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Research Article

EVALUATION OF NEUROPROTECTIVE AND ANTIOXIDANT POTENTIAL OF METHANOLIC LEAF EXTRACT OF *BIDENS PILOSA* AGAINST ALUMINIUM CHLORIDE-INDUCED COGNITIVE IMPAIRMENT AND NEUROTOXICITY IN WISTAR ALBINO RATS.**Deepika J K^{1*}, T Balasubramanian², Suresha B S³, Chintana B N⁴**^{1,4}PG Scholar, Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India – 571422.²Principal and Professor, Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India – 571422.³Professor and Head, Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India – 571422.**Abstract:**

Bidens pilosa known for its medicinal properties, methanolic leaf extract of this plant was evaluated for its neuroprotective effects against aluminium chloride induced Alzheimer's disease in Wistar albino rats and antioxidant activity using in-vitro method. The leaves of the plant *Bidens pilosa* was collected, shade dried and extracted using methanol by Soxhlet method. Antioxidant properties were evaluated using DPPH assay. Behavioural tests, including Elevated Plus Maze, Y Maze and Cook's pole method, were used to assess cognitive function in aluminium chloride-induced rats. Aluminium chloride administration produced significant cognitive impairment, as evidenced by increased transfer latency in the Elevated Plus Maze and escape latency in the Cook's pole test, along with reduced spontaneous alternation behaviour in the Y-maze test. Treatment with methanolic *Bidens pilosa* leaf extract at doses of 100 and 200 mg/kg improved the behavioural parameters compared with the Alzheimer's control group. Biochemical evaluation showed a marked reduction in catalase activity and protein concentration in the Alzheimer's control group compared with the normal control, whereas treatment with *Bidens pilosa* extract improved these parameters. The present study demonstrated that methanolic *Bidens pilosa* leaf extract exhibited a protective effect against aluminium chloride-induced Alzheimer's-like cognitive and biochemical alterations in rats. Treatment with *B. pilosa* improved learning and memory, enhanced antioxidant activity. These findings suggest that *B. pilosa* may possess potential neuroprotective and anti-Alzheimer's activity, which may be attributed to its antioxidant properties.

Keywords: Antialzheimer's activity, Antioxidant activity, *Bidens pilosa*, Aluminium chloride, Neurotoxicity, Oxidative stress.

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INTRODUCTION:

Alzheimer's disease (AD) is a chronic neurodegenerative disease with multiple etiologies that typically affects people over age 60, except when it is inherited in an autosomal dominant manner [1]. It has been stated that amyloid beta (A β) and tau proteins collectively stand out as the hallmark of progressive AD and the basis of most disease-modifying therapy [2]. The short-term memory impairment that characterizes the early stages of illness is followed by cognitive impairment, vocabulary loss, and concept loss [3]. Alzheimer's disease is one of the major healthcare challenges of the 21st century. It is characterized by a progressive decline in the individual's cognitive abilities. Worldwide, 55 million patients suffer from dementia [4]. Alois Alzheimer, a German psychiatrist, identified a rare type of mental illness in 1906 that affected Augustine Deter and was typified by progressive cognitive impairment, hallucinations, focal delusions, and symptoms of psychosocial incompetence [5]. The pathogenesis of AD is not yet clearly known. Probable mechanisms include the deposition of A β proteins and tau proteins in the cytoplasm of neurons, which leads to neuronal degeneration [6]. The accumulation of toxic amyloid beta-protein (A β) in the central nervous system (CNS) is the main cause of Alzheimer's disease. Extracellular abnormalities of A β levels in the brain may lead to the accumulation of A β , forming a structure rich in β -Sheet. After forming oligomers, they recombine into fibrils to form amyloid plaques. Moreover, the aggregation of oligomers is harmful to cells. In the brains of AD patients, oligomers penetrate the cell membrane, triggering a series of pathological events and ultimately leading to cell dysfunction and death [7]. Mitochondrial dysfunction and cellular energy deficits are increasingly thought to play a critical role in ageing and AD pathophysiology. Oxidative stress (OS), which occurs when there is an imbalance between oxidant and antioxidant levels in the cell, resulting in increased reactive oxygen species (ROS) production, is another important metabolic facet of AD pathology. Oxidative stress and microglia-mediated neuroinflammation adversely affect brain function and are now considered a hallmark feature of various neurodegenerative diseases, including AD. Evidence from brain imaging supports the role of neuroinflammation in the progression of dementia [8]. Progressive loss of cognitive functions can be caused by cerebral disorders like Alzheimer's disease or other factors such as intoxications, infections, abnormalities in the pulmonary and circulatory systems, which cause a reduction in the oxygen supply to the brain, nutritional deficiency, vitamin B₁₂ deficiency, tumours, and others. A patient suspected to have AD should undergo several tests, including neurological examination, magnetic resonance

