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Clinical Evaluation of Blood Glucose Regulation and Safety of *Cordyceps cicadae* Mycelium

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Abstract *Cordyceps cicadae* (*C. cicadae*) is one of the most valued traditional Chinese medicines and have been used for about 1600 years in China. It is edible and is used traditionally for vision improvement, renal protection, cancer treatment and regulation of blood cholesterol and blood glucose. The aim of this study was to evaluate the safety and to determine the effects of *Cordyceps cicadae* (*C. cicadae*) mycelium on hyperglycemic patients or type 2 diabetic patients by a single-blind, randomized, parallel-group, and placebo-controlled trial. In the treatment group, 22 subjects ingested a supplement containing freeze-dried *C. cicadae* mycelium powder (1050 mg/day) in hard capsule form, every day for 3 months. In the placebo group, 18 subjects ingested placebo capsules. Before supplementation, subjects completed a questionnaire to assess for personal data (age, medical history, medication record, physical activity). Subjects underwent blood pressure reading, anthropometric measurements, biochemical investigations, oral glucose tolerance test (OGTT) and urinalysis at baseline, the end of treatment period, and the end of follow-up period. Based on the results, freeze-dried *C. cicadae* mycelium powder could maintain blood glucose level in hyperglycemic patient or type 2 diabetic patients. Besides, no toxicity observed in kidney and liver. Results from the study do not raise concern with respect to possible use as a functional food ingredient. This study provides support for the use of *C. cicadae* fermentation product as a safe agent in functional food.

Keywords: *Cordyceps cicadae, blood glucose regulation, clinical trial, safety assessment, dietary supplement*

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1. Introduction

Diabetes is one of the most important topics in public health in the 21st century. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014 [1]. In Taiwan, prevalence of diabetes over 20 years of age was 12.7% in 2013 to 2014, suggesting that over 1.5 million people in Taiwan had diabetes [2]. WHO projects that diabetes will be the 7th leading cause of death in 2030 [3]. Besides, almost half of all deaths attributable to high blood glucose occur before the age of 70 years [1].

There is no cure for diabetes. The immediate goals are to stabilize the blood glucose and eliminate the symptoms of high blood glucose. The long-term goals of treatment are to prolong life, relieve symptoms, and prevent long-term complications such as blindness, kidney failure, heart disease, stroke and lower limb amputation. Diabetes can be treated and its consequences avoided or delayed with diet, physical activity, smoking cessation, and moderate alcohol use, medication and regular screening and treatment for complications [1]. Among these strategies for controlling diabetes, diet is the most important one to maintain a healthy body.

Cordyceps cicadae (*C. cicadae*) is one of the most valued traditional Chinese medicines and have been used for about 1,600 years in China. *C. cicadae* belongs to the family *Clavicipitaceae* and the genus *Cordyceps*, which strictly parasitize on cicada nymph or larva of *Cicada flammate*, *Platypleura kaempferi*, *Cryptotympana pustulata*

and *Patylomia pieli*. The host larva was consumed as nutrition and became a tightly packed mass of mycelium. Fine mycelia growing within nymph body are referred to as coremium. Stroma will develop on coremium and extruded from soil. Stroma usually has 3-4 branches in rod-like shape, with length of 4-10 cm, which form flower bud-shaped stroma from mouth, head or bottom of cicada larva. It is a wonderful biological complex of fungus and larva.

Modern pharmacological studies have indicated that *C. cicadae* exhibits a variety of biological functions, including vision improvement [4,5,6,7,8], renal protection [9,10,11], antitumor properties [12], hypocholesterolemic effect [13] and blood glucose regulation [14,15]. Song et al. demonstrated that administration of wild *C. cicadae* to diabetic ICR mice resulted in lowered blood glucose level [14]. Jhou et al. also found that pig fed with high-glucose diet containing *C. cicadae* mycelium for 28 days maintained blood glucose level [15].

