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MEMORY-ENHANCING EFFECT OF DONEPEZIL AND VITAMIN E COMBINATION IN SCOPOLAMINE-INDUCED AMNESIA

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Abstract

Scopolamine-induced amnesia is a standard rodent model for testing drugs that might improve memory, and it has been used to study cholinesterase inhibitors and antioxidants alike. This review summarises published work relevant to combining donepezil, an acetylcholinesterase inhibitor, with vitamin E, a lipid-soluble antioxidant, in that model. It covers the cholinergic and oxidative basis of scopolamine amnesia, the pharmacology of each agent, behavioural and biochemical outcome measures, and the logic and the limits of combination testing. The literature supports a plausible rationale but contains few direct combination studies, and the model captures only part of human dementia. The article is a narrative review and reports no new data.

1. INTRODUCTION

Forgetting is a common complaint and a frightening one when it becomes persistent. For the large group of people living with Alzheimer's disease, the available drugs ease symptoms for a time but do not stop the underlying loss of neurons. Donepezil is the most widely prescribed of these drugs, and it works by slowing the breakdown of acetylcholine in the brain [1, 2]. Its effect is real but modest, and that modesty has prompted repeated suggestions to add a second agent that acts through a different route. Antioxidants have drawn particular interest, and vitamin E is the best known of them [3, 4].

Before any such pairing reaches patients, it has to be tested in animals, and the test needs a model of memory failure that is quick, reproducible and sensitive to drugs. Scopolamine-induced amnesia fits that description. A single injection in a rat or mouse produces a transient deficit in learning that many cholinergic drugs can reverse, which makes the model a workhorse of early screening [5, 6]. Its simplicity is also the reason for caution, since a reversible pharmacological blockade is not the same thing as a progressive neurodegenerative disease [6, 7].

Dementia is a growing public health problem as populations age, and the failure rate of drug candidates is very high. Preclinical models serve as the first filter, and a considerable share of what is known about cholinergic and antioxidant approaches comes from them [4, 8]. Because the clinical stakes are high, the quality of that early evidence matters. Models that are cheap and quick are tempting, but poorly designed or poorly reported studies waste animals and mislead later work, which is a point the ARRIVE reporting guidelines and work on experimental design in laboratory animals were written to address [6, 9].

The question addressed here is a narrow one. What does the published literature say about the memory-enhancing effect of donepezil and vitamin E, given together, against scopolamine-induced amnesia? Direct reports of the exact combination are few, so the answer has to be assembled from three bodies of evidence: work on the scopolamine model itself, work on donepezil, and work on vitamin E in oxidative and cognitive settings. Each is summarised in turn, and the results are brought together in table 1, with the distribution of the evidence shown in fig. 1 [1, 3, 6].

This is a narrative review, and it reports no new data. It does not describe any experiment carried out by the authors, and it does not present doses or outcomes as findings of this article. Literature up to 2023 was drawn on, with priority given to primary experimental papers, major reviews and randomised clinical trials. Where the evidence is thin or inconsistent, the text says so. Methods are described in enough detail for a reader to see how an eventual combination study might be planned and reported [9, 10]. Doses and treatment schedules used in the animal literature are quoted only where they illustrate a general point, and none is offered as a recommendation, since suitable regimens depend on species, strain and the question asked.

Table 1: Models, outcome measures and evidence relevant to donepezil with vitamin E against scopolamine amnesia

Item	Reported feature or finding	Ref.
Scopolamine muscarinic antagonist	Blocks muscarinic receptors and impairs acquisition more than retrieval; accepted model of cholinergic deficit.	[5, 6, 7]
Morris water maze spatial memory	Escape latency and probe-trial time reveal deficits that cholinesterase inhibitors partly reverse.	[11, 12, 13]
Passive avoidance step-through	One-trial associative task; step-through latency falls after scopolamine and rises with cholinergic enhancement.	[6, 14]
Novel object recognition recognition memory	Preference for a new object; impaired by scopolamine and by oxidative insults, free of aversive stimuli.	[6, 15]
Donepezil reversible AChE inhibitor	Raises hippocampal acetylcholine in rats and reverses scopolamine deficits at low doses.	[1, 16, 17]
Donepezil trials Alzheimer's disease	Small benefits on cognition and global function; cholinergic adverse effects limit dose.	[2, 18, 19]
Lipid peroxidation TBARS assay	Malondialdehyde rises after scopolamine and falls with antioxidant treatment.	[20, 21]
Vitamin E alpha-tocopherol	Chain-breaking antioxidant that protected rats from age-related and amyloid-induced memory deficits.	[3, 22, 23]
Vitamin E trials dementia and MCI	Functional benefit in some trials; no delay of progression in mild cognitive impairment.	[24, 25, 26, 27]

