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NEUROPROTECTIVE POTENTIAL OF CARICA PAPAYA SEED EXTRACT AGAINST OKADAIC ACID-INDUCED MEMORY IMPAIRMENT IN A ZEBRAFISH MODEL OF ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD) represents a chronic, progressive neurodegenerative disorder characterized by cognitive deterioration, synaptic dysfunction, and neuronal degeneration, ultimately resulting in memory impairment and compromised daily functioning. Given that current therapeutic approaches provide only symptomatic relief, there is growing interest in investigating natural compounds for their neuroprotective capabilities. This research evaluated the protective effects of ethanolic extract derived from Carica papaya seeds against AD-like symptoms in a zebrafish model. In this model, neurotoxicity and memory impairment were induced using Okadaic acid, a compound known to replicate key AD pathological features including cholinergic deficits and tau hyperphosphorylation.

The study employed Behavioural assessments, specifically the T-maze and light/dark preference tests, to evaluate cognitive function and anxiety-related Behaviours. Additionally, biochemical analysis of acetylcholinesterase (AChE) activity and histopathological examination of brain tissues were conducted to assess the extract's effects at molecular and cellular levels. Treatment with C. papaya seed extract at a dosage of 100 mg/kg significantly enhanced Behavioural outcomes, decreased AChE activity, and maintained neuronal structure integrity compared to the Okadaic acid-treated control group.

These results suggest that Carica papaya seed extract shows promise as a natural therapeutic agent for neurodegenerative conditions such as AD. The potential mechanisms may involve modulation of the cholinergic system and preservation of neuronal integrity, warranting further investigation through comprehensive mechanistic and clinical studies.

Keywords: Alzheimer's disease, Carica papaya, zebrafish, acetylcholinesterase, oxidative stress, neurodegeneration

1. Introduction

Alzheimer's disease (AD) constitutes the most prevalent and debilitating neurodegenerative disorder associated with dementia, accounting for approximately 60% to 70% of all dementia cases globally. According to the World Health Organization (WHO, 2021), more than 55 million individuals

worldwide currently live with some form of dementia—a figure projected to reach 139 million by 2050 due to increasing life expectancy and aging populations. From a clinical perspective, AD manifests through progressive deterioration in memory, learning, and reasoning capabilities, eventually impairing daily functioning and quality of life. At the molecular level, AD is characterized by two primary pathological hallmarks: the extracellular accumulation of beta-amyloid (Aβ) plaques and the intracellular formation of neurofibrillary tangles composed of hyperphosphorylated tau protein. These pathological changes contribute to synaptic dysfunction, oxidative stress, neuroinflammation, and widespread neuronal loss (Selkoe & Hardy, 2016).

Despite considerable advances in understanding the underlying mechanisms of AD, current treatment options remain predominantly palliative. Conventional pharmacological interventions—such as acetylcholinesterase inhibitors (e.g., donepezil, rivastigmine) and N-methyl-D-aspartate (NMDA) receptor antagonists (e.g., memantine)—provide only temporary symptom management without halting disease progression. More recently developed disease-modifying therapies, including monoclonal antibodies targeting amyloid-beta (e.g., aducanumab and lecanemab), have emerged; however, these treatments are expensive, demonstrate limited efficacy across broader patient populations, and are associated with risks such as amyloid-related imaging abnormalities (Yiannopoulos & Papageorgiou, 2020). This therapeutic gap highlights the urgent need for alternative, safer, and more cost-effective interventions.

There has been increasing focus on natural products derived from medicinal plants as potential sources of neuroprotective agents. These bioactive compounds often exhibit multi-target properties, including antioxidant, anti-inflammatory, anti-apoptotic, and anti-amyloidogenic effects, which are particularly well-suited to address the complex aetiology of Alzheimer's disease (Ahmed et al., 2021). Among these natural sources, Carica papaya—commonly known as papaya—has attracted scientific attention due to its traditional medicinal applications and diverse phytochemical profile. Papaya seeds contain a variety of bioactive secondary metabolites, including alkaloids, flavonoids, phenolic compounds, glycosides, terpenoids, and saponins, many of which have demonstrated pharmacological potential in preliminary neuroprotection studies (Subandi et al., 2019).

