.153 in controls to 0.367 ± 0.278 with scopolamine. After treatment, GPX improved to 0.978 ± 0.188 with donepezil, 0.891 ± 0.2 with EEET at 200 mg/kg treated group, and 0.919 ± 0.111 with EEET at 400 mg/kg. Similarly, reduced glutathione (GSH) decreased from 2.14 ± 0.453 µg/mg protein in controls to 0.523 ± 0.0897 after scopolamine, while donepezil restored it to 2.3 ± 0.218, and EEET at both 200 mg/kg and 400 mg/kg improved it to 1.87 ± 0.346 and 2.29 ± 0.31, respectively. These results demonstrate that scopolamine induces oxidative damage by lowering antioxidant defences, while donepezil and EEET, especially at higher doses, effectively restore SOD, GPX, and GSH levels toward normal.



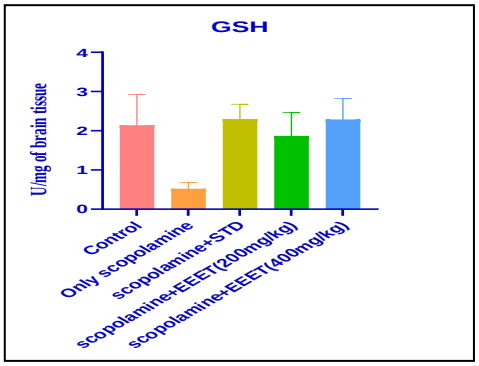




**Fig 15.** Effect of E. tectorius leaf extract (200&400 mg/kg) on brain antioxidant levels SOD, GPx, GSH. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

**Impact of Leaf Extract from E. tectorius on LPO (TBARS)**

Scopolamine treatment caused a marked increase in lipid peroxidation, with MDA levels rising from 0.18 ± 0.0392 nmol/mg protein in the control group to 0.95 ± 0.206 (P < 0.001), reflecting enhanced oxidative stress. Administration of donepezil lowered LPO to 0.11 ± 0.00549 (P > 0.05 vs. control), whereas 200 as well as 400 mg/kg of E. tectorius leaf extract (EEET) were decreased it to 0.34 ± 0.0149 and 0.21 ± 0.0233, respectively (P > 0.05 vs. control), indicating effective protection against scopolamine-induced oxidative damage.

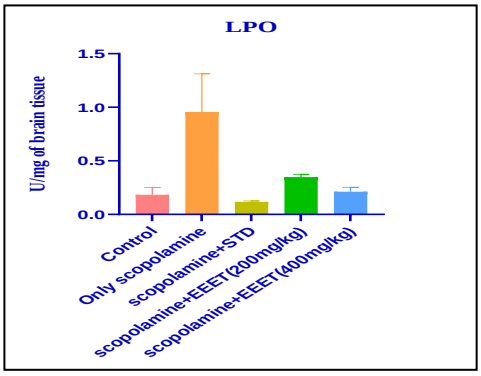



**Fig 15.** Effect of E. tectorius leaf extract (200&400 mg/kg) on LPO level. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

**Impact of Leaf Extract from E. tectorius on brain neurotransmitters**

Scopolamine treatment caused significant alterations in brain neurotransmitter levels. Dopamine decreased from 44.5 ± 0.289 ng/g in control rats to 27.6 ± 1.32 ng/g ($P < 0.001$), and serotonin dropped from 34.9 ± 0.12 to 21.9 ± 0.115 ng/g ($P < 0.001$). GABA levels also declined from 12.4 ± 0.0577 to 7.53 ± 0.186 ng/g, while glutamate increased from 5.4 ± 0.265 to 8.07 ± 0.291 ng/g ($P < 0.001$), indicating excitatory–inhibitory imbalance. Administration of donepezil or E. tectorius leaf extract (EEET) helped normalize these levels: dopamine reached 39.4 ± 0.418 and 38.6 ± 0.384 ng/g, serotonin 30.8 ± 0.145 and 29.4 ± 0.233 ng/g, GABA 10.9 ± 0.0577 and 11 ± 0.549 ng/g, and glutamate 4.17 ± 0.384 and 3.93 ± 0.437 ng/g, respectively, reflecting their neuroprotective potential against scopolamine-induced disturbances.