Studies have shown that the medicinal benefits of cultured mycelia are similarly effective as those found in the wild [16]. Because of the shortage of wild *C. cicadae*, it is desirable to produce *C. cicadae* by submerged fermentation in a batch stirred tank bioreactor for higher mycelial production in a shorter culture time with a less chance of contamination and a good reproducibility. Previous animal studies have shown that no toxic effect were observed in 28-day subacute toxicity test (rat) (unpublished data), 28-day subacute toxicity test (piglet) [17], 28-day subacute toxicity test (pig) [15], 3 different test systems of genotoxicity test [18], 90-day oral toxicity test (rats) [19], as well as teratogenicity test (data unpublished) of artificially cultivated *C. cicadae* mycelium. However, whether freeze-dried *C. cicadae* mycelium powder is safe for human consumption remains unknown. In addition, there are no reports specifically investigating the effects of *C. cicadae* on blood glucose regulation among hyperglycemic patients or type 2 diabetic patients. Therefore, the purpose was to evaluate edible safety and blood glucose regulation after intake of *C. cicadae* mycelium in hyperglycemic patients or type 2 diabetic patients.

2. Results

2.1. Participant Characteristics

The study was conducted between November 2012 and October 2013. Forty subjects (22 subjects taking freeze-dried *C. cicadae* mycelium powder capsules and 18 placebo subjects) who completed the study were included in the analysis. There were no significant differences between the randomized treatment groups at baseline (Table 1). Anthropometric data and blood pressure are presented in Table 2. Compared with M0, the body weight (64.5±10.8 kg vs.65.7±10.7 kg) and BMI (24.3±3.1 kg/m^2 vs.24.8±3.0 kg/m^2) at M3 were significantly reduced in the placebo group. There were no significant differences in body fat and blood pressure between M3 and M0 in the placebo group. On the other hand, compared with M0, the body weight (72.5±14.8 kg vs.73.3±15.5 kg), BMI (27.3±4.8 kg/m^2 vs.27.5±5.0 kg/m^2), systolic blood pressure (132.3±22.9 mmHg vs.141.5±21.8 mmHg) and diastolic blood pressure (80.5±13.7 mmHg vs.85.8±13.3 mmHg) at M3 were significantly lower in the treatment group. There was no significant differences in body fat between M3 and M0 in the treatment group.

Table 1. Age and anthropometric measurements of the study participant at baseline

Group Item	Treatment (n=22)	Placebo (n=18)
Age (years)	53.5 ± 8.8	58.1 ± 6.2
BH (cm)	162.9 ± 8.8	162.6 ± 6.8
BW (kg)	73.3 ± 15.5	65.7 ± 10.7
BMI (kg/m2)	27.5 ± 5.0	24.8 ± 3.0

Values are expressed as mean ± standard deviation (SD).
BH, Body height; BW, Body weight; BMI, Body mass index.

Table 2. Anthropometric measurements and blood pressure of the study participant

Group Item	Treatment (n=22)			P for trend	Placebo (n=18)			P for trend
	Month 0	Month 3	Month 4		Month 0	Month 3	Month 4	
BW (kg)	73.3 ± 15.5^{a}	72.5 ± 14.8$^{\dagger, b}$	72.6 ± 14.7$^{a, b}$	0.0354	65.7 ± 10.7^{a}	64.5 ± 10.8$^{\dagger, b}$	64.7 ± 11.2$^{\S, b}$	<0.0001
Difference M3-M0	-0.8 ± 1.5				-1.3 ± 1.3			0.6881
BMI (kg/m^2)	27.5 ± 5.0	27.3 ± 4.8†	27.3 ± 4.8	0.0651	24.8 ± 3.0^{a}	24.3 ± 3.1$^{\dagger, b}$	24.3 ± 3.1$^{\S, b}$	<0.0001
Difference M3-M0	-0.3 ± 0.6				-0.5 ± 0.6			0.6531
BF (%)	31.5 ± 7.1	31.2 ± 7.2	30.9 ± 6.9	0.6122	30.9 ± 4.4	30.4 ± 4.4	30.4 ± 5.0	0.0583
Difference M3-M0	-0.3 ± 3.1				-0.6 ± 1.4			0.8836
SBP (mmHg)	141.5 ± 21.8^{a}	132.3 ± 22.9$^{\dagger, b}$	135.1 ± 19.8$^{\S, b}$	0.0074	134.2 ± 26.3	128.7 ± 20.0	127.7 ± 29.2	0.1600
Difference M3-M0	-9.2±14.7				-5.4 ± 13.9			1.0000
DBP (mmHg)	85.8 ± 13.3^{a}	80.5 ± 13.7$^{\dagger, b}$	80.3 ± 11.3$^{\S, b}$	0.0027	75.4 ± 12.8	76.5 ± 12.3	76.3 ± 15.3	0.8837
Difference M3-M0	-5.3 ± 9.5				1.1 ± 8.4			0.5302

