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Assessment of the Nootropic Effects of *Tylophora indica* Leaves on Scopolamine-Induced Amnesia in Swiss Albino Mice

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ABSTRACT

Objective: This study investigates the nootropic effects of ethanolic extracts of *Tylophora indica* leaves on scopolamine-induced amnesia in Swiss albino mice. **Materials and methods:** Phytochemical studies were performed for ethanolic extraction of *Tylophora indica.* In vivo nootropic activity was evaluated using Morris water maze and Y maze apparatus. Ex vivo method was used to estimate the acetylcholinesterase level. The results were analyzed using one-way ANOVA and p values were calculated to find the significance of the results. **Results**: Phytochemical analysis confirmed the presence of alkaloids, carbohydrates, flavonoids, phenols, sterols, triterpenoids, proteins and amino acids in significant amounts. In vivo studies demonstrated that the ethanolic extract significantly decreased escape latency in the Morris water maze, decreased transfer latency in the Y maze and significant decrease in acetylcholinesterase level by ex vivo method compared to the standard nootropic agent, Piracetam. High dose leaves extract of *Tylophora indica* (400mg/kg) exhibited nootropic activity. Presence of phytochemicals such as alkaloids, flavonoids, sterols may contribute to the observed nootropic activity. **Conclusion:** From the study it is concluded that the ethanolic extract of *Tylophora indica* leaves possess beneficial effect against scopolamine induced amnesia proved by the valid data obtained from the *ex-vivo* and *in-vivo* evaluation. This study provides preliminary evidence that the ethanolic extract of *Tylophora indica* leaves exhibits potential nootropic effects against scopolamine-induced amnesia.

1. INTRODUCTION

Memory is a crucial brain function, essential for survival as it allows organisms to record and utilize experiences to adapt to environmental changes. Loss of memory and cognitive impaired functions are the major features of Alzheimer's disease (AD). Presence of acetylcholine within the neocortex is sufficient to ameliorate learning deficits and restore memory. Decreased cholinergic firing in brain, rise in oxidative stress, hypercholesterolemia, and neuroinflammatory reactions have been demonstrated to play an etiological role in memory decline. The central cholinergic system is involved in cognitive functions and plays an important role in learning and memory for humans and animals. (1)

Alzheimer's disease (AD), a progressive and irreversible neurodegenerative disorder, was first described by Dr. Alois Alzheimer in the early 1900s. It occurs gradually and results in cognitive impairment, unusual behaviour, personality changes, an ultimately death. Presently, it is the 4th leading cause of death in western countries, preceded only by heart disease, cancer and stroke.

India is now through a demographic shift, with an elderly population quickly increasing in India. In India, life expectancy has nearly doubled, rising from 36.98 years in 1950-1960 to 69.2 years in 2015-2020. In 2023 the census bureau has estimated 771 million persons aged 65+ years globally accounting for almost 10% of the world's population. This segment has been growing at an increasing rate and it expected to hit 16% in 2050, and eventually 24% by 2100. (2)

Alzheimer's is not a normal part of aging. Alzheimer's disease is a NDD which is associated with dementia. According to the World Health Organization, dementia is a syndrome in which there is deterioration in memory, thinking, behaviour and the ability to perform everyday activities. The estimated dementia prevalence for adult's ages 60+ in India is 7.4%. Dementia is more usual among females than in males and rural than in urban areas.

Alzheimer's worsens over time. Alzheimer's is a progressive disease, where dementia symptoms gradually worsen over a number of years. In early stages, memory loss is mild but with chronic-stage Alzheimer's, individuals lose the ability to carry on a conversation and respond to their environment.

Alzheimer's has no cure, but treatment anticholinesterase inhibitor and other drugs have been used in treating AD. There are used to extend which can only relieve symptoms. But side effects of neurodegenerative problems may show up in a later day. [3]

The most common cause of dementia in the elderly is probably Alzheimer's disease (AD), a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language.

Amnesia is the general term for a condition in which memory (either stored memories or the process of committing something to memory) is disturbed or lost, to a greater extent than simple everyday forgetting or absent mindedness.[4]

The introduction provides a comprehensive background on the importance of memory, the impact of Alzheimer's disease (AD), and the relevance of acetylcholine. However, it could benefit from a more focused narrative leading to the rationale for choosing *Tylophora indica. Tylophora indica* leaves have been chosen as the drug to perform the research after ethanolic extraction on its Nootropic activity based on its literature review.[5][6] Thus, the study pertains to evaluate the nootropic activity of leaves of *Tylophora indica* using Scopolamine induced amnesia in swiss albino mice.[7][8]

2. MATERIALS AND METHODS

2.1 PROCUREMENT OF *Tylophora indica* LEAVES

The plant leaves of *Tylophora indica* was collected from Xavier Research Foundation, Palayamkottai, Tamil Nadu (India) in the month of January 2024. The plant material was identified and authenticated by Dr. S. Mutheeswaran, Scientist.

