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Research Article

Pharmacological Evaluation of Neuroprotective Potential of Nelumbo Nucifera (Red Flower) Extract in Experimental Alzheimer's Disease in Rats

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Abstract:
Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, cholinergic dysfunction, and oxidative stress. The present study was undertaken to evaluate the neuroprotective potential of the red flower extract of Nelumbo nucifera in an aluminium chloride ($AlCl_3$)-induced experimental model of Alzheimer's disease in Wistar albino rats. The red flower extract was prepared and subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, alkaloids, phenolic compounds, tannins, glycosides, saponins, and terpenoids. An acute oral toxicity study conducted according to OECD guidelines demonstrated that the extract was safe up to 2000 mg/kg body weight. Based on this, doses of 100, 200, and 400 mg/kg were selected for the study. The animals were divided into six groups, including normal control, disease control, standard drug-treated group, and extract-treated groups. Cognitive function was assessed using the Morris Water Maze and Y-maze tests. The disease control group showed significant impairment in learning and memory, whereas treatment with Nelumbo nucifera extract significantly improved cognitive performance in a dose-dependent manner. Biochemical estimations revealed that aluminium chloride administration significantly increased acetylcholinesterase activity and lipid peroxidation (MDA levels), while decreasing antioxidant enzymes such as superoxide dismutase, catalase, and reduced glutathione. Treatment with Nelumbo nucifera extract significantly reversed these alterations, indicating restoration of cholinergic function and antioxidant defense. The extract also improved total protein levels, suggesting protection of neuronal integrity. Among the tested doses, the 400 mg/kg dose exhibited maximum neuroprotective effect and showed results comparable to the standard drug Donepezil. The observed effects may be attributed to the presence of bioactive phytoconstituents with antioxidant and cholinesterase inhibitory properties. In conclusion, the present study demonstrates that Nelumbo nucifera red flower extract possesses significant neuroprotective and anti-Alzheimer activity. It may serve as a promising natural therapeutic agent for the management of Alzheimer's disease. Further studies are required to elucidate its mechanism of action and clinical applicability.
Keywords: Nelumbo nucifera; Neuroprotection; Alzheimer's disease; Acetylcholinesterase inhibition.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a gradual decline in cognitive functions such as memory, reasoning, and behavior, ultimately resulting in functional dependence and death. It is the most common cause of dementia, accounting for approximately 60–70% of all dementia cases worldwide.[1]

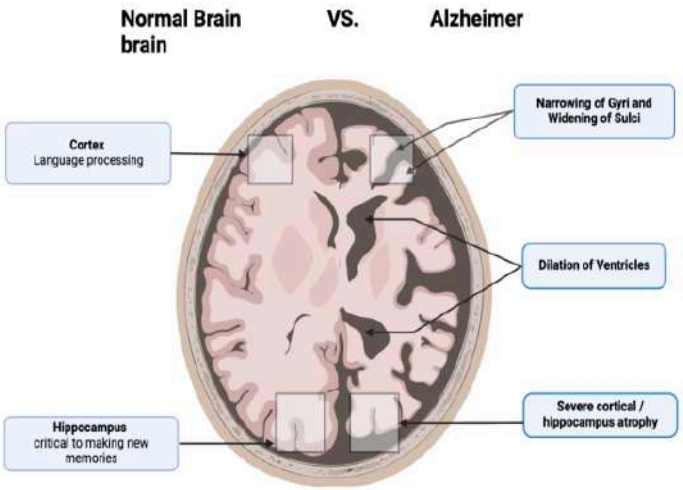

Figure 1: Normal Brain and Alzheimer

Pathophysiology of Alzheimer Disease

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by progressive synaptic dysfunction, neuronal loss, and cognitive impairment.

The underlying pathophysiology involves a complex interaction between genetic, molecular, and environmental factors**,** leading to accumulation of abnormal proteins, oxidative stress, and neuro inflammation. [2]

Plant Profile

Nelumbo nucifera Gaertn. is a well-known aquatic medicinal plant commonly referred to as the Sacred Lotus or Indian Lotus. It has a wide range of synonyms based on regional and traditional systems of medicine.[3]

Figure 2: Flower of Nelumbo nucifera

Key phytoconstituents [4]

Plant Part	Major Phytochemicals
Flowers	Quercetin, Kaempferol, Isorhamnetin, Nuciferine, Neferine, O-nornuciferine, Liriodenine
Leaves	Catechin, Myricetin, Isoquercitrin, Rutin, Luteolin
Rhizomes	Polyphenols, β-sitosterol, Palmitic acid, Linoleic acid, Vitamin C
Seeds	Nuciferine, Neferine, Liensinine, Flavonoid glycosides, Proteins
Stamens	Neferine, Anonaine, Armepavine, Norarmepavine

Pharmacological Activity[5-9]

1. Antioxidant activity:

Methanolic and ethanolic extracts of N. nucifera flowers and leaves exhibit strong DPPH, FRAP, and ABTS radical scavenging activities due to the presence of phenolic compounds.

2. Anti-inflammatory activity:

Extracts inhibit COX-2, TNF-α, and NF-κB pathways, suppressing microglial activation and neuroinflammation.

3. Antidiabetic and anti-obesity activity:

Leaf and seed extracts improve insulin sensitivity and lipid metabolism by modulating PPAR-γ and AMPK pathways.

4. Cardioprotective and hepatoprotective activity:

The rhizome and seed extracts protect against isoproterenol- and CCl_4-induced oxidative damage, improving antioxidant enzyme levels.

5. Anticancer potential:

Flavonoids and alkaloids from the petals induce apoptosis in human cancer cell lines by activating caspase pathways.

Methods and Materials

Plant Name: Nelumbo nucifera (Family: Nelumbonaceae)

Part Used: Flower

Collection Site: Fresh Flower were collected from local regions of Maharashtra (Mumbai) during the active growing season. The collected flowers was washed with distilled water to remove dust, shade-dried for 10–14 days at room temperature, and coarsely powdered using a mechanical grinder. The powder were stored in an airtight container protected from light and moisture until extraction.

