Section: Miscellaneous

Original Research Article

A COMPARATIVE EVALUATION OF CITRUS LIMON, DONEPEZIL AND EZETIMIBE IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

Fardan Qadeer[1], Fariha Fatima[1], Afroz Abidi[2], Ahmad Raza[3], Abeer Zubair Khan[4]

[1]*Associate Professor, Department of Pharmacology, Era's Lucknow Medical College&Hospital, Sarfarazganj, Lucknow, Uttar Pradesh, India*
[2]*Professor and Head, Department of Pharmacology, Era's Lucknow Medical College&Hospital, Sarfarazganj, Lucknow, Uttar Pradesh, India*
[3]*Senior Resident, Department of Pathology, MPMMCC, Varanasi, Uttar Pradesh, India*
[4]*Professor, Department of Anatomy, Integral Institute of Medical College, Lucknow, Uttar Pradesh, India*

Received *: 30/10/2025*
Received in revised form *: 21/12/2025*
Accepted *: 12/01/2026*

Corresponding Author:
Dr. Fariha Fatima,
Associate Professor, Department of Pharmacology, Era's Lucknow Medical College & Hospital, Lucknow, Uttar Pradesh, India.
Email: drfarihafshikoh@gmail.com

DOI: 10.70034/ijmedph.2026.1.158

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (1); 893-899

ABSTRACT

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory impairment. Current treatments provide only symptomatic relief, necessitating the exploration of alternative therapeutic approaches. This study aims to evaluate the neuroprotective effects of Citrus limon (CL) in comparison to Donepezil (a cholinesterase inhibitor) and Ezetimibe (a cholesterol-lowering agent) in an animal model of AD.

Materials and Methods: Male Wistar rats (n=30) were divided into five groups: Control, AD (untreated), Donepezil-treated, Ezetimibe-treated, and CL-treated. AD was induced using AlCl3. Treatment was administered daily for six weeks. Behavioural assessments (Morris water maze and cook's pole climbing response test) and oxidative stress markers were assessed.

Results: CL significantly improved cognitive function, reduced Aβ plaque deposition, and decreased oxidative stress markers, showing effects comparable to Donepezil. Ezetimibe also demonstrated neuroprotective properties, indicating a possible role of cholesterol metabolism in AD pathology.

Conclusion: CL exhibits promising neuroprotective effects, potentially serving as an adjunct therapy for AD. Further clinical studies are warranted to explore its therapeutic potential.

Keywords: Alzheimer's disease, Citrus limon, Donepezil, Ezetimibe, Neuroprotection, Oxidative stress, Beta-amyloid.

INTRODUCTION

Alzheimer's disease (AD) is a most common neurodegenerative disease characterized by a progressive decline in cognitive function. It is a debilitating condition affecting elderly people towards the end of their life. It affects about 17-25 million elderly people around the globe accounting for approximately 70% of dementia.[1] The prevalence of AD increases exponentially from approximately 2% at the age of 60–65 years to more than 30%–35% in people aged greater than 80 years.[2] According to the India Ageing Report 2023, the elderly population, which is growing at a faster rate of three percent, may up the burden of Alzheimer's in India, as the disease primarily occurs in patients over the age of 60. It noted that dementia prevalence is estimated at 7.4% for population aged greater than 60 years, with 8.8 million Indians in this age group living with dementia. This number is only expected to triple by 2050.[3]

The disease often starts with mild symptoms progressing towards moderate disease and finally culminating in severe and irreparable brain damage. There is a characteristic progressive decline in memory, thinking, language skills and learning capacity. The condition gradually worsens and leads to increasing dependence and to an akinetic mute state of the sufferer which signifies an end stage neurological disease.[4,5] The pathophysiology of AD is directly related to the cholinergic loss of neurons beginning in the hippocampal region which is involved in memory and learning and progressing towards the dilatation of ventricles and shrinkage of cortex.