Values are expressed as mean ± standard deviation (SD). † $p < 0.05$ vs. M0 (Wilcoxon signed-rank test), ‡ $p < 0.05$ vs. M3 (Wilcoxon signed-rank test), § $p < 0.05$ vs. M0 (Wilcoxon signed-rank test), * $p < 0.05$ vs. Placebo group (Wilcoxon rank sum test), ^{a,b}Groups that do not share the same letter are significant from each other (repeated ANOVA test, $p < 0.05$).
BW, Body weight; BMI, Body mass index; BF, Body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3. Clinical chemistry of the study participant

Group Item	Range	Treatmenr (n=22)			P for trend	Placebo (n=18)			P for trend
		Month 0	Month 3	Month 4		Month 0	Month 3	Month 4	
AC (mg/dL)	60~109	117.6 ± 35.9	119.6 ± 35.0	124.4 ± 40.4	0.4461	137.6 ± 68.0	132.3 ± 59.8	131.9 ± 45.7	0.8375
Difference M3-M0		2.0 ± 26.5				-5.3 ± 57.8			0.2094
HbA1c (%)	4.0~6.0	6.7 ± 0.9	6.9 ±0.9†	6.9 ±0.9	0.5564	6.9 ± 1.1	7.2 ± 1.6	7.2 ± 1.7	0.1618
Difference M3-M0		0.2 ± 1.2				0.3 ± 0.8			0.3023
Insulin (µIU/mL)	AC: 4~16	13.2 ± 8.6	12.0 ± 7.4	13.6 ± 7.8	0.4380	13.8 ± 10.3	10.7 ± 4.9	11.7 ± 8.1	0.1801
Difference M3-M0		-1.2 ± 6.7				-3.0 ± 8.9			0.2094
Fructosamine (µmol/ml)	118~351	305.5 ± 44.0	292.6 ± 45.4	313.8 ± 48.5‡	0.0643	284.7 ± 47.2^{c}	322.6 ± 77.8$^{\dagger, b}$	341.8 ± 69.4$^{\ddagger, \S, a}$	<0.0001
Difference M3-M0		-13.0 ± 43.9				37.9 ± 57.9			0.0597
TC (mg/dL)	120~200	205.9 ± 47.1	208.1 ± 50.4	209.7 ± 42.5	0.8339	206.8 ± 18.9	207.2 ± 39.8	202.4 ± 36.7	0.7189
Difference M3-M0		2.2 ± 38.4				0.4 ± 28.0			0.3341
TG (mg/dL)	<200	142.2 ± 84.8	130.6 ± 100.3	148.9 ± 91.0	0.3893	146.2 ± 116.0	130.6 ± 74.2	124.3 ± 56.2	0.5561
Difference M3-M0		-11.6 ± 68.6				-15.7 ± 115.1			1.0000
HDL-C (mg/dL)	>40	53.0 ± 17.7	51.8 ± 16.4	53.5 ± 15.6	>40	53.0 ± 17.7	59.3 ± 18.3$^{a, b}$	59.1 ± 17.8$^{\S, a}$	0.0448
Difference M3-M0		-1.2 ± 21.2				3.7 ± 7.9			0.0597
LDL-C (mg/dL)	<130	119.3 ± 39.1	123.2 ±38.4	120.8 ± 35.4	0.6986	121.4 ± 19.1	119.2 ± 32.8	117.2 ± 31.8	0.8005
Difference M3-M0		3.9 ± 27.3				-2.2 ± 26.9			0.3341
TC/HDL-C	<5	4.1 ± 1.1	4.2 ± 1.1	4.1 ± 1.1	0.5927	3.9 ± 0.9	3.7 ± 0.9	3.7 ± 1.0	0.3414
