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DONEPEZIL-MEDIATED NEUROPROTECTION IN MILD-TO-MODERATE ALZHEIMER'S DISEASE: A PROSPECTIVE STUDY OF COGNITIVE AND BIOMARKER OUTCOMES

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disorder marked by cognitive decline, oxidative stress, and neuroinflammation. Donepezil, an acetylcholinesterase inhibitor widely used for symptomatic management of mild-to-moderate Alzheimer's disease, may also exert neuroprotective effects via modulation of oxidative stress, inflammatory pathways, and neurotrophic factors; however, integrated clinical evaluation of these outcomes remains limited. This prospective observational study evaluated the efficacy, safety, and neuroprotective effects of donepezil (5 or 10 mg/day) in 60 patients with mild-to-moderate Alzheimer's disease over 3 months. Cognitive function was assessed using the MMSE and ADAS-Cog scales at baseline and monthly follow-ups, with clinical improvement rated using the Clinical Global Impression–Improvement (CGI-I) scale. Biochemical parameters such as malondialdehyde (MDA), superoxide dismutase (SOD), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and brain-derived neurotrophic factor (BDNF) were measured at baseline and follow-up, alongside adverse drug reactions. All 60 patients completed the study with no dropouts. MMSE scores rose from 15.67 ± 0.41 to 18.94 ± 0.34, while ADAS-Cog scores fell from 34.02 ± 0.58 to 29.41 ± 0.46 ($p < 0.001$). MDA, CRP, and TNF-α decreased significantly, whereas SOD and BDNF increased significantly (p

< 0.001), reflecting enhanced antioxidant defense and neurotrophic support. CGI-I assessment showed marked improvement in 73.3% of patients with no deterioration, and donepezil was well tolerated, with 55% reporting no adverse reactions and the rest experiencing only mild-to-moderate events. These findings indicate donepezil significantly improves cognitive function while reducing oxidative stress and inflammation and enhancing neurotrophic activity, with a favorable safety profile and multi-mechanistic neuroprotective effect in Alzheimer's disease management.

KEYWORDS: Alzheimer's disease; Donepezil; Neuroprotection; MMSE; Acetylcholinesterase inhibitor; Prospective observational study.

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent form of dementia and a leading cause of disability among older adults worldwide.[1,2] Characterised by the progressive accumulation of amyloid-β (Aβ) plaques and neurofibrillary tau tangles, the disorder initiates a cascade of cholinergic depletion, synaptic disintegration, oxidative neuronal injury, and neuroinflammation that collectively culminate in irreversible cognitive decline.[3-5] The global burden of AD is substantial: an estimated 55 million people were living with dementia in 2020, a number projected to exceed 139 million by 2050.[6] In India alone, approximately 4.4 million individuals are affected, with the prevalence expected to escalate sharply in tandem with the country's ageing demographic profile.[7]

Current pharmacotherapy for AD is anchored in two mechanistic classes: acetylcholinesterase inhibitors (AChEIs) such as donepezil, rivastigmine, and galantamine and the N-methyl-D-aspartate receptor antagonist memantine.[8] These agents provide symptomatic relief but do not arrest disease progression. Among available AChEIs, donepezil has emerged as the first-line agent owing to its high selectivity for central acetylcholinesterase, near-complete oral bioavailability (~100%), extended half-life (~70 hours) enabling once-daily dosing, and a well-established tolerability profile.[9,10] Recently approved disease-modifying immunotherapies targeting Aβ, such as lecanemab, hold promise but remain constrained by modest clinical benefit, significant adverse effects, and prohibitive cost.[11]

Beyond cholinergic augmentation, accumulating preclinical and clinical evidence suggests that donepezil may influence multiple pathophysiological pathways implicated in AD neurodegeneration.[12] These include modulation of oxidative stress through attenuation of

lipid peroxidation and enhancement of endogenous antioxidant enzyme activity;[13] suppression of neuroinflammatory cascades via reduction of pro-inflammatory cytokines such as TNF-α and inhibition of NF-κB signalling;[14] promotion of neurotrophic factor expression, particularly brain-derived neurotrophic factor (BDNF), which is critical for synaptic plasticity and neuronal survival;[15] and anti-apoptotic effects mediated through the PI3K/Akt pathway.[16] Despite these observations, the majority of evidence derives from in vitro models or transgenic animal experiments, with limited integration of cognitive, oxidative, inflammatory, and neurotrophic outcomes within a single prospective clinical evaluation.

