International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF NOOTROPIC ACTIVITY OF ETHANOLIC EXTRACT OF *EUPHORBIA CYATHOPHORA* STEMS ON MICE

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ABSTRACT

Euphorbia Cyathophora is a naturally occurring plant that is a member of the Euphorbiaceae family. This plant's stems have therapeutic qualities and are very nutritious. It is a native of Mexico. Although *Euphorbia Cyathophora* has many applications, its primary uses are in the treatment of skin infections, constipation, laxatives, and digestive issues. The current study provides an explanation for the ethanolic extract of *Euphorbia Cyathophora* (ECEE) stems' ability to improve memory. For nine days, the extract was taken orally in two doses of 100 mg and 200 mg per kg. The herbal extract enhanced memory and lessened the amnesia caused by diazepam (1 mg/kg). Behavioral models such as the Morris water maze, Labyrinth maze, and Hebb's William maze were used to describe memory improvement. Research has demonstrated that the existence of flavonoids improved memory.

Keywords: *Euphorbia Cyathophora*, Euphorbiaceae, Hebb's William maze, Labyrinth maze, Morri's water maze

INTRODUCTION:

Alzheimer's disease is the most prevalent type of dementia, With at least 27 million cases and 60–70% of all dementia cases. The incidence of this illness has a substantial effect on the patient's family in along with

having a large financial impact on society. From an anatomy perspective, AD is specified by two typical lesions: 1) Neurofibrillary tangles: these are intraneuronal deposits of phosphorylated tau

protein (P-tau); they consist of extracellular lesions called β-amyloid protein accumulation (Aβ42). Apart from raising the possibility of intraparenchymal spaces, β-amyloid protein deposition can also happen in arteries, arterioles, and capillary walls. This can result in amyloid cerebral angiopathy, a condition that impairs circulation and leads to degeneration of vascular wall components.

A language and visuospatial deficit follow an increasing decline in mental capacity and episodic memory in the typical presentation of AD. Behavioral disorders like depressive disorders, aggression, and apathy are often present alongside these changes. Notably, a sizable portion of AD patients do not fit the conventional amnestic profile and display non- amnestic deficits from the beginning of symptoms. When vascular and frontotemporal dementias are ruled out as additional potential causes, patients exhibiting typical disease features are most likely diagnosed with AD. Histologically identifying the neurofibrillary tangles and senile plaques through postmortem examination is typically the only way to reach a conclusive diagnosis of the disease [1].

Alzheimer's disease symptoms consist of the following:

- loss of memory
- deficiency in cognition

- issues with awareness
- issues at spatial consciousness
- difficulties with speech, language, communication
- personality or behaviour changes [2]

With Alzheimer's disease (AD) accounting for more than 80% of dementia cases in senior citizens worldwide, AD is the most typical neurodegenerative disease. The proportion of Indians 60 and older is predicted to increase to nearly 20% of the nation's the entire population by the year 2050 (319 million people), or 15.4% of all older people worldwide [3]. Alzheimer's dementia affects an estimated 6.7 million Americans who are 65 years of age or older. Alzheimer's condition was formally ranked as the 6th most common reason for mortality in the US [4].

The incidence of dementia associated with AD with a clinical manifestation rises with age. AD dementia is also more frequent in women than in men, according to a meta-analysis that found 7.13% of women and 3.31% of men were affected by the condition, as well as a systematic review in which all five studies that included occurrence in men and women stated an increased incidence in women [5].

Euphorbia Cyathophora, such as painted leaf, wild poinsettia, fire on the mountain, and Catalina dwarf poinsettia. *Euphorbia Cyathophora Murray* is a naturally occurring

species in Mexico that has the potential to be both ornamental and medicinal. Its characteristics include brightly colored bracts, black, cylindrical or ovoid seeds, and leaf dimorphism (oval, lanceolate, or sublinear, whole or sawed, smooth or with scare follicles) [6].

In folk medicine, *Euphorbia Cyathophora* is used mainly for Constipation, Asthma, Laxative, Wound healing ability, Digestive problems, Diarrhea, Fever, Snake bites, High cholesterol, Skin infections, treating worms, Rheumatism, some people used to cause vomiting [7].