Current treatment is aimed at alleviating its symptoms only failing to target its cure. For cognitive symptoms: augmentation of cholinergic transmission is currently the mainstay of therapy. Drugs used for this purpose are Cholinesterase inhibitors like Donepezil, Rivastigmine and Galantamine which are being used fervently in mild to moderate disease.[6] Cholinesterase inhibitors cannot reverse AD and they cannot stop the underlying destruction of neuronal cells, because dwindling brain cells produce less ACh as disease progresses, these medications eventually lose their effectiveness, hence, there is an urgent need to find and introduce new agents in the therapy of the disease.[7] It is proposed that Ezetimibe plays a beneficial role in AD pathology due to its potent cholesterol lowering mechanisms. It has been demonstrated in various studies that cholesterol levels alter APP and Aβ levels.[7]

These findings raise the possibility that treating human subjects with cholesterol lowering medications might decrease the risk of developing AD. Several studies revealed that there are certain fruits which have shown to possess powerful neuroprotective property. One such fruit being Citrus limon which apart from helping in conditions like hypertension, hypercholesterolemia, oxidative stress, hyperglycaemia and inflammation and has also shown to suppress PGE2 production and COX-2 expression in IL-1β stimulated SK-N-SH neuronal cells in previous studies and it may be useful in preventing the development and progression of AD.[8,9]

As AD is largely becoming a global problem worldwide affecting a vast number of population and exerting a huge financial burden on the health care system with no definitive treatment, the present research has been undertook in lieu of finding a solution to the above problem by exploring herbal preparations as well as medications which could either help prevent the disease or cure the existing pathological state. Therefore, to the best of our knowledge no such research has been carried out to study the effect of Citrus limon in ameliorating the effect of neurodegeneration similar to AD like pathology.

Moreover, its comparison has yet not been done with Ezetimibe in an experimental model of AD. Hence, the purpose of this study was to investigate the neuroprotective effect of Citrus limon in an Aluminium Chloride model of AD.

MATERIALS AND METHODS

Animals: Male Wistar Rats (Rattus norvegicus) of weight: 150-200gms were included in the study. The animals were maintained in cages, under a temperature of 25 ± 2°C and 45-55% relative humidity, with a 12-hour light/dark cycle. They were allowed food and water ad libitum. Animals were obtained from CPCSEA-certified animal house (IITR, Lucknow).

All experiments were performed after approval from Institutional Animal Ethics Committee of Eras' Lucknow Medical College (Approval No: ELMC/PHAR/IAEC-11) as per the guidelines of Animal Care by CPCSEA.

Induction of AD: Aluminium chloride (AlCl3) was extensively utilized for the stimulation of dementia in numerous animal models. Aluminium is a well-recognized neurotoxin and concerned with the pathological progression of numerous neurologic. Aluminium can act as a cross-linkers of amyloid β-protein and results in oligomerization, thus stimulating neurotoxicity.[10]

Aluminium chloride was prepared in saline 0.9%, and was administered in a dose of 100 mg/kg body weight orally via oral gavage tube for 6 weeks.

Standard Drugs: Donepezil was purchased from the authorised college Pharmacy and was pulverized and dissolved in distilled water to administer to experimental animals via oral gavage tube at a dose of 0.5mg/kg body weight orally for 6 weeks.[11]

Ezetimibe was purchased from the authorised college Pharmacy and was pulverized and dissolved in distilled water to administer to experimental animals via oral gavage tube at a dose of experimental animals at a dose of 1mg/kg/day.

Test drug: Citrus limon (lemon) was procured from the local market-Lucknow, Uttar Pradesh, and was authenticated by a botanist at NBRI, Lucknow. Dose (100mg/kg, p.o.) was given according to the previous studies.[12]

Method of preparation of Citrus limon peel Extract

The powdered peel of plant material (1900 g) was macerated and extracted in ethanol at room temperature (24 ± 3°C). The solvent was removed under vacuum conditions at temperatures below 40°C. The subsequent crude ethanol extract (EE; 13% yield) was stored at –18°C. It was then dissolved in distilled water overnight at room temperature and the yielded suspension was used per orally.[13]

Male Wistar rats (n=30) were used and divided into 5 groups of 6 animals each. The groups comprised of:

Groups	Intervention	Dose
Group I	Control	Non dementia (normal saline)
Group II	AD (untreated)	ALCL3. Induced Dementia (Untreated),
Group III	Donepezil	Donepezil (5 MG/KG, ORAL)
Group IV	Ezetimibe	Ezetimibe (10 MG/KG, ORAL)
Group V	Citrus limon	Citrus Limon Extract (100 MG/KG, ORAL)

Behavioral Assessments: Morris water maze (MWM) and cook's pole climbing apparatus. The rats were trained for 1 week prior to the start of the experiment. They were divided into 4 groups of 6 rats each. Total of 24 rats were taken. Behavioral assessment was done in rats at the start of the experiment i.e. at day 0.