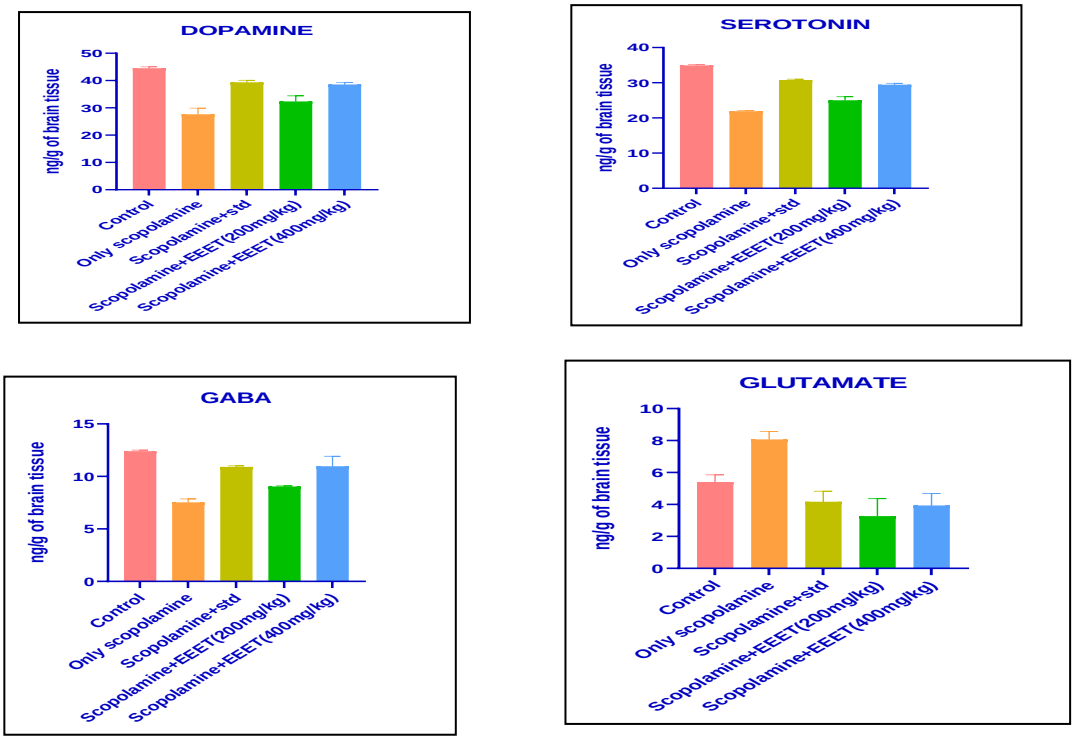



**Fig 16.** Effect of E. tectorius leaf extract (200&400 mg/kg) on brain neurotransmitters level. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

**Impact of Leaf Extract from E. tectorius on brain AchE level**

Scopolamine treatment significantly increased acetylcholinesterase (AchE) activity, rising from **0.18 ± 0.0057 mole/min/mg tissue** in controls to **0.393 ± 0.00882 ($P < 0.001$)**, indicating enhanced cholinergic degradation. Administration of donepezil reduced AchE activity to **0.21 ± 0.00577 ($P > 0.05$ vs. control)**, while E. tectorius

leaf extract (EEET) at 200 as well as 400 mg/kg lowered it to **0.28 ± 0.00577** and **0.217 ± 0.00882**, respectively, suggesting a protective effect in maintaining cholinergic function.

