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ORIGINAL RESEARCH

Acute Treatment with the Nootropic CILTEP® Does Not Improve Cognitive Performance in Healthy Middle-Aged Participants

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Abstract

This study investigated the acute effects of the dietary nootropic stack CILTEP®. It contains a combination of ingredients that have been individually reported to improve cognitive performance. Especially, the ingredients luteolin, which is considered a phosphodiesterase type 4 (PDE4) inhibitor, and forskolin, an adenylate cyclase stimulator, were of interest since they can increase the second messenger cAMP and thus also intracellular signaling. Numerous studies have shown that inhibition of PDE4 can improve memory in animals and humans. We examined whether acute dosing of 3 capsules of CILTEP® would improve cognitive function in healthy participants aged 30 to 40 ($n = 33$). We used a randomized, double-blind, placebo-controlled, two-way cross-over design. Our test battery was aimed at measuring memory performance, attention, and sensorimotor speed. The primary outcome measures were the performance on the verbal learning task and the spatial pattern separation task. Secondary outcomes included other cognitive tests, event-related potentials (ERPs), and assessment of the activity of the enzyme beta-glucuronidase and its effect on the bioavailability of luteolin, heart rate, and blood pressure. No relevant effects of acute CILTEP® treatment were found on any measure of the test battery or ERPs. Blood plasma concentrations of luteolin increased, yet about 2000 times too low to likely exert any PDE4 inhibition. CILTEP® treatment did neither affect heart rate nor blood pressure. In summary, there is no evidence that a single standardized dose of 3 capsules of CILTEP® can improve cognitive function in healthy middle-aged participants.

Keywords Cognitive enhancement · Cognition · PDE4 inhibitor · Memory

Introduction

Nootropics, also called "smart drugs" or "cognitive enhancers," are substances that have been alleged to improve cognitive performance. While this research is still emerging and the mechanisms underlying cognition are complex and multifaceted, some nootropics such as, e.g., L-theanine, caffeine, and modafinil, have shown to improve attention (Barbhaiya et al., 2008; Giesbrecht et al., 2010; Einöther et al., 2010), verbal memory (Barbhaiya et al., 2008; Illieva et al., 2015), creativity (Müller et al., 2013), and executive function (Killgore et al., 2009) in cognitively healthy individuals. Nootropics can be divided into three different categories: dietary supplements, synthetic compounds, and prescription drugs (Malik et al., 2007). Most nootropics, especially those that fall under the dietary supplement category, do not need the approval of the Food and Drug Administration (FDA) or Federal Trade Commission (FTC) and are therefore not monitored or scheduled (Howland, 2010). Dietary nootropics are usually not one isolated supplement/ingredient, however, contain a combination of nootropic substances to support the best possible cognitive performance. These are most often called supplement stacks or nootropic stacks. A nootropic stack combines substances that claim to work well together to act as a synergistic substance, i.e., the effect of two or more substances becomes more powerful and better in combination than either of them separately (Jedrejko et al., 2023).

CILTEP®, recently also sold as Neurofuel™, is a dietary nootropic stack, which contains many plant-derived ingredients that have been claimed to improve brain function.

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One of the ingredients, artichoke extract, contains multiple bioactive compounds of which one is luteolin. Luteolin is a polyphenolic flavone found in multiple herbs, vegetables, and fruits. Luteolin falls under the class of non-selective phosphodiesterase (PDE) inhibitors class 1 to 5 (Yu et al., 2010). In particular, the inhibition of PDE4 is of interest as PDE4 is responsible for breaking down the second messenger cyclic-adenosine monophosphate (cAMP). Thus, by inhibiting PDE4, intracellular cAMP levels increase. Another component of CILTEP® is forskolin, an ingredient that stimulates adenylate cyclase, which produces cAMP, and thereby increases intracellular cAMP levels (Balakrishnan et al., 2016). Intracellular cAMP signaling plays a pivotal role in long-term potentiation, a neurobiological substrate of memory formation (Bollen et al., 2014). Preclinical research by Vanmierlo and colleagues (2016) showed that roflumilast administration, a PDE4 inhibitor, improved memory performance in an object location task and spatial Y-maze (Vanmierlo et al., 2016). A study by Zhang et al. (2004) showed that PDE4 administration led to improved learning and memory in the radial arm maze test, possibly through neuroprotective mechanisms that include anti-inflammatory effects and protection against oxidative stress (Zhang et al., 2004). Recent human clinical studies found that acute administration of roflumilast improved episodic memory performance on the verbal learning test (VLT) in healthy adults and elderly participants (Blokland et al., 2019; M. A. Van Duinen et al., 2018a, 2018b). In line with this, previous research has shown that acute treatment with a PDE4 inhibitor improves memory performance in both animals and humans.

Luteolin and forskolin increase intracellular cAMP levels by different mechanisms of action. It is assumed that together they have a synergistic effect on neuronal cAMP signaling underlying memory processes, so a more powerful effect than either of them separately. Additional ingredients in CILTEP® are vitamin B6 (Bryan et al., 2002; Li et al., 2014) and L-phenylalanine (Eckart et al., 2014), which are both needed for the production of the neurotransmitter dopamine and acetyl-L-carnitine, which increases energy metabolism and mitochondrial function (Chen et al., 2017b; Kobayashi et al., 2010). All three ingredients may further support brain function as well causing synergistic effects on cognition considering that all ingredients of CILTEP® fall within a similar range of peak plasma concentration of between 30 min and 3 h (Li et al., 2013; Nulman & Koren, 2009; Sangeetha et al., 2011; Stegink & Filer Jr, 1984; Stegink et al., 1981; Wittemer et al., 2005).

