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Unveiling the Neurocognitive Potential: A Comprehensive Evaluation on Acetylcholine and Memory-Enhancing Activity of Ethanol Extract from *Ficus benghalensis in* Scopolamine-Induced Amnesia

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Sunkara Sivananda Swaroop[1*], Dudday Venkata Vyshnavi[1], Jeethuri Rajesh[1]

[1]Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research (Riper) – Autonomous, Chiyyedu (Post), Anantapuram, Andhra Pradesh, India-515721.

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ABSTRACT

Ficus benghalensis, is a medicinal plant with potential pharmacological properties. This study aimed to comprehensively evaluate the memory-enhancing activity of an ethanol extract from *Ficus benghalensis* and its impact on acetylcholine levels in a scopolamine-induced amnesia model. Albino Wister rats were used for the experiments. The ethanol extract from *Ficus benghalensis* was prepared and administered to the rats at a dose of 100, 200 mg/kg. The scopolamine-induced amnesia model was established by injecting scopolamine. The memory and cognitive function of the rat were assessed using appropriate behavioral tests, such as the elevated plus maze, and passive avoidance test. Acetylcholinesterase activity was measured using specific biochemical assays. The ethanol extract from *Ficus benghalensis* at a dose of 200 mg/kg exhibited significant memory enhancement in the scopolamine-induced amnesia model. The treated group showed improved performance compared to the control group. Furthermore, the extract demonstrated inhibitory effects on acetylcholineesterase activity, leading to increased levels of acetylcholine. These findings contribute to the understanding of the neurocognitive potential of *Ficus benghalensis* and its therapeutic implications in memory-related disorders.

INTRODUCTION

Ayurveda holds the unique position of being recognized as the "oldest documented medical system in existence, as well as the most ancient and all-encompassing spiritual teachings globally." Its foundation lies in the belief of establishing equilibrium between the interconnected aspects of the body and mind. By comprehending their body and mind, individuals are guided to form a harmonious connection with the natural world. Within Ayurvedic texts, one can find remedies for ailments related to aging, such as memory loss, osteoporosis, and diabetic wounds, which lack effective solutions in contemporary medicine [1].

Amnesia is a profound memory loss which is usually caused either by physical injury to the brain or by the ingestion of a toxic substance which affects the brain. In addition, the memory loss can be caused by a traumatic, emotional event, shock, illness or sometimes induced by anesthesia. A French psychologist Theodule-Armand Ribot was among the first scientists to study amnesia. Because of this, medical experts started to call the gradients of memory loss as Ribot gradients. He proposed Ribot's Law which states that there is a time gradient in retrograde amnesia. The law follows a logical progression of memory loss due to disease [2].

Alzheimer's disease is a degenerative brain condition characterized by a gradual and irreversible decline in memory, cognitive abilities, and the capacity to perform basic everyday tasks. Typically, symptoms of Alzheimer's manifest around the age of 65 or later. Although estimates vary, it is believed that over 5 million individuals in the United States may be affected by this disease. Presently, Alzheimer's disease is recognized as the sixth primary cause of death in the country. However, recent estimations suggest that it may rank as the third leading cause of death among older individuals, following closely behind heart disease and cancer [3].

The precise causes of Alzheimer's disease in the majority of cases are not yet fully understood by scientists. However, when it comes to early-onset Alzheimer's, it is often attributed to a genetic mutation. Late-onset Alzheimer's, on the other hand, is believed to stem from a multifaceted process of brain changes that develop gradually over several decades. The causes of late-onset Alzheimer's likely involve a combination of genetic, environmental, and lifestyle factors. It's important to note that the significance of each of these factors in terms of increasing or decreasing the risk of developing Alzheimer's can vary from person to person.

