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A SYSTEMATIC REVIEW OF *Azadirachta indica* FOR THE DEVELOPMENT OF AN IN-VITRO MODEL FOCUSING ON ALZHEIMER'S DISEASE

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ABSTRACT

The study focuses on the A SYSTEMATIC REVIEW OF *Azadirachta indica* FOR THE DEVELOPMENT OF AN IN-VITRO MODEL FOCUSING ON ALZHEIMER'S DISEASE. Alzheimer's disease is a progressive neurodegenerative disorder with limited effective treatments. Medicinal plants are being explored as alternative sources of neuroprotective agents. *Azadirachta indica* (Neem) is known for its antioxidant and anti-inflammatory properties, which may be beneficial in neurodegenerative conditions. This systematic review aims to analyze studies related to *Azadirachta indica* with relevance to Alzheimer's disease, focusing on information useful for the development of an in-vitro model. Published literature was reviewed to identify experimental approaches, biological targets, and cellular responses associated with Alzheimer's disease mechanisms. The review provides a structured summary of available evidence to support the design of in-vitro studies involving *Azadirachta indica*. This work may serve as a basic framework for future experimental research in Alzheimer's disease.

KEYWORDS: *Azadirachta indica*, Alzheimer's disease, Anti Alzhiemer's activity.

INTRODUCTION

Alzheimer's disease is a chronic, progressive neurological disorder that affects the brain and gradually destroys memory, thinking skills, and the ability to perform even the simplest daily tasks. It is the most common cause of dementia, contributing to approximately 60–80% of dementia cases worldwide.[29] Dementia is not a single disease but a general term used to describe a decline in cognitive function severe enough to interfere with daily life. Among all

types of dementia, Alzheimer's disease is the most widely recognized and studied due to its increasing prevalence and significant impact on individuals, families, and healthcare systems.[3]

The disease primarily affects older adults, typically those aged 65 and above. However, it is important to note that Alzheimer's is not a normal part of aging.[12] While aging increases the risk, the disease results from specific changes in the brain that go beyond typical age-related memory decline. In some cases, Alzheimer's can occur in younger individuals, known as early-onset Alzheimer's disease, which usually affects people under the age of 65. Although less common, early-onset Alzheimer's can be particularly challenging because it often affects individuals who are still working, raising families, and actively engaged in society.

Alzheimer's disease was first described in 1906 by German psychiatrist and neurologist Dr. Alois Alzheimer. He observed unusual changes in the brain of a woman who had died after experiencing severe memory loss, language difficulties, and unpredictable behavior. Upon examining her brain, he discovered abnormal clumps and tangled fibers, which are now known as amyloid plaques and neurofibrillary tangles.[1] These findings laid the foundation for understanding the biological basis of the disease, and since then, Alzheimer's has become a major focus of medical and scientific research.

One of the defining features of Alzheimer's disease is the accumulation of abnormal protein deposits in the brain. Beta-amyloid plaques form outside brain cells and interfere with communication between neurons, while tau tangles develop inside brain cells and disrupt the transport system that delivers nutrients and essential substances.[2] These abnormalities lead to the breakdown of neural connections and ultimately cause the death of brain cells. As the disease progresses, the brain shrinks significantly, particularly in regions responsible for memory, reasoning, and language.

The progression of Alzheimer's disease is typically divided into several stages, ranging from mild cognitive impairment to severe dementia.[9] In the early stages, individuals may experience subtle memory problems, such as forgetting recent conversations, misplacing items, or having difficulty finding the right words. These symptoms are often mild and may be mistaken for normal aging. However, as the disease advances, the symptoms become more noticeable and begin to interfere with daily activities.

In the middle stages of Alzheimer's disease, individuals may develop confusion, disorientation, and difficulty recognizing familiar people or places. They may struggle with tasks such as managing finances, traveling independently, or following instructions. Behavioral and psychological symptoms, including mood swings, anxiety, irritability, and depression, may also become more prominent. These changes can be distressing not only for the individual but also for family members and caregivers.