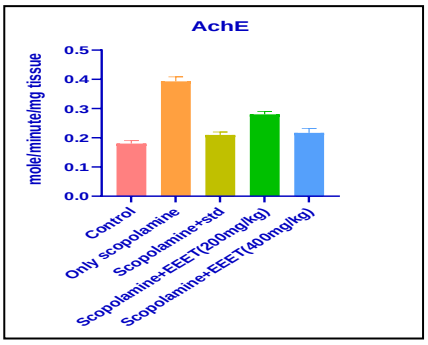
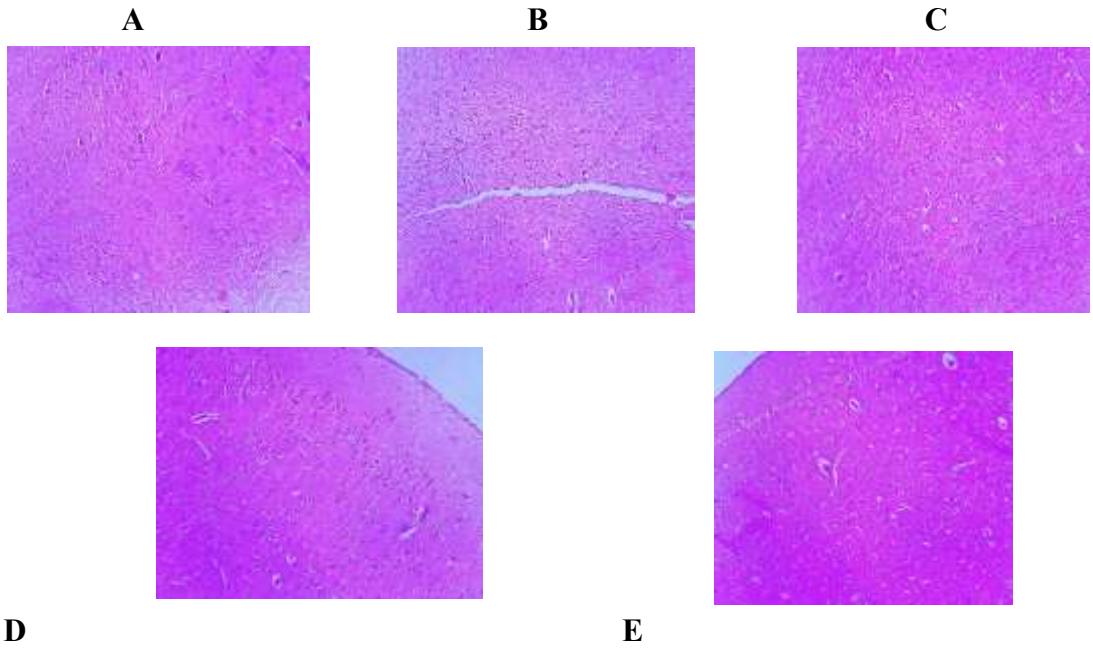
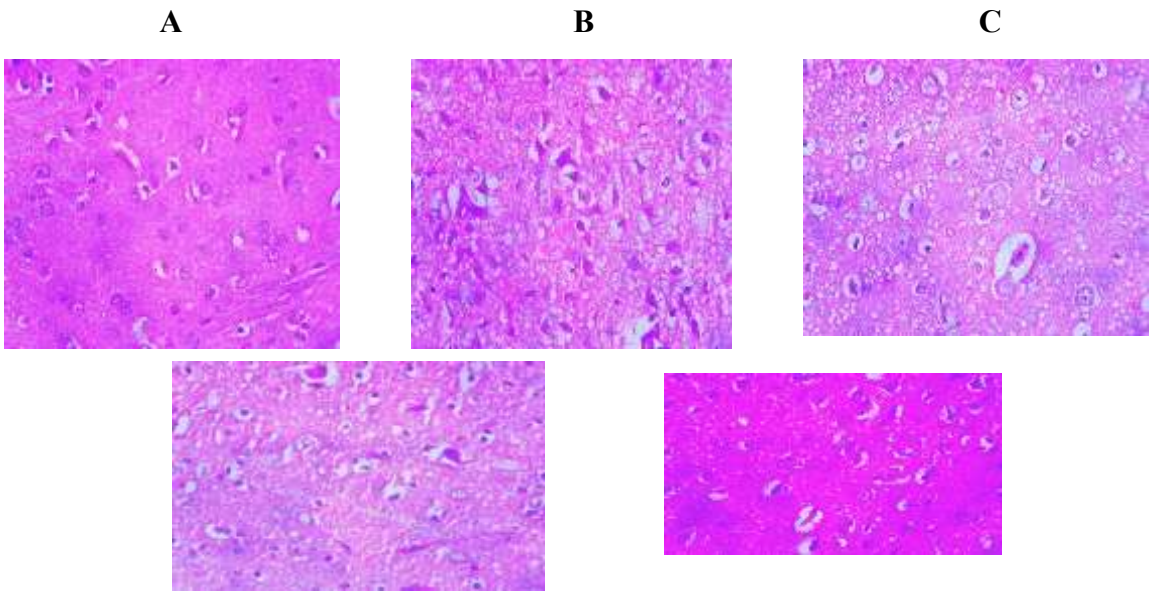



