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Neuroprotective activity of Aerva lanata extracts in Aluminium chloride induced Alzheimer's disease in Wistar rats

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ABSTRACT

Alzheimer's disease is a degenerative brain disorder that is associated with dementia that slowly erases memory, thinking, and behavioural skills, accounting for 60-70% of all cases affecting millions of people worldwide. Amyloid Beta forms extracellular neuritic plaques, and these neuritic plaques, along with intracellular NFTs, are the two hallmark features of Alzheimer's disease. Aluminium is one of the abundant elements. Aluminium exposure is associated with neurodegenerative diseases, particularly aluminium chloride ($AlCl_3$). In this study, the neuroprotective and therapeutic potential of *Aerva lanata* was investigated in $AlCl_3$-induced neurotoxicity in Wistar rats.

Thirty male Wistar rats were divided into 5 groups, with each group consisting of 6 rats treated for 28 days. The groups included control, $AlCl_3$, Standard, ethanolic, and aqueous extract-treated groups. Behavioural analysis was performed using the MWM Test. Biochemical examination of brain homogenates was performed to determine the activity of antioxidant markers and AChE activity as a measure of cholinergic function. Histopathological evaluations were also performed to assess tissue-level changes.

The administration of aluminium chloride led to notable cognitive deficits, elevated oxidative stress markers, increased AChE activity, and histopathological damage, confirming the successful induction of Alzheimer's like pathology. The *Aerva lanata* treatment, in particular its aqueous extract, significantly improved behavioural performance, reduced oxidative stress, suppressed AChE activity, and ameliorated histopathological damage. Both aqueous and ethanol extracts of *Aerva lanata* treated groups showed better efficacy compared to the standard treatment.

These findings suggest that *Aerva lanata*, especially in its aqueous form, offers multi-targeted neuroprotective effects against AD-like neurodegeneration through antioxidant and cholinergic modulating properties. This study highlights the therapeutic potential of *Aerva lanata* as a natural and effective candidate for the management of Alzheimer's disease. Further investigations are required to validate the mechanism and applicability in other AD models and human trials.

Keywords: *Alzheimer's disease, Aluminium chloride, Aerva lanata, Neurodegeneration*

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1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative illness and is the most prevalent type of dementia, accounting for 60–80% of all dementia cases. [1] Currently, over 46 million people suffer from dementia worldwide, and it is expected to increase threefold and reach 131.5 million cases globally by the year 2050. [2]

Depending upon the stage, it mainly affects cognitive ability and memory but also shows an impact on behavior and daily activities like mobility and speech [3]. AD is defined by the co-existence of intracellular β-amyloid plaques in the cortical and hippocampal regions of the brain and extracellular neurofibrillary tangles of hyperphosphorylated tau, neuroinflammation, dysfunction of cholinergic neurons due to which synthesis and secretion of acetylcholine decreases, and oxidative stress eventually leads to cognitive decline, neuronal damage, and brain atrophy. [4]

Amyloid-beta (Aβ) plaque accumulation is the characteristic feature of Alzheimer's disease. [5] They are formed by the aggregation of amyloid-beta peptides, produced from the cleavage of amyloid precursor protein (APP) by enzymes called secretases. In Alzheimer's, amyloid-beta peptides often form oligomers and larger fibrils, accumulated in the extracellular space of the brain.[6] These accumulated plaques are harmful to neurons, where they lead to disruption of synaptic function, and may trigger inflammatory responses that lead to further damage to brain tissue. These plaques are comprised of fibrils of amyloid-beta peptides (Aβ) that are cleaved from the larger transmembrane amyloid-ß precursor protein. [7]

Amyloid plaques and neurofibrillary tangles (NFTs) are two neuropathological characteristics of Alzheimer's [8]. The clinical signs of dementia were linked to the presence of pAβ in Aβ aggregates [9]. NFTs are argyrophilic hyperphosphorylated tau protein aggregates that form a β-pleated sheet shape. These protein aggregates are believed to impair cytoskeletal integrity, axonal transport, synapses, and neuronal function. [8] Intracellular accumulations of hyperphosphorylated tau protein (neurofibrillary tangles) are yet another key feature. [10] Tau normally stabilizes microtubules. However, in Alzheimer's, it becomes abnormally phosphorylated, which causes it to detach from the microtubules and aggregate into twisted tangles. These tangles disrupt cellular function and contribute to neuronal death. [11]

In the axon, soma, and dendrites, the infiltration of abnormally phosphorylated tau-protein is the first indicator of the tau pathology in a neuron [12]. Neurons are still morphologically normal at this point. Then, fibrillary tau-containing material emerges in the cytoplasm, progressively pushing the neuronal nucleus to the periphery until it is entirely extruded out of the cell body, and the cell dies. Upon neuronal death, the tangle stays behind as an extracellular ghost [13]. Additionally, tau can be found as neuropil threads within dendrites. [8]

Aluminium is a common element that is discharged throughout the environment through natural geological mechanisms and human activities. Aluminium is a risk factor for many age-related neurodegenerative disorders such as Alzheimer's disease. Aluminium chloride is a neurotoxicant that accumulates in the brain, affecting neurotransmission. The oxidative stress produced due to aluminium chloride contributes to the neuronal damage, leading to dysfunction in brain cells. Exposure to aluminium chloride leads to impaired learning and memory functions in animal models. Aluminium chloride ($AlCl_3$) was used to stimulate dementia in many animal models. As per reports, prolonged exposure to Aluminium chloride may induce neurodegenerative changes associated with dementia in Wistar rats. [14]

