경증의 간 기능 이상 소견을 보이는 성인에 대한 동충하초 추출물의 기능 및 안전성

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The Efficacy and Safety of *Cordyceps militaris* in Korean Adults Who Have Mild Liver Dysfunction

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Purpose: The aim of this study is to determine the efficacy and safety of *Cordyceps militaris* in Korean adults with mild liver dysfunction. *C. militaris* is a mushroom traditionally used for several clinical purposes in East Asian territory, including China, and has been found to be effective in improving liver function through animal studies.

Methods: The *C. militaris* group was administered 1.5 g/day of *C. militaris* (2 capsules per dose, twice per day) and the placebo group was administered the same volume of placebo. Laboratory test (white blood cell, hemoglobin, platelet, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltranspeptidase, lactic dehydrogenase, alkaline phosphatase, total bilirubin, blood urea nitrogen, creatinine), liver computed tomography (CT) were performed, and visual analogue scale score for subjective symptoms and fatigue severity scale were measured.

Results: In analysis of the liver CT scan at 8 weeks after administration compared to baseline, the mean ratio of change of Hounsfield unit of 8 segments of liver increased by an average of $21.43\% \pm 45.11\%$ in the *C. militaris* group and $9.64\% \pm 11.41\%$ in the placebo group. Others showed no statistically significant inter-group difference.

Conclusion: *C. militaris* extract was used safely as a functional food in patients with mild liver dysfunction, and is expected to protect against progression of fatty liver or cirrhosis caused by suppression of lipid accumulation in hepatocytes.

Key Words: *Cordyceps*, Cordycepin, Liver

INTRODUCTION

Cordyceps militaris, a mushroom in the category of ascomycetes, has been used for improving liver and renal functions, enhancing immunity through activation of basal metabolism in Asia. Recently, the attention to its ability of enhancing immunity is increasing.¹⁻⁷

Liver plays an important role in the human body and is involved in metabolism of protein synthesis, carbohydrate,

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lipid, hormone, vitamin and mineral, and production of bile acid, which helps digestion, including metabolism of endogenous substances as well as those absorbed from the outside.

In particular, liver protects the human body from a number of risk factors by detoxifying endogenous or exogenous toxins. Therefore, the risk of damage caused by alcohol, drugs, viruses, and toxins contained in the blood flow of the liver is higher because of its abundant distribution.

C. militaris has low content of carbohydrate, which is the energy source, and contains vitamin A and minerals in abundance compared to other *Cordyceps* spp.^{8,9} Cordycepin, secondary metabolite of *C. militaris*, is introduced as a physiologically active substance having the effect of fatigue recovery and liver protection. Awareness of *C. militaris* as a functional food is increasing.

As a result of research in terms of safety, it has been reported as safe based on toxicity data on acute single oral administration of *C. militaris*. Therefore, in the current situation, where there is no available medication for early care and treatment of liver injury which may lead to chronic liver disease, *Cordyceps* spp. was open to development as a functional food to improve liver function. While diverse types of animal testing on the effect of *C. militaris* on liver function have been conducted,¹⁰⁻¹² almost no testing has been conducted in human beings. Therefore, we studied the effect of *C. militaris* on liver function and its safety in human being.

MATERIALS AND METHODS

1. Methods

1) Participants

The participants were 20 to 65 years old adults with alanine aminotransferase (ALT) of 1.5 to 3 times the upper limit of normal, and not taking hepatotonics, drug that can affect the liver function, nutritional supplements, or alcohol for two weeks before screening.

Those with ALT more than three times the upper limit of normal, who had systemic disease, such as rheumatoid arthritis, metabolic syndrome, autoimmune disease, or malignancy, infectious disease, such as chronic hepatitis B or C,

or AIDS, severe infectious disease, such as pneumonia, or tuberculosis, severe hepatic failure, heart failure, renal failure, or history of hypersensitivity to functional food, or were allergic to mushrooms, including *Cordyceps* spp., were excluded from the experiment. Those considered unfit for the experiment by the doctor were also excluded.