Experimental Animals

- **Species:** Wistar albino rats
- **Weight:** 150–200 g
- **Sex:** Male Wistar rat
- **Housing:** Standard polypropylene cages (temperature 25 ± 2°C, humidity 60 ± 10%, 12 h light/dark cycle).
- **Diet:** Standard pellet diet and water ad libitum.
- **Ethical Clearance:** The study protocol were approved by the Institutional Animal Ethics Committee (IAEC)**,** in accordance with CCSEA guidelines (Govt. of India)**.[**10]

Acute Toxicity Study

The Conducted as per OECD Guideline 423 (Acute Oral Toxicity – Fixed Dose Method)**.** The hydroalcoholic extract were administered orally in graded doses up to 2000 mg/kg to groups of rats to determine the median lethal dose (LD_{50})**.** Animals were observed for 14 days for any signs of toxicity (behavioral, neurological, autonomic) and mortality. One-tenth of the maximum safe dose were selected as the therapeutic starting dose for neuroprotective potential evaluation. [11]

Induction of Alzheimer's Disease:

Alzheimer's disease were induced using Aluminium chloride ($AlCl_3$) at 150 mg/kg/day, intraperitoneally (i.p.) for 21 days**,** as reported in previous studies. [12]

Grouping of Animals

Table 1: A total of 36 Wistar albino rats were divided into six groups (n = 6 per group):[13]

Group	Treatment	Route of Administration	Dose (mg/kg)
Group I (Normal Group)	Normal Control	Orally	Vehicle only (food & water ad libitum)
Group II (Disease Group)	Disease Control	Orally	Aluminium chloride **(**$AlCl_3$) 150mg/kg Inducer
Group III (Standard Group)	Standard Drug (Donepezil)	Orally	5mg/kg Body weight
Group IV (Tretment Group)	Nelumbo nucifera Extract (Low Dose)	Orally	100mg/kg
Group V (Tretment Group)	Nelumbo nucifera Extract (Medium Dose)	Orally	200mg/kg
Group VI (Tretment Group)	Nelumbo nucifera Extract (High Dose)	Orally	400mg/kg

Behavioral Study

1. Morris Water Maze (MWM) Test:

The Morris Water Maze were used to evaluate spatial learning and memory in rats.

2. Y-Maze Test:

The Y-maze test was performed to assess working memory based on spontaneous alternation behavior.

Biochemical Parameters Estimation

After behavioral studies, rats was sacrificed, brains was isolated, rinsed in ice-cold saline, and homogenized (10% w/v) in ice-cold phosphate buffer (0.1 M; pH 7.4).

The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C and supernatant used for biochemical estimations. [14-15]

1. Estimation of Acetylcholinesterase (AChE) Activity
2. Lipid Peroxidation (MDA) Levels
3. Reduced Glutathione (GSH)
4. Superoxide Dismutase (SOD)
5. Catalase (CAT)
6. Total Protein Estimation Histopathological Examination

Principle

Histopathological examination is performed to evaluate microscopic structural changes in brain tissue associated with neurodegeneration and to assess the neuroprotective effect of Nelumbo nucifera extract against scopolamine-induced neuronal damage. In Alzheimer's disease models, histopathology helps identify neuronal degeneration, vacuolization, inflammatory changes, and alterations in hippocampal architecture.

Statistical Analysis

All experimental data was expressed as Mean ± Standard Error of Mean (SEM)**.** Statistical analysis were performed using GraphPad Prism / SPSS software. One-way Analysis of Variance (ANOVA) was used to compare different treatment groups.

Results

Pre-liminary Phytochemical Screening of Nelumbo nucifera Red Flower Extract

Table 2: Preliminary Phytochemical Screening of Nelumbo nucifera Red Flower Extract

S. No.	Phytochemical Constituents	Result
1	Alkaloids	+
2	Flavonoids	+
3	Phenolic compounds	+
4	Tannins	+
5	Glycosides	+
6	Saponins	+
7	Terpenoids	+
8	Carbohydrates	+
9	Proteins	–
10	Steroids	–

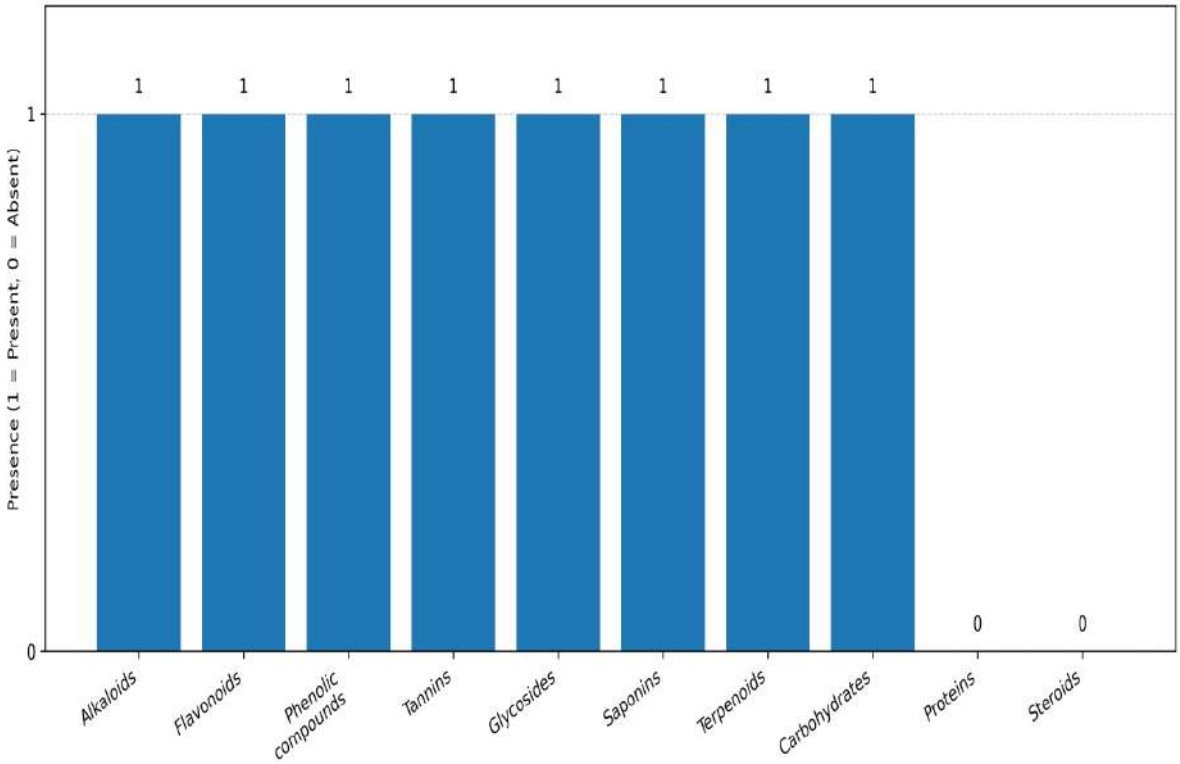

Figure 3: Preliminary Phytochemical Screening

Interpretation: The beneficial effects observed later in behavioral, biochemical, and histopathological parameters may be due to the combined action of flavonoids, alkaloids, phenolic compounds, tannins, glycosides, saponins, and terpenoids present in the extract.