2.2 PREPARATION OF EXTRACT [9] [10]

- Extraction was the preliminary step involved in the phytochemical studies.
- It was the separation of medicinally active portions of plant using selective solvents through Standard procedures.
- The leaves were cleaned, shade dried for about 15 days and the dried leaves was pulverized to a coarse powder by grinding in mixer and stored in an air tight container.

2.3 SOXHLET EXTRACTION METHOD [9] [11]

- A weighed quantity of the powder (1000 g) was passed through sieve number 40 and subjected to hot solvent extraction in a Soxhlet apparatus using ethanol at a temperature range of 60–80◦C, respectively.

- Before and after every extraction the powder bed was completely dried and weighed.

- The filtrate was evaporated to dryness at 40◦C under reduced pressure in a rotary vacuum evaporator.

- The percentage yield of ethanolic extract was calculated using the following formula.

%Yield = Weight of the Dry Extract/ Weight of the Dry Plant * 100

The extract was subjected to ex vivo and in vivo evaluation.

2.4 PHYTOCHEMICAL ANALYSIS [12]

2.4.1 Test for Alkaloids

- **Mayer's Test:** 0.5ml of sample was treated with 1ml of Mayer's reagent [Potassium mercuric iodide solution], presence of alkaloids produces cream colour precipitate.

- **Dragendroff's Test:** 1ml of the sample was treated with 1ml of Dragendroff's reagent [Potassium bismuth iodide solution], presence of alkaloids produces reddish brown precipitate.

- **Wagner's Test:** 0.5ml of the sample was treated with 0.5ml of Wagner's reagent [Solution of iodine in potassium iodide], presence of alkaloids produces reddish brown precipitate.

- **Hager's Test:** 1ml of the sample was treated with 0.5ml of Hager's reagent [Saturated solution of Picric acid], presence of alkaloids produces yellow colour precipitate.

2.4.2 Test for Carbohydrate

- **Molisch's Test:** To the 0.5ml of sample, few drops of alcoholic alpha- napthol was added and 0.2ml of concentrated sulfuric acid was added slowly through the sides of the test tube, a purple to violet colour ring appears at the junction.

- **Benedict's Test:** 1ml of sample was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath; presence of reducing sugars produces reddish brown precipitate.

- **Fehling's Test:** 1ml of sample was treated with few drops of Fehling's solution A and B, heat for few minutes, produces brick red precipitate.

- **Barfoed's Test:** 0.5ml of sample was treated with few drops of Barfoed's reagent, heat it for few minutes, produces red precipitate.

2.4.3 Test for Glycosides

- **Legal's Test:** 0.5ml of sample was treated with 0.3 ml of pyridine and alkaline sodium nitroprusside solution, presence of glycosides produces blood red colour.

- **Balijet Test:** 0.5ml of sample was treated with 0.3ml of sodium picrate; presence of glycosides produces yellow to orange colour.

- **Borntrager Test:** 1ml of sample was treated with 0.5ml of dilute sulphuric acid and boiled for few minutes, and then it was filtered. The filtrate was treated with ether or chloroform, to the organic layer few drops ammonia solution was added, presence of glycosides produces pink or violet colour.

2.4.4 Test for Cardiac Glycosides

- **Keller killani Test [Test for Deoxy sugars]:** 0.5ml sample was treated with 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. It was transferred to a small test tube and then 0.5ml of concentrated sulphuric acid was carefully added by the side of the test tube, blue colour forms in the acetic acid layer.

2.4.5 Test for Saponin

- **Foam froth Test:** 1ml of sample was treated with 10ml of water and boiled for few mins then it was filtered. The filtrate was shaken well and forms for the stable froth.

2.4.6 Test for Sterols

- **Salkowski Test:** To 0.5ml of sample about 0.3ml of chloroform with few drops of concentrated Sulphuric acid was added, shaken well and allowed to stand for some time, red

colour appeared at the lower layer indicating the presence of steroids, formation of yellow coloured lower layer indicates the presence of Triterpenoids.

- **Libermann Burchard Test:** 0.5ml of sample was treated with 0.3ml of chloroform, add small amount of acetic anhydride and concentrated sulphuric acid. The colour changes from red to bluish green.

2.4.7 Test for Tannins

- **Gelatin Test:** To 1ml of sample, 0.5ml of 1% gelatin and 10% sodium chloride was added indicates a white precipitate.

- **Lead acetate Test:** 1ml of sample was treated with 0.5ml of lead acetate solution formation of white precipitate indicates the presence of tannins.