The aim of this study is to explore the beneficial and therapeutic outcomes of *Aerva lanata* extract in the regression of neurodegenerative features of Alzheimer's dementia in $AlCl_3$ intoxicated Wistar rat models. Herbal plants have been used as traditional medicine for many years for their low cost and fewer side effects. *Aerva lanata* plant is a wolly, prostate undershrub in the family Amaranthaceae and occurs throughout India as a common weed in fields and waste places. The root has a camphor-like aroma. The plant has numerous medicinal effects due to the presence of many secondary metabolites found in it. *Aerva lanata* whole plant methanol extracts showed interesting antimicrobial activities against Bacillus subtilis, Staphylococcus aureus, Shigella dysenteriae, and many others. The seed and leaf extracts of *Aerva lanata* showed antiparasitic activity against the tapeworm. [15]

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Aerva lanata extract, Water, Ethanol, Aluminium chloride, Donepezil, Phosphate buffer, DTNB, Acetyl thio iodide, Pyragallol solution, Trichloro acetic acid, DMSO, Hydrogen peroxide.

2.2. Methodology

2.2.1 Experimental Animals

Thirty Wistar rats were used in this experiment. They were caged, provided with water, and fed commercially available rodent pellets under a standard laboratory environment. They were given a week to acclimate before the experiment began. The IAEC in the school of pharmacy, Anurag University, approved the experimental procedures. The animal ethical approval number is I/IAEC/AU/14/2025/WR♂. All the mentioned recommendations for animal care were followed as recommended by the CCSEA.

2.2.2 Experimental grouping and Treatment regimen

30 healthy Wistar male rats (270-350g) were divided into 5 groups, each comprising 6 rats (n=6/group) and aligned as follows [16]

Groups	Administration
1. Normal	Control (vehicle treated)
2. Negative	Aluminium chloride (100mg/kg Body weight)
3. Standard	Aluminium chloride(100mg/kg Body weight) + Donepezil (2.5mg/kg)
4. Ethanolic	Aluminium chloride(100mg/kg) + Ethanolic Extract (100mg/kg)
5. Aqueous	Aluminium chloride(100mg/kg) + Aqueous Extract (100mg/kg)

The dosage administration was based on the body weight (b. wt) of the rat. Rats in Group 1 (Control Group) were given distilled water for the entire duration of the experiment. This group served as a baseline to compare the effects of other treatment groups. Rats in group 2 were given aluminium chloride at a dose of 100 mg/kg body weight in distilled water for 28 days. Rats in group 3 received donepezil (2.5mg/kg) orally one hour prior to the administration of aluminium chloride. Rats in Groups 4 and 5 received ethanolic extract (100mg/kg) and aqueous extract (100mg/kg) before $AlCl_3$ administration.

The rats were monitored closely every day to ascertain their food consumption. At the conclusion of the study, all rats were subjected to behavioural tests before being euthanized. The blood and brain samples were promptly collected after the rats were anesthetized for subsequent pathological and biochemical examination.

2.2.3 Assessment of behavioural parameters in the rats

Behavioural assessment studies were conducted to examine the learning and memory abilities in rats utilizing the maze test. Morris water maze test (MWM) was employed to carry out the task with the rats.

2.2.4. Morris water navigation task

The rats were placed in a large circular pool filled with water and were allowed to swim to reach the end of a platform. [16] The time taken by the rats to reach the platform's end from various positions and escape from the water (escape latency - ELT) was recorded prior to the execution of treatment. After treatment, the rats' ELT to reach the platform was recorded. [17]

2.2.5 Evaluation of Antioxidant Markers

The evaluation of hippocampus tissue homogenate anti-oxidant markers (Glutathione, Catalase, Superoxide Dismutase [SOD]) was carried out using the UV spectroscopic method.

2.2.6 Tissue homogenate preparation

After behavioral assessment, rats were euthanized, and brains were extracted from the rats and stored in the refrigerator. Later, Refrigerated brain samples were divided into two symmetrical parts; the first half was kept in 10% saline for biochemical examination. The remaining half was preserved in 4% formaldehyde for histological examination. The tissues were thoroughly homogenized in PBS solution (pH 7.4) and centrifuged at high speed for 20 minutes at 4 °C. The resultant supernatant was analysed for different biochemical parameters. [18]

2.2.7 Superoxide Dismutase

The concentration of Superoxide Dismutase was assessed by its ability to inhibit superoxide-mediated reduction. [19] A single unit of SOD is defined as the amount of enzyme required to halve the oxidation of pyrogallol. The estimation of Superoxide dismutase was performed by taking the tissue solution and adding it to pyrogallol solution and PBS, and later analysed at 312 nm wavelength.

2.2.8 Reduced Glutathione

To prepare the reaction mixture, 1 ml of brain homogenisation is added to 1 ml of 10% trichloroacetic acid, later adding 0.5ml of DTNB and 4 ml of phosphate solution. The absorbance was observed at 412 nm [21], and the activity was expressed as nmol/min/mg protein

2.2.9 Catalase

One Catalase unit was defined as the quantity of enzyme required to break down 1µmol of hydrogen peroxide (H_2O_2) per minute.[20] The estimation of catalase (CAT) was done by adding the brain supernatant to the hydrogen peroxide and phosphate buffer, and later analyzing the absorbance at 240 nm. The CAT activity was expressed as µmol/min/mg.

