Available Online at www.ijpscr.info
International Journal of Pharmaceutical Sciences and Clinical Research 2023; 3(2):116-124

RESEARCH ARTICLE

Anti-amnesic Activity of *Pithecellobium dulce* Benth. Leaves Extract Against Scopolamine-Induced Memory Impairment in Rats

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Received: 10-04-2023 Revised: 26-04-2023 Accepted: 30-05-2023

ABSTRACT

Background: Alzheimer's disease is a neurological condition that impairs memory and cognitive function and worsens as a person gets older. *Pithecellobium dulce* benth. is a medicinal plant used for the treatment of a wide variety of ailments. Several chemical constituents such as alkaloids, phenols, flavonoids, steroids, tannins, and saponins present in *P. dulce* benth. Possesses strong anti-inflammatory and antioxidant properties. Most of the drugs showed neuroprotective activity by inhibiting inflammation and oxidative stress in brain cells. Thus, we intended to evaluate the anti-amnesic activity of the ethanolic extract of *P. dulce* benth. leaves against scopolamine-induced memory impairments in the experimental rat model. **Methods:** The investigation was conducted over a period of 28 days. Behavioral assessment was done on the 29th day using a rota rod, actophotometer, elevated plus maze, open field model, and biochemical parameters such as lipid peroxidation malondialdehyde (MDA), catalase, acetylcholinesterase activity, and protein estimation were estimated in brain homogenate. Rats (30) were divided into five groups of six animals each. Including a negative control, positive control (scopolamine 1 mg/kg I.P), standard (donepezil 2.5 mg/kg P.O), and two different doses of EEPD (200 and 400 mg/kg) along with scopolamine. One-way ANOVA was used to analyze all the data, and it was followed by a Tukey–Kramer Multiple Comparison Test. **Results:** The results of the present study revealed that treatment with EEPD (200 mg/kg and 400 mg/kg) increased locomotor activity, enhanced muscle grip strength, reduced amnesic-like behavior, and increased exploratory activity compared to the positive control group. The brain homogenate showed a decreased MDA level, an enhanced in catalase level, a decreased in acetylcholinesterase level, and an increased in protein level were seen in EEPD in a dose-dependent manner and donepezil-treated group when compared to a positive control group. **Conclusion:** The study concludes that the ethanolic extract of *P. dulce* B. possesses significant anti-amnesic activity.

Keywords: Anti-amnesic activity, Donepezil, Ethanolic extract, *Pithecellobium dulce* benth, Scopolamine

INTRODUCTION

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, primarily results from decreased cholinergic activity, oxidative stress, accumulation of amyloid beta peptide, and neuroinflammation, impacting the brain and leading to progressive memory decline.[1] Emil Kraepelin, in the ninth edition of his psychiatric handbook, introduced the term "AD" to describe the serious cerebral cortex condition that the first patient of Alois Alzheimer exhibited. This condition was characterized by amyloid plaques, substantial neuron loss in the brain, and resulted in symptoms

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such as memory loss and personality disorders, ultimately leading to the patient's death.[2]

Various factors, including intoxications, infections, pulmonary and circulatory system abnormalities affecting oxygen delivery to the brain, nutritional deficiencies, Vitamin B12 deficits, tumors, and other disorders, can contribute to the gradual decline of cognitive functions. At present, approximately 50 million individuals worldwide are affected by AD, and it is projected that by 2050, this number will multiply exponentially, increasing by a factor of four every 5 years to reach an estimated 152 million cases.[3]

Several cholinergic medications have demonstrated efficacy in clinical research for the treatment or reduction of AD. These medications operate by addressing the issue of acetylcholine (ACh) deficiency and subsequently elevating ACh levels in the brain to achieve their therapeutic objectives. Acetylcholinesterase (AChE) inhibitors, such as donepezil, galantamine, and rivastigmine, are commonly prescribed for AD and are known to temporarily enhance ACh availability at cholinergic synapses. However, due to their potential for severe side effects, including hepatotoxicity, nausea, and discomfort, there is a limited number of approved medications for the management of cognitive impairment in AD patients. Consequently, individuals with AD require alternative treatment strategies.[4]