2) Ethics consideration

To protect the participants ethically, the study was conducted under the approval of the Institutional Review Board of Bundang Jesaeng General Hospital (Seongnam, Korea) (IMG 12-03). The participants were given a full explanation of the purpose of the experiment, the efficacy of the experimental product, and its adverse reaction and gave written consent before starting the experiment.

3) Study design and treatments

The aim of this single center, randomized, double blind-ed, placebo controlled clinical trial was to determine the effects and safety of *C. militaris* for Korean adults with mild liver dysfunction. The study performed at Bundang Jesaeng Hospital between February 2013 and January 2014.

Laboratory test (white blood cell, hemoglobin, platelet, aspartate aminotransferase (AST), ALT, gamma glutamyl-transpeptidase (GGT), lactic dehydrogenase, alkaline phosphatase, total bilirubin, blood urea nitrogen, creatinine), liver computed tomography (CT), visual analogue scale (VAS) score of subjective symptoms, and fatigue severity scale (FSS) were measured to determine the effect of *C. militaris* on liver function and the adverse effects of administration were examined to determine its safety.

An independent statistician unrelated to the clinical trial used the Proc Plan procedure of SAS version 9.1 (SAS Institute, Cary, NC, USA) to randomly assign the participants before starting the trial.

The *C. militaris* group was given 1.5 g/day of *C. militaris* (2 capsules per dose, twice per day) and the placebo group was given the same volume of placebo for four weeks from day 0 to the closing day. Blood sampling was performed three times: before administration, after four weeks of administration, and after eight weeks of administration. CT scan was performed before administration and after eight

weeks of administration.

Two subjects dropped out of the *C. militaris* group and one subject dropped out of the placebo group. The dropout was excluded from the statistical analysis.

2. Materials

1) *Cordyceps militaris* extracts

C. militaris used in this study was supplied by Mushtech (Hoengseong, Korea). For species identification, DNA was extracted from *C. militaris* and fungal DNA was amplified using the highly conserved fungal rRNA gene primers (ITS1F and ITS4). To identify the isolates, sequences were subjected to the basic local alignment search tool (BLAST) search with the NCBI database (<http://www.ncbi.nlm.nih.gov/>). The BLAST search revealed a 99% similarity with the *C. militaris* ITS sequence.

Dried *C. militaris* was crushed, extracted in 50% ethanol at room temperature and at normal pressure for three days, filtered, concentrated, sterilized, and spray-dried. The major active component of *C. militaris* is cordycepin. Its content is approximately 1.9 mg/g, acceptable within a range (80% to 120%).

2) Tablet preparation

Each tablet contained 375 mg of dried extract of *C. militaris* and placebo was manufactured in the same volume, consisting mainly of microcrystalline cellulose and lactose.

3. Statistical analysis

All data were expressed as a mean $\pm$ standard error of the mean (SEM). The Hounsfield unit (HU) of liver CT and the ratio of change of laboratory test were analyzed using Wilcoxon rank sum test and two sample t-test. The normalized rate of laboratory test was evaluated using Pearson's chi-square test and Fisher's exact test. P-values <0.05 were considered statistically significant in safety test, <0.1 were considered statistically significant in function test.

RESULTS

1. Clinical characteristics of participants

Regarding the clinical characteristics of the participants,

there was no statistically significant inter-group difference in the mean age: 40.93 ± 10.08 years for the *C. militaris* group and 41.28 ± 10.46 years for the placebo group ($P=0.8570$). Men showed a higher rate than women in both groups, but there was no statistically significant inter-group difference ($P=0.9605$; Table 1).

2. Liver CT scan

In analysis of the liver CT scan at 8 weeks after administration compared to baseline, the mean ratio of change of HU of 8 segments of liver increased by an average of $21.43\% \pm 45.11\%$ in the *C. militaris* group and $9.64\% \pm 11.41\%$ in the placebo group, and showed a statistically significant difference (two sample t-test, $P=0.0987$). The mean ratio of change of HU of the caudate lobe increased by an average of $18.18\% \pm 34.16\%$ in the *C. militaris* group and $8.35\% \pm 15.77\%$ in the placebo group, which showed statistically significant difference in the two groups (two sample t-test, $P=0.0902$) but others showed no statistically significant inter-group difference (Table 2).