As per Ayurvedic texts, the Ficus genus, which includes *Ficus benghalensis* (Nyagrodha), Ficus glomerata/Ficus racemosa (Udumbara), Ficus lacor/Ficus retusa (Plaksha), and Ficus religiosa (Ashvattha), all possess latex. The bark and leaves of these plants are known for their astringent, haemostatic, anti-inflammatory, and antiseptic properties. They are commonly prescribed in the treatment of conditions such as diarrhea, dysentery, skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia, and deficient lactation. The Banyan tree, known for its immense size and wide-spreading branches, has been traditionally utilized in various health problems and diseases within the field of traditional medicine. [4,5]. The objective of this study is to provide a comprehensive overview of the traditional medicinal investigations conducted on the extract of *Ficus benghalensis* bark, specifically focusing on its reported anti-allergic and anti-stress activities. Previous research has demonstrated that both aqueous and methanolic extracts of *Ficus benghalensis* bark exhibit anti-helminthic properties [6,7]. Additionally, ethanolic and petroleum ether extracts of *Ficus benghalensis* have been found to possess anti-inflammatory activity, while the chloroform extract of its fruit shows anti-tumor and antimicrobial activities. The ethanolic extract of *Ficus benghalensis* root demonstrates anti-diarrheal properties [11,12]. Furthermore, extracts of *Ficus benghalensis* bark have been reported to exhibit analgesic and antipyretic activity [13,14], while extracts from its leaves and bark show anti-allelopathic effects. The extract of *Ficus benghalensis* bark also demonstrates hypolipidemic activity, and the methanolic extract exhibits immunomodulatory activity [15,16]. So, the present study was designed for evaluation of acetylcholine and memory enhancing activity with leaves of ethanolic extracts of *Ficus benghalensis* using Wistar albino rats.

MATERIALS AND METHODS

- **Plant material Collection and Extraction**

The Leaves of *Ficus benghalensis* were collected from the local gardens of Krishnamreddy Palli, Anantapur district, Once the collection process was completed, the leaves underwent a thorough cleaning procedure. Firstly, any visible dirt, debris, or insects were removed from the surface of the leaves by gently rinsing them under running water Following the cleaning process, the leaves were air-dried in a well-ventilated area, away from direct sunlight, until they reached a moisture-free state and then finely powdered and the resulting leaf powder was then sieved to ensure uniform particle size and remove any larger plant fragments.

In clean, wide-mouthed glass containers with tight-fitting lids, a measured quantity of leaf powder was placed. Suitable solvent, such as ethanol, is added to completely immerse the leaf powder, maintaining the desired extraction ratio. The mixture is thoroughly stirred to ensure proper wetting and dispersion of the powder. The containers were tightly sealed, labeled, and stored in cool, dark locations to allow for maceration over a predetermined period, typically 24 to 48 hours. After the maceration period, the liquid extract is separated from the solid residue by filtration using filter paper or appropriate filtration apparatus. Filtrate is collected, the extracts is concentrated using rotary evaporation to remove the solvent and obtain concentrated extract. Finally, the extract is subjected to freeze-drying to obtain dry extract powder, which was stored in airtight containers for further analysis and use.

- **Preliminary Phytochemical Screening**

The ethanolic extract of leaves of *Ficus benghalensis* was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponins, tannin, and glycosides. All the extracts subjected for estimation of tannin, and phenolic content [17].

1. Alkaloids:

a. Mayer's Test:

2-3 mL of the plant extract was taken in a test tube. Added a few drops of Mayer's reagent (potassium mercuric iodide) to the extract.

b. Wagner's Test:

A fresh solution of Wagner's reagent (iodine in potassium iodide) was prepared in a test tube. Added a few drops of the solution to the plant extract and observed for the formation of a reddish-brown precipitate, confirming the presence of alkaloids.

c. Dragendorff's Test:

Prepared Dragendorff's reagent (bismuth potassium iodide) in a test tube. Added a few drops of the reagent to the plant extract. and observed for the formation of an orange-red precipitate, indicating the presence of alkaloids.

