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Research Article

Effect of the Fungus *Isaria japonica* from the Silkworm on Cognitive Function in Older Adults with Mild Cognitive Decline: A Pilot Study

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Several *in vivo* studies report that the fungus *Isaria japonica* grown on silkworm pupae, classified as a *Cordycipitaceae*, plays a role in preventing memory deficits and may have a protective role against cognitive impairment. The present study investigated the cognitive effects and safety of *I. japonica* in older adults with mild cognitive decline. Intervention trial was conducted in subjects over 60 years of age with mild cognitive decline (Mini-Mental State Examination [MMSE] score: 20–27). The subjects consumed the *I. japonica* supplements twice per day for 12 weeks. The outcome measures included the MMSE score and blood test values before and after the intervention. A total of 25 subjects (aged 64–94 years) were eligible for the study. After the intervention, the MMSE score (mean ± standard deviation) significantly increased from 25.5 ± 1.6 to 27.1 ± 2.4 ($P < 0.0001$). The blood test revealed no change in any of the hematological and biochemical parameters evaluated. Also, no serious adverse events were reported. Despite several limitations in the study design, the findings of the present study suggest that the fungus *I. japonica* is safe to consume and it may improve cognitive function in older adults with mild cognitive decline.

Keywords: Clinical trial, *Isaria japonica* supplement, Mild cognitive decline, MMSE, Pilot study

Abbreviations Used: Alkaline phosphatase, ALP; Alanine aminotransferase, ALT; Aspartate transaminase, AST; Creatinine, CRE; C-reactive protein, CRP; γ-glutamyl transpeptidase, γ-GTP; Hematocrit, HCT; High-density lipoprotein cholesterol, HDL-C; Lactate dehydrogenase, LDH; Low-density lipoprotein cholesterol, LDL-C; Mean corpuscular hemoglobin, MCH; Mean corpuscular hemoglobin concentration, MCHC; Mild cognitive impairment, MCI; Mean corpuscular volume, MCV; Mini-Mental State Examination, MMSE; Platelet count, PLT; Red blood cell count, RBC; Standard deviation, SD; Total bilirubin, T-Bil; Total cholesterol, T-CHO; Triglyceride, TG; Total protein, TP; Uric acid, UA; White blood cell count, WBC

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INTRODUCTION

MCI is a clinical condition characterized by pronounced reduction in memory or other cognitive processes in comparison with normal age-related cognitive decline that does not meet the diagnostic criteria for dementia. Individuals with MCI are at higher risk for developing dementia, including Alzheimer disease (Ganguli et al., 2011; Petersen et al., 2001). Therefore, it is important to suppress cognitive decline with pharmaceutical or nonpharmaceutical interventions before dementia develops.

The *Cordycipitaceae* species is a caterpillar fungus, popularly named as "winter worm and summer grass," referred to as "Toh-Chu-Kasou" in Japanese, is a fungal parasite that grows on caterpillar of insects belonging to the family Ascomycetes. The *Cordycipitaceae* is classified as a medical mushroom and has long been used as food or traditional Chinese medicine in Asia, with approximately 400 different species reported worldwide (Wang and Yao, 2011). Recent studies have demonstrated multiple pharmacologic effects of *Cordycipitaceae*, such as anti-inflammatory, antioxidant, antiangiogenic, hypolipidemic, antiviral, antiaging, and immunomodulatory activities (Chen et al., 2012; Chen et al., 2010; Shin et al., 2003; Takano et al., 2005; Wang et al., 2009; Zhu et al., 2012). Moreover, the findings from several *in vivo* studies have shown that *Cordycipitaceae* plays a role in preventing memory deficits, and may protect against cognitive impairment or progression of dementia (Ji et al., 2009; Jin et al., 2004).

The fungus *Isaria japonica* (synonym: *Paecilomyces tenuipes*) from the silkworm has been classified as a species of *Cordycipitaneae* family. *I. japonica* is an entomopathogenic fungus specific for lepidopteran insects and belongs to Deuteromycotina (fungi imperfect). It also grows on larvae and pupae of the domestic silkworm (*Bombyx mori*). In aging mouse, treatment with *I. japonica* enhances spatial learning and ameliorates astrogliosis in the hippocampal CA3 area (Tsushima et al., 2010). These findings indicate that *I. japonica* may enhance recovery from central nervous system deficits and represents a promising astrocyte-targeting modulator that could play a role in preventing memory deficits and learning disorders in the aged brain (Robel et al., 2011; Tsushima et al., 2010). Acute toxicity tests in mice have also revealed the safety of *I. japonica;* even after continuous administration for 28 days, no deaths occurred and no histochemical abnormalities in the brain, heart, liver, kidney, and pancreas were detected (Sillapakong et al., 2015).