Evaluation of Oral Acute Toxicity Study: The oral acute toxicity study of Nelumbo nucifera red flower extract revealed no mortality or toxic symptoms in Wistar rats up to a dose of 2000 mg/kg, p.o**.** during the 14-day observation period. The extract was found to be safe, and the LD_{50} was considered to be greater than 2000 mg/kg.

Therefore, 100 mg/kg, 200 mg/kg, and 400 mg/kg were selected as low, medium, and high doses respectively for the pharmacological study.

Table 3: Observation of Acute Oral Toxicity Study of Nelumbo nucifera Red Flower Extract

S. No.	Parameter Observed	Observation
1	**Mortality**	No mortality observed
2	**Skin and fur**	Normal
3	**Eyes and mucous membrane**	Normal
4	**Tremors**	Absent
5	**Convulsions**	Absent
6	**Salivation**	Absent
7	**Diarrhea**	Absent
8	**Sleep**	Normal
9	**Coma**	Absent
10	**Locomotor activity**	Normal
11	**Food intake**	Normal
12	**Water intake**	Normal
13	**Body weight changes**	No significant abnormality

Interpretation: The absence of mortality and toxic manifestations in rats treated with Nelumbo nucifera red flower extract indicates that the extract has a wide margin of safety.

Behavioral Study Procedures

- **Morris Water Maze (MWM) Test**

Table 4: Effect of Nelumbo nucifera Red Flower Extract on Escape Latency Time (ELT) in Morris Water Maze Test

Group	Treatment	Dose (mg/kg)	Day 1 (sec)	Day 2 (sec)	Day 3 (sec)	Day 4 (sec)	Day 5 (sec)
Group I (Normal)	Normal Control	—	45 ± 2.1	35 ± 1.8	28 ± 1.5	22 ± 1.2	20 ± 1.0
Group II (Disease)	$AlCl_3$ Control	150	60 ± 2.5	62 ± 2.3	64 ± 2.4	66 ± 2.2	65 ± 2.1
Group III (Standard)	Donepezil	5	48 ± 2.0	36 ± 1.9	30 ± 1.6	26 ± 1.3	25 ± 1.2
Group IV (Low Dose)	Extract	100	55 ± 2.2	48 ± 2.0	44 ± 1.8	42 ± 1.6	45 ± 1.5
Group V (Medium Dose)	Extract	200	52 ± 2.1	44 ± 1.9	38 ± 1.7	36 ± 1.5	35 ± 1.4
Group VI (High Dose)	Extract	400	50 ± 2.0	40 ± 1.8	34 ± 1.6	30 ± 1.4	28 ± 1.3

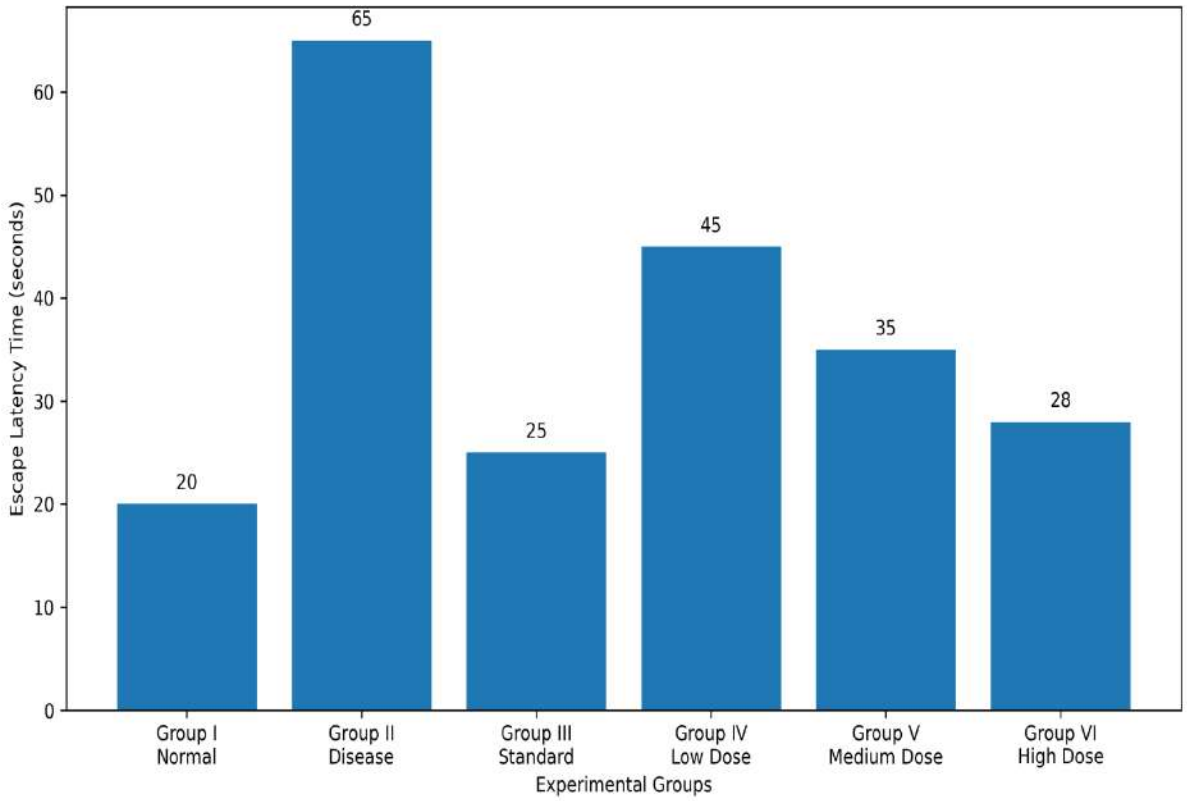

Figure 4: Effect of Nelumbo nucifera Red Flower Extract on Escape Latency Time (ELT) in Morris Water Maze Test

Interpretation

The Morris Water Maze test showed that the treatment with Nelumbo nucifera red flower extract resulted in a dose-dependent decrease in escape latency time and increase in target quadrant retention. The high-dose group showed results comparable to the standard drug Donepezil, indicating significant improvement in cognitive function.

