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Effect of Epimerized Catechins-Rich Green Tea Extract on Global Cognitive Function in Healthy Individuals: A Pilot Study

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Abstract Green tea consumption has been shown to have many beneficial effects on brain health. A high-temperature-processed green tea extract (HTP_GTE) were developed with high levels of epimerized catechins and its effects were investigated on brain functions. HTP_GTE was found to protects neuronal cells and reduces neurite length against oxidative stress-induced cell damage ($p \leq 0.001$). Furthermore, a double-blind, placebo-controlled, randomized clinical study using the Cambridge Neuropsychological Test Automated Battery (CANTAB) showed that daily administration of 500 mg HTP_GTE for 8 weeks significantly improved global cognitive functions in middle-aged healthy subjects (n=23; 40.9 ± 4.4 yrs) ($p = 0.049$ vs. placebo). These preliminary results suggest that HTP_GTE may have potential as a nutraceutical for cognitive improvement.

Keywords: *HTP_GTE, cognition, CANTAB, oxidative stress*

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1. Introduction

Green tea is one of the most widely consumed beverages in different regions of the world including East Asia. It is commonly assumed that tea drinking has various health benefits [1]. Indeed, numerous studies have reported that green tea (*Camellia sinensis*) is effective in preventing age-related chronic diseases, such as metabolic disease and neurocognitive disorders [2,3].

Particularly, the long-term consumption of green tea has been linked to the prevention of cognitive dysfunction and improved mental performance [4]. Several epidemiological studies and clinical trials have also shown that green tea consumption is inversely related to the risk of cognitive disorders [5]. Reference [6] showed an association between total tea consumption, including green tea, with better global cognition performances, memory, executive functions and processing speeds. Reference [7] also showed the association between green tea consumption and a lower prevalence of cognitive impairment. These researches suggest that tea consumption affects cognitive function; however, a few interventional studies have been reported, and these effects should be certified.

The beneficial effects of drinking green tea have been attributed to the presence of flavonoids (30% of the dry weigh of a leaf), including catechins and their derivatives [8]. Tea catechins are known to undergo epimerization—i.e., the conversion of tea catechins to their corresponding isomers—during manufacturing and brewing processes [9]. Thus, the profile of phenolic compounds in tea beverages differs from that of raw tea leaves. For green tea beverages, considerable amounts (approximately 50%) of catechins are epimerized at the 2-position, and (-)-catechin ((-)-C), (-)-gallocatechin ((-)-GC), (-)-catechin gallate ((-)-CG), and (-)-gallocatechin gallate ((-)-GCG) are also formed [10]. Some studies have revealed that catechin epimers show different cellular uptake abilities from their original catechins. Moreover, catechin epimers have different biological activities such as radical scavenging activity, cholesterol absorption and binding affinity with cellular proteins [11,12]. For example, GCG, a corresponding epimer of EGCG (Epigallocatechin gallate), has stronger free radical scavenging abilities than EGCG [13]. Although the consumption of canned and bottled tea drinks is increasing in Asian countries, less is known about the physiological effects of epimerized catechins.

Brain aging is a major global concern, and various efforts have been carried out to prevent it. With rapidly changing environments, many individuals have cognitive problems, and even young individuals report subjective memory complaints [14,15]. To investigate the effects of catechin epimers on neurocognitive functions, the green tea extract with a high content of catechin epimers was prepared, namely high-temperature-processed green tea extract (HTP_GTE), by heat treatment [16]. (Figure 1, Table S1; Online Supporting Information).

