IJBPAS, August, 2025, 14(8): 4550-4565

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# INVESTIGATING THE NEUROPROTECTIVE PROPERTIES OF *PISTIA STRATIOTES L.* AGAINST ALZHEIMER'S DISEASE INDUCED BY ALUMINUM CHLORIDE IN RAT MODEL

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**Received 10th April 2024; Revised 4th May2024; Accepted 7th Sept. 2024; Available online 1st Aug. 2025**

https://doi.org/10.31032/IJBPAS/2025/14.8.9348

## ABSTRACT

Alzheimer's disease (AD), a prevalent form of dementia in the elderly, is linked to brain damage from various processes, including neurotoxicity from substances like chemotherapeutic agents and heavy metals. This study investigates the neuroprotective properties of a methanolic extract of *Pistia stratiotes L.* in rats with AD induced by aluminum chloride ($AlCl_3$). Wistar rats (230-250 grams) received daily $AlCl_3$ (32.5 mg in saline) for 30 days to induce AD, alongside oral treatments of *Pistia stratiotes L.* extract in medium and high doses, and the standard drug Rivastigmine. Effectiveness was assessed through behavioral tests (motor and spatial learning, exploratory behavior, cognitive function), brain protein levels, and serum acetylcholinesterase activity. Behavioral, biochemical, and histopathological evaluations were conducted. The rotarod test indicated reduced muscle strength due to $AlCl_3$, which improved with *Pistia stratiotes L.* extract. In the Morris water maze test, the extract shortened the time to find the platform, countering the spatial learning deficit. The Hole board test showed increased head dipping (anxiety/exploratory behavior) in the $AlCl_3$ group, partially reduced by the extract. The extract also improved impaired cognitive function suggested by increased transfer latency time. Protein studies showed $AlCl_3$ led to increased brain protein levels and decreased acetylcholinesterase levels, effects reduced by the extract. Histopathological studies revealed significant damage in the AlCl3 group, with improvements seen with the extract. This study

suggests *Pistia stratiotes L*. as a neuroprotective agent, reducing oxidative stress from $AlCl_3$ accumulation and preserving acetylcholinesterase activity, indicating its therapeutic potential for neuroprotection.

**Keywords: Alzheimer's disease, AlCl3, *Pistia stratiotes L.*, Acetylcholinesterase, Neuroprotective, Total brain protein**

## 1. INTRODUCTION

The most common type of dementia, Alzheimer's disease (AD), is characterized by memory loss and attention problems. Its hallmark features include progressive cognitive decline and memory impairment. 44 million people worldwide were affected with AD in 2015. projected to triple by 2050, reaching 115 million individuals, with approximately 4.6 million new cases are reported globally annually **[1]**. A key pathological marker of AD involves the abnormal aggregation of beta-amyloid peptides, which correlates with increased oxidative stress and mitochondrial dysfunction **[2]**. Research efforts are exploring the potential protective effects of vitexin against this aggregation to mitigate its toxicity. While current treatments can alleviate symptoms, they do not halt or reverse disease progression, highlighting the need for novel approaches **[3]**. Given that genetic predisposition, environmental factors, and lifestyle choices contribute to AD susceptibility, this model is chosen for study. In the past decade, there has been significant progress in exploring the effects of flavonoids in mouse models of AD **[4]**. Numerous plants, fruits, and vegetables contain flavonoids, which are well-known for their wide range of pharmacological characteristics, including their anti-inflammatory, neuroprotective, and antioxidant capabilities. The fact that flavonoids can cross the blood-brain barrier is significant because it implies that they may have a direct effect on brain function and may work as a preventative measure to slow the onset of neurodegenerative illnesses like AD **[2]**. Several studies have shown that certain flavonoids, including vitexin, can mitigate beta-amyloid accumulation in laboratory experiments and animal models. Vitexin, present in various medicinal plants like *Ficus deltoida, Spirodela polyrhiza, and Acer palmatum,* exhibits antioxidant, anti-Alzheimer's, and neuroprotective properties. Studies suggest that vitexin enhances cell viability and ameliorates cognitive and locomotor functions in animal models, indicating promise in mitigating neurodegeneration**,** including the inhibition of beta-amyloid peptide toxicity and cholinesterase enzymes **[3]**. The substances found in *Pistia stratiotes L*., a floating aquatic plant, include flavonoids, phytosterols, glycosides, and

alkaloids **[5]**. Notably, the plant's vitexin has been found, indicating possible medicinal advantages. Traditional folklore suggests that infusions made from *P. stratiotes* leaves can treat various conditions **[6]**. Due to the limited literature on *Pistia stratiotes L.* and its rich source of unique active components, this study aims to address significant challenges posed by neurodegenerative diseases, notably Alzheimer's disease, in both basic science and clinical medicine **[7]**.