2. Flavonoids:

a. Shinoda Test:

A small amount of magnesium turnings were added to the plant extract in a test tube. Followed by a few drops of concentrated hydrochloric acid and for the formation of a pink, red, or magenta color, indicating the presence of flavonoids.

b. Ferric Chloride Test:

A few drops of the plant extract mixed with 2 drops of 10% ferric chloride solution in a test tube. And observed for the development of a color change, such as yellow, orange, or red, indicating the presence of flavonoids.

c. Lead Acetate Test:

Added a few drops of lead acetate solution to the plant extract in a test tube. Identification of the formation of a yellow precipitate, confirming the presence of flavonoids.

3.Saponins:

a. Froth Test:

Shook 5 mL of the plant extract vigorously in a test tube for a few minutes. And observed for the formation of a stable froth that persisted for at least 15 minutes, indicating the presence of saponins.

b. Hemolysis Test:

Mixed a small amount of the plant extract with a drop of blood on a glass slide. Observed for the formation of a clear zone around the mixture, indicating hemolysis and confirming the presence of saponins.

c. Foam Test:

Shook 5 mL of the plant extract vigorously with distilled water in a test. The formation of a persistent foam that remained for several minutes, indicating the presence of saponins.

4. Tannins:

a. Ferric Chloride Test:

Mixed a few drops of 10% ferric chloride solution with the plant extract in a test tube. Observed for the formation of a bluish-black or greenish-black color, indicating the presence of tannins.

b. Gelatin Test:

Mixed a small amount of gelatin solution with the plant extract in a test tube. Observed for the formation of a white precipitate or turbidity, indicating the presence of tannins.

c. Lead Acetate Test:

Added a few drops of lead acetate solution added to the plant extract in a test tube, observed for the formation of a white or creamy precipitate, confirming the presence of tannins.

5. Glycosides:

a. Legal's Test:

The plants extract treated with glacial acetic acid containing a few drops of ferric chloride solution. Added a few drops of concentrated sulfuric acid along the sides of the test tube.

b. Keller-Kiliani Test:

A few drops of the plant extract were mixed with glacial acetic acid and a small amount of concentrated sulfuric acid in a test tube. Heated the mixture gently and observed for the development of a red or violet color, indicating the presence of glycosides.

c. Baljet Test:

A few drops of the plant extract added to a test tube containing a mixture of sodium picrate and sodium hydroxide. Observed for the formation of a yellow or orange precipitate, confirming the presence of glycosides.

6. Phenolic compounds:

a.Ferric Chloride Test:

A few drops of a dilute solution of ferric chloride (FeCl3) are added to the test extract. A color change, usually from yellow to green, blue, purple, or reddish-brown, indicates the presence of phenolic compounds. The intensity of the color change can provide an estimation of the concentration of phenolics.

b.Shinoda Test:

A small amount of extract is treated with a few drops of concentrated hydrochloric acid (HCl) followed by a few drops of a 1% solution of sodium hydroxide (NaOH). A change in color, typically to red, pink, or magenta, indicates the presence of flavonoids.

c.Lead Acetate Test:

A small amount of the extract is mixed with a few drops of lead acetate solution (often referred to as lead subacetate solution) and observed for any color change. The formation of a black precipitate or a dark color indicates the presence of phenolic compounds.

- **Experimental animals**

The experiments were conducted using Wister rats weighing between 150-180 grams. Each group consisted of 8 animals. The rats were randomly placed and assigned to different treatment groups, and they were housed in polypropylene cages with paddy husk as bedding. The animals were kept in an environment with a temperature of 24 ± 2 degrees Celsius and a relative humidity ranging from 30% to 70%. A regular light-dark cycle of 12 hours of daylight followed by 12 hours of darkness was maintained for the rats.

All animals were allowed free access to water and fed. Ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC) (Reg.No.878/PO/Re/S/05/CPCSEA) constituted for animal experimentation as per CPCSEA guidelines.