Item	Reported feature or finding	Ref.
Combination design synergy analysis	Dose-response and isobolographic methods are needed to separate synergy from addition.	[4, 28, 29]

A note on terminology may help. Amnesia in this context means an experimentally induced and reversible failure of learning or recall, not the clinical syndrome. Cognition and memory are used interchangeably in many rodent papers, though the tests usually address specific processes such as spatial reference memory or recognition memory. The distinction becomes important later, when results from different tests are compared [6, 13]. Mixing the two meanings leads to claims about 'curing' memory loss that the models cannot support. Careful wording protects against that error, and it also helps readers who work in different disciplines to understand each other. Reviews that keep the terms separate are easier to apply [6, 13].

2. THE SCOPOLAMINE AMNESIA MODEL

Scopolamine is a non-selective muscarinic receptor antagonist that crosses the blood-brain barrier readily. The use of anticholinergic drugs to disrupt memory has a long history, and the classic human study by Drachman and Leavitt showed that scopolamine given to young volunteers produced memory deficits resembling those of normal ageing [5]. That observation fed directly into the cholinergic hypothesis of geriatric memory dysfunction, which proposed that a loss of cholinergic function in the ageing brain contributes to the decline in memory [8, 30]. The hypothesis has been refined since, but it still supplies the rationale for cholinesterase inhibitors [8].

In rodents, doses in the range of about 0.5 to 3 mg/kg given intraperitoneally some time before training or testing impair acquisition in many tasks, with a weaker effect on retrieval of an already learned response [6]. The review by Klinkenberg and Blokland remains the most thorough assessment. It concludes that scopolamine reliably disrupts attention, working memory and encoding, that cholinesterase inhibitors reverse these effects in a dose-dependent way, and that the model therefore has predictive validity for cholinergic drugs in particular [6]. The same analysis stresses that the evidence is far less convincing for drugs working through other mechanisms [6].

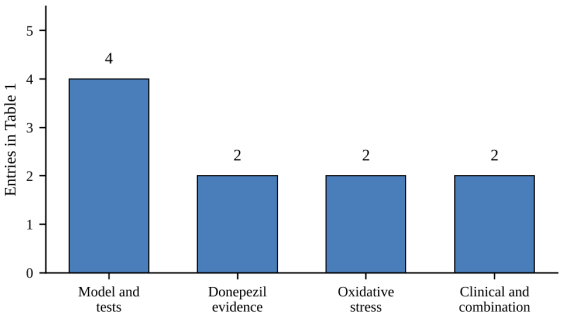


Fig. 1: Number of entries in table 1 by type of evidence

The model has real weaknesses. Scopolamine acts on peripheral muscarinic receptors as well as central ones, so tachycardia, dry mouth and altered locomotion can confound behaviour. A drug that reverses a performance deficit may do so by changing arousal, anxiety or sensory function and not memory itself [6]. Scopolamine also changes the activity of several other transmitter systems indirectly. Human studies using the drug report electroencephalographic and cognitive changes that resemble those of dementia in some respects but not all, and the reversible nature of the blockade makes it a poor mimic of a progressive disease [7].

Practical details of the model are less standardised than the literature suggests. Mice and rats differ in sensitivity, and so do strains, sexes and ages. Scopolamine hydrobromide and methylscopolamine behave differently because the second does not cross the blood-brain barrier, which makes it a useful control for peripheral effects [6]. The interval between injection and test is usually about 30 minutes, and doses that disturb locomotion or exploration can masquerade as memory deficits in some tasks [6, 12]. These variables explain part of the variation in published results and should be stated in any report [6, 7].

Oxidative stress is the aspect most relevant to a vitamin E study. Several experimental reports describe higher brain malondialdehyde and lower levels of superoxide dismutase and glutathione in

rodents given scopolamine, along with changes in acetylcholinesterase activity, and these changes can be moderated by antioxidant treatment [20, 21]. The finding gives the model a second, non-cholinergic dimension and opens it to antioxidants. It also complicates interpretation, because an antioxidant effect on behaviour may be secondary to cholinergic effects, or the reverse [20, 22]. Reports in the literature also note that the oxidative changes appear within hours, which suggests that they reflect acute neuronal stress and not only chronic damage [20, 22].