2.2.10 Determination of acetylcholinesterase (AchE) activity

In a falcon tube, 0.5ml of brain supernatant, 2.5ml of phosphate buffer (7.4), 0.1ml of 5,5 DTNB, and 20 microliter of acetyl thiocholine iodide were mixed. The absorbance was then recorded at 412 nm. [22] The enzyme inhibition percentage was calculated as follows:

$$Inhibition = 100 - \frac{change\ of\ sample\ absorbance}{change\ of\ blank\ absorbance} \times 100$$

2.2.11 Evaluation of Histopathological Alterations in rats induced with aluminium chloride

The other half of the brain sample was placed flat on a filter paper for the duration of 48 hrs, and later processed with alcohol for dehydration. The tissues were then soaked in paraffin wax for at least 30 min at 56 °C and were formed into a block. The block was cut into 4-micrometer-thick slices. H & E staining was used to examine the slices on glass slides, which were then examined under a microscope. [23]

2.3 Statistical Analysis

All the data were expressed as Mean ± S.E.M. A one-way analysis of variance (ANOVA) was used to test the significance of difference among the groups, followed by Tukey's test using SPSS software, and $p < 0.05$ was considered significant.

3. RESULTS

3.1 Morris Water Navigation task

3.1.1 Escape latency

Cognitive performance of rats in the Morris water Maze (MWM) test was examined by measuring escape latency for a duration of 5 days. As shown in Table 1 and Fig.1, rats that were exposed to $AlCl_3$ exhibited a greater increase in escape latency compared to the control group, which indicates impaired spatial learning and memory. In contrast, rats treated with standard, ethanolic extract, and aqueous extract of *Aerva lanata* showed a marked reduction in escape latency compared to the $AlCl_3$ group, showing improvement in learning performance.

Among the treatment groups, both the aqueous and ethanolic extracts were close to standard, showing a greater reduction in escape latency. However, the Aqueous extract of *Aerva lanata* displayed a more marked reduction in escape latency, approaching the efficacy of the standard.

Table 1: Latency to reach the platform

Day-wise averages					
	Normal	**Negative**	**Standard**	**Ethanolic**	**Aqueous**
Day-1	55.00	57.33	66.66	57.00	59.66
Day-2	43.33	49.67	45.00	48.33	40.33
Day-3	32.00	41.00	27.33	36.00	38.00
Day-4	35.67	37.67	22.33	31.66	27.67
Day-5	22.33	33.33	17.00	21.67	20.67
Mean	37.67	43.80	35.67	38.93	37.27
S.D	12.29	9.65	20.27	13.91	14.83

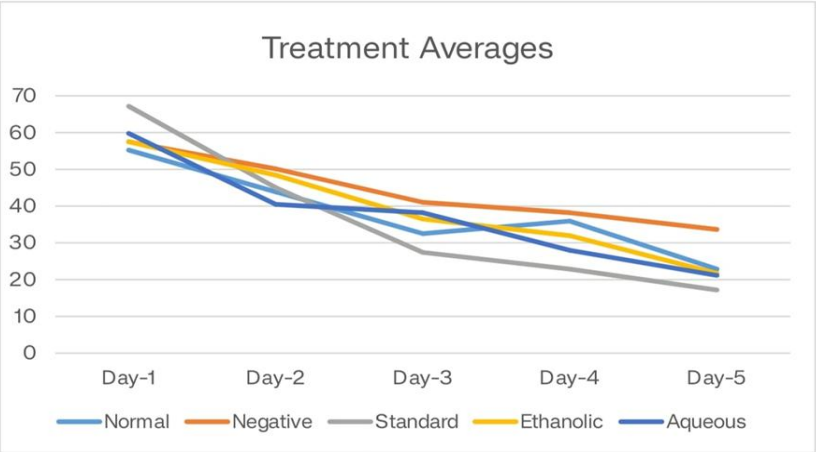

Fig.1- Represents the latency to reach the platform (sec) during the five-day training period after treatment. Data are presented as Mean ± Standard Deviation (SD). Statistical significance determined by ANOVA with significance levels - (p<0.01; *p<0.05) in 6 rats of each group.**

3.1.2 Probe Test

We conducted the probe test to evaluate the spatial memory retention of rats in 5 groups by measuring the time spent in the target quadrant where the platform was previously located. Rats of the Negative group ($AlCl_3$ group) spent notably less time in the target quadrant (16.667± 4.509) compared to the control group (24.467± 5.132), indicating impaired memory retention.

Significant improvements were shown by the standard (33.333 ± 4.041), ethanolic extract group (27.333 ± 3.512), and aqueous extract group (34.343 ± 5.132) (results shown in table 2 and Fig. 2). Notably, the aqueous extract treatment group exhibited the highest recovery, representing superior activity than standard group ($p<0.01$) strongly indicating the effectiveness of *Aerva lanata* in restoring memory function.

Table 2: Probe test

Groups	Time in target quadrant (sec)
1. Normal	24.467± 5.132
2. Negative	16.667 ± 4.509
3. Standard	33.333 ± 4.041
4. Ethanolic	27.333 ± 3.512**
5. Aqueous	34.343 ± 5.132***

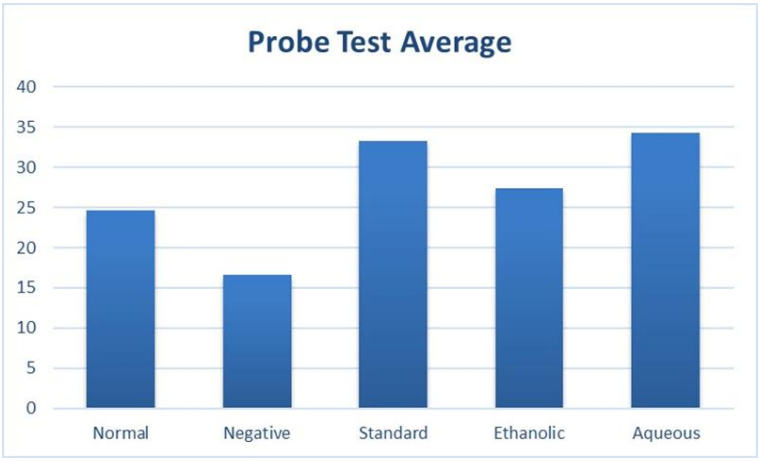

Fig.2 Time spent in target quadrant. Data are presented as Mean ± Standard Deviation (SD). Statistical significance determined by ANOVA with significance levels - (p<0.01; *p<0.05) in 6 rats of each group.**