## 2. METHODOLOGY

### 2.1 Plant collection and validation

*Pistia stratiotes L.* fresh plant. was collected in an area free of pesticides and other contaminants from Attibele area in Bangalore, Karnataka, the plant material in India was identified and authenticated. Dr. P. E. Rajshekaran with the herbarium reference number -PCOL/PS-432/WP/KCP/2021-22.

### 2.2 Preparation of methanolic extract of Pistia stratiotes L.

*Pistia stratiotes L.* was harvested as a fresh plant. After being collected, the plant material was dried in the shade and ground into a powder using a machine grinder. 50% alcohol was used during the extraction process, and 100 mg of the recently pulverized plant were placed in a Soxhlet device. Subsequently, the solvent evaporated at low temperature when there is less pressure. Before using the extract, it was examined **[7]**.

**Percentage yield: Practical yield / Theoretical yield × 100**

### 2.3 Preliminary phytochemical investigation *Pistia stratiotes L.* plant:

It was observed from the preliminary phytochemical screening of the *Pistia stratiotes L*. that plant showed presence of various biologically active constituents like flavonoids, glycosides, alkaloids, steroids, saponins and phenols are tested. It was observed that methanolic extract contain higher concentration of flavonoids component and selected this extract for further study **[8]**.

**2.4 Criteria for dose selection:** Acute oral toxicity has been already been done as per the OECD 425 guidelines and the dose was fixed to be used in chronic models of Aluminium chloride-induced Alzheimer's. **[9]**

**2.5. Experimental animals:** Wistar rats, weighing between 230 and 250 grams, were acquired from the Krupanidhi College of Pharmacy's animal house in Bangalore, India. Before the trial, they were kept in a building with good ventilation and given ten days to get adjusted to their surroundings. A 12-hour light/dark cycle was implemented to maintain a regulated temperature of 25±4°C and a relative humidity of 50–60% in the lab. Water and food were supplied in compliance with the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

recommendations. Under the reference number KCP/IAEC/PCOL/76/2021, the Institutional Ethical Committee authorized the experimental protocol.

### 3.1 Induction of Alzheimer's by Aluminium chloride

For thirty days, an oral dose of 32.5 mg/kg of aluminum chloride was used to induce Alzheimer's disease **[10]**. Pretreatment with medium- and high-dose methanolic extracts of *Pistia stratiotes L.* was initiated concurrently with aluminum chloride, once daily, with a 60-minute gap between administrations **[9]**. Rivastigmine (2.5 mg/kg p.o.) *or Pistia stratiotes L.* were administered to the test subjects along with aluminum chloride **[11]**. On days 1 and 30, behavioral tests such as the Rotarod, raised plus maze, Morri's water maze, and Hole board were carried out. Rats were given chloroform anesthesia, put to death, and their brains removed for additional examination and preservation at -20°C after passing behavioral tests.

### 3.2 Experimental Design

| | |
|---|---|
| **Group 1** | **Vehicle Control (rat was administered with a normal diet and was not given any treatment during the research study). [11]** |
| **Group 2** | **Positive Control (Aluminium chloride, 32.5 mg/kg *p.o*) [10]** |
| **Group 3** | **Medium dose (200mg/kg *p.o*) of methanolic extract of *Pistia stratiotes L.* along with Aluminium chloride [9]** |
| **Group 4** | **High dose (400 mg/kg *p.o*) of methanolic extract of *Pistia stratiotes L*. along with Aluminium chloride [9, 11]** |
| **Group 5** | **Standard drug (2.5mg/kg *p.o*) dose of Rivastigmine along with Aluminium Chloride [9]** |

## 4. BEHAVIOURAL PARAMETERS:

### 4.1. Rotarod Test: Motor Learning

The rotarod test is a method used to evaluate rats' motor coordination, where rats are trained on a rotating rod for two days, and assessed again on the first and 21st days to detect changes. **[12]**.

### 4.2. Morris water maze experiment

The Morris water maze is a sizable circular pool used to investigate the relationship between neurotransmitters and the effects of medication and spatial learning. It divides into four platform-filled quadrants, giving the animals 30 seconds to swim in each to assess hippocampus damage **[13]**.

### 4.3 Hole board test

The Hole Board Test evaluates exploratory behavior and anxiety levels in animals. It involves placing subjects in a box with 16 holes in the floor and observing their head dipping behavior. This behavior is directly linked to anxiety levels. Animals undergo a 2-day training period before the experiment. Head dipping frequency per 5-minute interval was recorded for 6 Subjects on the 1st and 21st days of testing **[14]**.

### 4.4 Elevated plus maze

The Elevated Plus Maze (EPM) measures Transfer Latency (TL), or the amount of time it takes an animal to go from an open arm to a closed arm with all four paws, in order to assess cognitive function. The maze comprises two open arms (50cm × 10cm) and two closed arms (40cm) elevated 50cm above the ground. Rats are familiarized with the maze before testing. Test days are on the 1st and 21st days, with results recorded in a dark, quiet environment **[15]**.