- **Potassium dichromate Test:** 0.5ml of sample was treated with 0.5ml of potassium dichromate solution; presence of tannins produces yellow precipitate.

- **Potassium ferric cyanide Test:** 1ml of sample was treated with 0.5ml potassium ferric cyanide solution and few drops of ammonia solution was added, forms red colour.

2.4.8 Test for Flavonoids

- **Shinoda Test (Magnesium Hydrochloride reduction Test):** To 1ml of sample, few fragments of magnesium ribbon were added, then few drops of concentrated hydrochloric acid were added, presence of flavonoids produces magenta colour.

- **Alkaline reagent Test:** To 2ml of sample, 1ml of sodium hydroxide was added, presence of flavonoids produce yellow colour.

- **Mineral acid Test:** To 1ml of sample, few drops of concentrated sulphuric acid was added, presence of flavonoids produces orange colour.

- **Boric acid Test:** To 0.5ml of sample, few drops of boric acid was added, presence of flavonoids produces yellow colour.

2.4.9 Test for Proteins and Amino acids

- **Millon's Test:** To 1ml of sample, 2ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) was added, white precipitate appears, which turns into red upon gentle heating.

- **Ninhydrin Test:** To 1ml of sample, 0.5ml of 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate) was added and boiled in a water bath; appearance of violet colour indicates the presence of amino acids and proteins.

- **Biuret Test:** To 1ml of sample, 1ml of 10% sodium hydroxide, 1% copper sulphate was added, appears violet colour.

- **Xanthoprotein Test:** To 0.5ml of sample, few drops of concentrate nitric acid was added, appears orange colour.

- **Tannic acid Test:** To 1ml of sample, 0.5ml of tannic acid solution was added appears white colour.

2.5 EXPERIMENTAL ANIMAL

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/20.09.2021 and 07/AEL/IAEC/MMC, Date:26/12/2023.

Albino Mice (25-30gm) was procured from Animal House, Madras Medical College, Chennai, Tamil Nadu, and India. It was acclimatized for laboratory condition for 7 days and randomly divided into five groups each having six animals. The animals were housed under standard laboratory conditions and maintained under a 12-h light- dark cycle and free access to drinking water and diet for one week. All the protocols in this study were approved by the animal ethics committee of Madras Medical College, Chennai.

2.6 Acute oral toxicity study [13]

An acute toxicity study was performed by Vipul Gujrati et al., it was found to be safe up to 2000mg/kg.

2.7 DRUG AND CHEMICALS

Scopolamine was purchased from greenmeds. Piracetam was purchased from local medical shop.

Based on literature review (Vipul Gujrati et al., 2007) upto 2000mg/kg of *Tylophora indica* the animal did not show any morbidity and mortality. So 1/5th and a/10th of the dose were selected for invivo memory enhancing activity.

2.8 GROUPING OF ANIMALS [14]

Total 30 animals (Swiss albino mice) was randomly divided into five different groups, each group containing 6 mice and treated for 14 days as follows; Scopolamine (1mg/kg) was administered at last day.

TABLE: 1

GROUP NO	GROUP	TREATMENT AND ROUTE OF ADMINISTRATION	DURATION
Group I	Control	Normal saline p.o	14 days
Group II	Negative control	Scopolamine (1 mg/kg) i.p	on 14th day(Single dose)
Group III	Standard control	Piracetam 200mg/kg i.p for 14 days+ Scopolamine (1mg/kg i.p) was given 1hr after drug extract on 14th day	14 days
Group IV	Treatment control (200mg)	Extract of *Tylophora indica* leaves p.o for 14 days + scopolamine (1mg/kg i.p) was given 1hr after drug extract on 14th day	14 days
Group V	Treatment control (400mg)	Extract of *Tylophora indica* leaves p.o for 14 days + scopolamine (1mg/kg i.p) was given 1hr after drug extract on 14th day	14 days

2.8.1 EXPERIMENTAL PROCEDURE

Induction of Amnesia [15]

Amnesia mainly induced by using Scopolamine into the mice. Scopolamine, also known as L-Duboisine, and Hyoscine, is an alkaloid drug with muscarinic antagonist effects.

Scopolamine was used as a standard/reference drug for inducing amnesia in man and animals. The effects are generally interpreted as a cholinergic deficit and related to the hypothesis that acetylcholine is involved in memory functions. Scopolamine, besides influencing learning and memory, affects various types of behaviour (e.g., loco motor activity, anxiety, attention).