imaging (MRI) for neurons, laboratory examinations such as vitamin B₁₂, and other tests besides the medical and family history of the patient. According to some studies, vitamin B₁₂ deficiency has long been known for its association with neurologic problems and increasing risks of AD [9].

Recent clinicopathological and radiological data suggest that there are close relationships between blood-brain barrier dysfunction and these established Alzheimer's biomarkers [10]. In current scenarios, herbal medicines have gained greater importance, mainly due to their safety, efficacy and easy availability as compared to synthetic drugs [11]. Various allopathic medicines are prescribed in AD, but they exert side effects. Therefore, herbal medicine could be a good source of drugs for the treatment of AD and memory deficit with fewer or no side effects [12]. Plants are a significant source of pharmacologically active secondary compounds that can be utilised in medicine to treat certain diseases and ailments, as well as to promote and maintain human health [13]. Medicinal plants have been extensively utilised in traditional medicine throughout history. Ethnobotanical information about these plants and their usage by indigenous groups is crucial for maintaining cultural legacy, protecting biodiversity, improving community healthcare, and advancing drug development [14]. Many herbal remedies are used in medical practice based on years of use; herbal preparations effectively prevent and treat a variety of ailments [15]. Natural resources, such as herbal plants rich in phytochemicals, are considered potential traditional therapies for chronic and infectious disorders. They are regarded as safer and more effective choices with fewer adverse effects than synthetic medications [16].

The genus *Bidens*, belonging to the Asteraceae family, includes around 230 species. *Bidens pilosa* L. is likely the most extensively studied species within its genus [17]. *B. pilosa* is a plant found in tropical and sub-tropical regions, commonly utilised in traditional medicine and contains a variety of phytochemicals [18]. *Bidens pilosa* was identified by Carl Linnaeus in 1753. This is a typical perennial plant that grows worldwide in both temperate and tropical climates. It has long been used in food and medicine without any noticeable side effects [19]. It is commonly known as: blackjack, amor seco, masaquía sillcao, chipaca, cadillo, picao preto, cuamba, Spanish needles, broom stick, beggar's ticks, cobbler's pegs, pitchforks, devil's needles, picao preto, and farmers' friends [20]. *Bidens pilosa* L. var. *radiata* (BPR), *B. pilosa* L. var. *pilosa* (BPP), and *B. pilosa* L. var. *minor* (BPM) are common variants of a plant [21]. *Bidens pilosa* is a medicinal plant recognised for its diverse collection of bioactive compounds. It contains a variety of phytochemicals that contribute to its pharmacological properties [22]. *Bidens pilosa* is abundant in

phytochemicals, including saponins, alkaloids, polyacetylenes, flavonoids, and diterpenes [23], phenolic acids, terpenoids, steroids, and other components, of which flavonoids and polyacetylenes are the main constituents [24]. Flavonoids such as quercetin, luteolin, and apigenin are present in *Bidens pilosa*. These compounds have been reported to protect cells from oxidative damage and prevent degenerative diseases such as cardiovascular disorders and neurodegenerative conditions [22]. Flavonoids also possess antioxidant, anti-amyloidogenic, and anti-inflammatory properties and regulate cell-signalling pathways [25]. This study aims to investigate the anti-Alzheimer's activity of *Bidens pilosa* in experimental animals.

