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Effects of the Combination Herbal Extract on Working Memory and White Matter Integrity in Healthy Individuals with Subjective Memory Complaints : A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Objectives The combination extract of four kinds of herbs, *Gastrodia elata*, *Liriope platyphylla*, *Dimocarpus longan*, and *Salvia miltiorrhiza*, has shown to have memory improving effects in mice. The aim of this study was to investigate the efficacy and safety of the herbal mixture for improving working memory as well as microstructural changes in white matter integrity in individuals with subjective memory complaints.

Methods Seventy-five individuals with subjective memory complaints were assigned to receive either placebo (n = 15) or herbal mixture (low-dose group, n = 30 and high-dose group, n = 30) supplementation in an 8-week, randomized, double-blind, placebo-controlled clinical trial. Changes in working memory performance and fractional anisotropy (FA) values reflecting white matter integrity from baseline to 8-week endpoint were assessed.

Results The herbal mixture group showed an increase in working memory performance compared to the placebo group (p for interaction = 0.001). In addition, the herbal mixture group showed an increase in FA values in the temporo-parietal regions (corrected $p < 0.05$), which are crucially involved in working memory function and are among the most affected regions in patients with cognitive impairments.

Conclusions Findings from this study indicate that the herbal mixture may be a promising therapeutic option for individuals with subjective memory complaints.

Key Words Combination herbal extract · Working memory · Diffusion tensor imaging · White matter integrity · Subjective memory complaints.

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Introduction

Subjective memory complaints, which are defined as self-reported cognitive and memory function problems, are common in middle-aged and elderly people.[1)] There is increasing evidence for the potential role of subjective memory complaints

in predicting the subsequent development of dementia.[2)3)] Individuals with subjective memory complaints also showed subtle functional deteriorations in various cognitive domains[4)5)] including the working memory domain.[6)] Working memory, as one of the core component of the fluid intelligence,[7)] has repeatedly been reported to have a significant role in daily functioning and quality of life.[8)9)] The clinical importance of this line of work points to the need of developing interventions with proven efficacy and safety for improving working memory in individuals with subjective memory complaints.

For proper functioning of working memory, multiple neural pathways are suggested to be involved.[10)11)] It has also been reported that the pathogenesis of subjective memory complaints is related to various brain factors.[1)] Herbal extracts, which are a mixture of various compounds with multiple targets, may be particularly useful for improving working memory in individuals with subjective memory complaints.[12)] In addition, natural substances or standardized herbal extracts, which have been suggested to reduce oxidative stress, would be beneficial to improve age-related cognitive deficits.[13)]

The herbal mixture (HX106) consists of water-soluble extracts from 4 plant sources: *Gastrodia elata* Blume (Rochidaceae family), *Liriope platyphylla* Wang et Tang (Liliaceae family), *Dimocarpus longan* Lour (Sapindaceae family), and *Salvia miltiorrhiza* Bunge (Lamiaceae family). Each plant species of HX106 has long been used for the treatment of selective cardiovascular, endocrinal, and neurologic diseases in traditional, oriental Korean medicine.[14-19)] Indeed, there is accumulating scientific evidence supporting the therapeutic effects of these plant extracts.[16)20)21)] A recent preclinical study has supported the memory-enhancing effects of HX106 in amyloid β-injected mice[13)] with neuroprotective effects.

In this randomized, double-blind, placebo-controlled clinical trial, we examined whether an 8-week oral administration of HX106 could improve working memory function in individuals with subjective memory complaints. We also sought to examine whether HX106 could increase white matter microstructural integrity in regions associated with working memory function, which has been known to be dynamically changing until the later stages of life.[10)22)23)]

Methods

Participants

Participants were recruited through local advertisements. The inclusion criteria were as follows : age between 20 and 60 years old, Global Deterioration Scale score of 2,[24)] one or more symptoms of subjective memory impairment,[25)26)] and high school or higher level of education. The exclusion criteria were as follows : current pregnancy or breast-feeding, evidence of neurologic or medical conditions, axis I mental disorders diagnosed by a board certified psychiatrist using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorder, 4th edition (SCID-IV), one or more major depressive episodes during last 12 months, mini-mental status examination score of 24 or less Clinical Dementia Rating score of 0.5 or more suggesting cognitive impairment beyond self-perceived subjective deficits,[27)] intelligence quotient of less than 80, any history of head trauma involving loss of consciousness or seizure, contraindications to magnetic resonance imaging, use of psychotropics in the past 3 months, use of oral contraceptives, and any plan of participation in other clinical trials during the study period that might affect the outcome of the present study. The study was conducted at Ewha Womans University. The study protocol was approved by the Institutional Review Board of Ewha Womans University. All participants provided written informed consent prior to study participation after detailed explanation from the board certified psychiatrists.

Production of quality standardized herbal formulation HX106

For the preparation of HX106 extract, rhizomata of *Gastrodia elata* Blume (2 g), radices of *Liriope platyphylla* Wang et Tang (10 g) and *Salvia miltiorrhiza* Bunge (6 g), and fructus of *Dimocarpus longan* Lour (6 g) were mixed, grinded, and water-extracted. Maltodextrin was added and the mixed solutions were spray dried. The HX106 extract was then filtered through polypropylene filter papers with 10-um pore size using a rotary evaporator (Eyela, Tokyo, Japan). The validation process to ensure the quality of HX106 extract is described in detail elsewhere.[13)]

A 590 mg tablet of HX106 included 300 mg of HX106 extract. Placebo tablets were not discernible by their taste, flavor, shape, or color. Detailed information on other adjuncts and the composition of the HX106 and placebo tablets can be found in the supporting information (Table S1). The tablets were manufactured at Insung Pharmaceuticals (Eumseong, Korea) with good manufacturing practice certificates for dietary supplements and medicines issued by the Korean Ministry of Food and Drug Safety (MFDS). Complete toxicology tests were performed (Table S2).