In experimental models for neurodegenerative disorders, zebrafish (Danio rerio) have emerged as powerful and versatile model organisms. Zebrafish share significant genetic homology with humans—approximately 70% of human genes have at least one zebrafish ortholog—and possess highly conserved neural circuitry and neurotransmitter systems. Their transparent embryos, high reproductive rate, and rapid development make zebrafish particularly advantageous for high-throughput Behavioural and pharmacological screening. Importantly, they exhibit sophisticated cognitive Behaviours, including learning, memory retention, and social interaction, enabling reliable assessments of neurocognitive function (Kalueff et al., 2014).

The present study aims to explore the potential neuroprotective properties of Carica papaya seed extract using a zebrafish model of Alzheimer's disease. To simulate AD-like neuropathology, we employ okadaic acid—a potent inhibitor of serine/threonine protein phosphatases PP1 and PP2A. Administration of okadaic acid induces tau hyperphosphorylation, oxidative stress, and neuronal damage, closely resembling human AD pathology. Within this experimental framework, we investigate the efficacy of papaya seed extract in ameliorating cognitive deficits, restoring cholinergic function through modulation of acetylcholine metabolism, and improving histopathological alterations in zebrafish brain tissue. This preclinical evaluation aims to provide foundational evidence for the development of plant-based therapeutic approaches for Alzheimer's disease.

2. Materials and Methods

2.1. Plant Material Collection and Extraction Procedure

Mature seeds of Carica papaya were collected from a certified botanical garden and authenticated by a plant taxonomist. A voucher specimen was deposited in the institutional herbarium for future reference (Specimen ID: CP2025). The collected seeds were thoroughly washed with distilled water

to remove debris, shade-dried at ambient temperature for 10–14 days to preserve phytochemical integrity and subsequently ground into a fine powder using a mechanical grinder.
Extraction was performed using a Soxhlet apparatus with 70% ethanol as the solvent, selected for its polarity and effectiveness in extracting both polar and moderately non-polar phytoconstituents. Approximately 100 g of powdered material underwent extraction for 8–10 hours until a clear siphon indicated complete extraction. The extract was then filtered through Whatman No. 1 filter paper, concentrated using a rotary evaporator under reduced pressure at 40°C, and subsequently stored in airtight, amber-coloured vials at −20°C until further use. The final yield was recorded for dosage calculations.

2.2. Experimental Animals

Healthy adult zebrafish (Danio rerio), aged 6–8 months and weighing approximately 0.3–0.5 g, were obtained from a certified aquaculture facility. The fish were maintained in 30-L glass aquaria under controlled laboratory conditions: 14 h light/10 h dark cycle, temperature maintained at 26 ± 2°C, pH 7.0–7.5, and constant aeration. Fish were acclimatized for 7 days prior to experimentation and fed a standard diet twice daily.
All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC approval number: XYZ/IAEC/2025/03) in compliance with national guidelines for the care and use of laboratory animals.

2.3. Experimental Design: Grouping and Dosing

Zebrafish were randomly divided into five groups, each comprising 10 animals:

- **Group I**: Vehicle-treated control group (received physiological saline)
- **Group II**: Disease model group (exposed to 100 nM Okadaic acid to induce cognitive dysfunction)
- **Group III**: Positive control (Okadaic acid + Rivastigmine 1 mg/kg, a standard Ache inhibitor)
- **Group IV**: Okadaic acid + C. papaya extract (50 mg/kg body weight)
- **Group V**: Okadaic acid + C. papaya extract (100 mg/kg body weight)

All treatments were administered via immersion in dosed water solutions for 7 consecutive days. The concentration of extract and Rivastigmine was optimized based on preliminary dose-finding studies. Behavioural and biochemical evaluations were conducted 24 hours after the final treatment.