MATERIAL AND METHODS:

Collection and authentication of plant material:

The fresh leaves of the plant *Bidens pilosa* were collected from Nagamangala, Mandya district, Karnataka. The leaves were identified, confirmed and authenticated by Dr Thejesh Kumar M.P., M.Sc., PhD, Co-ordinator, Department of Botany (PG), Bharathi College (PG & RC), Bharathinagara. The collected plant leaves were washed, shade-dried, subjected to size reduction into a fine powder.

Preparation of the plant leaf extract:

The leaves were cleaned with running tap water and rinsed with distilled water to remove debris and contaminants. The leaves were shade-dried at room temperature and powdered. The dried powder was taken and extracted with methanol for about 72 hrs at 40°C using a Soxhlet extractor. The obtained extract was evaporated using a rotating vacuum evaporator at 40°C, and then the concentrated extract was stored at 4°C for further use.

In vitro antioxidant assay:^[26]

The free radical scavenging activity of various concentrations of plant extract was measured in terms of DPPH scavenging assay.

DPPH assay of Bidens pilosa

The aliquots of 20, 40, 60, 80, and 100 µg were used to carry out the DPPH assay. To each test tube of standard ascorbic acid and extract, 1 mL of methanolic solution of DPPH was added. The reaction mixture was incubated in the dark at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was recorded at 517 nm using a UV-Visible spectrophotometer. 1mL of DPPH was taken as a control. % DPPH radical scavenging activity was calculated using the following equation.

$$\% \text{ RSA} = \{A_0 - A_1 / A_0\} * 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

Then % radical scavenging activity (RSA) was plotted against concentration, and from the graph, the IC_{50} value was calculated.

Evaluation of anti-Alzheimer's potential of *Bidens pilosa* leaf extract on aluminium chloride-induced Alzheimer's rat model:

Experimental animals:

Healthy Wistar albino male rats of approximately same age (10 to 12 weeks), weighing between 120-170 g were taken for evaluating anti-Alzheimer's activity. The animals were procured from Vertebrates, Magadi Road, Bangalore. The animals were acclimatized by keeping them in animal house facility of Bharathi College of Pharmacy, Bharathinagara. They were housed in polypropylene cages containing bedding material as husk and maintained under standard husbandry conditions and 12 hrs light and 12 hrs dark cycle. They were fed with commercial pelleted rat chow with water *ad libitum*. The animals were maintained in accordance with the CCSEA guidelines. The anti-Alzheimer's activity was conducted after obtaining the approval from Institutional Animal Ethic Committee (IAEC) with a reference no. BCP/IAEC/2025/03 dated 12/07/2025.

Experimental design:^[15]

A total of 30 Wistar albino rats weighing around 120-160 g will be divided into five groups, each group consisting of six animals.

Group 1: Normal control

Group 2: Alzheimer's control (AlCl₃ 175 mg/kg)

Group 3: Alzheimer's+ Rivastigmine (2.5 mg/kg)

Group 4: Alzheimer's + Extract (100 mg/kg)

Group 5: Alzheimer's + Extract (200 mg/kg)

Experimental procedure:

Alzheimer's was induced using aluminium chloride at 175 mg/kg body weight. The study was carried out for up to 36 days. As mentioned above, animals were weighed, recorded, numbered and divided into five groups of six animals each. Group 1: Animals received a standard pellet diet and purified water for 24 hrs over a period of 36 days and were considered the normal control group. Group 2: Animals received aluminium chloride (175 mg/kg) each day over a period of 20 days, and they were considered the Alzheimer's control group. Group 3, Group 4 and Group 5 animals received aluminium chloride (175 mg/kg body weight) each day over a period of 20 days. After that, standard dose (Rivastigmine 2.5 mg/kg) and leaf extract of *Bidens pilosa* at low dose (100 mg/kg) and high dose (200 mg/kg) were administered to Group 3, Group 4 and Group 5, respectively, for 11 days. After 24 hrs of the last treatment, all the animals were weighed, sacrificed, and the brain was collected.