2.9 EXTEROCEPTIVE BEHAVIORAL MODELS

2.9.1 MORRIS WATER MAZE [16] [17]

The procedure was to perform place navigation test from day 1 to 14, in which the escape latency the time required to escape onto the hidden platform was used to evaluate learning and memory function. Mice that found the platform were allowed to remain on the platform for 20 s and were then returned to the home cage. If the mice did not reach the platform within 120 s it was gently guided to the platform by the experimenter, where it remained for 20s. The last trial was regarded as the probe test on the day after removal of hidden platform which was performed to test the ability of the mice to find the removed platform by memory.

The water maze task was divided into two phases:

i. **Acquisition trials**: Each animal was subjected to four consecutive trials on each day (from 11th to 14th day) with an interval of 10 mins, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 s. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In a preliminary study, the trial was conducted to familiarize the mouse with the task and was not counted. Mouse was subjected to acquisition trials for 3consecutive days.

ii. **Retrieval trial:** On the 15th day, hidden platform was removed and each mouse was allowed to explore the pool for 90 sec. Mean time spent by the mouse in each of four quadrants was counted. The mean time spent by the mouse in target quadrant for searching the hidden platform was counted as an index of retrieval.

iii. Time to reach the hidden quadrant was counted (escape latency) for each trial.

2.9.2 Y-MAZE [18]

The behavioural test was conducted in large quite room. A stop watch was used to score the behaviours and all events are observed manually. Y maze was made up of three equally spaced arm as a, b, c, which are 120° from each other. It was used to access the spatial memory in mice. To test the spatial memory, the mice were baited with reward on one of the arm. After being able to freely explore the maze, the animal was discovering the treat and eats it. At a later point in time, place the animal in maze, if it reaches the arm in which the food bait was found. Based on spatial cues, it might know where to go from different starting point. To assess spatial memory, we measured the time taken by the mice to reach the correct arm and it's noted down. The floor of the apparatus is 5 cm wide and was levelled with saw shaves. Each mouse was stationed in one the arms and allowed to freely explore the apparatus.

2.9.3 NEUROTRANSMITTER ESTIMATION [19]

Acetylcholinesterase (AChE) Ellman's method

Acetylcholinesterase is an enzyme participating in cholinergic neurotransmission. It breaks down acetylcholine which ceases the neurotransmission process. The most common assay is based on Ellman's method using an alternative substrate acetylthiocholine and 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB). The reaction effects in production of 5-thio-2-nitrobenzoate has yellow colour due to the shift of electrons to the sulfur atom.

The animals were sacrificed; whole brains were removed quickly and placed in ice-cold saline. The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). 4ml aliquot of the homogenate was added to a cuvette containing 2.6ml phosphate buffer (0.1M, pH 8) and 100µl of 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB). The contents of the cuvette were mixed thoroughly and absorbance was measured at 412nm in a spectrophotometer. When absorbance reaches a stable value, it was recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine was added and change in absorbance was recorded. Change in the absorbance per minute was determined. Protein estimation done by folin methods. AChE activity was calculated using the following formula,

Calculations

The enzyme activity was calculated using the following formula

$$\delta O.D \times \text{Volume of Assay (5ml)}$$

Acetylcholinesterase activity (M/ml) R = E × mg of protein

Where, rate of enzyme activity in n' mole of acetylcholine iodide hydrolyzed / min/ mg E

Extinction coefficient13600 M-1cm-1

δO.D = change in absorbance

The final reading of enzyme activity was expressed as µ moles/min/mg tissue.

2.10 STATISTICAL ANALYSIS

All the values were expressed as mean ±SEM. The data was statistically analysed by one way ANOVA method. One way analysis of variance (ANOVA) was used to correlate the statistical difference between the variables. $P<0.05$, $P<0.001$, $P<0.0001$ is considered to be significant.

3. RESULTS AND DISCUSION

3.1 PHYTOCHEMICAL ANALYSIS

Tylophora indica leaves extract was subjected to phytochemical analysis for identification of phytoconstituents. The obtained results were illustrated in **Table no 2.**

TABLE: 2

S.NO	PHYTOCHEMICAL	RESULT
1	Tests for alkaloids	+
2	Test for carbohydrates	+
3	Test for glycosides	+
4	Test for cardiac glycosides	+
5	Test for saponin	+
6	Test for sterols and triterpenoids	+
7	Test for Tannins	-
8	Test for flavonoids	+
9	Test for proteins andamino acids	+

+ Present, - Absent

3.2 IN VIVO STUDIES

3.2.1 Evaluation of memory enhancing activity of TI by scopolamine induced model in mice using Morris water maze

The TI extract and piracetam was administered orally to animals for 14 successive days and on 14th day (acquisition) it was followed by scopolamine after 1 hr of administration of the extract. The obtained result of escape latency was illustrated in **Table No.3.**

