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Research Article

Effects of *Gastrodia elata* Blume on Cognitive Function Improvement: A 12 Weeks, Randomized, Double-Blind, Placebo-Controlled Pilot Study

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Abstract

Background and Objective: *Gastrodia elata* Blume (GEB) is a well-known herbal plant having medicinal value. This study was undertaken to assess the beneficial effects of GEB on cognitive performance in healthy late-middle-aged adults. This study investigated the influence of GEB on Brain Derived Neurotrophic Factor (BDNF) Val66Met genotype, which plays a key role in cognitive functioning. **Materials and Methods:** Memory function assessments and measurements of serum BDNF and C-Reactive Protein (CRP) levels were performed at baseline and after the 12 weeks intake of the test products. **Results:** The comparison of changes in cognitive function tests between the test and placebo groups revealed no notable difference. However, the effects of GEB on verbal and visual recognition memory were significant for participants with Val/Met polymorphism ($p < 0.05$). The serum BDNF and CRP levels didn't change markedly in the GEB group than in the placebo group. **Conclusion:** These observations suggested that supplementation of GEB for 12 weeks positively influenced memory function in subjects with Val/Met polymorphism.

Key words: *Gastrodia elata* blume, cognitive, computerized neuropsychological test, BDNF Val66Met genotype

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cognitive impairment is a significant health and social issue in the aging population. Cognitive decline is among the most feared aspects of growing old as it threatens the functional independence and quality of life in older adults and presents challenges to the health care system. The age-related decline is not uniform across the cognitive domains and some mental functions like numerical and verbal abilities and general knowledge could remain stable until very late in life¹. In contrast, attention, memory, processing speed and reasoning and executive function start to decline in middle adulthood and decline progressively until death²⁻⁵. Late-middle-aged adults are very keen on drugs, healthy diets and functional foods which enhance cognitive functioning. Functional foods targeting cognitive functioning have a rapidly growing demand due to increasing aging population and the need for well-being among elderly adults. The nutrients affecting the cognitive function are choline, curcumin, minerals (calcium, copper, iron, selenium and zinc), omega-3 fatty acids and vitamins (B, C, D and E)⁵. Functional foods approved as cognition-enhancing agents in Korea are angelica gigas Nakai root extract, platycodon grandiflorum extract, fermented milk with lactobacillus helveticus and Phosphatidylserine (PS). *Gastrodia elata* Blume (GEB) is a popular herbal medicine that is widely used to treat various conditions like paralysis, headache, dizziness, spasm and other disorders. It is found widely in hilly areas of China, Korea, Japan and eastern Asia. GEB as a herbal medicine has centuries of history and is listed in the Chinese Pharmacopoeia⁶. Gastrodin, a major bioactive component of GEB, is demonstrated to provide cognition-enhancing⁷⁻⁹, neuroprotective¹⁰, antidepressant¹¹ and anti-inflammatory effects¹². Previously, acute administration of gastrodin and p-hydroxybenzyl alcohol demonstrated prolonged cycloheximide- and apomorphine-induced step-through latency in a passive avoidance task and facilitated memory consolidation and retrieval but not acquisition⁷. However, 7 days gastrodin or p-hydroxybenzyl alcohol treatment remarkably prolonged the scopolamine-induced step-through latency during the passive avoidance task⁸. GEB also reduced focal ischemic brain injury through antioxidant related gene expressions¹³ or aluminum chloride-induced⁹ impairment in the passive avoidance test. More importantly, several investigations have demonstrated the potential value of GEB in treating Alzheimer's disease. GEB protects cells from amyloid β peptide-induced cell death via substantial hydroxyl radical scavenging activity¹⁴. Also, amyloid beta¹⁵⁻²⁵ and a rat model of Alzheimer's disease²⁶ showed that GEB treatment significantly affected choline

acetyltransferase expression in the medial septum and hippocampus and the activity of acetylcholinesterase in the prefrontal cortex²⁷. However, the effects of GEB on cognitive functions in human subjects have not been detailed. Here, we report the effects of GEB on cognitive functions in late-middle-aged healthy adults. Given that the presence of the Met allele of Brain Derived Neurotrophic Factor (BDNF) reduces cognitive performance in elderly adults²⁸, a sub-analysis was conducted exploring the effects of GEB depending on BDNF genotype. As secondary measures, serum BDNF and C-Reactive Protein (CRP) levels were measured during the screening and at the study endpoint.

MATERIALS AND METHODS

Subjects: This study was conducted according to the guidelines of the International Conference on Harmonization Good Clinical Practice (ICH GCP) and approved by the Institutional Review Board of Jeonbuk National University Hospital (approval number: CUH 2013-09-013-001). The clinical trial was conducted by following the provisions of the Helsinki Declaration and the standards for clinical trial management (IGCP). Subjects were recruited from December, 2013 to October, 2014 through internal advertising (internet postings on departmental home pages, brochures and posters) at the Clinical Trial Center for Functional Foods (CTCF2) at Jeonbuk National University Hospital.