Study design

A randomized, double-blind, placebo-controlled design was used, and participants were randomly assigned to receive either placebo (the placebo group, n = 15) or HX106 (the HX106

group, n = 60) daily for 8 weeks. For the HX106 group, two dose schedules were used in order to gain better understanding regarding the safety profile : a low-dose schedule with 600 mg (2 HX106 tablets and 2 placebo tablets) and a high-dose schedule with 1200 mg (4 HX106 tablets) of HX106 given daily. The sample size was determined based on a previous study on the effects of herbal extracts on working memory.[28]

Non-study personnel generated the randomization sequence and dispensed the supplements. Randomization sequence was generated using Stata 12.0 (StataCorp, College Station, TX, USA) statistical software with a 1 : 2 : 2 allocation (placebo : low-dose : high-dose groups). The unbalanced allocation may maximize the study's power for a finite sample size and increase recruitment rate by enhancing patient acceptability of the trial.[29] The daily dose of HX106 was determined based on the information from experiments in which rats showed significant working memory enhancement with 100 mg/kg or 200 mg/kg doses (Table S3 for equations to derive human equivalent doses). Since 2 g/kg administration to rats did not elicit any toxic effects in full toxicology battery recommended by the MFDS,[30] the 600 mg or 1200 mg doses were considered safe for use in humans (Table S3).

Participants visited the study sites on 5 different days : screening day, baseline, weeks 1, 4, and 8. During the screening visit, laboratory examinations including the complete blood cell count, differential white cell count, serum chemistry panel, urinalysis, and thyroid function test panel were performed. Urine pregnancy test was also performed on all women with reproductive capability. Electrocardiogram was also performed for screening purposes. Structured clinical interviews and the Korean version of the Wechsler Adult Intelligence Scale (K-WAIS) were performed. The Subjective Memory Complaints scale (SMC) was used for recruiting individuals with memory complaints.[31] Compliance was evaluated using the ingestion diary and tablet count.

Primary outcome measure : working memory assessments

To assess the working memory performance, four well-established tests, including the symbol span from the Wechsler Memory Scale-IV,[32] immediate recall domain from the Rey-Osterrieth Complex Figure Test (ROCF),[33] digit span, and letter number sequencing from the K-WAIS[33] were chosen. Participants were assessed using these tests at baseline and week 8 visit. These tests are standardized, well-established methods to evaluate working memory performance in clinical and research purposes.[32][33]

These tests were chosen considering that the study participants were healthy individuals with only subjective memory complaints and without apparent cognitive impairment. In order to avoid ceiling effects, the neuropsychological tests needed to be challenging for healthy individuals.[34][35] The appropriate tests should also have less habituation effect or learning effect.[34][36] This was particularly important for the current study because the participants were given the HX106 supplements for a relatively modest period of time, although this was one of the most widely used clinical trial periods for testing efficacy of nootropics.[37][38]

For proper operation of working memory, short-term memory is essential.[39] Visuospatial memory and auditory memory are two aspects of short term memory, which can be assessed with symbol span and digit span, respectively.[33][40] The immediate recall domain from the ROCF also examines visuospatial short-term memory.[41] Selective attention and mental manipulation of the stored information, which are also essential for working memory performance, were assessed using the letter-number sequencing and backward subtests of digit span.[42]

Each test score was adjusted with age,[43][44] sex,[44] intelligent quotient,[45] years of education,[46][47] and baseline test scores,[48][49] since these variables have been reported to affect the level of performance[34][43][46][47][50-52] in the tests included in the present study. The adjusted test scores were then standardized into z-scores using all participants' means and standard deviations. The relative improvement (positive z-scores) or decline (negative z-scores) in performance was measured in a unit-free manner using the obtained z-scores.[43] The individual z-scores of each test were averaged to the composite score for working memory domain,[53][54] which was used as the primary outcome measure.

Secondary outcome measure : white matter integrity assessment

Changes in voxel-wise FA values were used as the secondary outcome measure to assess the effects of HX106 on the white matter microstructural integrity. Detailed information on image data acquisition and the diffusion tensor imaging (DTI) processing pipeline are presented in the sections below.

Image data acquisition

DTI was performed at baseline and week 8 visits. Diffusion tensor images were acquired on the 3.0-tesla Siemens Magnetom Tim Trio system (Erlangen, Germany). Axially oriented, two-dimensional diffusion tensor images were acquired in 64 directions with b-values = 0 and 1000 s/mm^2 using the following parameters : field of view (FOV) = 20 cm ; slice thickness = 1.8 mm ; number of slices = 74 ; echo time (TE) = 94 ms ; repetition time (TR) = 9300 ms ; flip angle = 90° ; number of excitations (NEX) = 2. The axially oriented, two-dimensional proton-density and T2-weighted images were acquired using the

following parameters : FOV = 22 cm ; slice thickness = 1.5 mm ; number of slices = 100 ; TE = 13/133 ms ; TR = 5590 ms ; flip angle = 122° ; NEX = 1. The T1-weighted images were acquired with the following parameters : FOV = 22 cm ; slice thickness = 1 mm ; number of slices = 176 ; TR = 2250 ms ; TE = 2.7 ms ; flip angle = 9° ; NEX = 1.

The acquired T1, proton-density, and T2 images of all participants were carefully reviewed by an experienced neuroradiologist (S.M.L) to screen for clinically significant gross brain abnormalities.

Diffusion tensor image data preprocessing

The diffusion tensor images were processed to correct for eddy current effects and simple head motions using the FMRIB Software Library (FSL version 5.0.2.1 ; http://fsl.fmrib.ox.ac.uk/fsl). FA map for each diffusion tensor image was then calculated using FMRIB's Diffusion Toolbox (FDT version 3.0 ; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT).[55]

For longitudinal voxel-wise analysis of FA maps, we used modified Tract-Based Spatial Statistics procedures, which were optimized to minimize residual variation between images acquired at different time-points (TBSS version 1.2 ; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS).[55)56] First, for calculating base FA template images for each subject, the FA images of time-points 1 and 2 were halfway-registered using the skull-stripped b = 0 volumes. All these base FA template images were nonlinearly registered to the most representative base template image that was automatically identified (study-specific target image). This representative image was then nonlinearly registered to a $1 \times 1 \times 1$ mm^3 standard space (Montreal Neurological Institute 152). The matrix used for this registration process of the study-specific target image to standard space was applied to all FA images. Smoothing with Gaussian kernel (sigma = 2) was performed for all of these transformed images. Finally, all the processed images were projected onto the mean FA skeleton image, which was generated by averaging the registered base FA template images, skeletonizing to represent the center of white matter tracts, and then thresholding at FA > 0.20.