A notable gap persists in real-world, patient-centred research that simultaneously captures the biochemical correlates of donepezil therapy alongside established cognitive endpoints. Observational studies in ecologically valid clinical settings offer a complementary perspective to randomised controlled trials by reflecting the complexity of comorbid conditions, variable disease severity, and routine therapeutic practice. Moreover, correlating biochemical marker changes with cognitive outcomes can illuminate the mechanistic underpinnings of therapeutic response and identify candidates for biochemical monitoring in AD management.

The present study was therefore designed to address this gap by prospectively evaluating the effects of donepezil therapy on cognitive performance, oxidative stress markers (MDA, SOD), inflammatory biomarkers (CRP, TNF-α), and neurotrophic activity (BDNF) in a cohort of patients with mild-to-moderate AD attending a tertiary neurology outpatient department in Gujarat, India. The findings are expected to contribute to the evidence base supporting the neuroprotective clinical relevance of donepezil and to guide its rational use in the management of AD.

MATERIALS AND METHODS

Study Design and Setting

A prospective, single-centre observational study was conducted at the Outpatient Department of Neurology, PSM Hospital, Kalol, Gujarat, India, between January 2025 and April 2025. The hospital is equipped with standardised neurocognitive assessment instruments, validated biochemical assay laboratories, and appropriate patient monitoring infrastructure. This study design was selected because observational methodology is well-suited for assessing the real-

world pharmacological and biochemical impact of an established drug without altering routine clinical management, and it complements existing randomised trial evidence.[17]

Study Population

Patients clinically diagnosed with mild-to-moderate Alzheimer's disease in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) and the International Classification of Diseases, Tenth Revision (ICD-10) criteria were considered for enrolment. Eligible participants were aged 50 years or above, were already receiving donepezil therapy (5 mg/day or 10 mg/day) as part of their standard prescribed management, and were willing to provide written informed consent (or whose legal caregivers provided consent on their behalf).

Patients were excluded from participation if they had concurrent neurodegenerative disorders (e.g., Parkinson's disease, amyotrophic lateral sclerosis), were simultaneously receiving investigational agents or antioxidant nutritional supplements, exhibited severe hepatic, renal, or cardiovascular dysfunction, or had a documented history of psychiatric disorders that could confound cognitive assessment.

Sample Size

Sample size was estimated based on the primary endpoint of change in MMSE score over three months. Using a paired t-test framework, with an anticipated mean difference (Δ) of 2.0 points, an expected standard deviation (σ) of 3.0 points, 95% confidence level ($\alpha = 0.05$), and 80% statistical power ($Z\beta = 0.842$), the minimum required sample was calculated as approximately 23 participants. Accounting for 20% potential attrition, the target sample was set at a minimum of 28; however, 60 patients were ultimately enrolled over the study duration to enhance statistical robustness and generalisability.

Cognitive Assessment Tools

Cognitive function was evaluated using two validated, internationally standardised instruments. The Mini-Mental State Examination (MMSE) was used to assess global cognitive status across domains of orientation, registration, attention, calculation, recall, and language.[18] Scores range from 0 to 30, with higher scores indicating better cognitive function. The Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) was used as a domain-specific cognitive measure sensitive to change in AD, evaluating memory, language, praxis, and attention.[19] higher scores indicate greater cognitive impairment. Both

instruments were administered at baseline, Month 1, Month 2, and Month 3. Overall clinical improvement was rated at Month 3 using the Clinical Global Impression–Improvement (CGI-I) scale,[20] a seven-point clinician-rated scale ranging from 1 (very much improved) to 7 (very much worse).

Biochemical Procedures

Venous blood samples (approximately 5 mL, collected by antecubital venepuncture under standard aseptic conditions) were obtained from all participants at baseline and at the Month 3 follow-up visit. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at −80°C until assayed.

Oxidative stress was evaluated through measurement of malondialdehyde (MDA), a marker of lipid peroxidation, using the thiobarbituric acid reactive substances (TBARS) spectrophotometric assay,[21] and superoxide dismutase (SOD) activity by standard enzymatic spectrophotometry.[22] Neuroinflammation was characterised by quantification of high-sensitivity C-reactive protein (CRP) via immunoturbidimetry[23] and tumour necrosis factor-α (TNF-α) concentrations by commercially validated enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA).[24] Neurotrophic activity was assessed by measurement of BDNF levels using ELISA kits (Thermo Fisher Scientific, USA).[25] All assays were performed in duplicate following manufacturer instructions and previously validated methodologies.

Safety Monitoring

Adverse drug reactions (ADRs) were recorded at each clinical visit using structured case record forms and assessed for causality using the Naranjo Adverse Drug Reaction Probability Scale.[26] Vital signs (blood pressure, heart rate) and any patient-reported symptoms were documented systematically throughout the observation period.

