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Synergistic Effects of Sinomenine Hydrochloride and Low-Dose Donepezil on Cognitive Decline: Combined Modulation of Cholinergic Function and Nrf2-Mediated Antioxidant Response

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ABSTRACT

Background and Objective: Cognitive decline, a hallmark of neurodegenerative diseases such as Alzheimer's disease (AD), poses a major global health challenge. Current treatments like donepezil provide symptomatic relief but are limited by side effects and do not halt disease progression. This study aimed to investigate the synergistic effects of low-dose donepezil combined with sinomenine hydrochloride, a natural alkaloid with anti-inflammatory and neuroprotective properties, on cognitive deficits. **Materials and Methods:** An *in vivo* model of cognitive impairment was used to assess the effects of combination therapy on cholinergic function, oxidative stress, and the Nrf2-dependent anti-oxidative pathway. Behavioral tests evaluated spatial and recognition memory, while biochemical assays measured acetylcholine esterase (AChE), choline acetyltransferase (ChAT), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH). Expression of Nrf2 and its downstream targets, heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1), was analyzed. All data were analyzed using GraphPad Prism 9.0 and expressed as Mean±SEM, with significance determined at $p<0.05$ using one-way ANOVA (Tukey's *post hoc* test) and repeated-measures ANOVA for MWM escape latency. **Results:** Combined low-dose donepezil and sinomenine hydrochloride significantly improved spatial and recognition memory, restored AChE/ChAT balance, and reduced oxidative stress (MDA). The treatment robustly activated the Nrf2 pathway, increasing HO-1 and NQO1 expression and enhancing antioxidant capacity (SOD, GSH). These effects were superior to those observed with either agent alone, indicating a potent synergistic effect. **Conclusion:** The combination of low-dose donepezil and sinomenine hydrochloride synergistically improves cognitive function by modulating cholinergic neurotransmission and enhancing Nrf2-dependent antioxidant defenses. This strategy offers a promising approach to managing cognitive decline with potentially reduced side effects, warranting further investigation in clinical settings.

KEYWORDS

Cognitive decline, donepezil, sinomenine hydrochloride, cholinergic function, Nrf2, oxidative stress, synergy, neuroprotection

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INTRODUCTION

Cognitive decline, characterized by impairments in memory, learning, and executive functions, is a debilitating feature of various neurodegenerative disorders, most notably Alzheimer's disease (AD)[1]. The AD pathology is complex, involving the accumulation of amyloid-beta (Aβ) plaques, neurofibrillary tangles, cholinergic dysfunction, chronic neuroinflammation, and excessive oxidative stress[2,3]. The cholinergic hypothesis, positing that a deficit in acetylcholine (ACh) neurotransmission contributes significantly to cognitive impairment, has guided the development of current pharmacotherapies. Donepezil, a reversible acetylcholinesterase (AChE) inhibitor, enhances cholinergic transmission by preventing ACh breakdown, thereby offering symptomatic relief in AD patients[4]. However, donepezil's efficacy is limited, often associated with dose-dependent peripheral side effects (e.g., nausea, diarrhea, bradycardia), and it does not address the underlying neurodegenerative processes or halt disease progression[5]. This underscores the urgent need for novel therapeutic strategies that are more effective, have fewer side effects, and target multiple pathological mechanisms.

Emerging evidence suggests that oxidative stress plays a pivotal role in the initiation and progression of neurodegeneration by damaging cellular components and exacerbating inflammation[6]. The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is a master regulator of the cellular antioxidant response, governing the expression of a battery of cytoprotective genes, including heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1)[7]. Activation of Nrf2 enhances cellular resilience against oxidative insults and neuroinflammation, making it a highly attractive therapeutic target for neurodegenerative diseases[8]. Dysfunction of the Nrf2 pathway has been implicated in AD pathogenesis, further supporting its potential as a therapeutic avenue[9].

Sinomenine hydrochloride (Sinomenine) is an alkaloid derived from the root of *Sinomenium acutum*, a traditional Chinese medicinal plant. It is widely recognized for its anti-inflammatory, analgesic, and immunomodulatory properties, primarily through its ability to inhibit NF-κB signaling and cytokine production[10]. Recent studies have begun to uncover its neuroprotective potential, with some evidence suggesting it may attenuate neuroinflammation and oxidative stress in various neurological insult models[11,12]. While its direct impact on Nrf2-dependent antioxidant pathways in cognitive decline models is less explored, its known anti-inflammatory actions often converge with Nrf2 activation.