**Fig 16.** Effect of E. tectorius leaf extract (200&400 mg/kg) on brain neurotransmitters level. One-way analysis of variance was used to analyse the data, and the mean ± S.E.M. (n = 6 per group) is the outcome.

## 8.HISTOPATHOLOGICAL EVALUATION OF BRAIN



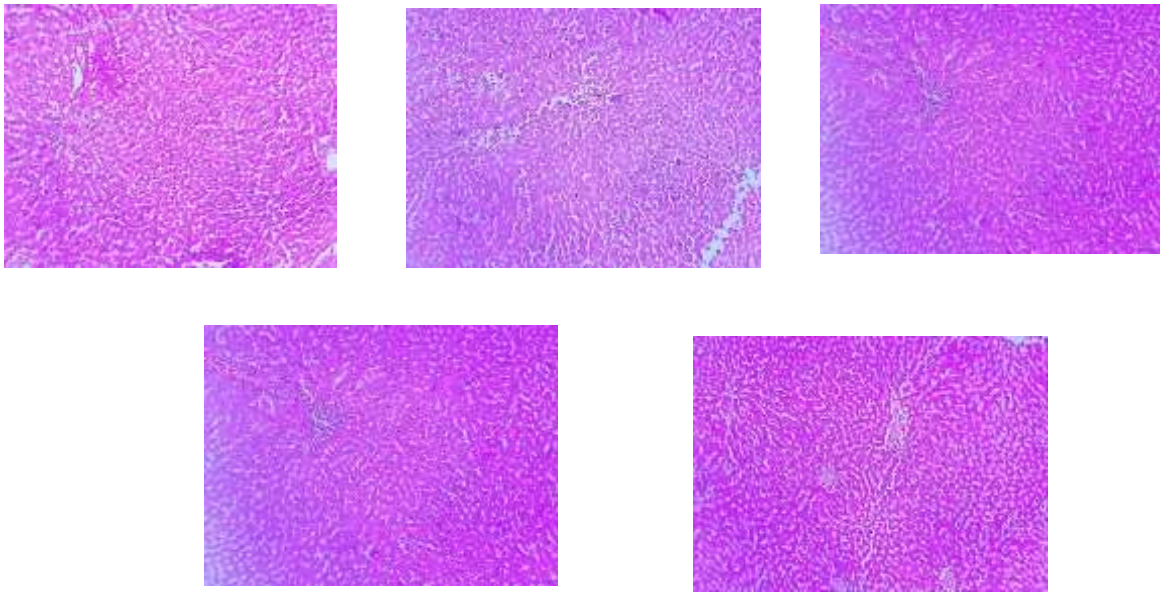

A) Shows normal cerebrum B) Shows mild gliosis C) shows normal cerebrum with fibrillary region D) shows mild gliosis E) Shows cerebral cortex with normal oligodendrocytes



A) shows normal cerebrum B) shows mild spacing with gliosis C) shows mild neuronal degeneration D) shows spacing of neurons with mild gliosis E) shows substantia nigra normal.