Statistical Analysis

All data were compiled in structured case record forms and analysed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard error of the mean (SEM) for continuous variables and as frequencies with percentages for categorical variables. The paired t-test was used to compare follow-up measurements with baseline values. Pearson correlation analysis was applied to explore associations between

cognitive and biochemical outcomes. A two-tailed p-value < 0.05 was considered statistically significant. Graphical representations were generated using GraphPad Prism v9.0.

Ethical Approval

The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC) prior to the commencement of the study (Approval No. IEC/PSM/2024-25). The research was conducted in full accordance with the ethical principles of the Declaration of Helsinki.[27] the International Conference on Harmonisation- Good Clinical Practice (ICH-GCP) guidelines, and the Ethical Guidelines for Biomedical Research on Human Participants of the Indian Council of Medical Research (ICMR, 2017).[28] Written informed consent was obtained from all participants or their legally authorised representatives. Participation was entirely voluntary; withdrawal at any stage carried no consequence for standard clinical care. Patient confidentiality and data anonymity were strictly maintained throughout the study.

RESULTS

Patient Demographics and Baseline Clinical Characteristics

A total of 60 patients with mild-to-moderate AD were enrolled; all completed the three-month follow-up period with no attritions. The mean age of the cohort was 68.17 ± 6.84 years, consistent with the established epidemiology of late-onset AD.[1] The cohort comprised 33 males (55%) and 27 females (45%), reflecting a relatively balanced sex distribution. Disease severity was evenly distributed, with 31 patients (51.7%) classified as mild and 29 (48.3%) as moderate AD. The most prevalent comorbidities were hypertension (30.0%) and diabetes mellitus (26.7%), which are well-recognised vascular risk modifiers in AD. A positive family history of dementia was identified in 36.7% of participants, underscoring the genetic susceptibility component in this cohort. Donepezil doses were similarly distributed between 5 mg/day (51.7%) and 10 mg/day (48.3%). Full baseline characteristics are presented in Table 1.

Table 1: Baseline Demographic and Clinical Profile of Study Participants (n = 60).

Parameter	Category	n	Percentage (%)
Gender	Male	33	55.0
	Female	27	45.0
Age (years)	Mean ± SD	68.17 ± 6.84	—
Weight (kg)	Mean ± SD	64.56 ± 8.12	—
Education	Primary	14	23.3
	Secondary	14	23.3
	Higher Secondary	15	25.0

	Graduate	17	28.3
AD Severity	Mild	31	51.7
	Moderate	29	48.3
Comorbidities	Hypertension only	18	30.0
	Diabetes only	16	26.7
	HTN + DM	14	23.3
	None	12	20.0
Family History of Dementia	Yes	22	36.7
	No	38	63.3
Donepezil Dose	5 mg/day	31	51.7
	10 mg/day	29	48.3
Therapy Duration at Baseline	1-2 months	26	43.3
	3-4 months	34	56.7

HTN = hypertension; DM = diabetes mellitus; AD = Alzheimer's disease. Age and weight expressed as mean ± SD.

Cognitive Outcomes

1. MMSE Scores

MMSE scores demonstrated a progressive and statistically significant increase across all follow-up time points. From a baseline value of 15.67 ± 0.41, scores rose to 16.82 ± 0.39 at Month 1 ($p < 0.05$), 17.76 ± 0.36 at Month 2 ($p < 0.01$), and 18.94 ± 0.34 at Month 3 ($p < 0.001$), representing an absolute improvement of 3.27 points over three months. These data are presented in Table 2.

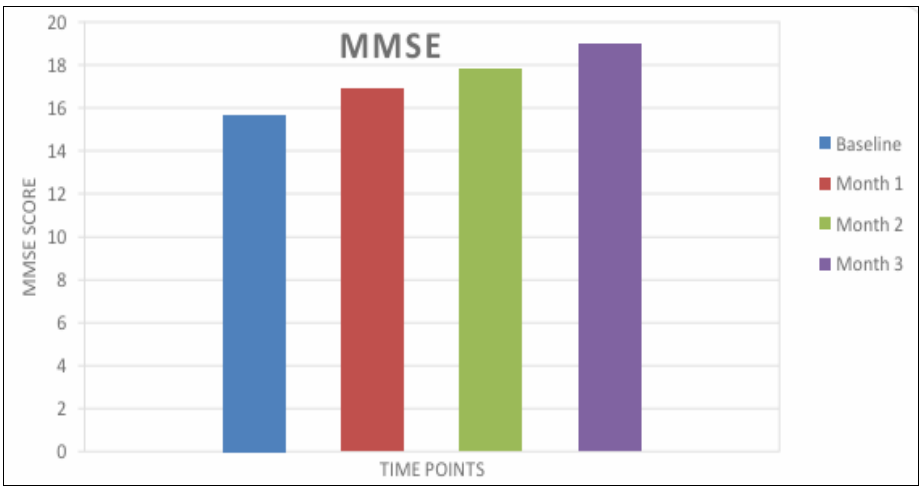

Fig. 1: MMSE Score Across Follow-up Visits (Baseline to Month 3).