The subjects signed informed consent and underwent a screening test. A total of 60 subjects were selected. Criteria for selecting subjects were as follows: All the participants in the study were 50-64 years old and were recruited via advertisements.

Inclusion criteria were, (1) Experience using computers and education above the middle school level, (2) Mini-mental state examination-Korean version²⁹ score ≥ 24 , (3) Bodyweight within 30% of the ideal bodyweight, (4) Understanding of the study's objectives and agreeing to abide by its rules during the study period.

Exclusion criteria were (1) Current diagnosis of axis I mental disorder or on treatment for any such disorder within the past 3 years, (2) Alcohol abuse or dependence within the past 3 months, (3) Gastrointestinal disease or history of gastrointestinal surgery which might affect the absorption of study materials, (4) Significant neurological (epilepsy, mental retardation or stroke) or medical (diabetes, hypertension or cardiovascular disease) illness, (5) Consumption of micronutrient supplements or herbal medicines during the 4 weeks preceding the start of the study, (6) Participation in other clinical trials for functional food

development within the past 2 months. Also, a psychiatric trainee performed interviews using the screening module of the structured clinical interview of the DSM-IV-NP³⁰ to screen axis I mental disorders in subjects. Written informed consent was obtained from the participants.

Study design: This study is a 12 weeks, double-blind, randomized controlled trial. During the screening period, physical examination, medical history recording, blood sampling for basic laboratory tests (BDNF genotyping and measurement of serum BDNF and CRP levels) and psychiatric interviews were conducted. For eligible individuals, data for cognitive function tests were obtained at baseline and after the 12 weeks treatment period, self-rating scales were completed at baseline and 6 and 12 weeks. Participants were then subjected randomly to the GEB (n = 30) or placebo (n = 30) condition using a Latin square design. All the participants were informed not to use any supplements or traditional medicine, avoid vigorous physical activity, refrain from alcohol consumption during the trial and have enough sleep one day before the baseline and endpoint assessment tests. Compliance was checked at each visit.

Test supplements: All samples were provided in a refined format by the muju cheonma cluster agency (Muju, Jeonbuk, Korea). Analysis of the surface components of GEB was performed as previously described in another preclinical test. Whole dried tubers of *Gastrodia elata* blume were hammered into smaller pieces and ground to a fine powder²⁶. GEB was administered orally three times a day in pills form, with a total daily dose of 13.5 g (containing GEB powder 12 g). The HPLC chromatogram of the GEB was shown in Fig. 1. The pills were made according to the standard manufacturing process. All ingredients were weighed accurately, passed through a sieve and mixed thoroughly. The powder blend was compressed using a rotary pills machine (PRESSIMA, KILIAN Tableting GmbH, Cologne, Germany). Placebo pills were made without GEB using the same preparation method. Placebo pills were identical to the active pills in color, shape and size. The composition of the test product was shown in Table 1.

Cognitive and clinical assessment

Cognitive function tests: The Computerized Neuropsychological Test (CNT) version 4.0 (MAXMEDICA Inc, Seoul, Korea) was used to assess the cognitive function^{18,31-34}. Primary output measures of this study were changes in cognitive function tests. Specifically, among the 18 sub-categories of the CNT, the auditory continuous-performance

test, Digit-Span Test (DST), Stroop test and verbal learning test (verbal LT) were performed as the appropriate tests for evaluation of cognitive function in this study³⁴. In addition, a Novel Object Recognition Test (NORT) was employed to assess visual recognition memory. The NORT procedure³⁵ was modified slightly to accommodate the age of the participants. Briefly, the NORT consisted of two phases. At 1st (encoding phase), subjects passively viewed about 30 images, where each image was shown for 500 ms with a 1,000 ms fixation cross between the images. On a white background, the images and fixation cross were displayed at the center of the screen. The selected images were all highly recognizable, readily nameable and neutral. In the 2nd (retrieval) phase, conducted 15 min after the 1st phase, participants completed the recognition memory task (60 images) where 30 were previously shown images and 30 were new images. Subjects were instructed to indicate whether each object was a member of the original set or a new object by key presses. The order of "old" and "new" images was randomized. The duration of image presentation was controlled by participants' response time, with no fixation during the retrieval block. Accuracy served as the primary variable of interest and was defined as the percentage of objects identified correctly as new. Participants completed 4 practice trials (with 4 images each) before the retrieval block began and were assigned randomly to one of 4 different versions of the task at baseline and at endpoint, thus, they were not tested with the same set of images more than once. The cognitive assessments were conducted by experienced research nurses who were blinded to the objectives of the study.