**HISTOPATHOLOGICAL EVALUATION OF LIVER**

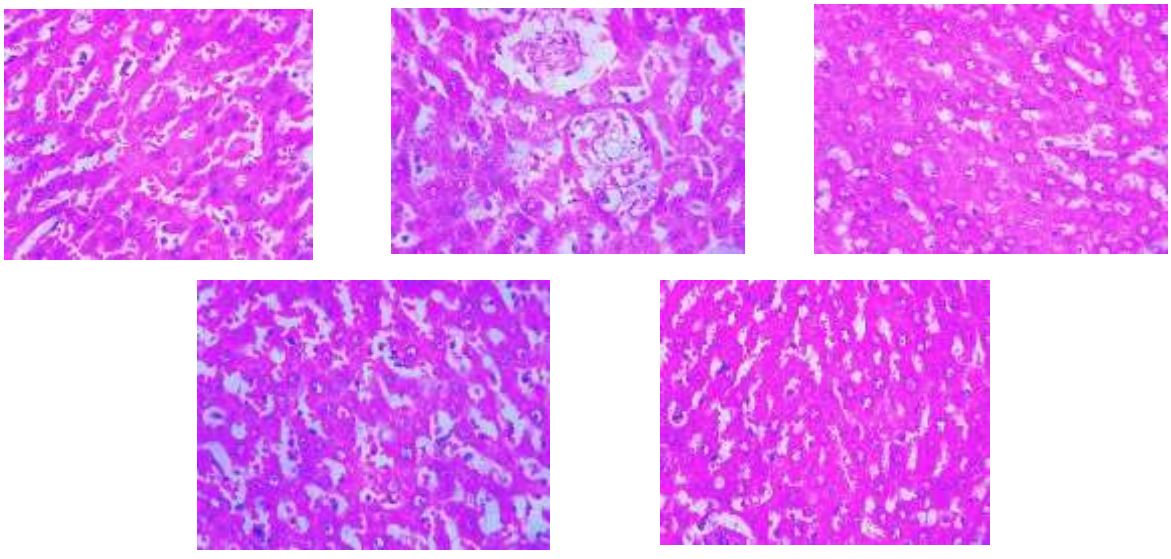
**A** **B** **C**



**D** **E**

A) Shows individual hepatocytes shows normal B) Shows sinusoidal dilatation with normal hepatocytes C) Shows lobular architecture with normal portal tract D) Shows sinusoidal dilatation with extravasated rbcs E) shows normal hepatocytes.