Scopolamine is also administered in humans in early-phase studies as a challenge test. Healthy volunteers receive a small dose so that a candidate procognitive drug can be tested for its ability to reverse the deficit before patient trials begin. The approach has had mixed results. Reversal of the deficit by donepezil is reasonably consistent, but reversal by drugs with other mechanisms is less reliable [6, 7]. The human challenge is therefore a useful bridge to the clinic for cholinergic agents but a weaker one for others [6, 7]. Doses, timing and the choice of memory task all differ between challenge studies, and so does the selection of volunteers, so pooled summaries of reversal effects should be read with care [6, 7]. Even so, the human work is valuable in showing that the cholinergic mechanism seen in animals also operates in people.

3. DONEPEZIL: PHARMACOLOGY AND EVIDENCE

Donepezil hydrochloride was developed in the late 1980s as a piperidine derivative with high selectivity for acetylcholinesterase over butyrylcholinesterase and a reversible mode of inhibition [1]. Its long plasma half-life in humans, of the order of three days, allows once-daily dosing. Microdialysis experiments in rats showed that donepezil raised extracellular acetylcholine in the hippocampus, the structure most closely tied to the memory tasks used in the scopolamine model [16, 17]. That result links the pharmacology directly to the behavioural endpoints of interest. It is also much less active against other cholinesterase-like enzymes, which helps to explain a tolerability profile that is gentler than that of earlier inhibitors such as tacrine.

In animals, donepezil reverses scopolamine-induced deficits across several tests, including passive avoidance, the water maze and recognition tasks, usually at doses in the low milligram per kilogram range [1, 6]. Reviews of the pharmacology emphasise that the effect follows an inverted-U relationship in some paradigms, so that high doses lose their advantage or cause cholinergic side effects such as tremor and salivation [1, 17]. For a combination study this matters, because a dose of donepezil that is itself already maximally effective leaves little room to show any additional gain from vitamin E [28, 29].

Clinical results are less dramatic than the animal data. In a 24-week randomised trial in mild to moderate Alzheimer's disease, donepezil at 5 and 10 mg daily improved scores on the cognitive subscale of the Alzheimer's Disease Assessment Scale and on clinician-rated global change relative to placebo [2]. The Cochrane review of the later trials found small benefits on cognition, daily living and global assessment, with nausea, diarrhoea, vomiting and insomnia more frequent than with placebo [18, 19]. These effects are measurable but modest, and they do not alter the course of the disease [17, 18].

The gap between animal and human efficacy has several explanations, and they are relevant to anyone designing a combination. The model examines a single pharmacological deficit in healthy animals, whereas patients lose neurons, synapses and several transmitter systems. Add-on strategies are therefore appealing in principle, and published reviews of combination treatment for Alzheimer's disease list antioxidants, memantine and other classes among the options considered [4, 26]. Few of those options have been tested rigorously in a model with matched pharmacological and oxidative components [4]. Reviews of Alzheimer's therapy also point out that benefits in animals are usually measured within hours or days of dosing, whereas human disease progresses over years, so the time scales hardly overlap [4, 17].

4. VITAMIN E AND OXIDATIVE STRESS

Vitamin E is a family of eight lipid-soluble compounds, the tocopherols and tocotrienols, of which alpha-tocopherol is the form retained in human tissues. Its best established function is as a chain-breaking antioxidant in membranes, where it donates a hydrogen atom to lipid peroxyl radicals and halts the propagation of lipid peroxidation [3]. The tocopheroxyl radical it forms can be regenerated by ascorbate, and this recycling helps to maintain the antioxidant capacity of cell membranes. Traber and Atkinson argue that this antioxidant function remains the most convincing basis for the vitamin's action, whatever other signalling roles have been proposed [3].

The brain is vulnerable to oxidative damage. It uses a large share of the body's oxygen, contains abundant polyunsaturated fatty acids that peroxidise easily, and has comparatively modest antioxidant defences [31]. Oxidative stress, defined as a disturbance of redox signalling and control with excess oxidants, has been proposed as a contributor to neuronal loss in ageing and in Alzheimer's disease [31]. Amyloid beta peptide has itself been linked to lipid peroxidation in affected brain regions, which offers a mechanism by which oxidative damage and the pathology of the disease might feed each other [32].