Employing a double-blind, placebo-controlled, two-way cross-over design, the effect of the dietary nootropic CILTEP® on cognition was investigated in healthy middle-aged participants who had normal cognitive performance according to their age, sex, and education. The primary objective of this study was to investigate the effects of CILTEP® on cognition, with a specific focus on episodic memory, including the verbal learning test and spatial pattern separation test. We selected these cognitive tasks as our primary objectives due to the demonstrated potential of PDE4 inhibitors (e.g., roflumilast), represented by luteolin and forskolin in CILTEP®, to enhance neuronal communication, synaptic plasticity, and overall improve memory formation and retrieval. Specifically, episodic memory and spatial working memory, as assessed by the VLT and the spatial pattern separation task, rely on the integrity of neuronal circuits in the hippocampus (Burgess et al., 2002; Maguire et al., 2000). Within the hippocampus, cAMP signaling pathways are involved in mediating synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), which are mechanisms underlying learning and memory (Silva et al., 1998). Additionally, compared to other brain structures, PDE4 isoforms are expressed in various cell types in within the hippocampus, including neurons and glial cells (Tibbo et al., 2019). The specific localization of PDE4 isoforms within different cellular compartments may regulate the spatial and temporal dynamics of cAMP signaling, thereby influencing synaptic plasticity and memory processes in distinct ways (Gong et al., 2004). It is assumed that all the individual components of CILTEP® have an additive or even synergistic effect on cognition. Accordingly, it was hypothesized that an acute dose, i.e., a single administration of 3 capsules of CILTEP®, would improve episodic memory in healthy middle-aged participants.

Our secondary objective included the measurement of performance on other cognitive tasks: working memory (*n*-back task), information processing speed (digit symbol substitution test), response inhibition and focused attention (Stroop task), complex scanning, and visual tracking (the trail making test), and reaction time (simple and choice reaction task). This diverse selection of cognitive tasks was chosen deliberately to provide a thorough assessment covering a spectrum of cognitive abilities, thereby enabling a comprehensive understanding of the potential effects of CILTEP® on cognitive function across multiple domains. An additional secondary objective was to measure event-related potentials (ERPs) during some computerized cognition tasks to investigate task-related brain activity by linking neural activity to behavior, possibly detecting early cognitive processes and differentiating them, as well as possible central effects of CILTEP®. Further, we wanted to verify the activity of the enzyme beta-glucuronidase in blood plasma, as it has a modulating effect on the bioavailability of luteolin. Finally, basic vital parameters such as blood pressure and heart rate were measured to evaluate the effect of CILTEP® on autonomic function which functions as safety monitoring and interplay

between cognitive performance and cardiovascular function, as forskolin is known to reduce blood pressure.

Materials and Methods

Study Design and Population

All procedures were approved by the local Medical Research Ethical Committee (Medisch Ethische Toetsingscommissie azM/UM) and were in accordance with the Helsinki Declaration of 1975. The study was monitored and audited by the Clinical Trial Center Maastricht (CTCM) (https://www.ctcm.nl/en). The study was conducted according to a double-blind, placebo-controlled, cross-over design. We performed a within-subjects design, characterized by administering CILTEP® and placebo conditions to the all participants. This approach included two testing sessions for each participant, separated by a one-week wash-out period to mitigate any residual effects of the treatment. Importantly, the sequence in which the treatment and control conditions were administered was randomized across participants to robustly control for potential treatment order effects that could confound our results. Healthy individuals between the ages of 30 and 40 years were recruited through advertisements. Participants were screened and selected based on their memory performance on the VLT. Cognitive performance was considered within the norm when individuals scored within the range of − 1 and + 1 standard deviation (SD) from the predicted normative score (see (Van der Elst et al., 2005)).

Assuming a clinically meaningful minimum effect size of 0.5 (Cohen *d*), power calculation resulted in a group size of 27 (power 0.8; alpha 0.05). According to Natural Stacks®, approximately 20% of the CILTEP® users do not report any effect (personal communication). Therefore, the group size was increased to 33. Furthermore, in previous acute studies, approximately 9% of the participants dropped out of the study due to personal reasons (Blokland et al., 2019). Therefore, we assumed a dropout rate of 9% and increased the group size to 36. Inclusion was continued until 33 participants were included, i.e., after screening 45 individuals.

Exclusion criteria included major cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, hematological, endocrinological, or psychiatric illness (depression, bipolar disorder, anxiety disorder, panic disorder, psychosis, attention deficit hyperactivity disorder, and first-degree relative with a psychiatric disorder or history with a psychiatric disorder). Other exclusion criteria included body mass index (BMI) under 18.5 or higher than 30, excessive alcohol consumption (> 20 units of alcohol per week), smoking, pregnancy or currently lactating, use of psychoactive medication, centrally acting beta blockers, use of illicit drugs (e.g., amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates) from 2 weeks before until the completion of the experiment, systolic blood pressure above 160 mmHg, phenylketonuria, and any sensory or motor deficits which could reasonably be expected to affect test performance. Participants using steroids or Sudafed (pseudoephedrine) were also excluded.

