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RESEARCH ARTICLE

Oral intake of carboxy alkyl ester improves attention: A randomized double-blind cross-over placebo-controlled study

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Abstract

Objective: To test the null hypothesis that oral intake of the dietary supplement carboxy alkyl ester (CAE) would have no effect on attention as revealed by mean rapid visual information processing (RVIP) scores.

Methods: In a randomized double-blind cross-over placebo-controlled trial, healthy participants (age 19–66 years) of both sexes were randomly assigned to consume 700 mg of CAE or 700 mg of placebo. They received baseline attention testing via the RVIP task. Then they consumed CAE or placebo followed by RVIP testing. Participants were then given a washout period where they did not consume CAE or placebo. Afterward, individuals who initially consumed CAE were given the placebo and those who initially consumed the placebo were given CAE. Finally, all participants were tested again via RVIP.

Results: A priori statistical computation revealed that 30-day oral intake of CAE improved mean RVIP test scores ($t = 2.4$, $p < .05$) relative to that at baseline, which resulted in a rejection of the null hypothesis.

Conclusions: Daily oral intake of the CAE dietary supplement may boost attention and further research is now needed to confirm this observation.

KEYWORDS
cognitive enhancer, psychotropic, smart pill

1 | INTRODUCTION

Attention has been described as both a filter to cognition (e.g., selective attention) and a resource allocator of cognitive processes (e.g., divided attention) (Wickens, 2021). Ultimately, it is the center of the psychological enterprise and improving attention is believed to affect other cognitive or psychological domains (Franke et al., 2014). Throughout history there has been an interest in improving attention among healthy individuals who are free from cognitive deficiencies (Napoletano et al., 2020). For instance, individuals may seek to improve attention to gain a selective advantage in endeavors that span education, recreation, occupation and athletics.

Studies designed to demonstrate improvement in cognitive functions including attention have deployed a large variety of approaches that can be categorized as pharmaceutical (e.g., prescription medication), non-pharmaceutical (e.g., caffeinated beverages) and behavioral (e.g., exercise, yoga and cognitive behavioral intervention) (Schifano et al., 2022). Such studies often suggest that attention can be improved among healthy individuals with relatively normal cognition (Adelhöfer et al., 2018). However, the studies generally suffer from a multiplicity problem characterized by an inflated type-I error (false-positive) rate (Albers, 2019). For instance, a given study may implement one independent variable (e.g., one pharmaceutical or one non-pharmaceutical) but deploy more than one dependent

variable such as attention, language, speech, learning, etc. This increases the likelihood of rejecting the null-hypothesis to perpetuate an inflated type-1 error rate. Other studies may implement one independent variable and one dependent variable but conduct subgroup analyses which again inflates the type-1 error rate. Multiplicity is also perpetuated when studies deploy more than one independent variable (e.g., two or more drugs) and/or more than one measurement (e.g., Stroop test, Conners continuous performance test, stop signal reaction time, etc.). Therefore, it remains unclear whether attention can be improved among relatively healthy individuals.

In the current study we deployed a randomized double-blind cross-over placebo-controlled study design. To constrain multiplicity, we used a single independent variable, a single dependent variable and one measurement. The aim of the study was to test the null hypothesis that 30-day oral intake of carboxy alkyl ester (CAE) would have no effect on attention as revealed by mean rapid visual information processing (RVIP) scores. CAEs are low molecular weight esters of quinic acid that are found in various healthy foods (e.g., vegetables and fruits), beverages (e.g., teas and coffee) and medicinal plants (e.g., *Uncaria tomentosa* and *Polypodium leucotomos*) (Guthrie et al., 2011). They have been standardized through a patented extraction and purification protocol that produces a formulation that enhances endogenous cellular repair mechanisms in humans and rodents (Guthrie, 2016). CAEs are the active ingredients of a number of plant-based therapies and in the literature these therapies have been given various names, such as C-MED-100®, AC-11®, Vincaria®, *U. tomentosa*, quinic acid, etc. The nature of the protocol used to extract CAEs from medicinal plants is important because tincture preparations can lead to a large variety of side effects and the presence of unknown phytoconstituents may also lead to side effects (Batiha et al., 2020). The current study used a decoction preparation that show no signs of toxicity at concentrations that exceed 8 g/kg (Batiha et al., 2020). It is commercially available as a dietary supplement, but it is also used in the management of a wide range of health conditions including cancer, inflammation and arthritis, allergies, viral infections, and hypertension (Batiha et al., 2020). Interestingly, most of these reported benefits have not been confirmed with randomized controlled trials. However, in the osteoarthritis field evidence to support the efficacy and tolerability of CAE has been demonstrated in multicenter randomized double-blind placebo-controlled human trials (Mehta et al., 2007; Miller et al., 2005; Piscoya et al., 2001). We speculate that the lack of utility-patent protection has limited commercial interest in further research and development of CAE as a medicinal treatment. Furthermore, no previous study has demonstrated efficacy in improving attention among relatively healthy participants (the focus of the current study).

In the gastrointestinal tract, esterases from *Lactobacillus gasseri*, *bifidobacterium* and *Escherichia coli* drive hydrolytic cleavage of the CAE molecule to produce quinic acid (Pero & Lund, 2011). Quinic acid stimulates the bacterial shikimate pathway which is also known as the common aromatic biosynthetic pathway (Pero, 2010). The shikimate pathway is a major biosynthetic cascade for producing aromatic amino acids (tryptophan, phenylalanine, and tyrosine). Aromatic amino acids are involved in the production of monoamine neurotransmitters (norepinephrine, dopamine and serotonin) that regulate the level and quality of attention (Kodama et al., 2002). Therefore, oral consumption of CAE may improve attention by increasing bioavailability of monoamine neurotransmitters. The current work is the first attempt at determining whether oral consumption of CAE may improve attention. Literature reviews and research studies have suggested that CAE can be used to prevent or limit neurocognitive deficits (Batiha et al., 2020; Castilhos et al., 2020; Quinn et al., 2004; Shi et al., 2013; Snow et al., 2019). However, the studies suffer from multiplicity and inconsistent results. What is needed is a study that interrogates the null hypothesis and constrain multiplicity by deploying a single independent variable, a single dependent variable, and one measurement.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This study was conducted as a randomized double-blind cross-over placebo-controlled trial. The study was conducted with the approval and oversight of the university's institutional review board (IRB1710775-2) in compliance with the United States Department Health and Human Services, Office for Human Research Protections Federalwide Assurance (FWA #00000357). The nutritional supplement CAE (trademark AC-11® and manufactured by Optigenex Inc.), served as the independent variable and mean attention scores on the RVIP task served as the dependent variable. The aim of the study was to test the null hypothesis that 30-day oral intake of CAE would have no effect on attention as revealed by mean RVIP scores. The experimental design allowed for direct testing of the planned hypothesis and provided an opportunity to rule-in or rule-out chance effects. For instance, within the same group of subjects, we could determine whether CAE influenced attention (aim of the present study) and determine whether the effect was real by evaluating if the placebo also had an effect on attention. Furthermore, the design allowed us to rule-in or rule-out any learning effects from taking the RVIP task more than once.