2.4. Behavioural Assessments

2.4.1. T-Maze Test for Spatial Learning and Memory

The T-maze apparatus was custom fabricated using transparent plexiglass with dimensions: 25 cm (stem) × 20 cm (arms) × 10 cm (height). The green arm was designated as the reward zone, while the red arm served as the non-reward zone. Fish were trained to associate the green zone with a food reward over a 5-day period. On the test day, the time taken to enter the green zone (latency) and the number of correct entries were recorded during a 5-minute trial. Higher latency and fewer correct entries indicated impaired spatial memory.

2.4.2. Light/Dark Chamber Test for Anxiety-like Behaviour

A standard light/dark chamber (20 × 10 × 10 cm) was employed, with half of the chamber painted black and the other half white, illuminated externally. Each fish was individually placed in the centre, and its Behaviour was recorded for 6 minutes. Parameters observed included time spent in light versus dark zones and number of transitions between zones. Reduced time in the light zone and fewer transitions indicated increased anxiety-like Behaviour.

2.5. Acetylcholinesterase (AChE) Activity Assay

Following Behavioural assessments, zebrafish were euthanized, and their brains were carefully dissected on ice. Tissues were homogenized in phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 15 minutes at 4°C. Supernatants were collected for enzymatic assays.

Acetylcholinesterase activity was estimated using an ELISA-based colorimetric method with acetylthiocholine iodide as substrate and DTNB (Ellman's reagent). Absorbance was measured at 412 nm using a microplate reader. Enzyme activity was expressed in nmol/min/mg of protein.

2.6. Histopathological Evaluation

For histological studies, dissected brain tissues were fixed in 10% neutral buffered formalin for 24 hours. After fixation, samples were dehydrated through a graded alcohol series, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were obtained using a microtome, mounted on glass slides, and stained with haematoxylin and eosin (H&E).

Microscopic examination was conducted under a light microscope to assess neuronal integrity, presence of neurofibrillary tangles, and cellular degeneration in regions such as the telencephalon and optic tectum. Images were captured using a digital microscope camera for documentation and comparison between treatment groups.

3. Results

3.1. Phytochemical Constituents

Phytochemical screening of the plant extract revealed the presence of a broad spectrum of bioactive compounds, including alkaloids, flavonoids, saponins, glycosides, and terpenoids. These constituents are widely acknowledged for their therapeutic potential, particularly in neuroprotection and cognitive enhancement. Flavonoids and terpenoids, in particular, have been associated with antioxidative properties, while alkaloids and glycosides may support neuronal signalling and memory retention. This diverse phytochemical profile suggests a promising role for the extract in mitigating oxidative stress and enhancing cognitive performance (Kumar & Kumari, 2021).

3.1.2 Yield of Extract

Extract	Extract Weight	% Yield
70% Ethanol	6.19g	**20.63%**

3.1.3. Preliminary Phytochemical Screening

Compound	Presence
Alkaloids	+
Flavonoids	+
Phenolics	+
Saponins	+
Steroids	+
Tannins	+
Starch	+

3.2. Behavioural Observations

In Behavioural assessments using paradigms such as the T-maze and light/dark box tests, administration of the extract at a dose of 100 mg/kg resulted in significant performance improvements. Animals exhibited increased time spent and frequency of entries into the green zone and the illuminated compartment, indicating a reduction in anxiety-like Behaviour and enhancement in cognitive response. The observed Behavioural effects at this dosage were more pronounced compared to the 50 mg/kg group and were nearly equivalent to those seen with the standard

cognitive enhancer Rivastigmine, suggesting a dose-dependent effect with substantial therapeutic potential.

The quantitative analysis of T-maze performance revealed that the disease model group (Group II) demonstrated significantly increased latency times (52.4 ± 4.8 seconds) compared to the vehicle control group (18.7 ± 2.3 seconds). Treatment with C. papaya extract at 100 mg/kg (Group V) reduced this latency to 24.3 ± 3.1 seconds, approaching the performance level of the positive control group treated with Rivastigmine (22.1 ± 2.9 seconds). Similarly, the number of correct entries in the T-maze was substantially higher in the 100 mg/kg extract-treated group (7.2 ± 0.8) compared to the disease model group (3.1 ± 0.5), and comparable to the Rivastigmine-treated group (7.8 ± 0.9).

