Ind. J. Pharm. Edu. Res., 2026; Krupapharmacon:133-140.
https://www.ijper.org

Original Article

Anti-Alzheimer's Potential of Flaxseed (*Linum usitatissumum*) in Aluminium Chloride Induced Alzheimer's Disease in Zebrafish Model

Ramadevi Pemmereddy[1], Swathi Boddupally[2], Namratha Sunkara[3], Priyanka Budime[4], Kusume Sireesha[5], Prathiksha Thakre[6], Meghana Rameshwaram[6], Pallavi Nagesh[7], Haritha Pasupulati[1,*]

[1]Department of Pharmacology, Bharat Institute of Pharmacy, Telangana, INDIA.
[2]Department of Pharmacy Practice, Bharat School of Pharmacy, Telangana, INDIA.
[3]Department of Pharmaceutical Analysis, Bharat Institute of Pharmacy, Telangana, INDIA.
[4]Department of Pharmaceutics, Bharat Institute of Pharmacy, Telangana, INDIA.
[5]Department of Pharmaceutical Chemistry, Bharat Institute of Pharmacy, Telangana, INDIA.
[6]Department of Pharmacy, Bharat School of Pharmacy, Telangana, INDIA.
[7]Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, INDIA.

ABSTRACT

Objectives: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder predominantly affecting the elderly population and is characterized by memory loss, cognitive impairment, and behavioural disturbances. Despite extensive research, currently approved therapies for AD are largely limited to symptomatic management and fail to modify the underlying disease pathology. Therefore, the identification of safe and effective disease-modifying agents remains a major therapeutic challenge. The present study aimed to establish an Alzheimer's-like cognitive impairment model in zebrafish using Aluminium Chloride ($AlCl_3$) and to evaluate the neuroprotective potential of Flax Seed Extract (FSE). **Materials and Methods:** $AlCl_3$ is a well-known neurotoxic agent that induces oxidative stress, cholinergic dysfunction, and neuronal damage, thereby replicating key pathological features of AD. Exposure to $AlCl_3$ resulted in significant deficits in cognitive performance, increased anxiety-like behaviour, and reduced locomotor activity in zebrafish, confirming the successful induction of Alzheimer's-like pathology. **Results:** Treatment with flaxseed extract significantly ameliorated the behavioural impairments in a dose-dependent manner, with higher doses showing superior protective effects. The improvement in memory and motor functions suggests a strong neuroprotective role of flaxseed against $AlCl_3$-induced neurotoxicity. These beneficial effects are likely attributed to the presence of bioactive phytochemicals such as flavonoids, phenols, tannins, and alkaloids, which are known to exhibit antioxidant, anti-inflammatory, and antiapoptotic activities. **Conclusion:** Overall, the findings validate the zebrafish $AlCl_3$-induced model as a reliable platform for preclinical evaluation of anti-Alzheimer's agents and highlight flaxseed as a promising, natural, and cost-effective therapeutic candidate for AD. Further studies focusing on molecular mechanisms, biomarker analysis, and clinical translation are warranted to substantiate these findings.

Keywords: Alzheimer's disease, Flax seed, Locomotor activity, Neuroprotective, Zebra fish.

Correspondence:
Dr. Haritha Pasupulati
Associate Professor, Department of Pharmacology, Bharat School of Pharmacy, Ibrahimpatnam-501510, Telangana, INDIA.
Email: pasupulatiharitha17@gmail.com

INTRODUCTION

Alzheimer's disease is a long-term, irreversible brain disease that gradually deteriorates thinking and memory abilities, ultimately making it difficult to do even the most basic tasks. Alzheimer's Disease (AD) is the most common cause of dementia and a sneaky neurodegenerative illness that presents one of the biggest public health issues of the twenty-first century.[1] The prevalence of this debilitating illness is predicted to reach 106.8 billion people by 2050 due to an aging global population; thus, it is critical to explore its complexity and seek out novel research and therapeutic strategies. Progressive functional decline is the hallmark of AD, which deprives people of their uniqueness, abilities, social impact, and treasured memories. The global dementia population was estimated to be 35.6 million in 2010, and it is predicted to grow every 20 years. By 2030, there will be 65.7 million AD patients, and by 2050, there will be 115.4 million, if appropriate preventative and treatment measures are not implemented. Between 2000 and 2019, the number of deaths in the US from AD reportedly rose by more than 145%, while deaths from stroke, heart disease, and HIV declined.[2] Together

DOI: 10.5530/ijper.krupapharmacon.30

with the disease's natural course, genetic and environmental risk factors might hasten cognitive impairment. People over 65 years are more likely to have AD, and the risk rises with age. The specific aetiology of Alzheimer's disease is unknown, although it involves a complex interaction of genetic, environmental, and behavioural influences.