Given the multi-factorial nature of cognitive decline and the limitations of mono-therapy, a combination approach targeting multiple pathological mechanisms concurrently is highly appealing[13]. We hypothesized that combining a low-dose of donepezil (to maintain cholinergic tone with reduced side effects) with sinomenine (to leverage its neuroprotective and antioxidative properties, potentially via Nrf2 activation) would yield synergistic benefits superior to either agent alone. This study aimed to investigate the synergistic effects of low-dose donepezil and sinomenine hydrochloride on cognitive function in an in vivo model of cognitive decline, focusing on their combined modulation of cholinergic neurotransmission and Nrf2-dependent anti-oxidative resilience.

MATERIALS AND METHODS

Study area and duration: This study was exclusively carried out within the accredited Animal Housing and Research Facility of the Department of Science Laboratory Technology, Delta Central Polytechnic, Ughelli, Delta State, Nigeria. The experimental procedures, including animal acclimatization, surgery, treatment administration, and behavioral testing as well as laboratory testing, were conducted over a definitive two-month period, from June, 2025 to August, 2025.

Animal model and experimental design: Male C57BL/6 mice (8-10 weeks old, 25-30 g) were purchased from Delta Central Polytechnic, Nigeria. Animals were housed in a controlled environment (22±2°C, 12 hrs light/dark cycle) with ad libitum access to food and water.

Ethical approval: All experimental procedures were approved by the Institutional Animal Care and Use Committee of Delta Central Polytechnic, Ughelli, Delta State, Nigeria and conformed to the NIH Guide for the Care and Use of Laboratory Animals.

Cognitive impairment was induced by intracerebroventricular (ICV) injection of amyloid-beta (Aβ)1-42. Briefly, mice were anesthetized with isoflurane, and Aβ1-42 (2 µg in 2 µL sterile saline, Sigma-Aldrich) was injected into the right lateral ventricle (coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to midline, 2.5 mm depth) using a stereotaxic apparatus[14]. Sham group animals received an equal volume of sterile saline.

Following a 7 day recovery period, mice were randomly assigned to five experimental groups (n = 10-12 per group):

- **Sham:** Saline ICV+Vehicle (oral gavage)
- **Model:** Aβ1-42 ICV+Vehicle (oral gavage)
- **Donepezil (DNP):** Aβ1-42 ICV+Donepezil (0.5 mg/kg, oral gavage)
- **Sinomenine (SNM):** Aβ1-42 ICV+Sinomenine HCl (20 mg/kg, oral gavage)
- **Combination (DNP+SNM):** Aβ1-42 ICV+Donepezil (0.5 mg/kg)+Sinomenine HCl (20 mg/kg, oral gavage)

Donepezil hydrochloride (Sigma-Aldrich) and Sinomenine hydrochloride (TargetMol, confirmed purity >98%) were dissolved in sterile saline immediately before administration. Treatments were administered daily via oral gavage for 28 consecutive days. Behavioral tests were performed during the last week of treatment.

Behavioral assessments

Morris water maze (MWM) test: A standard MWM apparatus (1.2 m diameter pool, 40 cm deep, filled with opaque water (22±1°C)) was used to assess spatial learning and memory[15]. A hidden platform (10 cm diameter) was submerged 1 cm below the water surface in one quadrant. Mice underwent 4 trials per day for 5 consecutive days, with a maximum trial duration of 60 sec. Escape latency (time to find the platform) and swimming speed were recorded. On day 6, a probe trial was conducted for 60 sec (platform removed) to assess spatial memory, measuring time spent in the target quadrant and the number of platform crossings.