MATERIALS AND METHODS

Plant material

Fresh stems of *Euphorbia Cyathophora* have been procured from the Chalapathi Institute of Pharmaceutical Sciences, Guntur. Plant-based matter was taxonomically identified and authenticated by Dr. P. Satyanarayana Raju Garu M. Sc., M. Phil., Ph. D from Department of Botany and Microbiology, Acharya Nagarjuna Nagar, AP on 21st November 2023.

The recently harvested stems were broken into tiny pieces and allowed to dry in the shade. The material that had fully dried was ground into a coarse powder using a mechanical grinder. It was then evenly powdered and kept in an airtight container after passing through 40 mesh sieves.

Preparation of the plant extract:

The fifty grams of botanical matter were extracted using the hot continuous Soxhlet extraction technique. The resulting substance was dried, concentrated, and filtered using a rotary evaporator. The extract was gathered, kept at a comfortable temperature in a container, and utilized in additional research projects.

Preliminary Phytochemical screening:

Different phytochemical constituents in *Euphorbia Cyathophora* were observed in the presence or absence using standard methods, including alkaloids (Wagner's test), flavonoids (Shinoda test), tannins (Ferric chloride test), triterpenes along with steroids (Liberman- Burchard's test), terpenoids (Salkowski test), glycosides (Borntrager's test), saponins (Foam test), and proteins (Biuret test).

DRUGS:

The drugs used within this research are diazepam injection, piracetam (Nootropil) (Dr. Reddy's Laboratories, Uttarakhand)

VEHICLE:

Plant extract (ECEE) was suspended in carboxy methyl cellulose and administered orally to mice. Piracetam and Diazepam were dissolved individually in normal saline and injected i. p. Volume of oral administration and i. p injection was 1ml/1000g of mice.

DRUG TREATMENT:

The mice in this investigation were divided into five groups in order to test their memory. Every group has five animals in it.

Group 1 was given only saline treatments and served as the control group. Group 2 is depicted as the inducing group, receiving only diazepam. Group 3 is given both standard medication piracetam and diazepam, representing the standard. As test groups, groups 4 and5 are given diazepam in varying dosages (100 mg and 200 mg/kg) of the extract.

For nine days in a row, all of the animals in the control group were given cars. ECEE was given orallyto Groups 3 and 4 for nine consecutive days, with water acting as the solvent. The training session with Hebbs William, the labyrinth maze, and the Morris Water Maze was presented to the mice thirty minutes after the last dose was administered (on the ninth day).

MORRIS WATER MAZE [8]

Morri"s water maze- (MWM) for mice considered of a circular pool (60 cm in diameter, 25 cm in height) filled to a depth of 20 cm with water maintained at 25 degrees Celsius. The water was made opaque with nontoxic white coloured dye. Through the assistance of 2 strings that were settled at right angles to one another on the pool's rim, the water chamber was split into 4 similar sections. Within the pool's target areas, an underwater structure measuring 6 cm by 6 cm and painted white was set up 1 cm below the liquid's surface. during the instructional period, the podium's location remained constant. Over the course of four consecutive days, every animal was put through four separate trails spaced five minutes apart. The method of instruction involved submerging the rodent carefully beneath the water within all the quadrants pointing toward the pool wall, through a different fall spot for every trail. The mice were given 120 seconds in which to find the submerged platform. The mouse was carefully directed onto the elevated surface and given twenty seconds to stay there in case it failed to locate it in a period of 120 seconds. The period of time it takes an individual to travel from it's beginning the quadrant to the go after quadrant's concealed framework is known as escape latency (EL). For every animal, EL collected data from the sixth to the ninth day. Every animal underwent a total of four days of learning trails; the desired quadrant stayed unchanged during the entire time, and the initial location was altered for every contact as detailed underneath.