Figure 1 illustrates the research design. Participants were randomized to one of two groups (CAE + PLACEBO or PLACEBO + CAE). No attempt was made to equalize the number of participants in each group, therefore random allocation resulted in 11 participants in the CAE + PLACEBO group and seven participants in the PLACEBO + CAE group. The CAE + PLACEBO group started the study by completing the RVIP task at baseline then they consumed CAE for 30 days (1-month). CAE consumption included oral intake of one 350 mg capsule twice daily (total of 700 mg/day) for 30 days. This daily regimen is based on the manufacturers recommended usage. Additionally, animal research has shown that 1 month of oral intake of CAE can provide trophic support to the nervous system, therefore a 30 days treatment duration was selected (Guthrie, 2012).

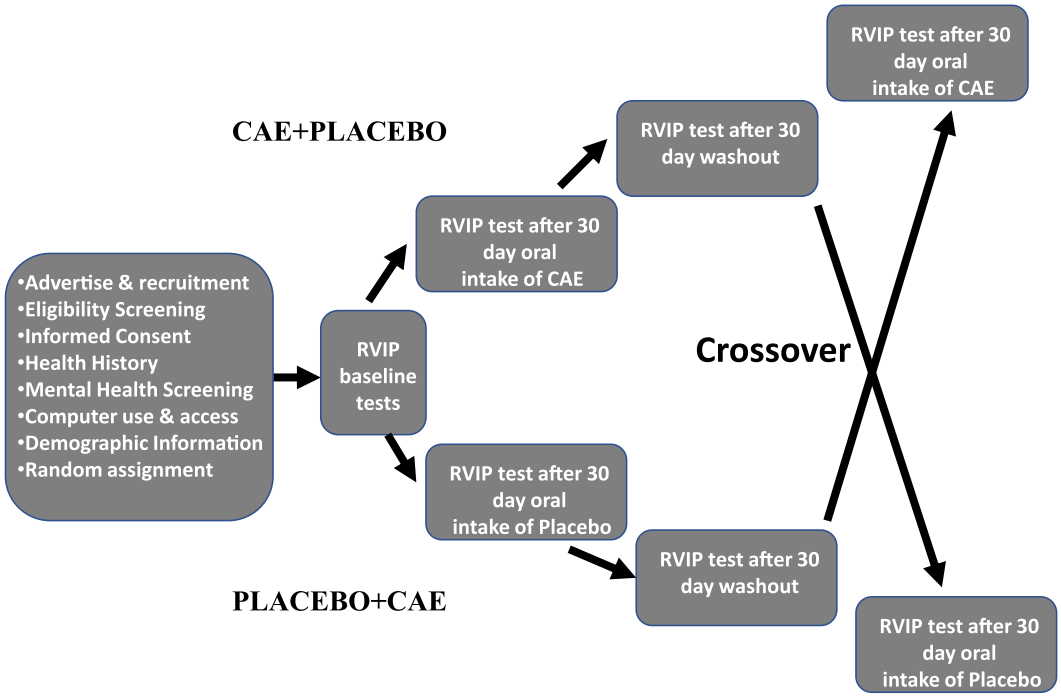

FIGURE 1 Research design. A randomized double-blind cross-over placebo-controlled trial. CAE, carboxy alkyl ester; RVIP, rapid visual information processing.

At the end of this month, participants took the RVIP task again to determine whether CAE induced an improvement in scores from baseline (primary outcome for the study). These same participants then experienced a washout period, where they did not take CAE for 1-month. Given that the participants consumed CAE for 30 days, a 30-day (1-month) washout period was assumed to be appropriate for this study. At the end of this washout period, the same participants took the RVIP task again. They then consumed the placebo (350 mg capsule twice daily, total of 700 mg/day) for 1-month and at the end of this month they took the RVIP task for the final time.

The PLACEBO + CAE group started the study by taking the RVIP task at baseline then they consumed placebo for 1-month. At the end of this month, they took the RVIP task again to determine whether the placebo had any positive effects relative to baseline. These same participants then experienced a washout period, where they did not take the placebo for 1-month. At the end of this washout period, the same participants took the RVIP task again. They then consumed CAE for 1-month and at the end of this month they took the RVIP task for the final time. All study staff (researchers) were blinded to the scoring of the RVIP assessments and tabulations. Unblinding occurred after test scoring, data tabulation, and statistical computation. Similarly, each participant was blinded to whether they were consuming CAE or the placebo. The CAE and placebo capsules were identical in appearance. The CAE capsules contained carboxy alkyl esters (active ingredient) and manioc maltodextrin (starch). CAE was extracted from the inner bark of the medicinal plant *U. tomentosa* via a patented decoction procedure (Guthrie et al., 2011). The placebo capsules were composed of manioc maltodextrin. The capsule materials were composed of titanium dioxide (food coloring).

2.2 | Participants

Adult (≥18 years old) female and male volunteers were invited to participate in the study from local media advertisements. All participants who completed the study were relatively healthy and free from known, (1) cognitive deficits, (2) concussions, (3) head injuries, (4) movement related pain, (5) dyslexia, (6) neurologic deficits, (7) psychiatric deficits, (8) psychologic symptoms, (9) motor deficits, (10) vision loss, (11) hearing loss, (12) cardiac deficits, (13) endocrine deficits, (14) high blood pressure, and (15) substance abuse. Participants were restricted in their consumption of dietary supplements, vitamins, caffeine, coffee, or tea during the study. To test the hypothesis a minimum sample size of 10 was desired because prior studies on dietary supplements have shown that a 5% improvement in cognitive function with a power of 0.80 can be expected with this sample size (Carswell et al., 2020). The focus of the hypothesis and the statistical analysis was on the CAE + PLACEBO group ($N = 11$) and the change in attention scores within this group before (baseline) and 1-month after CAE consumption (the planned comparison). The PLACEBO + CAE group ($N = 7$) was deployed to monitor the efficacy of the research design, and therefore not the focus of the hypothesis or statistical analysis.