Y-maze test: Spontaneous alternation behavior was used to assess spatial working memory in a Y-maze with three identical arms (30 cm long, 5 cm wide, 15 cm high)[16]. Each mouse was placed in the center of the maze and allowed to explore for 8 minutes. An "alternation" was defined as entry into three different arms in consecutive choices (e.g., ABC, BCA). The percentage of spontaneous alternation was calculated as:

$$\text{Spontaneous alternation (\%)} = \frac{\text{Number of alternations}}{\text{Total arm entries} - 2} \times 100$$

Novel object recognition (NOR) test: The NOR test evaluated recognition memory[17]. On day 1 (habituation), mice explored an empty open-field arena (40×40×40 cm) for 10 min. On day 2 (familiarization), two identical objects (familiar objects, A1 and A2) were placed in the arena, and mice

explored for 10 min. On day 3 (test), one familiar object (A1) was replaced by a novel object (B), and mice explored for 5 min. The time spent exploring each object was recorded. A discrimination index was calculated as:

$$\text{Discrimination index} = \frac{\text{Time exploring novel object - Time exploring familiar object}}{\text{Totaltime exploringboth object}}$$

Tissue preparation and biochemical assays: Following behavioral testing, animals were humanely euthanized by cervical dislocation under deep anesthesia. Brains were rapidly removed. The hippocampus and cerebral cortex were dissected, snap-frozen in liquid nitrogen, and stored at -80°C for biochemical analysis.

Cholinergic function assessment: Tissue homogenates from the hippocampus were prepared in saline (1:10 w/v).

- **AChE activity:** Measured using an Acetylcholinesterase Assay Kit (Abcam, ab138871) according to the manufacturer's protocol. Results were expressed as nmol/min/mg protein
- **Choline acetyltransferase (ChAT) levels:** Quantified using a Mouse ChAT ELISA Kit (R&D Systems, MCH00) following the manufacturer's instructions. Results were expressed as ng/mg protein

Oxidative stress markers: Hippocampal tissue homogenates were prepared in PBS.

- **Malondialdehyde (MDA) levels:** Assessed using a Lipid Peroxidation (MDA) Assay Kit (Abcam, ab118970). Results were expressed as nmol/mg protein
- **Superoxide dismutase (SOD) activity:** Measured using a Superoxide Dismutase Activity Assay Kit (Abcam, ab65354). Results were expressed as U/mg protein
- **Glutathione (GSH) levels:** Determined using a Glutathione Assay Kit (Sigma-Aldrich, CS0260). Results were expressed as µmol/mg protein

Nrf2 pathway markers (western blot analysis): Total protein was extracted from hippocampal tissues using RIPA buffer containing protease and phosphatase inhibitors. Nuclear and cytoplasmic fractions were separated using a Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific, 78833) to analyze nuclear Nrf2. Protein concentrations were determined using the BCA protein assay kit. Proteins (20-40 µg) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked with 5% skim milk and incubated overnight at 4°C with primary antibodies: anti-Nrf2 (1:1000, Abcam, ab137550), anti-HO-1 (1:1000, Abcam, ab13248), anti-NQO1 (1:1000, Abcam, ab80588), anti-Lamin B1 (1:1000, nuclear loading control, Abcam, ab16048), and anti-β-actin (1:5000, cytoplasmic loading control, Abcam, ab8227). After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5000, Abcam) for 1 hour at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system. Densitometric analysis was performed using ImageJ software.

Statistical analysis: All data were analyzed using GraphPad Prism 9.0 software. Data are presented as Mean±Standard Error of the Mean (SEM). Statistical significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons. MWM escape latency data were analyzed using repeated-measures ANOVA. A p-value<0.05 was considered statistically significant.

RESULTS

As shown in Table 1, Aβ1-42 injection significantly impaired spatial learning and memory in the Model group mice compared to the Sham group. Model mice exhibited significantly longer escape latencies to find the hidden platform across 5 training days ($p<0.001$) and spent less time in the target quadrant

Table 1: Effects of donepezil, sinomenine, and combination therapy on cognitive performance in Aβ1-42 induced mice

Group	MWM escape latency (Day 5, sec)	MWM target quadrant time (Probe trial, %)	Y-maze spontaneous alternation (%)	NOR discrimination index
Sham	10.2±1.1	45.1±2.3	75.8±1.9	0.72±0.04
Model	48.7±3.4$^{###}$	18.5±1.8$^{###}$	42.1±2.8$^{###}$	0.28±0.03$^{###}$
Donepezil	32.5±2.8*	28.9±2.1*	55.4±2.5*	0.45±0.04*
Sinomenine	34.1±2.9*	27.6±2.0*	53.7±2.7*	0.43±0.05*
Combination	16.8±1.5$^{**\#\#}$	41.5±2.5$^{**\#\#}$	71.2±2.1$^{**\#\#}$	0.68±0.05$^{**\#\#}$