The main drawback of the current treatments for Alzheimer's disease is their incapacity to alter the underlying disease process. Most licensed drugs like NMDA receptor antagonist and cholinesterase inhibitors simply provide symptomatic relief by momentarily enhancing or stabilizing cognitive abilities.[3] These effects are mild, though, and they usually go away as the illness worsens. Importantly, the total loss of memory, reasoning, and functional abilities is unabated because these medications do not stop, reverse, or considerably delay the neurodegenerative process. Another significant drawback is the lack of effectiveness of disease-modifying treatments that target tau tangles or beta-amyloid plaques.[4] Additionally, it is challenging to treat Alzheimer's with single-target therapy due to the disease's complexity and multifactorial character, includes amyloid deposition, tau pathology, neuroinflammation, oxidative stress, synaptic dysfunction, and vascular alterations. Effective management is further hampered by the lack of trustworthy biomarkers for early diagnosis and real-time tracking of illness progression or therapy response. Finally, despite documented differences in lifestyle, environmental, and genetic factors that affect treatment outcomes, customized medicine is not sufficiently included into the present strategy.[5] Therefore, more efficient, approachable, and comprehensive therapy plans that may address the various pathways behind Alzheimer's disease are desperately needed.

Natural compounds have demonstrated anti-oxidative and anti-neuroinflammatory properties, inhibiting the aggregation of Aβ and tau peptides and increasing cholinergic signalling. Recent research has demonstrated that several dietary factors lessen the incidence of Alzheimer's disease, motivating scientists to examine the benefits of phytoconstituents and extracted bioactive elements.[6,7] Flaxseeds, botanically known as *Linum usitatissimum*, are a small, oil-rich seed from the *Linaceae* family. Flaxseeds have a variety of pharmacological characteristics since they are high in dietary fiber, protein, polyphenols, lignans (especially seco isolariciresinol diglucoside, alpha- linolenic acid, an omega-3 fatty acid. They have long been utilized for their anti-inflammatory, laxative, cardioprotective, and antioxidant properties in a variety of medical systems, including Ayurveda and Unani.[8,9] Their potential to prevent and manage chronic diseases, including diabetes, cardiovascular disorders, and neurodegenerative ailments like Alzheimer's disease, has been brought to light by a wealth of scientific research in recent years. Flaxseeds are of particular interest in modern nutraceutical and pharmacological research due to their distinct phytochemical profile and health-promoting properties, which are being used more and more in functional meals, dietary supplements, and medicinal formulations. Due to its safety profile and natural origin, it is considered a potential adjunct or preventive strategy in the management of neurodegenerative diseases.[10]

Zebrafish (*Danio rerio*) can be used as an alternative animal model in neuroscience research. Their unique characteristics, such as rapid breeding, transparent embryos, and conserved genome, make them an attractive alternative to traditional mammalian models.[11] The zebrafish (*Danio rerio*) serves as an ideal vertebrate model for investigating neurodegenerative conditions like AD, owing to its genetic and neuroanatomical similarities to humans, along with the ease of conducting high-throughput behavioural and biochemical analyses.[12]

The current study seeks to validate flaxseed as a potential natural treatment agent for Alzheimer's disease by conducting a thorough preclinical study using the zebrafish model. This integrative approach may help uncover effective plant-based therapies for neurodegenerative diseases.

MATERIALS AND METHODS

Zebrafish maintenance

Zebrafish must be properly acclimatized to laboratory conditions. During this period, it is essential to monitor various physical, behavioural, and environmental parameters to ensure the health and well-being of the fish. Regular observation helps identify signs of stress, disease, or unsuitable environmental conditions.

Adult zebrafish (4-6 months old) are maintained in glass/acrylic tanks in regulated lab conditions, with 1-5 fish per Liter to reduce stress. Tanks have recirculating systems with weekly water changes, or daily 10-20% changes in static arrangements. Filtration maintains water quality by keeping ammonia/nitrite levels at 0 ppm and nitrates at less than 40 ppm. Fish are fed twice a day with a combination of dry feed and live/frozen food. Tanks need a 14:10 light-dark cycle to regulate their activity. Regular trash and biofilm removal is critical to maintaining cleanliness (Table 1). Fish are examined daily for health issues, and new arrivals are confined for 2-4 weeks.[13]

Water Quality Conditions for Zebrafish maintenance

Equipment's utilized for the study

- **Aquarium:** Zebrafish were housed in a glass aquarium measuring 30 cm x 15 cm x 15 cm, with a density of 5 fish per 3 L.
- **Aerator:** Aerators from the Sobo aquarium air pump-SB-548A were used to supply air into the water and keep the fish alive.
- **Animal feed:** Zebrafish were fed dry food that was available on a regular basis at the aquarium pet shop.