Self-rating scales: The Perceived Stress Scale (PSS)¹⁵ and Prospective and Retrospective Memory Questionnaire (PRMQ)¹⁶ were performed at baseline and at 6 and 12 weeks. The PSS is a set of 10 items measuring subjective perception of stress, with responses structured by a likert-type scale ranging from 0 (not at all) to 4 (always). The maximum possible PSS score is 40, with higher scores indicating greater subjective perception of stress. The PRMQ is a 16-item questionnaire with 2 items each in 8 categories reflecting different aspects of memory. The 8 categories are prospective short-term self-cued, prospective short-term environmentally cued, prospective long-term self-cued, prospective long-term environmentally cued, retrospective short-term self-cued, retrospective short-term environmentally cued, retrospective long-term self-cued and retrospective long-term environmentally cued memory. We used only the total PRMQ, prospective and retrospective raw scores converted to T scores using a computer program

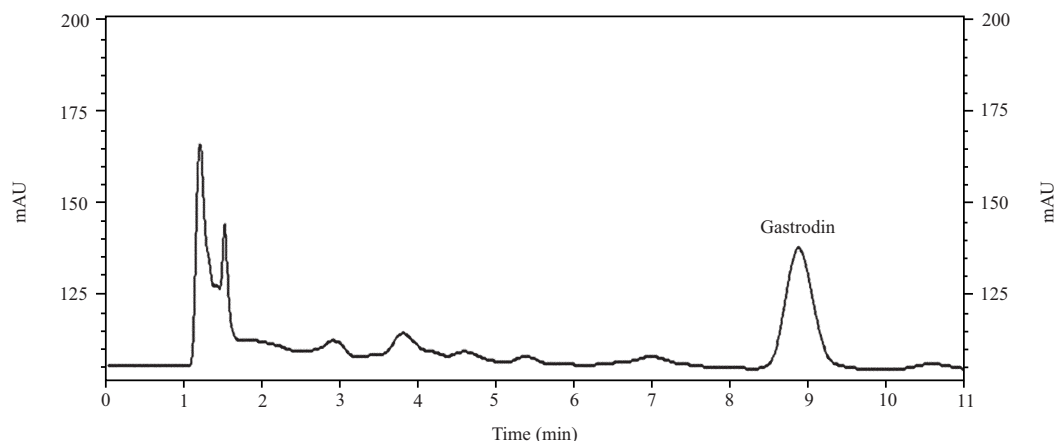


Fig 1: Representative chromatograms of GEB (*Gastrodia elata* blume powder) based on high-performance liquid chromatography (HPLC) analysis of gastrodin

Table 1: Composition of the test product

Components	Test GEB pill supplement (%)	Placebo pill supplement (%)
<i>Gastrodia elata</i> blume powder	89.5	-
Cacao powder	1.5	1.5
Wheat powder	8.5	98.0
Mint flavor powder	0.5	0.5
Total	100	100

GEB: *Gastrodia elata* blume powder

(www.psyc.abdn.ac.uk/homedir/jcrawford/prmq.htm). High T scores indicated better self-reported memory.

BDNF genotyping: Genomic DNA was isolated from whole blood samples (3 mL) using Easy-DNA Kit (Invitrogen, Boehringer Mannheim, San Diego, CA, USA). PCR was done using SolGent EF Taq Polymerase (SolGent, Daejeon, Korea) and amplification was under the following conditions: A hold step at 94°C for 5 min and 35 cycles of denaturation at 94°C for 30 sec followed by annealing at 72°C for 1 min. The extension was done at 72°C for 7 min. PCR products were purified using the MSNU030 plate (Millipore SAS, Molsheim, France) and sanger sequenced with the big dye terminator v3.1 sequencing kit and a 3730xlDNA analyzer (applied biosystems, Foster City, CA, USA). Targeted gene-specific primers used for amplification were as follows: Forward (F) 5'-ACTCTGGAGAGCGTGAAT-3' and reverse (R) 5'-ATACTGTCACACAGCTC-3'. Nucleotide sequences of PCR products were performed to standard sequencing at the MacroGen Co., Ltd., (Seoul, Republic of Korea). Nucleotide sequences of PCR products was performed by standard sequencing at the MacroGen Co., Ltd., (Seoul, Republic of Korea).