Data are expressed as Mean±SEM (n = 10-12 per group), $^{###}$p<0.001 vs Sham group, p<0.05 vs Model group; **p<0.01 vs Model group and $^{\#}$p<0.05 vs Donepezil or Sinomenine monotherapy group; $^{\#\#}$p<0.01 vs Donepezil or Sinomenine monotherapy group

Table 2: Effects of donepezil, sinomenine, and combination therapy on hippocampal cholinergic function

Group	AChE activity (nmoL/min/mg protein)	ChAT levels (ng/mg protein)
Sham	125.6±7.2	2.85±0.15
Model	289.4±14.5$^{###}$	1.12±0.09$^{###}$
Donepezil	198.3±11.8*	1.98±0.12*
Sinomenine	245.1±13.1*	1.55±0.10*
Combination	142.9±8.5$^{**\#\#}$	2.61±0.14$^{**\#\#}$

Data are expressed as Mean±SEM (n = 10 per group), $^{###}$p<0.001 vs Sham group, p<0.05 vs Model group; **p<0.01 vs Model group and $^{\#}$p<0.05 vs Donepezil or Sinomenine monotherapy group; $^{\#\#}$p<0.01 vs Donepezil or Sinomenine monotherapy group

during the probe trial (p<0.001). Donepezil and Sinomenine monotherapies partially but significantly improved these parameters. Crucially, the combination group (DNP+SNM) demonstrated a significantly shorter escape latency (p<0.01 vs. DNP or SNM alone) and spent markedly more time in the target quadrant during the probe trial (p<0.01 vs. DNP or SNM alone), reaching levels comparable to the Sham group. No significant differences in swimming speed were observed among groups (data not shown), indicating that motor function was not affected.

The Aβ1-42-induced Model group showed a significant reduction in spontaneous alternation percentage compared to the Sham group (p<0.001), indicating impaired spatial working memory (Table 1). Both Donepezil and Sinomenine treatment alone significantly mitigated this impairment (p<0.05 vs. Model). However, the combined DNP+SNM treatment led to a significantly greater increase in spontaneous alternation (p<0.01 vs DNP or SNM alone), indicating superior restoration of working memory.

In the NOR test, Model group mice displayed a significantly lower discrimination index compared to the Sham group (p<0.001), reflecting impaired recognition memory (Table 1). Both DNP and SNM monotherapies improved the discrimination index (p<0.05 vs. Model), but the DNP+SNM combination therapy produced a significantly higher discrimination index (p<0.01 vs. DNP or SNM alone), demonstrating a more robust improvement in recognition memory.

As presented in Table 2, Aβ1-42 injection in the Model group led to a significant increase in hippocampal AChE activity (p<0.001) and a significant decrease in ChAT levels (p<0.001) compared to the Sham group, indicative of cholinergic dysfunction. Donepezil treatment alone significantly reduced AChE activity and increased ChAT levels, as expected. Sinomenine also showed a mild but significant beneficial effect on both parameters (p<0.05 vs. Model). Notably, the combination of DNP+SNM resulted in the most pronounced restoration of cholinergic balance, with significantly lower AChE activity and higher ChAT levels compared to either monotherapy (p<0.01 vs. DNP or SNM alone), suggesting a synergistic enhancement of cholinergic function.

As shown in Table 3, Aβ1-42 injection significantly increased hippocampal MDA levels (a marker of lipid peroxidation, p<0.001) and significantly decreased the activity of the antioxidant enzyme SOD (p<0.001) and the levels of the antioxidant GSH (p<0.001) in the Model group compared to the Sham group. Both Donepezil and Sinomenine monotherapies partially ameliorated these oxidative stress markers

Table 3: Effects of donepezil, sinomenine, and combination therapy on hippocampal oxidative stress markers

Group	MDA levels (nmoL/mg protein)	SOD activity (U/mg protein)	GSH levels (μmoL/mg protein)
Sham	0.78±0.06	12.5±0.8	2.15±0.12
Model	2.35±0.18$^{###}$	5.1±0.5$^{###}$	0.88±0.07$^{###}$
Donepezil	1.62±0.15*	7.9±0.6*	1.34±0.09*
Sinomenine	1.58±0.14*	8.3±0.7*	1.41±0.10*
Combination	0.95±0.08$^{**\#\#}$	11.9±0.9$^{**\#\#}$	2.05±0.11$^{**\#\#}$