Table 2: MMSE Scores Across Follow-up Visits (n = 60).

Time Point	Mean ± SEM	p-value	Significance
Baseline (Reference)	15.67 ± 0.41	—	—
Month 1	16.82 ± 0.39	< 0.05	* Significant

Month 2	17.76 ± 0.36	< 0.01	** Very Significant
Month 3	18.94 ± 0.34	< 0.001	*** Highly Significant

*Data expressed as Mean ± SEM. Paired t-test with baseline as reference. *p < 0.05; **p < 0.01; ***p < 0.001.*

2. ADAS-Cog Scores

ADAS-Cog scores displayed a consistent and progressive decline, reflecting improvement in cognitive impairment severity. Baseline scores of 34.02 ± 0.58 decreased to 32.15 ± 0.53 at Month 1 ($p < 0.05$), 30.87 ± 0.49 at Month 2 ($p < 0.01$), and 29.41 ± 0.46 at Month 3 ($p < 0.001$), representing a reduction of 4.61 points. The inverse relationship observed between MMSE and ADAS-Cog trajectories validates the cognitive improvement observed across both instruments (Table 3).

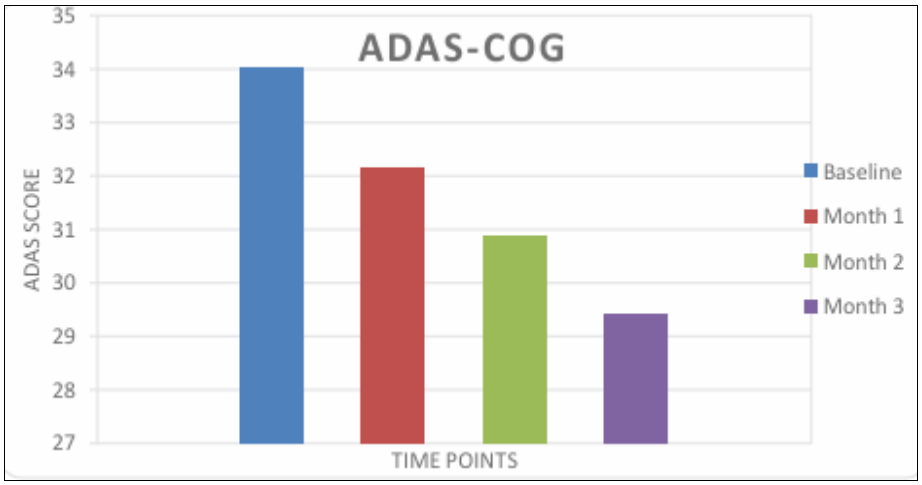

Fig. 2: ADAS-Cog Score Across Follow-up Visits (Baseline to Month 3).

Table 3: ADAS-Cog Scores Across Follow-up Visits (n = 60).

Time Point	Mean ± SEM	p-value	Significance
Baseline (Reference)	34.02 ± 0.58	—	—
Month 1	32.15 ± 0.53	< 0.05	* Significant
Month 2	30.87 ± 0.49	< 0.01	** Very Significant
Month 3	29.41 ± 0.46	< 0.001	*** Highly Significant

*Data expressed as Mean ± SEM. Paired t-test with baseline as reference. *p < 0.05; **p < 0.01; ***p < 0.001.*

3. Biochemical Marker Changes

Significant changes were observed across all measured biomarkers between baseline and Month 3. A comprehensive summary is presented in Table 4. MDA levels decreased progressively from 5.78 ± 0.21 nmol/mL at baseline to 4.32 ± 0.15 nmol/mL at Month 3 ($p <$

0.001), indicating a 25.3% reduction in lipid peroxidation. SOD activity increased from 97.6 ± 2.8 U/mL to 121.8 ± 2.2 U/mL ($p < 0.001$), reflecting a 24.8% enhancement of endogenous antioxidant capacity. CRP concentrations fell from 5.48 ± 0.26 mg/L to 3.32 ± 0.18 mg/L ($p < 0.001$), equating to a 39.4% reduction in systemic inflammatory burden. TNF-α levels declined from 19.6 ± 0.74 pg/mL to 11.4 ± 0.58 pg/mL ($p < 0.001$), representing a 41.8% decrease in this pro-inflammatory neurodestructive cytokine. BDNF concentrations increased substantially from 11.2 ± 0.48 ng/mL to 18.6 ± 0.57 ng/mL ($p < 0.001$), a 66.1% increase reflecting markedly enhanced neurotrophic support.