Figure 2 illustrates the flow of participants through the study. Participants were recruited for the study via digital and print announcements in Arizona USA. These announcements explicitly described the eligibility criteria, that participants must be free from the following: health problems, pregnancy, breastfeeding, daily coffee and/or tea drinking, neurologic, psychiatric, and psychological disorders. Furthermore, the announcements explicitly indicated that

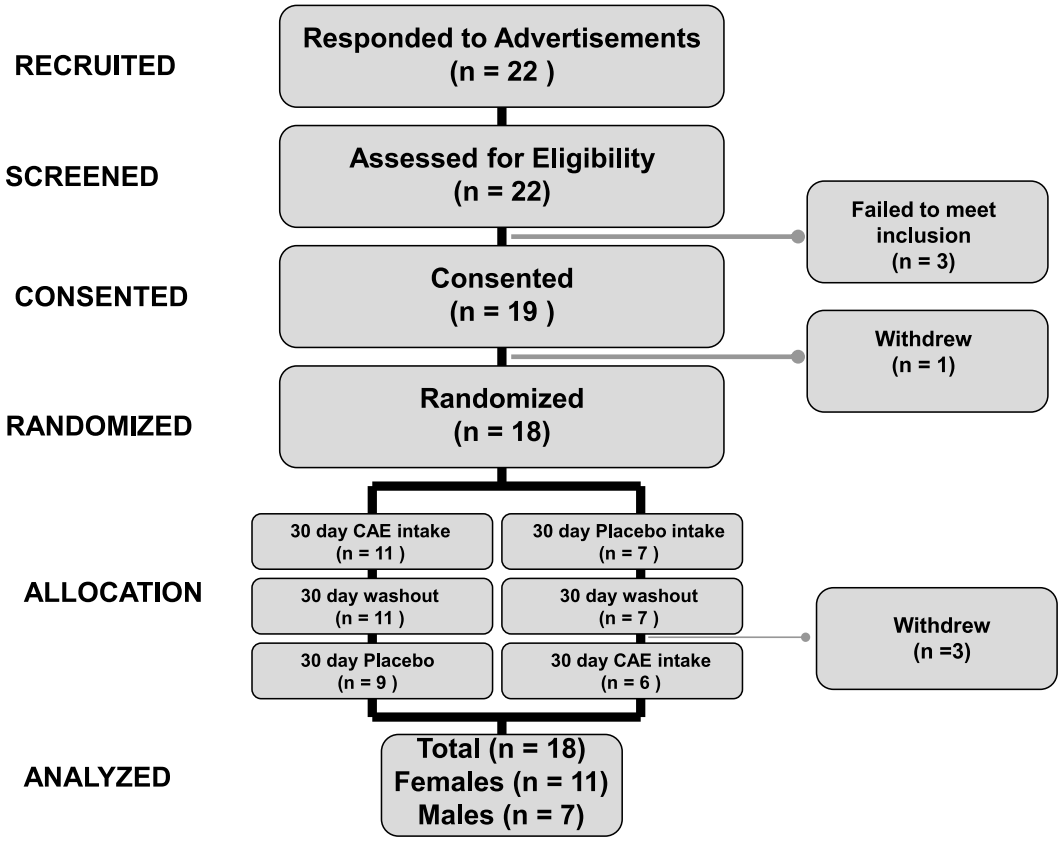

FIGURE 2 Flow of participants through the study. CAE, carboxy alkyl ester.

participants must have access to a computer for the study and they must exhibit the manual dexterity necessary to use the computer. Lastly, the announcements directed prospective participants to make an appointment for an initial intake interview. Prior to this initial interview each participant was randomly assigned to the CAE + PLACEBO group or the PLACEBO + CAE group via the random generator function in Microsoft Excel® v2209 (An et al., 2021). During the initial interview, the participants were screened for cognitive deficiency by taking and passing the mini-mental status examination (MMSE) (Folstein et al., 1975). Each participant was also queried to ascertain general health and neurologic status. Before a given participant could participate in the study, the participant had to achieve a passing score on the MMSE, then the participant was interviewed. During the interview the participant was asked if she/he was diagnosed with or believed to have any mental health issues or cognitive or neurologic conditions. Participants who indicated in the affirmative were excluded from entering the study. All participants who started and completed the study presented with relatively normal health and no known neurologic deficiencies. Additionally, participants were probed for eligibility for the study, and basic demographic data (sex, age, educational level, etc.) were collected from each participant (see Table 1). A total of 18 healthy participants of both sex (females, $n = 11$; males, $n = 7$) participated in the study. The participants ranged in age from 19 to 66 years old. They exhibited a range of educational achievements from high school to doctoral degrees. These participants self-identified as Caucasian, and Hispanic. All participants submitted informed written consent to participate in the study. To check compliance with the supplement the participants were required to maintain written logs of days and times they consumed the supplement. All participants, whether supplement or placebo, were 100% compliant. Participants had daily access to study staff where they had the opportunity to report any side effects and staff could monitor compliance. Furthermore, each participant received an exit interview which allowed for further compliance monitoring and reporting of side-effects. No adverse effects were encountered from participation in the current study.

2.3 | CANTAB: RVIP

In the current study attention was evaluated with an unsupervised computer automated assessment from the Cambridge Neuropsychological Test Automated Battery (CANTAB). CANTAB is published in over 2000 peer reviewed articles and is widely used in clinical, academic and pharmacologic research (Backx et al., 2020). The CANTAB assessment is fully automated (from training to testing to scoring and data tabulations) with visual on-screen and auditory voice-over guidance. The assessment began with a training paradigm to get participants familiar with the intended task. Once a given participant is trained on the task, then the assessment commences.

TABLE 1 Demographic characteristics of the PLACEBO + CAE and CAE + PLACEBO groups.

	PLACEBO + CAE	CAE + PLACEBO
Age in years, mean (*SD*)	53 (18)	43 (20)
Age range in years	21–66	19–66
Sex, number		
Female	4	7
Male	3	4
MMSE score, mean (*SD*)	34 (1)	33 (2)
Level of education/degrees, percent		
Ph.D.	14	9
M.S.	43	9
B.S.	14	27
Associates	0	36
High school	29	19
Race/Ethnicity, percent		
Caucasian	100	82
Hispanic	0	18
Computer experience in years, mean (*SD*)	27 (11)	22 (11)

Abbreviations: B.S., Bachelor of Science; CAE, carboxy alkyl ester; M.S., Master of Science; MMSE, mini-mental status examination; Ph.D., Doctor of Philosophy; *SD*, standard deviation.

The training and assessment are all unsupervised and artificial intelligence driven to remove bias induced by the interference of study staff (researchers). The neurocognitive assessment was the RVIP task. This assessment is designed to limit learning effects; therefore, it can be administered to the same participants over time. For each test session, test stimuli are presented at random from a large pool of stimuli or alternate test stimuli were selected which limits the possibility that a given participant will complete the same stimulus induced task more than once. This adaptive paradigm ensures little or no practice effects from taking the same assessment multiple times. Mixed-effects models, Bayesian analyses and Bland-Altman plots have shown that the RVIP is a valid test (Backx et al., 2020). Test retest reliability for the RVIP has yielded an interclass correlation coefficient of .67 and a Spearman correlation coefficient of .7 which exceeds the accepted reliability standard (≥0.60) in the literature (Karlsen et al., 2020).