Behavioural studies:

Behavioural tests were performed to functionally validate cognitive dysfunction amnesic models and to assess treatments. Methods to assess hippocampus-dependent memory functions were used.

Elevated Plus Maze:

The Elevated Plus Maze (EPM) is a widely used behavioural test for evaluating anxiety-like behaviour and learning and memory in rodents. The apparatus consists of two open and two closed arms, elevated 50 cm above the floor. Each arm measures 35×5 cm, with 15 cm high walls in the closed arms and a central area of 5×5 cm. The EPM test was conducted according to the methods described by Walf and Frye (2007) and Auti and Kulkarni (2019). The test was performed in two phases: acquisition phase, in which initial transfer latency (ITL) was recorded, and retention phase, in which memory was assessed the following day. Transfer latency was recorded after Alzheimer's induction to confirm cognitive impairment and after treatment to evaluate the effect of the extract. Each animal was placed in the open arm facing away from the central area, and the transfer latency was recorded as the time taken to enter the closed arm [27].

Cook's Pole Method:

The Cook's Pole Climbing test was used to assess conditioned avoidance response and long-term memory in experimental rats. The apparatus consisted of a wooden/Plexiglas box ($27 \times 27 \times 27$ cm) with a stainless-steel grid floor and a wooden pole ($10 \times 7 \times 1.7$ cm) positioned at the centre. The animals were acclimatised to the apparatus for 1 minute. A buzzer was sounded, followed 5 seconds later by a mild foot shock through the grid floor. The escape latency, defined as the time taken by the rat to climb onto the wooden pole, was recorded in seconds. The buzzer and shock were immediately stopped once the animal climbed the pole. A single trial was conducted as described by Sakurai et al. (2008), and escape latency was used as the endpoint for memory assessment [82].

Y Maze test:

The Y-maze test was used to evaluate short-term spatial working memory in experimental rats. The apparatus consisted of three arms arranged at 120° angles and designated as A, B, and C. Rats naturally tend to explore a new arm rather than immediately returning to a previously visited arm. This behaviour was assessed by measuring spontaneous alternation. Before testing, the apparatus was cleaned, and the animals were familiarised with the maze. Each rat was placed at the centre of the Y-maze and allowed to explore freely for 8 minutes. The sequence of arm entries was recorded, with an entry counted when all four paws entered the arm. The apparatus was cleaned, disinfected, and dried between trials to prevent olfactory cues. The percentage of spontaneous alternation was calculated using the formula:

$$\% \text{ alternation} = \left\{ \frac{\text{Number of alternation}}{\text{Total arm entries} - 2} \right\} \times 100$$

The % alternation was used as an indicator of spatial working memory and cognitive performance in the

aluminium chloride-induced Alzheimer's disease rat model [29].

Estimation of brain enzyme:**Catalase assay**

The activity of catalase in supernatants of brain tissue homogenates was measured. Catalase decomposes H_2O_2 to give water (H_2O) and oxygen (O_2) and oxidation of hydrogen donors. H_2O_2 shows a continuous increase in absorption with reducing wavelength in ultraviolet range. H_2O_2 decomposition can be directly followed by the decrease in absorbance at the wavelength of 240 nm. Absorbance difference per unit time is a measure of catalase enzyme activity using the molar extinction coefficient of $\text{H}_2\text{O}_2 = 43.6 \text{ M cm}^{-1}$. One mmol of H_2O_2 consumed / min/ mg of protein is equal to one unit of activity and this is expressed as U/mg protein. To 0.1 ml supernatant was added to cuvette containing 1.9 ml of phosphate buffer (50 mm, pH 7.0), and the reaction was initiated by adding 1 ml of H_2O_2 (10 mm in phosphate buffer). The change in absorbance was recorded for 1 min at 240 nm using spectrophotometer (Model UV-1650PC). Phosphate buffer was used as a blank. The catalase activity was calculated based on the rate of decomposition of H_2O_2 and was expressed as mmole/min/mg protein [30].