- **Toxicity studies**

According to the guidelines provided by the Organization for Economic Cooperation and Development (OECD) in their guideline 423, an acute oral toxicity study was conducted using Ethanolic Extract *Ficus benghalensis* (EEFB). The study involved administering EEFB

orally (p.o.) to test rats at various dose levels, namely 5, 50, 300, and 2000 mg/kg body weight. It was observed that when EEFB was administered at a dose of 2000 mg/kg body weight, there were no abnormal behavioral changes observed in the animals. Additionally, all the animals subjected to the test survived, indicating that EEFB exhibited low acute oral toxicity. Based on the results, the oral LD50 (the dose at which 50% of the animals would be expected to die) of EEFB in Rat was determined to be 100 mg/kg body weight.

- **Dose selection:**

To evaluate the potential anti-alzheimer's activity of EEFB (Ethanolic Extract *Ficus benghalensis* using animal models, two dose levels were selected. The dose selection was based on the acute toxicity study, ensuring a safe and effective range of doses. The low dose chosen was approximately one-twenth of the maximum dose administered during the acute toxicity study, which corresponded to 100 mg/kg. The high dose was selected to be twice that of the one-tenth dose, resulting in a dose of 200 mg/kg. This dose regimen allowed for the assessment of the antialzheimer's activity of EEFB in a range that considered both safety and potential therapeutic effects.

- **Induction of Memory impairment:**

In this experimental study, amnesia was induced in through scopolamine. Scopolamine, a muscarinic receptor antagonist, was used to disrupt cholinergic neurotransmission in the brain and impair memory function. Induction of memory impairment was employed by administration memory impairing dose of scopalamine 1mg\kg i.p , Intraperitoneal route for 14 days in each group of animal except Group 1 [18].

- **Grouping of animals**

  - Group 1- Normal control (Normal saline 1ml/kg for 14 days)
  - Group 2 - Treated with scopolamine 1 mg/kg alone i.p. (negative control group).
  - Group 3- Treated with scopolamine 1 mg/kg + piracetam 200mg/kg i.p. (standard group/ positive control).
  - Group 4- Treated with scopolamine 1 mg/kg + EEFB 100mg/kg P.O
  - Group 5: Treated with scopolamine 1 mg/kg + EEFB 200mg/kg P.O

- **Evaluation of memory enhancing activity**

1. Elevated plus maze:

The EPM (elevated plus maze) apparatus utilized in the experiment was constructed from black plywood and consisted of two opposing open arms and enclosed arms. The open arms measured 50 cm in length, 10 cm in width, and had no height enclosure. The enclosed arms, on the other hand, were 50 cm in length, 10 cm in width, and 40 cm in height. The central platform was an open square measuring 10 cm in length and 10 cm in width. The maze was elevated 80 cm above the floor. During the acquisition trial, each rat was placed individually at the end of one of the open arms, facing away from the central platform. The time it took for the rat to move from the open arm to either of the enclosed arms was recorded by two experimenters. To mark the entry into the enclosed arm, all four paws of the rat needed to cross the line separating the central square from the open arms. The same procedure was repeated for the 24-hour retention trial. Rats that took longer than 60 seconds to enter the enclosed arm during the acquisition trial or fell off the maze at any point were excluded from the experiment. A shorter transfer latency, or the time taken to enter the enclosed arm, was considered as an indication of improved memory [19].

2. Passive avoidance test

The experimental setup involved a two-chamber box, comprising a light chamber connected to a dark chamber that contained an electrified grid capable of delivering electric shocks to the animal. Basal readings were initially recorded. A rat was then placed in the light chamber, and the duration of time it spent in that chamber was observed. The transfer latency (TL) was measured as the time taken by the animal to enter the dark chamber with all four legs inside, starting from the moment it was placed in the light chamber. On day 14, 30 minutes after the administration of scopolamine, the TL was noted. Furthermore, the TL was measured again 24 hours later on day 15. [20].