The RVIP assessment evaluates sustained attention (Cabeça et al., 2018). At a rate of 100 digits per minute, 2–9 digits are presented successively in pseudorandom order. Participants are tasked with motor responses to target sequences, such as three consecutive odd or three even digits (3-5-7, 2-4-6, 4-6-8, etc.) as quickly as possible. Stimulus duration was 600 ms with no interstimulus intervals. Target sequences may be one or multiple simultaneous sequences. Outcome measure was the mean latency (in ms) of responses to targets (Cabeça et al., 2018).

2.4 | Statistical analyses

The aim of the study was to test the null-hypothesis that 30-day oral intake of CAE would have no effect on attention as revealed by mean RVIP scores. Therefore, planned comparison between baseline attention scores and attentions scores after 30 days (1 month) of daily CAE consumption served are the primary outcome for the current study. Statistical *t*-tests (independent samples) were deployed to detect statistically significant differences between baseline scores and attention scores after 1 month of CAE intake. Such a priori comparisons provide a higher statistical power in testing the pre-planned hypothesis than the more generic *F*-test from an analysis of variance followed by post hoc comparisons. To rule-in or rule-out learning effects from taking the RVIP task multiple times and to further judge the efficacy of the experimental design, unplanned comparisons were made. Unplanned comparisons were conducted as follows: baseline scores versus scores at 2-months after CAE consumption; baseline scores versus scores at 3-months after CAE consumption; baseline scores versus scores after placebo consumption; and CAE scores versus placebo scores. A p-value of <.05 was used as the significance criterion. To constrain multiplicity, we used a single independent variable (CAE), a single dependent variable (attention), one measurement (RVIP latency), one group (CAE + PLACEBO) and one time point comparison (baseline to the first 30 days of CAE consumption) to make statistical conclusions about the hypothesis (Blakesley et al., 2009; Frane, 2015). A professional graphing and statistical software suite (GraphPad Software, Inc.) was deployed for all graphing and statistical computations.

3 | RESULTS

Three participants withdrew early from the study after participating for 2 months due to undisclosed personal reasons. Two of these participants were from the CAE + PLACEBO group and one was from the PLACEBO + CAE group. Table 1 reveals that there were differences between the CAE + PLACEBO group and the PLACEBO + CAE group. The CAE + PLACEBO group was on average 10 years younger, composed of more females, less educated, more ethnically diverse and had less computer experience.

Figure 3 reveals that 30-day oral intake of CAE improved attention above baseline levels (lower scores equal better performance). In this figure average baseline attention scores ($\bar{x}$ = 544; s = 113) are displayed as well as the average attention scores for participants who consumed CAE ($\bar{x}$ = 479; s = 52) or the placebo ($\bar{x}$ = 534; s = 101). The group who consumed CAE showed an improvement in attention compared to baseline while the group who consumed the placebo showed less improvement. This suggests that oral intake of CAE was sufficient to improve attention. Furthermore, the group who consumed CAE exhibited better attention scores than the group who consumed the placebo. Therefore, 30-day CAE intake enhanced attention above baseline and this effect was not due to a learning effect.

A statistical computation was conducted, and the result confirmed the conclusion that 30-day CAE intake improved attention. Planned comparison was between baseline and the treatment condition. Comparing mean baseline attention scores to mean attention scores after 30-day oral intake of CAE resulted in statistically significant improvement ($t[46] = 3.356$, $p = .0016$; two-tailed). Unplanned statistical comparison between baseline attention scores and attention scores after placebo intake resulted in no statistically significant improvement ($t[35] = 0.7345$, $p = .4675$; two-tailed). Additionally, an unplanned comparison between the CAE group and the placebo group revealed statistically significant improvement in attention scores relative to that of placebo ($t[47] = 2.068$, $p = .0442$; two-tailed).

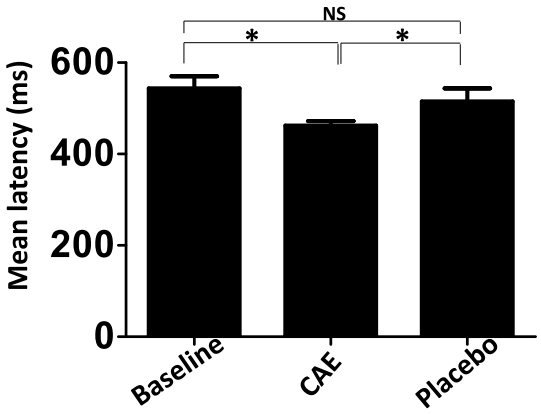

FIGURE 3 CAE intake improves attention. This panel shows that 30-day oral intake of CAE resulted in a statistically significant improvement in mean latency (lower scores equal better performance) compared to baseline (at the beginning of the study). Note that oral intake of the placebo resulted in no statistically significant improvement compared to baseline. Furthermore, CAE intake produced scores that were statistically better than that of placebo. Bars, mean ± S.E.; CAE, carboxy alkyl ester; ms, milliseconds; NS, not statistically significant; *$p < .05$ or statistically significant.

Figure 4 reveals additional data that confirmed that the positive effect of CAE on attention was not due to a learning effect. Figure 4a shows the results for a group of participants who received placebo the first month of the study and their attention scores ($\bar{x} = 534$; $s = 101$) were similar to baseline scores ($\bar{x} = 544$; $s = 113$). This indicates that taking the RVIP task twice did not produce a significant learning effect. These same participants then went through a month-long washout period (break or rest period). After this washout period their scores ($\bar{x} = 531$; $s = 128$) did not significantly change and remained close to that at baseline. However, when CAE was introduced and the same participants consumed CAE for 1-month, there was a significant improvement in their attention scores ($\bar{x} = 478$; $s = 152$) compared to baseline scores. Figure 4b shows the results for a group of participants who received CAE the first month of the study and their attention scores ($\bar{x} = 479$; $s = 52$) were improved relative to their scores at baseline. These same participants then went through a month-long washout period (break or rest period). After this washout period their scores ($\bar{x} = 473$; $s = 65$) remained improved relative to baseline. Interestingly, when placebo was introduced and the same participants consumed the placebo for 1-month, their scores ($\bar{x} = 431$; $s = 40$) continued to be better than that at baseline.