Experimental evidence supports a protective role for vitamin E. Rats made cognitively impaired by oxidative stress and ageing showed less impairment when given vitamin E, together with lower markers of lipid peroxidation in the brain [22]. In another study, alpha-tocopherol protected rats against learning and memory deficits induced by amyloid beta, and the protection accompanied reduced oxidative markers in tissue [23]. Both studies point to a link between oxidative status and performance in memory tasks. They do not prove that oxidative damage is the cause of the deficit, since vitamin E has effects on membrane properties and signalling too [3, 23].

There is an indirect link between oxidative damage and cholinergic function that makes the pairing more than a convenient guess. Oxidative stress can damage membrane proteins and impair the synthesis and release of acetylcholine, and cholinergic neurons are thought to be particularly exposed to such injury in the ageing brain [31, 32]. If so, protecting those neurons from oxidative damage could in principle sustain the response to a cholinesterase inhibitor. The argument is a reasonable one but rests mainly on cell and tissue studies, and direct evidence in behaving animals is scarce [22, 23, 32].

Human trials have been much less encouraging than the animal work. In moderately severe Alzheimer's disease, alpha-tocopherol at 2000 IU daily slowed the time to loss of ability to perform basic activities, institutionalisation or death in one controlled trial [24]. In mild cognitive impairment, however, vitamin E did not slow progression to Alzheimer's disease, while donepezil delayed it for the first year [25]. A later trial in mild to moderate disease found slower functional decline with vitamin E than with placebo [26]. A Cochrane analysis concluded that evidence of benefit is inconsistent and of low quality [27].

Safety is a limit on how far these doses can be pushed. A meta-analysis of randomised trials suggested that supplementation at 400 IU daily or more was associated with higher all-cause mortality, although the finding has been contested and later work has not settled the matter [33]. This is a reminder that antioxidants are not harmless simply because they occur in food. Preclinical dose choices that cannot be translated into tolerable human doses have limited value, and a careful study would report the dose in terms that allow such a comparison [33, 34].

Different forms of the vitamin are not interchangeable. Synthetic alpha-tocopherol contains a mixture of stereoisomers, while the natural form is a single stereoisomer retained preferentially by the hepatic tocopherol transfer protein. Studies that do not state the form, ester and vehicle used make it harder to compare results, and oil-based vehicles themselves can influence behaviour in rodents when given repeatedly [3, 34]. Studies that report only the dose in milligrams per kilogram, without these details, leave the reader unable to judge how much vitamin was

actually delivered to the brain, where concentrations rise slowly and depend on the diet of the animals.

5. OUTCOME MEASURES AND METHODS

Behavioural tests in the scopolamine model assess different aspects of memory. The Morris water maze measures spatial learning through the time an animal takes to find a hidden platform, with a probe trial that tests memory for its location [11, 12]. Passive avoidance uses the latency to enter a compartment where the animal has received a mild footshock, and so tests an associative memory acquired in a single trial [14]. Novel object recognition exploits the rodent's tendency to spend more time with a new object, and it does not depend on aversive stimuli [15]. Each task depends differently on the hippocampus and cortex, and they respond differently to cholinergic blockade [13, 35].

Acetylcholine's role in these tasks has been mapped in some detail. Cholinergic projections to the hippocampus and cortex are thought to adjust the balance between encoding of new information and retrieval of stored information, with high acetylcholine levels favouring encoding [35]. Deiana and colleagues reviewed the evidence that cholinergic manipulation affects spatial learning, and reported that the effect depends on the type of task, the site of action and the timing of treatment [13]. These findings help to explain why scopolamine impairs acquisition most strongly and why the pattern of reversal by a drug depends on when it is given [13, 35].

Biochemical endpoints complement the behaviour. Acetylcholinesterase activity in brain homogenates is usually measured with the colorimetric method of Ellman and colleagues, which follows the reaction of thiocholine with a chromogen [36]. Lipid peroxidation is commonly assessed by the thiobarbituric acid reactive substances assay, which estimates malondialdehyde and is simple but not fully specific [21]. Antioxidant enzymes and reduced glutathione are measured with further assays. Put simply, these measures allow a study to ask whether any behavioural benefit parallels a cholinergic change, an oxidative change, or both [21, 36]. Immunohistochemical and gene-expression readouts add detail but are used less often, and they are costly for small studies.