The rats were pretreated with the test and standard drugs for 5 days, following which AlCl3 (100 mg/kg orally) was administered in all the groups and behavioral assessment on the Cook's Pole Climbing Response apparatus and Morris Water Maze Response was carried out behavioral assessment was done in all the groups as well the rats from all the groups were sacrificed following anesthesia by i.p. ketamine and brains were quickly excised. The hippocampi were isolated and processed for enzyme assays.

Following methods to assess hippocampus-dependent memory functions were used:[14]

The Morris water maze (MWM) is a test of spatial learning for rodents that relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials and reference memory is determined. The maze consists of a circular pool (1.2 m in diameter and 0.47 m high) made of white plastic. The pool was filled to a depth of 20 cm with water (24°C-25°C) that was made opaque by the addition of any non-toxic substance like milk powder or any coloring reagent. An escape platform (10 cm in diameter), made of white plastic with a grooved surface for a better grip, is submerged 0.5 cm under the water level.

Procedure: The procedure was taken as per Vorhees C V (2006) with slight modifications. Rats were trained prior to the start of the experiment for 1 week. The water in the maze was made opaque by adding sufficient quantities of milk powder to it. Animals were placed in the maze and allowed to explore the maze to find the hidden platform. Time taken by the rat to find the hidden platform was noted in seconds known as the "escape latency." [15]

Analysis was done at day 0, 3 & 6 weeks. Escape latency in seconds was then recorded and taken as an end point

Cook's Pole Climbing Apparatus: The rats were trained for conditioned avoidance response by using Cook's Pole Climbing Apparatus.[16]

Procedure: Rats were trained 1 week prior to the start of the experiment. Each rat was allowed to acclimatize and explore the apparatus for 1 minute. The buzzer was then sounded. 5 seconds after switching on the buzzer, mild electric shocks were administered through the stainless-steel grid floor. The time taken by the rat to climb the wooden pole in the center known as "escape latency" is recorded. As soon as the rat climbed the pole, both the buzzer and foot-shock were switched off.

Rats with escape latency within 5 seconds were included in the experiment. Escape latency in seconds was recorded as end point measure. Each rat was allowed to acclimatize for 2 min and was then exposed to a buzzer noise. After 5 s of putting on the buzzer, mild electric shocks were given through the stainless-steel grid floor. The magnitude of the voltage was adequate (10 V) to stimulate the rat to escape from the floor and climb the pole. As soon as the rat climbed the pole, both the buzzer and foot-shock button were switched off. At least 10 such trials were given to each rat at an interval of 1 min per day for 10 days. After about 10 days training schedule, most of the rats learned to climb the pole within 5 s of starting the buzzer to avoid the electric foot shocks. After behavioral assessments, rats were sacrificed and brains were quickly excised. The hippocampi were isolated, weighed, and homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4). Homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was used for enzyme assays.

- Superoxide Dismutase (SOD) activity was measured using a commercially available WST-based colorimetric assay kit (e.g., Cayman Chemical or Sigma-Aldrich), which detects the inhibition of superoxide-induced formazan dye formation. Absorbance was read at 450 nm.[17]
- Malondialdehyde (MDA), a marker of lipid peroxidation, was assessed using the thiobarbituric acid reactive substances (TBARS) assay. Samples were incubated with thiobarbituric acid reagent at 95°C for 15 minutes, cooled, and absorbance was measured at 532 nm.[18]
- Glutathione Peroxidase (GPx) activity was determined using a coupled enzymatic method based on NADPH oxidation, with absorbance measured at 340 nm. Commercial kits (e.g., Cayman Chemical) were used as per manufacturer's protocol.[19]

Statistical analysis: The data obtained was tabulated and subjected to descriptive analysis. The different groups were compared using ANOVA (Analysis of Variance) followed by Post Tests. All statistical analysis were done using Graph pad Prism software (version 6.02) p value < 0.05 was considered as significant.