Table 1. Baseline characteristics

Characteristic	<i>C. militaris</i> group (n=28)	Placebo group (n=29)	P-value
Age (y)	40.93 ± 10.08	41.28 ± 10.46	0.8570
Sex			0.9605
Male	25 (89.3)	25 (86.2)	
Female	3 (10.7)	4 (13.8)	
Laboratory test			
WBC ($10^3/\mu\text{L}$)	7.00 ± 1.86	6.70 ± 1.29	0.4833
Hemoglobin (g/dL)	15.37 ± 1.11	15.02 ± 1.01	0.2198
Platelet ($10^3/\mu\text{L}$)	244.11 ± 51.46	234.69 ± 51.84	0.4942
BUN (mg/dL)	13.68 ± 3.69	14.59 ± 4.63	0.4187
Creatinine (mg/dL)	1.12 ± 0.22	1.17 ± 0.15	0.2954
LDH (IU/L)	370.17 ± 73.16	364.32 ± 61.20	0.7442
ALT (IU/L)	77.07 ± 14.20	76.10 ± 15.80	0.8086
AST (IU/L)	48.18 ± 16.91	51.38 ± 20.42	0.5215
GGT (IU/L)	126.18 ± 113.45	69.03 ± 51.82	0.0199
ALP (IU/L)	206.93 ± 66.60	228.93 ± 65.85	0.2152
Total bilirubin (mg/dL)	0.77 ± 0.42	0.79 ± 0.36	0.8203

Values are presented as mean $\pm$ standard error of the mean or number (%).

C. militaris = *Cordyceps militaris*; WBC = white blood cell; BUN = blood urea nitrogen; LDH = lactic dehydrogenase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyltranspeptidase; ALP = alanine aminotransferase.

3. ALT, AST, and GGT

The ratio of change of ALT at 4 weeks after administration compared to baseline was 19.07 ± 24.12 in the *C. militaris* group and 16.61 ± 40.36 in the placebo group. The ratio increased in *C. militaris* group as well as placebo group, but there was no statistically significant inter-group difference (Table 3).

The normalized rate of ALT at 4 weeks after administration compared to baseline was 17.9% in the *C. militaris* group and 34.5% in the placebo group. The placebo group showed a higher normalized rate than the *C. militaris*

group, but there was no statistically significant inter-group difference (Table 4).

The results of AST and GGT did not differ from ALT, and also there was no statistically significant inter-group difference between *C. militaris* group and placebo group (Table 3, 4).

4. VAS and FSS

1) VAS

In analysis of VAS score of subjective symptoms at 4 and 8 weeks after administration compared to baseline, chronic fatigue, general weakness, and abdominal bloating increased in the *C. militaris* group, but there was no statistically sig-

Table 2. The mean ratio (%) of change of HU of liver CT at 8 weeks after administration compared to baseline

Segment	<i>C. militaris</i> group (n=28)	Placebo group (n=29)	P-value
Total	21.43 ± 45.11	9.64 ± 11.41	0.0987
Caudate lobe	18.18 ± 34.16	8.35 ± 15.77	0.0902
Superior subsegment of the lateral segment	28.59 ± 83.17	17.72 ± 24.17	0.2594
Inferior subsegment of the lateral segment	69.08 ± 355.89	8.86 ± 29.45	0.1944
Left medial segment	14.05 ± 46.34	10.46 ± 19.73	0.3559
Inferior subsegment of the anterior segment	17.66 ± 49.49	12.33 ± 15.63	0.2981
Inferior subsegment of the posterior segment	28.09 ± 65.10	7.77 ± 17.79	0.0637
Superior subsegment of the posterior segment	-40.59 ± 245.63	19.47 ± 32.47	0.8908
Superior subsegment of the anterior segment	42.03 ± 131.00	10.74 ± 21.50	0.1154

Values are presented as mean $\pm$ standard error of the mean.