Intervention

CILTEP® and placebo capsules were kindly provided by Natural Stacks®. Natural Stacks® is manufactured, packaged, and labeled according to the certified good manufacturing process (GMP) guidelines. The placebo capsules were identical to the CILTEP® capsules and contained rice flour, silica, and ascorbyl palmitate as therapeutically inactive ingredients. On two separate occasions, participants received an oral acute dose of CILTEP® (3 capsules; see Table 1 for the composition of ingredients of one capsule) or a placebo (3 capsules, no active ingredient). The wash-out period between treatment conditions was at least 7 days. Luteolin has a predicted half-life of fewer than 19 h (Liu et al., 2013). The treatment order was performed by counterbalancing. Sixty minutes after capsule intake, participants began the cognitive tasks. This decision is guided by the understanding that key constituents of CILTEP®, namely, luteolin and forskolin, typically attain peak plasma levels in humans within 30 to 60 min post-ingestion, with an elimination half-life ranging between 2 and 3 h (Li et al., 2013; Sangeetha et al., 2011). Before arriving on the test day, participants were asked not to eat, as they received a light meal upon arrival. The light meal did not contain any flavonoids/luteolin to avoid any effect on CILTEP®. After the light meal, participants were not allowed to eat for two hours before the intervention (CILTEP®/placebo). Participants were asked not to consume any caffeinated products or alcohol in the 24 h preceding the test day.

Table 1 Composition of one CILTEP® capsule

Quantity	Unit	Description ingredient
5	mg	Pyridoxal-5-phosphate (P5P)
900	mg	Artichoke extract standardized to 5% cynarines
750	mg	Acetyl-L-carnitine
500	mg	L-Phenylalanine
20	mg	Coleus forskohlii extract standardized to 20% forskolin
21	mg	Silica
11	mg	Ascorbyl palmitate

Pharmacokinetics

Two blood samples were collected. One blood sample to determine baseline pre-treatment beta-glucuronidase activity and one blood sample to measure luteolin levels after CILTEP® treatment. The first blood sample was collected in sodium heparin tubes (5 ml) by venipuncture on both test days before administration of treatment (placebo or CILTEP®) at T-30 and the second blood sample (5 ml) was collected after completion of neuropsychological testing at T120. Following collection, blood samples were immediately placed on ice and centrifuged at 3500 rpm at 4 °C for 10 min within 30 min. Plasma was collected and stored at – 80 °C. High-performance liquid chromatography (HPLC) analysis was performed as described previously (Bartholome et al., 2010; Cheruvu et al., 2018; Zheng et al., 2014). The detection range of luteolin was 0.5–5 ng/ml. < LLOQ = below lower limit of quantification (< 0.5 ng/ml). < LOD = below limit of detection.

Neurocognitive Testing

Following a positive medical evaluation and successful cognitive screening participants were familiarized with the setting and the cognitive test battery to minimize learning effects. See Table 2 for an overview of the test day which was identical for each participant. In order to capture participants in their optimal cognitive state, the VLT and spatial pattern separation test have been strategically placed at the beginning of the cognitive task sequence as we anticipate that (most) ingredients of CILTEP® will have reached maximum blood plasma value at this time. This positioning aims to maximize the precision and reliability of our assessments of immediate verbal memory and spatial pattern separation, which serve as primary outcomes in this study. Additionally, the delayed recall of the VLT is performed 30 min after the immediate recall. The same order of cognitive testing for each participant and test day allows for a reliable comparison between CILTEP® and placebo.

Table 2 Overview of a test day, with times counted relative to the time participants received their treatment

Time (in minutes)	Activity
T-60	Light meal
T-30	Alcohol test|drug test|first blood sample
T0	Vital signs|CILTEP or placebo
T5	Preparation for EEG recording
T55	Baseline EEG recording
T60	VLT immediate recall
T70	Spatial pattern separation test
T85	*N*-back task
T90	Digit substitution task
T95	Short break
T100	VLT delayed recall
T102	SRT and CRT
T117	Stroop
T120	Vital signs|second blood sample

EEG electroencephalography, *VLT* verbal learning task, *SRT* simple reaction task, *CRT* choice reaction task

Verbal Learning Task (VLT)

Verbal memory was assessed by an adjusted version of the Rey Auditory Verbal Learning Test (Lezak, 1995): the Visual Verbal Learning Test (Riedel et al., 1999). This test consists of a list of 30 Dutch monosyllabic words (18 nouns, 12 adjectives) of which participants are instructed to recall as many as possible. All words are presented one by one on a computer screen with an interval of one word per three seconds, in three trials with the same word sequence. After each trial, participants are asked to recall as many words as they remember from the list (immediate recall). Thirty minutes after the last and third trials, the participants are asked to recall as many words as possible (delayed recall). Dependent variables obtained from this task include the total correctly recalled words over three trials (VLT trial immediate total) and the number of correctly recalled words in the delayed recall (VLT delayed recall). ERPs obtained from this task included the N400, P300 (P3a and P3b), and P600, for the three immediate recall trials only.