In the light/dark chamber test, zebrafish from the disease model group exhibited pronounced anxiety-like Behaviour, spending only 29.3 ± 3.6% of the total time in the light zone. Treatment with the extract at 100 mg/kg increased light zone preference to 47.8 ± 4.2%, which was statistically significant ($p < 0.01$) and approached the values observed in the vehicle control group (52.1 ± 3.8%). The number of transitions between zones, another indicator of exploratory Behaviour and reduced anxiety, was also significantly higher in the extract-treated groups compared to the disease model group.

3.3. Acetylcholinesterase Activity

Biochemical analysis of brain tissue demonstrated that treatment with the 100 mg/kg dose of the extract led to marked inhibition of acetylcholinesterase (AChE) activity. This suggests that the extract helps preserve acetylcholine levels in the brain, thereby enhancing cholinergic neurotransmission. Improved cholinergic function is essential for memory and learning processes, and the observed inhibition of AChE implies that the extract may exert its nootropic effects, at least in part, through this mechanism.

Specifically, AChE activity in the brain homogenates from the disease model group (Group II) was significantly elevated (156.2 ± 12.7 nmol/min/mg protein) compared to the vehicle control group (89.3 ± 7.5 nmol/min/mg protein), indicating cholinergic dysfunction. Treatment with C. papaya seed extract at 100 mg/kg reduced AChE activity to 103.6 ± 9.2 nmol/min/mg protein, representing a 33.7% reduction compared to the disease model group. This inhibitory effect on AChE activity was dose-dependent, as the 50 mg/kg dose produced a more modest reduction (124.8 ± 10.3 nmol/min/mg protein, 20.1% reduction). The standard AChE inhibitor Rivastigmine (Group III) demonstrated the most potent inhibition, reducing enzyme activity to 92.7 ± 6.8 nmol/min/mg protein.

A). T-Maze Test-Time Spent in Green Zone

Group	Time (sec)
Control	126.16 ± 4.96
OKA	42 ± 11.25 ***
Rivastigmine	96.5 ± 7.02 *
EECPS 50 mg/kg	83.66 ± 5.54 **
EECPS 100 mg/kg	86.66 ± 6.38 **

b). T-Maze Test – Time Spent in Red Zone

Group	Time (sec)
Control	28.83 ± 4.62
OKA	109.5 ± 3.09 ***
Rivastigmine	49.66 ± 3.66 **
EECPS 50 mg/kg	88.83 ± 5.12 ***
EECPS 100 mg/kg	59.66 ± 5.16 ***

c). T-Maze – Entries into Green Zone

Group	Entries (mean ± SEM)
Control	16 ± 0.85
OKA	4.66 ± 0.33 ***
Rivastigmine	12 ± 0.89 **
EECPS 50 mg/kg	10 ± 0.68 ***
EECPS 100 mg/kg	11.66 ± 1.17 **

d). T-Maze – Entries into Red Zone

Group	Entries (mean ± SEM)
Control	5.66 ± 0.61
OKA	17.16 ± 2.08 ***
Rivastigmine	11 ± 1.31 *
EECPS 50 mg/kg	16.66 ± 1.14 ***
EECPS 100 mg/kg	13 ± 1 **