3.2 Antioxidant Enzyme Activities

3.2.1 Superoxide Dismutase (SOD)

Table 3 and Fig.3 illustrate the estimation of SOD (superoxide dismutase). The SOD activity was notably lower in the negative group ($AlCl_3$) (0.92 ± 0.2957 units/min/mg) compared to the control group (2.37 ± 0.929 units/min/mg). Treatment with aqueous extract significantly increased SOD levels (2.10 ± 0.721 units/min/mg) near the standard, while ethanolic extract showed a moderate effect (1.27 ± 0.4726 units/min/mg).

Table 3: Estimation of SOD (superoxide dismutase)

Groups	SOD(units/min/mg)
1. Normal	2.37 ± 0.929**
2. Negative	0.92 ± 0.2957
3. Standard	3.40 ± 0.7057
4. Ethanolic	1.27 ± 0.4726
5. Aqueous	2.10 ± 0.721**

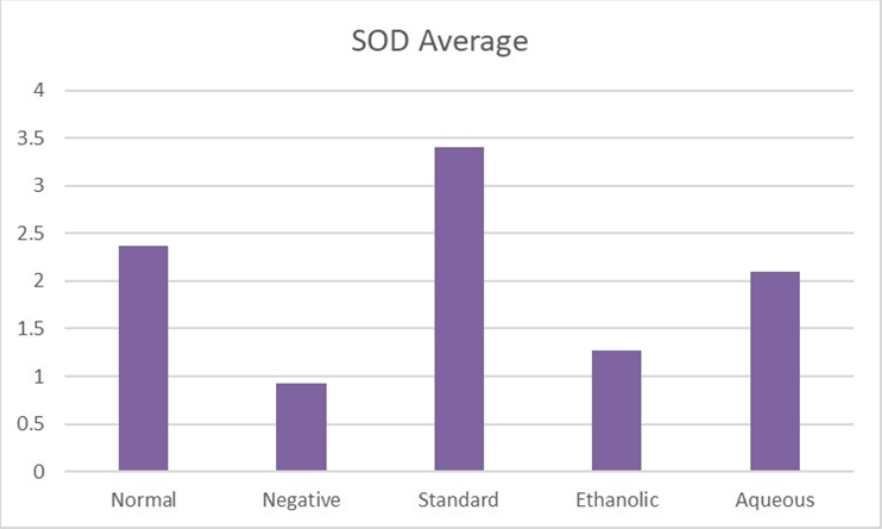

Fig.3 SOD levels in brain homogenates in units/min/mg protein. Data are presented as Mean ± Standard Deviation (SD). Statistical significance determined by ANOVA with significance levels - (p<0.01; *p<0.05) in 6 rats of each group.**

3.2.2 Glutathione (GSH)

The GSH concentration significantly decreased in the Negative group ($AlCl_3$) (19.633 ± 0.946), while the aqueous extract-treated group and the ethanolic extract-treated group indicated elevated GSH levels compared to the toxic group. This investigation suggests the potential of *Aerva lanata* in restoring redox balance in neuronal tissues.

Table 4: Estimation of GSH (glutathione)

Groups	GSH(nmol/min/mg protein)
1. Normal	31.167 ± 2.59**
2. Negative	19.633 ± 0.946
3. Standard	33.7 ± 2.002
4. Ethanolic	22.433 ± 1.347*
5. Aqueous	25.967 ± 0.981*

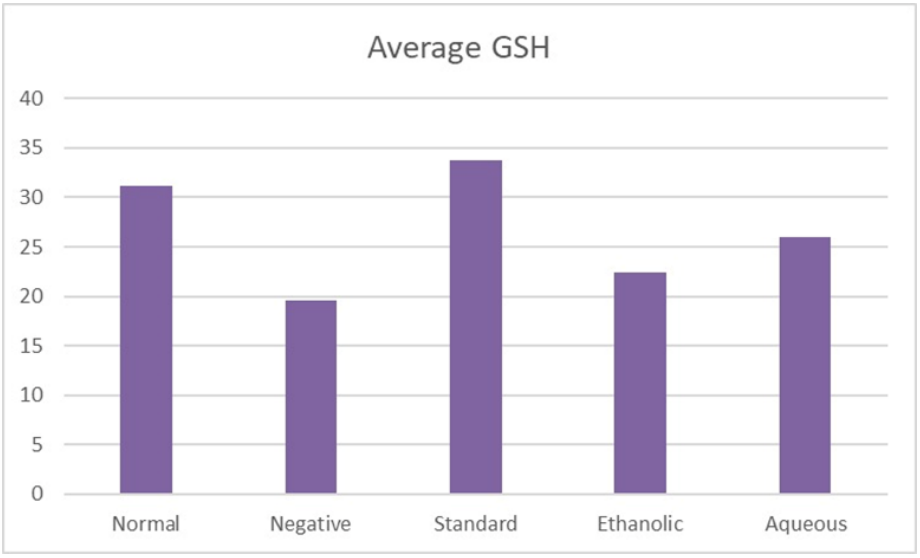

Fig.4 GSH levels in brain homogenates in units/min/mg protein. Data are presented as Mean ± Standard Deviation (SD). Statistical significance determined by ANOVA with significance levels - (p<0.01; *p<0.05) in 6 rats of each group.**

3.2.3 Catalase (CAT)

Table 5 and Fig. 5 represent the catalase activity, which was suppressed in the negative group (AlCl3-treated) (18.6 ± 0.889 µmol/min/g), compared to the control group (21.33 ± 1.242 µmol/min/g). Aqueous extract restored CAT levels (20.58 ± 3.092 µmol/min/g), and less improvement is seen in the ethanolic group.