TABLE: 3 TREATMENT EFFECT ON 14TH DAY (BY MORRIS WATER MAZE)

GROUPS	DRUG TREATMENT	ACQUISITION-14TH DAY ESCAPE LATENCY
GROUP I	Control (Vehicle treated)	16.166±1.077
GROUP II	Scopolamine (1mg/kg i.p) (Negative control)	21.666±1.256###
GROUP III	Scopolamine (1mg/kg) + piracetam (200mg/kg i.p)	7.83±0.703****
GROUP IV	Scopolamine (1mg/kg) + low dose of EETI (200mg/kg p.o)	10.833±0.477****
GROUP V	Scopolamine (1mg/kg) + high of EETI(400mg/kg p.o)	9.833±0.600****

Values are expressed as Mean ± SEM (n=6).

###**p<0.001** compared with Control.

****p<0.0001** compared with Negative control.

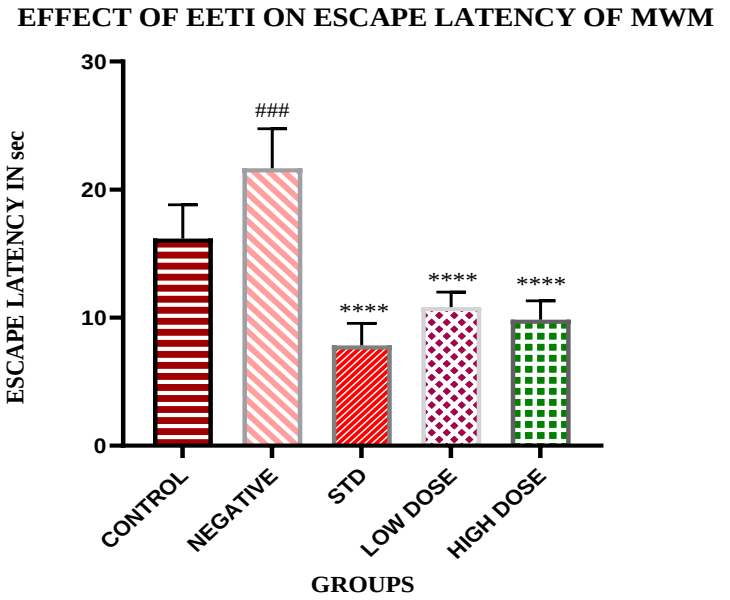

FIGURE: 1

The memory enhancing effects of *Tylophora indica* are presented in table 3. It was observed that when scopolamine administered, it has significantly (P<0.001) increased escape latency value (21.666±1.256) as compared to the normal group (16.166±1.077).

When the piracetam was administered for fourteen days at the dose of 200mg/kg, it has significantly (P<0.0001) decreased escape latency value (7.83±0.703) as compared to the scopolamine treated group.

It was observed that administration of low dose (200mg/kg) and high dose (400mg/kg) of ethanol extract *Tylophora indica* of resulted in significant decreased escape latency value (for low dose 10.833±0.477, for high dose 9.833±0.600) as compared to the scopolamine treated group. It has shown effect similar to that of piracetam.

There was an increase in escape latency in negative control group when compared with the control group (P<0.001). The high dose of *Tylophora indica* extract (400mg/kg) exhibited significant nootropic activity and the significance value of (P<0.0001) as shown in table 3.

3.2.2 Evaluation of memory enhancing activity of TI by scopolamine induced model in mice using Morris water maze

The TI extract and piracetam was administered orally to animals for 14 successive days followed by scopolamine after 1 hr of administration of extract on 14th day. On day 15 also, the escape latency was observed. The obtained result was illustrated in **Table no. 4.**

TABLE: 4 TREATMENT EFFECT ON 15TH DAY (BY MORRIS WATER MAZE)

GROUPS	DRUG TREATMENT	RETENTION-15TH DAY ESCAPE LATENCY
GROUP I	Control (vehicle treated)	17.666±0.494
GROUP II	Scopolamine (1mg/kg) (Negative control)	25.166±1.166$^{####}$
GROUP III	Scopolamine (1mg/kg) + piracetam (200mg/kg i.p)	7.666±0.494****
GROUP IV	Scopolamine (1mg/kg) + low dose of EETI (200mg/kg p.o)	13.5±0.763****
GROUP V	Scopolamine (1mg/kg) + high dose of EETI (400mg/kg p.o)	12.5±0.763****

Values are expressed as Mean ± SEM (n=6).

$^{####}$**p<0.0001** compared with Control.