Serum levels of BDNF and CRP and laboratory testing: Blood samples (10 mL) were drawn at screening and after the

12 weeks treatment from the cubital vein into a serum separator tube to minimize the effects of possible circadian variations. Blood samples was collected from all subjects after at least 12 hrs of overnight fasting. These samples were drawn between 7 and 9 am after overnight fasting. Collected blood samples were centrifuged at 4°C (1,000×g for 15 min) after allowing the blood to clot for 30 min. Serum was aliquoted and stored at -80°C. Serum BDNF and CRP levels were determined using the Quantikine® Human BDNF immunoassay kit (R and D Systems, Minneapolis, MN, USA) and the Quantikine® ELISA kit (Human C-Reactive Protein/CRP Immunoassay, R and D Systems), respectively. All samples were analyzed in triplicate in one session by an investigator blinded to the experimental design and objectives. During the screening period and after the 12 weeks treatment, basic laboratory tests, such as blood cell count, electrolyte measurement, liver function test, urinalysis and electrocardiogram were performed.

Sample size: The sample size calculation for this study was based on the assumption that the variation in the Rapid Visual Information-Processing (RVIP) task average correct response test after 12 weeks of intake in the GEB group would be 3.61, the variation in the placebo group would be 1.0 and the standard deviation would be 2.89. The number of subjects

required was calculated as described by Chung *et al.*³⁶. The number of subjects required in each group to achieve 80% power for a 5% significance level with a 2 sided test was 20 persons per group. Considering an estimated dropout rate of 30%, the required sample size was determined to be 60 subjects in total.

Statistical analysis: Cognitive test and biomarker data were analyzed using two-way (treatment×time) repeated-measures Analysis of Variance (ANOVA) and an independent t-test. For independent t-tests, changes in measurements over the 12 weeks study period were determined by calculating the difference between the pre- and post-intervention measurements in each group. Paired t-tests were performed for within-group analysis. All data are presented as means with standard deviations. As the CRP levels were not distributed normally, the data were log-transformed. Significance was set at a $p < 0.05$.

RESULTS

Characteristics of the participants: A total of 78 subjects were screened and among them, 60 subjects were eligible. All the selected participants were randomly divided into GEB (n = 30) and placebo (n = 30) groups. No dropout occurred due to high compliance, no withdrawal of consent or violation of the protocol during the study. The compliance rates in the GEB and placebo groups were 93.2 and 94.6%, respectively. Demographic characteristics and other variables did not differ significantly between the groups (Table 2).

Primary outcomes: We observed marked improvement in most verbal LT and stroop test (error) items at the endpoint compared with baseline in the GEB and placebo groups (Table 3). However, no significant difference was observed from baseline to endpoint in both groups. Two-way repeated-measures ANOVA demonstrated that the main effect of time was significant for the verbal LT, DST and stroop test, whereas

no significant difference for the main effects of the treatment and time×treatment interaction was observed.

Secondary outcomes: For the sub-analysis exploring the effects of GEB according to BDNF genotype, only data for the Val/Met polymorphism were analyzed because of too few numbers in other genotypes. Significant improvement was seen in most verbal LT and DST (forward) items and NORT accuracy in the GEB and placebo groups after the 12 weeks treatment (Fig. 2). Comparison of changes between the groups revealed significant differences in the verbal LT B score and NORT accuracy. Analysis of treatment effects over time demonstrated significant time effects for most verbal LT and stroop test (error) items and significant time×interactions for the verbal LT B score and NORT accuracy. The latter findings indicate significant improvements in the VLT B and NORT accuracy in the GEB group than the placebo group. As a result of the genotype analysis of the study subjects, only 42 out of 60 subjects were able to obtain results, of which were Val/Met (n = 31), Val/Val (n = 5) and Met/Met (n = 6). The number of samples in the Val/Val and Met/Met groups were small. Thus, the effect analysis was performed only in the Val/Met group.

The self-rating PSS and PRMQ scales were not significant within- and between-group for participants as initially assigned and those with the Val/Met polymorphism. BDNF levels showed appreciable reductions from screening to the endpoint in both groups as initially assigned but only in the placebo group among subjects with the Val/Met polymorphism (Table 4). No significant change in the CRP level from screening to endpoint was observed in either group as initially assigned or in subjects with the Val/Met polymorphism. Change in BDNF and CRP levels between the groups as initially assigned and in subjects with the Val/Met polymorphism showed no significant difference (Supplementary Table 1). Two-way repeated-measures ANOVA also demonstrated no significant time×treatment interaction in participants as initially assigned and with the Val/Met polymorphism.