Table 5: Estimation of Catalase (CAT)

Groups	CAT(µmol/min/g protein)
1. Normal	21.33 ± 1.242**
2. Negative	18.6 ± 0.889
3. Standard	24.367 ± 1.504
4. Ethanolic	18.013 ± 1.556
5. Aqueous	20.58 ± 3.092**

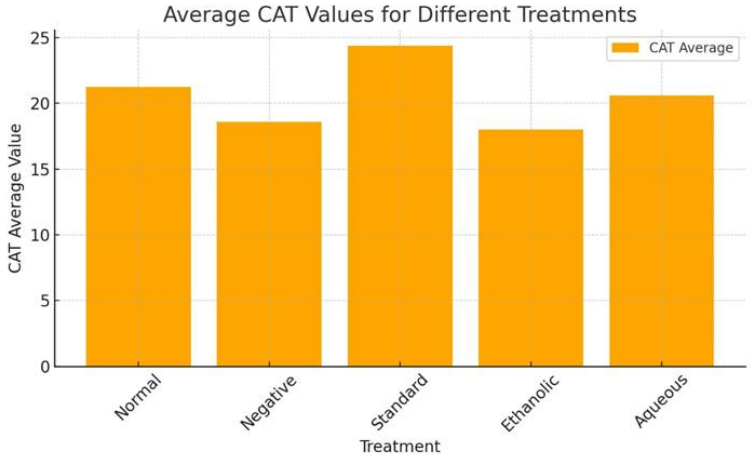

Fig.5 Catalase levels in brain homogenates in units/min/mg protein. Data are presented as Mean ± Standard Deviation (SD). Statistical significance determined by ANOVA with significance levels - (p<0.01; *p<0.05) in 6 rats of each group.**

3.3 Acetylcholinesterase activity

Cholinergic dysfunction was measured through brain AChE activity. AChE activity was notably elevated in the Negative (Alcl3-treated rats) group (9.533 ± 0.603 µg/min/g protein) compared to the control (6.533 ± 0.751 µg/min/g protein). Aqueous extract (4.933 ± 1.106 µg/min/g protein) and standard group (4.453 ± 0.683 µg/min/g protein) have shown significantly reduced AChE activity; the aqueous extract treatment group showed its effect very near to the standard. While the ethanolic extract (6.967 ± 0.802 µg/min/g protein) showed moderate inhibition. This supports the cholinergic restorative effect of *Aerva lanata*, particularly with the aqueous formulation.

Table 6: Estimation of AChE

Groups	AChE (µg/min/g protein)
1. Normal	6.533 ± 0.751
2. Negative	9.533 ± 0.603
3. Standard	4.453 ± 0.683
4. Ethanolic	6.967 ± 0.802*
5. Aqueous	4.933 ± 1.106**

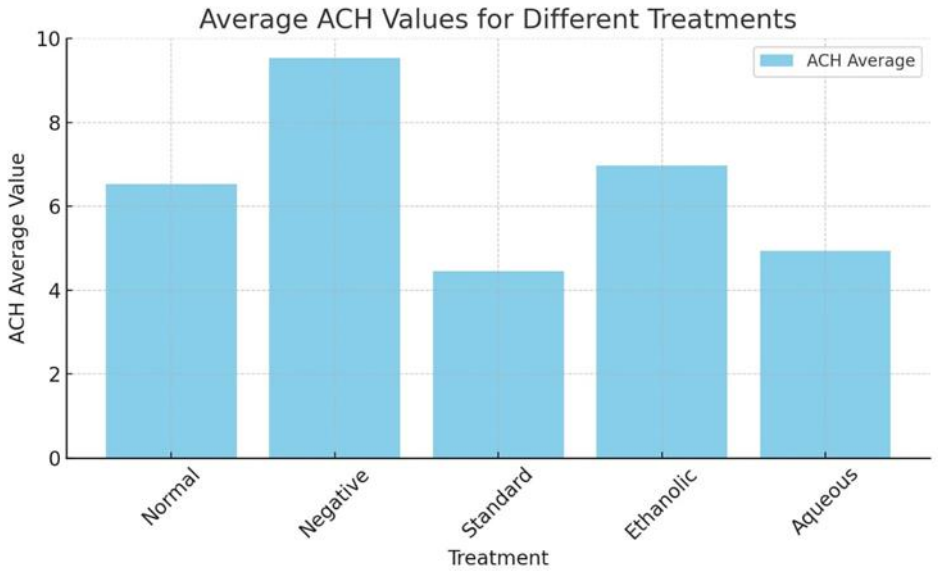

Fig.6 AChE levels in brain homogenates in units/min/mg protein. Data are presented as Mean ± Standard Deviation (SD). Statistical significance determined by ANOVA with significance levels - (p<0.01; *p<0.05) in 6 rats of each group.**

3.4 Effect of *Aerva lanata* extract treatment on the brain histopathology of the $AlCl_3$-induced rats

Figures 7 & 8 represent the control group having normal brain architecture with no signs of neurodegeneration, which serves as a baseline showing intact histological features. Fig. 9 & 10 indicate the Negative group, which clearly shows $AlCl_3$-induced neurotoxicity, having neurodegeneration in the cerebral cortex and reduced cell count in the granular layer, degeneration of Purkinje cells, and vascular congestion, which mimics the Alzheimer-like histopathological pattern. Figs 11 and 12 represent the standard group, indicating partial reversal of neurodegenerative damage, improving histological integrity, used as a therapeutic benchmark. Figs 13 & 14 represent the ethanolic extract-treated group having minimal neurodegeneration in the cerebral cortex region and mild degeneration in the granular layer. The structural integrity is largely preserved with only mild changes, indicating histopathological recovery. Figs 15 & 16 represent the Aqueous extract-treated group, with no evident neurodegeneration and minimal degeneration in the granular layer, indicating the most potent neuroprotective effect, closely resembling the control group with well-preserved architecture and negligible signs of damage.