Day 1 Q1 Q2 Q3 Q4 Day 2 Q2 Q3 Q4 Q1 Day 3 Q3 Q4 Q1 Q2 Day 4 Q4 Q1 Q2 Q3

The scaffolding was taken down on day 5, or the tenth day of the medication treatment, and the mice travelled around the zones for a duration of three hundred seconds. The average amount of time spent in each of these quadrants—Q1, Q2, and Q3—was noted. The measure of recovery or recall was defined as the average amount time that was used searching to find the missing

framework in the intended quadrant. The individual being observed maintained a similar posture at all times the angle of the water's maze in relation to other testing things was carefully monitored.
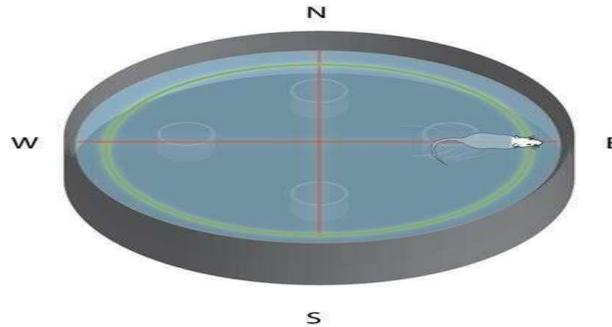

Figure 1: Morris Water Maze

LABRYNTH MAZE [9]

Labyrinth maze is another best-known device for studying the learning, remembering (memory) and reasoning in animals. Labyrinth maze contains channel or tunnel which is formed by number of Y figures for exploring by the animals like mice and rats the mazes are connected alternatively oppositeto each other in a continuous line and ending of mazes is like cone shape.

There are up to five phases in the mazes, which are organized in a pattern of zigzags. The dimensions of the various stages were roughly 15 cm by 30 cm. The equipment covering the mazes has an upper roofing that is roughly sixty centimetres broad and 55 centimetres long aswell. Three rooms make up the labyrinth maze, just like in a rectangular maze.

1. Chamber- A wherein an animal is situated. Its door that slides can be widened for letting the mice into the maze.

2. The maze that the animal must investigate (chamber C)

3. Chamber- B at the conclusion of the labyrinth where the prize is located.

Each of these maze divisions has its own hinge. Top covers 50 keep the conditions beneath the maze consistent and stop external stimuli or clues from reaching the animal.

Whenever a rodent is put inside the box, a power supply indicates the situation. The vibrations of the detector layers in rooms A and B detect the arrival of rodents by blinking a lamp at the animal's existence measure and recording times that are not seen on the electronic shows.

Procedure:

- A set of rats is taught how to carry out the pharmacological activity using tools.

- Prior to entering the labyrinth, close the passageway entrance leading to chamber A.

- Once the animal is in room A, the screen indicates that it is prepared and the A indicator lights up.

- Shut the upper lids of all 3 sections; do not open the equipment so the animal can get used to its new surroundings within the maze.

- Once the animal has had enough time to adjust to its new surroundings, clear the sliding door.

- As soon as the animal enters the maze and exits its space, the "A" light will go out. The "C" light will begin to glow at the same moment as the counter begins to countdown the seconds that have passed.

- From the moment as the animal goes into chamber B, the final area, the recording will stop and the B indicator will light up.

- The letter B denotes the conclusion of the section and the procedure's finish; the timer's collected analyzing will be the total amount of seconds the animal spent Going through.

Figure 2: Labrynth Maze

HEBB- WILLIAMS MAZE [10]

Hebb- William's maze is behavioural model for measuring memory. It is partitioned into 3 compartments: an animal chamber (or start box), which is connected to the middle chamber (or exploratory area), and a reward chamber at the other side of the maze in which the reward (food) is provided. Before the experiment animals are habituated to the maze for significant results. On the first day (i.e., 8th of drug treatment), the rat was allowed in the animal chamber or initiate box and the entrance were opened to let the animal enter the next chamber, but as soon as the animal moved, the door was securely locked to keep it from coming back in. Here time is taken as the parameter to measure the activity.

Time is carried by the animal to go from the start box to the prize chamber was noted on 1st day (training session) every animal is permitted to spend three minutes exploring the maze with all of the doors accessible prior to going back to its own cage. Holding onto this learned task (memory) was looked at 24 hrs after 1st day trail (i.e., 9th day, 24 hrs after last dose).

i) Chamber A, wherein the rat is placed. When the sliding entrance opens up, the rodent may enter the maze.

ii) Chamber C the maze the creature needs to

examine.

iii) Chamber B, at the opposite side of the maze where the prize resides. The time

taken by rat to traverse the maze is noted.