Difference M3-M0		0.1 ± 0.6				-0.3 ± 0.5*			0.0074
AST (U/L)	8~38	29.1 ± 10.7	30.9 ± 10.7	27.9 ± 8.0‡	0.1304	27.5 ± 17.4	27.5 ± 17.0	36.9 ± 35.6	0.3291
Difference M3-M0		1.7 ± 6.8				0.0 ± 8.9			0.7442
ALT (U/L)	4~44	30.5 ± 21.8	30.4 ± 15.1	28.2 ± 15.8	0.6840	28.7 ± 25.0	28.7 ± 23.4	43.6 ± 56.7	0.2804
Difference M3-M0		-0.2 ± 16.3				0.0 ± 7.0			0.5196
BIT (mg/dL)	0.2~1.4	0.9 ± 0.3	0.8 ± 0.3	0.9 ± 0.4	0.7486	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	0.3075
Difference M3-M0		-0.1 ± 0.3				0.1 ± 0.2*			0.0195
γ-GT (U/L)	<52	27.3 ± 13.5	27.1 ± 9.7	26.6 ± 10.6	0.9387	32.1 ± 26.9	30.4 ± 24.1	43.3 ± 58.5§	0.4650
Difference M3-M0		-0.2 ± 9.6				-1.6 ± 6.1			0.2612
BUN (mg/dL)	6~23	15.0 ± 3.4	14.6 ± 3.2	13.7 ± 2.9§	0.1678	16.4 ± 3.3^{a}	15.1 ± 4.2$^{a, b}$	14.1 ± 3.6^{b}	0.0312
Difference M3-M0		-0.4 ± 3.7				-1.3 ± 3.3			0.0597
Cr (mg/dL)	0.6~1.2	0.8 ± 0.2	0.9 ± 0.2†	0.9 ± 0.2	0.2723	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.7438
Difference M3-M0		0.0 ± 0.2				0.0 ± 0.1			0.1195
UA (mg/dL)	2.6~7.5	6.0 ± 1.5	5.9 ± 1.4	5.9 ± 1.5	0.9866	5.1 ± 1.4^{a}	4.8 ± 1.2$^{a, b}$	4.8 ± 1.1$^{\S, b}$	0.0426
Difference M3-M0		0.0 ± 0.8				-0.3 ± 0.7			0.3072
TP (g/dL)	6.7~8.3	7.3 ± 0.3^{b}	7.5 ± 0.3$^{a, b}$	7.6 ± 0.5$^{\S, a}$	0.0320	7.2 ± 0.4	7.4 ± 0.5	7.4 ± 0.4§	0.0811
Difference M3-M0		0.1 ± 0.4				0.2 ± 0.5			0.2092
Alb (g/dL)	3.8~5.3	4.3 ± 0.2^{b}	4.5 ± 0.3$^{\dagger, a}$	4.5 ± 0.3$^{\S, a}$	0.0024	4.3 ± 0.2	4.4 ± 0.3	4.4 ± 0.2§	0.0661
Difference M3-M0		0.2 ± 0.3				0.1 ± 0.2			0.5612
LDH (U/L)	313~618	530.4 ± 105.8	518.5 ± 106.4	501.9 ± 99.1	0.1640	465.9 ± 78.5	449.7 ± 61.6	495.4 ± 135.7	0.2422
Difference M3-M0		-11.9 ± 74.0				-16.2 ± 50.8			1.0000

Values are expressed as mean ± standard deviation (SD). † $p < 0.05$ vs. M0 (Wilcoxon signed-rank test), ‡ $p < 0.05$ vs. M3 (Wilcoxon signed-rank test), § $p < 0.05$ vs. M0 (Wilcoxon signed-rank test), * $p < 0.05$ vs. Placebo group (Wilcoxon rank sum test), ^{a,b,c}Groups that do not share the same letter are significant from each other (repeated ANOVA test, $p < 0.05$).