******p<0.0001** compared with Negative control.

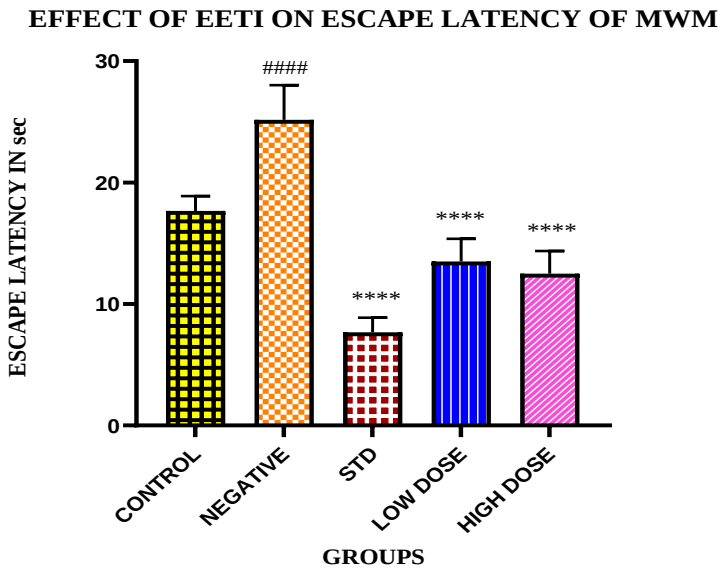

FIGURE: 2

On 15th day the memory enhancing effects of *Tylophora indica* were presented in table 4. It was observed that negative control group significantly **(P<0.001)** increased escape latency value (25.166±1.166) as compared to the normal group (17.666±0.494).

When the piracetam was administered for fourteen days at the dose of 200mg/kg, it has significantly **(P<0.0001)** decreased escape latency value (7.666±0.494) as compared to the scopolamine treated group.

It was observed that administration of low dose (200mg/kg) and high dose (400mg/kg) of ethanol extract *Tylophora indica* of resulted in significant decreased escape latency value (for low dose 13.5±0.763, for high dose 12.5±0.763) as compared to the scopolamine treated group. It has shown effect similar to that of piracetam.

There was an increase in escape latency in negative control group when compared with the control group **(P<0.0001).** The high dose of *Tylophora indica* extract (400mg/kg) exhibited significant nootropic activity and the significance value of **(P<0.0001)** as shown in table 4.

3.2.3 Evaluation of memory enhancing activity of TI by scopolamine induced model in mice using Y maze

The TI extract and piracetam was administered orally to animals for 14 successive days and on 14th day (acquisition) it was followed by scopolamine after 1 hr of administration of the extract. The obtained result was illustrated in **Table No.5.**

TABLE: 5 TREATMENT EFFECT ON 14TH DAY (Y MAZE)

GROUPS	DRUG TREATMENT	ACQUISITION-14TH DAY TRANSFER LATENCY
GROUP I	Control (Vehicle treated)	46±2.09
GROUP II	Scopolamine (1mg/kg i.p) (Negative control)	56.833±1.046####
GROUP III	Scopolamine (1mg/kg) + piracetam (200mg/kg i.p)	11.833±1.108****
GROUP IV	Scopolamine (1mg/kg) + low dose of EETI (200mg/kg p.o)	18.666±1.145****
GROUP V	Scopolamine (1mg/kg)) + high dose of EETI (400mg/kg p.o)	17±0.96****

Values are expressed as Mean ± SEM (n=6).

####**p<0.0001** compared with Control.

****p<0.0001** compared with Negative control.

EFFECT OF EETI ON TRANSFER LATENCY OF Y MAZE

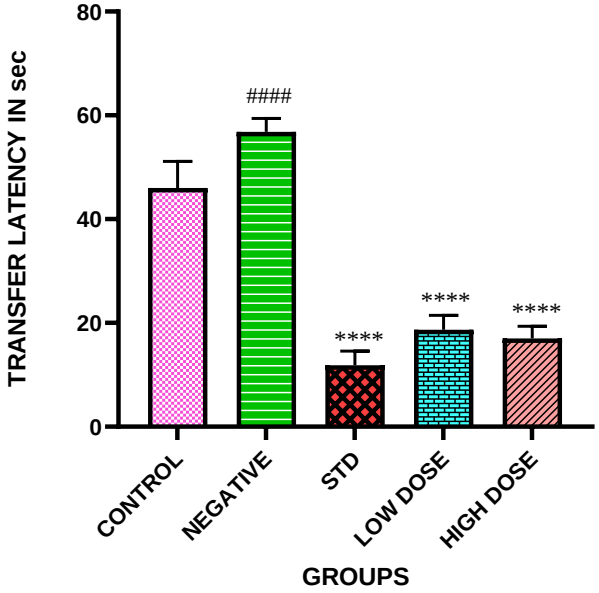

FIGURE: 3

The memory enhancing effects of *Tylophora indica* are presented in table 5. It was observed that when scopolamine administered, it has significantly **(P<0.0001)** increased transfer latency value (56.833±1.046) as compared to the normal group (46±2.09).