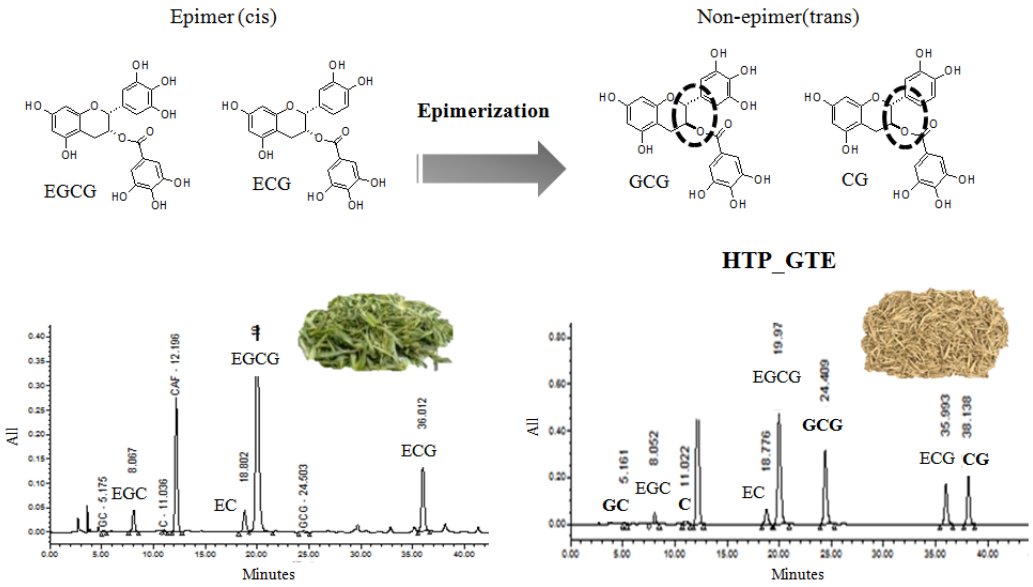

Figure 1. Bioactive chemical components of HTP_GTE with catechin epimerization

Catechin epimers from HTP_GTE were previously reported that are transported via the blood-brain barrier (BBB) with the highest permeability rates at the lowest concentration, and had free radical scavenging activity and anti-amyloidogenic activity, suggesting a potential neuroprotective role of HTP_GTE [17,18]. Furthermore, dietary supplementation of a HTP_GTE recently showed that it attenuates several cognitive-decline phenotypes in a model mouse of Alzheimer's disease, suggesting that HTP_GTE could act as a useful natural functional agent for the prevention of degenerative cognitive decline [19].

In this study, the protective effects of HTP_GTE on neuronal oxidative damage via *in vitro* studies and on cognitive function via a clinical study were examined. The *in vitro* findings revealed that HTP_GTE protects neuronal cells and preserves neurite length from oxidative stress in differentiated human neuroblastoma cell, SH-SY5Y. Furthermore, HTP_GTE were found to improves cognitive functions in healthy middle-aged subjects who have subjective memory complaints through a double-blind, placebo-controlled randomized study using the Cambridge Neuropsychological Test Automated Battery (CANTAB) [20].

2. Materials and Methods

2.1. Test Substances

Fresh green tea (*Camellia sinensis*, CS) leaves were collected in the spring from Osulloc Tea Garden in Jeju, Korea and were dried. The dried CS leaves were extracted twice with 50% aqueous ethanol and incubated at 100°C (1.2 atm) under aqueous conditions for 5 h to gain HTP_GTE. The catechin contents in HTP_GTE were measured and analyzed by high-performance liquid chromatography (HPLC) with a photodiode array (PDA) detector (Alliance 2695 system, Waters) using a Thermo Syncronis C18 column (250 × 4.6 mm, I.D., 5 μm; Thermo Fisher Scientific Inc.) HTP_GTE contained almost the same amount of epicatechins and catechin epimers (Table 1, Figure S1; Online Supporting Information).

Table 1. Catechins and their Epimers content in HTP_GTE

	Content (mg/g HTP_GTE)
(Catechin epimers)	
Catechin	14.8 ± 0.6
Catechin gallate	13.2 ± 0.2
Gallocatechin	46.4 ± 1.3
Gallocatechin gallate	56.4 ± 0.4
Sum of catechin epimers	*130.8 ± 2.5*
(Epicatechins)	
Epicatechin	9.2 ± 0.4
Epicatechin gallate	13.0 ± 0.0
Epigallocatechin	32.8 ± 1.0
Epigallocatechin gallate	53.2 ± 0.5
Sum of epicatechins	*108.2 ± 1.9*
Total catechins	**239.6 ± 0.8**
Caffeine	38.6 ± 0.5
L-theanine	11.2 ± 0.5

2.2. DPPH Scavenging Assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was calculated from the residual DPPH concentration, as estimated by a spectrophotometric method [21]. In brief, 180 μL of 222 μM DPPH (Sigma, St Louis., MO) was placed in a 96 well plate, and 20 μL of EGCG, GCG (Sigma, St Louis., MO; final concentration 10 μM), and a combination of the two (final concentration 5 μM each) in ethanol were added to initiate the DPPH quenching. The quenching reaction was performed for 30 min incubation at room temperature (RT) and absorbance at 517 nm was measured using a spectrometer. The residual DPPH is expressed as a percentage of the control and ethanol was used as a vehicle control.

2.3. Cell Culture

SH-SY5Y human neuroblastoma cells were obtained from the Korean Cell Line Bank (22266). SH-SY5Y cells were grown to confluence in complete culture medium consisting of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12 (Ham); Gibco, Waltham, MA; 11330-032) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT; SH30919.03),

100 units/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 and 95% air at 37 °C. The medium was refreshed every 2 days. To induce neurogenesis, SH-SY5Y cells were incubated in DMEM/F12 containing 3% FBS and 10 μM all-trans retinoic acid (RA) for 5 days. The differentiation medium was changed every 2 days.

2.4. Cell Viability Assay

To test the protective effects of HTP_GTE on oxidative stress, differentiated SH-SY5Y cells were preincubated with DMSO or 10 μg/ml of HTP_GTE for 24 hr before treatment with 100 μM hydrogen peroxide (H_2O_2). After 30 min, the cell viability was determined using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS; Promega, Madison, WI; G3582).

2.5. Neurite Outgrowth Analysis

Neurite outgrowth analysis was performed as described previously by Kim and Yoo [22]. Briefly, the neurite length was determined by manually measuring images taken from a phase-contrast microscope using ImageJ (NIH, Bethesda, MD). SH-SY5Y cells were plated onto a Nunc™ Lab-Tek™ II CC2™ Chamber Slide (Nunc, Waltham, MA; 154852) and were differentiated into neuronal cells for 5 days. Differentiated SH-SY5Y cells were incubated with DMSO or 10 μg/ml of HTP_GTE for 24 hr. After 30 min of 100 μM H_2O_2 treatment, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight at 4 °C and then washed with PBS. In total, 50 neurites were measured for each group.