In the late stages of the disease, the impact becomes severe and debilitating. Individuals lose the ability to communicate effectively, require assistance with basic activities such as eating, dressing, and bathing, and may experience significant physical decline.[12]. Functions such as walking, swallowing, and bladder control may also be affected. At this stage, full-time care and support are essential, and the burden on caregivers becomes extremely high.

Alzheimer's disease affects more than just memory; it has a wide range of cognitive, behavioral, and physical effects. Memory loss is often the earliest and most noticeable symptom, particularly short-term memory loss. Individuals may repeatedly ask the same questions, forget recent events, or have difficulty remembering names and faces. As the disease

progresses, long-term memory is also affected, and individuals may forget important life events or fail to recognize close family members.

In addition to memory loss, Alzheimer's disease impairs reasoning and decision-making abilities.[11] Individuals may find it difficult to plan activities, solve problems, or make judgments. Simple tasks such as cooking, following a recipe, or managing daily schedules can become challenging. Language difficulties are also common, with individuals struggling to find the right words, follow conversations, or understand written and spoken language.[27]

Another significant aspect of Alzheimer's disease is the change in behavior and personality. Individuals may become more withdrawn, suspicious, or anxious. They may experience mood swings, agitation, or even hallucinations and paranoia in advanced stages. These changes can be difficult for loved ones to understand and manage, often leading to emotional stress and frustration.

Spatial awareness and coordination may also be affected in individuals with Alzheimer's disease. They may have difficulty judging distances, recognizing objects, or navigating familiar environments. This can increase the risk of falls, accidents, and getting lost, even in places they have known for years. As a result, safety becomes a major concern, and constant supervision may be required.

Several risk factors contribute to the development of Alzheimer's disease. Age is the most significant factor, with the risk increasing as a person grows older.[13] Genetics also play an important role, particularly in cases of early-onset Alzheimer's. Certain genes, such as the APOE-e4 gene, have been linked to a higher risk of developing the disease. Family history, therefore, can be an important indicator of susceptibility.[8]

Lifestyle and environmental factors are also believed to influence the risk of Alzheimer's disease. Conditions such as high blood pressure, diabetes, obesity, and heart disease can increase the likelihood of developing cognitive decline. Unhealthy habits such as smoking, lack of physical activity, poor diet, and limited mental stimulation may also contribute to brain changes associated with Alzheimer's. On the other hand, maintaining a healthy lifestyle, engaging in regular physical exercise, staying mentally active, and having strong social connections may help reduce the risk.

Diagnosing Alzheimer's disease involves a comprehensive evaluation that includes medical history, physical examination, cognitive and neurological tests, and brain imaging techniques. Doctors may also use laboratory tests to rule out other possible causes of memory loss, such as vitamin deficiencies or thyroid disorders. Early diagnosis is crucial because it allows individuals and their families to plan for the future, seek appropriate treatment, and make lifestyle changes that may help slow the progression of the disease.

Although there is currently no cure for Alzheimer's disease, various treatments are available to help manage symptoms and improve quality of life. Medications can help regulate brain chemicals involved in memory and thinking, while non-drug therapies such as cognitive stimulation, physical activity, and behavioral interventions can provide additional support. Caregiver education and support are also essential components of effective management, as caregivers play a vital role in the well-being of individuals with Alzheimer's.

The impact of Alzheimer's disease extends beyond the individual to affect families, caregivers, and society as a whole. Caring for someone with Alzheimer's can be physically, emotionally, and financially demanding. Caregivers often

experience stress, fatigue, and burnout, highlighting the need for support systems and healthcare resources. On a larger scale, the growing number of Alzheimer's cases places a significant burden on healthcare systems and economies worldwide.[29]

In conclusion, Alzheimer's disease is a complex and challenging condition that requires increased awareness, understanding, and research. Its progressive nature and widespread impact make it one of the most important public health issues of our time. By studying its stages, signs, and symptoms, as well as its risk factors and management strategies, we can better understand the disease and work toward improving the lives of those affected by it. This project aims to provide a comprehensive overview of Alzheimer's disease, focusing on its progression, characteristics, and the challenges it presents to individuals and society.