Figure 3: Hebbs Williams Maze

RESULTS

Preliminary phytochemical screening

The result of phytochemical screening shows the existence of alkaloids, glycosides, carbohydrates, flavonoids, phenols, proteins, saponins, tannins by utilizing conventional techniques.

Hebb's William maze:

Effect of transfer latency (using Hebb's-williams maze)

Time required to arrive at the reward chamber is described as latency and it explains about recollections of the rodents on the 9th day.

ECEE (100mg/kg and 200mg/kg) administered orally for 9 days have shown substantial impact on latency of transfers equal to standard piracetam.

Amnesia was induced by diazepam (1mg/kg) i. p. have increased the latency scores of creatures that were significantly reduced by standard piracetam and ECEE. ($p<0.0001$) represent notable decrease in latency scores which consequently represents improvement in memory levels which were decreased by diazepam (1mg/kg i. p.).

Table 1: Latency scores of Hebbs William maze

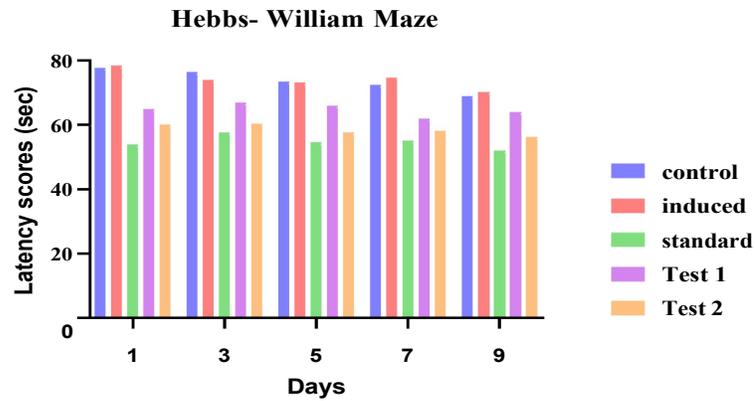| Days	Control	Induced	Standard	Test 1	Test 2
1	77.75±1.31	78.50±1.04ⁿˢ	54.00±1.22****	65.00±1.54****	60.25±1.08****
3	76.50±1.04	74.00±1.47ⁿˢ	57.75±1.25****	67.00±1.32****	60.50±1.65****
5	73.50±0.85	74.25±1.75ⁿˢ	54.75±0.85****	66.00±1.03****	57.75±1.47****
7	72.50±1.04	74.25±1.75ⁿˢ	55.25±1.10****	62.00±1.65****	58.25±0.64****
9	69.00±1.08	70.00±1.58ⁿˢ	52.00±0.91****	64.00±0.40****	56.00±1.43****

Hebbs- William Maze

Figure 4: Hebbs Williams Maze Graph
Data represent Mean ±SEM, (n=5). Two- way Anova followed by Tukey's multiple comparisons test.Significance was shown at $p < 0.0001$ in contrast to control. **** denotes $p < 0.0001$ which is compared with control group of mice.

Labyrinth maze

Time required for arriving at the reward chamber is described as latency and it explains about memories of the mice on the 9th day. ECEE (100mg/kg and 200mg/kg) administered orally for 9days have shown A major effect on a latency period of transfers equal to standard piracetam. Amnesia was induced by diazepam (1mg/kg) i. p. have increased the latency scores of mice that were significantly reduced by standard piracetam and ECEE. ($p < 0.0001$) represent significant reduction in latency scores which in turn represents improvement in memory levels which were decreased by diazepam (1mg/kg i. p.).