Safety assessments

At week 1, 4, and 8 visits, laboratory examinations including the complete blood cell count, differential white cell count, urinalysis, urine pregnancy tests (only in cases of woman participants), and the serum chemistry panel were performed. All laboratory examinations were performed at an independent reference lab certified by the College of America Pathology-Laboratory Accreditation Program and German External Quality Assessment Scheme (Green Cross Laboratories, Yongin, Gyeonggi-do, South Korea). At every visit, neurological and physical examinations including vital sign check were performed. The Udvalg for Kliniske Undersogelser (UKU) side effect rating scale[57] was administered at week 1, 4, and 8 visits. At week 8 visit, electrocardiogram and the thyroid function test battery were repeated.

Statistical analysis

To compare the demographic and clinical characteristics between the HX106 group and the placebo group, independent t-test and Fisher's exact test were used for continuous and categorical variables, respectively.

The efficacy of HX106 on working memory performance enhancement was tested using the linear mixed-effects model with the interaction term between time and group.[58] The model included the dependent variable of composite scores for working memory domain and independent variables of 'group', 'time', and 'group-by-time interaction' as fixed effects and 'participant' as a random effect.[59] Analyses were performed in the intent-to-treat (ITT) population.[29]

For the DTI analysis, a four-dimensional FA image of difference was produced by calculating differences in the skeletonized FA values of time-point 2 from those of time-point 1. These difference maps were compared between groups using the Randomise software implemented in the FSL (version 2.9, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise). To resolve multiple comparison issue that is inherent in voxel-wise brain image analysis, a cluster-based thresholding method was used.[60] A commonly used cluster-forming threshold of $t = 2$ or $t = 3$[61)62] and a cluster-wise significance level of $p < 0.05$ were used with 5,000 permutations. Mean FA values from the clusters formed at the threshold of $t = 3$ were extracted for further post hoc analyses. The Pearson correlation analysis was used to test whether the magnitude of changes in FA values in the clusters were correlated with that of working memory performance changes.

Results

Participant characteristics

A total of 107 participants were screened and 75 (70.1%) individuals were randomized into the study groups. Baseline characteristics of the study participants are shown in Table 1. There were not significant differences in demographic variables across groups.

The participant flow diagram is shown in Fig. 1. Following the baseline visit, clinically non-significant brain abnormalities were found in 3 participants and they were included in the study. Fifty-two participants in the HX106 group (86.7%) and 15 participants in the placebo group (100%) completed the tri-

al. No participants were excluded due to poor compliance. Safety and efficacy assessments were made on an ITT basis for the original 75 study participants.

Primary outcome measure

During the 8-week administration period, working memory performance did not significantly change in the placebo group

Table 1. Demographic and clinical characteristics of participants

	HX106 high-dose group (n = 30)	HX106 low-dose group (n = 30)	Placebo group (n = 15)	p
Age, yr, mean (SD)	42.5 (11.2)	37.6 (11.7)	40.6 (12.7)	0.27
Women, n (%)	17 (56.7)	18 (60.0)	4 (26.7)	0.09
Subjective Memory Complaints scale score, mean (SD)	7.9 (3.2)	7.9 (3.1)	8.9 (4.1)	0.60
Mini-Mental Status Examination total score, mean (SD)	8.6 (1.3)	8.6 (1.3)	28.9 (1.3)	0.72

SD : standard deviation

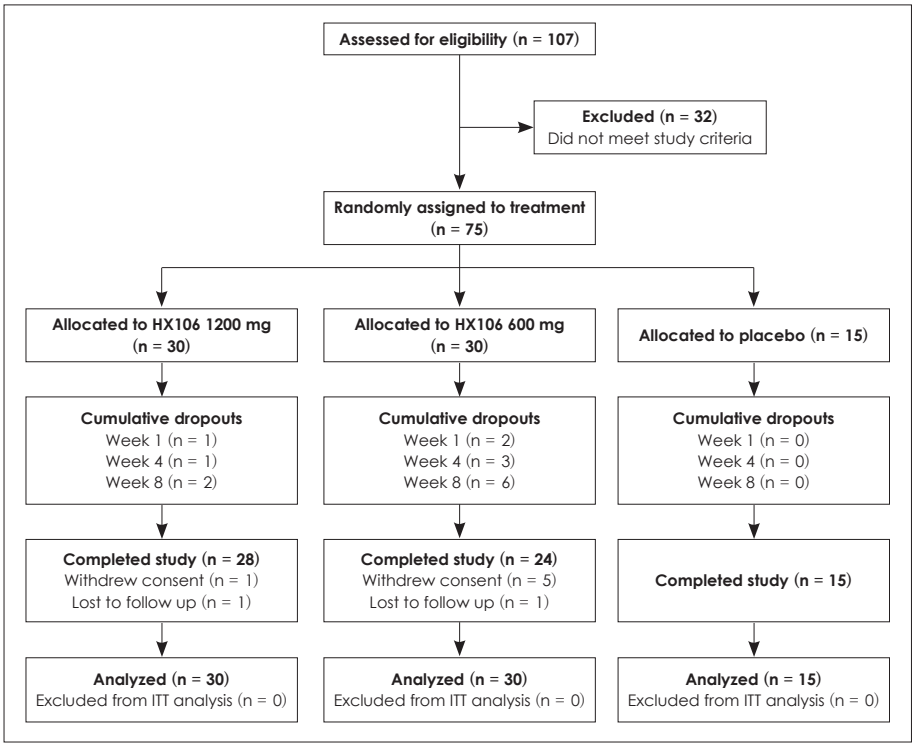


Fig. 1. Participant flow diagram. Flow diagram of the progress through the phases of a randomized, double-blind, placebo-controlled trial investigating the effects of HX106 on working memory. ITT : intent-to-treat.