Taxonomic history of Azadirachta indica

Azadirachta indica, commonly known as neem, is a highly valued medicinal and evergreen tree widely distributed in tropical and subtropical regions. The scientific description of this plant was first provided in 1830 by the French botanist **Adrien-Henri de Jussieu**, whose abbreviation "A. Juss." is used as the species authority. Before its formal classification, the plant had already been recognized for centuries in traditional systems of medicine such as Ayurveda, Unani, and Siddha.[5,6]

Initially, *Azadirachta indica* was grouped under the genus *Melia* due to similarities in morphological features such as compound leaves and general tree structure. Early botanists referred to it as *Melia azadirachta*, reflecting its close resemblance to species like *Melia azedarach* (chinaberry tree). However, as botanical science advanced, taxonomists began to notice significant differences in reproductive structures, fruit characteristics, and chemical composition.

The separation of *Azadirachta* from *Melia* was based on detailed morphological and anatomical studies. Key distinguishing features included differences in leaf arrangement, the structure of flowers, and the nature of the fruit and seeds. For example, neem produces smooth, olive-like drupes, whereas *Melia* species typically bear more segmented fruits. Additionally, the presence of unique bioactive compounds such as azadirachtin in neem further supported its classification as a distinct genus.[5,14]

The name *Azadirachta* is derived from the Persian phrase **"Azad Dirakht,"** meaning "free tree" or "noble tree," reflecting its importance and widespread use in traditional cultures. The species name *indica* indicates its origin in the Indian subcontinent, where the tree has been cultivated and revered for thousands of years.[5]

From a taxonomic perspective, *Azadirachta indica* is classified as follows:

- **Kingdom**: Plantae
- **Subkingdom**: Tracheobionta (vascular plants)
- **Superdivision**: Spermatophyta (seed plants)
- **Division**: Magnoliophyta (flowering plants)
- **Class**: Magnoliopsida (dicotyledons)
- **Order**: Sapindales
- **Family**: Meliaceae
- **Genus**: *Azadirachta*
- **Species**: *Azadirachta indica* A. Juss.

The family Meliaceae, commonly known as the mahogany family, includes economically important trees such as mahogany (*Swietenia*) and cedar (*Cedrela*). Members of this family are typically characterized by pinnate leaves, small fragrant flowers, and woody fruits.

Historically, neem has been extensively documented in ancient Indian texts for its medicinal and agricultural uses. Sanskrit literature refers to it as "Nimba," and it has been described as a "sarva roga nivarini," meaning "the curer of all diseases." Ancient practitioners utilized various parts of the tree—including leaves, bark, seeds, and oil—for treating infections, skin disorders, and other ailments.[5,6]

During the colonial period, European scientists and botanists became interested in neem due to its wide range of applications. This led to further scientific investigations into its chemical constituents and biological properties. In the 20th century, neem gained global attention for its natural pesticidal properties, particularly due to the discovery of azadirachtin, a powerful insect growth regulator.[5,14]

Modern taxonomic studies, including molecular and phylogenetic analyses, have confirmed the distinct position of *Azadirachta indica* within the Meliaceae family. These studies have also helped clarify its evolutionary relationships with other genera in the family, reinforcing its classification as a unique and important species.[14]

In conclusion, the taxonomy of *Azadirachta indica* has evolved over time through careful observation, comparison, and scientific advancement. From its initial misclassification under *Melia* to its recognition as a separate genus, neem's taxonomic history reflects the progress of botanical science. Today, it is universally accepted as *Azadirachta indica* A. Juss., a species of immense ecological, medicinal, and economic significance.[14]

MATERIALS AND METHODS

Chemicals and reagents

The leaves of *Azadirachta indica* were collected from the malappuram district, kerala, India, during the month of October 2025. The plant materials were identified and authenticated by sree neelakanda govt Sanskrit college, pattambi, Palakkad, kerala-679306. Voucher specimens were kept in our laboratory for future reference.