3. Brain Acetyl cholinesterase Activity:

The procedure to estimate brain acetylcholinesterase was conducted following the method. In summary, 0.4 mL of brain homogenate was combined with 2.6 mL of phosphate buffer in a test tube. Then, 0.1 mL of DTNB reagent was added to the mixture, and the absorbance at 412 nm was recorded. Afterward, 0.02 mL of acetylcholine iodide solution was introduced,

and the absorbance was noted again 15 minutes later. The change in absorbance per minute was calculated to determine the activity of brain acetylcholinesterase[21].

The rate of hydrolysis of substrate was calculated using following formula:

R = change in absorbance/min ×5.74 × 10−4/C0,

R = rate of hydrolysis of acetylcholine iodide/min/mg tissue,

C0 =weight of tissue homogenate in mg/mL.

RESULTS AND DISCUSSION

a. Preliminary phytochemical screening:

The extract contains alkaloids, flavonoids, phenolic compounds, tannins, and phenols. These constituents are known to possess various biological activities and can contribute to the potential therapeutic effects of the extract[Table1,2].

b. Effect of EEFB (Ethanolic extract of *Ficus benghalensis*) on transfer latency (TL) in elevated plus maze model:

In Elevated plus maze TL for all the groups noted on day 14 was comparable to each other. Table 2 shows Scopolamine induced group 2 showed significantly high TL on day 15 when compared to the rats in the control group. The rats which received extracts 100mg/kg and 200mg/kg showed a decrease in TL compared to induce control and values were comparable to control group. Administration of piracetam (standard) exhibited further decrease in TL (compared to scopolamine group).

c. Effect of EEFB on transfer latency (TL) in passive avoidance test:-

In passive avoidance test TL for all the groups noted on day 14 was comparable to each other. Scopolamine induced group 2 showed significantly decrease in TL on day 15 when compared to the rats in the control group. The rats which received extract showed an increase in TL compared to induce control and values were comparable to control group. Table 4 Administration of piracetam (standard) exhibited further increase in TL (compared to scopolamine group). 5th Group 200mg/kg shown marked increase in latency time than group 4 (i.e, 100mg/kg).

d. Effect of EEFB on brain acetylcholine esterase (ACHE) activity:

The results showed that the normal control group exhibited an ACHE activity of 53 m mol/min/mg of tissue. Table.5 shows in the negative control group, the ACHE activity was slightly higher at 55 m mol/min/mg of tissue. In the standard control group, where a specific standard was used, the AChE activity was significantly lower, measuring only 30 m mol/min/mg of tissue. This suggests that the standard control compound had an inhibitory effect on ACHE activity.

When the extract of a plant named EEFB (100 mg/kg) , the ACHE activity decreased to 36 m mol/min/mg of tissue. Furthermore, a higher dosage of EEFB (200 mg/kg) resulted in a further decrease in AChE activity, measuring 30 m mol/min/mg of tissue. These findings indicate that the EEFB extract had an inhibitory effect on AChE activity. Which would be having phenomenal effect on the brain acetyl choline levels leading the exerting its neurocognitive effects by increasing the acetylcholine levels by mitigating the acetylcholine esterase activity.

CONCLUSION:-

In conclusion, the obtained results indicate that the EEFB extract exhibits potential memory and learning-enhancing activity through the inhibition of acetylcholinesterase (ACHE). AChE plays a crucial role in the breakdown of acetylcholine, a neurotransmitter involved in cognitive functions. By inhibiting ACHE, the extract increases the levels of acetylcholine, leading to improved cognitive performance. The significant decrease in ACHE activity observed in the EEFB-treated groups suggests that the extract contains bioactive compounds that effectively modulate this enzymatic activity. These findings support the notion that the EEFB extract may hold promise as a natural source for the development of cognitive enhancers. Further studies are warranted to elucidate the specific mechanisms of action and to explore the potential therapeutic applications of this extract in memory and learning disorders.