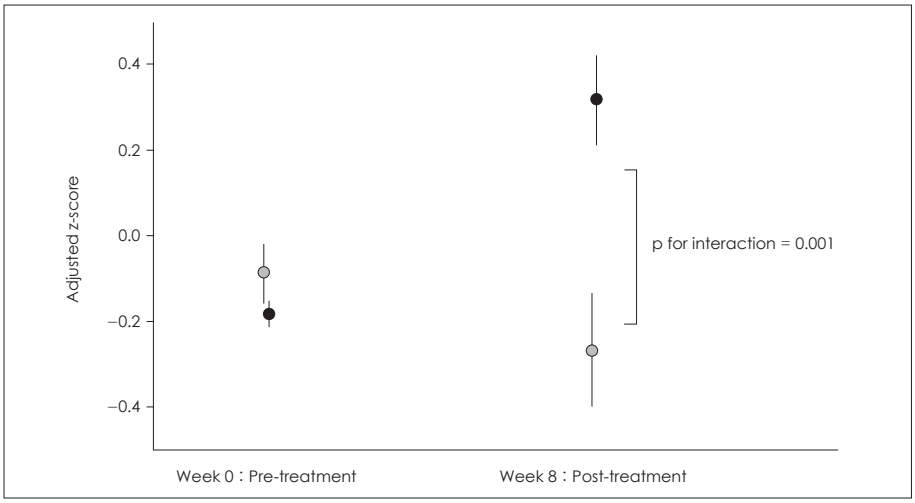


Fig. 2. Effects of HX106 administration on working memory performance enhancement. The changes in adjusted z-scores of the performance level in the working memory domain in pre- and post-administration for each group are shown as scatter plots. The error bars represent standard errors of the mean values. Blue and gray dots indicate the HX106 group and the placebo group, respectively.

(p for time effect = 0.20). The HX106 group showed a significance improvement in working memory performance (p for time effect < 0.001). The extent of working memory performance enhancement was greater in the HX106 group as compared to the placebo group (p for interaction = 0.001) (Fig. 2, S1). Each dose group showed significantly greater improvement in working memory performance than the placebo group [p for interaction = 0.003 (600 mg) and p for interaction = 0.001 (1200 mg)] (Fig. S2).

Secondary outcome measure

There were significant differences in FA value changes between the placebo group and the HX106 groups in three regions (Fig. 3). Regions showing increased FA values in the HX106 group relative to the placebo group were primarily located in the white matter of the bilateral temporal and the right parietal regions. Detailed information for each region is presented in Table 2. The magnitude of changes in the mean FA values in the region of significant differences in the left hemisphere, which included the inferior fronto-occipital fasciculus and the inferior longitudinal fasciculus, correlated with the magnitude of changes in working memory performance (r = 0.30, p = 0.01).

Safety measures

None of the participants experienced drug-related serious adverse events. The potential side effects reported by the participants are listed in Table 3, S4.

There were no significant differences in the proportions of participants who complained about any of the side effects listed in the UKU side effect rating scale[57] (placebo group : n = 12, 80.0% ; HX106 high-dose group : n = 22, 73.3% ; HX106 low-dose group : n = 23, 76.7%) (Fisher's exact test, p = 0.94). The dropout rate was not significantly different across the groups (Fisher's exact test, p = 0.10).

There were no significant differences in changes of systolic or diastolic blood pressure, pulse rate, or body weight between individuals receiving HX106 and those receiving placebo (Table S5). No participants showed clinically important abnormalities in laboratory evaluations. There were no laboratory values which showed significantly different changes between individuals taking HX106 and those receiving the placebo (Table S5).

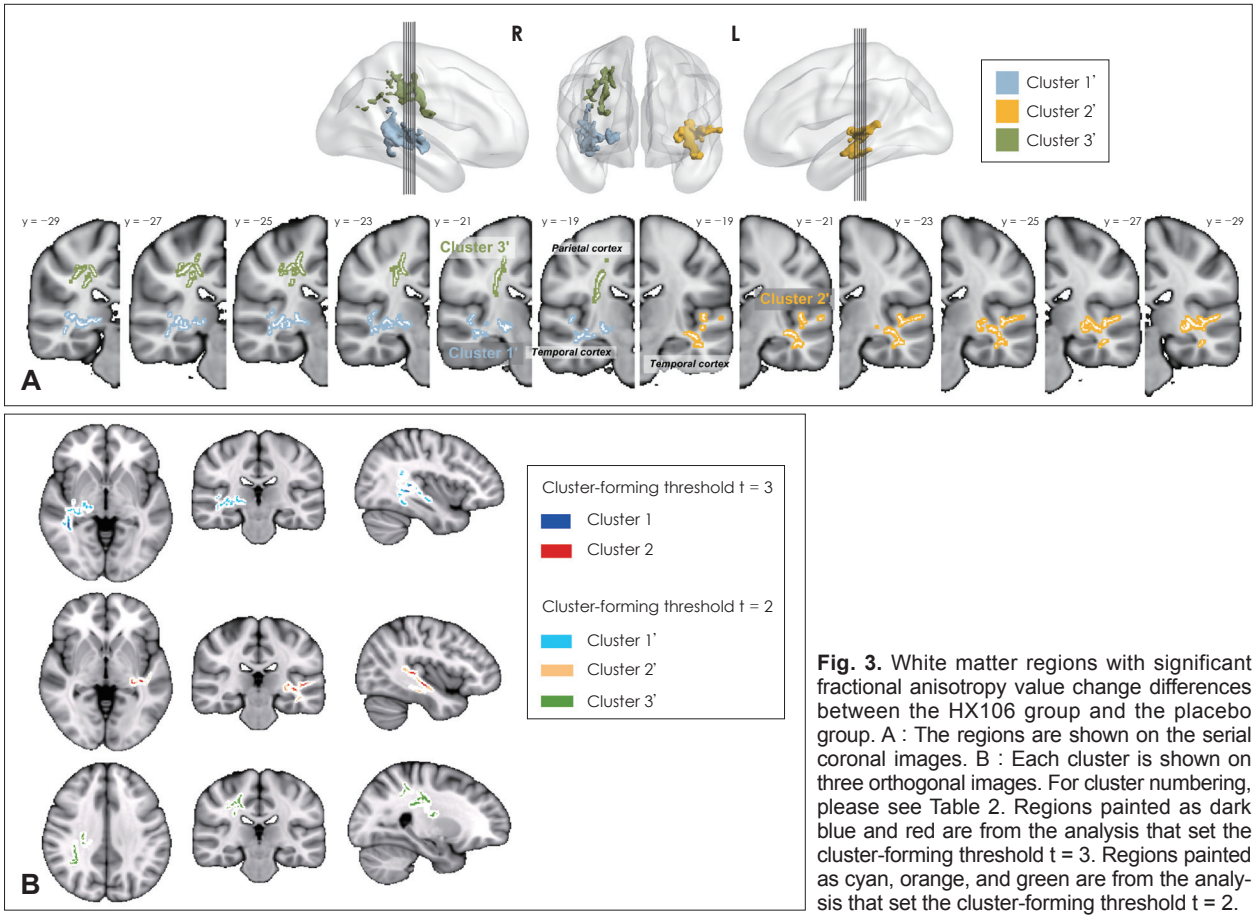
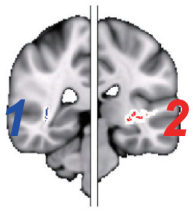
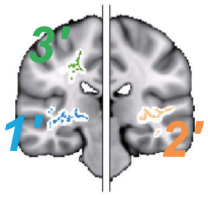