**5.TISSUE PARAMETERS**

**5.1. Total brain protein**

On the thirty-first day following the behavioral testing, biochemical assays were carried out with saline-rubbed brains and euthanized animals. Supernatant from centrifuging brain samples was used to evaluate acetylcholinesterase activity and total brain protein. The samples were homogenized in 0.1M phosphate buffer and compared to a blank **[10]**.

**6. SERUM PARAMETERS**

**6.1 Acetylcholinesterase assay**

The Ellman technique was used to investigate AchE levels in brain tissues. The percentage of the control was used to express the results **[16]**.

**7. HISTOPATHOLOGICAL STUDIES**

On the 31st day, the experiment concluded with the collection of brain samples from the test animals, which were then preserved in 10% formalin and forwarded to the laboratory for additional analysis **[17]**.

**8.STATISTICAL ANALYSIS**

A one-way ANOVA was used for statistical analysis, and the Dunnett test was used for post hoc testing. The results were presented as mean ± SEM, with $P<0.05$ being deemed significant.

**9. RESULTS**

**9.1 Preparation of *Pistia stratiotes L.* methanolic extract and its yield**

By using Soxhlet extraction, *Pistia stratiotes L.*'s methanolic extract was created. A yield of 2.98% was obtained after the solvent was evaporated at lower pressure.

**9.2 Behavioural assessments**

**9.2.1 Rotarod Test (Motor learning)**

Effect of extract from *Pistia stratiotes L*. on rats' motor learning in Alzheimer's disease produced by $AlCl_3$ using Rotarod apparatus. The data (n = 6) are shown as mean ± SEM. Significant differences are denoted by the following: **p < 0.01, ***p < 0.0001 compared to the diseased control group, and ###p < 0.0001 compared to the vehicle control. Standard Error of the Mean is abbreviated as SEM, and aluminum chloride as $AlCl_3$ **(Graph 1)**.

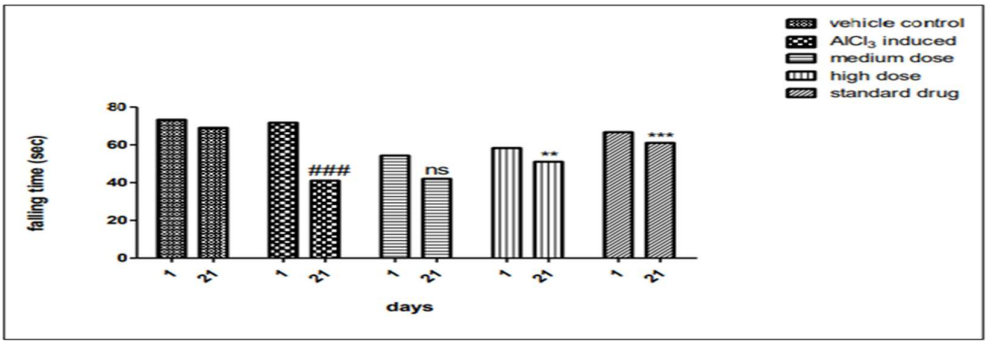
**9.2.2 Morri's water maze test**

*Pistia stratiotes L.* extract's effect on the Morri's water maze test in rats with Alzheimer's disease induced by AlCl3. Mean ± SEM (n = 6) is the data's expression. Notable variations are denoted by ###p < 0.0001 in relation to the vehicle control, and

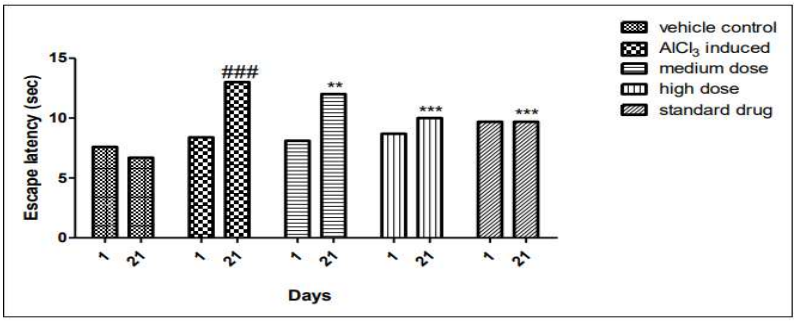
**p^0.001, ***p < 0.0001 in relation to the sick control group. Standard Error of the Mean (SEM); AlCl3, Aluminum Chloride **(Graph 2)**.