Y-Maze Test

Table 5: Effect of Nelumbo nucifera Red Flower Extract on Y-maze Performance

Group	Treatment	Dose (mg/kg)	Total Arm Entries	Spontaneous Alternations	% Alternation
Group I (Normal Group)	Normal Control	—	20 ± 1.2	13 ± 0.8	72 ± 2.1
Group II (Disease Group)	$AlCl_3$ Control	150	18 ± 1.0	6 ± 0.5	38 ± 1.8
Group III (Standard Group)	Donepezil	5	19 ± 1.1	12 ± 0.7	68 ± 2.0
Group IV (Low Dose Group)	Extract	100	18 ± 1.0	8 ± 0.6	50 ± 1.9
Group V (Medium Dose Group)	Extract	200	19 ± 1.1	10 ± 0.7	58 ± 2.0
Group VI (High Dose Group)	Extract	400	20 ± 1.2	11 ± 0.7	65 ± 2.1

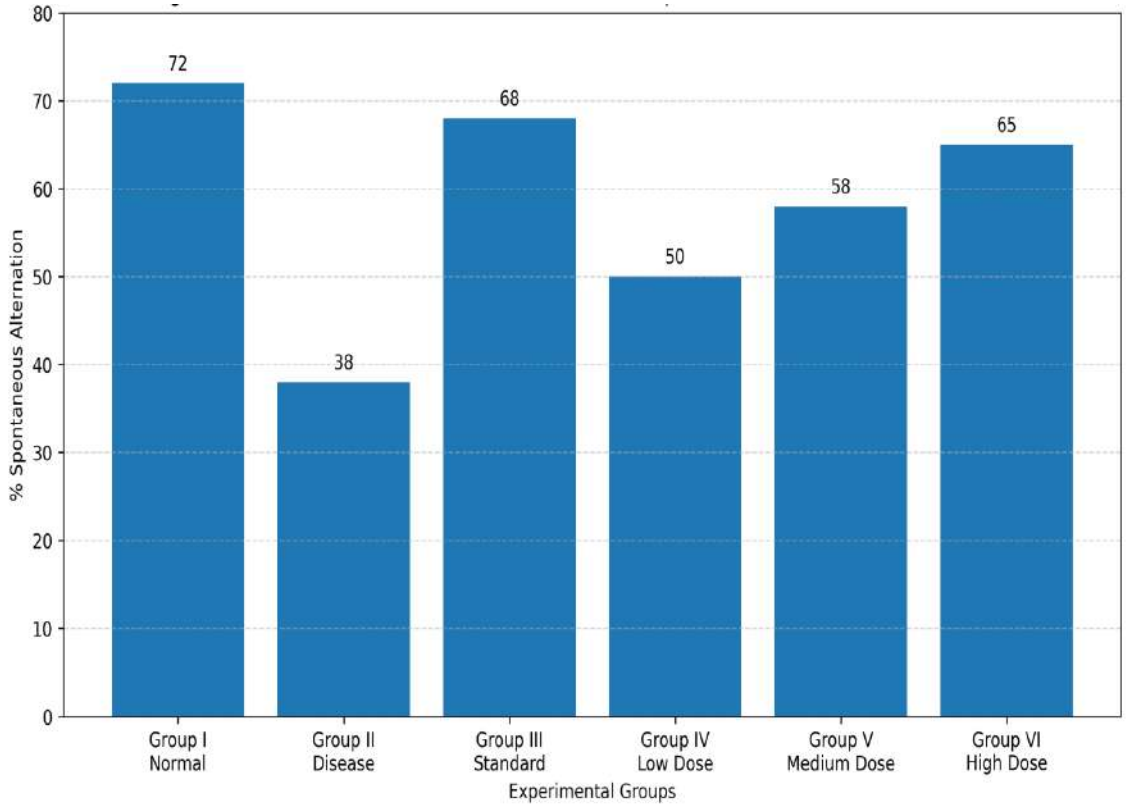

Figure 5- Effect of Nelumbo nucifera Red Flower Extract on Y-maze Performance

Interpretation:

The graph shows that the disease group had the lowest percentage spontaneous alternation, confirming aluminium chloride-induced cognitive dysfunction.

The standard group showed marked improvement. Among the extract-treated groups, the low-dose group showed mild improvement, the medium-dose group showed moderate improvement, and the high-dose group showed significant restoration of working memory, with values approaching the standard group.

Biochemical Estimations

- **Estimation of Acetylcholinesterase (AChE) Activity**

Table 6: Effect of Nelumbo nucifera Red Flower Extract on Brain Acetylcholinesterase Activity

Group	**Treatment**	**Dose (mg/kg)**	**AChE Activity (µmol/ min/mg protein)**
Group I (Normal Group)	Normal Control	—	2.15 ± 0.12
Group II (Disease Group)	$AlCl_3$ Control	150	5.82 ± 0.25
Group III (Standard Group)	Donepezil	5	2.48 ± 0.14
Group IV (Low Dose Group)	Extract	100	4.32 ± 0.20
Group V (Medium Dose Group)	Extract	200	3.45 ± 0.18
Group VI (High Dose Group)	Extract	400	2.76 ± 0.16