Fig. 3. White matter regions with significant fractional anisotropy value change differences between the HX106 group and the placebo group. A : The regions are shown on the serial coronal images. B : Each cluster is shown on three orthogonal images. For cluster numbering, please see Table 2. Regions painted as dark blue and red are from the analysis that set the cluster-forming threshold t = 3. Regions painted as cyan, orange, and green are from the analysis that set the cluster-forming threshold t = 2.

Table 2. Detailed information on clusters of significant between-group differences in white matter integrity changes

		Cluster size, mm^3	Anatomical location* (% of voxels included in the tract)	Corresponding cortical area	Maximum t values	MNI coordinate†
Cluster-forming t = 3						
	Cluster 1	87	Inferior fronto-occipital fasciculus (59.8) Inferior longitudinal fasciculus (40.2)	Right temporal cortex	4.33	X = −39.0 Y = −35.0 Z = 2.0
	Cluster 2	289	Inferior fronto-occipital fasciculus (39.8) Inferior longitudinal fasciculus (38.4)	Left temporal cortex	5.35	X = −42.0 Y = −21.0 Z = −11.0
Cluster-forming t = 2						
	Cluster 1'	1341	Inferior fronto-occipital fasciculus (32.9) Inferior longitudinal fasciculus (23.2) Superior longitudinal fasciculus, temporal part (12.2)	Right temporal cortex	4.33	X = −39.0 Y = −35.0 Z = 2.0
	Cluster 2'	903	Inferior longitudinal fasciculus (40.3)	Left temporal cortex	5.35	X = −42.0 Y = −21.0 Z = −11.0
	Cluster 3'	1341	Superior longitudinal fasciculus (44.6) Corticospinal tract (39.7)	Right parietal cortex	4.94	X = −32.0 Y = −31.0 Z = 38.0

* : To identify the anatomical locations of each cluster, Johns Hopkins University white matter tractography atlas was used.[74] Only tracts that occupy more than 10% of the cluster mass are presented. Multiple comparison correction was performed. The percentages of cluster mass occupancy of each tract are given in parentheses. † : Coordinates are for the maximum t of the clusters. MNI : Montreal Neurological Institutes

Table 3. Side effects reported by more than 15% of participants overall

	HX106		Placebo (n = 15)	p
	High-dose (n = 30)	Low-dose (n = 30)		
Increased fatigability, n (%)	5 (16.7)	11 (36.7)	4 (26.7)	0.23
Sleepiness/sedation, n (%)	3 (10.0)	9 (30.0)	2 (13.3)	0.15
Pruritus, n (%)	7 (23.3)	4 (13.3)	2 (13.3)	0.67
Headache, n (%)	6 (20.0)	6 (20.0)	5 (33.3)	0.56

Percentages were calculated by using the numbers of participants who reported side effects scored as 1 or higher according to the Udvalg for Kliniske Undersogelser (UKU) side effect rating scale,[57] regardless of physician's judgment of causality. Only side effects that occurred in more than 15% of participants overall are presented here. For the complete list, please see the supporting information (Table S4).

Discussion

This study was a randomized, double-blind, placebo-controlled clinical trial that investigated the effects of HX106 in healthy individuals who reported subjective memory complaints. These findings are consistent with pre-clinical data that have shown memory-enhancing effects of HX106 administration in amyloid β-injected mice.[13] In addition, enhanced working memory was associated with increased white matter integrity, as indicated by higher FA values, in the temporal-parietal regions in the HX106 group compared with the placebo group.

Since the efficiency of working memory function is essential for monitoring and processing the environmental information,[6] individuals with subjective memory complaints, who often suffer from working memory deficits, may have problems in everyday activities and real-life decision making. The effects of HX106 administration on working memory enhancement may have clinical implication in that it might enhance the quality of life in individuals with subjective memory impairment.

Interestingly, the 8-week administration of HX106 was associated with increased white matter integrity, particularly in the medial temporal regions which are preferentially involved in memory function.[63] A previous neuroimaging study reported that white matter lesions in the temporal region were associated with subjective memory complaints.[64] HX106 might improve working memory function by inducing microstructural changes in the temporal white matter. HX106 administration increased white matter integrity within the superior longitudinal fasciculus connecting the prefrontal and posterior associative areas including the parietal and occipital lobes.[65][66] This association fi-

ber is one of the most commonly affected white matter tracts in patients with Alzheimer's dementia and frontotemporal dementia.[67] Posterior involvement in the white matter regions has also frequently been observed in patients with dementia.[67][68] In contrast, the changes resulting from normal aging process appear to occur more in the anterior regions of the white matter.[68] Although the mechanisms underlying the regional specificity of HX106 administration remains to be elucidated, HX106 administration may help to prevent pathological white matter degeneration in the posterior region of the superior longitudinal fasciculus.