**9.2.3 Hole board test**

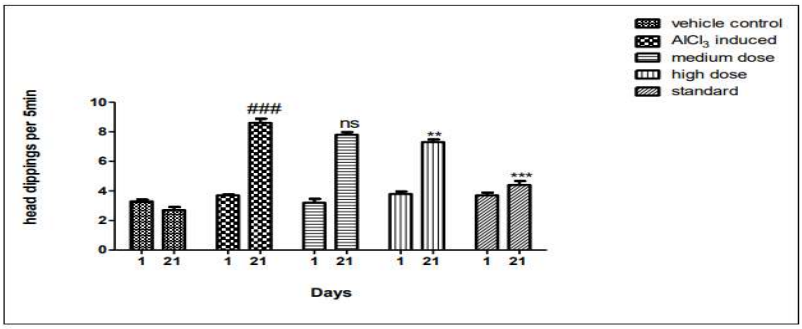
Using a Hole Board device, the impact of *Pistia stratiotes L.* extract on rats' exploratory behavior was evaluated. The effect of the extract on the exploratory behavior of rats treated with aluminum chloride ($AlCl_3$)-induced Alzheimer's disease was also assessed in this investigation. For n = 6, the data are given as mean ± SEM. When compared to the vehicle control group, the statistical significance was recorded as ###p < 0.0001, and when compared to the diseased control group, it was marked as **p < 0.001, ***p < 0.0001. Standard Error of the Mean is referred to as SEM **(Graph 3)**.




**Graph 1: Graphical representation of Effect of *Pistia Stratiotes L.* extract on motor learning of rats using Rotarod Apparatus**




**Graph 2: Graphical representation of Effect of *Pistia Stratiotes L.* extract on spatial learning of rats using Morri's water maze test**




**Graph 3: Graphical representation of Effect of *Pistia Stratiotes L.* extract on exploratory behaviour of rats using Hole board apparatus**

### 9.2.4 Elevated plus maze

The Elevated Plus Maze test was used to assess the impact of *Pistia stratiotes L*. extract on rats' transfer latency in an Alzheimer's disease model caused by AlCl3. The data (n = 6) are shown as mean ± SEM. The following comparisons show statistical significance: *p < 0.05, **p < 0.01, ***p < 0.0001 when compared with the diseased control group, and ###p < 0.0001 when compared with the vehicle control. SEM is for Standard Error of the Mean, while $AlCl_3$ stands for aluminum chloride **(Graph 4)**.

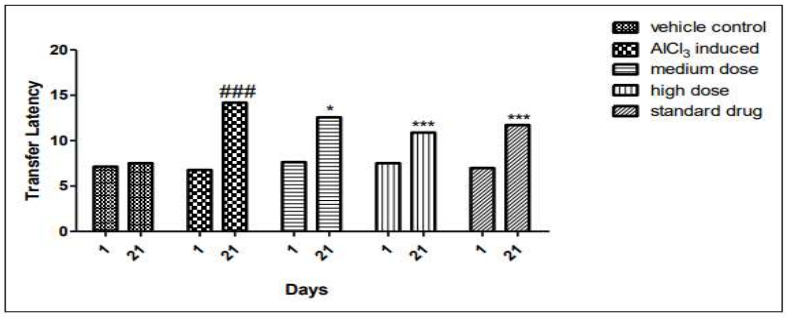



**Figure 4: Graph showing the impact of an extract from *Pistia stratiotes L.* on rats' transfer latency using the Elevated Plus Maze Test**

## 10.TISSUE PARAMETERS

### 10.1. Total brain protein

When compared to the vehicle control group, the animals treated with aluminum chloride showed a considerable increase in protein levels. In contrast to the disease-induced group, treatment with *Pistia stratiotes L*. extract at doses of 200 and 400 mg/kg markedly reduced protein levels. The *Pistia stratiotes L*. extract treatment yielded satisfactory results, comparable to those of the Rivastigmine-treated group, effectively reducing total brain protein levels **(Table 1)**.

**Table 1: Effect of *Pistia Stratiotes L.* extract on Total brain protein concentration of rats.**

| Treatment groups | Concentration (g / dl) (6 animals) | | | | | | Mean ± SEM |
|---|---|---|---|---|---|---|---|
| Vehicle control | 0.11 | 0.04 | 0.10 | 0.075 | 0.08 | 0.09 | 0.083 ± 0.010 |
| $AlCl_3$ induced | 0.14 | 0.50 | 0.60 | 0.30 | 0.20 | 0.20 | 0.323 ± 0.076### |
| Medium dose | 0.23 | 0.16 | 0.15 | 0.17 | 0.22 | 0.24 | 0.195 ± 0.016ns |
| High dose | 0.22 | 0.15 | 0.14 | 0.17 | 0.23 | 0.22 | 0.188 ± 0.016* |
| Standard drug | 0.22 | 0.14 | 0.10 | 0.13 | 0.15 | 0.17 | 0.15 ± 0.015** |

The present study reports the mean ± standard error of mean (n = 6) regarding the impact of *Pistia stratiotes L.* extract on the concentration of total brain protein in rats suffering from Alzheimer's disease induced by AlCl3. *p<0.05, **p < 0.01, and ###pˆ 0.0001 compared to the vehicle control group are indications of significant differences. Aluminum chloride is represented by the symbol $AlCl_3$, and the Standard Error of the Mean is denoted by SEM.