These results conclude the potential of *Aerva lanata* extracts, mainly the Aqueous extract, preserving neuronal integrity

and suggest that *Aerva lanata* possesses substantial neuroprotective properties.

Group I (Control group)

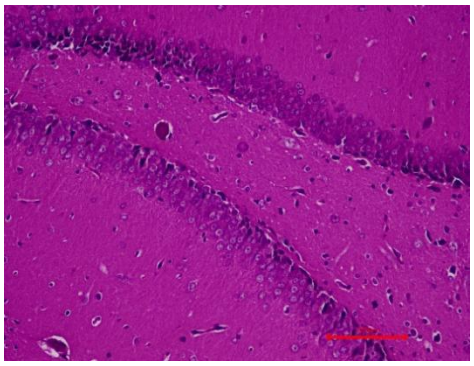

Fig.7. Cerebral cortex

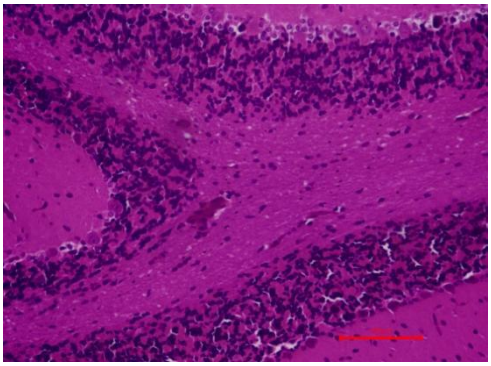

Fig.8. Hippocampus

Group-II (Negative group)

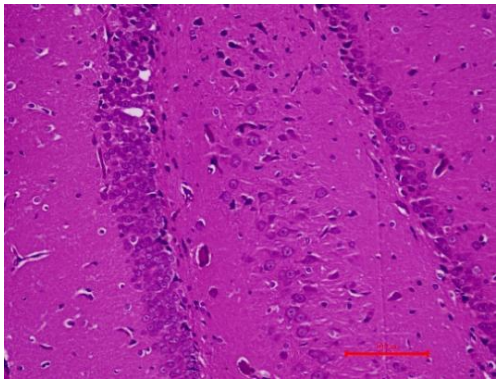

Fig.9. Cerebral cortex

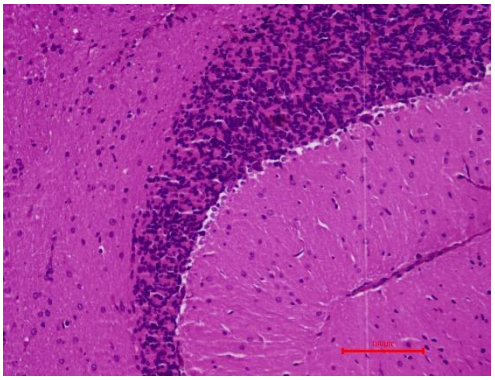

Fig.10. Hippocampus

Group-III (Standard group)

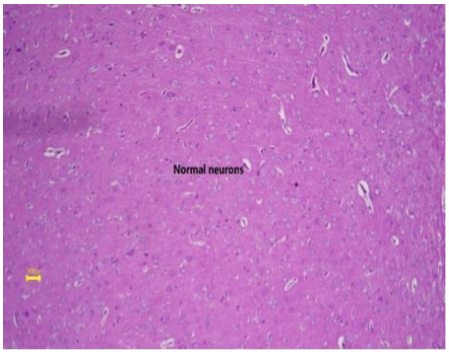

Fig.11. Cerebral cortex

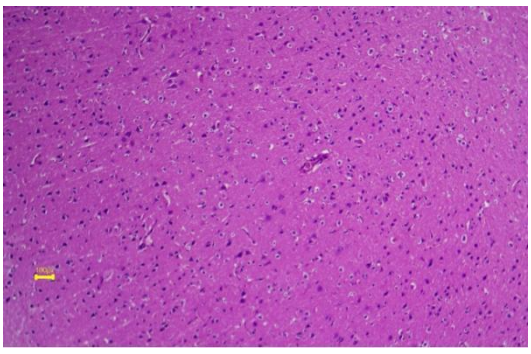

Fig.12. Hippocampus

Group-IV (Ethanolic extract-treated group)

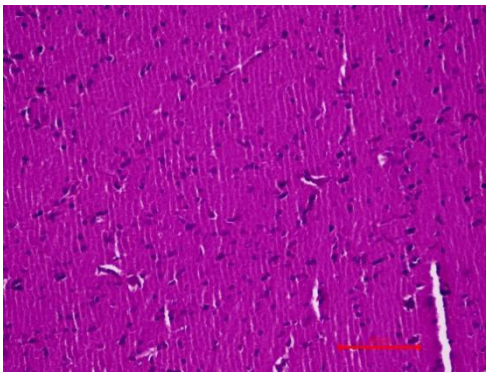

Fig.13. Cerebral cortex

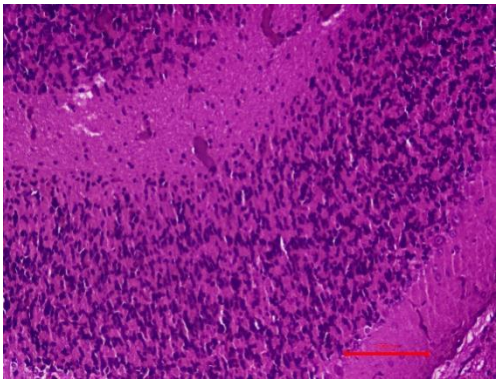

Fig.14. Hippocampus

Group-V (Aqueous extract-treated group)