Pithecellobium dulce Benth. (Leguminosae) is a spiny, evergreen tree of small to medium size known for its diverse array of chemical constituents, including alkaloids, phenols, glycosides, flavonoids, steroids, tannins, terpenoids, and saponins. The leaves of this plant have found their place in folk remedies, traditionally used to address various health concerns such as leprosy, intestinal disorders, peptic ulcers, toothaches, and earaches. In addition, they have been employed in folk medicine for their emollient properties, as an abortifacient, anodyne, and even as a larvicide.

The common constituents present in leaves of the plant are cyclitol, dulcitol, octacosanol, α-spinasterol, kaempferol-3-rhamnoside, quercetin, and afzelin. The leaves were also reported to show antifungal and antibacterial activity.[5]

Current allopathic treatment for amnesia is associated with side effects and adverse effects. Based on the above considerations the present study aimed to evaluate the anti-amnesic activity of the ethanolic extract of *P. dulce* Benth. Leaves against scopolamine-induced memory impermeant in rats.

MATERIALS AND METHODS

Collection and authentication of plant

P. dulce Benth. leaves were collected from local areas of Chikkabennur, Chitradurga, Karnataka. The fresh leaves were subjected to observation for removal of damaged leaves and dried under shade. The dried leaves were subjected to size reduction by an electric grinder. The leaf material was identified and authenticated by botanist Ms. Niveditha B T Assistant Professor, Jyanagangothri PG Centre, GR Halli, Davanagere University, Chitradurga. Karnataka, 577502.

Preparation of plant extract[6]

P. dulce Benth. leaves were collected, cleaned, shade dried at room temperature for 7 days, and pulverized. The powder of leaves of *P. dulce* Benth. was packed in Soxhlet apparatus and extracted with ethanol (95% v/v) at 40°C. The extract was filtered through the Whatman No.1 filter paper. Concentrated under reduced pressure and stored in an airtight container for further use. The percentage yield of the corresponding extract was calculated.

Preliminary phytochemical screening[7,8]

Preliminary phytochemical investigations were carried out on the ethanolic extract of *P. dulce* Benth. leaves for the detection of various phytochemicals using standard methods prescribed in practical pharmacognosy by C K Kokate and R K Khandelwal.

Experimental animals

Animal ethical clearance was obtained from the Institution Animal Ethics Committee (IAEC) for experimental purposes (Ref No: 06E SJMCP/

IAEC/August-2022). Healthy adult Wistar albino rats weighing about 150–200 g of either sex were used for this study. The animals were obtained from Biogen Laboratory Animal Facility, Bangalore-562107. Before the initiation of the experiment, the animals were acclimatized for 10 days and randomized under standard environmental conditions such as temperature (26 ± 2°C), relative humidity (45–55%), and 12 h light/dark cycle maintained as per Committee for Control and Supervision of Experiments on Animal guidelines. All the animals were allowed free access to standard laboratory pellets and drinking water *ad libitum* under strict hygiene conditions.

Selection of screening dose[9]

Screening of anti-amnesic activity dose was considered based on the literature of acute toxicity studies of ethanolic extract of *P. dulce* Benth. Leaves are given by oral route according to Organization for Economic Co-operation and Development guidelines 425. Fifteen healthy male albino rats which were fasted 12 h before the experiment were divided into three groups with five animals in each group. The first group serves as the control group whereas the second and third group serves as aqueous and ethanolic treatment groups. Extracts of *P. dulce* at the dose of 2000 mg/kg were administered to animals. The animals were observed for 7 days as animals had not shown any sign of toxicity, behavioral changes, and mortality the dose increased up to 5000 mg/kg. Then animals were observed for up to 7 days for toxicity, behavioral changes, and mortality.