Even though the mechanism that underlie enhancement of white matter integrity and working memory is unclear, the effects of active components included in HX106 may in part account for these changes. A growing body of evidence has documented the neuroprotective effects of these herbal compounds and suggests that they may be natural nootropic agents.[69]

A previous study that examined the effects of *Gastrodia elata* Blume reported neuroprotective effects on neuronal cell death induced by amyloid β-peptide.[70] This *in vitro* study showed that the ethyl ether fraction of Gastrodia elata Blume dramatically reduced the amount of cell death caused by amyloid β-peptide in IMR-32 neuroblastoma cells. In another study, elevated gene expression levels of the antioxidant proteins such as protein disulfide isomerase and 1-Cys peroxiredoxin in damaged neural cells have been identified.[21]

In addition to its antioxidant effects, *Liriope platyphylla* Wang et Tang has improved learning and memory in mice and this effect is suggested to be mediated, in part, by brain-derived neurotrophic factor (BDNF) or nerve growth factor expression.[71] Extracts from *Dimocarpus longan* Lour flower have been reported to show antioxidative and anti-inflammatory activities.[20][72] Lastly, *Salvia miltiorrhiza* Bunge, a plant widely used for its clinical efficacy of blood circulation and cardio vascular diseases in Asian countries, has been increasingly gaining attention for its neuroprotective effects.[16] The study demonstrated that mice with Aβ25-35 peptide-induced Alzheimer's disease, when treated with Salvianolic acid B (SalB), a polyphenolic compound from *Salvia miltiorrhiza* Bunge, showed memory enhancement, measured by the passive avoidance task. Moreover, SalB administration reduced the number of activated microglia and astrocytes and improved the decreased levels of choline acetyltransferase and BDNF protein.

There were no differences in the overall rates of adverse events between the HX106 group and the placebo group. All adverse events reported by the study participants were mild and improved without specific interventions. Furthermore, both of two dose schedules of HX106 administration (600 and 1200 mg) showed excellent safety and tolerability profiles, which was comparable to those of the placebo group. This result is not surprising because these 4 plant species have long been used as nutritional supplements or herbal therapeutics in Asia.[14][16-18]

Since HX106 is a combination herbal extract, the use of reproducible techniques to prepare the reagent is important to ensure reliable potency and safety.[73] In this study, a cell-based bioassay was employed to ensure the validity of quality and consistent composition of HX106 and to identify the pharmacologically active components of the HX106 extract.[13]

Both HX106 subgroups according to the dose schedule (600 and 1200 mg) showed an increase in working memory performance compared to the placebo group. Although this subgroup analysis was aimed to assess safety profiles across different doses, we found that there were no differences in the magnitude of working memory improvement between the low- and high-dose groups. Although challenging tests were carefully chosen to avoid the ceiling effects, they may partly explain the absence of dose-dependent cognitive enhancing effects of HX106 administration. A relatively small sample size of each HX106 subgroup may also contribute to these findings. Future studies with a lager sample size will be necessary to find the optimal dosage of HX106.

The present study suggests that HX106 administration has beneficial effects on working memory performance and also on white matter integrity of the temporo-parietal regions in individuals with subjective memory complaints. Subjective memory complaints are often regarded as one of the earliest presenting symptoms of dementia.[1] Therefore, HX106 may provide a promising therapeutic option for healthy individuals without clinically evident cognitive impairment because HX106 has a high safety profile. Given the relatively potent effects of HX106 administration, it may be worth considering a long-term clinical study to investigate its effect on dementia or prevention for mild cognitive impairment.

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Conflicts of interest

DSL's current affiliation is with ViroMed, which holds the patent rights to HX106. Other authors have no potential conflicts of interest.

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■ Supplement ■

Supporting Information Referred to in the Materials and Methods Section

Table S1. The composition profile of the HX106 and placebo tablets

Ingredients	Content (%)
HX106 tablet (590 mg)	
HX106 (herbal extracts : maltodextrin = 7 : 3)	72.7
Microcrystalline cellulose	12.6
Lactose	6.3
Titanium dioxide	4.5
Silicon dioxide	1.9
Sodium carboxymethyl cellulose	1.0
Magnesium stearate	1.0
Gardenia yellow	0.1
Placebo tablet (510 mg)	
Microcrystalline cellulose	83.7
Lactose	7.2
Titanium dioxide	4.5
Silicon dioxide	2.1
Sodium carboxymethyl cellulose	1.2
Magnesium stearate	1.2
Gardenia yellow	0.1

Percentages may not add up 100% due to rounding

Table S2. Toxicology profile of HX106

Test group	HX106 dosage	Route of administration	Results
Single-dose acute oral toxicity			
Sprague-Dawley rats		P.O.	
Control Male : 5 Female : 5	0 g/10 mL/kg		Death : none Clinical signs : not observed Behavioral abnormalities : not observed Body weight : no significant changes Autopsy and microscopic examination : no gross or histopathologic changes
Test Male : 5 Female : 5	2 g/10 mL/kg		
Beagle dogs Male : 2 Female : 2	2 g/5 mL/kg	P.O.	Death : none Clinical signs : not observed Behavioral abnormalities : not observed Body weight : no significant changes Autopsy and microscopic examination : no gross or histopathologic changes
Sub-chronic oral toxicity			
Sprague-dawley rats		P.O.	
Control Male : 10 Female : 10	0 g/5 mL/kg/day for 91 consecutive days		Death : occurred in one male rat at day 14 (autopsy indicated that the cause of death would be accidental intratracheal administration of HX106) Clinical signs : not observed Behavioral abnormalities : not observed Body weight, amount of water and feed ingestion, feed efficiency : no significant changes Laboratory findings : no clinically significant changes. Autopsy and microscopic examination : no clinically meaningful gross or histopathologic changes Eye inspection : no abnormalities
Test Male : 10 Female : 10	2 g/5 mL/kg/day for 91 consecutive days		
Mutagenicity and genotoxicity			
Salmonella typhimurium, Strain TA98, TA100, TA1535, TA1537 Escherichia coli, Strain WP2 (trp, uvrA)	0, 312.5, 625.0, 1250.0, 2500.0, and 5000.0 ug /100 uL/plate		Suppression with cell proliferation : not observed No mutagenicity
Chinese hamster lung fibroblast cell	0, 1250.0, 2500.0, and 5000.0 ug/mL in media		No genotoxicity
ICR mice (male) Negative control : 5 Low HX106 : 5 Medium HX106 : 5 High HX106 : 5	0, 0.5, 1.0, and 2.0 g/5 mL/kg/day for 4 consecutive days	P.O.	No significant differences in the polychromatic erythrocytes with micronuclei (MNPCE) frequencies in 2000 polychromatic erythrocytes (PCE) PCE frequencies / (PCE + normochromatic erythrocytes) : no significant differences No evidence supporting erythrocyte micronucleus induction by HX106