Table 2: Demographic characteristic of study participants

	GEB groups (n = 30)	Placebo groups (n = 30)	Total (n = 60)	p-value
Sex (male/female) (%)	5 (16.7)/25 (83.3)	5 (16.7)/25 (83.3)	10 (16.7)/50 (83.3)	1.000 ¹
Academic ability (years)	12.33±2.07	12.37±2.31	12.35±2.18	0.953 ²
Age (years)	55.73±3.24	55.8±4.61	55.77±3.95	0.949 ²
Alcohol amount consumed (units/month)	1.68±3.53	2.88±9.05	2.28±63.84	0.502 ²
Smoker (yes/no) (%)	2 (5.7)/33 (94.3)	2 (5.7)/33 (94.3)	4 (5.7)/66 (94.3)	1.000 ¹
No. cigarettes per day	0.00±0.00	0.13±0.73	0.07±0.52	0.326 ²
Caffeine (cup/week)	8.63±9.10	5.27±6.03	6.95±7.84	0.096 ²
MMSE-K total	28.17±1.51	27.93±1.34	28.05±1.42	0.529 ²

Values are presented as Mean±SD or numbers (percentages), ¹Analyzed by chi-square test, ²Analyzed by independent t-test, GEB: *Gastrodia elata* blume powder and MMSE-K: Mini-mental status examination-Korean (unit, 10 g of pure alcohol)

Table 3: Changes in neurocognitive function tests before and after 12 weeks of subjects

	GEB groups (n=30)					Placebo groups (n=30)					p ³		
	Baseline	12 weeks	Change value	p ¹		Baseline	12 weeks	Change value	p ¹	p ²	Time	Group	Time*group
CNT													
Verbal LT A1	6.37±1.69	7.70±1.99	1.33±2.01	0.001		5.63±1.33	7.53±2.32	1.90±2.20	0.000	0.302	0.000	0.262	0.302
Verbal LT A2	8.60±1.61	9.20±2.75	0.60±3.01	0.284		8.63±1.92	10.00±2.07	1.37±1.99	0.001	0.250	0.004	0.347	0.250
Verbal LT A3	9.97±2.16	11.10±2.17	1.13±2.11	0.006		9.50±2.16	11.17±2.05	1.67±2.06	0.000	0.326	0.000	0.679	0.326
Verbal LT A5	11.37±1.8	12.57±1.55	1.20±1.42	0.000		11.30±1.70	12.23±1.74	0.93±2.05	0.019	0.561	0.000	0.602	0.561
Verbal LT B ⁴	5.27±1.80	6.20±1.40	0.93±1.87	0.011		5.00±1.48	5.73±1.48	0.73±1.80	0.033	0.675	0.001	0.232	0.675
Verbal LT A20 (delayed recall) ⁵	9.23±3.02	11.37±2.43	2.13±2.30	0.000		9.13±3.17	11.20±2.06	2.07±2.49	0.000	0.915	0.000	0.832	0.915
Digit span test													
Forward	10.17±2.88	10.60±2.97	0.59±2.26	0.173		10.03±2.94	11.03±3.46	1.00±2.69	0.051	0.526	0.017	0.926	0.526
Backward	8.10±3.28	8.03±2.72	-0.10±2.57	0.830		8.00±2.61	8.23±2.46	0.23±2.06	0.540	0.580	0.831	0.922	0.580
Stroop test (word color)													
Reaction time	43.13±25.04	36.03±12.18	-5.10±19.52	0.163		3.09±19.70	36.89±13.08	-1.20±17.70	0.713	0.421	0.915	0.789	0.421
Error	2.13±4.55	0.60±2.04	-1.53±3.00	0.009		1.00±1.58	0.13±0.35	-0.87±1.66	0.008	0.291	0.000	0.187	0.291
Auditory CPT													
FAR	0.01±0.01	0.01±0.01	0.00±0.01	0.586		0.01±0.02	0.01±0.012	0.00±0.01	0.152	0.409	0.141	0.182	0.425
Sensitivity ⁶	0.99±0.01	0.99±0.02	0.00±0.02	0.674		0.99±0.02	0.99±0.01	0.00±0.01	0.197	0.250	0.579	0.343	0.237
Reaction time	0.64±0.04	0.64±0.05	0.00±0.03	0.712		0.66±0.04	0.65±0.03	0.01±0.03	0.097	0.159	0.387	0.326	0.159
NORT accuracy	27.7±3.29	28.13±2.15	0.43±2.40	0.331		27.67±2.17	26.93±3.46	0.73±3.71	0.288	0.155	0.712	0.317	0.154
Values are presented as Mean±SD; ¹ Analyzed by a paired t-test; ² Analyzed by independent t-test; ³ Two-way RM ANOVA; ⁴ After the 5th trial (A5), a new interference list (list B) was presented and recalled; ⁵ Total number of words recalled after a 20 min delay (verbal LT A20 delayed recall); ⁶ Sensitivity = 0.5+(HR-FAR)/(1+HR-FAR)/(1+HR-FAR)/4HR(1-FAR); GEB: <i>Gastrodia elata</i> blume powder, CNT: Computerized neuropsychological test; Verbal LT: Verbal learning test, CPT: Continuous performance test, HR: Hit rate (correct response/135), FAR: False alarm rate, HR (1) and NORT: Novel object recognition test													