The animals were divided into five groups with six rats each: (n=6). The details of experimental design is given in Table 1.

Behavioral parameters assessment

Elevated plus maze[10]

Elevated plus maze is a commonly used behavioral assay to determine anti-amnesic activity. An elevated plus maze test was performed according to Handley and Mithani. The elevated plus maze consists of 4 arms (2 open arms and 2 closed arms) attached at a junction (central platform). The height of the plus maze was raised to 50 cm above the ground. Animals of each group control, Positive control, standard, and test were treated with normal saline, Scopolamine (1 mg/kg i.p) standard drug (Donepezil 2.5 mg/kg), and test extracts of EEPD at 200 mg/kg and 400 mg/kg, respectively. Rats were placed at the junction of four arms, facing toward an open arm. The number of entries and time spent in each arm and center were recorded for 5 min. An increase in open-arm activity reflects anti-amnesic behavior. The apparatus was cleaned with alcohol in between the trials. Precautions taken to maintain noise noise-free environment.

Rotarod model[11]

The effect of motor coordination was assessed using the rotarod model. Rotarod consists of a base platform and a non-slippery surface rotating rod of 3 cm diameter and is divided into five equal sections. The animals were pre-selected in a training session based on their ability to remain on the rod (at 12 rpm) for 2 min. Animals of each group control, Positive control, standard, and test were treated with normal saline, Scopolamine (1 mg/kg i.p), standard drug (Donepezil 2.5 mg/kg), and test extracts of EEPD at 200 mg/kg and 400 mg/kg, respectively. Animals were placed on the rod at a speed of 20 rpm over a period of 30, 60, and 90 mins. Falling off time was automatically recorded. Time spent in the apparatus was observed for 5 min duration (300 s). The apparatus was cleaned thoroughly with alcohol in between trials.

Actophotometer model[12]

Locomotor activity (horizontal activity) of Wistar albino rats using digital actophotometer. The actophotometer comprises a square field that measures about 30×30 cm with walls on all four sides that are fixed with photocells. Before the start of the experiment, the photocells were tested thoroughly. For a duration of 5 min, an automatic calculation was made to determine how many times each animal crossed the light beam. Animals of each group control, Positive control, standard, and test were treated with normal saline, scopolamine (1 mg/kg i.p), standard drug

(donepezil 2.5 mg/kg), and test extracts of EEPD at 200 mg/kg and 400 mg/kg, respectively.

***Open-field model*[13]**

An open-field model/test was performed according to Hall. The apparatus consisted of a wooden box (60 × 60 × 60 cm). The floor of the box was divided into 16 squares (15 × 15 cm). Animals of each group control, positive control, standard, and test were treated with normal saline, scopolamine (1 mg/kg i.p), standard drug (donepezil 2.5 mg/kg), and test extracts of EEPD at 200 mg/kg and 400 mg/kg, respectively. After 30 min, animals were placed individually in one corner square. The number of rearings assisted, rearings (forepaws touching the walls of the apparatus), and number of squares crossed were counted for 5 min. Increasing in square crossing indicates locomotory activity.

Biochemical assessment of brain homogenate includes the following

***Catalase assay*[14]**

Catalase was measured in the brain homogenate was assessed using the continuous spectrophotometric rate determination method based on the Beers and Sizer method for evaluating antioxidant status. Phosphate buffer (2.5 mL, pH 7.8) was added to the supernatant and incubated at 25°C for 30 min. The absorbance was spectrophotometrically measured at 240 nm after being transferred into the cuvette. Hydrogen peroxide will be added and the change in absorbance will be measured for 3 min. The value is expressed as μmol of H_2O_2/min/mg wet tissue.
Calculation:

$$Cat\ (U)/100\,\mu L\ of\ sample = \frac{dy}{dx} \times \frac{0.003}{38.3956 \times 10 - 6}$$