2.6. Participants

To gain insights into the involvement of HTP_GTE in brain functions, clinical experiments were conducted. Because cognitive functions were gradually decreased with aging and the accumulation of oxidative stress, middle-aged (MA) subjects (40.9 ± 4.4 yrs), who begin to have cognitive complaints with aging, and old-aged (OA) subjects (61.5 ± 3.4 yrs), who have relatively low cognitive functions were recruited. This study examined whether supplementation of HTP_GTE improved mental state and cognitive functions.

In total, forty-four Korean subjects participated in this study. Twenty-three (10 females, 40.9 ± 4.4 yrs) and twenty-one (15 females, 61.5 ± 3.4 yrs) healthy adults were recruited in the MA and OA subjects, respectively. They were randomly assigned to the HTP_GTE or placebo groups (HTP_GTE group: MA=13, OA=11). Their cognitive ability was normal, but they had subjective cognitive complaints in their everyday lives. Exclusion criteria included neuropsychological disorders that can affect cognitive function. More detailed information of inclusion and exclusion criteria are described in Table S2 (Online Supporting Information). Any functional foods containing green tea extract were restricted during the study period. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study. This protocol was approved by the Amorepacific R&D Institutional Review Board (IRB: 2017-1CR-N030P). In addition, this study was registered in the clinical research information service (CRIS; registration number: KCT0004174), Republic of Korea (URL link: https://cris.nih.go.kr/cris/en/). This study was registered retrospectively because of proprietary issues of the HTP_GTE at the time of recruitment.

2.7. Clinical Study Design

Participants took two capsules (total 500 mg) of HTP_GTE or placebo (microcrystalline cellulose) daily for 8 weeks. The dose of 500 mg was determined as described by Nair and Shery [23] and Korea Food and Drug Administration (KFDA). They were required to write a daily record of consumption to check their compliance. Subjective questionnaires and cognitive function assessments were performed at baseline and 8 weeks after treatment. All participants responded to the Korean version of the subjective memory complaints questionnaire (SMCQ) [24] and perceived stress scale (PSS) [25] to check their psychological well-being. Only the OA subjects underwent the Korean Montreal Cognitive Assessment (K-MoCA) [26] at the baseline period to make sure that cognitive levels are not different between groups. The background characteristics of the participants at baseline are shown in Table 2. In the analysis, only twenty-two MA subject data were included because one MA subject from HTP_GTE group didn't participate during follow-up period (8 week). Study protocol is described in Figure 2.

Table 2. Demographic and Summary of Cognitive Function from Subjective Questionnaires of Participants at Baseline

	Middle-aged		Old-aged	
	HTP_GTE (n=13)	Placebo (n=10)	HTP_GTE (n=11)	Placebo (n=10)
Age	41.3 ± 4.6	40.3 ± 4.2	61.8 ± 4.0	61.1 ± 2.7
Gender, M/F	5/8	8/2	2/9	4/6
SMCQ	6.5 ± 2.8	6.3 ± 4.5	4.6 ± 3.8	4.5 ± 2.8
PSS	19 ± 5.7	18 ± 5.1	19 ± 6.2	17 ± 3.9
K-MoCA	-	-	25.7 ± 1.7	26 ± 1.5

All values are expressed as the means ± SD. SMCQ = subjective memory complaints questionnaire, PSS = perceived stress scale, K-MoCA = Korean Montreal Cognitive Assessment.