When the piracetam was administered for fourteen days at the dose of 200mg/kg, it has significantly **(P<0.0001)** decreased transfer latency value (11.833±1.108) as compared to the scopolamine treated group.

It was observed that administration of low dose (200mg/kg) and high dose (400mg/kg) of ethanol extract *Tylophora indica* of resulted in significant decreased transfer latency value (for low dose 18.666±1.145, for high dose 17±0.96) as compared to the scopolamine treated group. It has shown effect similar to that of piracetam.

There was an increase in transfer latency in negative control group when compared with the control group **(P<0.0001).** The high dose of *Tylophora indica* extract (400mg/kg) exhibited significant nootropic activity and the significance value of **(P<0.0001)** as shown in table 5.

3.2.4 Evaluation of memory enhancing activity of TI by scopolamine induced model in mice using Y maze

The TI extract and piracetam was administered orally to animals for 14 successive days followed by scopolamine after 1 hr of administration of extract on 14th day. On day 15 also, the transfer latency was observed. The obtained result was illustrated in **Table No**.6.

TABLE: 6 TREATMENT EFFECT ON 15TH DAY (Y MAZE)

GROUPS	DRUG TREATMENT	RETENTION-15TH DAY TRANSFER LATENCY
GROUP I	Control (Vehicle treated)	52.833±0.945
GROUP II	Scopolamine (1mg/kg i.p) (Negative control)	58.833±0.666$^{###}$
GROUP III	Scopolamine (1mg/kg) + piracetam (200mg/kg i.p)	15±0.577****
GROUP IV	Scopolamine (1mg/kg) + low dose of EETI (200mg/kg p.o)	28±0.966****
GROUP V	Scopolamine (1mg/kg)) + high dose of EETI (400mg/kg p.o)	22.5±0.763****

Values are expressed as Mean ± SEM (n=6).

$^{###}$p<0.001 compared with Control.

****p<0.0001 compared with Negative control.

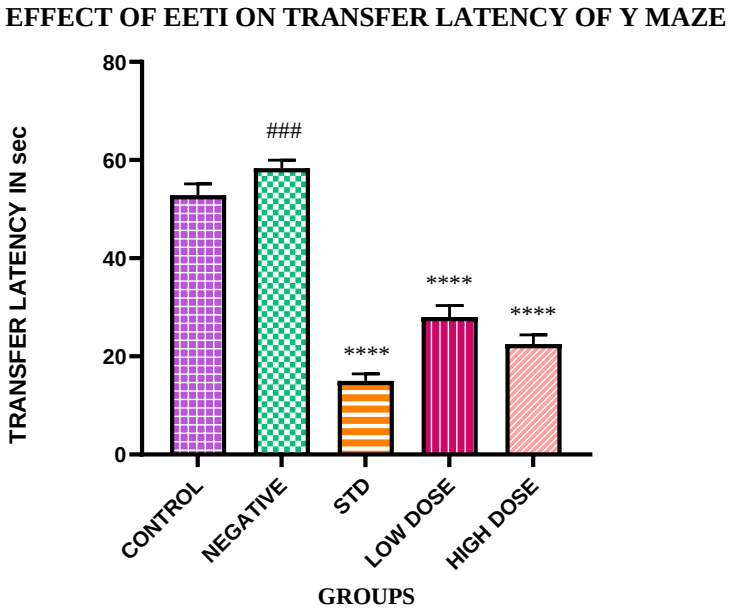

FIGURE: 4

On 15th day the memory enhancing effects of *Tylophora indica* were presented in table 6. It was observed that negative control group significantly (P<0.001) increased transfer latency value (58.833±0.666) as compared to the normal group (52.833±0.945).

When the piracetam was administered for fourteen days at the dose of 200mg/kg, it has significantly **(P<0.0001)** decreased transfer latency value (15±0.577) as compared to the scopolamine treated group.

It was observed that administration of low dose (200mg/kg) and high dose (400mg/kg) of ethanol extract *Tylophora indica* of resulted in significant decreased transfer latency value (for low dose 28±0.966, for high dose 22.5±0.763) as compared to the scopolamine treated group. It has shown effect similar to that of piracetam.

There was an increase in transfer latency in negative control group when compared with the control group (P<0.001). The high dose of *Tylophora indica* extract (400mg/kg) exhibited significant nootropic activity and the significance value of **(P<0.0001)** as shown in table 6.