Unplanned statistical computations were conducted on the data in Figure 4. There was no statistically significant ($t[6] = 0.9216$, $p = .3923$; two-tailed) difference between baseline attention scores and attention scores following 30 days placebo treatment. Similarly, there was no statistically significant difference ($t[5] = 1.563$, $p = .1787$; two-

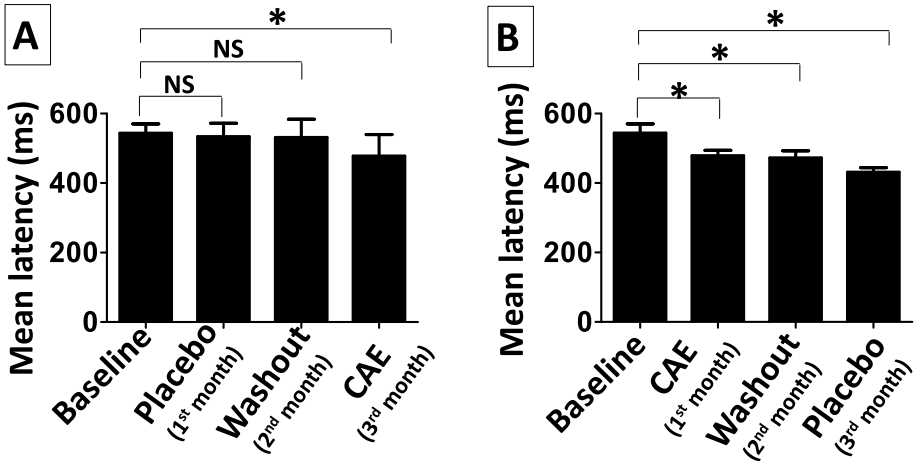

FIGURE 4 CAE improved attention is not due to a learning effect. Panel (a) shows the participants who experienced daily (1st month) oral intake of the placebo, then they experienced 30 days of no treatment (washout period: 2nd month) and lastly, these same participants took CAE for 30 days (3rd month). Note that the only statistically significant improvement in attention occurred at 3 months due to CAE intake (lower scores equal better performance). Panel (b) shows the participants who experienced daily (1st month) oral intake of CAE, then they experienced 30 days of no treatment (washout period: 2nd month) and lastly, these same participants took the placebo for 30 days (3rd month). Note that CAE intake improved attention after 1 month of treatment and this improvement continued out to 3 months. Bars, mean ± S.E.; CAE, carboxy alkyl ester; ms, milliseconds; NS, not statistically significant; *$p < .05$ or statistically significant.

tailed) between baseline attention scores and attention scores following the washout period. However, there was a statistically significant difference ($t[8] = 3.468$, $p = .0085$; two-tailed) between baseline attention scores and attention scores following CAE intake. Interestingly, there was a slight difference between placebo (1st month) and CAE (3rd month), however this difference was not statistically significant ($t[5] = 2.088$, $p = .0911$; two-tailed) which suggest little or no learning effect after taking the RVIP multiple times. Furthermore (Figure 4b), there was a statistically significant ($t[10] = 2.376$, $p = .0389$; two-tailed) difference between baseline attention scores and attention scores after 30 days CAE intake. Similarly, there was a statistically significant difference ($t[9] = 2.388$, $p = .0407$; two-tailed) between baseline attention scores and attention scores following the washout period. Additionally, there was a statistically significant difference ($t[8] = 3.468$, $p = .0085$; two-tailed) between baseline attention scores and attention scores following placebo intake. Interestingly, there was a difference between CAE (1st month) and placebo (3rd month), and this difference was statistically significant ($t[8] = 3.106$, $p = .0145$; two-tailed) which suggests continued improvement in attention months after cessation of CAE intake.

4 | DISCUSSION

The aim of this study was to test the null hypothesis that 30-day oral intake of CAE would have no effect on attention as revealed by mean RVIP scores. Planned comparison statistical testing revealed that 30-day oral intake of CAE resulted in a significant improvement in attention relative to baseline (Figure 3), therefore, the null hypothesis can be rejected. Unplanned statistical testing revealed that oral intake of the placebo failed to produce a significant improvement in attention scores relative to baseline (Figure 3), which suggest that the improvement observed after CAE intake was not due to a learning effect. Furthermore, CAE intake produced significantly better attention scores than that of placebo intake (Figure 3). CAE intake exhibited improvement in attention scores among the two groups assessed in the current study. For instance, CAE intake improved attention scores in the CAE + PLACEBO group which consisted of participants who were on average younger, less educated, and more ethnically diverse (Table 1 and Figure 4b). CAE intake also improved attention scores among the PLACEBO + CAE group which consisted of participants who were, on average, older (10 years older), more educated (more doctoral and M.S. degrees) and less ethnically diverse (100% self-identify as Caucasian). The combined results suggest that CAE consumption demonstrated consistent improvement in attention scores regardless of whether it was consumed early (1st month) or late (3rd month) during the study (Figure 4) and a boost in attention can be observed across different demographic groups.

Placebo intake failed to demonstrate consistent improvement in attention scores (Figure 4). For instance, placebo intake alone could not significantly improve attention scores. However, combining CAE intake (1st month) with placebo intake (3rd month) resulted in a significant improvement. Here, the consumption of CAE in the 1st month resulted in a significant improvement in attention scores and this improvement continued even after placebo consumption 3-month later. This suggests that the CAE induced improvement is sustainable months after initial consumption. The underlying mechanism to account for this sustained improvement is currently unresolved and additional research on the neurobiology of CAE on attention is now needed.

4.1 | Neurobiology of CAE on attention

The exact mechanism by which oral intake of CAE results in a sustained boost in attention is not entirely resolved. However, oral intake of CAE results in cleavage of the quinic acid moiety by esterases from enteric bacteria. The free quinic acid is then metabolized to produce chorismate which serves as a major substrate in the production of aromatic amino acids (tyrosine, tryptophan and phenylalanine) (Pero, 2010). Tyrosine which can be indirectly synthesized from phenylalanine is then converted to levodopa (L-DOPA) to produce dopamine and hydroxylation of dopamine produces norepinephrine (Fitzgerald, 2020). Tryptophan is metabolized to form 5-hydroxytryptophan which is used in the production of serotonin (Andreou et al., 2014). Therefore, oral consumption of CAE increases bioavailability of the monoamine neurotransmitters, dopamine, norepinephrine and serotonin (Mondanelli et al., 2022; Mondanelli & Volpi, 2021; Pero, 2010). This is particularly important because these neurotransmitters regulate attention. For instance, norepinephrine is involved in regulating the level of attention while dopamine and serotonin are involved in regulating the type of attention (Kodama et al., 2002). Therefore, it is conceivable that the improvement in attention observed in the current study was related to an increase in bioavailability of neurotransmitters that directly improved attention. This may have implications for psychiatric and neurologic conditions such as attention-deficit/hyperactivity disorder (ADHD) and dementia.