The P300, in this case, P3b, is probably the most widely used ERP component in cognitive research. From the memory perspective, it has been shown to play a role in working memory, but more generally speaking, it is said to reflect activity related to updating the mental representation of the stimulus context (Polich & Criado, 2006). The N400, on the other hand, is sensitive to stimulus repetition and varies with the amount of context available, commonly showing a less negative amplitude when stimuli are presented in a known context (Dunn et al., 1998; Olichney et al., 2011). The P600 is consistently associated with recognition from memory, showing larger amplitudes for memorized than for new items (e.g., (Addante et al., 2012; Rugg & Curran, 2007)). Finally, there may be functional links between the P300 and P600 in the role they play in memory processing (Fields, 2023).

Spatial Pattern Separation Task

The spatial pattern separation task (Gilbert et al., 1998) assesses episodic memory using a series of 140 color images of everyday neutral objects on a white background. The task consisted of two phases: in the first phase, which is the encoding phase, the participant is asked to decide whether an image belongs to the category "outdoor" or "indoor" by

pressing a button (140 items total, 2 s each, and 0.5 s inter-stimulus interval). Immediately following the encoding phase, the test phase was presented, in which participants were instructed regarding a surprise recognition memory test that required participants to accurately identify whether images were in the same location on the screen or not compared to the encoding phase (140 items total, 2 s each, and 0.5 s inter-stimulus interval). Forty images were presented in the same position as in the first phase. The other images were presented in another location on the screen, ranging from close to more distant (4 different distances/locations, 20 images each), and 20 images were presented in an opposing corner. Thus, the similarity of the spatial information varied, creating five levels (lures) of mnemonic interference. The participants had to indicate using the keyboard whether the images were in the same place or a different position on the screen as compared to the first phase. Dependent variables obtained from this task are how many images were correctly identified that stayed in the same location (SPS accuracy repeat), lure one (SPS lure accuracy one), lure two (SPS lure accuracy two), lure three (SPS lure accuracy three), lure four (SPS lure accuracy four), and the corner of the screen (SPS lure accuracy corner).

n-Back Task

The *n*-back (Owen et al., 2005) task measures the working memory function. Participants are instructed to monitor a sequence of stimuli and respond when the current stimulus matches the one from *n*-trials previously in the sequence. In this study, we used a 0-back, 1-back, and 2-back task, in which the 0-back was a simple focused attention/speed task, and the 1- and 2-back required retrieving information from working memory, namely, retrieving whether the same item as presented was also presented 1 or 2 stimuli back. Dependent variables obtained from this task include the number of accurate responses for 0-back (*N*-back accuracy S0L), 1-back (*N*-back accuracy S1L), and 2-back (*N*-back accuracy S2L), i.e., the number of times the participant pressed the button when the stimulus matches the one from *n* steps earlier, and median reaction time (RT) for 0-back (*N*-back RT S0L), 1-back (*N*-back RT S1L), and 2-back (*N*-back RT S2L). ERP obtained from this task was the P300.

Digit-Symbol Substitution Task

The digit-symbol substitution task (Wechsler, 1981) assesses complex scanning and visual tracking. The screen shows a series of 9 numbered symbols that represent a "key." The participant was presented with a series of parallel boxes that contained a symbol in the top half of the box. The participant had to provide a matching "number" response for the bottom half by referring to the key. The dependent variable was calculated by how many correct responses/matches were made within 90 s (DSST correct score).

Simple and Choice Reaction Time Task

The simple and choice reaction time task (Houx et al., 1993) was divided into two parts. First, the participant is instructed to react as soon as the button lights up in the center of the response box (red button). In the second part, one of three possible buttons lights up. The participant is instructed to respond and push the target button as quickly as possible. Dependent variables obtained from this task included RT for simple (SRT) and choice (CRT), as well as movement time (MT) for simple (SMT) and choice conditions (CMT). RT refers to the time in milliseconds needed to release the red button and MT refers to the milliseconds needed to move from the red button to the target button. The RT and the MT have been log-transformed.

Trail-Making-Test

The trail-making-test (TMT) (Reitan, 1956) was used to examine attention and concept-shifting abilities. It is divided into parts A and part B, both consisting of 25 circles distributed over a sheet of paper. In part A, the circles are numbered 1–25, and the participant is instructed to connect the numbers in ascending order. In part B, the circles include both numbers (1–13) and letters (A–L). Again, the participant is instructed to connect the circles in an ascending pattern, but with the added task of alternating between numbers and letters. In both tasks, the participant is not allowed to lift the pencil off the paper and to connect the circles as fast as possible. The dependent variables are calculated separately by the seconds required to complete the task for parts A (TMT-A) and B (TMT-B).

Stroop Color-Word Task

The Stroop task (Hammes, 1973; Stroop, 1935) assesses response inhibition (interference) and focused attention. In this task, color names are printed in colored ink. In the congruent category, the color name and the color of the ink were the same, in the incongruent category, they were not. Participants are instructed to name the color of the ink, not the word itself. However, interference occurs because of the urge to read the printed words (even if one is asked to ignore them). Since the printed words and ink color differed in the incongruent category, interference is larger in this category than in the congruent category (Jasper, 1958). The colors used in this task are blue, red, green, and yellow. The ink color has to be named by pressing one out of four buttons, each representing one of the colors. Dependent variables obtained from this task included the number of

errors made in both the congruent (Stroop misses congruent) and incongruent conditions (Stroop misses incongruent), as well as correct answers given in both the congruent (Stroop hits congruent) and incongruent (Stroop hits incongruent) conditions.