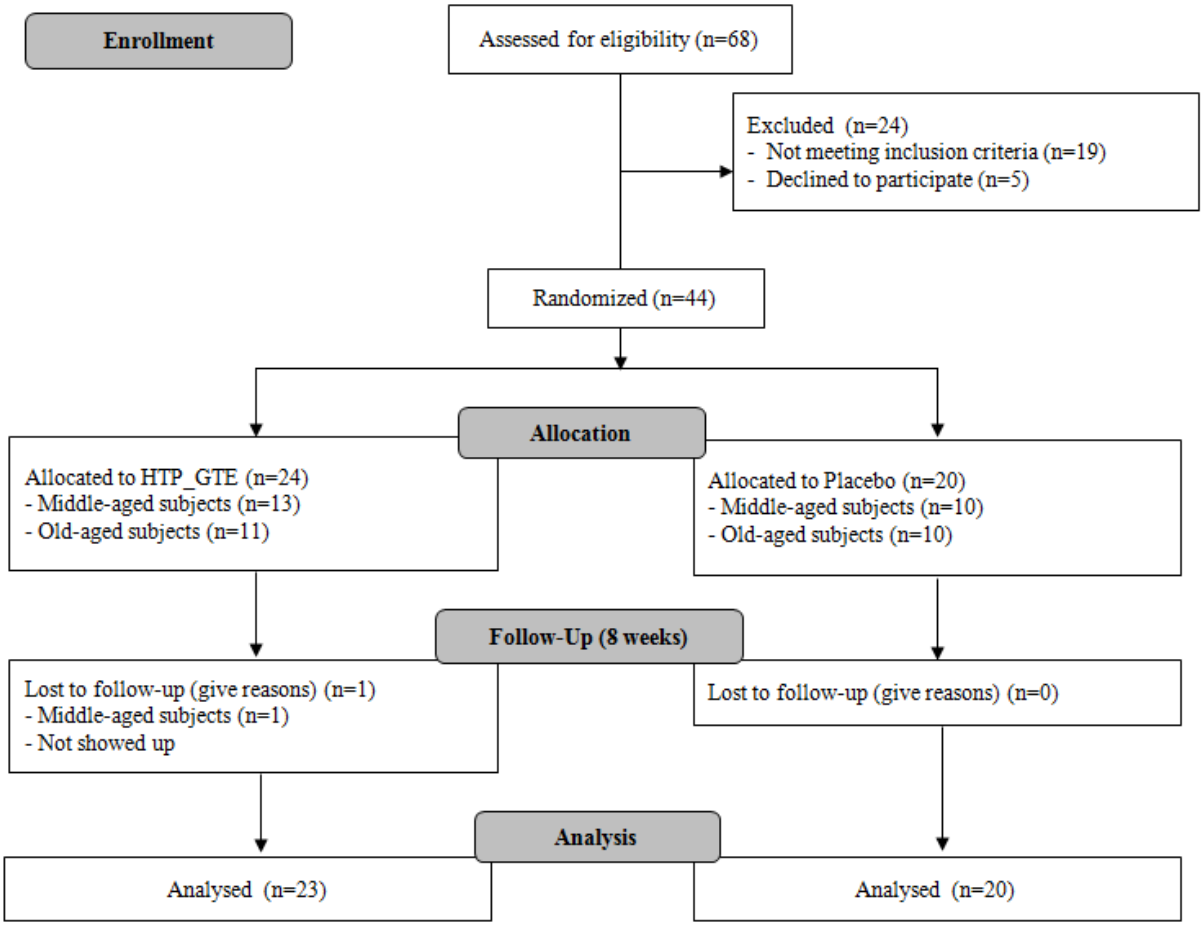

Figure 2. Study flow diagram

2.8. Cognitive Measurements

Cognitive functions were assessed using subtasks in a well-validated computerized software program from the Cambridge Neuropsychological Test Automated Battery (CANTAB) measurements. The CANTAB has been widely employed with sensitivity for measuring cognitive performance, including in healthy individuals. The following five tasks that are known to be sensitive for measuring global cognitive function [27] were used to assess different cognitive domains: motor screening task (MOT) to assess sensorimotor function and comprehension, reaction time (RTI) to assess processing speed and psychomotor speed, rapid visual information processing (RVP) to assess sustained attention, paired associates learning (PAL) to assess visual episodic memory, and spatial working memory (SWM) to assess working memory and strategy.

The primary endpoint adopted the changed composite Z score, which was calculated from the five key outcome measures (median reaction time from RTI; sensitivity to the target sequence from RVP; adjusted total errors from PAL; between errors and strategy from SWM) except MOT. Each outcome measure was standardized into the Z score using the mean and standard deviation of the placebo group at baseline [28]. The individual Z score was then averaged to generate a composite score from the CANTAB measurement. Each individual Z score is shown in Table S3 (Online Supporting Information).

2.9. Statistical Analysis

The results using cells are presented as the means ± s.e.m. Statistical significance was assessed by the two-tailed Student's t-test and one-way analysis of variance (ANOVA) followed by Newman Keuls post-hoc test using GraphPad Prism 5.0 (GraphPad Software). When cells were used for experiments, triplicates per group were chosen. Differences were considered statistically significant at $p \leq 0.05$.

Two-way repeated measure ANOVA was applied for the between-subjects factor treat (HTP_GTE and placebo) and within-subject factor time (0 week and 8 week) to assess the treat-by-time interaction effect. The statistical significance ($p \leq 0.05$) of the CANTAB composite Z score differences between 0 weeks and 8 weeks in each group were determined using the Wilcoxon paired test. Between-group (HTP_GTE vs. placebo) differences were analyzed using the Mann-Whitney U test. All statistical analyses were performed using IBM SPSS Statistics version 2010 (Chicago, IL).

3. Results and Discussion

3.1. Effects of HTP_GTE on Neuronal Cell Viability and Neurite Outgrowth Length

The antioxidant effects of EGCG and its iso-epimer, GCG were first evaluated in this study by analyzing their

DPPH radical scavenging activities (Figure 3A). EGCG or GCG at 10 μM and equal molar combinations of two (5 μM each, a total concentration of 10 μM) were used. EGCG or GCG exhibited similar DPPH scavenging rate (51.67 ± 0.07 % and 50.92 ± 0.97 %, respectively). Surprisingly, the combinations of EGCG and GCG showed stronger DPPH scavenging rate than either EGCG or GCG alone (69.27 ± 3.56 %; respectively $p \leq 0.01$). These findings suggested that the combinations of EGCG and GCG could exhibit a more potent antioxidant activity than either EGCG or GCG alone. Therefore, the HTP_GTE, which contains almost equal amounts of EGCG and GCG, was adopted and effects on oxidative stress-related neuronal cell damage were investigated. Remarkably, preincubation of HTP_GTE abolished hydrogen peroxide (H_2O_2)-mediated SH-SY5Y cell death (Figure 3B). Moreover, HTP_GTE preserved neurite destruction induced by H_2O_2 treatment (Figure 3C). These results indicate that HTP_GTE has protective effects on oxidative stress-induced neuronal cell death as well as the reduction of neuronal projections.