Determination of total protein by Lowry's method [31]

The Lowry protein assay is a sensitive colorimetric method used to estimate protein concentration in biological samples. It is based on the reaction of proteins with alkaline copper reagent followed by the Folin–Ciocalteu reagent, producing a bluish colour proportional to the protein concentration. The absorbance is measured at 750 nm using a spectrophotometer.

Reagents Required:

1. Reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide.
2. Reagent B: 0.5% copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 1% potassium sodium tartrate.
3. Alkaline copper reagent (Reagent C): Prepared freshly by mixing 50 mL of Reagent A with 1 mL of Reagent B.
4. Diluted Folin–Ciocalteu reagent (Reagent D): Folin–Ciocalteu reagent diluted with an equal volume of 0.1 N NaOH.
5. Standard: Bovine serum albumin (BSA) working standard containing 200 $\mu\text{g/mL}$ protein.

BSA standards (0.2–1.0 mL) and 1 mL of sample were taken in separate tubes and made up to 1 mL with distilled water. 5 mL of alkaline copper reagent was added, mixed, and incubated for 10 minutes. Then, 0.5 mL of diluted Folin–Ciocalteu reagent was added, mixed, and incubated in the dark for 30 minutes. Absorbance was measured at 750 nm against

the blank. Protein concentration was determined from the BSA standard curve.

Statistical analysis:

All the values were expressed as mean $\pm$ S.E.M and statistical significance was analysed using one-way ANOVA followed by Dunnett's multiple comparison test. p-values <0.0001 were considered very highly significant, <0.001 considered highly significant, <0.01 were considered moderately significant and <0.05 considered significant.

RESULT AND DISCUSSION

In the DPPH assay, both ascorbic acid and *B. pilosa* extract demonstrated concentration-dependent

increases in percentage inhibition. The % reactive scavenging activity (RSA) produced by *B. pilosa* increased from 32.3% at 20 $\mu\text{g/mL}$ to 89.43% at 100 $\mu\text{g/mL}$, while ascorbic acid increased from 49.2% to 97.87% over the same concentration range. The calculated IC_{50} values were 41.05 $\mu\text{g/mL}$ for *B. pilosa* and 24.38 $\mu\text{g/mL}$ for ascorbic acid. Thus, the DPPH findings provide evidence for the antioxidant potential of the methanolic leaf extract of *Bidens pilosa*. DPPH radical scavenging potential of Ascorbic acid and *B. pilosa* leaf extract shown in table 1 and figure 1.

Table 1: DPPH radical scavenging potential of Ascorbic acid and *B. pilosa* leaf extract.

Sample	Concentration ($\mu\text{g/mL}$)	% RSA	IC_{50} value in
Standard Ascorbic acid	20	49.2	24.38 $\mu\text{g/mL}$
	40	58.13	
	60	70.36	
	80	85.33	
	100	97.87	
<i>B. pilosa</i> leaf extract	20	32.3	41.05 $\mu\text{g/mL}$
	40	51.2	
	60	65.23	
	80	79.28	
	100	89.43	

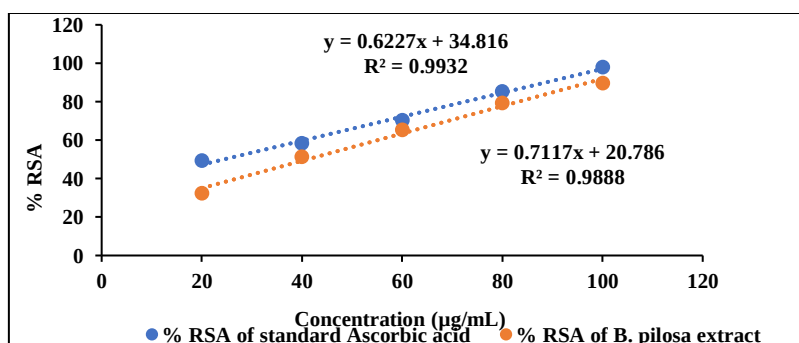


Figure 1: DPPH radical scavenging potential of *B. pilosa* leaf extract.