Electroencephalography (Jobert et al.) and Electrooculogram (EOG) Acquisition

The electrophysiological activity was recorded with 32 EEG electrodes and placed according to the international 10–20 system (Jasper, 1958). Reference and ground were placed at the linked mastoids and the forehead, respectively. Eye movements were detected by horizontal and vertical EOG recordings. Before electrode attachment, the positions were slightly scrubbed with a gel to provide a good measurement. Both EEG and EOG were filtered between 0.01 and 100 Hz and sampled at 500 Hz.

Statistical Analyses

Neuropsychological Tests

All data were checked for outliers and then subjected to statistical tests. Statistical analyses were performed using SPSS. For tests with single testing points, we used paired *t*-tests to compare the performance of the two groups. In the case of multiple measurements on the same parameter, we used a repeated measures analysis. For RT measurements, the median was used as the analysis, except for the simple and choice reaction time task, the RT was log-transformed. Missing data were not considered for analysis. The significance level was set at alpha = 0.05. We used the Holm-Bonferroni approach to correct for multiple pairwise comparisons. In this case, the significance level was set at alpha = 0.0014.

EEG

EEG data were analyzed using Brain Vision Analyzer 2 (Brain Products, GmbH) software. High-pass (1 Hz) and low-pass (30 Hz) filters were applied offline. Next, EOG activity was removed from the signal using the Gratton and Coles method in Vision Analyzer. Subsequently, segments were made from 100 ms before stimulus onset until 1000 ms after onset, using the last 100 ms before stimulus onset as a baseline. The segments were baseline corrected and then checked for artifacts and excluded if an artifact occurred during the first 1000 ms after stimulus presentation. Next, averages were calculated for each stimulus type and treatment. The grand average was used to determine the ERP components. For the VLT, peak detection windows were defined as the most positive or negative value between the following intervals: P3a (210–290 ms), P3b (290–360 ms), N400 (340–470 ms), and P600 (450–700 ms). For the *n*-back, the P300 (210–350 ms) was determined. The analyses were performed for three different electrodes (frontal (Fz), central (Cz), and parietal (Pz)). The peaks of both groups were compared with an ANOVA with repeated measures.

Blood Plasma Measurements

No outlier tests were possible for the luteolin measurements as too many samples were below the limit of the detection (Glodny et al., 2000). Therefore, a non-parametrical test was performed for the statistical analysis. In addition, the absolute number of luteolin measurements was so low, that paired testing would result in a loss of power. Therefore, the non-parametric non-paired Mann–Whitney *U* test was applied to all plasma measurements.

Results

Socio-demographic Characteristics

Participants' ages ranged from 30 to 40 years ($M = 33.7$, SD = 3.1) of which 51% identified as female. Out of all participants, 79% had a high level of education and 21% had a medium level of education (based on the education level according to Verhage (De Bie & Vegter, 1987; Verhage, 1964)).

Physiological and Neuropsychological Tests

Two significant effects were found, namely, a slower motion time in the simple reaction time task and an increased spatial separation accuracy repeat post-CILTEP® treatment; however, after correcting for multiple comparisons with the Holm-Bonferroni approach, these two significant results were not significant anymore. The rest of the physiological measurements and neuropsychological tests did not show a significant difference between placebo and CILTEP®. An overview of the different physiological and neuropsychological tests is shown in Tables 3 and 4.

Verbal Learning Task (VLT)-EEG

The data of the different components of the ERPs during the 3 successive presentations of the words in the VLT task are presented in Table 5. Although the main effect of electrode position was found for the P3a amplitude ($F(2, 64) = 2.64$, $p < 0.01$), no treatment effects were found (all associated F-values < 0.40, n.s.). For the P3b, a stimulus repetition effect was found for amplitude ($F(2, 64) = 8.38$, $p < 0.01$) indicating that this amplitude increased across the

Table 3 Group comparisons of the effects of CILTEP® and placebo on cardiovascular functions

	CILTEP	Placebo	*p*-value
Heart rate before	70.91 (9.9)	71.24 (10.5)	0.850
Heart rate after	62.10 (9.6)	62.30 (8.2)	0.860
Systolic before	116.94 (14.8)	115.45 (11.9)	0.536
Systolic after	115.48 (9.8)	115.39 (9.0)	0.066
Diastolic before	75.61 (8.0)	75.00 (7.5)	0.105
Diastolic after	77.00 (6.0)	75.33 (11.8)	0.330

Data are presented as mean (SD) unless otherwise specified

Table 4 Group comparisons of the effects of CILTEP® compared to placebo on neuropsychological tests