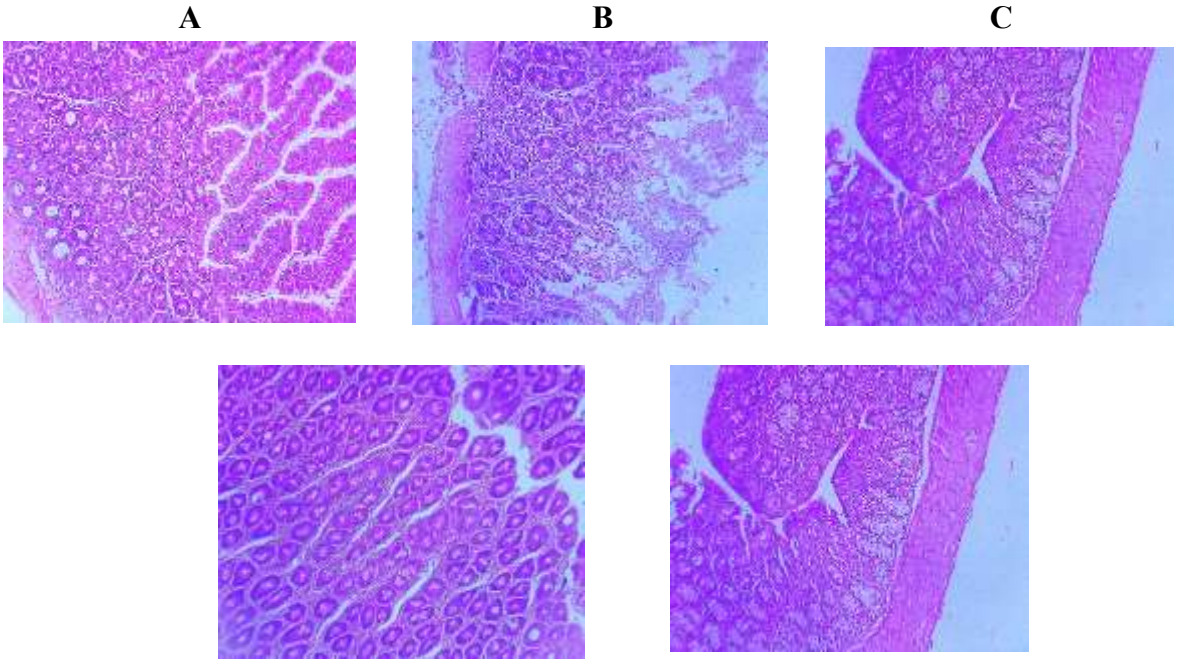
**A** **B** **C**



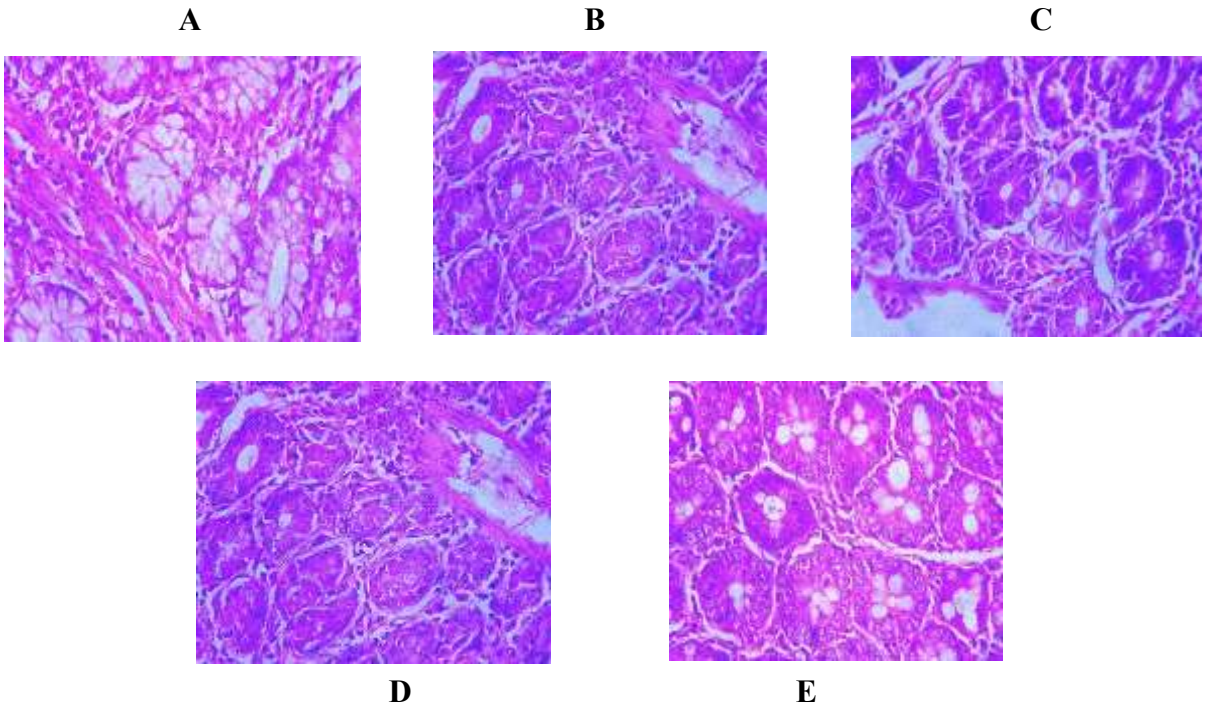
**D** **E**

A) shows individual hepatocytes normal B) shows central vein dilatation and congestion C) shows focal mild cytoplasmic vacuolation D) shows mild interface hepatitis E) shows lobular architecture.