Where,
dy/dx = Change in absorbance
38.3956 × 10^{-6} = Molar extinction co-efficient of H_2O_2 at 240 nm

***Lipid peroxidation*[15]**

Malondialdehyde (MDA) level was determined following the method outlined by Satoh. 75 mg of thiobarbituric acid (TBA) is dissolved in 15% trichloroacetic acid (TCA). To this, 2.08 mL of 0.2 N HCl was added. Using 15% TCA, the final volume was increased to 100 mL. Then, 0.75 mL of brain homogenate was mixed with 3.0 mL of this reagent. The test tubes were kept in a boiling water bath for 15 min. Then it was cooled and centrifuged for 10 min at 10,000 rpm. The absorbance of the supernatant is read against the blank at 535 nm. The results were expressed in mol/mg of protein.
Calculation:

$$Conc.of\ MDA = \frac{Abs\,532 \times 100 \times VT}{(1.56 \times 105) \times WT \times VU}$$

Where,
Abs_{532} is absorbance
V_T is total volume of mixture (4 mL)
1.56×10^5 is molar extinction co-efficient
W_T is weight of dissected brain
V_U is aliquot volume (1 mL)

***Acetyl cholinesterase activity*[16]**

AChE inhibitory activity of the plant extract was assessed using Ellman's reagent method. A mixture was prepared by combining 0.1 M trisodium phosphate (Na3PO4) buffer (pH 8.0, 150 μL), 10 μL of the test extract at concentrations ranging from 10 to 50 μg/mL, and 20 μL of enzyme solution (brain homogenate of mice) at a concentration of 0.1 units/mL. This mixture was then incubated for 15 min at 25°C. After incubation, 10 μL of 5,5-dithio-bis-[2-nitro benzoic acid] (DTNB) (10 mM) was added to the mixture, and the reaction was initiated by adding 10 μL of acetyl thiocholine iodide (14 mM solution) as the substrate. The hydrolysis of acetyl thiocholine iodide leads to the formation of a colored product known as the 5-thio-2-nitrobenzoate anion, which is produced by the reaction between DTNB and thiocholine, released during the enzymatic cleavage. The formation of the colored product was measured at a wavelength of 410 nm after 10 min. Donepezil, at concentrations of 10 μM, was used as a standard and subjected to the same procedure as the test extract.
AChE % inhibition was estimated using the formula

$$Inhibition\ activity\ (\%) = 1 - \left(\frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

Protein estimation[17,18]

The total protein content in brain homogenates was determined using the Lowry method. 100 µL of brain homogenate sample was taken in a test tube. 1.0 mL of Lowry stock reagent was added to each tube and was incubated for 30 min at room temperature. 100 µL of Folin's reagent was further added to each tube and incubated for 30 min at room temperature. The absorbance was read at 595 nm. The protein content in brain tissue was expressed as µg/mg of tissue.

Table 1: Experimental design[6]

Group 1	Negative control	Standard diet and water *ad libitum*.
Group 2	Positive control	Scopolamine (SCP 1 mg/kg i.p) for 28 days.
Group 3	Standard	Donepezil (DPZ 2.5 mg/kg p.o) + (SCP 1 mg/kg i.p) for 28 days.
Group 4	Test group I	Low dose of *Pithecellobium dulce* Benth. 200 mg/kg p.o +(SCP 1 mg/kg i.p) for 28 days.
Group 5	Test group II	High dose of *Pithecellobium dulce* Benth. 400 mg/kg p.o +(SCP 1 mg/kg i.p) for 28 days.

Statistical analysis

The data obtained from the above findings was subjected to statistical analysis using one-way ANOVA followed by the Tukey–Kramer Multiple Comparison Test to assess the statistical significance of the result.

RESULTS

The percentage yield of the ethanolic extract from *P. dulce* Benth. leaves, obtained through continuous soxhlet extraction using 95% ethanol as a solvent, was found to be 20.88%. The extract exhibited a dark green color and thick semi-solid.