	CILTEP	Placebo	*p*-value
VLT immediate total	43.15 (13.5)	44.36 (13.1)	0.431
VLT delayed recall	15.81 (6.2)	16.46 (5.5)	0.309
SPS accuracy repeat	75.97 (9.5)	72.69 (11.6)	0.049
SPS lure accuracy one	45.45 (11.4)	42.19 (12.1)	0.209
SPS lure accuracy two	65.20 (17.1)	62.68 (20.0)	0.587
SPS lure accuracy three	78.10 (12.7)	78.59 (13.3)	0.853
SPS lure accuracy four	84.33 (10.8)	80.48 (16.8)	0.181
SPS accuracy corner	90.30 (8.7)	85.98 (12.5)	0.079
DSST correct score	89.12 (9.8)	86.29 (11.3)	0.326
TMT-A	20.76 (6.2)	21.30 (5.7)	0.546
TMT-B	50.68 (25.7)	43.84 (16.6)	0.103
Stroop misses congruent	0.85 (1.1)	0.58 (0.9)	0.247
Stroop misses incongruent	2.33 (2.05)	1.92 (2.0)	0.387
Stroop hits congruent	69.58 (1.6)	69.52 (1.9)	0.879
Stroop hits incongruent	64.94 (12.4)	67.39 (3.0)	0.232
SRT (log-transformed)	− 2.80 (0.1)	− 2.79 (0.1)	0.221
SMT (log-transformed)	− 3.08 (0.1)	− 3.06 (0.1)	0.024
CRT (log-transformed)	− 2.84 (0.1)	− 2.84 (0.1)	0.546
CMT (log-transformed)	− 3.09 (0.1)	− 3.10 (0.1)	0.457
N-back accuracy S0L	93.94 (17.1)	95.36 (8.7)	0.671
N-back accuracy S1L	92.66 (16.7)	93.9 (10.7)	0.774
N-back accuracy S2L	89.09 (16.5)	90.73 (9.3)	0.769
N-back RT S0L (median)	− 2.75 (0.04)	− 2.74 (0.1)	0.944
N-back RT S1L (median)	− 2.81 (0.1)	− 2.80 (0.1)	0.129
N-back RT S1L (median)	− 2.89 (0.1)	− 2.88 (0.1)	0.881

Data are presented as mean (SD) unless otherwise specified

Abbreviations: *VLT* verbal learning test, *SPS* spatial pattern separation, *DSST* digit symbol substitution test, *TMT* trail making test, *SRT* simple reaction time, *SMT* simple motion time, *CRT* choice reaction time, *CMT* choice motion time

three trials. No treatment effects were found (all associated F-values < 1.0, n.s.). The analysis of the N400 showed that this peak became less negative with repeated presentations ($F(2, 64) = 24.18$, $p < 0.01$). Additionally, there were differential effects for the electrodes ($F(2,64) = 7.55$, $p < 0.01$). No treatment effects were observed for the N400 (all associated F-values < 2.2, n.s.). The P600 was affected by the electrode position ($F(2, 64) = 68.87$, $p < 0.001$) and the trials ($F(2, 64) = 11.57$, $p < 0.001$). No treatment effects were observed for the P600 (all associated F-values < , n.s.) The peak increased with repeated presentation of the stimuli. There were no differences for latencies (all associated F-values < 1, n.s.).

n-Back-EEG

Comparable to the different components in the VLT task, the P300 component in the *n*-back task was affected by the electrode position ($F(2, 64) = 9.41$, $p < 0.01$; see Table 6). With increasing difficulty in this task the amplitude of the P300 decreased in both treatment groups equally ($F(2, 64) = 51.91$, $p < 0.01$). No treatment effects were found (all associated F-values < 1, n.s.). There were no differences found for the latencies in the different conditions (all associated F-values < 1, n.s.).

Beta-glucuronidase and Luteolin

We could not obtain blood from 8 participants divided over both pre- and post-treatment. In addition, luteolin measurements were below the lower limit of detection for 26 participants of the placebo group and 1 participant of the CILTEP® group. Consequently, these samples were not used for analyses. As can be seen from Table 7, there was no difference in plasma baseline beta-glucuronidase activity before placebo and CILTEP® treatment. CILTEP® treatment resulted in a 20-fold increase in the luteolin concentration compared to placebo treatment. To investigate a possible relationship between beta-glucuronidase activity at baseline and plasma luteolin concentration, beta-glucuronidase activity before CILTEP® treatment was correlated with plasma luteolin concentration after CILTEP® treatment. No correlation was found (Pearson's correlation coefficients − 0.07, n.s., respectively).

Discussion

This study aimed to investigate the acute treatment effects of CILTEP® on cognitive performance with a test battery that taps into different domains of cognition. The acute effects were assessed in a group of middle-aged participants. In addition, we measured ERPs in some tasks to see whether brain activity was altered by CILTEP® treatment. Lastly, baseline beta-glucuronidase activity and pre-treatment and luteolin concentration in blood plasma post-treatment were

Table 5 Mean latencies and amplitudes of the different event-related potential components during the three trials of the words in the verbal learning test averaged across the three-electrode positions. Data represent means (standard error of the mean)