RESULTS

Table 1: Effect of Treatments on Escape Latency in Alzheimer's Disease Model Over Time

Time Point	Group	Escape Latency (s)	F-value	t-value (vs AD Untreated)	p-value
Day 0	Control	15.2 ± 1.3	–	–	0.95
	AD (Untreated)	15.4 ± 1.5	–	–	0.95
	Donepezil	15.3 ± 1.4	–	–	0.95
	Ezetimibe	15.6 ± 1.3	–	–	0.95
	Citrus limon	15.5 ± 1.2	–	–	0.95
Week 3	Control	15.0 ± 1.2*	28.46	6.23	$p < 0.001$
	AD (Untreated)	32.1 ± 2.1⁺	–	Reference	–
	Donepezil	19.2 ± 1.8*	28.46	5.42	$p < 0.001$

	Ezetimibe	20.0 ± 2.0*	28.46	4.97	$p < 0.001$
	Citrus limon	22.2 ± 1.7*	28.46	4.36	$p < 0.01$
Week 6	Control	14.7 ± 1.2*	31.82	6.75	$p < 0.001$
	AD (Untreated)	38.6 ± 2.5⁺	–	Reference	–
	Donepezil	17.4 ± 1.4*	31.82	7.12	$p < 0.001$
	Ezetimibe	19.3 ± 2.1*	31.82	6.48	$p < 0.001$
	Citrus limon	19.6 ± 1.7*	31.82	6.10	$p < 0.001$

Data are presented as Mean ± SD (n = 6 per group).One-way ANOVA was performed at each time point to assess overall differences among groups. Post-hoc pairwise comparisons versus the AD (Untreated) group were conducted using Tukey's multiple comparison test; corresponding t-values and p-values are reported. $p < 0.05$ is considered statistically significant. "–" indicates not applicable (e.g., no self-comparison for the AD untreated group or baseline/pre-intervention).

Figure 1: Graphical Representation Of Effect Of Treatments On Escape Latency In Alzheimer's Disease Model Over Time

Graphical representation of the effect of treatments on escape latency in the Morris Water Maze test at Day 0, Week 3, and Week 6. Data: Mean ± SD (n = 6); significance assessed using one-way ANOVA followed by Tukey's post-hoc test.

A one-way ANOVA revealed significant differences among groups at Week 3 ($F(4,25) = 28.46$, $p < 0.001$) and Week 6 ($F(4,25) = 31.82$, $p < 0.001$).
At baseline (Day 0), all groups showed comparable escape latencies (≈ 15 s; $p = 0.95$), confirming uniform pre-treatment learning ability.
By Week 3, the untreated Alzheimer's disease (AD) group exhibited a marked increase in escape latency (32.1 ± 2.1 s), indicating impaired spatial memory. In contrast, all treatment groups demonstrated significant improvement compared with the AD group:
Control: $t(10) = 6.23$, $p = 0.0001$; Donepezil: $t(10) = 5.42$, $p = 0.0003$; Ezetimibe: $t(10) = 4.97$, $p = 0.0006$; Citrus limon: $t(10) = 4.36$, $p = 0.0014$.
At Week 6, escape latency further worsened in the untreated AD group (38.6 ± 2.5 s), while the treated groups showed sustained improvement:
Control: $t(10) = 6.75$, $p = 0.00005$; Donepezil: $t(10) = 7.12$, $p = 0.00004$; Ezetimibe: $t(10) = 6.48$, $p = 0.00007$; Citrus limon: $t(10) = 6.10$, $p = 0.0001$.
These results indicate that Citrus limon significantly reduced escape latency, exhibiting neuroprotective efficacy comparable to Donepezil and Ezetimibe in improving spatial learning and memory retention.