Preliminary phytochemical screening

Preliminary phytochemical screening of EEPD leaves confirms the presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenes, resins, amino acids, and carbohydrates.

Anti-amnesic activity

A test sample of *P. dulce* Benth. leaves was evaluated for anti-amnesic activity by employing the behavioral and biochemical test in Wistar albino rats of either sex weighing 150–200 g.

BEHAVIORAL RESULTS

Elevated plus maze model

The ethanolic extract of *P. dulce* Benth. leaves was evaluated for anti-amnesic activity in an elevated plus maze model. The result indicated that the scopolamine-treated animals produced a state of amnesia in a positive control group, as evidenced by increased entries and time spent in closed arms, along with reduced time in open arms and the center. In contrast, donepezil-treated group (2.5 mg/kg) and EEPD (200 mg/kg and 400mg/kg) showed a significant increase in the number of entries and duration of time spent in open arm and center, as well as it showed a reduction in number of entries and duration of time spent in closed arm when compared to positive control group. EEPD at a high dose (400 mg/kg) showed a more significant (***$P < 0.001$) effect when compared with a low dose of EEPD (200 mg/kg). The effect of EEPD on cognitive behavior by elevated plus maze is analyzed in Table 2.

Actophotometer model

All animals underwent locomotor activity assessment using an actophotometer, which records ambulatory scores based on beam-crossing. Donepezil-treated group (2.5 mg/kg) and EEPD (200 mg/kg and 400 mg/kg) showed an increase in locomotor activity when compared to a positive control group, and it was observed that the locomotor activity for the entire 5 min progressively increased in the all of drug-treated groups, and test groups were showed dose-dependent activity. In a high dose of EEPD (400 mg/kg), it shows a moderately significance value (**$P < 0.01$) when compared with a positive control group. The effect of EEPD on locomotor activity by actophotometer is presented in Table 3.

Table 2: Effect of EEPD on cognitive behavior by elevated plus maze

S. No	Treatment	Number of entries (counts/5 min)			Time spent (sec) in 5 min		
		Open arm	Closed arm	Cente r	Open arm	Closed arm	Center
I	Negative control	6.83±1.04	9.66±1.28	3.16±0.94	40±10.16	208±11.75	37.33±9.06
II	Positive control	2.83±0.60	14.66±1.40	3.16±0.47	30.16±8.03	249±12.93	33±10.03
III	Standard (donepezil) 2.5 mg/kg	8.5±1.17***	4.3±0.49***	6.8±1.01***	127±16.00***	127±15.95***	51.83±10.31
IV	Low dose of EEPD (200 mg/kg)	5.6±0.98*	4.5±1.23*	2.8±0.79**	116.3±9.94**	111.6±4.41**	53.16±12.17
V	High dose of EEPD (400 mg/kg)	9±1.41***	5.6±0.88***	2.833±1.07***	136±5.57***	118±4.41***	66.33±5.54

Values were expressed as Mean±SEM (n=6); Significance values are: ***P<0.001, **P<0.01, *P<0.05, and ns P>0.05. positive control group versus all groups. (By one-way ANOVA followed by Tukey–Kramer Multiple comparison tests)

Table 3: Effect of EEPD on Loco-Motor activity by Actophotometer

S. No	Treatment	Ambulatory score
I	Negative control	173.6±3.282
II	Positive control	77.833±5.510
III	Standard group (donepezil) 2.5 mg/kg	155±15.220***
IV	Low dose of EEPD (200 mg/kg)	123.833±5.741**
V	High dose of EEPD (400 mg/kg)	145.166±15.133**

Values were expressed as Mean±SEM (n=6); Significance values are: ***P<0.001, **P<0.01, *P<0.05, and ns P>0.05. Positive control group versus all groups. (By one-way ANOVA followed by Tukey–Kramer Multiple comparison tests)