Table S3. Human equivalent dose calculation

Rat average body weight	250 g
Rat caloric expenditure	150 kcal
Effective dose in rats	100–200 mg/kg/day
Dose per calorie of diet	0.17–0.33 mg/250 g/day/kcal (= 25–50 / 150 kcal)
Human equivalent dose	416–833 mg/60 kg/day (= 0.33×2500 kcal)

Supporting Information Referred to in the Results Section

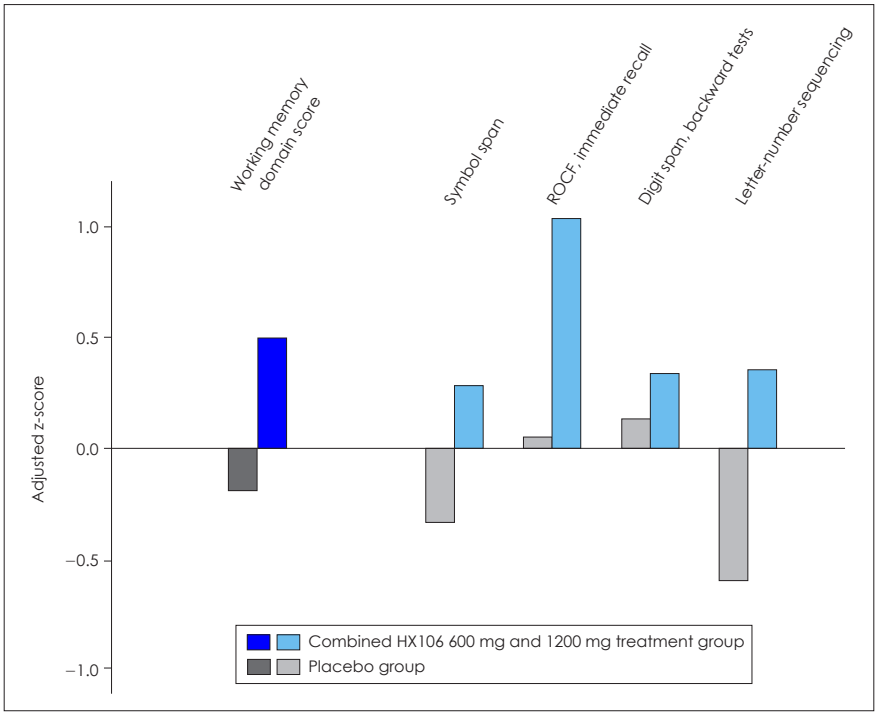

Fig. S1. Changes of adjusted z-scores of each test. The change values in adjusted z-scores of the performance level in the working memory domain from pre-administration to post-administration are shown as bar graphs. ROCF : Rey-Osterrieth Complex Figure Test.

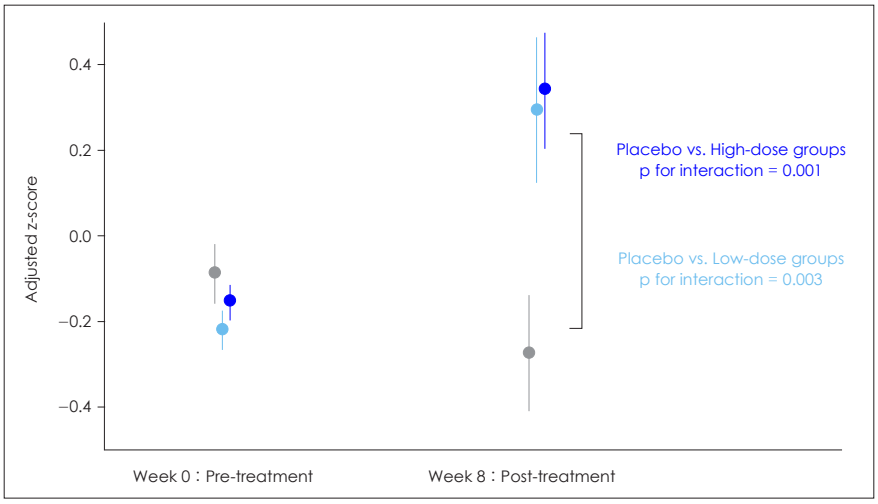

Fig. S2. Effects of HX106 low- (600 mg) or high-dose (1200 mg) on working memory performance enhancement. The changes in adjusted z-scores of the performance level in working memory in pre-treatment and post-treatment for each group are shown as scatter plots. The error bars represent standard errors around the mean values. Light blue or dark blue and gray dots indicate low- (600 mg) or high-dose (1200 mg) of the HX106 group and the placebo group, respectively.