## 11. SERUM PARAMETERS

### 11.1. Assay for acetylcholinesterase

When compared to vehicle control animals, the brains of animals treated with aluminum chloride exhibited a substantial increase in AChE activity ($p < 0.05$). Comparing the treated animals to the disease control group, the administration of *Pistia stratiotes L.* extract effectively inhibited the increase in AChE activity caused by the administration of 400 mg/kg of aluminum chloride ($p < 0.05$). The group treated with *Pistia stratiotes L.* extract had similar outcomes to the group treated with rivastigmine **(Table 2)**.

**Table 2: Effect of *Pistia Stratiotes L.* extract on acetylcholinesterase levels in rat brain.**

| reatment group | OD Value at 412nm (6 animals) (Day 31) | | | | | | Mean ± SEM |
|---|---|---|---|---|---|---|---|
| Vehicle control | 0.49 | 0.45 | 0.45 | 0.49 | 0.52 | 0.45 | 0.47 ± 0.012 |
| $AlCl_3$ induced | 0.66 | 0.63 | 0.62 | 0.68 | 0.70 | 0.66 | 0.65 ± 0.012### |
| Medium dose | 0.65 | 0.68 | 0.62 | 0.72 | 0.50 | 0.66 | 0.63 ± 0.030ns |
| High dose | 0.45 | 0.52 | 0.58 | 0.50 | 0.62 | 0.66 | 0.55 ± 0.032* |
| Standard drug | 0.42 | 0.40 | 0.48 | 0.50 | 0.57 | 0.52 | 0.48 ± 0.025** |

*Pistia stratiotes L.* extract's effect on the acetylcholinesterase assay in rats with Alzheimer's disease induced by AlCl3. As mean ± SEM (n = 6), the data are displayed. When comparing the vehicle control group to the sick control group, ***$p < 0.0001$ and *$p < 0.05$, respectively, showed statistical significance. Aluminum chloride is referred to as $AlCl_3$, and standard error mean is represented by SEM.

## 12.HISTOPATHOLOGICAL ANALYSIS:

### 12.1. Histology report of vehicle control rat brain

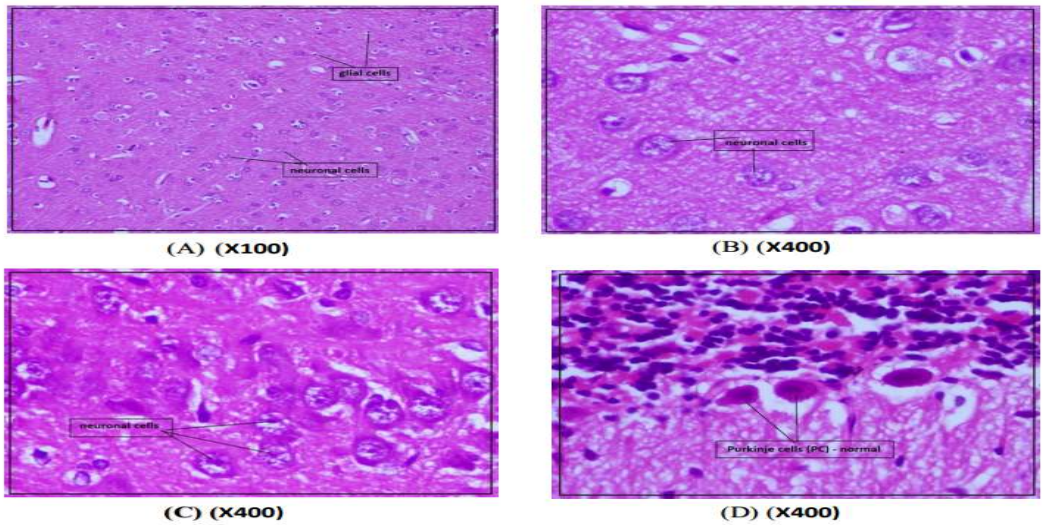



**Figure 8: Histology of rat brain showing normal morphology (cortical region, hippocampus region and pyramidal region)**
**A. Cortical region showing Glial cell & neuronal cell normal morphology (X100)**
**B. Cortical region showing neuronal cell normal morphology (X400)**
**C. Hippocampus region showing neuronal cell normal morphology (X400)**
**D. Purkinje cells (PC) in a pyramidal region with normal shape (X400)**

**13.2. Analysis using histology to determine how aluminum chloride ($AlCl_3$) affects the rat brain's development of Alzheimer's disease**