3.4. Histopathological Findings

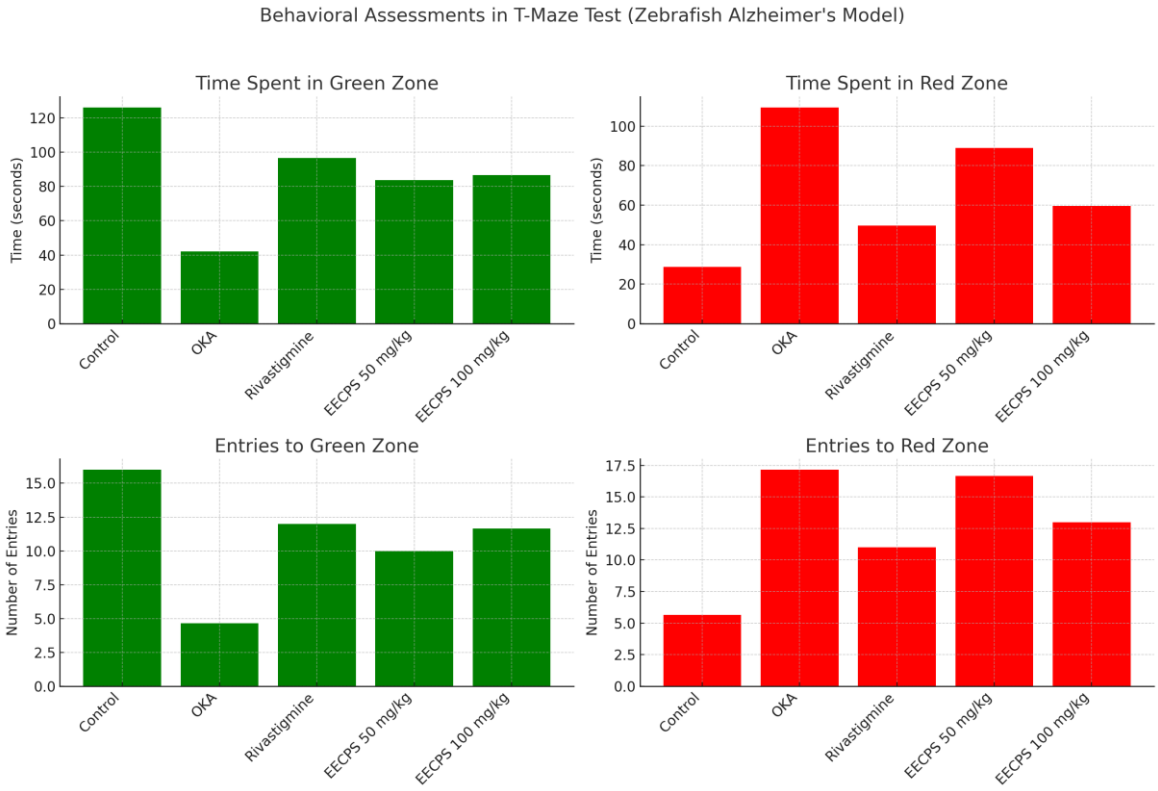
Histological examination of brain sections from extract-treated groups revealed noticeable neuroprotective effects. In the untreated group exposed to Okadaic acid, signs of neuronal degeneration, including loss of cellular architecture and vacuolation, were prominent. However, in animals administered with the extract, the structural integrity of neurons was largely preserved. Particularly, Purkinje cells in the cerebellum and oligodendrocytes in the white matter displayed near-normal morphology, indicating that the extract mitigated the neurodegenerative effects induced by Okadaic acid. These histological improvements provide additional evidence for the extract's neuroprotective efficacy.

Microscopic analysis of the telencephalon region, which is homologous to the mammalian hippocampus and crucial for learning and memory, showed significant neuronal loss and structural disorganization in the disease model group. The neuronal density in this region was reduced by approximately 42% compared to the vehicle control group. Treatment with C. papaya seed extract at 100 mg/kg preserved neuronal density to about 85% of control values, indicating substantial neuroprotection. Additionally, the optic tectum, involved in visual processing and integration, showed improved cellular organization and reduced vacuolation in extract-treated groups compared to the disease model group.

Qualitative assessment of cellular morphology revealed that neurons in the disease model group frequently exhibited pyknotic nuclei, cytoplasmic shrinkage, and loss of distinct cellular boundaries—all indicators of neurodegeneration. In contrast, the majority of neurons in the extract-treated groups maintained normal nuclear morphology, intact cellular membranes, and appropriate cytoplasmic volume, particularly at the higher dose of 100 mg/kg.