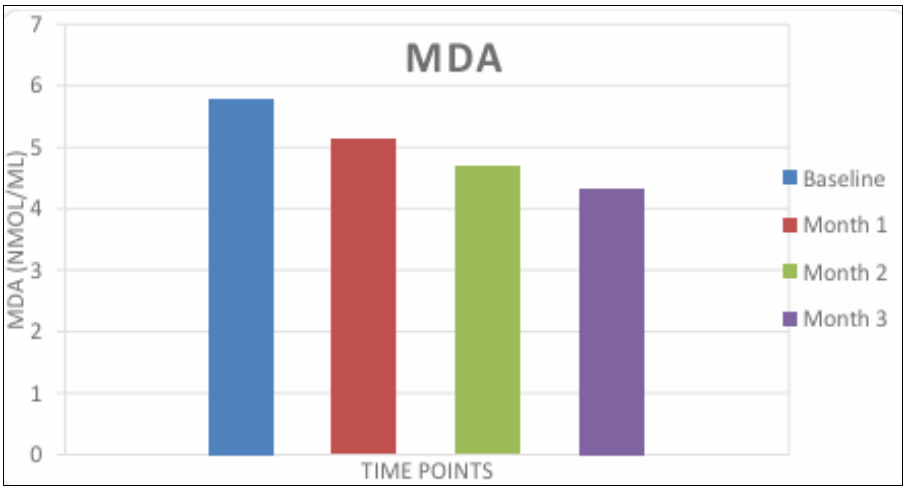

Fig. 3: MDA Levels Across Follow-up Visits.

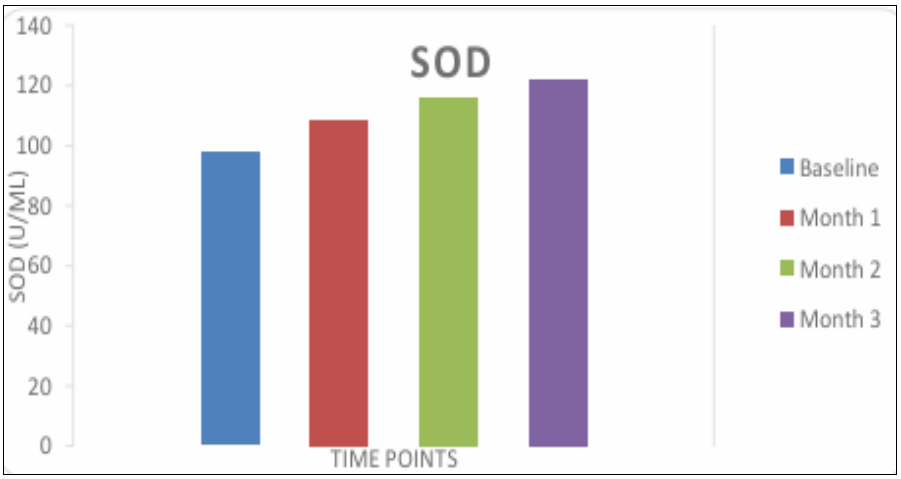

Fig. 4: SOD Activity Across Follow-up Visits.

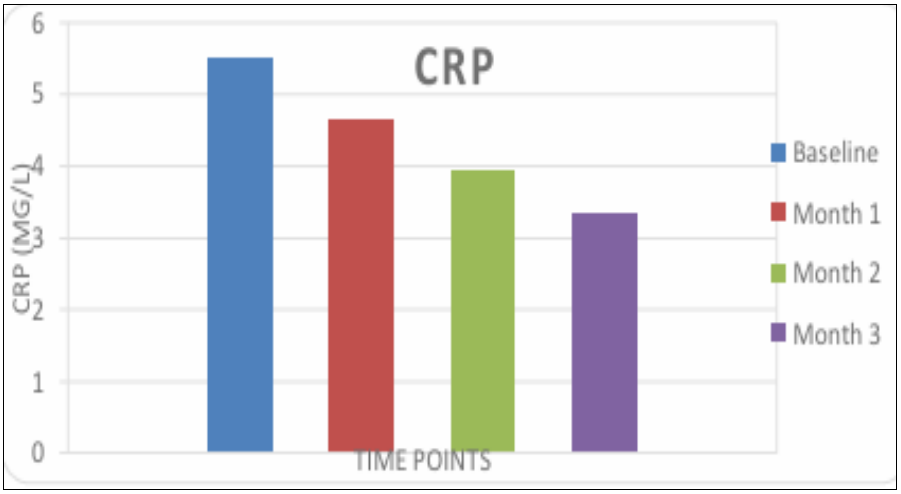

Fig. 5: CRP Levels Across Follow-up Visits.