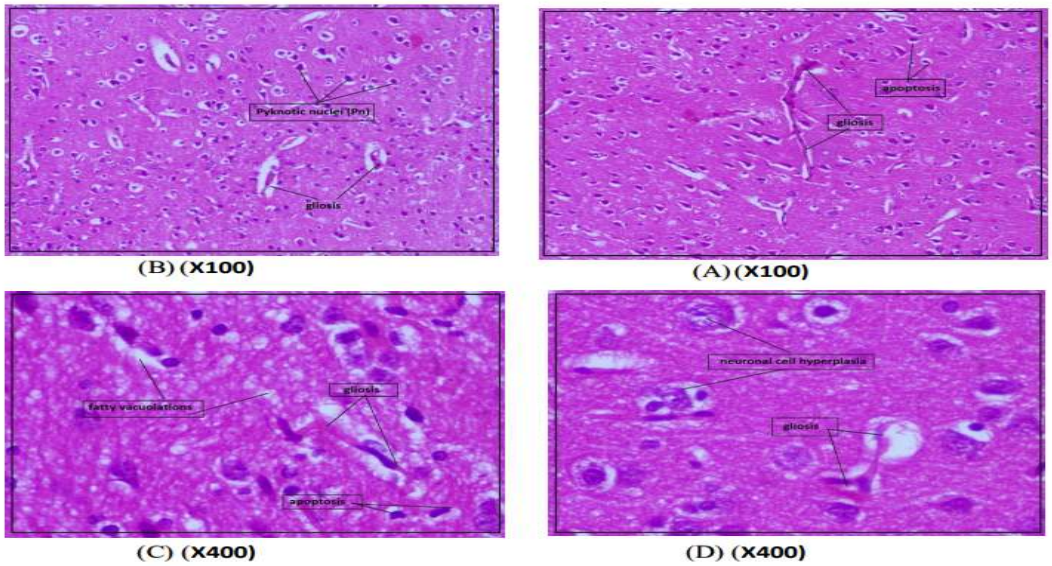



**Figure 9: Histology of rat brain showing Moderate Gliosis, Pyknotic nuclei (Pn), apoptosis, fatty vacuolations and neuronal cell hyperplasia in Cortical region.**
**A. Cortical region showing moderate Pyknotic nuclei (X100)**
**B. Cortical region showing moderate Gliosis and Apoptosis (X100)**
**C. Cortical region showing moderate Fatty vacuolations, Gliosis and Apoptosis (X400)**
**D. Cortical regions showing moderate Neuronal cell hyperplasia (X400)**

**13.3. The effect of *Pistia stratiotes L.* extract on Alzheimer's disease in rats caused by aluminum chloride ($AlCl_3$) was examined histologically.**

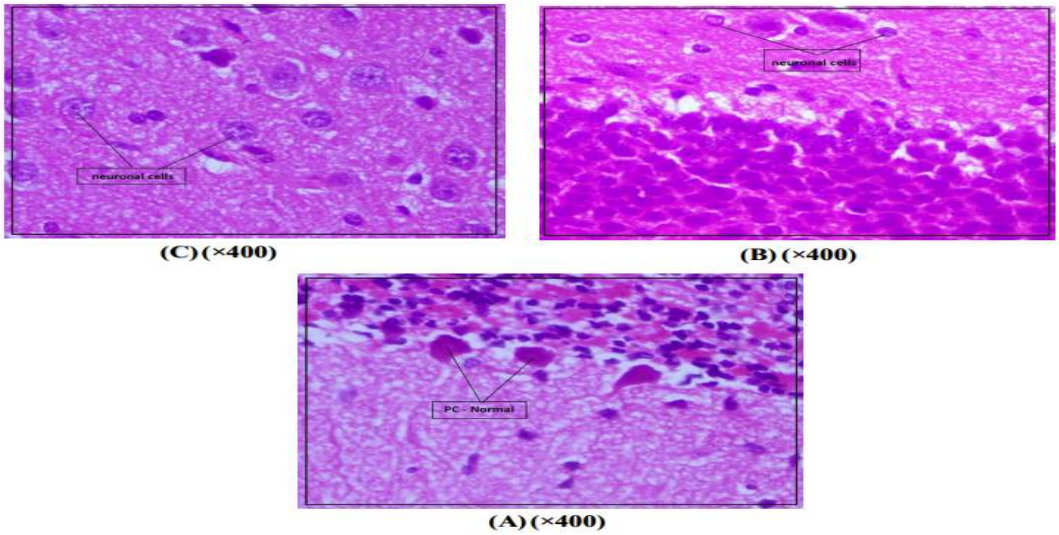



**Figure 10: Histology of rat brain showing normal morphology (cortical region, hippocampus region and pyramidal region)**
**A. Cortical region showing neuronal cell normal morphology (X400)**
**B. Hippocampus region showing neuronal cell normal morphology (X400)**
**C. Pyramidal region showing PC normal morphology (X400)**

### 13.4. Histological analysis of the impact of Rivastigmine, a standard drug, on the effects of aluminum chloride-induced Alzheimer's in rat brains