A combination study needs more groups than a single-drug study, and the groups multiply quickly. A sound layout would include a vehicle control, a scopolamine-only group, donepezil alone, vitamin E alone and the combination, with a positive control if comparison with a known reference is wanted. Animals should be allocated at random, the observer should be blind to treatment, and the sample size should be justified in advance [9, 10]. Route, vehicle, interval between the last dose and testing, and the order of tests all need recording, since each can alter results, and animal care should follow accepted guidelines [34].

Reporting quality is a recurring problem in this literature. The ARRIVE guidelines ask authors to describe randomisation, blinding, sample size justification, exclusion criteria and the exact conditions of testing, and work on the design and conduct of animal experiments stresses the same items [9], while analyses of statistical power in neuroscience show how little a small study can establish [10]. Many scopolamine studies give little of this information, and use small groups without a stated power calculation. Housing and handling follow national guidance on laboratory animal care, which also sets limits on the severity of procedures [34]. Without transparent reporting, a modest effect in a small study is hard to trust [9, 10].

6. RATIONALE AND EVIDENCE FOR THE COMBINATION

The case for combining donepezil with vitamin E rests on complementary mechanisms. Donepezil raises acetylcholine by inhibiting its breakdown, which addresses the transmitter deficit produced by scopolamine. Vitamin E addresses the oxidative changes that accompany scopolamine treatment and neurodegeneration. If both contribute to the behavioural deficit, then acting on both might give more than either alone [1, 3, 20]. The reasoning is plausible, and it is the basis for

most proposals to combine cholinesterase inhibitors and antioxidants. It is still only a hypothesis until direct tests are run [4]. It is also a reasonable argument for testing both at doses below those that each would need alone.

What does exist? The clinical trial comparing donepezil, vitamin E and placebo in mild cognitive impairment did not test the two together, but it did show that the cholinesterase inhibitor delayed progression for a year, and that vitamin E did not [25]. Experimental papers have studied antioxidants alongside cholinesterase inhibitors in scopolamine models, usually reporting that the combination improved behaviour and lowered malondialdehyde more than either agent alone. Direct studies of donepezil with vitamin E specifically are limited in number, differ in design and doses, and few meet reporting standards [9, 10]. Conclusions should therefore be tentative.

Expected patterns of results deserve thought before an experiment is run. If donepezil alone already restores performance close to the control level, a ceiling effect hides any gain from adding vitamin E, and the study cannot answer its own question. If a submaximal dose of donepezil is used, an apparent improvement with vitamin E becomes possible, but then it is essential to include vitamin E alone as a control, because an effect on its own would complicate the reading of the combination [6, 28, 29]. These design choices, rather than the compounds, often decide whether a study is informative.

A proper test of the combination requires a statistical design that separates a true interaction from simple addition. Isobolographic analysis and the median-effect method of Chou and Talalay were created to quantify synergy, additivity or antagonism between two drugs [28, 29]. Both need dose-response curves for each agent alone and in fixed ratio, so a design with only single dose levels of each drug and their mixture cannot show synergy. Many reports describe an enhanced effect of a combination as synergistic with no such analysis, and the word should be treated with suspicion in the absence of one [28, 29].

A second concern is pharmacokinetic interaction. Vitamin E is lipophilic and absorbed with fat, and it could in principle alter exposure to a co-administered drug. Donepezil is metabolised mainly by CYP2D6 and CYP3A4, and no strong interaction with vitamin E has been described, but this has not been investigated systematically in the scopolamine model [1, 17]. Measuring plasma or brain levels of donepezil in the combined group would remove the doubt. Without such data, an apparently greater effect might reflect altered exposure and not an interaction of mechanisms [17, 28].

Time-course also matters. Scopolamine acts for a few hours, and donepezil is given shortly before it, but antioxidant protection through vitamin E usually needs repeated dosing over days or weeks to raise tissue levels. A design that gives vitamin E for a single day and sees no benefit says little. A pre-treatment period of one to several weeks is common in the studies that report positive effects, and it should be documented clearly when results are compared [3, 22, 23]. Sampling brain tissue at the same time point after the final dose for both agents allows biochemical and behavioural results to be read together, which is neater than combining them across studies.

7. SAFETY AND TRANSLATION

Translating any positive result into practice calls for caution. The adverse effects of donepezil are cholinergic and include nausea, vomiting, diarrhoea, muscle cramps, bradycardia and sleep disturbance, and they limit dose escalation in some patients [2, 18, 19]. Vitamin E is better tolerated at moderate doses, but higher doses carry concern about bleeding risk and mortality [33]. A combination should therefore be judged by whether it permits a lower dose of donepezil with equal benefit, or a higher overall effect at the same level of side effects, and not by efficacy alone [18, 33].