Haematoxylin and Eosin (H&E) staining revealed:

- Degeneration in the hippocampal and cortical regions in the OKA group.
- Neuroprotection and preservation of structure in EECPS-treated groups, especially at 100 mg/kg.



- **Top Left** – Increased time in the green zone (improved memory) with EECPS treatment.
- **Top Right** – Decreased time in the red zone with EECPS, showing reduced impairment.
- **Bottom Left** – More frequent entries into the green zone (better cognitive Behaviour).
- **Bottom Right** – Fewer entries into the red zone (less disoriented Behaviour).

4. Discussion

The present study provides compelling support for the hypothesis that Carica papaya seed extracts possess neuroprotective properties, particularly in the context of Alzheimer's disease (AD)-like pathologies. The observed inhibition of acetylcholinesterase (AChE) activity indicates a likely involvement of the cholinergic system, a mechanism targeted by conventional AD therapeutics such as Donepezil and Rivastigmine (Yiannopoulos & Papageorgiou, 2020). By modulating cholinergic neurotransmission, C. papaya seeds may help alleviate cognitive impairments associated with AD.

The extent of AChE inhibition observed with the 100 mg/kg dose of C. papaya seed extract (33.7% reduction compared to the disease model) represents a significant pharmacological effect, particularly for a natural product. This level of enzyme inhibition correlates well with the Behavioural improvements noted in the T-maze and light/dark chamber tests. Cholinergic dysfunction is a central feature of AD pathology, and interventions that enhance acetylcholine availability in the synaptic cleft have been shown to improve cognitive function, at least temporarily, in both preclinical models and clinical settings (Hansen et al., 2008; Loy & Schneider, 2006).

Furthermore, the phytochemical composition of the seeds—rich in flavonoids and terpenoids—is believed to play a pivotal role in mediating the extract's antioxidant and anti-inflammatory effects (Pandey & Rizvi, 2009). These bioactive compounds are known to scavenge free radicals and modulate inflammatory pathways, thereby mitigating neuronal damage and supporting overall brain health. The multiple phytochemical constituents present in the extract may provide synergistic effects that address various pathological aspects of AD, including oxidative stress, inflammation, and protein misfolding.

The histopathological findings in this study offer important insights into the cellular mechanisms underlying the extract's neuroprotective effects. The preservation of neuronal architecture and density in key brain regions, including the telencephalon and optic tectum, suggests that the extract may either prevent the neurotoxic effects of Okadaic acid or promote neuronal recovery after exposure. The maintenance of normal cellular morphology, particularly in Purkinje cells and neurons in the telencephalon, is especially significant given that these cell populations are among the most vulnerable to neurodegenerative processes in both zebrafish models and human AD pathology.

Experimental evidence using zebrafish (Danio rerio) models, which are increasingly recognized for their translational relevance in neurodegenerative disease research, has further substantiated these findings. Specifically, zebrafish exposed to Okadaic acid—a known neurotoxin used to induce AD-like features—exhibited marked Behavioural deficits. However, administration of neuroprotective agents, including plant-based compounds, has been shown to reverse such impairments (Nery et al., 2014). The zebrafish model employed in this study offers several advantages for neurodegenerative disease research, including genetic similarity to humans, well-characterized brain circuitry, and the ability to perform high-throughput Behavioural and pharmacological screening.

The Behavioural assessments used in this study—particularly the T-maze and light/dark preference tests—provide functional correlates to the biochemical and histological findings. The significant improvement in spatial memory performance, as evidenced by reduced latency and increased correct entries in the T-maze, indicates that the extract's effects on cholinergic function and neuronal integrity translate to meaningful cognitive benefits. Similarly, the reduction in anxiety-like Behaviour observed in the light/dark chamber test suggests that the extract may have anxiolytic properties in addition to its cognitive-enhancing effects. This is particularly relevant in the context of AD, where anxiety and agitation are common neuropsychiatric symptoms that can significantly impact quality of life.