The chemicals and reagents used in the present study include α-naphthol, concentrated sulphuric acid, Fehling's solution A and B, Benedict's reagent, Barfoed's reagent, Dragendorff's reagent, Wagner's reagent, Mayer's reagent, Hager's reagent, chloroform, acetic anhydride, pyridine, sodium nitroprusside, sodium picrate, dilute sulphuric acid, ether, ammonia, acetic acid, ferric chloride, distilled water, magnesium turnings, hydrochloric acid, tin granules, thionyl chloride, 5% ferric chloride solution, lead acetate solution, gelatin solution, bromine water, dilute iodine solution, dilute nitric acid, ethanol (95%), chloroform water (0.1%) and gum acacia,MTT reagent (5 mg/ml), and dimethyl sulfoxide(DMSO).

1. COLLECTION OF THE PLANT

Fresh leaves of Azadirachta indica (5kg) were collected and shade-dried to remove moisture.

2. PHYSICOCHEMICAL EVALUATION

Loss on drying

Loss on drying is the loss of mass expressed as percent w/w, the prescribed quantity of the fresh plant materials of the two plants were shade dried and the percentage of loss on drying the fresh plant materials was calculated.

Determination of foreign matter

Approximately 100g of the dried plant material was spread in a thin layer and examined macroscopically for the presence of foreign materials, including molds, insects, and other animal contaminants. Any foreign matter was separated and weighed to calculate the percentage of contamination relative to the initial sample weight.

Determination of moisture content

An accurately weighed quantity of the sample was placed in a tared evaporating dish and dried in an oven at 105ºC for a duration of five hours. The sample was weighed until a constant weight was achieved, and the percentage of volatile matter was calculated based on the weight loss.

Determination of Total ash value

To determine the total ash content, 2g of the ground air-dried plant material was accurately weighed into a previously ignited and tared crucible. The sample was spread in an even layer and ignited by gradually increasing the heat to 500-600º C until it became white, indicating the absence of carbon. For samples where carbon-free ash could not be obtained, the residue was moistened with a saturated solution of ammonium nitrate before final ignition. The crucible was cooled in a desiccator, weighed, and the total ash percentage was calculated relative to the air-dried material.

Determination of acid insoluble ash value

The acid-insoluble ash was determined by boiling the total ash residue with 25ml of dilute hydrochloric acid for five minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited in the original crucible until a constant weight was reached. This value was used to estimate the amount of silica and siliceous earth present in the sample.

Determination of Extractive Values

The extractive values were determined to estimate the amount of active constituents soluble in specific solvents. For the alcohol-soluble extractive, 5g of the air-dried drug was macerated with 100ml of alcohol in a closed flask for 24 hours, followed by filtration and evaporation of the filtrate to dryness. A similar procedure was followed for the water-soluble extractive using 0.1% chloroform water as the solvent. The percentage of each extractive was then calculated based on the weight of the air-dried drug.

3. PREPARATION OF EXTRACTS

Plant material and extraction

The granulated dried leaves of *Azadirachta indica* was packed in a Soxhlet apparatus and subjected to continuous hot percolation for using 450 ml of ethanol (95 % v/v) as solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 75 g, 15 % w/w). The extract was suspended in 5 % gum acacia and used for further experiments.

The ethanolic extract of the dried leaves of *Azadirachta indica* were stored in air-tight containers and stored in desicators.[5]

4. PRELIMINARY PHYTOCHEMICAL SCREENING

The alcoholic extract of the leaves of *Azadirachta indica* were screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc.