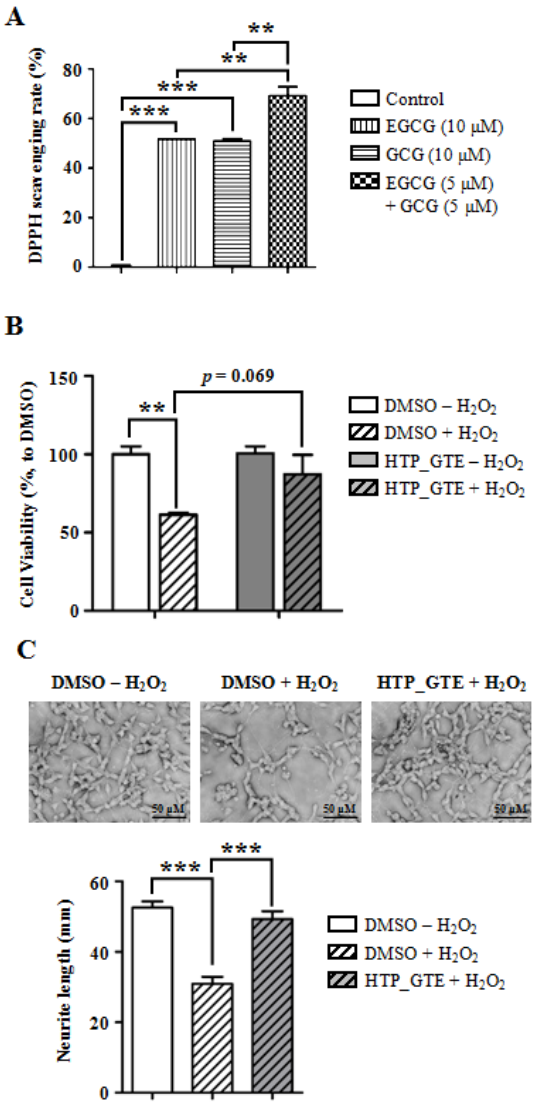

Figure 3. HTP_GTE protects neuronal cells from oxidative stress. (A) DPPH scavenging assay of EGCG, GCG, and a combination of the two. (B) Cell viability assay. (C) Microscopic view of SH-SY5Y cells and quantification of neurite length. The results are expressed as the means ± s.e.m. ** $p \leq 0.01$ and *** $p \leq 0.001$

Given that oxidative stress participates in neurodegeneration and brain aging, the reduction of oxidative stress is considered as a good target to cure the aging-related decrease in cognitive function [29]. It has been shown that green tea or green tea polyphenol including EGCG consumption reduces several oxidative stress markers and improves cognitive functions [30-36]. However, the effect of epimerized catechins including GCG or high dose of GCG containing extract on either oxidative stress or cognitive functions were not well elucidated yet.

3.2. Effects of HTP_GTE on Subjective Feeling and Cognition

3.2.1. Assessment of Psychological Well-being

The influence of HTP_GTE on mental health was tested first. There were no significant differences between the HTP_GTE and placebo groups on both the SMCQ and PSS measurements in MA subjects. Regarding the within-group differences, however, only the HTP_GTE group showed decreased stress levels (0 week, 20 ± 5.8; 8 week, 16 ± 5.3; marginal significance, $p = 0.059$) after 8 weeks. Although the differences were not significant, the HTP_GTE group reported fewer subjective memory complaints after 8 weeks of treatment (0 week, 6.3 ± 2.8; 8 week, 4.9 ± 2.7; $p = 0.122$) (Table 3). In contrast with the middle-aged subjects, old-aged subjects showed no significant differences between the placebo and HTP_GTE groups. Regarding the within-group differences, only the placebo group showed significance (0 week, 17 ± 3.9; 8 week, 20 ± 5.7; $p = 0.021$), but their stress levels rather increased 8 weeks later (Table 3).

Tea consumption is known to relieve mental stress [37]. For instance, black tea has shown stress recovery effects in a healthy population [38] by reducing blood pressure, heart rate, subjective stress ratings and even cortisol activation. In addition, a cohort study in Japan showed an inverse association between green tea consumption and psychological distress [39]. Furthermore, EGCG administration showed increased human electroencephalogram (EEG) activity as well as self-rated calmness and decreased self-rated stress [40], indicating that EGCG promotes relaxed and attentive states. They also showed that these effects were accompanied by increased alpha, beta and theta activities. However, most studies examining the psychological effects of green tea have focused on L-theanine or EGCG, which are constituents of green tea [41,42]. Although these results only showed marginal significance in the MA subjects with their stress level, it also provide the possibility that HTP_GTE, which contains high levels of epimerized catechins, may enhance psychological well-being.

3.2.2. Assessment of Global Cognitive Functions

The changes in global cognitive function induced by HTP_GTE using CANTAB were examined next. The interaction effects were checked before analyzing global cognitive function in MA subjects. The interaction effect between treat and time was marginally significant ($F_{(1,21)} = 4.2$, $p = 0.054$), and the main effect was significant for time ($F_{(1,21)} = 15.2$, $p = 0.001$). These results indicate that the differential effect of HTP_GTE over 8 weeks is dependent on the group.