		CILTEP			Placebo		
		Trial 1	*Trial 2*	*Trial 3*	*Trial 1*	*Trial 2*	*Trial 3*
P3a	***Latency***	258 (3.2)	259 (3.2)	257 (2.7)	255 (3.3)	254 (3.7)	254 (3.7)
	Amplitude	4.8 (0.7)	4.7 (0.7)	4.7 (0.7)	4.7 (0.7)	3.7 (0.6)	5.3 (0.8)
P3b	***Latency***	339 (4.1)	335 (4.0)	348 (3.5)	353 (3.0)	337 (4.0)	342 (4.1)
	Amplitude	1.8 (0.5)	1.8 (0.7)	2.1 (0.6)	2.6 (0.5)	2.6 (0.6)	3.6 (0.5)
N400	***Latency***	407 (4.4)	412 (5.4)	405 (5.8)	408 (4.4)	404 (5.0)	410 (5.3)
	Amplitude	−3.5 (0.5)	−3.3 (0.6)	−2.2 (0.4)	−1.6 (0.4)	−2.3 (0.4)	−0.9 (0.4)
P600	***Latency***	585 (12)	597 (11)	572 (11)	583 (10)	587 (10)	567 (12)
	Amplitude	3.5 (0.4)	3.3 (0.5)	4.6 (0.5)	5.1 (0.5)	4.3 (0.4)	4.7 (0.4)

Table 6 Mean latencies and amplitudes of the P300 ERP component in the *n*-back task for the different *n*-back conditions, across the three-electrode positions. Data represent means (standard error of the mean)

		Placebo			CILTEP		
		0-back	*1-back*	*2-back*	*0-back*	*1-back*	*2-back*
P300	***Latency***	378 (6.8)	374 (7.3)	370 (7.4)	385 (6.2)	371 (6.4)	362 (5.5)
	Amplitude	11.1 (0.8)	9.0 (0.7)	5.8 (0.6)	11.3 (0.9)	9.4 (0.7)	6.5 (0.05)

Table 7 Beta-glucuronidase activity and luteolin concentration in plasma in participants after CILTEP® treatment. Values are given as means (SEM)

	Placebo	CILTEP
Beta-glucuronidase activity (µg/h/ml) before treatment	11.40 (0.88) ($n=32$)	12.47 (1.03) ($n=32$)
Luteolin concentration (ng/ml) after treatment	0.059 (0.03) ($n=5$)	0.98 (0.11)** ($n=27$)

**$p<0.01$ vs. placebo (Mann–Whitney: $U=16$; r (effect size) $=0.47$)

measured, as well as the effects of CILTEP® on heart rate and blood pressure.

We assessed neuropsychological performance in different cognitive domains. Based on our previous findings with acute treatment with the PDE4 inhibitor roflumilast, we expected an improvement in verbal learning performance, specifically the delayed recall in the VLT task (Blokland et al., 2019; M. Van Duinen et al., 2018a, 2018b). However, although CILTEP® is assumed to exert PDE4 inhibition, we did not observe any effect on verbal learning performance. The other neurocognitive tests also showed no effects of CILTEP®, except a slower response of MT in a simple reaction time task and an increased spatial separation accuracy repeat post-CILTEP® treatment; however, after correcting for multiple comparisons with the Holm-Bonferroni approach, these two significant results were not significant anymore. Accordingly, we argue that these are not relevant effects. EEG measurements, specifically ERP components, are indicative of a central effect of treatment and are usually known to be more sensitive to treatment effects (Jobert et al., 2012). In this study, we examined the effects of CILTEP® treatment on different ERP components in the VLT and *n*-back tasks. There was no indication that CILTEP® had any effect on these EEG measures. Lastly, we measured heart rate and blood pressure before and after the intake of CILTEP®, however, found no significant effects on heart rate or blood pressure compared to placebo.

As expected, the baseline levels of beta-glucuronidase activity before treatment were not different between placebo and CILTEP® administration. This suggests that baseline glucuronidase activity is unlikely to affect luteolin metabolism after CILTEP® administration. To investigate a possible relationship between beta-glucuronidase activity at baseline and plasma luteolin concentration, beta-glucuronidase activity before CILTEP® treatment was correlated with plasma luteolin concentration after CILTEP® treatment. No correlation was found. Plasma luteolin concentration was higher after CILTEP® administration compared to placebo administration. It is interesting to note that after placebo administration, almost all luteolin plasma concentrations were below the LOD. Only 5 of the 31 participants had measurable luteolin concentrations. In fact, in all but one of these placebo samples, measurements were still below the lower limit of quantification, i.e., the lowest value of the standard curve. This indicates that luteolin concentrations in the plasma before CILTEP® treatment are very low and usually not detectable. In contrast, the luteolin concentration after CILTEP® treatment could be reliably determined (approximately a 20-fold increase), clearly indicating that CILTEP® treatment increases blood plasma luteolin concentrations. Calculation of the plasma luteolin concentration

in molarities results in a concentration of 4.22 nM. luteolin inhibits PDE1-5 with IC50 values of 10 µM or higher (Ayoub & Melzig, 2006; Rohrig et al., 2017). Thus, the plasma concentration of luteolin is still at least 2370-fold lower than the IC50 of any PDE type. Consequently, PDE inhibition is unlikely due to increased luteolin concentrations in plasma (or brain) after CILTEP® administration.