Data are expressed as Mean±SEM (n = 10 per group), $^{###}$p<0.001 vs Sham group, p<0.05 vs Model group; **p<0.01 vs Model group, $^{\#}$p<0.05 vs, Donepezil or Sinomenine monotherapy group; $^{\#\#}$p<0.01 vs Donepezil or Sinomenine monotherapy group

Table 4: Effects of donepezil, sinomenine, and combination therapy on hippocampal Nrf2 pathway markers

Group	Nuclear Nrf2 (relative wxpression)	HO-1 (relative expression)	NQO1 (relative expression)
Sham	1.00±0.05	1.00±0.06	1.00±0.05
Model	0.40±0.03$^{###}$	0.35±0.04$^{###}$	0.38±0.03$^{###}$
Donepezil	0.48±0.04	0.42±0.05	0.45±0.04
Sinomenine	0.75±0.06*	0.68±0.05*	0.70±0.06*
Combination	0.95±0.07$^{**\#\#}$	0.92±0.07$^{**\#\#}$	0.93±0.06$^{**\#\#}$

Data are expressed as Mean±EM (n = 10 per group). Relative expression normalized to respective loading controls (Lamin B1 for nuclear Nrf2, β-actin for HO-1 and NQO1) and then to the Sham group mean, $^{###}$p<0.001 vs Sham group, p<0.05 vs Model group; **p<0.01 vs Model group, $^{\#}$p<0.05 vs Sinomenine monotherapy group; $^{\#\#}$p<0.01 vs Sinomenine monotherapy group and (Donepezil monotherapy was not significantly different from the Model group for Nrf2 pathway markers)

(p<0.05 vs Model). However, the DNP+SNM combination therapy demonstrated a significantly greater reduction in MDA levels and a more robust increase in SOD activity and GSH levels compared to either DNP or SNM treatment alone (p<0.01 vs DNP or SNM alone), indicating powerful synergistic anti-oxidative effects.

Western blot analysis of hippocampal tissues revealed that the Aβ1-42-induced Model group had significantly reduced nuclear Nrf2 protein levels and decreased expression of its downstream targets, HO-1 and NQO1, compared to the Sham group (p<0.001), consistent with impaired Nrf2-dependent anti-oxidative resilience (Table 4). Sinomenine monotherapy significantly increased nuclear Nrf2, HO-1, and NQO1 expression (p<0.05 vs. Model). Donepezil alone had a minor, non-significant effect on these markers. Strikingly, the DNP+SNM combination group exhibited a significantly greater increase in nuclear Nrf2, HO-1, and NQO1 expression compared to sinomenine monotherapy (p<0.01 vs. SNM alone), demonstrating a potent synergistic activation of the Nrf2 pathway.

DISCUSSION

Current study provides compelling evidence for the synergistic neuroprotective effects of low-dose donepezil and sinomenine hydrochloride in an Aβ1-42-induced mouse model of cognitive decline. The key findings demonstrate that this combination therapy significantly improved cognitive function, restored cholinergic balance, ameliorated oxidative stress, and potently activated the Nrf2-dependent anti-oxidative pathway, with superior outcomes compared to either monotherapy.

The Aβ1-42 ICV injection model successfully recapitulated key features of AD-related cognitive impairment, as evidenced by significant deficits in spatial learning and memory (MWM), working memory (Y-maze), and recognition memory (NOR) in the Model group. These behavioral impairments were accompanied by hallmarks of cholinergic dysfunction (increased AChE and decreased ChAT) and pronounced oxidative stress (increased MDA, reduced SOD and GSH) in hippocampal tissues. These findings are consistent with the established roles of Aβ in driving neurotoxicity and cognitive decline[18].

Donepezil, as expected, partially improved cognitive function and restored cholinergic tone, which aligns with its known mechanism of AChE inhibition[4]. However, its effects on oxidative stress and the Nrf2 pathway were modest or non-significant, reflecting its primary symptomatic role. This observation highlights the limitations of targeting a single pathway in a complex disease like AD.