The dry food has 30% crude protein, 4% crude fat, 5% crude fibre, 12% crude ash, and 10% moisture.

- **Micropipette:** Throughout the study, thermoscientific fine pipettes with 100 µL and 1000 µL capacities were utilised.
- **Novel tank diving test apparatus:** 15 L of acrylic tank.
- **T-Maze test apparatus:** Glass rod and T- shaped container.

Preparation of Flax Seed Extract (FSE) by Maceration Technique

Raw flaxseeds were dried in a hot air oven for two hours to remove moisture. After cooling to room temperature, the seeds were ground into a fine powder using a mechanical grinder. In the proper container, 50 mL of 70% ethanol was mixed to 5 g of precisely weighed flaxseed powder. To aid in the initial extraction process, the mixture was placed in a dark environment for 2 hr. To enhance the extraction process, the mixture was then placed on a water bath and heated progressively for ½ hr. The solvent was evaporated from the resulting mixture using a rotary evaporator for three days, or until all of the ethanol was gone. The final dry extract was collected and stored in an airtight container.

Preliminary phytochemical Screening of FSE

Test for Alkaloids- Mayer's test

1g of flaxseed powder mixed with 70% of 10 mL ethanol. Few drops of potassium iodide added to 2-3 mL of extract solution. The cream-coloured precipitate indicates the presence of alkaloids.[14]

Test for Flavonoids- Alkaline Reagent Test

To an aliquot of the FSE (2-3 mL), few drops of normal sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.[15]

Test for Phenols- Ferric chloride test

1 g of FSE mixed with 70% of 10 mL ethanol. 0.5 mL of $FeCl_3$ (w/v) solution was added to 2 mL of flaxseed extract solution, the production of a strong colour indicates the presence of phenols.[16]

Test for Tannins-Lead Acetate test

1% lead acetate solution was added to an aliquot of the FSE solution; white or cream coloured precipitate indicate the presence of tannins.[17]

Experimental Design

Adult zebrafish weighing 0.5 g were randomly allocated into six groups of six individuals each. All of the animals were divided into groups in housing tanks and acclimatised in the laboratory for 5 days.

Induction of Alzheimer's disease by Aluminium Chloride ($AlCl_3$) by Immersion method

Alzheimer's-like symptoms will be chemically induced using $AlCl_3$, a neurotoxin known to mimic AD pathophysiology by promoting oxidative stress, beta-amyloid accumulation, and cholinergic dysfunction.[18,19] Induction will be carried out by the immersion method. In the immersion protocol, zebrafish will be exposed to a 150 mg/L $AlCl_3$ solution for 30 min per day over a period of 7 days. A separate control group of zebrafish will not be exposed to the neurotoxin and will serve as the baseline reference for behavioural and biochemical comparisons.

Experimental grouping

Adult zebrafish weighing 0.5 g were randomly allocated into six groups of six individuals each. All the animals were separated into different groups in housing tanks and kept for acclimatization in the laboratory for 5 days. FSE was given at a dose of 80 mg/kg body weight, which has been demonstrated in prior research to dramatically alter lipid profiles and control blood pressure in rats weighing about 150 g. The same dose (80 mg/kg) was given via the immersion method in this investigation, with the dosage being modified based on the body weight of the zebrafish. To guarantee

Table 1: Water quality conditions of zebrafish housing tank.

Parameter	Ideal Range	Why It Matters
Temperature	26°C-28.5°C	Controls metabolism, breeding, and immunity.
pH	6.8-7.5	Too acidic or basic conditions cause stress and damage to tissues.
Ammonia (NH_3)	0 ppm	Ammonia is toxic even at low levels.
Nitrite (NO_2^-)	0 ppm	Nitrite is toxic; disrupts oxygen transport in blood.
Nitrate (NO_3^-)	< 40 ppm	High levels affect long-term health.
Dissolved Oxygen	≥ 6 mg/L	Vital for respiration and survival.
Conductivity	300 - 1500 µS/cm	Reflects ion balance in water; important for osmoregulation.

accurate dosing, the necessary amount of extract was diluted in 1 L of water and administered using a 100-1000 µL micropipette. The treatment groups (n=6) with FSE were administered to the zebrafish 2 hr before the commencement of the experiment (Table 2, Figure 1).

Behavioural Assessments

Behavioural parameters were evaluated using the novel tank diving test, T-maze test, and locomotor activity assessment across the control and all experimental treatment groups.