Research has shown that lower levels of serotonin and its precursor tryptophan are associated with ADHD (Mette et al., 2013). Indeed, serotonin regulates attention via the default mode network which is comprised of the posterior cingulate cortex, the anterior cingulate cortex, medial prefrontal cortex, medial temporal lobes, angular gyrus, and the precuneus (Weinberg-Wolf et al., 2018). Additionally, genetic variants within serotonin pathways are associated with ADHD (van Rooij et al., 2015) and a selective serotonergic drug (Fluoxetine) has shown some efficacy in improving inattention among ADHD patients by regulating the default mode network (Carlisi et al., 2016; Quintana et al., 2007). Similarly, lower levels of dopamine and norepinephrine are also associated with the development of ADHD in both humans and animals (González-Martínez et al., 2023). Here, dopamine and norepinephrine modulate a fronto-striato-cerebellar circuit that underlies prefrontal executive functions that are often impaired in ADHD patients (del Campo

et al., 2011). Furthermore, medications use to treat ADHD, such as methylphenidate, dextroamphetamine and atomoxetine act by increasing levels of dopamine and norepinephrine (Williams et al., 2023). Given that oral consumption of CAE can increase systemic levels of tryptophan, serotonin, dopamine, and norepinephrine, then it is possible that CAE could serve as an alternative and/or complement to current allopathic substances in the management of ADHD. This conclusion could be extended to dementia. For example, pre-clinical experiments have shown that CAE and other phytochemicals can inhibit or reduce beta-amyloid protein containing plaques and tau protein containing tangles that contribute to Alzheimer's disease (Snow et al., 2019).

Pathological perturbations of neurotransmitter systems are believed to play a role in the development of Alzheimer's neuropathology and includes, loss of monoaminergic neurons, decreased monoamine levels, decreased γ-Aminobutyric acid (GABA) levels, dysfunction of glutamatergic neurons, and loss of cholinergic neurons (Yang et al., 2023). Both animal and human studies have demonstrated a role for monoamine neurotransmitters in the development of Alzheimer's disease (Reddy et al., 2021). Unlike glutamate and GABA, which are fast neurotransmitters, monoamine neurotransmitters act via metabotropic receptors and thus move more slowly. However, monoamine receptors (e.g., serotonin) couple with GABA, glutamate and acetylcholine receptors to form heterodimers that interact with various other neurotransmitters networks (Joshi et al., 2020). Therefore, it is possible that oral intake of CAE may increase bioavailability of monoamines neurotransmitters that interact with GABA, glutamate, and acetylcholine receptors to limit the progression of dementia. However, future human research is now needed to demonstrate the efficacy of CAE in the clinical management of ADHD and/or dementia.

4.2 | Study limitations

Given the experimental design and the results from the current study, the following recommendations should be considered in future research on the efficacy of CAE intake on attention. Future research will need to be conducted with a larger sample size than the sample size used in the current study. This will serve to further verify the main outcome from the current study. Fortunately, the participants in the current study were highly motivated which resulted in 100% compliance; however, a larger study could be limited by the compliance rate. Additionally, the current study confirmed the lack of side effects from daily intake of CAE, which further supports the use of CAE among larger groups of participants. It is important to emphasize that the CAE extraction protocol is important in the development of side effects. For instance, alcohol-based extraction protocols will contain oxindole alkaloids and may results in several side effects, including diarrhea, nausea, kidney dysfunction, endocrine and liver effects (Batiha et al., 2020). However, hot water extracts similar to the decoction used in the current study are known to exhibit no side effects with excellent tolerability (Batiha et al., 2020; Mehta et al., 2007).

The present work was only focused on attention, but other cognitive domains might also be relevant and should be pursued in the future. Although the current study did not significantly alter, compare, or monitor the diets of the participants, future research may seek to specifically explore the degree to which diet is an important variable. Additionally, future research may seek to focus on a more selective demographic. For example, college students often misuse caffeine and prescription drugs to improve cognition and attention. CAE may prove to be a more natural and safe means of improving academic performance among college students. The ability of CAE to sustain improvements in attention months after oral intake deserves additional research. The methods employed in the current research provided no insights on the mechanism underlying a sustained effect. Therefore, future research may benefit from serial monitoring of systemic aromatic amino acids and/or monoamine neurotransmitters.

5 | CONCLUSION

The present study was the first to demonstrate an association between CAE intake and improvement in attention. This improvement or boost in attention was revealed in a research design that constrained statistical multiplicity, and thus elevates the importance of the results. Research on the use of pharmaceuticals, non-pharmaceuticals and/or behavioral approaches to improve attention are frequently limited by statistical multiplicity, yet no systematic quantitative or qualitative review that appropriately address this issue has immerged in the literature. Therefore, such systematic reviews are a needed future direction in this line of research. Additionally, future research is needed to further support the notion that the dietary supplement, CAE, may improve attention.

AUTHOR CONTRIBUTIONS

O'neil W. Guthrie: Conceptualization; formal analysis; writing - original draft; writing - review & editing; funding acquisition; project administration; methodology; resources; software; supervision; validation. **Li Yang**: Data curation; formal analysis; writing - review & editing; methodology; investigation; validation; visualization.

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CONFLICT OF INTEREST STATEMENT

Author (OWG) is the inventor of several patents that include carboxy alkyl esters and serves on the advisory board of Optigenex Inc. Optigenex Inc. manufactured carboxy alkyl esters and sponsored the study.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data was created or analyzed in this study.

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REFERENCES

Adelhöfer, N., Gohil, K., Passow, S., Teufert, B., Roessner, V., Li, S.-C., & Beste, C. (2018). The system-neurophysiological basis for how methylphenidate modulates perceptual-attentional conflicts during auditory processing. *Human Brain Mapping*, *39*(12), 5050–5061. https://doi.org/10.1002/hbm.24344

Albers, C. (2019). The problem with unadjusted multiple and sequential statistical testing. *Nature Communications*, *10*(1), Article 1. https://doi.org/10.1038/s41467-019-09941-0

An, S., Huang, H., Wang, H., & Jiang, Y. (2021). Prophylactically injection of Nicorandil to reduce no-reflow phenomenon during PCI in acute STEMI patients. *Medicine*, *100*(15), e25500. https://doi.org/10.1097/MD.0000000000025500

Andreou, D., Söderman, E., Axelsson, T., Sedvall, G. C., Terenius, L., Agartz, I., & Jönsson, E. G. (2014). Polymorphisms in genes implicated in dopamine, serotonin and noradrenalin metabolism suggest association with cerebrospinal fluid monoamine metabolite concentrations in psychosis. *Behavioral and Brain Functions*, *10*(1), 26. https://doi.org/10.1186/1744-9081-10-26