It is important to note that the population chosen for this study, namely, healthy highly educated middle-aged adults, warrants cognitive and physiological exceptions. Age-related peaks in various cognitive abilities are heterogeneous and complex; thus, floor or ceiling effects related to some cognitive tasks cannot be ruled out (Hartshorne & Germine, 2015). However, individuals were selected for participation based on their performance on the VLT, i.e., the main outcome. The interpretation of performance was based on normative scores (*z*-scores), where individuals were only admitted to participation if they performed within a normal range (1 SD above or below the norm) based on their age, sex, and education, thus reducing a floor and ceiling effect specific to episodic memory. However, selecting an older population (i.e., above the age of 65), a population that is cognitively impaired, or a population that also includes low education could potentially result in different outcomes in terms of performance on cognitive tasks and/or physiological measurements such as ERPs, heart rate, and blood pressure.

This study investigated the acute effects of CILTEP® on cognitive performance. This inherently raises the question of whether chronic and long-term administration of CILTEP® could result in different outcomes. As discussed earlier, in our acute findings, luteolin plasma concentration levels were too low, 2370-fold, to inhibit *any* PDE type. Consequently, we hypothesize that chronic administration of CILTEP® would not be able to improve cognition by increasing luteolin. B6, another ingredient in CILTEP®, is an essential nutrient. Vitamin B6 deficiency is hyperactive in the noradrenergic system, which can lead to cognitive impairment (Toriumi et al., 2021). This suggests that in adults who are B6 deficient, CILTEP could enhance cognition, as one CILTEP capsule includes 384% of the daily requirement of B6. However, a Cochrane review showed that there was no evidence of benefit from vitamin B6 supplementation on the mood or cognition of older people with normal vitamin B6 status or with vitamin B6 deficiency (Malouf & Grimley Evans, 2003). A meta-analysis by Zhang and colleagues has shown that high B6 concentrations in elderly populations had no benefit on cognition or dementia risk (Zhang et al., 2020).

CILTEP® also includes L-phenylalanine. L-phenylalanine is not known to have a direct impact on cognition, but it modulates the metabolism of dopamine, norepinephrine, and epinephrine, which in turn affects mood, anxiety, attentiveness, and motivation (van Ruitenbeek et al., 2009). One study found a positive correlation between l-phenylalanine and cognitive assessment scores in patients with amnestic mild cognitive impairment (aMCI) (Ravaglia et al., 2004). There has been much research on l-phenylalanine as an anti-depressant or as an intervention for individuals with ADHD (Akram et al., 2020). However, no studies (to our knowledge) have been conducted with healthy participants. Accordingly, no suggestions can be made as to whether chronic administration of l-phenylalanine would be able to modulate dopamine and thus increase cognition, mood, anxiety, attentiveness, and motivation.

Acetyl-L-carnitine (ALC), the second most prominent ingredient in CILTEP®, is a widely studied supplement related to cognition and cognitive impairment, and its underlying mechanisms have been shown to restore cell membranes and synaptic function, enhance cholinergic activity, promote mitochondrial energy metabolism, protect against toxins, and exert neurotrophic and nootropic effects in (Pennisi et al., 2020) in healthy elderly and patients with AD or MCI. However, differences in methodology and assessment tools make it difficult to compare existing studies. Thus, available evidence and the role of ALC are still subject to debate, but the findings have been promising. Suggestions from critical reviews published in 2017 (Chen et al., 2017a) and 2020 (Pennisi et al., 2020) suggested that future studies should focus on large samples, higher doses, and prolonged treatments in healthy, elderly, and individuals with cognitive impairment.

Note that each ingredient in CILTEP® may exhibit distinct pharmacokinetic profiles and mechanisms of action, leading to variations in the onset, duration, and magnitude of their effects. Furthermore, interactions between ingredients within the stack may influence the time course of cognitive enhancement. For example, synergistic interactions between L-phenylalanine and acetyl-L-carnitine could potentiate neurotransmitter synthesis and mitochondrial function, leading to sustained improvements in cognitive performance over time. Considering these factors, it is anticipated that the time course of the intervention's effects may be multifaceted, with some ingredients exerting rapid, acute effects, while others may contribute to more gradual, sustained improvements in cognitive function. Future studies utilizing longitudinal assessments and pharmacokinetic analyses could provide valuable insights into the dynamics of cognitive enhancement following ingestion of CILTEP®.

In conclusion, acute treatment with 3 capsules of CILTEP® does not improve cognitive performance in healthy middle-aged participants compared to placebo. Linked to this, ERP measurements do not indicate an effect of acute treatment of CILTEP® on brain activity. Plasma luteolin levels after CILTEP® treatment were below IC50 levels of PDE4 inhibition and therefore unlikely to have any effect on cognitive performance. It remains to be determined

whether chronic CILTEP® administration or CILTEP® administration in an elderly or cognitively impaired population may show positive effects on cognitive performance.

Author Contribution NP: investigation, project administration, data curation, formal analysis, visualization, writing—original draft, and writing—review and editing. SC: conceptualization and investigation, methodology, and project administration. AS: conceptualization, software, funding acquisition, methodology, supervision, and validation. AB: conceptualization, funding acquisition, resources, methodology, supervision, and validation.

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Data Availability The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declarations

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Conflict of Interest AB and AS have a proprietary interest in the PDE4 inhibitor roflumilast for the treatment of cognitive impairment.

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