Novel tank diving test

A 15-L tank was used, into which 6 L of water (approximately 10 cm in height) was added. The tank was vertically divided into three equal sections, with the top 5 cm and bottom 5 cm designated as separate zones. The water temperature was maintained at 28°C throughout the experiment. After 1 hr of FSE and $AlCl_3$ administration, each fish from the respective groups was subjected to the Novel Tank Diving Test for a duration of 6 min (Figure 2).

Record or observe (manually) and analyse the number of times the fish enters the top and bottom zones, as well as the time spent in the top zone, the freezing time, and the distance travelled.[20]

T-maze test

The T-maze equipment was built with transparent acrylic glass sheet. The maze's dimensions were 50 cm x 10 cm x 10 cm for the long arm, 20 cm x 10 cm x 10 cm for the two short arms, and a 10 cm x 10 cm x 10 cm start box at the stem's foot. The two short arms, left and right, are covered with green and red sleeves, respectively. The maze was filled with water to a height of 6 cm, and the temperature of the water was kept constant at 28°C during the experiment.[21]

Training and testing sessions were used to conduct the evaluation. The training sessions lasted 6 days and included a test session on the 7th day. The test session animals are placed in the start arm for 1 min. The blocking door was opened and then closed once the fish was present. Fish enters the short arms, and a blocking door located at the intersection of the two short arms and one long arm is closed. This allows the fish to swim for a total of 5 min inside the short arms (red and green arms). The total number of entries into short arms were calculated. After 6 days of training, a test session was conducted on the 7th day. The technique involved without blocking any doorways and the behavioral responses were recorded (Figure 3).

Locomotor Activity

Adult zebrafish are individually placed in a transparent tank or beaker with a defined water volume. The fish are acclimated for 5 min to minimize stress-induced movement. Locomotor activity is observed for a fixed duration (6 min) (Figure 4). Distance traveled, movements of fishes and freezing time are analyzed for behavioral assessment (Table 3).[22]

Table 2: Zebrafish grouping and treatment.

Groups	Number of zebra fish (n=6)	TREATMENT
I	6	Control (water)
II	6	150 mg $AlCl_3$ (dissolved in tank)
III	6	150 mg $AlCl_3$ + Low dose FSE (30 µg)
IV	6	150 mg $AlCl_3$ + Medium dose FSE (50 µg)
V	6	150 mg $AlCl_3$ + High dose FSE (70 µg)

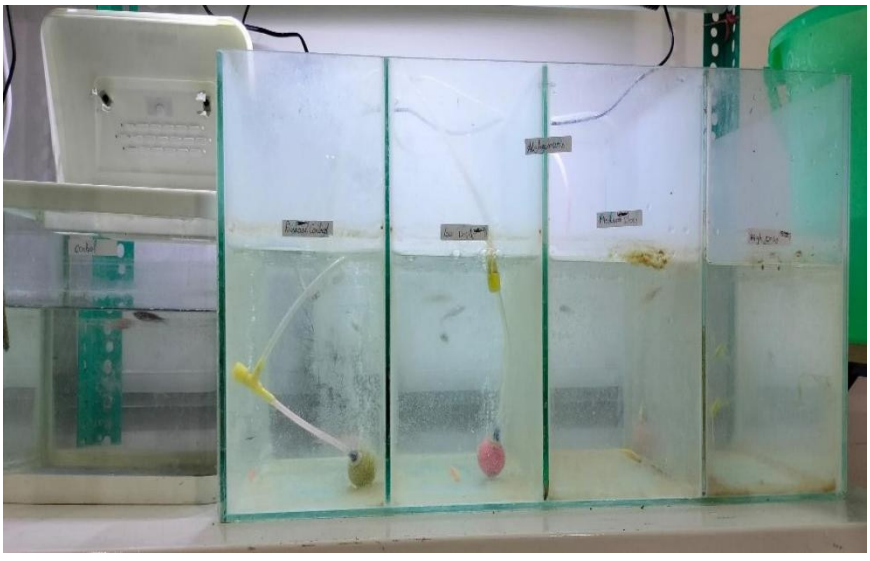

Figure 1: Zebrafish grouping.

RESULTS AND DISCUSSION

Qualitative Screening of phytochemicals

The FSE was screened for the Phytochemical Constituents by Mayers test, alkaline reagent test, ferric chloride test and lead acetate test and notice that the FSE comprises of alkaloids, flavonoids, phenols and tannins (Table 4).

Novel Tank diving test

The novel tank diving test behavioural analysis revealed clear distinctions in locomotion and cognitive activity across different zebrafish groups exposed to FSE, following Alzheimer's disease induction (Table 5).

Normal Control Group (no disease induced) displayed healthy exploration behavior, evidenced by the highest total distance travelled (2.10±0.01 m), frequent entries into the top zone (8±0.02), and a long top-zone residence time (240±1.23 sec). The low freezing time (30±0.12 sec) and a consistent average speed (0.035±1.02 m/s) further indicated an absence of anxiety or cognitive dysfunction.