AC, Glucose fasting; HbA1c, glycated hemoglobin; TC, total cholesterol; TG, triglycerol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; AST, Aspartate transaminase; ALT, Alanine transaminase; BIT, Bilirubin total; γ-GT, gamma-glutamyltranspeptidase; BUN, blood nitrogen urea; Cr,creatinine; UA, uric acid; TP, total protein; Alb, albumin; LDH, lacticdehydrogenase.

2.2. Clinical Chemistry

Table 3 shows the results for clinical chemistry during M0 to M4. In the placebo group, there is no significant difference in clinical chemistry between M3 and M0 except that fructosamine (322.6±77.8 µmol/ml vs. 284.7±47.2 µmol/ml) of M3 was significantly higher compared with that of M0 but was still within the normal range. In the treatment group, there is no significant difference in clinical chemistry between M3 and M0 except that HbA1c (6.9±0.9% vs. 6.7±0.9%), creatinine l vs. 4.3±0.2 g/dl) of M3 were significantly higher

compared with those of M0. In the treatment group, creatinine and albumin of M3 were both within the normal range; HbA1c of M3 was lightly elevated but was still close to that of M0.

2.3. Hematology

Hematology of the study participant demonstrated in Table 4. No significant difference was found in the placebo group between M0 and M3 except that MCH (29.0±3.4 pg vs. 29.3±3.3 pg) and MCHC (32.8±1.5% vs. 33.4±1.4%) at M3 were significantly reduced compared with those at M0 but were still within the normal range. On the other hand, no significant difference was found in the treatment group between M0 and M3 except that MCH (29.0±2.5 pg vs. 29.3±2.5 pg) and MCHC (33.0±1.1% vs. 33.4±1.0%) at M3 were significantly reduced compared with those at M0 but were still within the normal range.

Table 4. Hematology of the study participant

Group Item	Range	Treatmenr (n=22)			P for trend	Placebo (n=18)			P for trend
		Month 0	Month 3	Month 4		Month 0	Month 3	Month 4	
WBC (cumm)	4000~11000	6184.5 ± 1366.5	6356.4 ± 1539.0	6053.6 ±1549.8	0.7192	5768.3 ± 1457.6	5906.7 ± 1486.0	5538.8 ± 1761.9	0.5957
Difference M3-M0		171.8 ± 1881.3				138.3 ± 774.5			1.0000
RBC (MIL/cumm)	3.7~6.2	4.9 ± 0.8	4.9 ± 0.7	4.9 ± 0.7	0.9876	4.7 ± 0.6	4.7 ± 0.6	4.6 ± 0.6‡	0.2311
Difference M3-M0		0.0 ± 0.5				0.1 ± 0.3			0.0991
Hb (gm/dL)	11.3~18.3	14.3 ± 1.6	14.2 ± 1.4	14.2 ± 1.5	0.8425	13.6 ± 1.9	13.7 ± 2.1	13.4 ± 2.4	0.2239
Difference M3-M0		-0.2 ± 1.5				0.1 ± 0.7			0.6649
HCT (%)	33~53	42.8 ± 4.3	42.9 ± 3.9	43.2 ± 3.9	0.8910	40.7 ± 4.6	41.6 ± 5.3	40.7 ± 5.8	0.1849
Difference M3-M0		0.1 ± 4.2				0.8 ± 1.9			0.0597
MCV (fl)	79~99	87.8 ± 6.8	87.9 ± 6.5	88.4 ± 7.0	0.5905	87.6 ± 7.9^{a}	88.0 ± 7.8^{a}	88.5 ± 7.8$^{‡, §, b}$	0.0483
Difference M3-M0		0.2 ± 3.4				0.4 ± 1.6			0.5302
MCH (pg)	26~34	29.3 ± 2.5	29.0 ± 2.5†	29.0 ± 2.6	0.5790	29.3 ± 3.3^{a}	29.0 ± 3.4$^{†, b}$	29.0 ± 3.5$^{a, b}$	0.0429
Difference M3-M0		-0.3 ± 1.5				-0.3 ± 0.6			0.7336
MCHC (%)	30~36	33.4 ± 1.0^{a}	33.0 ± 1.1$^{†, a, b}$	32.8 ± 1.0	0.0073	33.4 ± 1.4^{a}	32.8 ± 1.5$^{†, b}$	32.7 ± 1.6$^{§, b}$	0.0002
Difference M3-M0		-0.4 ± 1.0				-0.6 ± 0.6			0.8286
PLA (1000/cumm)	120~400	219.0 ± 65.9	231.2 ± 66.8	243.0 ± 50.9	0.1893	235.6 ± 51.0^{b}	259.7 ± 64.9$^{a, b}$	280.2 ± 71.9$^{§, a}$	0.0091
Difference M3-M0		12.2 ± 73.7				24.1 ± 61.3			0.5302