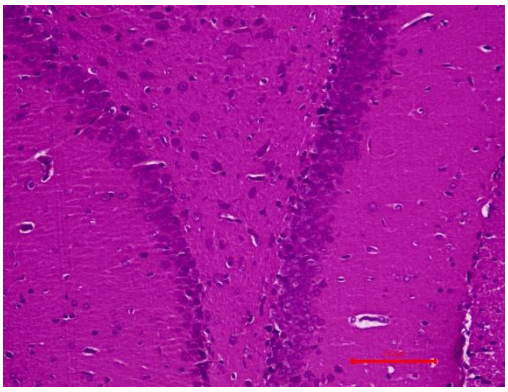

Fig.15. Cerebral cortex

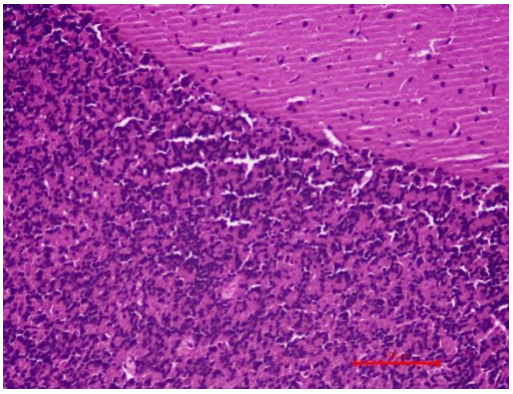

Fig.16. Hippocampus

4. DISCUSSION

Our current study was aimed at assessing the neuroprotective potential of *Aerva lanata* extracts in AlCl3-induced neurotoxicity rat models, by an approach of traditional medicinal use and exploring its effectiveness in Alzheimer's disease management. *Aerva lanata*, known for its ethnobotanical relevance and rich phytochemical composition [24], exhibited significant beneficial effects on cognitive, oxidative, and cholinergic disturbances induced by $AlCl_3$.

Behavioural assessment using the MWM test demonstrated pronounced cognitive impairment in $AlCl_3$-exposed rats. Treatment with *Aerva lanata*, particularly the aqueous extract, significantly improved escape latency and target quadrant retention times, indicating a restorative effect on spatial learning and memory functions. These observations align with the hypothesis that phytoconstituents of *Aerva lanata* contribute to cognitive enhancement.

In Alzheimer's disease, along with other neurodegenerative diseases, oxidative stress plays an essential role. [25] $AlCl_3$ strongly damages and reduces the effectiveness of antioxidant defence mechanisms, as seen by decreased SOD, GSH, and CAT activities in Figs 3, 5, and 6, respectively. *Aerva lanata* extracts effectively decreased these oxidative deficits, with the aqueous extract showing superior activity in restoring antioxidant enzyme activities. The abundance of alkaloids, cardiac glycosides, and saponins in *Aerva lanata* might be the reason for its potent anti-oxidative properties, neutralizing reactive oxygen species (ROS) and minimizing oxidative stress damage.

Cholinergic dysfunction, shown by elevated AChE activity, is the hallmark of Alzheimer's pathology. Our study has shown that *Aerva lanata* ethanolic and aqueous extracts strongly suppressed AChE activity, preserving acetylcholine levels and thereby improving cholinergic neurotransmission, promising *Aerva lanata*'s therapeutic activity.

Notably, histopathological studies proved these biochemical and behavioural findings. In the Negative group (Figs 9 &10), the neurodegeneration was evident along with degeneration of Purkinje cells, mimicking the Alzheimer's like neurodegenerative changes. In contrast, the aqueous extract treated group (Fig. 10 & 11) displayed preserved cortical and hippocampal architecture with minimal signs of degeneration, the ethanolic group displayed partial protection with mild changes, while the standard group showed moderate histopathological recovery.

Overall, all of these findings suggest that *Aerva lanata* exerts multi-targeted neuroprotection by significantly reducing oxidative stress by increasing antioxidant defences, modulating cholinergic neurotransmission. In particular, the aqueous extract demonstrated better efficacy over the ethanolic extract, potentially due to better bioavailability or a higher concentration of active phyto constituents.

5. CONCLUSION

In summary, both the extracts of *Aerva lanata, i.e.,* ethanolic and aqueous extracts, effectively suppressed $AlCl_3$-induced cognitive deficits, neuronal damage, and cholinergic dysfunction through inhibition of Reactive oxygen species (ROS); this protective activity is attributed to its antioxidant property.

In Alzheimer's disease management, these findings suggest the potential of *Aerva lanata* extracts as a promising neuroprotective agent, especially in its aqueous form. However, further studies are essential to confirm the anti-Alzheimer's activity of *Aerva lanata* against other models to validate and elucidate the precise mechanism underlying its neuroprotective efficacy, paving the way for clinical translation.