Table 1: **preliminary phytochemical screening**

Sn.No	Constituents	Presence (+)/absence(-)
1	Alkaloids	+
2	flavnoids	+
3	saponins	-
4	Phenolic Compounds	+
5	Tannins	+
6	glycosides	-
7	phenols	+

Table 2: Total phenol, flavonoid and tannin content of ethanolic extract of leaves of *Ficus bengalensis*

Extract	Total phenol mg GA(gallicacid0/g of extract	Total flavonoid mg Ru(Rutin)/g of extract	Total tannin mg TA(tannic acid)/g of extract
Ethanolic extract	54	42	48

Table 3: Effect of EEFB on transfer latency using Elevated plus maze:

S.no	Drug Treatment	Transfer latency (sec)	
		After 30 mins (on day 14)	After 24 Hrs (on day 15)
1	Normal control	12.0±0.21	37.6±0.12
2	Negative control	43.0±0.12	178±0.37
3	Standard control	17.6±0.3	42.0±0.19
4	EEFB 100mg/kg P.O	18±0.41	51±0.32
5	EEFB 200mg/kg P.O	16.8±0.17	48±0.14

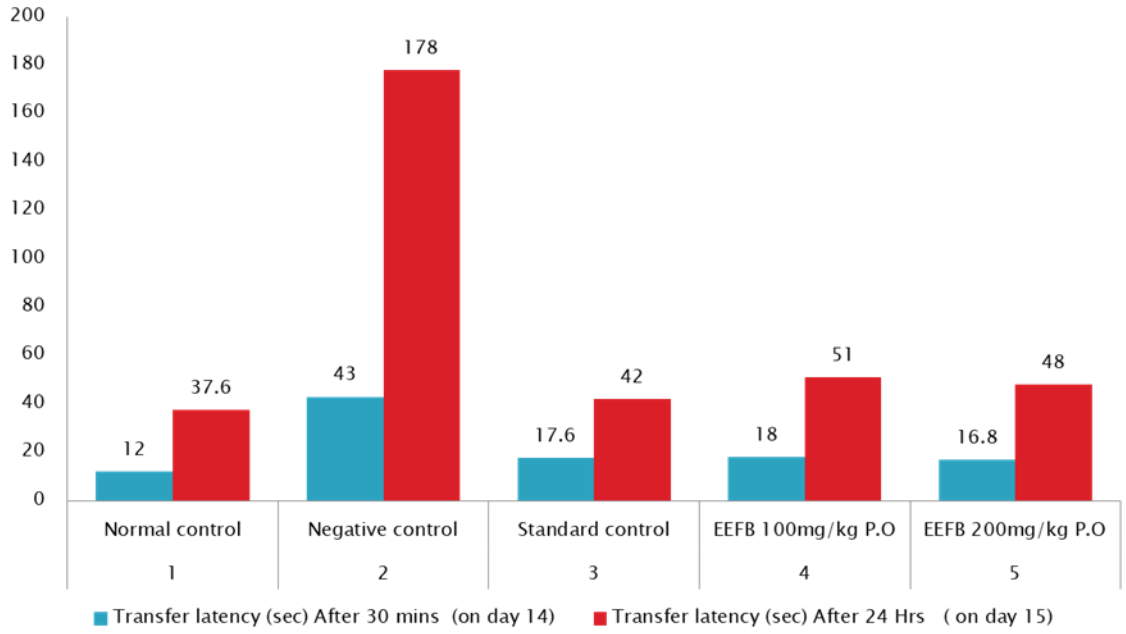

Fig 1: Effect on transfer latency by using Elevated plus maze model

Table 4: Effect of EEFB on Transfer latency in seconds in the model of passive avoidance test

S.no	Drug Treatment	Transfer latency (sec)	
		Day 14	Day 15
1	Normal control	38.6±0.04	258.14±0.22
2	Negative control	37.8±0.15	69±0.31
3	Standard Control	34.4±0.13	198±0.14
4	EEFB 100mg/kg P.O	37.4±0.32	101±0.54
5	EEFB 200mg/kg P.O	38.7±0.27	142±0.16