Disease Control Group (Alzheimer's-induced, untreated) showed marked cognitive and behavioral impairment. The fishes of this group exhibited minimal movement (1.05±0.14 m), rarely entered the top zone (only 2±0.03 times), and spent only 110 sec in the top zone, accompanied by excessive freezing behavior (110±1.41 sec). These findings reflect severe anxiety-like behavior and cognitive deficits typical of Alzheimer's pathology in zebrafish.

Low dose FSE Group showed moderate improvement over disease control. While the total distance increased to 1.35±0.002 m and top zone entries 4±0.561, the fish still displayed relatively high freezing time (85±0.25 sec) and a modest speed (0.023±1.124 m/s). This suggests partial neuroprotection, though not sufficient to fully restore normal behavior.

Medium dose FSE Group exhibited notable behavioral recovery. The zebrafish traveled 1.65±0.08 m, entered the top zone 6±0.96 times, and spent 190±0.81 sec exploring the top zone. The freezing time dropped to 60±0.031 sec, showing reduced anxiety and improved spatial memory, indicating effective partial reversal of Alzheimer-like symptoms at this dosage.

High dose FSE Group (70 µg) showed behavior almost indistinguishable from the normal control. These fish covered 1.80±0.162 m, entered the top zone 7±0.231 times, and spent 200±2.12 sec there. The freezing time was minimal (28±0.12 sec) and average speed (0.030±0.02 m/s) was near normal. This suggests that the high concentration of FSE effectively restored normal cognitive and exploratory function, demonstrating strong anti-Alzheimer's potential (Table 5).

The statistical analysis depicts that the high concentration of FSE effectively restored normal cognitive and exploratory function compared with that of disease control group. The effects were dose-dependent, with the high-dose FSE demonstrating the most pronounced recovery. It markedly enhanced locomotor and swimming performance, improved exploratory behavior, and produced the greatest reduction in freezing time, indicating superior neuroprotective potential at the highest dose.

All Values are expressed as Mean±SEM, n=6, Statistical analysis was carried out using One way ANOVA followed by *post hoc* Dunnett's test. $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ versus Disease Control

T-maze test

Control group having significant number of entries into green arm whereas the number of entries into green arm by $AlCl_3$ treated group is less than control group which indicates induction

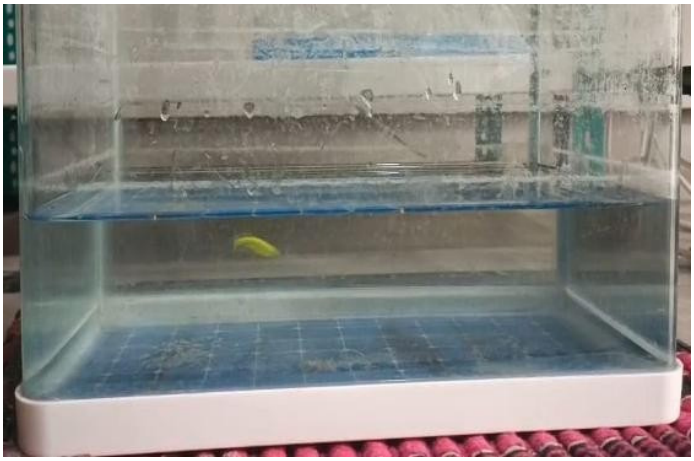

Figure 2: Novel tank diving test.

Figure 3: T-maze test glass apparatus.

Table 3: Parameters for the behavioral assessments.

Behavioral test	Parameter	Measurement outcome
Novel Tank Diving Test	Time spent in top zone (s)	Exploratory behavior and anxiety reduction
	Time spent in bottom zone (s)	Anxiety-like response
	Number of entries to top zone	Adaptation to novel environment
	Freezing duration (s)	Fear and stress response
T-Maze Test	Transfer latency (s)	Learning performance
	Total number of entries into short arms	Cognitive function
Locomotor Activity Test	Total distance travelled	General locomotor activity
	Swimming speed (cm/s)	Motor coordination
	Number of movements	Behavioural activity level

Table 4: Phytochemical screening test results.

Sl. No.	Test	Result
1.	Alkaloids	✓
2.	Flavonoids	✓
3.	Phenols	✓
4.	Tannins	✓

of AD. Disease control group entered lesser number of times into green arm than control group. The number of entries into green arm by Low dose FSE treatment group is less than medium dose FSE treatment group. If the dose of FSE is increased to 70 µg in $AlCl_3$ treated zebrafish group, it is having lesser number of entries into green arm of T-maze than Control group. This Results indicates that better memory retention when the dose of FSE at 70 µg administered to zebrafish (Table 6). The FSE significantly improved cognitive behavior in $AlCl_3$-induced zebrafish in a dose-dependent manner. Increased green arm entries and reduced latency suggest improved memory and learning, likely due to flaxseed's antioxidant, anti-inflammatory, and neuroprotective properties (Figure 5).