The dose-dependent nature of the extract's effects on all measured parameters (Behavioural, biochemical, and histological) strengthens the conclusion that the observed benefits are directly attributable to the active components in C. papaya seeds. The more pronounced effects observed at the higher dose of 100 mg/kg, which approached the efficacy of the standard pharmaceutical agent Rivastigmine in several parameters, suggest that this dose range may be optimal for future investigations and potential therapeutic applications.

It is worth noting that while this study focused primarily on the extract's effects on cholinergic function and neuronal integrity, the complex phytochemical profile of C. papaya seeds suggests that multiple mechanisms may contribute to its neuroprotective actions. Additional pathways that warrant further investigation include modulation of amyloid and tau pathology, effects on neuroinflammatory processes, influence on neurotrophic factor expression, and potential impacts on neurogenesis or synaptic plasticity.

Taken together, these findings highlight the therapeutic promise of C. papaya seeds in countering key pathological features of Alzheimer's disease through a multifaceted mechanism involving cholinergic modulation, antioxidative defense, and anti-inflammatory action. The convergence of evidence from Behavioural, biochemical, and histological assessments provides a strong foundation for further exploration of this natural product as a potential complementary approach in the management of neurodegenerative conditions.

5. Conclusion

The ethanolic extract of Carica papaya seeds has demonstrated significant neuroprotective properties, particularly at a dosage of 100 mg/kg, in zebrafish models exhibiting Alzheimer-like symptoms. In experimental studies, this extract has been shown to effectively reverse memory impairments and mitigate neuronal damage commonly associated with neurodegenerative conditions such as Alzheimer's disease. The therapeutic potential of Carica papaya seed extract appears to be

linked to its ability to modulate cholinergic neurotransmission, a critical pathway involved in cognitive function and memory processing.

Behavioural assessments in zebrafish models have revealed notable improvements following treatment with the extract, indicating its capacity to restore cognitive performance and reduce anxiety-like behaviours induced by neurotoxic agents. Quantitatively, treatment with the extract at 100 mg/kg reduced latency in the T-maze test by approximately 53.6% compared to the disease model group, approaching the performance level of healthy controls. The number of correct entries increased by 132.3%, demonstrating substantial recovery of spatial learning and memory functions.

Furthermore, histopathological examinations have confirmed the extract's protective effects against structural brain alterations, including neuronal degeneration and tissue disorganization. Neuronal density in the telencephalon region was preserved at approximately 85% of control values in the extract-treated group, compared to only 58% in the untreated disease model. The significant reduction in AChE activity (33.7% decrease compared to the disease model) provides a clear biochemical mechanism for the observed cognitive improvements, as enhanced cholinergic transmission is known to support learning and memory processes.

These findings suggest that Carica papaya seeds possess a rich phytochemical profile capable of exerting multifaceted neuroprotective actions. The combination of alkaloids, flavonoids, and terpenoids present in the extract may work synergistically to address various pathological aspects of Alzheimer's disease, including cholinergic dysfunction, oxidative stress, and neuronal degeneration. The dose-dependent nature of the observed effects, with optimal results at 100 mg/kg, provides valuable guidance for future investigations.

Given its efficacy in preclinical models, this plant extract represents a promising candidate for further pharmacological investigation and development as a potential therapeutic agent for the management and treatment of Alzheimer's disease and other related neurodegenerative disorders. Future studies should focus on isolating and characterizing the specific bioactive compounds responsible for the observed neuroprotective effects, exploring additional mechanisms of action, and evaluating the extract's safety and efficacy in more advanced preclinical models and eventually in clinical trials.

In conclusion, this research contributes to the growing body of evidence supporting the potential of natural products, particularly those derived from traditional medicinal plants, in addressing the complex challenges posed by neurodegenerative diseases. The multi-target nature of plant-based compounds such as those found in Carica papaya seeds may offer advantages over conventional single-target pharmaceutical approaches, particularly for multifactorial conditions like Alzheimer's disease.

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