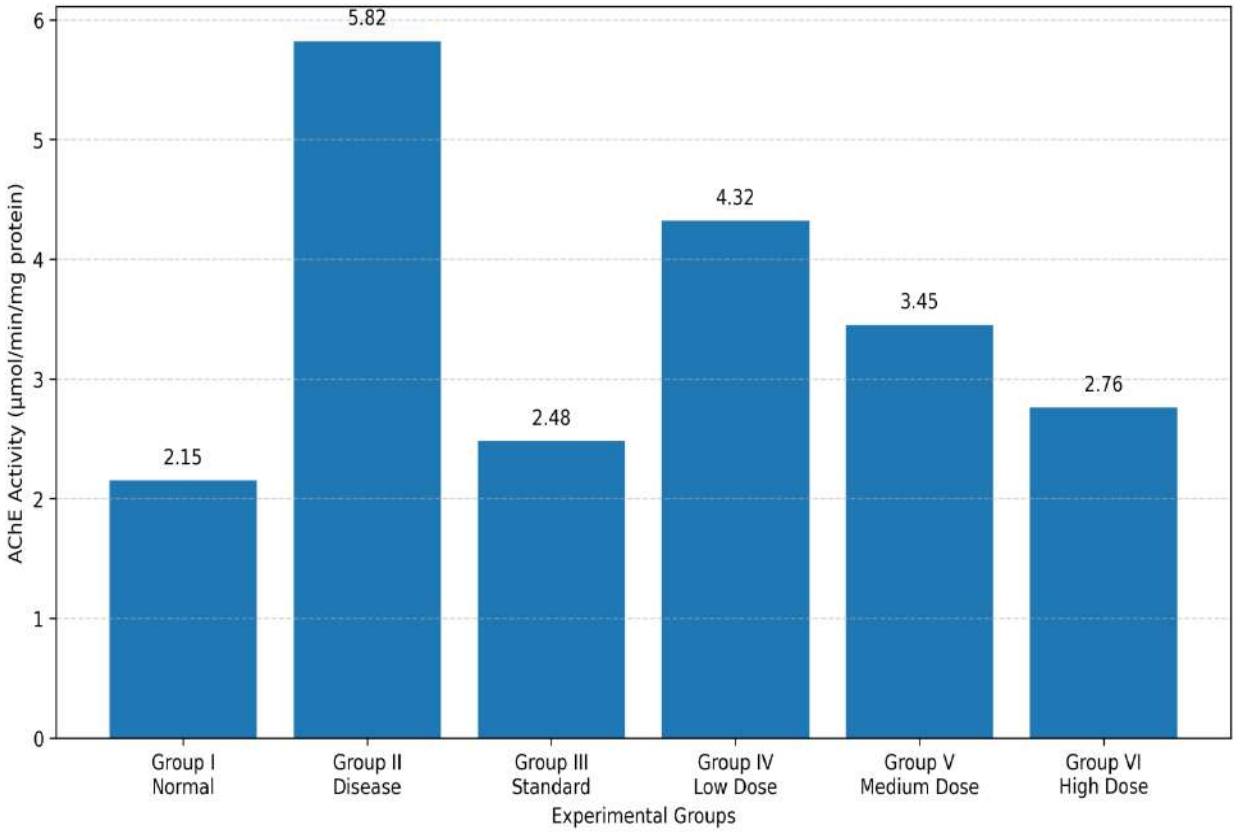

Figure 6: Effect of Nelumbo nucifera Red Flower Extract on Brain Acetylcholinesterase Activity

Interpretation

The graph clearly shows that aluminium chloride administration significantly increased AChE activity in the disease group, reflecting impaired cholinergic neurotransmission. Treatment with Nelumbo nucifera red flower extract resulted in a dose-dependent decrease in AChE activity. The high-dose group exhibited maximum reduction, with values approaching those of the standard group, suggesting significant improvement in cholinergic function and potential anti-Alzheimer activity.

Estimation of Lipid Peroxidation (MDA) Levels

Table 7: Effect of Nelumbo nucifera Extract on Lipid Peroxidation (MDA Levels)

Group	Treatment	Dose (mg/kg)	MDA (nmol/mg protein)
Group I (Normal Group)	Normal Control	—	1.85 ± 0.10
Group II (Disease Group)	$AlCl_3$ Control	150	5.96 ± 0.25
Group III (Standard Group)	Donepezil	5	2.15 ± 0.12
Group IV (Low Dose Group)	Extract	100	4.32 ± 0.20
Group V (Medium Dose Group)	Extract	200	3.28 ± 0.18
Group VI (High Dose Group)	Extract	400	2.42 ± 0.14

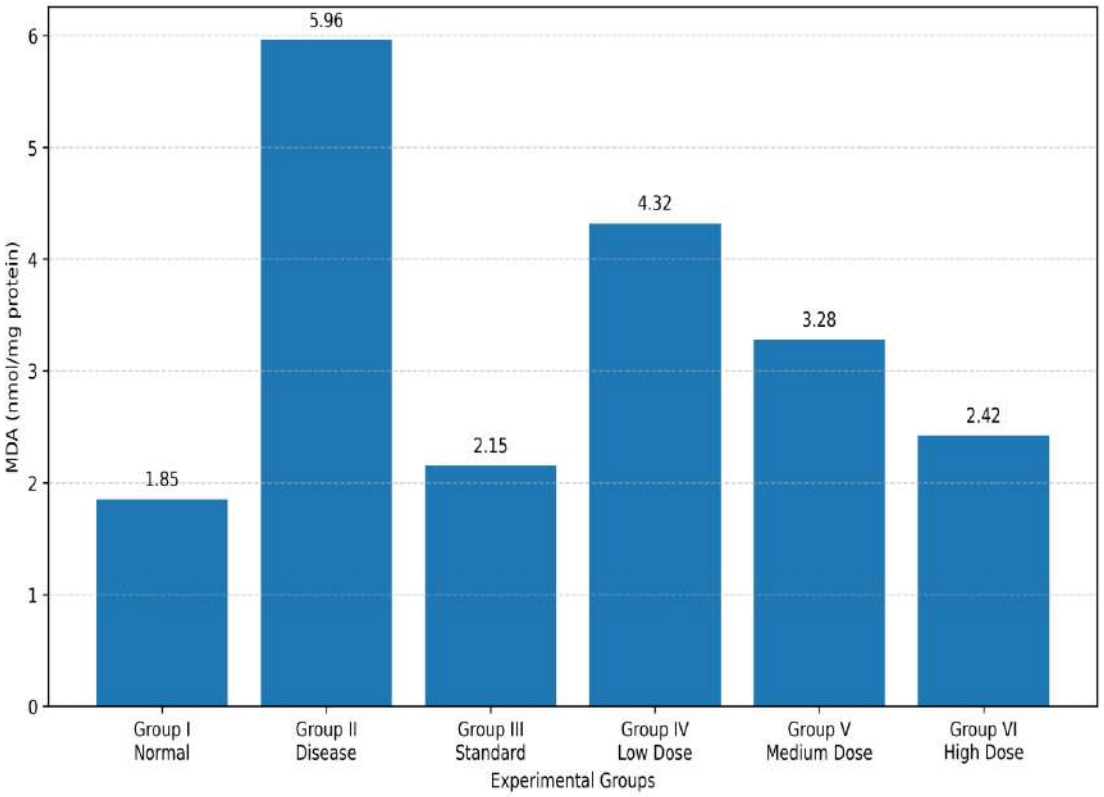

Figure 7: Effect of Nelumbo nucifera Extract on Lipid Peroxidation (MDA Levels)

Interpretation:

The graph shows that the disease group had the highest MDA level, confirming severe oxidative stress induced by aluminium chloride. Treatment with Nelumbo nucifera red flower extract significantly reduced MDA levels in all treated groups. The high-dose group showed the maximum reduction and was close to the standard group, suggesting strong antioxidant and neuroprotective activity.