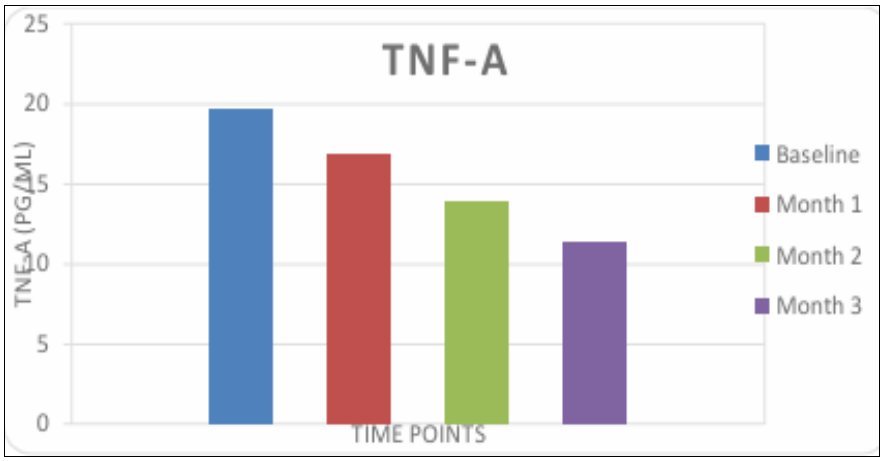

Fig. 6: TNF-α Levels Across Follow-up Visits.

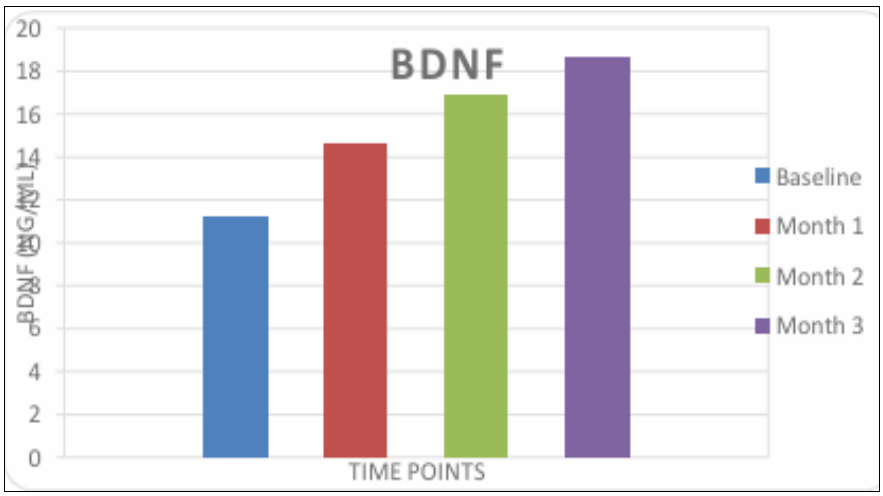

Fig. 7: BDNF Levels Across Follow-up Visits.

Table 4: Biochemical Marker Changes Across Follow-up Visits (n = 60).

Biomarker	Baseline	Month 1	Month 2	Month 3	p-value
MDA (nmol/mL)	5.78 ± 0.21	5.12 ± 0.19	4.68 ± 0.17	4.32 ± 0.15	< 0.001
SOD (U/mL)	97.6 ± 2.8	108.4 ± 2.6	115.9 ± 2.4	121.8 ± 2.2	< 0.001
CRP (mg/L)	5.48 ± 0.26	4.62 ± 0.23	3.92 ± 0.21	3.32 ± 0.18	< 0.001
TNF-α (pg/mL)	19.6 ± 0.74	16.8 ± 0.68	13.9 ± 0.62	11.4 ± 0.58	< 0.001
BDNF (ng/mL)	11.2 ± 0.48	14.6 ± 0.52	16.9 ± 0.55	18.6 ± 0.57	< 0.001

*MDA = malondialdehyde; SOD = superoxide dismutase; CRP = C-reactive protein; TNF-α = tumour necrosis factor-alpha; BDNF = brain-derived neurotrophic factor. Data expressed as Mean ± SEM. Paired t-test (baseline vs Month 3). ***p < 0.001.*

4. Clinical Global Impression–Improvement (CGI-I)

At the Month 3 assessment, 73.3% of patients demonstrated marked global clinical improvement: 30.0% were rated as 'very much improved' and 43.3% as 'much improved'. A further 26.7% were classified as 'minimally improved', and no patient experienced clinical deterioration or remained unchanged (Table 5). The CGI-I outcomes corroborated the statistically significant improvements documented in cognitive and biochemical assessments, providing convergent evidence of clinical benefit.