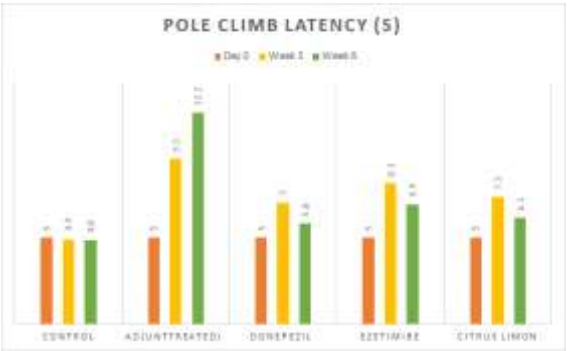

Figure 2: Graphical Representation of Effect of Treatments on Cook's Pole Climb Latency in Alzheimer's Disease Model

Graphical representation of the effect of treatments on Cook's Pole Climbing latency in the Morris Water Maze test at Day 0, Week 3, and Week 6. Data: Mean ± SD (n = 6); significance assessed using one-way ANOVA followed by Tukey's post-hoc test.

No intergroup differences were noted at baseline (Day 0, $p = 0.95$). By Week 3, a significant increase in pole climb latency was observed in the AD group (9.50 ± 1.81 s), confirming neuromotor and learning impairment. ANOVA revealed highly significant differences among groups ($F(4,25) = 26.84$, $p < 0.001$). Post-hoc comparisons demonstrated improved performance with all treatments:
Control: $t(10) = 5.03$, $p = 0.0002$; Donepezil: $t(10) = 4.49$, $p = 0.0005$; Ezetimibe: $t(10) = 4.79$, $p = 0.0003$; Citrus limon: $t(10) = 5.31$, $p = 0.0001$.
At Week 6, further deterioration in the untreated AD group (12.20 ± 1.52 s) contrasted with significant improvement in all treated groups ($F(4,25) = 30.21$, $p < 0.001$):
Control: $t(10) = 6.33$, $p = 0.00006$; Donepezil: $t(10) = 6.44$, $p = 0.00005$; Ezetimibe: $t(10) = 4.22$, $p = 0.001$; Citrus limon: $t(10) = 5.53$, $p = 0.0001$.
Both Donepezil and Citrus limon demonstrated substantial improvement in avoidance response and neuromotor coordination relative to the AD group, indicating preserved cognitive and motor function.

Table 2: Effect of Treatments on Cook's Pole Climb Latency in Alzheimer's Disease Model Over Time

Time Point	Group	Pole Climb Latency (Mean ± SD)	F-value	t-value (vs AD)	p-value
Day 0	Control	5.00 ± 0.35	–	–	0.95
	AD Untreated	5.02 ± 0.62	–	–	0.95
	Donepezil	4.98 ± 0.48	–	–	0.95
	Ezetimibe	5.00 ± 0.80	–	–	0.95
	Citrus limon	5.00 ± 0.28	–	–	0.95
Week 3	Control	4.88 ± 0.58*	26.84	5.03	<0.001
	AD Untreated	9.50 ± 1.81	26.84	Reference	–
	Donepezil	6.98 ± 1.02*	26.84	4.49	<0.001
	Ezetimibe	8.12 ± 1.31*	26.84	4.79	<0.001
	Citrus limon	7.28 ± 1.16*	26.84	5.31	<0.001
Week 6	Control	4.80 ± 0.51*	30.21	6.33	<0.001
	AD Untreated	12.20 ± 1.52	30.21	Reference	–
	Donepezil	5.78 ± 1.06*	30.21	6.44	<0.001
	Ezetimibe	6.90 ± 1.41*	30.21	4.22	<0.001
	Citrus limon	6.10 ± 0.53*	30.21	5.53	<0.001

Data are presented as Mean ± SD (n = 6 per group).One-way ANOVA was performed at each time point to assess overall differences among groups. Post-hoc pairwise comparisons versus the AD (Untreated) group were conducted using Tukey's multiple comparison test; corresponding t-values and p-values are reported. $p < 0.05$ is considered statistically significant. "–" indicates not applicable (e.g., no self-comparison for the AD untreated group or baseline/pre-intervention).