Estimation of Reduced Glutathione (GSH) Levels

Table 8: Effect of Nelumbo nucifera Red Flower Extract on Reduced Glutathione (GSH) Levels

Group	Treatment	Dose (mg/kg)	GSH (µmol/mg protein)
Group I (Normal Group)	Normal Control	—	6.82 ± 0.30
Group II (Disease Group)	$AlCl_3$ Control	150	2.15 ± 0.18
Group III (Standard Group)	Donepezil	5	6.25 ± 0.28
Group IV (Low Dose Group)	Extract	100	3.85 ± 0.22
Group V (Medium Dose Group)	Extract	200	4.92 ± 0.25
Group VI (High Dose Group)	Extract	400	5.78 ± 0.27

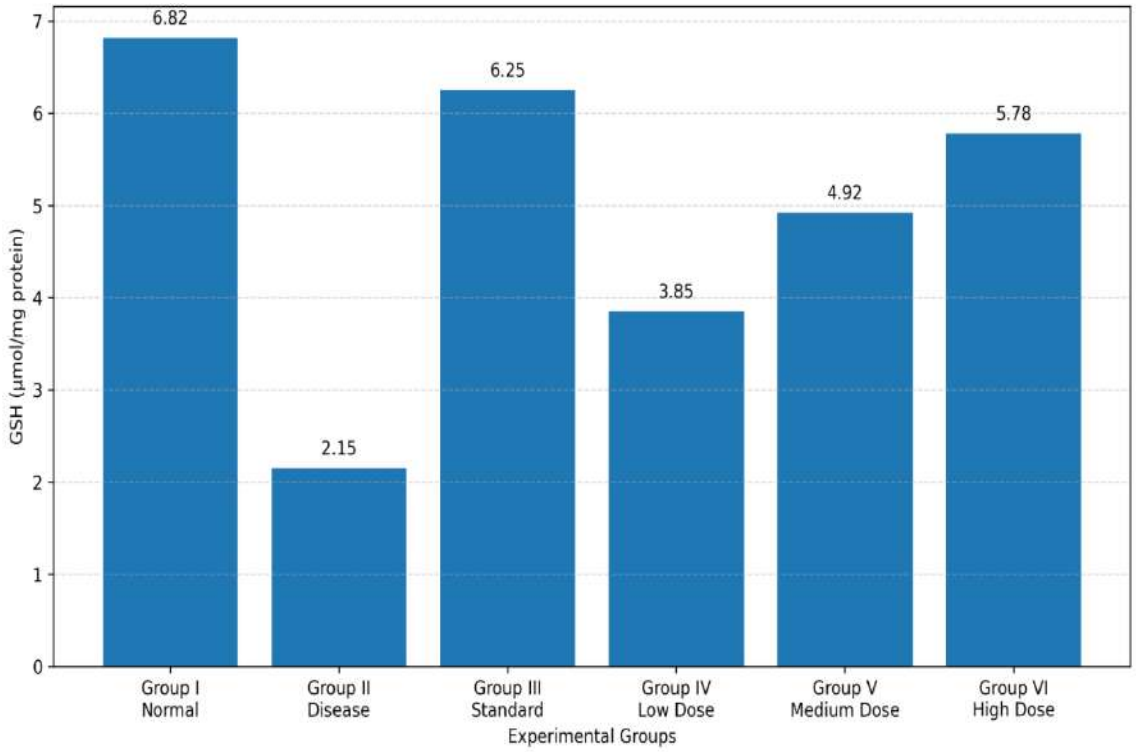

Figure 8: Effect of Nelumbo nucifera Red Flower Extract on Reduced Glutathione (GSH) Levels

Interpretation: The graph shows that the disease group exhibited the lowest GSH levels, confirming depletion of endogenous antioxidant defense due to aluminium chloride-induced oxidative stress. Treatment with Nelumbo nucifera red flower extract resulted in a dose-dependent increase in GSH levels. The high-dose group showed maximum restoration, with values approaching the standard group, indicating strong antioxidant and neuroprotective activity.

Estimation of Superoxide Dismutase (SOD) Levels

Table 9: Effect of Nelumbo nucifera Red Flower Extract on Superoxide Dismutase (SOD) Levels

Group	Treatment	Dose (mg/kg)	SOD (U/mg protein)
Group I (Normal Group)	Normal Control	—	8.45 ± 0.35
Group II (Disease Group)	$AlCl_3$ Control	150	3.12 ± 0.22
Group III (Standard Group)	Donepezil	5	7.96 ± 0.32
Group IV (Low Dose Group)	Extract	100	4.86 ± 0.25
Group V (Medium Dose Group)	Extract	200	6.18 ± 0.28
Group VI (High Dose Group)	Extract	400	7.35 ± 0.30