**HISTOPATHOLOGICAL EVALUATION OF COLON**




A) Shows normal glands B) Shows Surface epithelial loss with lamina propria show inflammatory infiltrates c) Shows normal mucosal epithelium D) Shows surface epithelial loss with lamina propria shows inflammatory infiltrates E) shows normal mucosal epithelium.




A)Shows muscular layer B) Shows lamina propria shows mild inflammatory infiltrates C) Shows normal crypts D) 40x shows lamina propria shows scattered inflammatory infiltrates E) Shows normal mucosal glands with crypt architecture.

## 9.DISCUSSION

Dementia is the sixth greatest cause of death, affecting 55 to 57 million individuals globally and accounting for roughly 10 million new cases annually. Its frequency is predicted to nearly quadruple to over 150 million

by 2050, with low- and middle-income countries accounting for most of the growth, since they account for over 60% of current instances. Research suggests that up to 45% of instances may be avoided by targeting 14 risk factors that can be changed throughout one's life, including smoking, Type 2 diabetes, depressive disorders, loneliness, elevated cholesterol levels, high blood pressure, obesity, hearing loss, and vision impairment.

In this study we used ethanolic leaf extract of *Elaeocarpus tectorius* as the test drug. Cognitive function may be improved by ET (Elaeocarpus tectorius) ethanolic extract. By inhibiting AChE, improving cholinergic tone, and stimulating neurotrophic pathways (such as CREB/BDNF), scopolamine-induced memory deficits can be reversed. Numerous physiological processes, including antiviral, antibacterial, antioxidant, and anti-inflammatory properties, are exhibited by these species. In order to determine if an ethanolic extract of Elaeocarpus tectorius leaves may ameliorate cognitive impairments in a Wistar rat model of scopolamine-induced dementia, this study was conducted. Behaviour, brain neurotransmitters, haematological, and biochemical indicators are utilized to assess how well an ethanolic extract of Elaeocarpus tectorius leaves might improve cognitive impairments.

**_INVITRO_ ANTIOXIDANT STUDIES- DPPH radical scavenging assay**

Both ascorbic acid (vitamin C) and the ethanol extract (ET) demonstrated concentration-dependent enhancements in antioxidant activity, according to the DPPH radical scavenging experiment. Ascorbic acid achieved 90% inhibition and ET reached 85% at 100 µg/mL. The ET's moderate antioxidant efficacy was shown by its $IC_{50}$ value of 28.8 µg/mL. This implies that ascorbic acid is still more effective than ET even though the latter has notable radical scavenging activity.

**PHARMACOLOGICAL EVALUATION**

**Behavioural parameters:**

Animals in the scopolamine-only (negative control) group showed clear signs of cognitive impairment, including decreased step-down latency in passive avoidance and increased escape latency in Morri's water maze. In contrast, rats who receive scopolamine after a 14-day pretreatment with 200 and 400 mg/kg of the plant extract perform noticeably better on behavioural tasks.

• Open Field Test: reduced anxiety-like behaviour and normal locomotor activity.

In contrast to scopolamine controls, Novel Object Recognition (NORT) restored recognition memory.

• Improved fear-associated memory is indicated by an enhanced step-down delay on the passive avoidance test.

• Morris Water Maze (MWM): longer duration in the target quadrant and reduced escape latency.

• Y Maze: higher frequencies of spontaneous alternation.

**BODY WEIGHT ANALYSIS:**

While the EEET-treated group's body weight greatly increased, the disease-untreated group's body weight significantly decreased.