Table 3: Effect of Treatments on Oxidative Stress Markers (SOD, MDA, GPx) in Alzheimer's Disease Model

SOD (U/mg)					
	Control	**AD(untreated)**	**Donepezil**	**Ezetimibe**	**Citrus limon**
Day 0	5.8 ± 0.3	5.6 ± 0.3	5.5 ± 0.3	5.6 ± 0.3	5.6 ± 0.3
Week 3	5.0 ± 0.3	3.5 ± 0.3+	4.7 ± 0.3	4.8 ± 0.3	5.0 ± 0.3
Week 6	5.4± 0.3	2.4 ± 0.3+	5.1 ± 0.3*	4.3 ± 0.3*	5.2 ± 0.3*
MDA (nmol/mg)					
	Control	**AD(untreated)**	**Donepezil**	**Ezetimibe**	**Citrus limon**
Day 0	2.5 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.2
Week 3	2.5 ± 0.2	4.0 ± 0.3+	3.3 ± 0.3	3.5 ± 0.3	3.2 ± 0.2
Week 6	2.5 ± 0.2	5.2 ± 0.4+	2.9 ± 0.3*	3.8 ± 0.3*	3.0 ± 0.2*
GPx (U/mg)					
	Control	**AD(untreated)**	**Donepezil**	**Ezetimibe**	**Citrus limon**
Day 0	3.2 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.2
Week 3	3.2 ± 0.2	2.3 ± 0.2	2.7 ± 0.2	2.6 ± 0.2	2.8 ± 0.2
Week 6	3.2 ± 0.2	1.8 ± 0.2+	3.0 ± 0.2*	2.5 ± 0.2*	3.1 ± 0.2*
(F-range = 25.4–34.2, $p < 0.001$).					

+ Significant change compared with Day 0 ($p < 0.05$).
*Significant difference compared with AD (Untreated) group ($p < 0.05$).
Statistical analysis: Data are mean ± SEM (n = 6). One-way ANOVA followed by Tukey's multiple comparison test. Significance was considered at $p < 0.05$.

One-way ANOVA revealed significant intergroup variation in antioxidant enzyme activities and lipid peroxidation markers at Week 6 (F-range = 25.4–34.2, $p < 0.001$).
Superoxide Dismutase (SOD): AD group showed a marked reduction (2.4 ± 0.3 U/mg) compared to control (5.4 ± 0.3 U/mg; $p < 0.001$). Donepezil, Ezetimibe, and Citrus limon significantly restored SOD activity (5.1 ± 0.3, 4.3 ± 0.3, and 5.2 ± 0.3 U/mg, respectively; all $p < 0.01$ vs AD).
Malondialdehyde (MDA): MDA levels increased in the AD group (5.2 ± 0.4 nmol/mg) versus control (2.5 ± 0.2 nmol/mg; $p < 0.001$). Treatments significantly attenuated lipid peroxidation, with the lowest values in Donepezil (2.9 ± 0.3) and Citrus limon (3.0 ± 0.2) groups.
Glutathione Peroxidase (GPx): GPx activity declined in AD rats (1.8 ± 0.2 U/mg), while treatment with Donepezil (3.0 ± 0.2), Ezetimibe (2.5 ± 0.2), and Citrus limon (3.1 ± 0.2) restored antioxidant function (all $p < 0.01$ vs AD).

DISCUSSION

This study demonstrates that Citrus limon exerts significant neuroprotective effects in an $AlCl_3$-induced rat model of Alzheimer's disease (AD), with cognitive, biochemical, and histological improvements that are comparable to Donepezil and Ezetimibe. The findings add to the growing body of literature supporting the role of phytochemicals in managing neurodegenerative conditions like AD.[20]
The untreated AD group showed marked impairments in memory and learning, reflected by increased escape latency in the Morris Water Maze (MWM) and prolonged response in the Cook's pole climbing test. These behavioural changes were associated with reduced antioxidant enzyme activity (SOD, GPx) and elevated MDA levels, suggesting

increased oxidative stress—a hallmark of AD pathology. $AlCl_3$ is a widely accepted neurotoxin used to simulate AD-like symptoms due to its ability to promote Aβ aggregation and induce oxidative damage in the brain.[20]

Donepezil showed the expected improvement in both behavioural and oxidative parameters, affirming its role as a cholinesterase inhibitor that enhances central cholinergic neurotransmission. However, its benefits are largely symptomatic and diminish as the disease progresses due to irreversible neuronal loss.[11]

Ezetimibe also showed moderate cognitive and histological improvement. Recent studies suggest that cholesterol metabolism is intricately linked to Aβ generation, as cholesterol-rich lipid rafts promote amyloidogenic processing of amyloid precursor protein (APP). Ezetimibe, by reducing cholesterol absorption, may indirectly downregulate Aβ production and reduce neuroinflammation.[21]