Table 2: Latency scores of Labyrinth maze

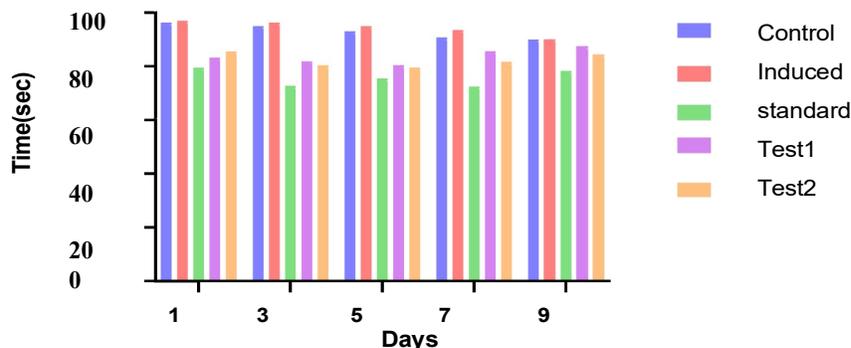| Days	Control	Induced	Standard	Test 1	Test 2
1	96.25±0.85	97.00±0.91[ns]	79.50±0.64****	83.25±0.52***	85.56±0.65****
3	95.00±1.08	96.25±1.25[ns]	72.75±1.10****	81.85±0.42***	80.36±1.71****
5	93.00±1.80	95.00±1.29[ns]	75.50±1.19****	80.46±1.56***	79.62±1.34****
7	90.75±0.75	93.50±1.32[ns]	72.50±0.86****	85.64±0.92***	81.73±0.23****
9	90.00±1.22	89.75±1.25[ns]	78.00±0.91****	87.52±1.15***	84.34±1.09****

Labyrinth Maze

Figure 5: Labyrinth Maze Graph
Data represent Mean ±SEM, (n=5). Two- way Anova followed by Tukey's multiple comparisons test. Significance was shown at $p < 0.0001$ when compared with control. **** denotes $p < 0.0001$ which is compared with control group of mice and *** denotes 0.0004.

Morris water maze

The learning and memory enhancement activity of leaf extract of *Euphorbia Cyathophora* using morris water maze.

Table 3: Learning Scores And Memory Scores By Morri's Water Maze

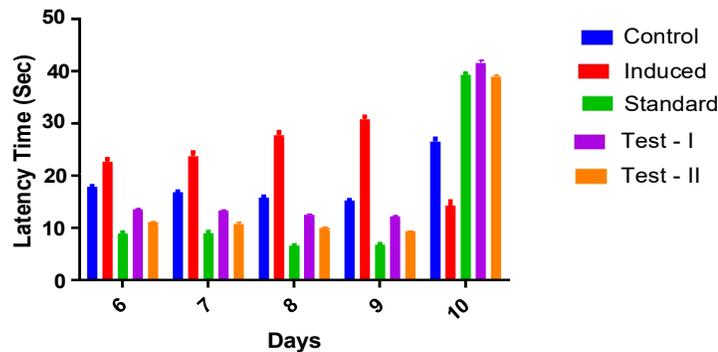| Days	Control	Induced	Standard	Test 1	Test 2
6	17.85±0.17	22.65±0.56	8.92±0.21	13.55±0.13	11.05±0.10
7	16.80±0.17	23.70±0.81	8.95±0.27	13.25±0.16	10.77±0.22
8	15.80±0.21	27.77±0.61	6.57±0.12	12.47±0.14	9.97±0.12
9	15.22±0.11	30.75±0.59	6.80±0.12	12.17±0.11	9.35±0.06
10	26.50±0.64	14.25±0.85	39.30±0.33	41.55±0.51	38.95±0.25

Figure 6: Morris Water Maze Graph

DISCUSSION

Due to the high level of flavonoids, a class of phytochemical constituent, the ECEE has demonstrated a significant effect at a dose of 200 mg/kg, which is comparable to the standard piracetam 400 mg/kg. According to the research, ECEE exhibits standard-level useful memory enhancement activity. The Hebb's William maze, the labyrinth maze, and the Morris water maze are common techniques used to assess memory.

STATISTICALANALYSIS

The mean standard error (SEM) accustomed to represent all of the above results. Two-way ANOVA was used to analyse the data, and Tukey's multiple comparison test was then performed. A P value of less than 0.0001 was deemed statistically significant.

CONCLUSION

Euphorbia Cyathophora stem extract was used to standardize and assess behavior models for research on medications or illnesses that impact cognitive functions. The Labyrinth maze, Hebb's William maze, and Morri's water maze has demonstrated noteworthy activity that improves memory in response to extract of ethanol of *Euphorbia Cyathophora*. The process underlying memory enhancement activity may be discovered through more research on ECEE.

ACKNOWLEDGEMENTS

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