Values are presented as Mean±SD, ¹Analyzed by a paired t-test, ²Analyzed by independent t-test, ³Two-way RM ANOVA, ⁴After the 5th trial (A5), a new interference list (list B) was presented and recalled, ⁵Total number of words recalled after a 20 min delay (verbal LT A20 delayed recall), ⁶Sensitivity = 0.5+(HR-FAR) (1+HR-FAR)/4HR(1-FAR), GEB: *Gastrodia elata* blume powder, CNT: Computerized neuropsychological test, Verbal LT: Verbal learning test, CPT: Continuous performance test, HR: Hit rate (correct response/135), FAR: False alarm rate, HR (1) and NORT: Novel object recognition test

Table 4: Comparison of BDNF and CRP levels between GEB and placebo groups

	GEB (n = 30)					Placebo (n = 30)					p ³		
	Baseline	12 week	Change value	p ¹		Baseline	12 week	Change value	p ¹	p ²	Time	Group	Time*group
BDNF (pg mL ⁻¹)	25563.3±4915.6	24417.1±4592.6	-1146.3±2363.5	0.013		25549.2±4770.56	23724.7±4141.5	-1824.5±1953.1	0.000	0.231	0.000	0.761	0.231
CRP (mg mL ⁻¹)	0.53±0.57	0.47±0.43	-0.06±0.52	0.544		0.39±0.44	0.44±0.28	0.05±0.44	0.550	0.393	0.941	0.389	0.393
logCRP	-1.08±0.99	-1.06±0.78	-1.97±1.11	0.869		-1.31±0.85	-1.04±0.71	-1.98±1.33	0.058	0.982	0.126	0.586	0.120

Values are presented as Mean± SD, ¹Change between baseline and 12 weeks analyzed by paired t-test, ²Change value compared between groups by independent t-test, ³Two-way RM-ANOVA.

GEB: *Gastrodia elata* blume powder, BDNF: Brain-derived neurotrophic factor and CRP: C-Reactive protein

Values are presented as Mean±SD, Change between baseline and 12 weeks analyzed by paired t-test, ²Change value compared between groups by independent t-test, ³Two-way RM-ANOVA, GEB: *Gastrodia elata* blume powder, BDNF: Brain-derived neurotrophic factor and CRP: C-Reactive protein

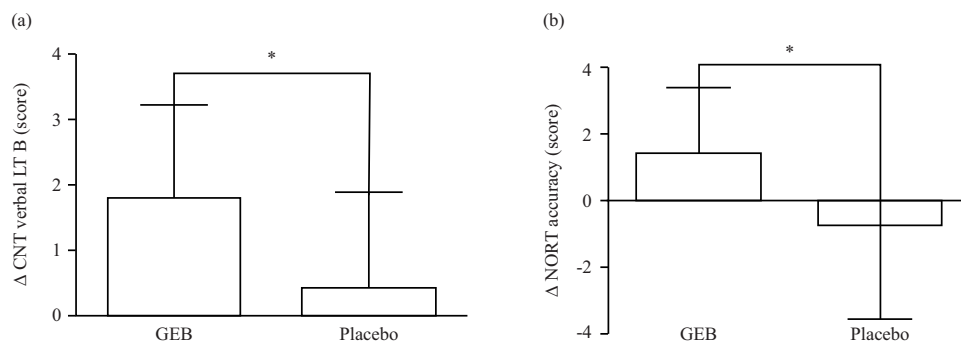


Fig. 2(a-b): Changes in (a) CNT verbal LT B and (b) NORT accuracy of the subjects with Val/Met polymorphism

There was a difference in the changes of verbal learning test (verbal LT) and novel object recognition test score (NORT accuracy) between the GEB and placebo groups. The verbal LT B and NORT accuracy score was significantly increased in the GEB group after 12 weeks, * $p < 0.05$, CNT verbal LT B (score): After the 5th trial (verbal LT A5), a new interference list (list B words) was presented and recalled

Safety and adverse events: Subjects in this study showed no significant changes or differences in safety indicators such as laboratory tests, electrocardiograms, or vital signs (BP and pulse) during the study ($p > 0.05$). All laboratory test items were within the normal range and no side effects were observed (Supplementary Table 2).