The most significant outcome of this study is the observed neuroprotective effect of Citrus limon, which was associated with enhanced antioxidant activity, reduced lipid peroxidation, and preserved hippocampal morphology. Recent research has confirmed that citrus flavonoids like hesperidin and limonene can cross the blood-brain barrier and exert neuroprotective actions by modulating oxidative stress, mitochondrial dysfunction, and inflammatory cascades,[22] Specifically, hesperidin has been shown to attenuate memory deficits and reduce Aβ deposition in rodent AD models.[23]

Moreover, the improved GPx and SOD levels in the Citrus limon group suggest that its phytoconstituents may activate endogenous antioxidant systems—potentially through the Nrf2-ARE pathway, which is increasingly recognized as a target for combating neurodegenerative stressors.[24]

Future Perspectives: While this study provides robust preclinical evidence for the efficacy of Citrus limon in AD, further work is needed to isolate its active compounds, delineate their exact mechanisms, and evaluate their pharmacokinetics. Large-scale animal studies and eventual human clinical trials are essential to validate its therapeutic potential and establish it as a safe adjunct or alternative to conventional AD therapies.

Future research should focus on the molecular mechanisms underlying its neuroprotective effects and clinical trials to validate its efficacy in humans.

CONCLUSION

Citrus limon demonstrates promising neuroprotective properties comparable to Donepezil in an animal model of AD. Ezetimibe also shows potential benefits, highlighting the role of cholesterol metabolism in AD. These findings warrant further clinical investigation into CL as a natural therapeutic agent for AD management.

REFERENCES

1. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002 Jul 19;297(5580):353–6. doi:10.1126/science.1072994. Erratum in: Science. 2002 Sep 27;297(5590):2209. PMID: 12130773.
2. Zhang H, Tahami Monfared AA, Zhang Q, Honig LS. Incidence and prevalence of Alzheimer's disease in Medicare beneficiaries. Neurol Ther. 2025 Feb;14(1):319–33. doi:10.1007/s40120-024-00695-6. Epub 2024 Dec 19. PMID: 39699743; PMCID: PMC11762046.
3. United Nations Population Fund (UNFPA), India; International Institute for Population Sciences (IIPS). India Ageing Report 2023. New Delhi: UNFPA India; 2023.
4. Alzheimer's Association. 2023 Alzheimer's disease facts and figures. Alzheimers Dement. 2023 Apr;19(4):1598–695. doi:10.1002/alz.13016.
5. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018 Jan;25(1):59–70. doi:10.1111/ene.13439. Epub 2017 Oct 19. PMID: 28872215.
6. Cummings J, Aisen PS, DuBois B, Frölich L, Jack CR Jr, Jones RW, et al. Drug development in Alzheimer's disease: the path to 2025. Alzheimers Res Ther. 2016 Sep 20;8(1):39. doi:10.1186/s13195-016-0207-9. PMID: 27646601; PMCID: PMC5028936.
7. Dalla Y, Singh N, Jaggi AS, Singh D, Ghulati P. Potential of ezetimibe in memory deficits associated with dementia of Alzheimer's type in mice. Indian J Pharmacol. 2009 Dec;41(6):262–7. doi:10.4103/0253-7613.59925. PMID: 20407557; PMCID: PMC2846500.
8. Scott HD, Laake K. Statins for the prevention of Alzheimer's disease. Cochrane Database Syst Rev. 2001;(4):CD003160. doi:10.1002/14651858.CD003160. Update in: Cochrane Database Syst Rev. 2009 Apr 15;(2):CD003160. doi:10.1002/14651858.CD003160.pub2. PMID: 11687176.
9. Raimondo S et al. Anti-inflammatory properties of lemon-derived extracellular vesicles are achieved through the inhibition of ERK/NF-κB signalling pathways. J Cell Mol Med. 2022 Aug;26(15):4195–209. doi:10.1111/jcmm.17404. Epub 2022 Jul 4. PMID: 35789531; PMCID: PMC9344827.
10. Maya S, Prakash T, Madhu KD, Goli D. Multifaceted effects of aluminium in neurodegenerative diseases: a review. Biomed Pharmacother. 2016 Oct;83:746–54. doi:10.1016/j.biopha.2016.07.035. Epub 2016 Jul 29. PMID: 27479193.
11. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain. 2018 Jul 1;141(7):1917–33. doi:10.1093/brain/awy132. PMID: 29850777; PMCID: PMC6022632.
12. Khan RA, Riaz A. Behavioral effects of Citrus limon in rats. Metab Brain Dis. 2015 Apr;30(2):589–96. doi:10.1007/s11011-014-9616-2. Epub 2014 Sep 17. PMID: 25227172.
13. Papoutsis K, Pristijono P, Golding JB, Stathopoulos CE, Scarlett CJ, Bowyer MC, et al. Impact of different solvents on the recovery of bioactive compounds and antioxidant properties from lemon (Citrus limon L.) pomace waste. Food Sci Biotechnol. 2016 Aug 31;25(4):971–7. doi:10.1007/s10068-016-0158-8. PMID: 30263362; PMCID: PMC6049099.
14. Wahl D, Coogan SC, Solon-Biet SM, de Cabo R, Haran JB, Raubenheimer D, et al. Cognitive and behavioral evaluation of nutritional interventions in rodent models of brain aging and dementia. Clin Interv Aging. 2017 Sep 8;12:1419–28. doi:10.2147/CIA.S145247. PMID: 28932108; PMCID: PMC5598548.
15. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006;1(2):848–58. doi:10.1038/nprot.2006.116. PMID: 17406317; PMCID: PMC2895266.
16. Krupa N, Hegde K. Evaluation of anti-psychotic potential of ethanolic extract of Crossandra infundibuliformis leaves on