Polypharmacy is a practical issue in the older adults who would be the likely users. Many commonly prescribed drugs carry anticholinergic activity, including some antihistamines, bladder antimuscarinics and tricyclic antidepressants, and in effect they

reproduce a mild version of the scopolamine challenge that cholinesterase inhibitors are meant to correct [5, 18]. A patient given donepezil alongside such a drug receives opposing actions, and the net benefit is likely to be reduced. Reviewing the whole medication list is, in this sense, a cheaper way of supporting cholinergic function than adding a second agent [18, 19].

The clinical literature gives limited support for the combination being tried. The only large trial that placed donepezil and vitamin E in the same design did so in mild cognitive impairment as separate arms, and the result favoured donepezil alone for a limited period [25]. The trial of vitamin E and memantine in established Alzheimer's disease suggested functional benefit with vitamin E, which is the strongest positive human signal, but cannot be generalised to cognitive endpoints or to other combinations [26]. Cummings and colleagues, reviewing treatment combinations, note that most proposed pairings have never been tested in properly designed human trials [4].

8. LIMITATIONS AND FUTURE DIRECTIONS

This review has several limits. The evidence on donepezil together with vitamin E in scopolamine-induced amnesia is sparse, and much of the argument has been built from adjacent studies, which may not transfer. Most rodent reports use young, healthy animals of one sex, short treatment periods and a single behavioural test, and they seldom report whether the person scoring behaviour was unaware of the group allocation [6, 9, 10]. Publication bias towards positive findings is likely in a field where a reversal of the scopolamine deficit is a common and easy result to obtain [6, 10].

The translational gap deserves direct comment. Procognitive compounds have repeatedly shown activity in scopolamine models and then failed to help patients, and the history of drug development in Alzheimer's disease is dominated by such disappointments [4, 6]. Part of the explanation is that the model responds to almost any increase in cholinergic tone, so a positive result has low discriminating power. Part is that clinical benefit must be shown on function and cognition over months, and not on the success of a rat in a maze over minutes. Even the one modest positive signal for vitamin E came from a trial in established disease and not from prevention [24, 26].

Future work could improve on this in practical ways. Studies should use dose-response designs capable of testing interaction, include a donepezil-alone arm at an effective but not maximal dose, measure drug and vitamin levels, and combine at least two memory tests with biochemical endpoints [21, 28, 29, 36]. Models with a chronic, progressive component, such as amyloid beta infusion or transgenic animals, would address the main criticism of the scopolamine model [4, 23]. Pre-registration and adherence to reporting guidelines would make small studies easier to evaluate and combine [9, 10].

9. CONCLUSION

The published literature gives a reasoned but unproven case for combining donepezil and vitamin E against scopolamine-induced amnesia. Scopolamine produces a cholinergic deficit with an oxidative component, donepezil addresses the first, and vitamin E addresses the second, so the two mechanisms are complementary on paper [1, 3, 5, 6, 20]. The behavioural and biochemical tools for testing the idea are well established and inexpensive, which makes the shortage of rigorous combination studies the more striking [11, 21, 36]. That makes the pairing a sound subject for a well-controlled experiment, and not yet a basis for any claim.

Human data should temper enthusiasm. Donepezil gives small, consistent benefits, while vitamin E has produced mixed results and carries a mortality signal at high doses [18, 24, 25, 33]. A well-designed study would test interaction with proper dose-response methods, report to ARRIVE standards, and be honest about how little a reversible pharmacological model can say about a progressive disease [6, 9, 28, 29]. Until such work appears, claims for a memory-enhancing combination remain a hypothesis worth testing and not a result to be relied upon [4]. Pharmacists and clinicians can help by keeping questions about

supplements and unproven combinations anchored to this kind of evidence.

10. ABBREVIATIONS

AChE-Acetylcholinesterase, ARRIVE-Animal Research: Reporting of In Vivo Experiments, CYP-Cytochrome P450, IU-International unit, MCI-Mild cognitive impairment, MDA-Malondialdehyde, SOD-Superoxide dismutase, TBARS-Thiobarbituric acid reactive substances

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Nil

12. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

13. CONFLICT OF INTERESTS

Declared none

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