Aluminium chloride administration produced marked cognitive impairment in experimental rats. In the Elevated Plus Maze (EPM), the Alzheimer's control group showed increased transfer latency compared with the normal control, indicating impaired learning and memory. Treatment with *Bidens pilosa* methanolic leaf extract at 100 and 200 mg/kg reduced transfer latency, with a more pronounced improvement at the higher dose, suggesting enhanced memory retention (Table 2 and Figure2). Similarly, in the Cook's pole climbing test, aluminium chloride increased escape latency, indicating impaired

conditioned avoidance learning. Treatment with *B. pilosa* extract reduced escape latency compared with the Alzheimer's control group, demonstrating improvement in learning and memory, particularly at the higher dose (Table 2 and Figure 3). In the Y-maze test, aluminium chloride-induced rats exhibited reduced spontaneous alternation behaviour, indicating impaired spatial working memory. Administration of *B. pilosa* extract increased spontaneous alternation compared with the disease control, with greater improvement observed at 200 mg/kg (Table 2 and Figure 4).

Table 2: Behaviour assessment data.

Animal groups (n=6)	TL before treatment (sec)	TL after treatment (sec)	EL before treatment (sec)	EL after treatment (sec)	% Alternation
Normal control	16 ± 1.03	13.16 ± 0.59	12 ± 0.5	12 ± 0.82	81.81 ± 1.47
Alzheimer's control	45.83 ± 5.59 ###	44.20 ± 1.37 ###	37.3 ± 3.4 #####	37.33 ± 3.68 #####	48.25 ± 2.67 #####
Alzheimer's + Standard Rivastigmine	44.20 ± 4.5 ###	14.50 ± 0.76 ****	36.6 ± 2.47 #####	17 ± 0.93 ****	73.93 ± 3.56 ***
Alzheimer's + Extract (100 mg/kg)	43.80 ± 4.03 ###	26.33 ± 2.15 **	37.5 ± 3.17 #####	24.50 ± 2.19 *	69.47 ± 6.18 **
Alzheimer's + Extract (200 mg/kg)	44.60 ± 5.10 ###	24 ± 2.24 ***	37.6 ± 1.8 #####	21.83 ± 1.07 **	76.28 ± 4.67 ***

Data are presented as mean ± SEM values, n=6. Data were analyzed by one-way ANOVA followed by the Dunnet's multiple comparison test and the significance of results showed as #####p<0.0001, and ###p<0.001, when compared with normal control and ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 when compared with the Alzheimer's control.

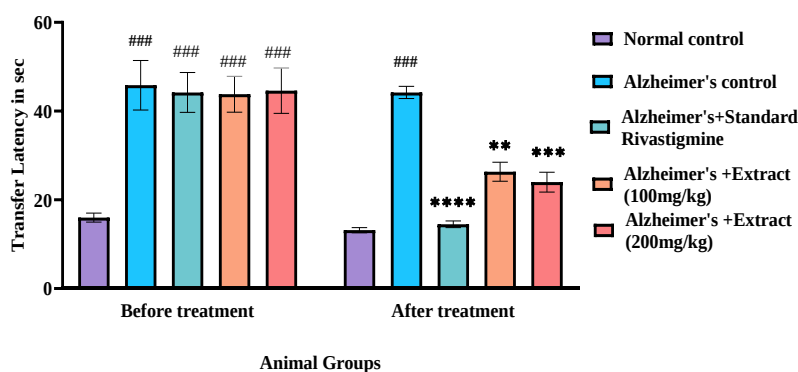


Figure 2: Effect of *B. pilosa* leaf extract on before-treatment and after-treatment transfer latency in the Elevated Plus Maze test in aluminium chloride-induced Alzheimer's rats.