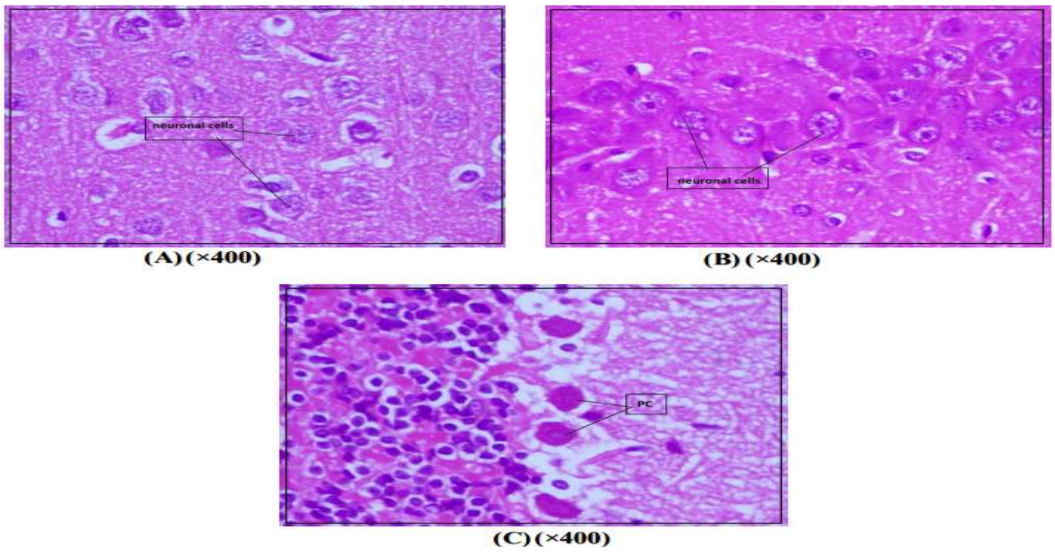



**Figure 11: Histology of rat brain showing normal morphology (cortical region, hippocampus region and pyramidal region)**
**A. Cortical region showing neuronal cell normal morphology (X400) (A) (×400) (B) (×400) (C) (×400)**
**B. Hippocampus region showing neuronal cell normal morphology (X400)**
**C. Pyramidal region showing PC normal morphology (X400)**

## 14. DISCUSSION

The purpose of the current study was to investigate the neuroprotective effects of *Pistia stratiotes L.* on Alzheimer's disease in Wistar rats that was caused by aluminum chloride. Neurodegenerative diseases, including Alzheimer's disease, pose significant challenges to global healthcare due to their high prevalence among dementias. Age-related increases in the prevalence of Alzheimer's disease have afflicted 35.6 million individuals worldwide; by 2030, that figure is expected to rise to 65.7 million, and by 2050, it is expected to reach 115.4 million. according to Anders and Martin. Aluminum is known to contribute to Alzheimer's disease by promoting oxidative damage to cellular lipids, proteins, and DNA **[18]**. Due to its ability to increase oxidative damage in the brain, cause neuronal inflammation, and impair working memory, visuoperception, attention, and semantic memory, aluminum has been connected to the onset of neurodegenerative disorders and cognitive impairments. Aluminum also influences alterations in cholinergic and noradrenergic neurotransmission and functionally modulates the blood-brain barrier **[19]**. Alzheimer's disease typically starts affecting individuals older than 65 years old and is a major contributor to dementia. It features a significant reduction in brain cholinergic activity. Key neuropathological indicators

include intracellular neurofibrillary tangles and extracellular amyloid-beta plaque deposits. The disease progressively impairs memory, judgment, decision-making, spatial orientation, and language skills. As Alzheimer's progresses, it leads to memory loss and cognitive issues such as agnosia and aphasia, severely disrupting daily and personal activities. The risk of developing the disease doubles every five years **[20]**. Initially, *Pistia stratiotes L.* was collected, identified, and subjected to extraction using alcohol. The alcoholic extract underwent phytochemical analysis, demonstrating the existence of several substances that are physiologically active, including phenols, alkaloids, glycosides, flavonoids, steroids, and saponins. Following this initial phytochemical screening, behavioral evaluations were conducted **[7]**. The current study demonstrated that administering aluminum chloride (AlCl3) led to a progressive decline in rats' motor coordination. The Rotarod test, which assesses motor learning by recording the time it takes for rats to fall, showed that an increase in falling time indicates improved motor learning. Rats treated with the extract from *Pistia stratiotes L.* demonstrated a substantially longer falling time than those that received aluminum chloride treatment, suggesting an enhancement in motor learning **[3]**. The recent study revealed that administration of AlCl3 led to a progressive decline in motor coordination in rats. The Rotarod test, which assesses motor learning by timing how long rats can stay on a rotating rod, was employed. In this test, longer falling times indicate better motor learning. Rats treated with the extract of *Pistia stratiotes L.* demonstrated significantly longer falling times compared to those given aluminium chloride, suggesting enhanced motor learning. The Morris Water Maze (MWM) examination evaluates cognitive functions, specifically spatial learning, by timing how long it takes the rats to locate a submerged platform, known as escape latency (EL). A reduction in EL signifies an enhancement of spatial memory and learning. In this study, rats that received *Pistia stratiotes L.* extract demonstrated a substantial reduction in EL as compared to the group given aluminum chloride treatment, indicating enhanced memory and cognitive functions **[21]**. Buildup of aluminum in the brain disrupts the function of important signaling molecules like cyclic GMP, leading to Dysfunction in the Glutamate-nitric oxide-cyclic GMP pathway. This Interference with this pathway leads to cognitive impairments and neurobehavioral deficits in animals. However, Therapy with high doses of *Pistia stratiotes L.* has been shown to reverse spatial cognitive impairments. In the Hole board test, which assesses anxiety-related responses, an increased number of head dips