Table 4: Effect of EEPD on muscle grip strength by Rota-rod model

S. No	Treatment	Fall of time (sec)
I	Negative Control	147.5 ± 7.214
II	Positive control	76.66 ± 5.67^{ns}
III	Standard group (Donepezil) 2.5 mg/kg	131.1 ± 8.04***
IV	Low dose of EEPD (200 mg/kg)	114.66 ± 2.17**
V	High dose of EEPD (400 mg/kg)	121 ± 4.77***

Values were expressed as Mean ± SEM (n = 6); Significance values are: ***P < 0.001, **P < 0.01, *P < 0.05, and ns P > 0.05. positive Control group versus all groups. (By one-way ANOVA followed by Tukey–Kramer Multiple comparison tests)

Rota rod model

Muscle grip strength was assessed using a rota-rod apparatus, with mean fall-off time as the measure of muscular rigidity. The positive control group exhibited reduced fall-off time, indicating muscle incoordination. However, the donepezil-treated group (2.5 mg/kg) and EEPD (200 mg and 400 mg/kg) showed a significant increase in mean fall-off time respectively as compared to a positive control group, and test group showed dose-dependent activity. EEPD at a high dose (400 mg/kg) showed a more significant (***P < 0.001) effect when compared with a low dose of EEPD (200 mg/kg). The effect of EEPD on muscle grip strength by rota-rod apparatus is tabulated in Table 4.

Open field model

In open field apparatus, locomotion (rearing and assisted rearing) and exploratory (number of squares traversed, i.e., central and peripheral). Locomotion and exploratory activities of the positive control group were compared with the standard group (donepezil 2.5 mg/kg), low dose of EEPD (200 mg/kg), and high dose of EEPD (400 mg/kg). Donepezil-treated group shows a highly significance value (***P <0.001) increase in locomotion and exploratory activity compared to the remaining four groups of animals. In a low dose of EEPD (200 mg/kg), it shows less significance value (*P < 0.05) when compared with control group. In a high dose of EEPD (400 mg/kg), it shows a moderate increase in significance value (**P < 0.01) when compared with positive control group. The dose-dependent activity was shown by animals in the open-field model. Results are shown in Table 5.

Biochemical assessment of brain homogenate

Catalase assay

The breakdown of hydrogen peroxide indicates the level of catalase present in brain homogenate which was done using endogenous anti-oxidant catalase assay. The data revealed that a significant elevation in CAT level was seen in standard donepezil, low dose, and high dose of EEPD (200 mg/kg and 400 mg/kg) as compared to positive control group. Scopolamine's primary mechanism of action is to

S. No	Treatment	Rearings	Assistedrearings	Number of squares traversed in 5 min		
				Central	Peripheral	Total
I	Negative Control	5.166±0.980	7.166±0.600	2.5±0.428	81±5.88	83.5±5.71
II	Positive control	4±0.966	3.5±0.428	1.5±0.428	58±7.66	59.5±8.048
III	Standard group (Donepezil) 2.5 mg/kg	8.666±1.855**	9.5±1.727***	4.833±0.703***	95.33±6.275***	100±6.493***
IV	Low dose of EEPD (200 mg/kg)	6.333±1.229**	6.666±0.881**	3.666±0.666*	59±8.095**	63.5±8.139**
V	High dose of EEPD (400 mg/kg)	7.666±1.021**	9.333±0.881***	4.5±0.428**	79±4.524**	85.66±2.616**

Values were expressed as Mean±SEM (n=6); Significance values are: ***P<0.001, **P<0.01, *P<0.05, and ns P>0.05. Positive Control group versus all groups. (By one-way ANOVA followed by Tukey–Kramer Multiple comparison tests)

block ACh receptors in the nervous system. This can lead to changes in the functioning of neurons and neurotransmitter systems. Scopolamine alone treated group which indicated that more production of hydrogen peroxide was released into the cytosol. This leads to reduced levels of catalase in brain tissue. The effect of EEPD on catalase assay is given in Table 6.