Backx, R., Skirrow, C., Dente, P., Barnett, J. H., & Cormack, F. K. (2020). Comparing web-based and lab-based cognitive assessment using the Cambridge neuropsychological test automated Battery: A within-subjects counterbalanced study. *Journal of Medical Internet Research*, *22*(8), e16792. https://doi.org/10.2196/16792

Batiha, G. E.-S., Magdy Beshbishy, A., Wasef, L., Elewa, Y. H. A., Abd El-Hack, M. E., Taha, A. E., Al-Sagheer, A. A., Devkota, H. P., & Tufarelli, V. (2020). *Uncaria tomentosa* (Willd. ex Schult.) DC.: A review on chemical constituents and biological activities. *Applied Sciences*, *10*(8), Article 8. https://doi.org/10.3390/app10082668

Blakesley, R. E., Mazumdar, S., Dew, M. A., Houck, P. R., Tang, G., Reynolds, C. F., III, & Butters, M. A. (2009). Comparisons of methods for multiple hypothesis testing in neuropsychological research. *Neuropsychology*, *23*(2), 255–264. https://doi.org/10.1037/a0012850

Cabeça, H. L. S., Rocha, L. C., Sabbá, A. F., Tomás, A. M., Bento-Torres, N. V. O., Anthony, D. C., & Diniz, C. W. P. (2018). The subtleties of cognitive decline in multiple sclerosis: An exploratory study using hierarchichal cluster analysis of CANTAB results. *BMC Neurology*, *18*(1), 140. https://doi.org/10.1186/s12883-018-1141-1

Carlisi, C. O., Chantiluke, K., Norman, L., Christakou, A., Barrett, N., Giampietro, V., Brammer, M., Simmons, A., & Rubia, K. (2016). The effects of acute fluoxetine administration on temporal discounting in youth with ADHD. *Psychological Medicine*, *46*(6), 1197–1209. https://doi.org/10.1017/S0033291715002731

Carswell, A. T., Howland, K., Martinez-Gonzalez, B., Baron, P., & Davison, G. (2020). The effect of caffeine on cognitive performance is influenced by CYP1A2 but not ADORA2A genotype, yet neither genotype affects exercise performance in healthy adults. *European Journal of Applied Physiology*, *120*(7), 1495–1508. https://doi.org/10.1007/s00421-020-04384-8

Castilhos, L. G., Oliveira, J. S., Adefegha, S. A., Manzoni, A. G., Passos, D. F., Assmann, C. E., Silveira, L. L., Trelles, K. B., Kronbauer, M., Doleski, P. H., Bremm, J. M., Braun, J., Abdalla, F. H., Gonçalves, J. F., Andrade, C. M., Cruz, I. B. M., Burger, M. E., & Leal, D. B. R. (2020). *Uncaria tomentosa* improves cognition, memory and learning in middle-aged rats. *Experimental Gerontology*, *138*, 111016. https://doi.org/10.1016/j.exger.2020.111016

del Campo, N., Chamberlain, S. R., Sahakian, B. J., & Robbins, T. W. (2011). The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biological Psychiatry*, *69*(12), e145–e157. https://doi.org/10.1016/j.biopsych.2011.02.036

Fitzgerald, P. J. (2020). Neurodining: Common dietary factors may be substrates in novel biosynthetic pathways for monoaminergic neurotransmitters. *Medical Hypotheses*, *138*, 109618. https://doi.org/10.1016/j.mehy.2020.109618

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, *12*(3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6

Frane, A. V. (2015). Planned hypothesis tests are not necessarily exempt from multiplicity adjustment. *Journal of Research Practice*, *11*(1). https://eric.ed.gov/?id=EJ1083896

Franke, A. G., Bagusat, C., Rust, S., Engel, A., & Lieb, K. (2014). Substances used and prevalence rates of pharmacological cognitive enhancement among healthy subjects. *European Archives of Psychiatry and Clinical Neuroscience*, *264*(Suppl 1), S83–S90. https://doi.org/10.1007/s00406-014-0537-1

González-Martínez, Á., Muñiz de Miguel, S., Graña, N., Costas, X., & Diéguez, F. J. (2023). Serotonin and dopamine blood levels in ADHD-like dogs. *Animals*, *13*(6), Article 6. https://doi.org/10.3390/ani13061037

Guthrie, O. W. (2012). Dynamic compartmentalization of DNA repair proteins within spiral ganglion neurons in response to noise stress. *International Journal of Neuroscience*, *122*(12), 757–766. https://doi.org/10.3109/00207454.2012.721828

Guthrie, O. W. (2016). Preservation of neural sensitivity after noise-induced suppression of sensory function. *Journal of the American Academy of Audiology*, *27*(1), 49–61. https://doi.org/10.3766/jaaa.15047

Guthrie, O. W., Gearhart, C. A., Fulton, S., & Fechter, L. D. (2011). Carboxy alkyl esters of *Uncaria tomentosa* augment recovery of sensorineural functions following noise injury. *Brain Research*, *1407*, 97–106. https://doi.org/10.1016/j.brainres.2011.06.044

Joshi, A., Wang, D.-H., Watterson, S., McClean, P. L., Behera, C. K., Sharp, T., & Wong-Lin, K. (2020). Opportunities for multiscale computational modelling of serotonergic drug effects in Alzheimer's disease. *Neuropharmacology*, *174*, 108118. https://doi.org/10.1016/j.neuropharm.2020.108118

Karlsen, R. H., Karr, J. E., Saksvik, S. B., Lundervold, A. J., Hjemdal, O., Olsen, A., Iverson, G. L., & Skandsen, T. (2020). Examining 3-month test-retest reliability and reliable change using the Cambridge Neuropsychological Test Automated Battery. *Applied Neuropsychology: Adult*, *0*(0), 1–9. https://doi.org/10.1080/23279095.2020.1722126

Kodama, T., Honda, Y., Watanabe, M., & Hikosaka, K. (2002). Release of neurotransmitters in the monkey frontal cortex is related to level of attention. *Psychiatry and Clinical Neurosciences*, *56*(3), 341–342. https://doi.org/10.1046/j.1440-1819.2002.00977.x

Mehta, K., Gala, J., Bhasale, S., Naik, S., Modak, M., Thakur, H., Deo, N., & Miller, M. J. (2007). Comparison of glucosamine sulfate and a polyherbal supplement for the relief of osteoarthritis of the knee: A randomized controlled trial [ISRCTN25438351]. *BMC Complementary and Alternative Medicine*, *7*(1), 34. https://doi.org/10.1186/1472-6882-7-34