HU = Hounsfield unit; CT = computed tomography; *C. militaris* = *Cordyceps militaris*.

Table 3. The ratio (%) of change of ALT, AST, and GGT at 4 and 8 weeks after administration compared to baseline

Liver function test		<i>C. militaris</i> group (n=28)	Placebo group (n=29)	P-value
ALT	4 wk	19.07 ± 24.12	16.61 ± 40.36	0.3903
	8 wk	20.10 ± 25.30	17.92 ± 35.60	0.3951
AST	4 wk	16.82 ± 20.90	20.35 ± 27.21	0.7076
	8 wk	16.75 ± 23.43	10.36 ± 65.64	0.3125
GGT	4 wk	7.20 ± 24.34	5.57 ± 22.76	0.3981
	8 wk	3.70 ± 31.56	4.38 ± 26.11	0.5351

Values are presented as mean $\pm$ standard error of the mean.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyltranspeptidase; *C. militaris* = *Cordyceps militaris*.

Table 4. The normalized rate of ALT, AST, and GGT at 4 and 8 weeks after administration compared to baseline

Liver function test		<i>C. militaris</i> group (n=28)	Placebo group (n=29)	P-value
ALT	4 wk	5 (17.9)	10 (34.5)	0.2609
	8 wk	7 (25.0)	5 (17.2)	0.6941
AST	4 wk	19 (67.9)	22 (75.9)	0.7057
	8 wk	19 (67.9)	20 (69.0)	1.0000
GGT	4 wk	17 (60.7)	23 (79.3)	0.2133
	8 wk	17 (60.7)	25 (86.2)	0.0595

Values are presented as number (%).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyltranspeptidase; *C. militaris* = *Cordyceps militaris*.

Table 5. The ratio (%) of change of VAS score of subjective symptoms at 4 and 8 weeks after administration compared to baseline

Subjective symptom		<i>C. militaris</i> group (n=28)	Placebo group (n=29)	P-value
Fatigue	4 wk	1.12 ± 91.71	2.62 ± 69.13	0.5275
	8 wk	23.57 ± 75.87	28.62 ± 32.04	0.6266
General weakness	4 wk	19.52 ± 35.43	16.12 ± 60.80	0.3981
	8 wk	26.79 ± 33.34	25.35 ± 52.40	0.4508
Dyspepsia	4 wk	3.02 ± 56.64	-5.11 ± 60.96	0.3019
	8 wk	12.92 ± 54.17	-7.82 ± 84.70	0.1374
Nausea, vomiting	4 wk	-43.69 ± 100.58	4.13 ± 43.62	0.9868
	8 wk	-41.25 ± 111.83	-4.71 ± 62.41	0.9315
Poor oral intake	4 wk	-66.31 ± 187.03	-16.59 ± 81.49	0.8980
	8 wk	-18.77 ± 82.14	-28.86 ± 121.57	0.3571
Abdominal discomfort	4 wk	-31.64 ± 111.36	1.12 ± 57.82	0.9134
	8 wk	-55.36 ± 166.96	-2.61 ± 78.80	0.9312
Abdominal bloating	4 wk	12.35 ± 34.34	12.24 ± 47.38	0.4959
	8 wk	12.60 ± 57.21	11.62 ± 62.36	0.4756

Values are presented as mean $\pm$ standard error of the mean.

VAS = visual analogue scale; *C. militaris* = *Cordyceps militaris*.