experimental animals. Int J Creative Res Thoughts. 2024 Nov;12(11):447–52.
17. Peskin AV, Winterbourn CC. Assay of superoxide dismutase activity in a plate assay using WST-1. Free Radic Biol Med. 2017 Feb;103:188–91. doi:10.1016/j.freeradbiomed.2016.12.033. Epub 2016 Dec 23. Erratum in: Free Radic Biol Med. 2017 Nov;112:631. doi:10.1016/j.freeradbiomed.2017.06.001. PMID: 28017897.
18. Leon JADD, Borges CR. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J Vis Exp. 2020 May 12;(159):e61122. doi:10.3791/61122. PMID: 32478759; PMCID: PMC9617585.
19. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973 Feb 9;179(4073):588–90. doi:10.1126/science.179.4073.588. PMID: 4686466.
20. Kawahara M, Kato-Negishi M, Tanaka K. Neurotoxicity of aluminum and its link to neurodegenerative diseases. Metallomics Res. 2022;1(1):47–65. doi:10.11299/metallomicsresearch.MR202104.
21. Liu LC, Liang JY, Liu YH, Liu B, Dong XH, Cai WH, et al. The intersection of cerebral cholesterol metabolism and Alzheimer's disease: mechanisms and therapeutic prospects. Heliyon. 2024 Apr 30;10(9):e30523. doi:10.1016/j.heliyon.2024.e30523. PMID: 38726205; PMCID: PMC11079309.
22. Sairazi NSM, Sirajudeen KN. Natural products and their bioactive compounds: neuroprotective potentials against neurodegenerative diseases. Evid Based Complement Alternat Med. 2020;2020:6565396. doi:10.1155/2020/6565396. PMID: 32612476; PMCID: PMC7328445.
23. Olasehinde TA, Ekundayo TC, Ijabadeniyi OA, Olaniran AO. The impact of hesperidin on cognitive deficit and neurobehavioural disorders: a systematic review and meta-analysis of preclinical individual studies. Curr Behav Neurosci Rep. 2024 Dec;11(4):246–59. doi:10.1007/s40473-024-00202-1.
24. Sidiropoulou GA, Metaxas A, Kourti M. Natural antioxidants that act against Alzheimer's disease through modulation of the NRF2 pathway: a focus on their molecular mechanisms of action. Front Endocrinol (Lausanne). 2023 Jul 3;14:1217730. doi:10.3389/fendo.2023.1217730. PMID: 37520888; PMCID: PMC10384122.