**ORGAN WEIGHT ANALYSIS:**

Administering scopolamine causes brain shrinkage, which is seen in the reduction of hippocampal volume and total brain weight. It also damages gut health by increasing permeability, crypt loss, and villus atrophy. By restoring brain weight and bringing the hippocampus's sections area and perimeter back to normal, donepezil therapy successfully counteracts these effects. Furthermore, natural substances such as Elaeocarpus have shown protective properties against scopolamine-induced hippocampus atrophy and brain weight loss. Scopolamine does not significantly alter the liver, and remedies like donepezil or plant-based microbiome-restoring medicines can repair damage to the gut by restoring its morphology and barrier function, which most likely corresponds to the gut tissue's restored integrity.

### *IN VIVO* ANTIOXIDANT STUDIES:

Disease groups show a significant increase in lipid peroxidation (LPO) and a decrease in CAT, GSH, GPx, and SOD levels in brain tissues. When compared to 200 mg/kg, the treatment group's high dose of EEET (400 mg/kg) greatly reduces the amount of lipid peroxidation (LPO) while dramatically increasing the levels of CAT, GSH, and GPx in brain tissues. The synthesis of proteins was a significant phenomenon. Pathways essential for formation of memories and synaptic plasticity were affected by the disruption of protein synthesis in the brain, particularly in the area known as the hippocampus (Scopolamine suppresses the phosphorylation of ERK proteins and CREB and lowers the level of BDNF protein expression in the hippocampus). The treatment group restored the scopolamine-reduced p-ERK/p-CREB/BDNF level, frequently through increased protein synthesis.

### BRAIN NEUROTRANSMITTERS:

By upsetting the neurotransmitter equilibrium in the brain, scopolamine treatment reduces memory. It dramatically increases the activity of acetylcholinesterase (AchE), which lowers acetylcholine (Ach) levels, which are essential for memory and learning. Scopolamine further impairs memory function by decreasing the amount of gamma-aminobutyric (GABA), the main inhibitory neurotransmitter, and raising glutamate levels, which is an excitatory neurotransmitter that can be hazardous at high quantities. These effects may be reversed by administering 400 mg/kg of Elaeocarpus extract (EEET). EEET mitigates excitotoxicity by lowering glutamate levels and dramatically reducing AchE activity, which raises Ach levels. EEET also raises GABA levels, which may indicate that it maintains neurotransmitter balance and improves memory. Studies showing that natural substances can alter neurotransmitter networks and provide neuroprotective advantages in models of scopolamine-induced cognitive impairment corroborate these conclusions.

### HISTOPATHOLOGICAL STUDIES

The disease group exhibits focal cryptitis and epithelial loss in the colon, central venous dilatation and congestion in the liver, and moderate gliosis with hippocampal vacuolation and neuronal spacing in the brain. In contrast, the therapy group has a normal hippocampal region in the brain, normal hepatocytes, and the Portal triad, which includes normal colonel mucosa, preserved lamina propria in the colon, and periportal inflammation in the liver.

## 10.CONCLUSION

The ethanolic extract of *Elaeocarpus tectorius* (ET) leaves has demonstrated significant potential in mitigating cognitive deficits associated with scopolamine-induced dementia in Wistar rats. This extract, rich in flavonoids, terpenoids, and phenolic compounds, exhibits potent antioxidant properties, effectively reducing oxidative stress and lipid peroxidation. Biochemical analyses revealed that ET administration restored neurotransmitter balance by inhibiting acetylcholinesterase (AChE) activity and modulating glutamate and gamma-aminobutyric acid (GABA) levels. Additionally, ET enhanced antioxidant defences by elevating catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) levels. Histopathological examinations confirmed the protective effects of ET on neuronal structures, while behavioural assessments demonstrated significant improvements in memory and learning, as evidenced by enhanced performance in the Morris water maze test. Additionally, ET reduced systemic inflammation and improved general brain function by supporting gut health and the microbiota–gut–brain axis. Collectively, these findings underscore the therapeutic promise of *Elaeocarpus tectorius* as a natural, multi-target agent with antioxidant, anti-inflammatory, and cholinergic-modulating properties for mitigating dementia and related neurodegenerative disorders.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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