Test for carbohydrates

1. **Molisch Test:** It consisted of treating the compounds of α-naphthol and concentrated sulphuric acid along the sides of the test tube. Purple color or reddish violet color was produced at the junction between two liquids.
2. **Fehling's Test:** Equal quantity of Fehling's solution A and B were added. Heated gently, brick red precipitate was obtained.
3. **Benedict's test:** To the 5ml of Benedict's reagent, were added 8 drops of solution under examination. Mixed well, the mixture was boiled vigorously for two minutes and then cooled. Red precipitate was obtained.
4. **Barfoed's test:** To the 5ml of the Barfoed's solution added 0.5ml of solution under examination, heated to boiling, red precipitate of copper oxide was obtained.

Test for Alkaloids

1. **Dragendroff's Test:** To the extract, 1ml of Dragendroff's reagent was added Orange red precipitate was produced.
2. **Wagner's test:** To the extract Wagner reagent was added. Reddish brown precipitate was produced.
3. **Mayer's Test:** To the extract 1ml or 2ml of Mayer's reagent was added. Dull white precipitate was produced.
4. **Hager's Test:** To the extract 3ml of Hager's reagent was added. Yellow precipitate was produced.

Test for Steroids and Sterols

1. **Liebermann Burchard test:** The test sample was dissolved in 2ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.
2. **Salkowski test:** The sample of test solution was dissolved in chloroform and equal volume of conc. sulphuric acid was added. Bluish red, cherry red and purple color is noted in chloroform layer, whereas acid assumed marked green fluorescence.

Test for Glycosides

1. **Legal's test:** Sample was dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red color was produced.
2. **Baljet test:** To the drug sample, sodium picrate solution was added. Yellow to orange color was produced.
3. **Borntrager test:** Few ml of dilute sulphuric acid was added to the test solution. Boiled filtered and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet color was produced in organic layer.
4. **Killer Killani test:** Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color was produced which gradually became blue.

Test for Saponins

Foam test: About 1ml of alcoholic sample was diluted separately with distilled water to 20ml and shaken in graduated cylinder for 15 minutes.1 cm layer of foam indicated the presence of saponins.

6.7.6 Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid were added. Red color was produced.

Test for Tri-terpenoids:

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution was added. Pink color was produced which indicated the presence of triterpenoids.

Tests for Tannins and Phenolic Compounds

The Phenol content in the raw material of two plants extracts was estimated spectroscopically.

To 2-3 ml of extract, add few drops of following reagents were added

a) 5% $FeCl_3$ solution : Deep blue-black color.
b) Lead acetate solution : White precipitate.
c) Gelatin solution : White precipitate
d) Bromine water : Decoloration of bromine water.
e) Acetic acid solution : Red color solution
f) Dilute iodine solution : Transient red color.
g) Dilute HNO_3 : Reddish to yellow color.

5. IN-VITRO SCREENING

MTT assay

The MTT assay is a colorimetric assay for measuring cell metabolic activity by detecting the conversion of yellow tetrazolium salt (MTT) to purple formazan crystal by mitochondrial enzymes in metabolically active cells.[15,24] It is to assess cell viability, proliferation, and cytotoxicity in research. It measures the reduction of a yellow tetrazolium salt (MTT) to purple formazan crystals by mitochondrial enzymes in metabolically active cells.

Cell culture and treatment

The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. PC12 and N2a cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin. For the experiments, cells were seeded into 96-well and 24-well culture plates for MTT/ROS and MDA assays, respectively. For apoptosis studies, cells were seeded at a density of 1×10^5 cells/well in 24-well plates. All treatments were carried out in triplicate.

Cells were pretreated with ethanolic extract of *Azadirachta indica* leaves (25–200 µg/ml) for 2 hours. Following pretreatment, the cells were incubated for 24 hours in the presence of the extract with or without amyloid-β peptide, which was used as the inducer of neurotoxicity.