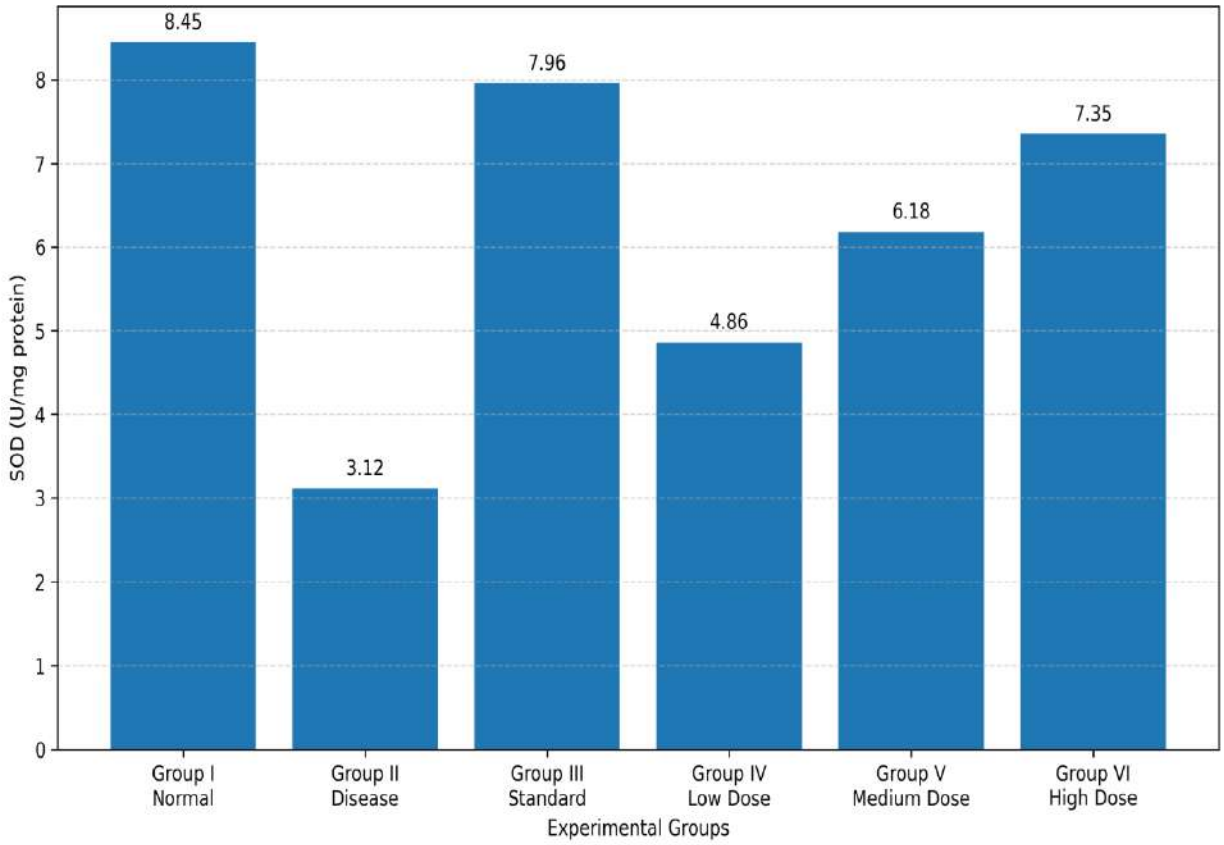

Figure 9 Effect of Nelumbo nucifera Red Flower Extract on Superoxide Dismutase (SOD) Levels

Interpretation

The graph clearly shows that the disease group exhibited the lowest SOD activity, confirming severe oxidative stress induced by aluminium chloride. Treatment with Nelumbo nucifera red flower extract resulted in a dose-dependent increase in SOD levels. The high-dose group showed maximum restoration, with values approaching those of the standard group, indicating strong antioxidant and neuroprotective activity.

Estimation of Catalase (CAT) Levels

Table 10: Effect of Nelumbo nucifera Red Flower Extract on Catalase (CAT) Levels

Group	Treatment	Dose (mg/kg)	CAT (µmol/min/mg protein)
Group I (Normal Group)	Normal Control	—	55.82 ± 2.10
Group II (Disease Group)	$AlCl_3$ Control	150	22.45 ± 1.85
Group III (Standard Group)	Donepezil	5	52.36 ± 2.05
Group IV (Low Dose Group)	Extract	100	32.18 ± 1.92
Group V (Medium Dose Group)	Extract	200	42.65 ± 2.00
Group VI (High Dose Group)	Extract	400	49.28 ± 2.08

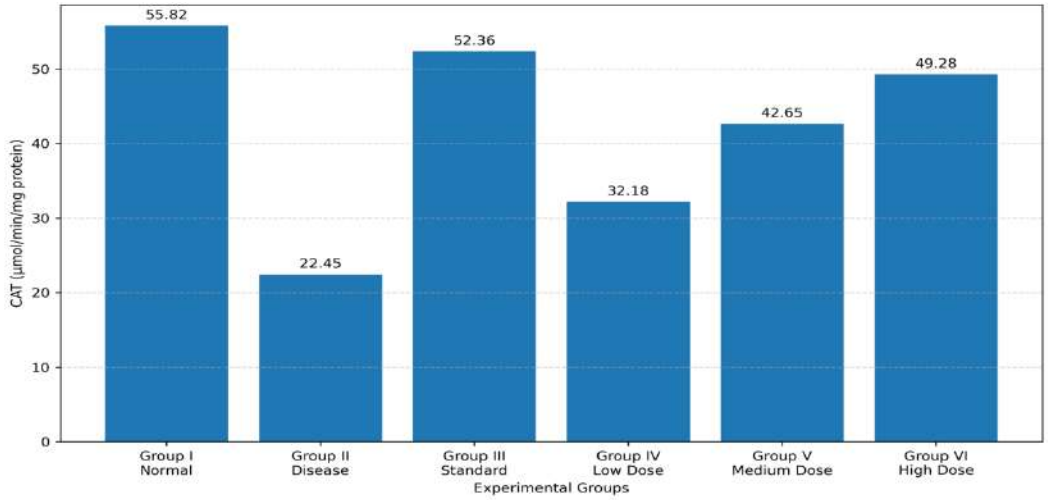

Figure 10: Effect of Nelumbo nucifera Red Flower Extract on Catalase (CAT) Levels

Interpretation

The graph clearly indicates that the disease group exhibited the lowest catalase activity due to aluminium chloride-induced oxidative stress. Treatment with Nelumbo nucifera red flower extract resulted in a dose-dependent increase in catalase levels. The high-dose group showed maximum restoration, with values approaching the standard group, indicating strong antioxidant and neuroprotective activity.

Estimation of Total Protein

Table 11: Effect of Nelumbo nucifera Red Flower Extract on Total Protein Levels

Group	Treatment	Dose (mg/kg)	Total Protein (mg/g tissue)
Group I (Normal Group)	Normal Control	—	68.45 ± 2.15
Group II (Disease Group)	$AlCl_3$ Control	150	38.62 ± 1.92
Group III (Standard Group)	Donepezil	5	64.28 ± 2.08
Group IV (Low Dose Group)	Extract	100	46.75 ± 1.98
Group V (Medium Dose Group)	Extract	200	55.82 ± 2.04
Group VI (High Dose Group)	Extract	400	61.46 ± 2.10