DISCUSSION

Here, in this investigation, an effort was made to determine the influence of GEB treatment on cognitive performance and serum BDNF and CRP levels in healthy late-middle-aged adults. The results showed a positive effect of GEB on verbal and visual recognition memory in participants with the Val/Met polymorphism. As the full analysis set ($n = 60$), both GEB and placebo groups showed significant improvement at the endpoint in the verbal LT and stroop tests. These results could reflect practice effects from the repetition of the same tests rather than true improvement in cognitive functioning. Practice effects may appear even after 6¹⁷ or 12 months¹⁸. They may be greater for tests requiring complex cognitive processing and those where the formulation of a strategy might aid performance than for tests such as the trail making, stroop and verbal fluency tests, which measure simpler cognitive functions¹⁸. Notably, a comparison of changes between the groups revealed no marked difference in any test and also, observed no significant time $\times$ treatment interaction. These results indicated that the GEB pill does not have superior effects on cognitive functioning than the placebo. Nevertheless, we observed different results in sub-analyses in subjects with the Val/Met polymorphism. Comparison of changes in values between the groups and the analysis of treatment effects over time demonstrated significant improvements in verbal LT B

score and NORT accuracy in the GEB group than in the placebo group. These results suggest that intake of the GEB pill is beneficial in improving short-term verbal memory and long-term visual recognition memory in subjects with the Val/Met polymorphism. Healthy young Chinese adults carrying the homozygous Met allele of BDNF have been reported to have smaller brain volumes in the frontal, temporal, cingulate and insular cortices¹⁹. Also, the presence of the Met allele is reported to be associated with reduced cognitive performance in elderly adults²⁰. These findings suggested that the genetic effects on cognition are more pronounced, particularly for those carrying the Met allele of BDNF. The Val/Met polymorphism was reported to be the most frequent genotype of the BDNF Val 66Met polymorphism among the Korean participants²¹. Taken together, the results of sub-analyses imply that intake of the GEB pill could be beneficial, especially for the subjects with the Val/Met polymorphism, the most prevalent genotype in Korea, who are at high risk of cognitive decline with aging. However, recent evidence links the Met allele to enhanced cognitive performance and brain activation^{22,23}. BDNF is a key protein in the regulation of neuronal survival, differentiation and synaptic plasticity and it seems to be important for learning and memory function. Spatial memory improvement induced by flavonoid-rich foods has been reported to be associated with increased expression of BDNF in the hippocampus²⁴. High-fat meals resulted decreased plasma BDNF levels in young healthy male subjects²⁵. Lower serum levels of BDNF were associated with smaller hippocampi and poorer memory in elderly adults³⁷. CRP is an acute-phase inflammatory factor that sensitively reflects the systemic inflammation situation³⁸. Associations between cognitive decline and elevated peripheral inflammatory marker concentrations, including CRP and interleukin-6, have been reported in cross-sectional studies of general populations³⁹ and in people with Alzheimer's disease

and mild cognitive impairment⁴⁰. Based on these associations, we hypothesized that intake of the GEB pill might affect serum levels of BDNF or CRP, resulting in the enhancement of cognitive functioning. However, we found no significant difference in BDNF or CRP level between the groups as initially assigned or in subjects with the Val/Met polymorphism. These results indicate that some cognitive improvement seen in participants with the Val/Met polymorphism taking the GEB pill for 12 weeks are not related to changes in serum BDNF and CRP levels.

This study has several limitations. The first is the appropriateness of the cognitive tests that were selected, in one of thereview⁴¹ identified memory tasks and other tasks that required greater cognitive load as the cognitive modality most sensitive to macronutrient manipulations. In that respect, a mental fatigue test requiring substantial cognitive effort could be the best choice. However as our objective was to evaluate the effects of nutritional intervention on diverse domains of cognitive function, we did not select cognitive tests based on task loading. Second, the positive results obtained in subjects with the Val/Met polymorphism should be interpreted with caution because participants with other BDNF genotypes were not included in the analysis. Therefore, further investigation of these findings is strongly warranted. Third, the sample size was relatively small and limited to late-middle-aged adults. As mean changes in scores in the present study were small with relatively large standard deviations, increasing the sample size would lower the probability of type II (false negative) error. Nevertheless, the strengths of the present study are its double-blind, randomized, controlled design and the attempt to explore mechanisms underpinning cognitive improvement.

CONCLUSION

In conclusion, administration of GEB for 12 weeks in healthy late-middle-aged adults produced beneficial effects on memory in subjects with the Val/Met polymorphism but not in subjects initially assigned to the treatment. GEB intake produced no significant change in the serum level of BDNF or CRP compared with the placebo. Results suggest that GEB intake improves cognitive function in a specific population and that this improvement is mediated by factors other than BDNF and CRP.

SIGNIFICANCE STATEMENT

The results showed a positive effect of GEB on verbal and visual recognition memory in subjects with the Val/Met

polymorphism ($p < 0.05$). The serum BDNF and CRP levels did not change markedly in the GEB group than in the placebo group ($p > 0.05$). These observations suggest that supplementation of GEB for 12 weeks positively influenced memory function in subjects with Val/Met polymorphism.