Table 3. Psychological Well-Being Questionnaire Results

	Middle-aged			Old-aged		
	HTP_GTE (n=12)	Placebo (n=10)	*p* value[1]	HTP_GTE (n=11)	Placebo (n=10)	*p* value[1]
SMCQ						
0 week	6.3 ± 2.8	6.3 ± 4.5	0.821	4.6 ± 3.8	4.5 ± 2.8	0.918
8 week	4.9 ± 2.7	6.6 ± 4.0	0.582	4.7 ± 3.7	3.9 ± 3.1	0.468
p value[2]	0.122	0.878		0.644	0.402	
PSS						
0 week	20 ± 5.8	18 ± 5.1	0.539	19 ± 6.2	17 ± 3.9	0.387
8 week	16 ± 5.3	14 ± 2.7	0.381	18 ± 6.0	20 ± 5.7	0.282
p value[2]	0.059†	0.152		0.623	0.021*	

All values are expressed as the means ± SD. [1] Mann-Whitney U test. [2] Wilcoxon paired test. † $p \leq 0.1$, * $p \leq 0.05$, and ** $p \leq 0.01$.

Table 4. CANTAB Composite Z Score Results

	Middle-aged			Old-aged		
	HTP_GTE (n=12)	Placebo (n=10)	*p* value[1]	HTP_GTE (n=11)	Placebo (n=10)	*p* value[1]
0 week	0.1 ± 0.49	0.01 ± 0.4	0.83	-0.24 ± 0.7	0 ± 0.57	0.5
8 week	0.82 ± 0.6	0.23 ± 0.76	0.049*	-0.24 ± 0.68	0.12 ± 0.53	0.26
Change	0.72 ± 0.58	0.22 ± 0.57	0.088†	0 ± 0.61	0.12 ± 0.41	0.6
p value[2]	0.004**	0.33		0.79	0.21	

All values are expressed as the means ± SD. [1] Mann-Whitney U test. [2] Wilcoxon paired test. † $p \leq 0.1$, * $p \leq 0.05$, and ** $p \leq 0.01$.

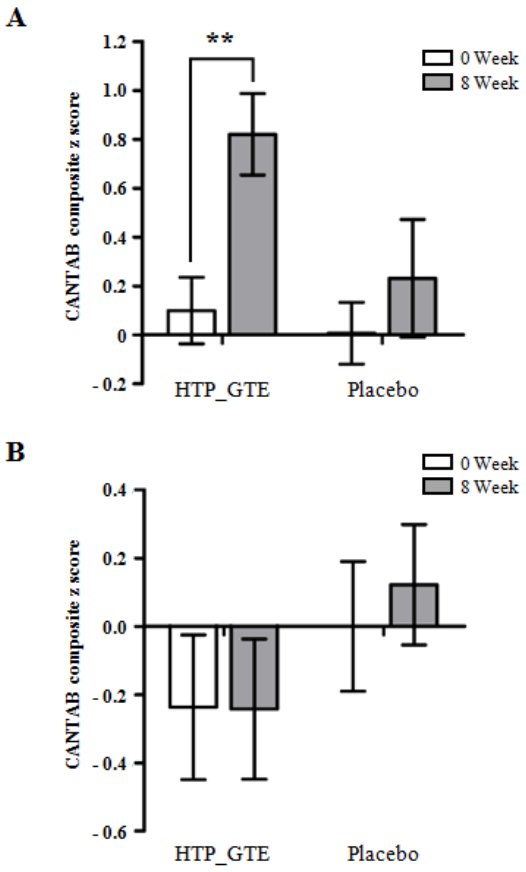

Figure 4. CANTAB composite Z score at baseline (0 week) and 8 weeks after treatment in the (A) MA and (B) OA subjects. The results are expressed as the means ± s.e.m. ** $p \leq 0.01$

The global cognitive function was represented by the CANTAB composite Z score; surprisingly, the HTP_GTE group showed significant improvement in middle-aged subjects after 8 weeks (0 week, 0.1 ± 0.49; 8 week, 0.82 ± 0.6; Change, 0.72 ± 0.58; p = 0.004) (Table 4 & Figure 4A). Additionally, five individual Z scores were also increased after 8 weeks in the HTP_GTE group, and the differences were all significant except for the SWM between errors (Table S3; Online Supporting Information). Between-group (HTP_GTE vs. placebo) differences also showed marginal significance (HTP_GTE, 0.72 ± 0.58; Placebo, 0.22 ± 0.57; p = 0.088), with Z score changes from 0 to 8 weeks (Table 4). The RTI reaction time only showed significant between-group differences (HTP_GTE, 0.60 ± 0.82; Placebo, -0.61 ± 1.06; p = 0.008) among the five individual outcome measures (Table S3; Online Supporting Information).