Values are expressed as mean ± standard deviation (SD). † $p < 0.05$ vs. M0 (Wilcoxon signed-rank test), ‡ $p < 0.05$ vs. M3 (Wilcoxon signed-rank test), § $p < 0.05$ vs. M0 (Wilcoxon signed-rank test), * $p < 0.05$ vs. Placebo group (Wilcoxon rank sum test), ^{a,b}Groups that do not share the same letter are significant from each other (repeated ANOVA test, $p < 0.05$).
WBC, white blood cell count; RBC, red blood cell count; Hb, Hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean cell hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLA, platelet count.

Table 5. Oral glucose tolerance test in the study participant

Group Item	Unit	Treatment (n=22)		P for trend	Placebo (n=18)		P for trend
		Month 0	Month 3		Month 0	Month 3	
0 min	mg/dL	126.3 ± 35.7	127.6 ± 31.9	0.7405	140.7 ± 51.5	138.4 ± 55.6	0.8326
Difference M3- M0		1.4 ± 19.1			-2.3 ± 45.0		0.0597
30 min	mg/dL	211.1 ± 41.0	214.8 ±46.4	0.5001	235.3 ± 63.2	242.5 ± 76.9	0.1926
Difference M3- M0		3.7 ±23.4			14.4 ± 43.7		0.8573
60 min	mg/dL	243.7 ± 55.3	248.4 ± 73.2	0.5696	283.6 ± 70.4	270.1 ± 81.7	0.5665
Difference M3- M0		4.7 ± 38.4			-6.8 ± 47.7		0.3341
90 min	mg/dL	229.6 ± 81.5	225.3 ± 83.3	0.7450	291.3 ± 79.6	261.9 ± 103.2	0.2167
Difference M3- M0		-3.8 ± 52.3			-20.5 ± 65.8		0.3341
120 min	mg/dL	201.6 ± 86.9	205.8 ± 85.5	0.7705	259.8 ± 92.5	238.1 ± 100.3	0.1411
Difference M3- M0		4.2 ± 66.4			-21.7 ± 59.7		0.2094

Values are expressed as mean ± standard deviation (SD).

2.4. Oral Glucose Tolerance Test (OGTT)

Table 5 shows results of the oral glucose tolerance test (OGTT). There was no significant differences observed in the placebo group between M3 and M0. On the other hand, there was also no significant differences observed in the treatment group between M3 and M0.

3. Discussion

Type 2 diabetes, a chronic disease occurs when the body cannot effectively use the insulin it produces [20], is largely the result of unhealthy diet, excess body weight and physical inactivity. In Taiwan, diabetes ranked fifth

among the top 10 causes of deaths in 2014, causing 9,845 deaths in a year [1]. In almost all high-income countries, diabetes is a leading cause of cardiovascular disease, blindness, kidney failure, and lower limb amputation. Maintaining blood glucose levels, blood pressure, and cholesterol at or close to normal can help delay or prevent diabetes complications.

During the study period, no treatment-related changes were observed in body weight, BMI, body fat, OGTT and urinalysis in the treatment and placebo groups. In the treatment group, taking freeze-dried *C. cicadae* mycelium powder capsules for 3 consecutive months slightly lowered systolic blood pressure and diastolic blood pressure to bear normal levels in hyperglycemic patient and type 2 diabetic patients.