REFERENCES

[1] Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, Vargas-Rodríguez I, Cadena-Suárez AR, Sánchez-Garibay C, Pozo-Molina G, Méndez-Catalá CF, Cardenas-Aguayo M-d-C, Diaz-Cintra S, et al. Alzheimer's Disease: An Updated Overview of Its Genetics. International Journal of Molecular Sciences. 2023; 24(4):3754. https://doi.org/10.3390/ijms24043754

[2] Prince, Martin & Wimo, Anders & Guerchet, Maëlenn & Ali, Gemma-Claire & Wu, Yu-Tzu & Prina, Matthew. (2015). World Alzheimer Report 2015. The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Cost, and Trends.

[3] Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018;25(1):59-70. doi:10.1111/ene.13439

[4] Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. Molecules. 2020;25(24):5789. Published 2020 Dec 8. doi:10.3390/molecules25245789

[5] Veurink G, Perry G, Singh SK. 2020 Role of antioxidants and a nutrient rich diet in Alzheimer's disease. Open Biol.10: 200084. http://dx.doi.org/10.1098/rsob.200084

[6] Wolfe MS. Probing Mechanisms and Therapeutic Potential of γ-Secretase in Alzheimer's Disease. Molecules. 2021; 26(2):388. https://doi.org/10.3390/molecules26020388

[7] Louise C Serpell,Alzheimer's amyloid fibrils: structure and assembly, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Volume 1502, Issue 1, 2000, Pages 16-30, ISSN 0925-4439, https://doi.org/10.1016/S0925-4439(00)00029-6.

[8] Thal DR, Attems J, Ewers M. Spreading of amyloid, tau, and microvascular pathology in Alzheimer's disease: findings from neuropathological and neuroimaging studies. J Alzheimers Dis. 2014;42 Suppl 4:S421-S429. doi:10.3233/JAD-141461

[9] Ajeet Rijal Upadhaya, Irina Kosterin, Sathish Kumar, Christine A. F. von Arnim, Haruyasu Yamaguchi, Marcus Fändrich, Jochen Walter, Dietmar Rudolf Thal, Biochemical stages of amyloid-β peptide aggregation and accumulation in the human brain and their association with symptomatic and pathologically preclinical Alzheimer's disease, Brain, Volume 137, Issue 3, March 2014, Pages 887–903, https://doi.org/10.1093/brain/awt362

[10] Fatima SJ, Prasad DK. Enhanced Neuroprotection by Diosgenin and Pterostilbene Combination Against Neurotoxicity Induced by Amyloid-B 1-42 in SH-SY5Y Differentiated Cell Models. Annals of Neurosciences. 2025 Jul 24:09727531251356049. https://doi.org/10.1177/09727531251356049

[11] Khan K, Emad NA, Sultana Y. Inducing Agents for Alzheimer's Disease in Animal Models. J Explor Res Pharmacol. 2024;9(3):169-179. doi: 10.14218/JERP.2023.00028.

[12] Rawat P, Sehar U, Bisht J, Selman A, Culberson J, Reddy PH. Phosphorylated Tau in Alzheimer's Disease and Other Tauopathies. Int J Mol Sci. 2022;23(21):12841. Published 2022 Oct 25. doi:10.3390/ijms232112841f

[13] Fatima SJ, Prasad DK. Unveiling neuroprotective mechanisms of diosgenin and pterostilbene in diabetes-associated Alzheimer's disease through multi-target molecular docking approach. Journal of Herbmed Pharmacology. 2024 Oct 1;13(4):659-73. doi: 10.34172/jhp.2024.52606

[14] Rahman A, Banu Z*. Impact of Aluminium Chloride (AlCl3) on Brain Function: A Review of Neurotoxic Mechanisms and Implications for Alzheimer's Disease. Trends in Pharmaceutical Sciences. 2024;10(4):355-366. doi: 10.30476/tips.2024.103641.1253

[15] Goyal M, Pareek A, Nagori BP, Sasmal D. Aerva lanata: A review on phytochemistry and pharmacological aspects. Pharmacogn Rev. 2011;5(10):195-198. doi:10.4103/0973-7847.91120

[16] Di Shunan, Miao Yu, Huibo Guan, Yanyan Zhou, Neuroprotective effect of Betalain against AlCl3-induced Alzheimer's disease in Sprague Dawley Rats via putative modulation of oxidative stress and nuclear factor kappa B (NF-κB) signaling pathway, Biomedicine & Pharmacotherapy,Volume 137, 2021, 111369, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2021.111369.

[17] Touqeer Ahmed, Anwarul-Hassan Gilani, Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease, Pharmacology Biochemistry and Behavior, Volume 91, Issue 4, 2009, Pages 554-559, ISSN 0091-3057, https://doi.org/10.1016/j.pbb.2008.09.010.

[18] Smith, Kerri. (2014). Tissue sample preparation in bioanalytical assays.

[19] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-474. doi:10.1111/j.1432-1033.1974.tb03714.x

[20] Gsell W, Conrad R, Hickethier M, et al. Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of Alzheimer type. J Neurochem. 1995;64(3):1216-1223. doi:10.1046/j.1471-4159.1995.64031216.x

[21] ELLMAN GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-77. doi:10.1016/0003-9861(59)90090-6

[22] ELLMAN GL, COURTNEY KD, ANDRES V Jr, FEATHER-STONE RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95. doi:10.1016/0006-2952(61)90145-9

[23] Ahmed, Edrissi. (2022). Routine histological techniques.

[24] Ankul Singh S, Gowri K, Chitra V. A Review on Phytochemical Constituents and Pharmacological Activities of the plant: Aerva lanata. Research J. Pharm. and Tech 2020; 13(3):1580-1586. doi: 10.5958/0974-360X.2020.00286.3

[25] Huang WJ, Zhang X, Chen WW. Role of oxidative stress in Alzheimer's disease. Biomed Rep. 2016;4(5):519-522. doi:10.3892/br.2016.630