Cell viability assay

Cell viability was assessed using the MTT assay.[15] MTT reagent was prepared in phosphate-buffered saline (5 mg/ml) and added to each well at a final concentration of 0.05%. After incubation for 3 hours at 37 °C, the formed formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm with 620 nm as background using a Stat FAX 303 plate reader. Cell viability was expressed as a percentage relative to control cells.

Formula

Percentage cell viability(%)=(Test OD/Control OD)×100

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL PARAMETERS OF THE PLANT

Table 1: physico-chemical parameters (after shade drying) of the plant.

S. NO	Name Of the plant	Foreign matter	Moisture content	Total Ash	Acid insoluble ash	EtOH Soluble Extractive value	Water soluble Extractive value
1.	Azadirachta indica	1.2%	8%	7%	1.3%	10%	15%

The preliminary phytochemical analysis of fraction of *Azadirachta indica* showed presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate.

PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical analysis of fraction of *Azadirachta indica* showed presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate.

Table 2: Phytochemical screening of ethanolic extract of *Azadirechta indiga.*

Constituents	Test	*Azadirachta indica*
Carbohydrates	Molisch Test Fehling's Test Benedict's test: Barfoed's test:	+ + + –
Alkaloids	Dragendroff's Test Wagner's test Mayer's Test Hager's Test	+ + + +
Steroids and Sterols	Liebermann Burchard test Salkowski test	- +
Glycosides	Legal's test Baljet test Borntrager test Killer Killani test	+ + – +
Saponins	Foam test	+
Flavonoids	Shinoda test	+
Tri-terpenoids	In the test tube, 2 or 3 granules of tin+2ml of thionyl chloride solution and test solutionis added.➔Pink color	+

+ Present, - Absent

Phytochemical constituents of the various extracts

The extracts of the parts of the two plants were subjected to the phytochemical studies. They revealed the presence of the following constituents:

A.indica: The Alcoholic extract revealed the presence of glycosides, tannins, steroids ,flavonoids and carbohydrates

MTT ASSAY

Neuroprotective effect of *Azadirachta indica* against amyloid-β-induced cytotoxicity

To evaluate the neuroprotective effect of *Azadirachta indica* extract, PC12 and N2a cells were treated with different concentrations (6–200 µg/ml), and cell viability was assessed. Treatment with the extract alone did not show any significant cytotoxic effect, indicating that it was safe for both cell lines.

Exposure to amyloid-β significantly reduced cell viability compared to control cells ($p < 0.001$), confirming amyloid-β–induced cytotoxicity. Pre-treatment with *Azadirachta indica* extract significantly increased cell viability in a concentration-dependent manner in PC12 cells when compared to the amyloid-β–treated group ($p < 0.05$–0.001).

Similarly, in N2a cells, the extract effectively attenuated amyloid-β–induced cell death, as demonstrated by a significant improvement in cell viability at higher concentrations ($p < 0.01$–0.001).

These findings suggest that *Azadirachta indica* extract exhibits significant neuroprotective activity against amyloid-β–induced neuronal damage in PC12 and N2a cell lines.

Table 3: Effect of *Azadirachta indica* leaf extract on amyloid-β induced cytotoxicity in PC12 cells using MTT assay.

Treatment	Concentration(µg/ml)	Cell viability(%± SEM)	significance
Control	-	100	-
amyloid-β	-	52.4 ± 1.2%	$p < 0.001$
Extract + amyloid-β	12 µg/ml	69.2 ± 2%	$p < 0.05$
Extract + amyloid-β	25 µg/ml	73.6 ± 2.9%	$p < 0.01$
Extract + amyloid-β	50 µg/ml	79.1± 3.1%	$p < 0.001$
Extract + amyloid-β	100 µg/ml	83.5 ± 3.8%	$p < 0.001$
Extract + amyloid-β	200 µg/ml	88.2 ± 3.0%	$p < 0.001$

Table 4: Effect of Azadirachta indica leaf extract on amyloid-β induced cytotoxicity in N2a cells using MTT assay.