per 5 minutes is observed in animals exposed to aluminum chloride, indicating heightened anxiety and reduced exploratory behavior. Conversely, animals treated with a high dose of *Pistia stratiotes L*. exhibited a reduction in the number of head dips per 5 minutes, suggesting a decrease in anxiety levels. The Elevated Plus Maze Test (EPM) results indicated that rats treated with aluminum chloride exhibited a notable rise in transfer latency relative to control animals. However, compared to the rats treated with aluminum chloride, a methanolic extract of *Pistia stratiotes L.* administered at doses of 200 and 400 mg/kg resulted in a considerable reduction in transfer latency in a dose-dependent manner **[1]**. One Additionally, table 1's findings showed that rats treated with aluminum chloride had higher total brain protein levels in their brain homogenates than did the animals in the vehicle control group. When compared to the protein levels in rats treated with aluminum chloride, treatment with the methanolic extract of *Pistia stratiotes L*. at 200 and 400 mg/kg dosages significantly and dose-dependently lowered these levels **[22]**. Lima LK *et al*. reported that vitexin can cover both the formation and the toxicity induced by beta-amyloid peptide, which are key factors in Alzheimer's Disease (AD) pathogenesis. Vitexin's effects at safe concentrations help reduce neural degeneration and enhance cognitive function by inhibiting Acetylcholinesterase. Rats treated with AlCl3 had significantly higher levels of brain acetylcholinesterase than vehicle control animals, according to **Tables 2 and 3**. However, in comparison to the rats treated with aluminum chloride, treatment with the methanolic extract of *Pistia stratiotes L.* at dosages of 200 and 400 mg/kg dramatically reduced the levels of acetylcholinesterase in the brain in a dose-dependent way. Histopathological evaluations were conducted to assess the cytoprotective activity of *Pistia stratiotes L*. against AD induced by aluminum chloride in rat models. These evaluations revealed that treatment with *Pistia stratiotes L.* mitigated aluminum chloride-induced brain changes, including reducing oxidative stress and neuroinflammation. Notable improvements were observed in three regions of the rat brain, characterized by reductions in oxidative damage, gliosis, apoptosis, and fatty vacuolar changes. In the study, the rat brains affected by aluminum chloride ($AlCl_3$), which induces Alzheimer-like symptoms, showed significant pathological changes. These changes included pyknotic nuclear condensation, severe hyperplastic fatty vacuolar alterations, inflammation, and congestion in the cortical and hippocampal regions. Additionally, hyperplasia of Purkinje cells was observed in the pyramidal region. However, Within the rat cohort administered

the medication, there was a noticeable improvement across all three brain regions. Compared to the severity induced by $AlCl_3$, the drug-treated brains demonstrated a substantial reduction in damage, with the histological architecture of the brain largely returning to near-normal morphology. These findings strongly suggest that vitexin, a compound derived from *Pistia stratiotes L.,* is effective as a neuroprotective agent **[23]**. The observed improvements in the treated rat brains were satisfactory across all evaluated parameters, comparing favorably both to the rivastigmine is the usual treatment for Alzheimer's disease, and the model is induced by aluminum chloride. This underscores the potential of vitexin as a beneficial treatment for neurodegenerative conditions similar to Alzheimer's disease **[1]**.

## 14. CONCLUSION

The phytochemical screening of *Pistia stratiotes L.'s* alcoholic extract unveiled Alkaloids, glycosides, flavonoids, steroids, saponins, and phenols are all present. Concurrent administration of this extract exhibited dose-dependent effects, such as increased falling time in the Rotarod Test, decreased head dipping in the Hole Board Test, extended Escape Latency in the Morris Water Maze Test, and reduced latency during transfers in the Elevated Plus Maze Exam. Additionally, treatment with the extract led to dose-dependent decreases in total brain protein levels and Acetylcholinesterase activity. Consequently, it may be said that *Pistia stratiotes L.*'s methanolic extract has neuroprotective qualities.

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