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Supplementary Table 1: Comparison of BDNF and CRP levels of the subjects with val/met polymorphism

	GEB groups				Placebo groups			
	Baseline	12 weeks	Change value	p ¹	Baseline	12 weeks	Change value	p ¹
BDNF (pg mL ⁻¹)	25557.6±3650.1	23908.3±3668.5	-1649.3±2975.6	0.069	26191.9±4319.2	24330.8±4009.9	-1861.1±1984.0	0.001
CRP (mg mL ⁻¹)	0.58±0.45	0.66±0.56	0.52±0.14	0.583	0.47±0.54	0.40±0.28	0.50±0.12	0.573
logCRP	-0.90±0.96	-0.72±0.84	-1.83±1.40	0.379	-1.20±0.97	-1.16±0.76	-2.30±1.60	0.528
p ³								
	p ²		Time		Groups		Time*group	
BDNF (pg mL ⁻¹)	0.813		0.000		0.703		0.813	
CRP (mg mL ⁻¹)	0.428		0.947		0.209		0.428	
logCRP	0.528		0.428		0.220		0.625	

Values are presented as Mean±SD for the GEB (n = 13) and placebo (n = 18) groups, ¹Change between baseline and 12 weeks analyzed by paired t-test, ²Change value compared between groups by independent t-test, ³Two-way RM-ANOVA, GEB: *Gastrodia elata* blume powder, BDNF: Brain-derived neurotrophic factor and CRP: C-reactive protein

Supplementary Table 2: Laboratory profiles of study subjects

Laboratory profiles (standard range)	GEB groups (n = 30)			Placebo groups (n = 30)			
	Baseline	12 Weeks	p-value ¹	Baseline	12 Weeks	p-value ¹	p-value ²
WBC (4.8-10.8×10 ³ µL ⁻¹)	5.0±1.2	5.2±1.1	0.185	4.9±1.2	5.2±0.93	0.250	0.864
RBC (4.2-5.4×10 ⁶ µL ⁻¹)	4.3±0.4	4.3±0.3	0.316	4.3±0.3	4.3±0.3	0.295	0.143
Hemoglobin (12-16 g dL ⁻¹)	13.4±1.1	13.3±0.9	0.106	13.4±1.0	13.8±0.9	0.798	0.195
Hematocrit (37-47%)	40.1±2.8	39.3±2.6	0.002	39.8±2.9	39.3±2.4	0.043	0.370
Platelet (130-450×10 ³ µL ⁻¹)	238.3±49.6	240.9±50.8	0.597	249.1±50.8	252.6±43.4	0.497	0.893
ALP (45-129 IU L ⁻¹)	78.3±18.6	7.3±19.8	0.526	70.8±18.6	74.9±221.1	0.201	0.232
GGT (8-48 IU L ⁻¹)	20.3±21.3	20.2±16.5	0.953	19.3±11.3	20.2±11.9	0.691	0.906
AST (12-33 IU L ⁻¹)	22.0±4.6	22.51±7.9	0.704	21.8±3.9	21.6±3.6	0.842	0.609
ALT (5-35 IU L ⁻¹)	21.4±10.2	21.1±12.9	0.867	19.0±6.7	18.4±4.9	0.220	0.913
Total bilirubin (0.2-1.2 mg dL ⁻¹)	0.86±0.2	0.78±0.2	0.037	0.86±0.3	0.87±0.3	0.803	0.381
Total protein (6.7-8.3 g dL ⁻¹)	7.4±0.4	7.4±0.4	0.320	7.5±0.4	7.5±0.4	1.000	0.296
Albumin (3.5-5.3 g dL ⁻¹)	4.5±0.2	4.4±0.3	0.004	4.5±0.2	4.5±0.2	0.293	0.571
BUN (8-23 mg dL ⁻¹)	14.2±2.9	15.6±3.6	0.0075	17.0±3.9	16.6±4.1	0.559	0.021
Creatinine (0.7-1.7 mg dL ⁻¹)	0.7±0.2	0.7±0.2	0.96	0.6±0.1	0.7±0.1	0.006	0.009
Glucose (74-106 mg dL ⁻¹)	99.5±9.8	94.2±11.5	0.006	96.0±10.5	95.0±8.6	0.514	0.560
eGER (-ml/min/1)	106.9±9.0	105.9±6.0	0.528	106.7±10.1	105.2±7.7	0.391	0.812
SG (1.005-1.030)	1.02±0.01	1.02±0.01	0.551	1.02±0.01	1.02±0.01	0.259	0.533
pH (4.5-9.0)	6.1±0.8	6.2±0.8	0.519	6.0±0.9	6.4±0.9	0.058	0.450

Values are presented as Mean±SD, ¹Analyzed by a paired t-test, ²Analyzed by a linear mixed model of differences between groups, GEB: *Gastrodia elata* blume powder, WBC: White blood cell, RBC: Red blood cell, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase, AST: Aspartate transaminase, ALT: Alanine transaminase, BUN: Blood urea nitrogen and SG: Specific gravity