A few parameters in clinical biochemistry and hematology analysis showed significant differences between M3 and M0 in both treatment and control groups, including HbA1c, fructosamine, creatinine, albumin, MCH and MCHC. Fructosamine, creatinine, albumin, MCH, MCHC in both treatment and placebo groups were all within the normal range. A serum fructosamine level, similar to a HbA1c level, enables assessment of long-term glycemic control in patients with diabetes [21,22]. It should be noted that we found a near-significant reduction in the level of fructosamine in the treatment group ($p=0.0643$). In addition, the level of fructosamine in the treatment group were almost but not quite significant less than that of the control group ($p=0.0597$), suggesting that *C. cicadae* mycelium may exhibits beneficial effect on long-term blood glucose regulation. HbA1c of M3 was lightly elevated but was close to that of M0 in the treatment group. A major diabetes study, the Diabetes Control and Complications Trial (DCCT), found that people who keep their HbA1c levels close to 7% have a much better chance of delaying or preventing complications that affect the eyes, kidneys, and nerves than people with HbA1c of approximately 9% [23]. Therefore, HbA1c goal for people with type 2 diabetes is less than 7%. The HbA1c levels in treatment group were all less than 7% and were all lower than those at M0, M3 and M4 compared with placebo group.

AST, ALT, BIT, γ-GT are useful biomarkers of liver injury in a patient with some degree of intact liver function [24]. There were no significant differences in AST, ALT, BIT, and γ-GT between M3 and M0 in both the placebo and treatment groups and all values were within the normal range. Kidney function tests are simple blood tests and urine tests that to measure kidney function, damage and detect abnormalities. There were no significant differences in BUN, Cr, UA and urinalysis between M3 and M0 in both the placebo and treatment groups and all values were within the normal range. These results suggest that no significant toxicity occurred in liver and kidney. In addition, Wang et al. indicated that using the ratio of total to HDL cholesterol as the initial screening tool for predicting the risk for coronary heart disease is better than current guidelines for lipid management in Chinese population [25]. There was no significant difference in TC/HDL-C between M3 and M0 in both treatment and control group.

Our unpublished data demonstrated that *C. cicadae* mycelium (1000 μg/ml) has the capability to increases glucose uptake in human hepatocarcinoma SK-Hep-1 cells at 20 h of incubation. However, the mechanism of blood glucose homeostasis and the bioactive compounds responsible for these beneficial effects remain unknown.

According to the results, taking freeze-dried *C. cicadae* mycelium powder capsules for 3 consecutive months resulted in stable blood glucose and lipid levels, and no significant toxicity occurred in liver and kidney.

4. Materials and Methods

4.1. Materials

The test article used in the present study was freeze-dried *C. cicadae* mycelium powder capsule. *C. cicadae* (MU30106) procured from the Bioresource Collection and Research Center at the Food Industry Research and Development Institute(Hsinchu, Taiwan) was grown on potato dextrose agar at 25°C for 5 days, transferred to a 2.0-L flask containing 1.0 L of PDB, and incubated at 25°C on a rotary shaker at 120 rpm for 5 days. The fermented broth (1.0 L) was inoculated into a 200-L fermentor (BioTop, Taichung, Taiwan) with 60% working volume (2% glucose, 1% yeast extract, 1% soybean powder; pH 6.0), and agitated at 60 rpm with an aeration rate of 0.5 vvm at 25°C for 3 days. The submerged mycelial culture was heated at 100°C for1 h, freeze dried, and ground to powder. Powders are used to make capsules and store at room temperature.

4.2. Subjects

Forty subjects recruited into this study in 2012 to 2013 were divided into two groups: a treatment group who received three freeze-dried *C. cicadae* mycelium powder capsules daily (22 subjects) and a placebo group who received three starch capsules daily (18 subjects) in a randomized study. Healthy individuals, outpatients, patients with chronic disease, and elderly residents of long-term care facilities were reviewed. The volunteers were required to meet the following inclusion criteria: (1) aged over 20 years; (2) body mass index (BMI) greater than 15 kg/m2; (3) stable vital sign; (4) mild diabetic patients or healthy individuals with an glycated hemoglobin (HbA1c)more than 6%; (5) being willing to cooperate with dietary and testing requirements. Subjects having an HbA1c between 6% and 7% and not receiving treatment were included. Subjects with an HbA1c more than 7% were asked to continue their regular medications. All subjects provided written informed consent. Exclusion criteria were: patients with advanced cancer, liver cirrhosis, renal failure, or uremia.