Table 5: CGI-I Category Distribution at Month 3 (n = 60).

CGI-I Category	n (Total = 60)	Percentage (%)
Very Much Improved	18	30.0
Much Improved	26	43.3
Minimally Improved	16	26.7
No Change / Worsened	0	0.0

5. Overall Outcome Summary

Table 6 presents an integrated summary of treatment response across all evaluated parameters at Month 3. All seven assessed outcomes — MMSE, ADAS-Cog, MDA, SOD, CRP, TNF-α, and BDNF — demonstrated statistically highly significant changes ($p < 0.001$), collectively confirming the multi-target pharmacological activity of donepezil in this real-world cohort.

Table 6: Summary of Treatment Response Across All Parameters (n = 60).

Parameter	Direction of Change	p-value	Significance
MMSE	↑ Improved	< 0.001	*** Highly Significant
ADAS-Cog	↓ Reduced	< 0.001	*** Highly Significant
MDA	↓ Reduced	< 0.001	*** Highly Significant
SOD	↑ Increased	< 0.001	*** Highly Significant
CRP	↓ Reduced	< 0.001	*** Highly Significant
TNF-α	↓ Reduced	< 0.001	*** Highly Significant
BDNF	↑ Increased	< 0.001	*** Highly Significant

*Paired t-test; baseline vs Month 3. All values expressed as Mean ± SEM. ***p < 0.001.*

6. Safety and Tolerability

Donepezil was well tolerated in the study cohort. The majority of patients (55.0%) reported no adverse drug reactions during the observation period. Among the 45.0% who experienced an adverse event, nausea was the most frequent (20.0%), followed by dizziness (15.0%) and headache (10.0%). All adverse events were categorised as mild to moderate in severity; none required dose modification, treatment discontinuation, or hospitalisation. No serious or unexpected adverse events were recorded. Table 7 presents the complete safety and tolerability profile.

Table 7: Safety and Tolerability Profile of Donepezil Therapy (n = 60).

Adverse Event	n	Percentage (%)	Severity
No ADR	33	55.0	—
Nausea	12	20.0	Mild
Dizziness	9	15.0	Mild–Moderate
Headache	6	10.0	Mild

DISCUSSION

The present study provides an integrated, multi-domain evaluation of donepezil's pharmacological and biochemical effects in a real-world cohort of patients with mild-to-moderate Alzheimer's disease. The principal finding is that three months of donepezil therapy produced statistically highly significant, progressive improvements in cognitive function, concurrent with substantial attenuation of oxidative stress and neuroinflammation, and a marked increase in neurotrophic factor levels. These outcomes, observed across seven distinct parameters ($p < 0.001$ for each), collectively establish a compelling evidence base for donepezil's multi-mechanistic neuroprotective action.

Cognitive Outcomes

The progressive and time-dependent improvements in MMSE and ADAS-Cog scores are consistent with the established pharmacodynamics of donepezil as a reversible AChEI [29,30] By inhibiting acetylcholinesterase, donepezil prolongs the availability of acetylcholine at synaptic clefts in the hippocampus and cortex, regions critically implicated in memory encoding and executive function.[31] The 3.27-point improvement in MMSE and 4.61-point reduction in ADAS-Cog observed in the present study are clinically meaningful and align favourably with findings from landmark randomised controlled trials.[29,30] and real-world

observational cohorts.[32] The inverse, congruent trends in both scales strengthen the internal validity of the cognitive observations. The time-dependent improvement pattern further suggests that sustained pharmacological exposure is requisite for maximal cholinergic benefit, an implication relevant to treatment adherence counselling.

Oxidative Stress Modulation

The significant reduction in MDA, a canonical marker of lipid peroxidation and membrane oxidative damage — alongside enhanced SOD activity provides compelling evidence that donepezil influences the redox environment in AD patients. Oxidative stress occupies a central and early role in AD pathogenesis, driving Aβ aggregation, tau hyperphosphorylation, and neuronal apoptosis[33,34] The antioxidant effects of donepezil have been attributed to multiple mechanisms: direct scavenging of reactive oxygen species, enhancement of mitochondrial function by stabilising the mitochondrial membrane potential, and Nrf2 pathway activation promoting phase II detoxifying enzyme synthesis[13,35] These results are consistent with preclinical observations in rodent Aβ neurotoxicity models[36] and in vitro studies demonstrating reduced lipid peroxidation and improved SOD activity in donepezil-treated neuronal cultures.[13] The current data extend these findings to a clinical population, supporting the translational relevance of the antioxidant mechanism.