All Values are expressed as Mean±SEM, n=6, Statistical analysis was carried out using One way ANOVA followed by *post hoc* Dunnett's test. $^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ versus Disease Control.

Locomotor activity

The disease control group showed a marked reduction in locomotor performance compared with the normal control group, as evidenced by decreased distance travelled (1.05±0.01 m vs. 2.10±0.01 m) and movement duration (190±2.89 s vs. 270±3.21 s), along with a significant increase in freezing time (110±2.31 s vs. 30±1.54 s). These findings indicate impaired motor activity, increased anxiety-like behaviour, and cognitive dysfunction following $AlCl_3$ induction. Treatment with the test compound produced a dose-dependent improvement in behavioural performance. The treated groups exhibited progressive increases in distance travelled and movement duration, accompanied by a reduction in freezing time compared with the disease control group. Among the treatment groups, the High dose FSE treatment group showed the most significant neuroprotective effect,

Table 5: Measurement outcomes of zebrafish by Novel tank diving test.

Parameters	Normal Control	Disease Control	$AlCl_3$ + Low dose FSE (30 µg)	$AlCl_3$ + Medium dose FSE (50 µg)	$AlCl_3$ + High dose FSE (70 µg)
Distance Travelled (m)	2.10±0.01	1.05±0.14	1.35±0.002[a]	1.65±0.008[a]	1.80±0.162[a]
Average Speed (m/s) (60 sec trial)	0.035±1.02	0.017±0.09	0.023±1.124[a]	0.027±0.142[a]	0.030±0.02[a]
Entries into Top Zone	8±0.02	2±0.03	4±0.561[b]	6±0.96[a]	7±0.231[a]
Freezing Time (sec)	30±0.12	110±1.41	85±0.25[b]	60±0.031[a]	28±0.12[a]
Time in Top Zone-Green zone (sec)	240±1.23	120±0.12	170±1.34[a]	190±0.81[a]	200±2.12[a]

Table 6: Measurement outcomes of zebrafish by T-maze test.

Sl. No.	Parameters	Normal Control	Disease control (150 mg $AlCl_3$)	$AlCl_3$ + Low dose FSE (30 µg)	$AlCl_3$ + Medium dose FSE (50 µg)	$AlCl_3$ + High dose FSE (70 µg)
1.	Latency to enter an arm (sec)	10±0.58	180±2.67	120±2.10[a]	60±1.53[a]	15±0.86[a]
2.	No. of entries to green arm	30±0.58	11±0.37	19±0.52[a]	23±0.42[a]	25±0.52[a]
3.	No. of entries to red arm	10±0.47	17±0.52	15±0.52[c]	12±0.52[a]	11±0.33[a]

Table 7: Measurement outcomes of zebrafish by Locomotor activity test.

Parameter	Normal Control	Disease Control	$AlCl_3$ + Low dose FSE (30 µg)	$AlCl_3$ + Medium dose FSE (50 µg)	$AlCl_3$ + High dose FSE (70 µg)
Distance Travelled (m)	2.10±0.01	1.05±0.01	1.35±0.03a	1.65±0.002a	1.80±0.005a
Movements (sec)	270±3.21	190±2.89	215±2.69a	240±2.16a	260±2.1a
Freezing Time (sec)	30±1.54	110±2.31	85±1.47a	60±1.41a	25±1.83a

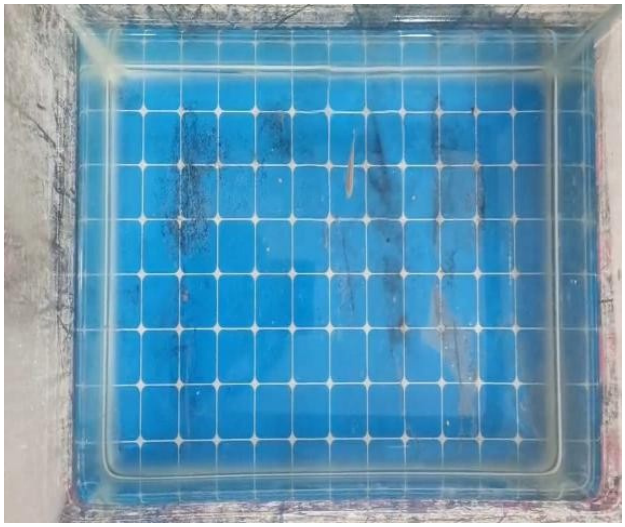

Figure 4: Locomotor activity test.