Treatment	Concentration(µg/ml)	Cell viability(%± SEM)	significance
Control	-	100	-
amyloid-β	-	51.3 ± 2.9%	($p < 0.01$).
Extract + amyloid-β	25 µg/ml	67.9 ± 2.8%	($p < 0.05$)
Extract + amyloid-β	50 µg/ml	74.3 ± 2.4%	($p < 0.001$)
Extract + amyloid-β	100 µg/ml	81.6 ± 2.7%	($p < 0.001$)
Extract + amyloid-β	200 µg/ml	87.4± 3.1%	($p < 0.001$)

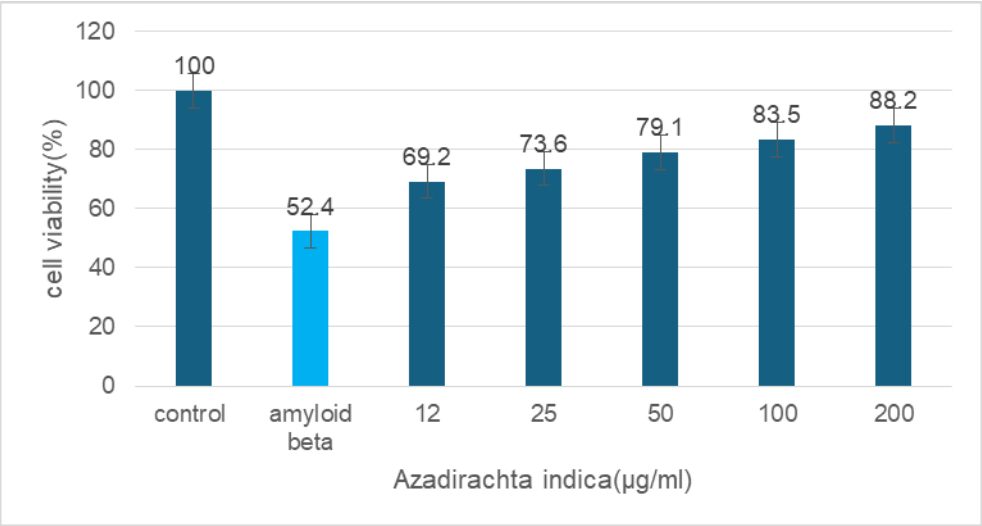

Figure 1: Effect of *Azadirachta indica* on cell viability of amyloid-β-injured PC12 cells. Cells were pretreated with different concentrations of the extract for 2 hours and then exposed to amyloid-β for 24 hours. Cell viability was quantified using the MTT assay.