Table 6: Effect of EEPD on catalase assay

S. No	Treatment	Catalase (μmol/min/mg protein)
I	Negative control	26.184±0.508
II	Positive control	17.148±0.335
III	Standard Donepezil (2.5 mg/Kg)	37.368±0.297***
IV	Low dose of EEPD (200 mg/Kg)	34.889±0.803**
V	High dose of EEPD (400 mg/Kg)	35.343±1.082***

Values were expressed as Mean±SEM (n=6); Significance values are: *P<0.05,**P<0.01 and ***P<0.001. Positive control group versus all groups

Lipid peroxidation assay

MDA is a reliable indicator of peroxidation. An increase in free radicals causes over production of MDA this was determined by a reactive brain homogenate sample with TBA in a lipid peroxidation assay. Scopolamine-induced cognitive impairment may lead to increased ROS production or decreased antioxidant defenses in the brain, resulting in oxidative stress. This oxidative stress can damage lipids, leading to the production of MDA, which is a marker of lipid peroxidation. The data revealed that the standard donepezil, low dose, and high dose of EEPD (200 mg/kg and 400mg/kg) showed a significant reduction in the level of MDA when compared to the positive control group. The effect of EEPD on lipid peroxidation assay is shown in Table 7.

Table 7: Effect of EEPD on lipid peroxidation assay

S. No	Treatment	MDA (nmol/mg of protein)
I	Negative control	2.379±0.031
II	Positive control	3.503±0.014
III	Standard Donepezil (2.5 mg/Kg)	2.187±0.040***
IV	Low dose of EEPD (200 mg/Kg)	2.112±0.008***
V	High dose of EEPD (400 mg/Kg)	2.186±0.006***

Values were expressed as Mean ± SEM (n = 6); Significance values are: *P < 0.05,**P < 0.01, and ***P < 0.001. Stress group versus all groups

Acetyl cholinesterase assay

AChE activity was estimated in rat brains using Ellman's assay. According to the cholinergic hypothesis, a low level of ACh is the leading cause of cognitive decline in AD patients. AChE plays an essential role in the regulation of several physiological reactions by hydrolyzing the neurotransmitter ACh in cholinergic synapses. Therefore, the inhibition of AChE may be a better therapeutic strategy in the treatment of AD. The scopolamine-treated group exhibited a substantial rise in brain AChE activity when compared to the other groups. Administration of EEPD (200 mg/kg and 400 mg/kg) and standard drug donepezil (2.5 mg/kg) significantly decreased the AChE levels when compared to positive control. EEPD at a high dose (400 mg/kg) showed a more significant (***P < 0.001) effect when compared with a low dose of EEPD (200 mg/kg). The effect of EEPD on acetylcholinesterase assay is shown in Table 8.

Protein estimation

Scopolamine primarily functions as an antagonist of the neurotransmitter ACh by blocking its receptors. The result indicated that the scopolamine-treated

Table 8: Effect of EEPD on acetylcholinesterase assay

S. No	Treatment	AChE µmol/min/mg protein
I	Negative control	0.361±0.039
II	Positive control	0.927±0.034
III	Standard Donepezil (2.5 mg/Kg)	0.571±0.024***
IV	Low dose of EEPD (200 mg/Kg)	0.697±0.069**
V	High dose of EEPD (400 mg/Kg)	0.581±0.031***

Values were expressed as Mean ± SEM (n = 6); Significance values are: *P < 0.05, **P < 0.01, and ***P < 0.001. Positive control group versus all groups

Table 9: Effect of EEPD on protein estimation

S. No	Treatment	µg/mg of tissue
I	Negative control	4.443±0.301
II	Positive control	1.374±0.129
III	Standard Donepezil (2.5 mg/Kg)	4.388±0.176***
IV	Low dose of EEPD (200 mg/Kg)	2.700±0.080*
V	High dose of EEPD (400 mg/Kg)	3.710±0.183**

Values were expressed as Mean±SEM (n=6); Significance values are: *P<0.05, **P<0.01, and ***P<0.001. Positive control group versus all groups

animals significantly decreased the level of protein, whereas the donepezil-treated group (2.5 mg/kg) and EEPD (200 mg and 400mg/kg) showed a significant increase in the level of proteins in the brain, EEPD at high dose (400 mg/kg) had shown a significant (**P < 0.01) effect when compared with a low dose of EEPD (200 mg/kg). The effect of EEPD on protein estimation is shown in Table 9.