4.3. A Placebo-controlled, Randomized Study in Patients with Type 2 Diabetes

This study was conducted in accordance with the "Safety Evaluation Methods for Health Food. DOH, Taiwan, R.O.C.". All study protocols were approved by the TMU-Joint Institutional Review Board (No. 201204027). At baseline, personal data (age, medical history, medication record, physical activity) of all

participants were inquired about in a questionnaire. During intervention period, subjects were asked not to change their dietary habits. Participants were instructed to take three capsules per day, each capsule containing either 350 mg freeze-dried *C. cicadae* mycelium powder or 350 mg placebo (starch). During follow-up period, subjects were asked to stop taking capsules and not to change their dietary habits.

Subjects were underwent blood pressure reading, anthropometric measurements, biochemical investigations including clinical chemistry and complete blood count (CBC), oral glucose tolerance test (OGTT) and urinalysis at baseline (month 0, M0), the end of treatment period (month 3, M3), and the end of follow-up period (month 4, M4).

All laboratory investigations were performed by medical technologist. Anthropometric measurements include height, weight, and body fat. Blood samples were collected by lancing device after an overnight fast of at least 10 h. Clinical chemistry includes glucose fasting (AC), HbA1c, insulin, fructosamine, total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), aspartate transaminase (AST), alanine transaminase, ALT), bilirubin total (BIT), gamma-glutamyltranspeptidase (γ-GT), blood nitrogen urea (BUN), creatinine (Cr), uric acid (UA), total protein (TP), albumin (Alb), lactic dehydrogenase (LDH). CBC includes white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLA). OGTT were evaluated with a glucometer (Accu-chek Go, Roche).

4.4. Statistical Analysis

Values are expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) and Student's t-test for comparisons of group means. Changes in quantitative parameters from baseline within each group were assessed using Wilcoxon signed-rank test. Differences between groups at M0, M3 and M4analyzed using Wilcoxon rank sum test. All statistical analyses were performed using SAS; a P value < 0.05 is considered statistically significant.

5. Conclusions

We demonstrated that freeze-dried *C. cicadae* mycelium powder could maintain blood glucose level in hyperglycemic patient or type 2 diabetic patients. Besides, no toxicity observed in kidney and liver. Results from the study do not raise concern with respect to possible use as a functional food ingredient. Results of this study provide support for the use of *C. cicadae* fermentation product as a safe agent in functional food.

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Author Contributions

C.-C.C. and S.-H.Y. conceived and designed the experiments; T.-C.W. and C.-L.H. performed the experiments; Y.-T.T. analyzed the data; J.-H.H. and S.-H.Y. contributed reagents/materials/analysis tools; B.-Y.J. wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

Alb	Albumin
AC	Glucose fasting
ALT	Alanine transaminase
ANOVA	Analyzed using one-way analysis of variance
AST	Aspartate transaminase
BIT	Bilirubin total
BMI	Body mass index
BUN	Blood nitrogen urea
CBC	Complete blood count
C. cicadae	*Cordyceps cicadae*
Cr	creatinine
DCCT	Diabetes Control and Complications Trial
Hb	Hemoglobin
HbA1c	Glycated hemoglobin
HCT	Hematocrit
HDL-C	High density lipoprotein-cholesterol
LDH	Lactic dehydrogenase
LDL-C	Low density lipoprotein-cholesterol
MCH	Mean cell hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
OGTT	Oral glucose tolerance test
PLA	Platelet count
RBC	Red blood cell count
γ-GT	Gamma-glutamyltranspeptidase
SD	Standard deviation
TC	Total cholesterol
TG	Triglyceride
TP	Total protein
UA	Uric acid
WBC	White blood cell count

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