Although several *in vivo* studies have demonstrated that *I. japonica* improves brain function, the beneficial effects of *I. japonica* against brain disorders or cognitive dysfunction in humans and its safety remains largely unexplored. Thus, the present pilot study investigated the effects of *I. japonica* on cognitive dysfunction and its safety in older adults with mild cognitive decline.

MATERIALS AND METHODS

Study Design and Subjects

The present study was conducted as a prospective, single-arm, open-label, 12-week intervention trial for older adults over 60 years of age having awareness of mild cognitive decline. Thirty subjects living in Sendai city, Miyagi, Japan were recruited for this study from January to February 2019 using posters describing the outline of the study. The inclusion criteria of the subjects were as follows: Mini-Mental State Examination Japanese version (MMSE-J) score ranging from 20 to 27 and taking no supplements that affect cognitive function, such as *Ginkgo biloba* extract, docosahexaenoic acid, vitamin E, lactic acid bacteria extract, green tea extract, plasmalogen extract, or others, from 1 week before to the start of the intervention. Exclusion criteria included the following: subjects on dementia therapeutics such as donepezil hydrochloride, rivastigmine, galantamine, and memantine; having cognitive decline related to side effects of a drug or disease such as neurodegenerative disease; having an allergy to silkworm or mushrooms; and having systemic chronic infectious disease, heart disease, hepatic dysfunction, kidney disease, and any other serious disease. For all eligible subjects, written informed consent was obtained prior to their participation in the study. The study protocol was approved by the Ethics Committee of the University of Shizuoka (No. 30–33, allowed on January 11, 2019), and also registered as a clinical trial in the University Hospital Information Network (UMIN No. 000035765).

Intervention

All enrolled subjects were required to consume *I. japonica* capsules at the rate of four capsules twice per day (1.6 g/day) in the morning and evening over the course of 12 weeks. The major components of each capsule (800 mg) included 31 mg *β-glucan*, 13 mg free arginine, and 4 mg ergosterol, as well as a single peptide, which was detected by matrix-assisted laser desorption/ionization-time of flight mass spectrometry analysis (Suzuki et al., 2017). The *I. japonica* supplement manufactured by Biococoon Laboratories, Inc. (Tanagura Factory, Fukushima, Japan) was used for the intervention group. These supplements are commercially available in Japan.

Outcome Measures

For evaluation of the effectiveness of the *I. japonica* supplement to improve cognitive function, the MMSE-J, which is an interviewer format questionnaire that tests five domains (orientation, memory registration, attention and calculation, memory recall, and language), was administered before and after the intervention by trained medical researchers who had no knowledge of treatment. The MMSE score ranges from 0 to 30 points, with a lower score denoting more impaired cognition (Folstein et al., 1975; Ideno et al., 2012). For evaluation of the safety of the intervention, hematologic and biochemical blood tests were performed before and after the intervention. All blood test measurements were outsourced to Hoken Kagaku, Inc. and a reference range was used to evaluate the efficacy and safety based on the judgment of independent physicians. The occurrence of adverse events was monitored throughout the intervention period. All subjects were required to report any adverse events in report form and all reports were evaluated by independent physicians to determine the presence or absence of adverse events at the end of the intervention period. For monitoring the intervention, all subjects were required to complete the supplement intake diary to confirm their adherence. Moreover, all subjects were instructed to avoid changing their lifestyle, including diet or exercise, as much as possible during the intervention period.