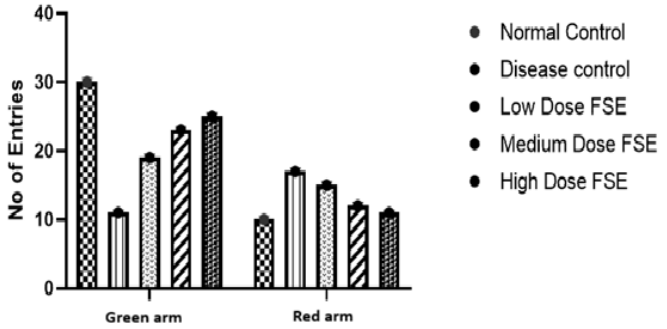

Figure 5: Graphical representation of fishes entering no of times into green arm and red arm.

restoring locomotor activity close to normal levels and markedly reducing freezing behaviour. This suggests effective attenuation of $AlCl_3$-induced neurobehavioral deficits with the FSE (Table 7).

All Values are expressed as Mean±SEM, n=6, Statistical analysis was carried out using One way ANOVA followed by *post hoc* Dunnett's test. [a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$ versus Disease Control.

Overall, the results indicate that treatment significantly improves locomotor activity, reduces anxiety-like behaviour, and protects against aluminium-induced neurotoxicity, supporting its neuroprotective potential.

CONCLUSION

This study uses an $AlCl_3$-induced zebrafish model to show that flaxseed (*Linum usitatissimum*) extract can reduce Alzheimer's disease like symptoms. Alzheimer's disease is difficult to treat with current pharmaceutical medications, which mostly provide symptomatic relief without slowing neurodegeneration. This gap has sparked greater interest in natural substances like flaxseed, which have antioxidant, anti-inflammatory, and neuroprotective qualities. In this study, zebrafish exposed to 150 mg/L $AlCl_3$ displayed significant behavioural changes that characterize AD

pathology, such as decreased locomotor activity, longer freezing time, and poor memory, as indicated by the Novel Tank Diving Test, Locomotor Activity Test, and T-maze assessments. Amyloid-beta buildup, cholinergic transmission disruption, and oxidative stress caused by $AlCl_3$ are thought to be the causes of these deficits. Treatment with flaxseed extract (FSE), however, dramatically and dose-dependently reversed these effects. Notably, zebrafish exhibited near-normal behaviour after being exposed to a high dose FSEwhich included improved spatial learning, decreased freezing time, and increased exploratory behaviour. These results were corroborated by the T-maze test, which revealed that those treated with flaxseed had better memory recall. Zebra fish treated with 70 µg of FSE showed improved memory and learning skills, as evidenced by reduced latency and more entry into the proper arm.

This group demonstrated mobility and cognitive measures that were similar to those of the control group, demonstrating the potential of flaxseed as a neurotherapeutic agent. The presence of important substances such flavonoids, phenols, alkaloids, and tannins, all of which are known to have neuroprotective effects which was verified by phytochemical screening. By reducing oxidative stress, maintaining brain structure, and improving neurotransmitter function, these components most likely help to explain the noted cognitive gains.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Pharmacology, Bharath Institutions Pharmacy, Mangalpalle, Hyderabad for their support in conducting this study.

ABBREVIATIONS

AD: Alzheimer's disease; **$AlCl_3$:** Aluminium chloride; **µg:** Micro gram; **FSE:** Flax Seed Extract; **NMDA:** N-methyl D- Aspartic acid; **°C:** Degree Celsius; **%:** Percentage; **ppm:** Parts per million; **mg/L:** Micro gram/ milli liter; **µS/cm:** microSiemens per centimeter; **cm:** Centi meter; **µl:** Micro liter; **mL:** Milli liter; **$FeCl_3$:** Ferric chloride; **w/v:** Weight/ volume; g: Grams; cm/s: Centi meter/ seconds; **m/sec:** meters/ seconds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The current work shows that in a zebrafish model of Alzheimer's disease caused by $AlCl_3$, flaxseed extract has notable neuroprotective effects. According to behavioural experiments, flaxseed enhanced cognitive performance, decreased anxiety-like behaviour, and restored locomotor activity, especially at larger dosages. These results demonstrate the zebrafish model as a useful platform for preclinical neurological research and indicate the potential of flaxseed as a natural, affordable therapeutic agent for treating Alzheimer's disease.