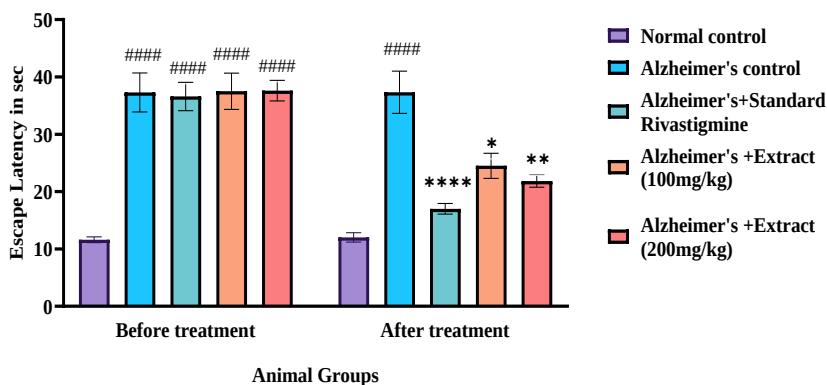


Figure 3: Effect of *B. pilosa* leaf extract on escape latency before treatment and after treatment. Escape Latency in Cook's Pole test in aluminium chloride-induced Alzheimer's rats.

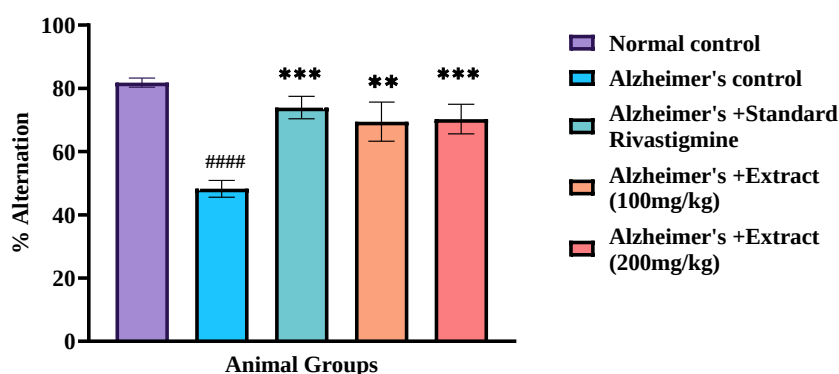


Figure 4: Effect of *B. pilosa* leaf extract on % Alternation in Y maze.

Oxidative stress was evaluated by measuring catalase activity. Catalase levels in normal control rats (Group 1) were found to be 220.99 ± 4.94 U/mg of protein. Alzheimer's control rats (Group 2) showed a significant decrease ($p < 0.01$) in catalase levels in the brain as compared to the normal control group. Rats treated with the standard drug rivastigmine (Group 3) showed a significant increase ($p < 0.05$) in catalase levels in the brain when compared to Group 2. Treatment of the rats with *B. pilosa* (Groups 4 and 5) resulted in a significant ($p < 0.05$) increase in catalase levels in the brain when compared to Alzheimer's control rats (Group 2) table and figure. The reduction in catalase activity in the Alzheimer's control group indicates impairment of endogenous antioxidant defence under the experimental disease condition. Since catalase contributes to the detoxification of

hydrogen peroxide, reduced activity may reflect increased oxidative burden. The progressive restoration of catalase activity following *B. pilosa* treatment, particularly at 200 mg/kg, suggests improvement of the endogenous antioxidant defence system. This finding is also consistent with the *in-vitro* antioxidant results, indicating that the extract may exert neuroprotective effects partly through decrease of oxidative stress (Table 3 and Figure 5). Brain protein concentration was reduced in the Alzheimer's control group ($p < 0.0001$) compared with the normal control. Treatment with Rivastigmine and *B. pilosa* extract (100 and 200 mg/kg) increased protein levels towards normal, with greater improvement at 200 mg/kg, suggesting a protective effect against aluminium chloride-induced neuronal protein alterations (Table 3 and Figure 6).