Statistical Analyses

Since this was an exploratory clinical study, sample size calculation was not conducted but was set based on other preliminary trials related to cognitive dysfunction (Ide et al., 2014). Descriptive statistics for all analyses are expressed as mean ± SD for continuous variables, and absolute and relative frequencies for nominal and ordinal variables. For statistical analysis, Student's paired *t*-test or a Wilcoxon-signed rank test was used for estimation of changes in all variables during the 12-week intervention. A P-value of less than 0.05 was considered to indicate a statistical significance. All statistical analyses were conducted using the statistical analysis program R (version 3.4.2, R Development Core Team 2018, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

From a total of 30 subjects initially recruited, 25 subjects (10 women; 40% and 15 men; 60%) fulfilled the study criteria and were enrolled into the study; the remaining 5 candidate subjects were excluded because their baseline MMSE-J scores were over 27 or they did not meet the other inclusion criteria. After enrolling

in the study, none of the subjects dropped out. The age of the subjects ranged from 64 to 93 years, with a mean ± SD age of 78.8 ± 7.5 years. The distribution of the total MMSE-J scores at baseline was as follows: 23 (92.0%) subjects with a score of 24–27 (MCI) and 2 subjects (8.0%) with a score of 20–23 (mild and moderate dementia). All of the 25 subjects completed the 12-week intervention and mean (±SD) adherence to the intervention was 87.5 ± 8.0%. Changes in the MMSE score, including the specific cognitive domain score, before and after the 12-week intervention are shown in Figure 1 and Table 1.

The mean (±SD) total MMSE score was significantly increased after the 12-week intervention from 25.5 ± 1.6 to 27.1 ± 2.4. The scores of the short term memory domain and the attention and calculation domain significantly increased; from 4.6 ± 1.0 to 5.0 ± 1.0 in the short term memory domain and from 2.5 ± 1.1 to 3.5 ± 1.6 in the attention and calculation domain. The results of the hematologic and biochemical blood tests are shown in Table 2. There were slight changes after the 12-week intervention in the following variables: Hct, MCV, MCH, AST, ALT, CRE, HDL-C, and LDL-C. Since these differences were within the determined reference range, they were judged as not clinically relevant; also, there was no unusual fluctuation in the individual data. There were no reports of serious adverse events, only mild diarrhea was reported in two subjects during the study period.

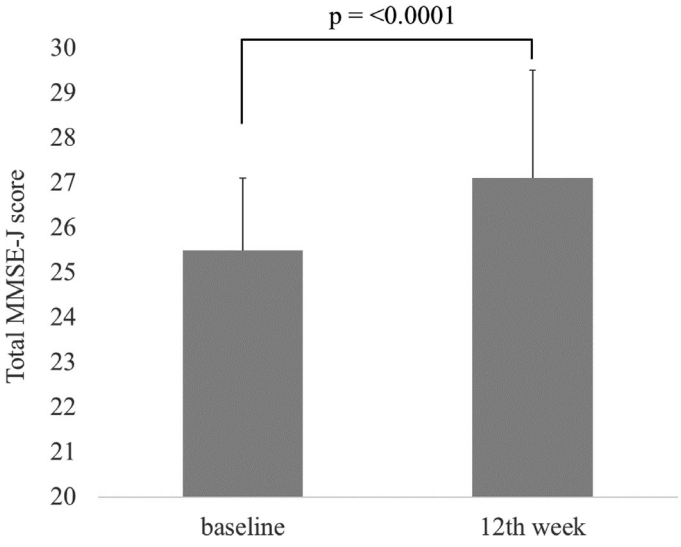

Figure 1 | Comparison of total MMSE scores of subjects before and after intervention. Error bars, standard deviation (n = 25); P-value, calculated by paired t-test.

Table 1 | Comparison of specific cognitive domain scores of subjects before and after intervention.

Variables	Baseline	12th week	P-values
Orientation (max, 10)	9.4 ± 1.2	9.6 ± 1.3	0.323
Short-term memory (max, 6)	4.6 ± 1	5.0 ± 1.0	0.02
Attention and calculation (max, 5)	2.5 ± 1.1	3.5 ± 1.6	0.001
Language (max, 8)	8.0 ± 0.2	8 ± 0	0.33
Visual construction (max, 1)	1 ± 0	1 ± 0	n.a.

Values: mean ± SD. Each P-value was calculated by paired t-test.

Table 2 | Hematologic and blood biochemistry values. The data are presented as mean ± SD.