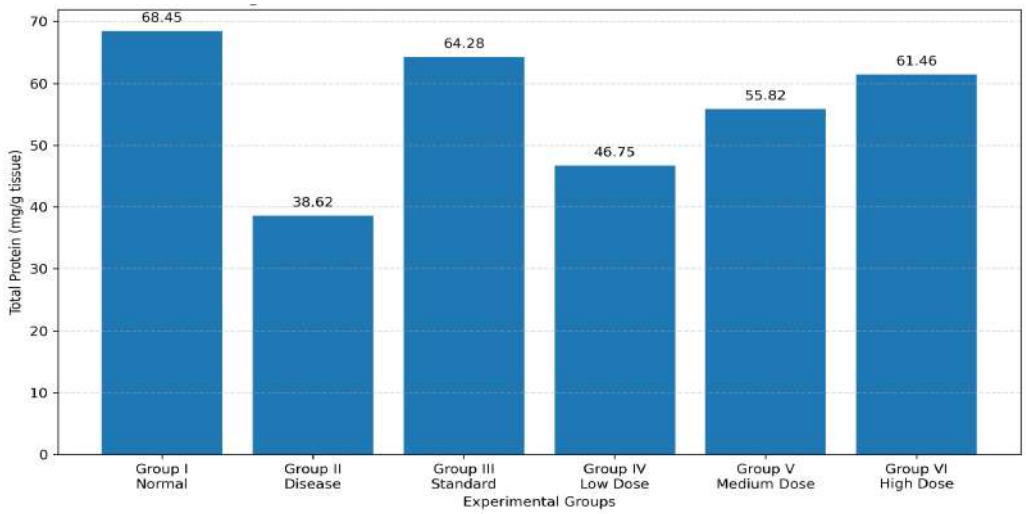

Figure 11: Effect of Nelumbo nucifera Red Flower Extract on Total Protein Levels

Interpretation

The graph clearly shows that the disease group exhibited the lowest total protein levels due to aluminium chloride-induced neurotoxicity. Treatment with Nelumbo nucifera red flower extract resulted in a dose-dependent increase in total protein content. The high-dose group showed maximum restoration, with values approaching the standard group, indicating protective effects on brain tissue and improved cellular integrity.

Histopathological Examination of Brain Tissue

Histopathological examination of brain tissue was carried out to evaluate the protective effect of Nelumbo nucifera red flower extract against aluminium chloride-induced neuronal damage in experimental Alzheimer's disease. Brain sections, particularly from the hippocampal and cerebral cortical regions, were isolated, fixed in 10% formalin, processed routinely, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope for pathological alterations such as neuronal degeneration, pyknosis, vacuolation, inflammatory cell infiltration, and disruption of normal neuronal architecture.

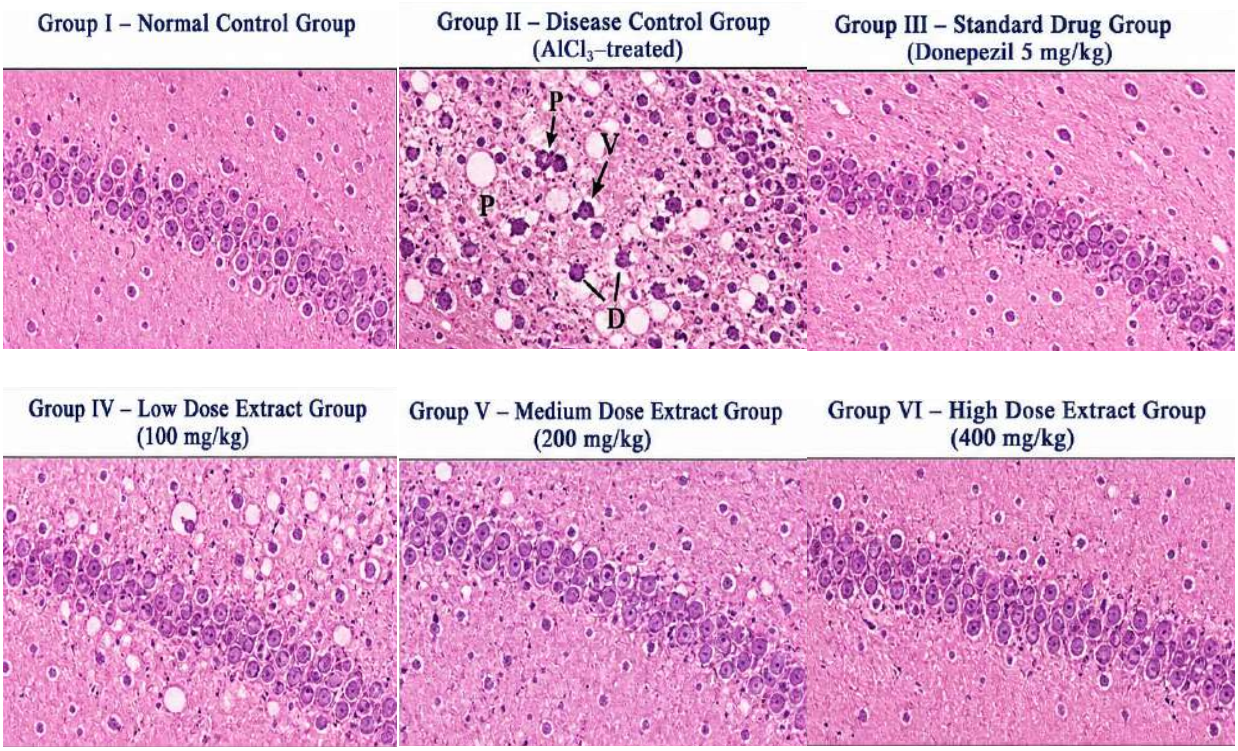

Figure 12: Effect of Nelumbo nucifera Red Flower Extract on Histopathology Parameters

Overall Histopathological Interpretation

Histopathological evaluation revealed that aluminium chloride administration caused severe neuronal damage and degeneration in the disease control group. Treatment with Nelumbo nucifera red flower extract produced dose-dependent protection against neuronal injury. The extract significantly restored normal brain architecture, reduced neuronal degeneration, and preserved hippocampal integrity.

The high-dose group showed maximum protection and results comparable to the standard drug Donepezil, confirming the neuroprotective potential of the extract.

Discussion

The present study was designed to evaluate the neuroprotective potential of the red flower extract of Nelumbo nucifera against aluminium chloride ($AlCl_3$)-induced experimental Alzheimer's disease in Wistar albino rats.

Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive decline, cholinergic dysfunction, oxidative stress, and neuronal degeneration.

The experimental model using aluminium chloride is well established for inducing Alzheimer-like pathology, including memory impairment, oxidative damage, and biochemical alterations. The results obtained in the present study clearly demonstrate that Nelumbo nucifera extract exhibits significant neuroprotective and anti-Alzheimer activity.

Conclusion

The present study concludes that the red flower extract of Nelumbo nucifera possesses significant neuroprotective activity against aluminium chloride-induced experimental Alzheimer's disease in Wistar albino rats. Administration of aluminium chloride produced marked cognitive impairment, cholinergic dysfunction, oxidative stress, and biochemical alterations, as evidenced by poor performance in behavioral tests, increased acetylcholinesterase activity, elevated lipid peroxidation, reduced antioxidant enzyme levels, and decreased total protein content. These findings confirmed the successful induction of Alzheimer-like pathology in the experimental animals. Treatment with Nelumbo nucifera red flower extract produced significant and dose-dependent improvement in all evaluated parameters.

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