Neuroinflammatory Modulation

The significant reductions in CRP and TNF-α observed at Month 3 indicate that donepezil suppresses both systemic and neuroinflammatory processes. Neuroinflammation, driven by activated microglia and reactive astrocytes, is now recognised as a principal driver of synaptic dysfunction and neurodegeneration in AD.[37] TNF-α, a pleiotropic pro-inflammatory cytokine, promotes neuronal apoptosis, exacerbates tau pathology, and impairs synaptic plasticity; its reduction therefore has direct neuroprotective implications.[14,38] Donepezil has been shown to suppress microglial NF-κB activation and reduce gliosis-associated neuroinflammatory burden in experimental models,[14,39] effects that the present study corroborates in a clinical setting. The reduction in CRP, a sensitive systemic inflammatory marker associated with AD severity and progression,[37] may reflect both peripheral and central anti-inflammatory effects of the drug. These findings support the proposal that cholinergic augmentation through the cholinergic anti-inflammatory pathway mediated by α7-nicotinic acetylcholine receptors on macrophages and microglia — may mechanistically underlie the observed attenuation of inflammatory mediators.[40]

Neurotrophic Upregulation

The most substantial biochemical finding of the present study was the 66.1% increase in BDNF concentrations at Month 3. BDNF is an indispensable mediator of hippocampal neuroplasticity, long-term potentiation, and adult neurogenesis, and its deficiency is closely associated with synaptic deterioration and cognitive decline in AD.[15,41] Donepezil has been demonstrated to upregulate BDNF expression and activate its receptor TrkB in hippocampal neurons.[42] enhancing signalling pathways that promote neuronal survival and memory consolidation. These effects are thought to be mediated partly through enhanced cholinergic input to BDNF-expressing neurons and partly through anti-apoptotic signalling via the PI3K/Akt cascade.[16,43] The magnitude of BDNF upregulation observed in the present cohort is consistent with experimental data and suggests that this neurotrophic effect may translate into durable cognitive and synaptic benefits beyond the observation window. Longitudinal studies measuring BDNF trajectories alongside neuroimaging endpoints would be valuable in characterising this relationship further.

CGI-I and Safety

The CGI-I finding that 73.3% of patients demonstrated marked global improvement provides clinically relevant corroboration of the quantitative biochemical and cognitive data. This global clinician-rated measure integrates the holistic treatment response and is less susceptible to scale-specific measurement variance, thereby strengthening the ecological validity of the study. The safety profile characterised by the absence of serious adverse events and by predominantly mild, transient gastrointestinal and autonomic symptoms in a minority of patients is consistent with the established tolerability of donepezil in geriatric population[44, 45] and supports its suitability for long-term use.

LIMITATIONS

The present study has several limitations that should be acknowledged. The observational single-centre design without a placebo control group precludes definitive causal attribution of biochemical changes exclusively to donepezil, as natural disease course and concurrent management of comorbidities may have contributed to observed outcomes. The three-month follow-up period, while sufficient to detect early and meaningful pharmacological effects, does not capture long-term trajectory or sustainability of the observed neuroprotective benefits. The sample was drawn from a single tertiary facility in Gujarat, India, which may limit generalisability to more diverse clinical settings. Additionally, the study did not include

measurement of glutathione (GSH) or interleukin-6 (IL-6), which would have provided a more comprehensive characterisation of the antioxidant and inflammatory profiles. Future multicentre randomised investigations with extended follow-up, incorporating neuroimaging biomarkers and a broader panel of molecular markers, are warranted to confirm and expand upon these findings.

CONCLUSION

This prospective observational study demonstrates that donepezil therapy produces significant, progressive improvement in cognitive function, as reflected by increasing MMSE and declining ADAS-Cog scores over three months, in patients with mild-to-moderate Alzheimer's disease. Concurrent with these cognitive gains, donepezil substantially attenuated oxidative stress (reduced MDA, enhanced SOD), suppressed neuroinflammatory mediators (CRP, TNF-α), and markedly elevated BDNF levels, collectively reflecting a multi-mechanistic neuroprotective pharmacological profile. Clinical global assessment confirmed marked improvement in 73.3% of patients, with no cases of deterioration. The drug was well tolerated, with only mild, transient adverse events in a minority of patients. These findings reinforce donepezil's role as the pharmacological cornerstone of AD management and provide clinically grounded evidence that its therapeutic benefits extend beyond cholinergic augmentation to encompass attenuation of the oxidative, inflammatory, and neurotrophic pathological mechanisms central to AD pathogenesis. Biochemical parameter monitoring alongside established cognitive scales may, in the future, serve as a more holistic framework for evaluating and individualising treatment response in Alzheimer's disease.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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