3.3 EX VIVO STUDY

3.3.1 Estimation of AChE activity of EETI by scopolamine induced model in mice

All the animals were sacrificed after the *invivo* study and the brain was isolated for measuring the Acetylcholinesterase activity using *exvivo* method. The obtained result was expressed in **Table No.7.**

TABLE: 7 EFFECT OF EETI ON ACETYLCHOLINESTERASE ACTIVITY

GROUPS	DRUG TREATMENT	ACETYLCHOLINESTERASE ACTIVITY (µ moles)
GROUP I	Control (Vehicle treated)	55.166±1.579
GROUP II	Scopolamine (1mg/kg i.p) (Negative control)	69.833±1.137####
GROUP III	Scopolamine (1mg/kg) + piracetam (200mg/kg i.p)	20.5±0.763****
GROUP IV	Scopolamine (1mg/kg) + low dose of EETI (200mg/kg p.o)	38±1.290****
GROUP V	Scopolamine (1mg/kg) + high dose of EETI (400mg/kg p.o)	29.333±1.054****

Values are expressed as Mean ± SEM (n=6)

####**p<0.0001** compared with Control.

******p<0.0001** compared with Negative control.

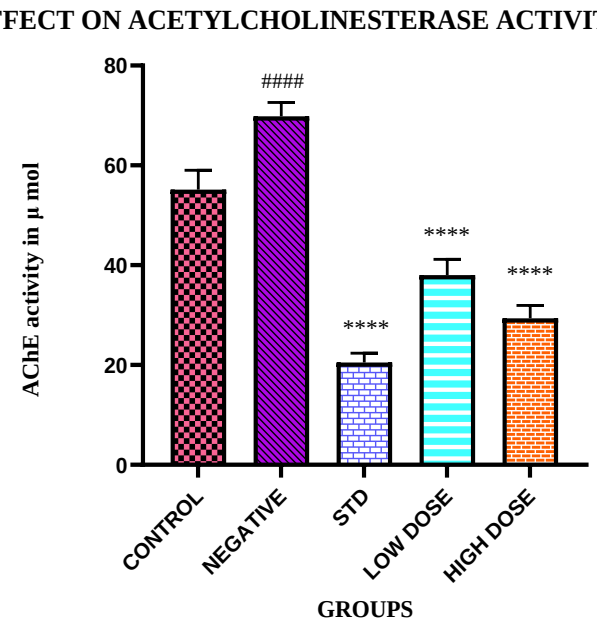

FIGURE: 5

In this study level of AChE in the whole brain homogenate of all group animals, this was used to assess the nootropic activity. The effects of *Tylophora indica* extract on

acetylcholinesterase were presented in table 7. It was observed that negative control group has significantly **(P<0.0001)** increased AChE value (69.833±1.137) as compared to the normal group (55.166±1.579).

The piracetam administered group has significantly **(P<0.0001)** decreased AChE value (20.5±0.76) as compared to the scopolamine treated group.

It was observed that low dose (200mg/kg) and high dose (400mg/kg) of EECE administered group resulted in significant decreased AChE value (for low dose 38±1.290, for high dose 29.333±1.054) as compared to the scopolamine treated group. It has shown similar effect to that of piracetam.

There was an increased AChE value in negative control group when compared with the control group **(P<0.0001).** The high dose of *Tylophora indica* extract (400mg/kg) exhibited significant nootropic activity (P<0.0001) as shown in table 7.

The nootropic effects observed might be attributed to the presence of flavonoids and alkaloids, which have been reported to possess neuroprotective properties.

CONCLUSION

The phytochemical constituents such as alkaloids, sterols and flavonoids present in *Tylophora indica* may improve memory by inhibiting the enzyme Acetylcholinesterase. This process may inhibited by EETI which also provides neuroprotection due to its phytoconstituents.

In the present study, ethanol extract of *Tylophora indica* significantly decreased the AChE level in the mice whole brain homogenate, indicating its potential in the attenuation of severity of Alzheimer's disease.

In the present study was showed that ethanol extract of *Tylophora indica* exhibited,

- Decreased escape latency and transfer latency value
- Decreased acetylcholinesterase enzyme levels

The phytoconstituent **quercetin, kaempferol and sigmasterol** present in the *Tylophora indica* extract, which may be the responsibility for memory enhancement activity.Thus a combination of anticholinesterase activity and presence of kaempferol, quercetin and

sigmasterol exhibited by *Tylophora indica* may be eventually responsible for memory improving effect was observed in the present study.

The *Tylophora indica* ethanol extract contains majorly flavonoids, alkaloids and sterols which may responsible for the nootropic effect. The extract evaluated that is safer to animal inhibiting AChEs level facilitating Ach level to improve memory enhancement. This study suggests that the ethanolic extract of *Tylophora indica* leaves may have nootropic effects, providing a potential therapeutic option for amnesia, warranting further research.

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