Table 3: Effect of *B. pilosa* leaf extract on catalase activity and protein concentration

Animal groups (n=6)	Catalase activity (U/mg)	Protein concentration (mg/dL)
Normal control	220.99 ± 4.94	1.37 ± 0.03
Alzheimer's control	166.09 ± 11.54 ##	0.87 ± 0.06 ####
Alzheimer's + Standard Rivastigmine	201.62 ± 6.25 *	1.29 ± 0.04 ***
Alzheimer's + Extract (100 mg/kg)	189.81 ± 4.96 *	1.16 ± 0.03 **
Alzheimer's + Extract (200 mg/kg)	195.04 ± 6.17 *	1.27 ± 0.04 ***

Data are expressed in mean $\pm$ SEM values, n=6. Data were analyzed by one-way ANOVA followed by the Dunnett's multiple comparison test and the significance of results showed as #### $p < 0.01$, ## $p < 0.01$ when compared with normal control group and *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with the Alzheimer's control group.

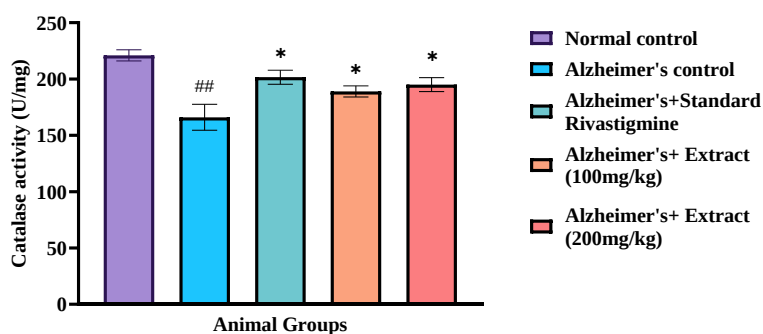


Figure 5: Effect of *Bidens pilosa* leaf extract on catalase activity in rat brain.

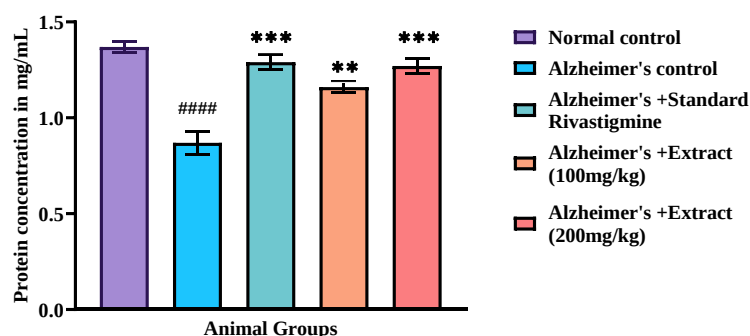


Figure 6: Effect of *B. pilosa* extract on protein concentration.

CONCLUSION:

The present study demonstrated that methanolic *Bidens pilosa* leaf extract possesses promising neuroprotective and anti-Alzheimer's potential against aluminium chloride-induced cognitive impairment in rats. Treatment with *B. pilosa* at 100 and 200 mg/kg improved learning and memory, as evidenced by reduced transfer and escape latencies in the Elevated Plus Maze and Cook's pole tests and increased spontaneous alternation in the Y-maze. The extract also improved catalase activity and brain protein concentration compared with the Alzheimer's control group. Overall, the findings suggest that *B. pilosa* may provide neuroprotection through its antioxidant potential, with the 200 mg/kg dose showing greater improvement in the assessed parameters. Further studies are necessary to establish its precise mechanism of action and therapeutic potential.

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