On the other hand, both the interaction ($F_{(1,18)}$ = 0.29, p = 0.6) and main ($F_{(1,18)}$ = 0.24, p = 0.63) effects were not significantly different in the OA subjects. In addition, the within- and between-group differences were not significant in the old-aged subjects (Table 4 & Figure 4B). Only the RVP measure showed a significant increase after 8 weeks in the HTP_GTE group (0 week, -0.50 ± 1.31; 8 week, 0.40 ± 1.07; Change, 0.90 ± 0.98; p = 0.016) (Table S3; Online Supporting Information). During aging with decreased brain functions, cognitive or memory complaints are common among middle-aged individuals (from their 30s to 50s). These individuals are concerned with their diminishing cognition and/or memory function, and even having dementia in the late stages of their lives. Therefore, it has been interesting to examine ways to protect against cognitive dysfunction. Despite the attention to brain health for middle-aged individuals, most clinical studies with green tea have been performed in elderly populations, cognitively impaired people or patients. In this regard, these results have a meaningful

implication for the middle-aged population who have mild subjective cognitive complaints.

CANTAB has been applied to multiple studies to evaluate the effects of supplements on cognitive enhancement among various target groups [43,44]. It was also used to determine the effects of EGCG treatment on neuropsychological performance in Down Syndrome patients aged 14 to 29 yrs, showing the significant effects of EGCG on working and episodic memory [45]. With these previous studies, CANTAB was adapted in this study to assess cognitive functions and composed four tasks—RTI, RVP, SWM, and PAL—to create an index score for global cognitive function because CANTAB does not provide a total score. Using the index score from CANTAB, the effects of HTP_GTE on global cognitive functions can be evaluated. In this context, this study is the first report to show the HTP_GTE-mediated improvement of cognitive functions in a healthy middle-aged population using CANTAB.

Many studies have shown the effects of green tea consumption on health and cognition in elderly people. Although this study showed no effects on global cognitive function in the OA subjects, the RVP outcome, which is frequently used in green tea effect studies on cognitive function [46], showed a significant increase in the OA subjects who consumed HTP_GTE. These results imply the functional role of HTP_GTE on attention and memory in the OA subjects, as this task represents sustained attention and a working memory. It is clearly important to verify the effects of HTP_GTE on global cognition in OA subjects in future studies.

4. Conclusion

In conclusion, this study showed that HTP_GTE, which contains almost equal amounts of catechins (EGCG, EGC, ECG, EC) and epimerized catechins (GCG, GC, CG, C), improved some cognitive functions including memory and attention in middle-aged subjects, as represented by the CANTAB composite score. The beneficial effects of HTP_GTE on cognitive functions might be due to, at least in part, its ability to prevent oxidative stress, although the precise underlying mechanisms are yet to be identified. Further investigations in human clinical studies with a larger sample size and comparative studies on HTP_GTE and EGCG are also needed to corroborate the functional role of newly developed HTP_GTE.

Conflict of Interests

This study was financially supported by the Amorepacific corporation (project number: R18E000002).

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Supporting Information

Table S1. Catechin Profiles of HTP_GTE and Green Tea Beverages

	HTP_GTE (%w/w)	Green tea beverage [b] (%w/w)
Catechin	1.5	1.3
Catechin gallate	1.2	1.2
Gallocatechin	4.6	3.8
Gallocatechin gallate	5.6	4.1
Epicatechin	0.9	1.0
Epicatechin gallate	1.3	0.9
Epigallocatechin	3.3	2.0
Epigallocatechin gallate	5.3	3.0
Total catechin[a]	23.7	17.3

[a] Sum of eight catechins
[b] Kao Corporation.

Table S2. Inclusion/Exclusion Criteria

Inclusion criteria	Exclusion criteria
1) People who fully understood about the test and voluntarily signed on a consent form	1) People who don't want to participate or didn't singed on a consent form
2) Healthy individual above 30 in their age	2) People who take herb or supplement (Anticoagulants / Antiplatelets, Functional Herbs and Supplements, Caffeine-Containing Herbs and Supplements, Bitter Orange, Creatine, Ephedra, Genistein Herbs and Supplements, Hepatotoxic Herbs and Supplements, Iron, Folic Acid, Calcium and Magnesium)
3) People who are willing to participate in the test	3) People who take medication that interact with green tea
4) People who report subjective cognitive impairment	4) People who have following disorder: Anemia, Anxiety Disorder, Bleeding Disorder, Cardiovascular Disease, Diabetes, Irritable Target Syndrome (IBS), Glaucoma, Hypertension, Liver Disease, Osteoporosis
	5) Pregnant and lactating women
	6) People who have liver disease
	7) People who have iron deficiency
	8) People who take supplement which can enhance cognitive function or memory
	9) If we decide it is difficult for them to participate