Variables	Baseline	12th week
WBC (μL)	6156 ± 1242	6148 ± 1427.2
RBC (*10^6/μL)	443.6 ± 40.5	437.0 ± 45.4
Hct (%)	41.9 ± 3.5	40.8 ± 3.8
MCV (fL)	94.8 ± 4.0	93.6 ± 4.3
MCH (pg)	31.1 ± 1.5	30.9 ± 1.4
MCHC (%)	32.8 ± 0.77	33.0 ± 0.72
Plt (*10^6/μL)	23.1 ± 4.8	22.7 ± 5.2
ALP (U/L)	239.8 ± 76.2	239.5 ± 89.6
AST (GOT) (U/L)	25.1 ± 6.1	29.1 ± 7.3
ALT (GPT) (U/L)	20.8 ± 12.5	31.7 ± 17.9
LDH (U/L)	203.4 ± 32.8	213.7 ± 22.4
γ-GTP (U/L)	27.2 ± 20.1	33.0 ± 29.5
T-Bil (mg/dL)	0.52 ± 0.25	0.56 ± 0.23
TP (g/dL)	7.3 ± 0.40	7.2 ± 0.40
CRE (Cre) (mg/dL)	0.76 ± 0.19	0.79 ± 0.20
UA (mg/dL)	5.4 ± 1.4	5.5 ± 1.4
T-Cho (mg/dL)	205.6 ± 18.2	190.6 ± 22.0
HDL-C (mg/dL)	56.3 ± 11.7	51.3 ± 10.4
LDL-C (mg/dL)	121.3 ± 16.2	111.1 ± 16.4
TG (mg/dL)	171.5 ± 82.6	150.2 ± 57.4
CRP (mg/dL)	0.08 ± 0.07	0.08 ± 0.09

DISCUSSION

The aim of the present exploratory study was to evaluate the effects of the fungus *I. japonica* on cognitive function in older adults with mild cognitive decline. We found that intake of the *I. japonica* supplements significantly attenuated cognitive dysfunction on the basis of the MMSE scores. The scores in both the short term memory domain and attention and calculation domain of the MMSE were especially improved.

Administration of the *I. japonica* supplements in an aging mouse model was shown to promote the recovery of central nervous system deficits (Tsushima et al., 2010), which may be related to its activity as a promising astrocyte-targeting modulator (Robel et al., 2011). Recent studies of dementia and aging have focused on glia, especially astrocytes and microglia, which have long been thought to merely support neurons, but are now known to have distinct physiological roles in synaptic function, the blood–brain barrier, and neurovascular coupling (Blanco-Suarez at al., 2017; Garwood et al., 2017; Robel and Sontheimer, 2016). The extract of *Cordycipitaneae* is also reported to improve or enhance learning and memory in mice (Cai et al., 2013; Ji et al., 2009). *Cordycipitaneae* may alleviate short term memory impairment by

increasing the neurotrophic factors such as brain-derived neurotrophic factor and tropomyosin receptor kinase B (Lee et al., 2016). Only a few clinical studies, however, have evaluated the clinical effects of *Cordycipitaneae* on cognitive function and no conclusive evidence has been provided. The results of the present study report the cognition-enhancing effects of *I. japonica*. Although the safety of *I. japonica* was reported on the basis of an acute toxicity test in mice, there are no such reports in humans, especially with reference to older adults with mild cognitive decline (Sillapakong et al., 2015). Even though all the subjects in the present study were older adults of over 60 years of age, there were no changes in the blood test values from baseline and no interruption due to adverse events during the intervention, which is important information for future research development.

Although, the present study provides valuable information, there are some limitations due to the study design. First, the present study was an uncontrolled, single-arm, and open-label study. The subjects might have taken the *I. japonica* supplements believing that it might improve cognitive function, which may have induced a placebo effect on the MMSE score or other variables. Also, the implementation of the MMSE score was confounded by repeated MMSE test itself. Therefore, on the basis of this study alone we cannot definitively conclude that *I. japonica* effectively improves cognitive function. Second, the present study included only a small sample size in a specific area of Sendai city, which limits the generalizability of the findings. Although the subjects were widely and randomly recruited using posters, healthy subjects with a high interest in research may have participated in the study. Third, the study lasted only 12 weeks with no follow-up, which limits conclusions regarding the effects of long-term intake. Generally, cognitive decline must be evaluated over a long period of time, and the length of the follow-up period is an important factor. The present study period was based on a similar previous preliminary study; a longer period of investigation is needed to investigate the detailed effects of *I. japonica* on cognitive decline. Despite several limitations, however, the present study indicates that *I. japonica* may have potential as a new candidate supplement for improving cognitive decline. Further studies are necessary to elucidate the effects of *I. japonica* and address the limitations of the present study in a randomized controlled study with a larger and more diverse population across a long-term follow-up period.

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CONFLICT OF INTEREST

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