REFERENCES

1. Murphy MP, LeVine III H. Alzheimer's disease and the amyloid-β peptide. Journal of Alzheimer's disease. 2010;19(1):311-23.
2. Vejandla B, Savani S, Appalaneni R, Veeravalli RS, Gude SS. Alzheimer's disease: The past, present, and future of a globally progressive disease. Cureus 16: e51705 [Internet]. 2024.
3. Liu J, Chang L, Song Y, Li H, Wu Y. The role of NMDA receptors in Alzheimer's disease. Frontiers in neuroscience. 2019;13:43.
4. Pathan A. Limitations of Alzheimer's disease medications. NeuroPharmac J. 2023; 8:11-7.
5. Ornish D, Madison C, Kivipelto M, Kemp C, McCulloch CE, Galasko D, Artz J, Rentz D, Lin J, Norman K, Ornish A. Effects of intensive lifestyle changes on the progression of mild cognitive impairment or early dementia due to Alzheimer's disease: a randomized, controlled clinical trial. Alzheimer's research and therapy. 2024;16(1):122.
6. Noori T, Dehpour AR, Sureda A, Sobarzo-Sanchez E, Shirooie S. Role of natural products for the treatment of Alzheimer's disease. European journal of pharmacology. 2021;898:173974.
7. Wightman EL. Potential benefits of phytochemicals against Alzheimer's disease. Proceedings of the Nutrition Society. 2017;76(2):106-12.
8. Pansare TA, Panchaware P, Makadi A, Jankar S, Verma D. Ayurvedic nutritional, phytochemical, therapeutic and pharmacological overview on atasi *(Linum usitatissimum linn*). World J Pharm Res. 2020;9(15):388-413.
9. Soni RP, Katoch M, Kumar A, Verma P. Flaxseed-Composition and its health benefits. Res. Environ. Life Sci. 2016;9:310-6.
10. Mishra DK, Awasthi H, Srivastava D, Fatima Z, Mohapatra L. Flaxseed oil: Safeguarding neurological health through apoptosis and oxidative damage defense. Current Functional Foods. 2025;3(3):E26668629319735.
11. Gerhard GS. Comparative aspects of zebrafish (*Danio rerio*) as a model for aging research. Experimental Gerontology. 2003;38(11-12):1333-41.
12. Chia K, Klingseisen A, Sieger D, Priller J. Zebrafish as a model organism for neurodegenerative disease. Frontiers in molecular neuroscience. 2022;15:940484.
13. Aleström P, D'Angelo L, Midtlyng PJ, Schorderet DF, Schulte-Merker S, Sohm F, Warner S. Zebrafish: Housing and husbandry recommendations. Laboratory animals. 2020;54(3):213-24.
14. Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of phytology. 2011;3(12).
15. Garg P, Garg R. Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extract. Pharma Innov J. 2019;8(2):16-21.
16. Shah RK, Yadav RN. Qualitative phytochemical analysis and estimation of total phenols and flavonoids in leaf extract of *Sarcochlamys pulcherrima* wedd. Glob J Biosci Biotechnol. 2015;4(1):81-4.
17. Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Arumugam P, Sagadevan E. Analysis of total phenols, total tannins and screening of phytocomponents in *Indigofera aspalathoides* (Shivanar Vembu) Vahl EX DC. Journal of chemical and pharmaceutical research. 2012;4(6):3259-62.
18. Nadiga AP, Krishna KL. A novel Zebrafish model of Alzheimer's disease by Aluminium chloride; involving nitro-oxidative stress, neuroinflammation and cholinergic pathway. European Journal of Pharmacology. 2024;965:176332.
19. Kaur K, Narang RK, Singh S. AlCl3 induced learning and memory deficit in zebrafish. Neurotoxicology. 2022;92:67-76.
20. Farías-Cea A, Leal C, Hödar-Salazar M, Esparza E, Martínez-Duran L, Fuentes I, Iturriaga-Vásquez P. Behavioral study of 3-and 5-halocytisine derivatives in zebrafish using the novel tank diving test (NTT). International Journal of Molecular Sciences. 2023;24(13):10635.
21. Lamb EA, Jouandot DJ, Echevarria DJ. The utility of the T-maze in assessing learning, memory, and models of neurological disorders in the zebrafish. Behaviour. 2012;149(10-12):1081-97.
22. Padilla S, Hunter DL, Padnos B, Frady S, MacPhail RC. Assessing locomotor activity in larval zebrafish: Influence of extrinsic and intrinsic variables. Neurotoxicology and teratology. 2011;33(6):624-30.

Cite this article: Pemmereddy R, Boddupally S, Sunkara N, Priyanka B, Sireesha K, Prathiksha D, *et al*. Anti-Alzheimer's Potential of Flaxseed (*Linum usitatissumum*) in Aluminium Chloride Induced Alzheimer's Disease in Zebrafish Model. Indian J of Pharmaceutical Education and Research. Krupapharmacon 2026:133-40.