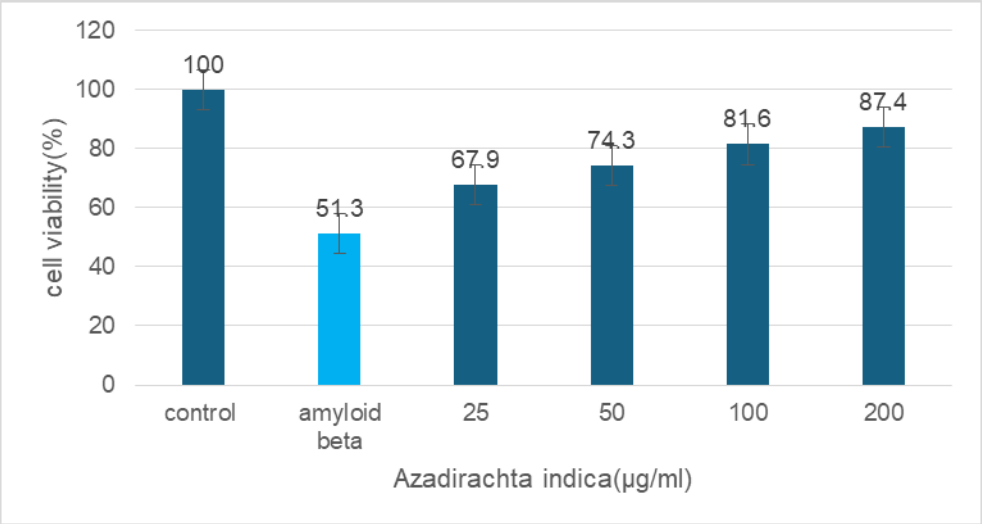

Figure 2: Effect of *Azadirachta indica* on cell viability of amyloid-β-injured N2a cells. Cells were pretreated with different concentrations of the extract for 2 hours and then exposed to amyloid-β for 24 hours. Cell viability was quantified using the MTT assay.

DISCUSSION

The present study demonstrates that *Azadirachta indica* exhibits significant neuroprotective effects against amyloid-β-induced cytotoxicity in PC12 and N2a cell lines in a dose-dependent manner. Amyloid-β is a key pathological factor in Alzheimer's disease and is known to induce neuronal damage through oxidative stress, mitochondrial dysfunction, and activation of apoptotic pathways.

PC12 and N2a cells are widely used neuronal models for studying Alzheimer's disease-related neurotoxicity. In the present investigation, amyloid-β exposure markedly reduced cell viability, confirming its neurotoxic potential. Pretreatment with *Azadirachta indica* extract significantly improved cell survival, suggesting its ability to counteract amyloid-β-mediated neuronal damage.

The neuroprotective effect of *Azadirachta indica* may be attributed to its rich phytochemical content, including flavonoids, limonoids, and phenolic compounds, which are known to possess antioxidant and anti-inflammatory properties.[14,18] By reducing oxidative stress and preventing neuronal apoptosis, *Azadirachta indica* may target multiple pathological pathways involved in Alzheimer's disease.

Overall, these findings support the potential application of *Azadirachta indica* in the development of in-vitro models for Alzheimer's disease and justify further mechanistic and in-vivo studies to validate its therapeutic relevance.

CONCLUSION

Alzheimer's disease is a complex and progressive neurodegenerative disorder characterized by multiple pathological mechanisms including β-amyloid plaque accumulation, tau hyperphosphorylation, cholinergic dysfunction, oxidative stress, mitochondrial impairment, neuroinflammation, and synaptic loss. Current pharmacological therapies provide only symptomatic relief and are associated with limited efficacy and adverse effects, highlighting the need for safer and more effective therapeutic alternatives.

The present systematic review evaluated the potential of *Azadirachta indica* (Neem) in the context of Alzheimer's disease, with particular emphasis on its relevance for the development of in-vitro experimental models. Evidence from the reviewed studies demonstrates that *Azadirachta indica* possesses significant antioxidant, anti-inflammatory, anti-apoptotic, and neuroprotective properties, largely attributed to its rich phytochemical profile, including flavonoids, limonoids, alkaloids, glycosides, and terpenoids. These bioactive constituents are shown to effectively reduce oxidative stress, modulate inflammatory mediators, improve mitochondrial function, and protect neuronal cells from excitotoxic and oxidative damage.

In-vitro studies reviewed in this work further support the ability of *Azadirachta indica* extracts and isolated compounds such as nimbolide to enhance neuronal cell viability, reduce reactive oxygen species production, and prevent apoptosis in neuronal cell lines. Such effects directly target key pathological pathways involved in Alzheimer's disease, making *Azadirachta indica* a promising candidate for cell-based neuroprotective assays.

Overall, this systematic review provides a structured scientific basis for the use of *Azadirachta indica* in the development of in-vitro models relevant to Alzheimer's disease research. The findings support its potential as a multitarget neuroprotective agent and justify further experimental validation using well-designed in-vitro and in-vivo studies. Future research focusing on standardized extracts, dose optimization, and molecular mechanisms will be essential to translate these findings into potential therapeutic applications for Alzheimer's disease.

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