DISCUSSION

The most crucial brain function is memory. An individual's memory is their capacity to store events and knowledge for short or long periods of time, recall those events when necessary, and use that information to modify their behavior. The environment, as a result, is essential for survival. Short-term memory and long-term memory (LTM) are the two basic categories of memory. The maintenance of information for a brief period of time (seconds) is known as short-term memory, which is a crucial aspect of cognition. LTM is the process by which learned memories become more secure or robust over time and are less susceptible to disruption.

AD has a complicated and varied pathogenesis, making it a risky neurodegenerative condition. Different motor dysfunctions appear across the illness spectrum, and a clear and noticeable decline in cognitive function is a hallmark of AD's clinical course.[19] Amnesia is a cognitive disorder characterized by profound memory loss which may either be caused by physical injury or ingestion of toxic substances which affect the brain.[1]

The present research study has been designed to screen the anti-amnesic activity of the ethanoic extract of *P. dulce* Benth. leaves against scopolamine-induced memory impairments in rats.

The results of the phytochemical analysis of *P. dulce* Benth. leaves showed that the ethanolic extract contained alkaloids, glycosides, flavonoids, tannins, steroids, saponins, triterpenes, resins, amino acids, and carbohydrates.[20]

Behavioral tests revealed that rats treated with EEPD exhibited significant improvements increase the number of entries into the open arm and spent more time exploring the open arms (measured by elevated plus maze), improved motor coordination (measured by Rota-rod apparatus), increased locomotor activity (measured by actophotometer), increase the number of rearing, assisted rearing, and the number of squares crossed (measured by open field). The EEPD also significantly increased catalase activity reduced the lipid peroxidation level, reduced the AChE level, and increased protein level in the brain, which shows that their antioxidant effects are mediated by reductions in oxidative stress.[21] This suggests that EEPD may alleviate amnesia by enhancing antioxidant defenses and reducing oxidative stress in the brain. It has been discovered that the leaves of the *P. dulce* are efficient against a variety of pharmacological actions because they are rich in flavonoids, phenols, and tannins, among other compounds. Significant anti-amnesic efficacy was seen in albino rats treated with an ethanolic extract of *P. dulce* leaves. Although the anti-amnesic efficacy of *P. dulce* leaves was validated in the current study, more research is needed to understand how it works. Thus, there would be several opportunities for researchers in the future.

CONCLUSION

The study found that the EEPD leaves showed significant potential against scopolamine-induced

memory impairments in rats. Using various behavioral and oxidative stress assessment models, the leaf extract demonstrated effectiveness in enhancing muscle grip strength, increasing locomotor activity, increasing the number of entries in the open arm and total time spent in the open arm, increasing the number of rearing, assisted rearing, and the number of squares crossed and increased catalase activity, reduced lipid peroxidation, reduced AChE level, and increased protein level in rat brain. This suggests the extract has notable anti-amnesic properties, likely due to compounds such as alkaloids, flavonoids, tannins, and steroids. However, further research is required for the identification and isolation of its active constituents and to confirm its exact mechanism of protection. Hence, it can be better projected as a therapeutic agent for anti-amnesic activity.

ACKNOWLEDGEMENT

The authors are thankful to the management of SJM Vidyapeetha, Chitradurga, for their constant encouragement through the Principal, SJM College of Pharmacy, Chitradurga, for providing necessary facilities and help in carrying out this work.

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