Table S4. Full list of potential side effects reported by participants*

	HX106 high-dose group (n = 30)	HX106 low-dose group (n = 30)	Placebo group (n = 15)	p
Psychic symptoms, n (%)				
Concentration difficulties	1 (3.3)	1 (3.3)	0 (0.0)	1.00
Increased fatigability	5 (16.7)	11 (36.7)	4 (26.7)	0.23
Sleepiness/sedation	3 (10.0)	9 (30.0)	2 (13.3)	0.15
Failing memory	1 (3.3)	3 (10.0)	1 (6.7)	0.84
Depression	1 (3.3)	4 (13.3)	3 (20.0)	0.18
Tension/inner unrest	1 (3.3)	0 (0.0)	2 (13.3)	0.10
Increased duration of sleep	2 (6.7)	2 (6.7)	1 (6.7)	1.00
Reduced duration of sleep	2 (6.7)	7 (23.3)	1 (6.7)	0.14
Increased dream activity	2 (6.7)	5 (16.7)	1 (6.7)	0.55
Neurologic symptoms, n (%)				
Dystonia	1 (3.3)	0 (0.0)	2 (13.3)	0.10
Rigidity	2 (6.7)	2 (6.7)	2 (13.3)	0.74
Tremor	1 (3.3)	0 (0.0)	0 (0.0)	1.00
Akathisia	1 (3.3)	0 (0.0)	0 (0.0)	1.00
Paresthesia	0 (0.0)	3 (10.0)	2 (13.3)	0.17
Autonomic symptoms, n (%)				
Accommodation disturbances	0 (0.0)	3 (10.0)	2 (13.3)	0.17
Increased salivation	1 (3.3)	0 (0.0)	0 (0.0)	1.00
Reduced salivation	1 (3.3)	0 (0.0)	1 (6.7)	0.68
Nausea/vomiting	2 (6.7)	2 (6.7)	2 (13.3)	0.74
Diarrhea	0 (0.0)	1 (3.3)	2 (13.3)	0.10
Constipation	2 (6.7)	4 (13.3)	0 (0.0)	0.31
Micturition disturbances	0 (0.0)	0 (0.0)	1 (6.7)	0.20
Polyuria/polydipsia	4 (13.3)	1 (3.3)	1 (6.7)	0.43
Orthostatic dizziness	2 (6.7)	5 (16.7)	2 (13.3)	0.53
Palpitations/tachycardia	2 (6.7)	3 (10.0)	1 (6.7)	1.00
Increased tendency to sweating	1 (3.3)	2 (6.7)	2 (13.3)	0.42
Other symptoms, n (%)				
Rash	3 (10.0)	0 (0.0)	2 (13.3)	0.17
Morbilliform	2 (6.7)	0 (0.0)	1 (6.7)	0.41
Urticarial	1 (3.3)	0 (0.0)	0 (0.0)	1.00
Cannot be classified	0 (0.0)	0 (0.0)	1 (6.7)	0.20
Pruritus	7 (23.3)	4 (13.3)	2 (13.3)	0.67
Weight gain	3 (10.0)	6 (20.0)	0 (0.0)	0.21
Weight loss	0 (0.0)	4 (13.3)	2 (13.3)	0.09
Increased sexual desire	0 (0.0)	0 (0.0)	1 (6.7)	0.20
Diminished sexual desire	1 (3.3)	0 (0.0)	0 (0.0)	1.00
Dry vagina	1 (3.3)	0 (0.0)	0 (0.0)	1.00
Headache	6 (20.0)	6 (20.0)	5 (33.3)	0.56

* : Percentages were calculated by using the numbers of participants who reported side effects scored as 1 or higher according to the Udvalg for Kliniske Undersogelser (UKU) side effect rating scale,[57] regardless of physician's judgment of causality. Fisher's exact tests were used to compare the frequency among groups

Table S5. Statistics for differences in changes of values from physical examinations and laboratory evaluations between the HX106 group and the placebo group*

	Combined HX106 vs. placebo		HX106 high-dose vs. placebo		HX106 low-dose vs. placebo	
	z	p	z	p	z	p
Physical examinations						
Systolic blood pressure	−0.84	0.40	−1.34	0.18	−0.22	0.83
Diastolic blood pressure	−0.81	0.42	−0.29	0.77	−1.18	0.24
Pulse rate	0.82	0.41	−0.10	0.92	1.55	0.12
Body temperature	0.19	0.85	0.19	0.85	0.13	0.90
Weight	0.27	0.79	0.63	0.53	−0.22	0.83
Complete blood cell counts						
WBC	−0.12	0.91	−0.91	0.36	0.61	0.54
ANC	−0.12	0.90	−1.27	0.20	0.96	0.34
Platelet	−0.15	0.88	−1.46	0.14	1.06	0.29
Hemoglobin	0.64	0.52	1.29	0.20	−0.10	0.92
Hematocrit	0.85	0.39	1.24	0.22	0.34	0.74
Chemistry panel						
Creatinine	0.91	0.36	1.00	0.32	0.68	0.50
ALT	−0.45	0.66	−0.43	0.67	−0.47	0.64
AST	0.25	0.80	−0.35	0.73	0.59	0.56
ALP	−1.71	0.09	−1.26	0.21	−1.90	0.06
Total bilirubin	0.64	0.52	0.73	0.46	0.38	0.71
Total protein	<0.01	1.00	0.06	0.95	−0.02	0.99
Albumin	0.27	0.79	0.43	0.67	0.10	0.92
Total cholesterol	−0.85	0.39	−0.59	0.56	−0.93	0.35
Uric acid	1.33	0.18	1.61	0.11	0.83	0.41
BUN	0.69	0.49	0.09	0.93	1.33	0.18
Glucose	1.29	0.20	0.99	0.32	1.22	0.22
Phosphorus	−0.42	0.67	−0.44	0.66	−0.25	0.81
Calcium	0.94	0.35	1.01	0.31	0.86	0.39
Thyroid function test						
TSH	0.08	0.93	0.09	0.93	0.06	0.95
T4	0.04	0.97	0.23	0.82	−0.26	0.79
T3	−0.30	0.77	0.14	0.89	−0.77	0.44

* : For values from physical examinations, complete blood cell counts, and serum chemistry panel, those acquired at visits of baseline, weeks 1, 4, and 8 were used. For values from thyroid function test, those acquired at baseline and week 8 visits were used. Linear mixed effects regression analysis was used to test whether individuals who received HX106 showed significantly greater increase or decrease of laboratory values. z and p values for time by group interaction term are presented. WBC : white blood cell, ANC : absolute neutrophil count, ALT : alanine transaminase, AST : aspartate transaminase, ALP : alkaline phosphatase, BUN : blood urea nitrogen, TSH : thyroid stimulating hormone