Table S3. Individual Z Score of Each CANTAB Outcome Measure

	Middle-aged			Old-aged		
	HTP_GTE (n=12)	Placebo (n=10)	*p* value[1]	HTP_GTE (n=11)	Placebo (n=10)	*p* value[1]
RTI[a]						
0 week	-0.06±1.07	0±1.00	0.927	-0.13±1.56	0±1.00	0.710
8 week	0.54±1.21	-0.61±1.22	0.015*	-0.38±1.39	0.56±1.02	0.095†
Change	0.60±0.82	-0.61±1.06	0.008**	-0.25±0.86	0.56±0.71	0.175
p value[2]	0.036*	0.114		0.374	0.110	
RVP[b]						
0 week	0.27±1.23	0±1.00	0.556	-0.50±1.31	0±1.00	0.261
8 week	1.21±0.62	0.45±0.80	0.017*	0.40±1.07	0.36±1.31	0.824
Change	0.95±1.04	0.40±0.70	0.148	0.90±0.98	0.36±1.29	0.370
p value[2]	0.003**	0.066†		0.016*	0.314	
PAL[c]						
0 week	0.30±1.09	0±1.00	0.483	0±0.95	0±1.00	1.000
8 week	1.02±0.74	0.76±0.92	0.483	0.30±0.96	-0.14±1.23	0.552
Change	0.71±1.18	0.76±1.36	0.832	0.30±0.86	-0.14±1.16	0.370
p value[2]	0.059†	0.114		0.283	0.594	
SWM[d]						
0 week	0.25±0.70	0±1.00	0.738	-0.47±0.73	0±1.00	0.295
8 week	0.46±0.64	0.02±1.24	0.605	-0.67±0.81	0.28±0.96	0.012*
Change	0.20±0.67	0.02±1.23	0.738	-0.20±0.91	0.28±1.32	0.412
p value[2]	0.241	0.593		0.514	0.513	
SWM[e]						
0 week	-0.27±1.00	0±1.00	0.446	-0.09±0.92	0±1.00	1.000
8 week	0.87±1.37	0.58±1.61	0.563	-0.86±0.87	-0.44±0.95	0.456
Change	1.14±1.77	0.58±1.16	0.446	-0.77±1.13	-0.44±1.26	0.882
p value[2]	0.036*	0.143		0.034*	0.320	

All values are expressed as the means±SD. [1] Mann-Whitney U test. [2] Wilcoxon paired test. [a] median reaction time; [b] sensitivity to target sequence; [c] adjusted total errors; [d] between errors; and [e] strategy. † $p \leq 0.1$, * $p \leq 0.05$, and ** $p \leq 0.01$.

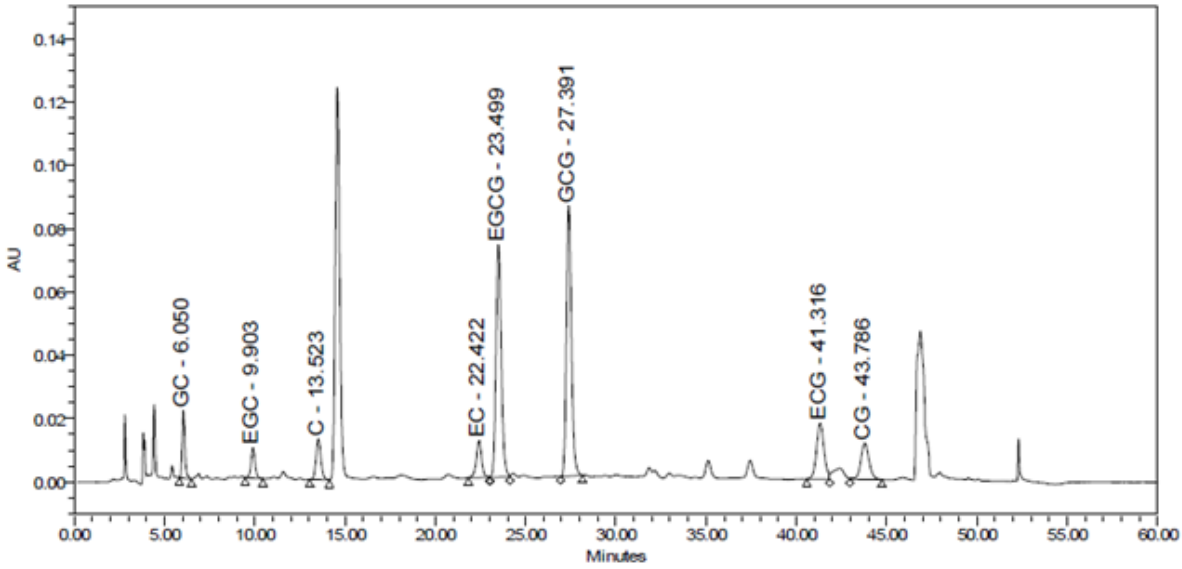

Figure S1. The representative HPLC chromatogram of catechin compounds in high-temperature-processed green tea extract (HTP_GTE). HTP_GTE was standardized using reverse-phase high-performance liquid chromatography (HPLC)-PDA (Alliance 2695 system, Waters) system. Separation was carried out using a Thermo Syncronis C18 column (250 × 4.6 mm, I.D., 5μm; Thermo Fisher Scientific Inc.). The mobile phases were 0.1% acetic acid in water for solvent A and acetonitrile for solvent B. The gradient elution was 90% A + 10% B at 0-10 min, 85% A + 15% B at 10-30 min, 80% A + 20% B at 30-53 min, 5% A + 95% B at 53 -55 min, 90% A + 10% B at 55-60 min with a flow rate of 1.0 mL/min. The injection volume of sample was 20 μl, and the wavelength of UV was 280 nm. Each peak was named by corresponding compound obtained by standard curve. EGCG, (-)-epigallocatechin 3-O-gallate; GCG, (-)-gallocatechin 3-O-gallate; EGC, (-)-epigallocatechin; GC, (-)-gallocatechin; ECG, (-)-epicatechin 3-O-gallate; CG, (+)-catechin 3-O-gallate; EC, (-)-epicatechin; C, catechin