Mette, C., Zimmermann, M., Grabemann, M., Abdel-Hamid, M., Uekermann, J., Biskup, C. S., Wiltfang, J., Zepf, F. D., & Kis, B. (2013). The impact of acute tryptophan depletion on attentional performance in adult patients with ADHD. *Acta Psychiatrica Scandinavica*, *128*(2), 124–132. https://doi.org/10.1111/acps.12090

Miller, M. J., Mehta, K., Kunte, S., Raut, V., Gala, J., Dhumale, R., Shukla, A., Tupalli, H., Parikh, H., Bobrowski, P., & Chaudhary, J. (2005). Early relief of osteoarthritis symptoms with a natural mineral supplement and a herbomineral combination: A randomized controlled trial [ISRCTN38432711]. *Journal of Inflammation*, *2*(1), 11. https://doi.org/10.1186/1476-9255-2-11

Mondanelli, G., & Volpi, C. (2021). The double life of serotonin metabolites: In the mood for joining neuronal and immune systems. *Current*

Opinion in Immunology, *70*, 1–6. https://doi.org/10.1016/j.coi.2020.11.008

Mondanelli, G., Volpi, C., & Orabona, C. (2022). Decoding the complex crossroad of tryptophan metabolic pathways. *International Journal of Molecular Sciences*, *23*(2), Article 2. https://doi.org/10.3390/ijms23020787

Napoletano, F., Schifano, F., Corkery, J. M., Guirguis, A., Arillotta, D., Zangani, C., & Vento, A. (2020). The psychonauts' world of cognitive enhancers. *Frontiers in Psychiatry*, *11*, 546796. https://doi.org/10.3389/fpsyt.2020.546796

Pero, R. W. (2010). Health consequences of catabolic synthesis of hippuric acid in humans. *Current Clinical Pharmacology*, *5*(1), 67–73. https://doi.org/10.2174/157488410790410588

Pero, R. W., & Lund, H. (2011). Quantitative analytical method development for the assessment of bioactive quinic acid-type esters and free quinic acid in dietary supplements. *International Journal of Biotechnology & Biochemistry*, *7*(2), 293–305.

Piscoya, J., Rodriguez, Z., Bustamante, S. A., Okuhama, N. N., Miller, M. J. S., & Sandoval, M. (2001). Efficacy and safety of freeze-dried cat's claw in osteoarthritis of the knee: Mechanisms of action of the species *Uncaria guianensis*. *Inflammation Research*, *50*(9), 442–448. https://doi.org/10.1007/PL00000268

Quinn, J., Kaye, J., Montine, T., & Stackman, R. (2004). Phytochemicals in Alzheimer disease: The development of clinical trials. *Pharmaceutical Biology*, *42*(sup1), 64–73. https://doi.org/10.3109/138802090893531

Quintana, H., Butterbaugh, G. J., Purnell, W., & Layman, A. K. (2007). Fluoxetine monotherapy in attention-deficit/hyperactivity disorder and comorbid non-bipolar mood disorders in children and adolescents. *Child Psychiatry and Human Development*, *37*(3), 241–253. https://doi.org/10.1007/s10578-006-0032-7

Reddy, A. P., Yin, X., Sawant, N., & Reddy, P. H. (2021). Protective effects of antidepressant citalopram against abnormal APP processing and amyloid beta-induced mitochondrial dynamics, biogenesis, mitophagy and synaptic toxicities in Alzheimer's disease. *Human Molecular Genetics*, *30*(10), 847–864. https://doi.org/10.1093/hmg/ddab054

Schifano, F., Catalani, V., Sharif, S., Napoletano, F., Corkery, J. M., Arillotta, D., Fergus, S., Vento, A., & Guirguis, A. (2022). Benefits and harms of "smart drugs" (nootropics) in healthy individuals. *Drugs*, *82*(6), 633–647. https://doi.org/10.1007/s40265-022-01701-7

Shi, Z., Lu, Z., Zhao, Y., Wang, Y., Zhao-Wilson, X., Guan, P., Duan, X., Chang, Y.-Z., & Zhao, B. (2013). Neuroprotective effects of aqueous extracts of *Uncaria tomentosa*: Insights from 6-OHDA induced cell damage and transgenic *Caenorhabditis elegans* model. *Neurochemistry International*, *62*(7), 940–947. https://doi.org/10.1016/j.neuint.2013.03.001

Snow, A. D., Castillo, G. M., Nguyen, B. P., Choi, P. Y., Cummings, J. A., Cam, J., Hu, Q., Lake, T., Pan, W., Kastin, A. J., Kirschner, D. A., Wood, S. G., Rockenstein, E., Masliah, E., Lorimer, S., Tanzi, R. E., & Larsen, L. (2019). The Amazon rain forest plant *Uncaria tomentosa* (cat's claw) and its specific proanthocyanidin constituents are potent inhibitors and reducers of both brain plaques and tangles. *Scientific Reports*, *9*(1), Article 1. https://doi.org/10.1038/s41598-019-38645-0

van Rooij, D., Hartman, C. A., van Donkelaar, M. M. J., Bralten, J., von Rhein, D., Hakobjan, M., Franke, B., Heslenfeld, D. J., Oosterlaan, J., Rommelse, N., Buitelaar, J. K., & Hoekstra, P. J. (2015). Variation in serotonin neurotransmission genes affects neural activation during response inhibition in adolescents and young adults with ADHD and healthy controls. *World Journal of Biological Psychiatry*, *16*(8), 625–634. https://doi.org/10.3109/15622975.2015.1067371

Weinberg-Wolf, H., Fagan, N. A., Anderson, G. M., Tringides, M., Dal Monte, O., & Chang, S. W. C. (2018). The effects of 5-hydroxytryptophan on attention and central serotonin neurochemistry in the rhesus macaque. *Neuropsychopharmacology*, *43*(7), Article 7. https://doi.org/10.1038/s41386-017-0003-7

Wickens, C. (2021). Attention: Theory, principles, models and applications. *International Journal of Human-Computer Interaction*, *37*(5), 403–417. https://doi.org/10.1080/10447318.2021.1874741

Williams, O. C., Prasad, S., McCrary, A., Jordan, E., Sachdeva, V., Deva, S., Kumar, H., Mehta, J., Neupane, P., & Gupta, A. (2023). Adult attention deficit hyperactivity disorder: A comprehensive review. *Annals of Medicine and Surgery*, *85*(5), 1802–1810. https://doi.org/10.1097/MS9.0000000000000631

Yang, Z., Zou, Y., & Wang, L. (2023). Neurotransmitters in prevention and treatment of Alzheimer's disease. *International Journal of Molecular Sciences*, *24*(4), Article 4. https://doi.org/10.3390/ijms24043841

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