Table 6. The ratio (%) of change of FSS at 4 and 8 weeks after administration compared to baseline

FSS category		<i>C. militaris</i> group (n=28)	Placebo group (n=29)	P-value
Decreased desire	4 wk	16.14±32.65	20.50±53.56	0.6444
	8 wk	30.73±42.85	36.24±27.76	0.7156
Fatigue during exercise	4 wk	0.36±41.88	6.37±49.26	0.6894
	8 wk	26.24±30.81	19.39±36.19	0.2222
Easy fatigue	4 wk	6.10±39.65	20.71±36.09	0.9241
	8 wk	26.18±39.28	28.65±50.29	0.5817
Difficulty of phy- sical activity	4 wk	-6.61±63.70	14.93±42.80	0.9290
	8 wk	19.32±49.97	19.53±40.14	0.5070
Frequent fatigue	4 wk	-13.10±65.36	-1.95±41.66	0.7759
	8 wk	5.99±45.27	5.29±42.69	0.4759
Continuity of phy- sical activity	4 wk	-23.00±55.98	3.45±44.24	0.9732
	8 wk	-1.53±53.24	-7.47±54.21	0.3390
Job performance	4 wk	-20.24±55.27	2.07±54.46	0.9346
	8 wk	-3.63±62.55	0.40±45.96	0.6084
Give up of difficult problem	4 wk	-22.32±82.49	-1.14±55.85	0.8682
	8 wk	7.56±74.25	10.58±46.40	0.5724
Family and social life	4 wk	-31.85±88.67	2.13±52.90	0.9563
	8 wk	-8.94±71.51	0.06±68.49	0.6851

Values are presented as mean±standard error of the mean.

FSS = fatigue severity scale; *C. militaris* = *Cordyceps militaris*.

nificant inter-group difference (Table 5).

2) FSS

In measurement using a questionnaire composed of 9 items, there was no statistically significant inter-group difference in all items (Table 6).

5. Adverse effect

No case of serious adverse effects related to administration was reported during the study (8 weeks). In addition, no clinically significant difference in clinical indexes, including vital signs and other diagnostic blood test, was observed between before and after administration.

DISCUSSION

C. militaris is a mushroom traditionally used in several area of East Asian territory. Lots of studies have been reported diverse results such as improving liver and renal functions, enhancing immunity through activation of basal metabolism.

Cordyceps spp. contains various chemical with biological

activities, such as cordycepin, cordycepic acid, and guanosine, are regarded as the most important substances working these pharmaceutical activities.¹³

Low density on liver CT scan indicates accumulation of fat, such as triglyceride, in hepatocytes,¹⁴ causing failure of hepatocytes and can lead to fibrosis or cirrhosis if lasting a long time.¹⁵ In this study, the HU of the liver CT scan showed significant improvement in the *C. militaris* group compared to the placebo group, as a result of ingestion of *C. militaris* extract for 8 weeks by male and female adults with mild liver dysfunction (*C. militaris* 21.43%±45.11% vs. placebo 9.64%±11.41%; two sample t-test, P=0.0987). These results may be caused by a suppression effect of *C. militaris* extract on accumulation of fat in hepatocytes.

On the other hand, the normalization rate of serum level of ALT, AST, and GGT showed no significant inter-group difference after administration compared to baseline. Serum level of ALT, AST, and GGT showed a tendency to increase at every point but without statistical significance. It is believed that *C. militaris* extract does not have hepatotoxicity compared to placebo, but additional studies will be needed.

On the VAS score, none of the 7 subjective symptoms associated with liver function showed statistically significant inter-group difference, and the FSS questionnaire consisting of 9 items showed no statistically significant inter-group difference either. Thus there was no improving effect according to intake of *C. militaris* extract. However, because our participants had only mild liver dysfunction and their symptoms were mild, it is possible that the effect of *C. militaris* extract on this group was insufficient. Also, it is possible that the SEM was overestimated, because our sample size is small.

There was no adverse effect during the study period and no abnormal laboratory tests. Thus *C. militaris* extract was safe to administer to the human body. As a result of this study, *C. militaris* extract was a safe functional food that improved liver CT scan for male and female adults with mild liver dysfunction.

This study is meaningful in that liver CT scan was used for functional testing. As a result, *C. militaris* extract was used safely in patients with mild liver dysfunction as a func-

tional food, and is expected to protect against progression of fatty liver or cirrhosis caused by suppression of lipid accumulation in hepatocytes. However, because we studied adults with only mild liver dysfunction, it is possible that its effect of improving liver function was insufficient. Therefore, further study aimed at moderate to severe liver dysfunction will be needed.

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