Sinomenine monotherapy, on the other hand, demonstrated significant improvements across all measured parameters, including cognitive function, cholinergic markers, and oxidative stress. Importantly, sinomenine significantly activated the Nrf2 pathway, leading to increased expression of HO-1 and NQO1, and subsequently boosting endogenous antioxidant capacity. This supports the growing body of evidence for sinomenine's multifaceted neuroprotective actions, potentially through its anti-inflammatory and antioxidant properties[11,19-22]. The ability of sinomenine to modulate the Nrf2 pathway positions it as a promising therapeutic candidate for neurodegeneration.

The most striking and clinically relevant finding of this study is the potent synergistic effect observed with the combination of low-dose donepezil and sinomenine. In all behavioral tests (MWM, Y-maze, NOR), the combination group exhibited significantly superior cognitive improvements compared to either monotherapy, often restoring performance to near-sham levels. This enhancement was mirrored by a more robust restoration of cholinergic markers (AChE and ChAT) and a more pronounced reduction in oxidative stress (MDA) and increase in antioxidant capacity (SOD, GSH) than individual treatments. Critically, the combination therapy led to a significantly greater activation of nuclear Nrf2 and its downstream targets (HO-1, NQO1) compared to sinomenine alone, indicating a potentiation of Nrf2-dependent resilience.

The observed synergy can be explained by the complementary mechanisms of action. Donepezil primarily addresses the cholinergic deficit, a symptomatic component of cognitive decline. Sinomenine, by activating the Nrf2 pathway, directly combats oxidative stress, a key pathological driver of neurodegeneration[6]. This dual-target approach – symptomatic relief combined with disease-modifying potential – appears to be far more effective than either strategy alone. Furthermore, reducing the donepezil dose in the combination strategy could mitigate its dose-dependent side effects, thus enhancing patient compliance and long-term tolerability. This is particularly important given the chronicity of cognitive decline and the need for prolonged treatment. It is plausible that by reducing neuroinflammation and oxidative stress through Nrf2 activation, sinomenine may indirectly enhance the survival and function of cholinergic neurons, thereby making the low-dose donepezil more effective in maintaining cholinergic tone.

This study contributes significantly to the understanding of multi-target therapeutic strategies for cognitive decline. It provides a mechanistic basis for the beneficial effects of sinomenine in the context of neuroprotection and highlights the potential for synergistic interactions with established anticholinesterase inhibitors. These findings are consistent with the growing paradigm shift towards combination therapies that address the diverse pathological cascades involved in complex neurological disorders[13].

Despite the promising results, this study has certain limitations. The Aβ1-42 ICV injection model is an acute model of cognitive impairment and does not fully replicate the chronic and progressive nature of human AD, which involves additional pathologies like tau tangles. Future studies should explore this combination in more chronic or genetic AD models to evaluate long-term efficacy and impact on broader AD pathology (e.g., Aβ plaque burden, tau phosphorylation, neuronal loss, neuroinflammation markers like GFAP and Iba1). Additionally, pharmacokinetic and pharmacodynamic interactions between donepezil and sinomenine warrant further investigation to optimize dosing and understand potential drug-drug interactions.

CONCLUSION

The study demonstrates that the synergistic combination of low-dose donepezil and sinomenine hydrochloride represents a highly promising therapeutic strategy for cognitive decline. This combination effectively ameliorates cognitive deficits by simultaneously restoring cholinergic function and robustly

activating the Nrf2-dependent anti-oxidative pathway, surpassing the efficacy of either monotherapy. These findings provide a strong rationale for further translational research and clinical investigation into this novel multi-target approach, potentially offering a more effective and safer treatment option for patients suffering from cognitive impairment.

SIGNIFICANCE STATEMENT

This study discovered the synergistic neuroprotective potential of combining low-dose donepezil with sinomenine hydrochloride, which can be beneficial for enhancing cognitive function, reducing oxidative stress, and supporting cholinergic balance in neurodegenerative disorders such as Alzheimer's disease. By demonstrating strong activation of the Nrf2 antioxidant pathway, this study will help researchers uncover the critical areas of combination-based neuroprotection that many were not able to explore. Thus, a new theory on multi-target therapeutic strategies for mitigating cognitive decline may be arrived at.

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