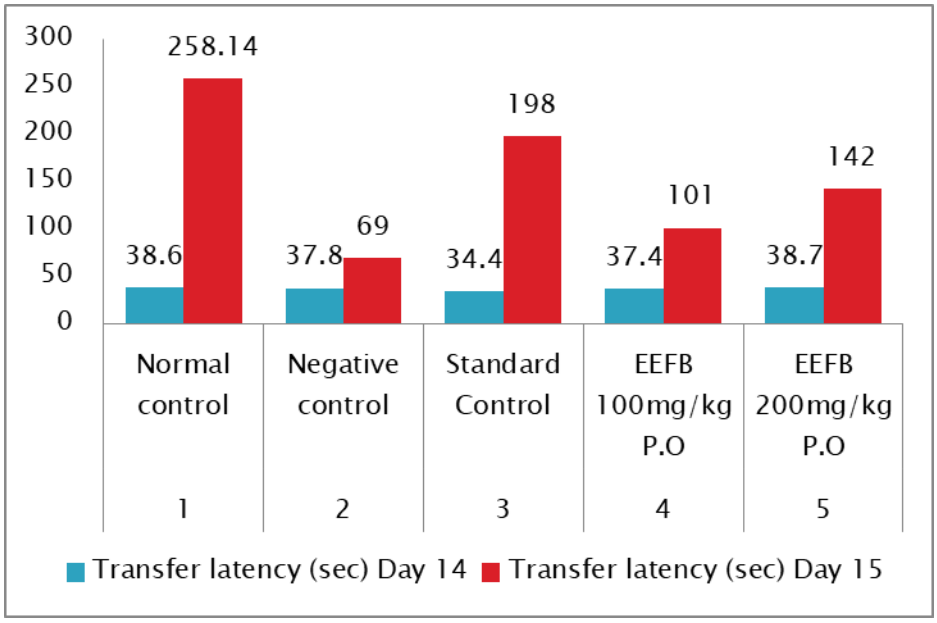

Fig 2: Effect of EEFB on Transfer latency in seconds in the model of passive avoidance test

Table 5: Effect of EEFB on brain acetyl choline esterase activity

Group	Acetylcholine esterase activity m mol/min/mg of tissue
Normal control	53
Negative control	55
Standard control	30
EEFB (100 mg/kg)	36
EEFB (200 mg/kg)	30

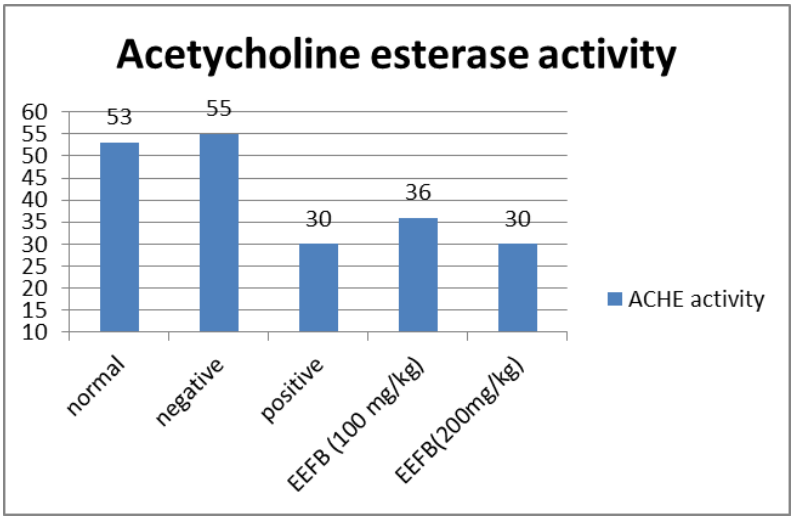

Fig 3: Effect of EEFB on brain acetyl choline esterase activity

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