

IMPROVEMENT IN IMMUNE FUNCTION BY SUPPLEMENT CONTAINED β -GLUCANS

— A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY —

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Abstract

Objectives: The objective of this research was to investigate the effectiveness of daily ingestion of Reishi tablet, which contains β -glucans, on the immunological vigor of human.

Methods: In this randomized, placebo-controlled, double-blind trial, 27 people were subjected. The SIV score (combination of the number of T cells, naive T cells, CD8⁺CD28⁺ T cells, B cells and NK cells, and ratios of CD4/CD8 T cell and naive/memory T cell) and blood pressure were measured as its primary outcomes.

Results: 6 subjects were withdrawn due to personal reasons and the remaining 21 subjects completed the study. Significant differences of T lymphocyte, T cells, CD8⁺CD28⁺ T cells and the systolic blood pressure were observed between test and placebo samples after 12 weeks of ingestion.

Conclusion: We found out that the ingestion of the processed food of Reishi (*Ganoderma lucidum*) containing β -glucans for 12 weeks contributed to improving the immunological vigor of human and the systolic blood pressure. In addition, no safety-related matter occurred during the 12-week test period.

Key words: Reishi, *Ganoderma lucidum*, β -glucan, immunological vigor, immune, SBP

1. INTRODUCTION

Modern Japanese society has been generally called a “super-aging society”, after a ratio of the number of elderly people aged 65 years older compared to the total population, became 23% in 2010¹⁾. Coupled with that, the medical spending of all the public is growing steadily, and “Self-medication” is now receiving plenty of attention as one of the measures to cope with this problem. According to WHO, self-medication is defined as “the selection and use of medicines by individuals to treat self-recognized illnesses or symptoms”²⁾. A typical example of this definition includes utilizing OTC (over-the-counter) medicine with the correct knowledge. In addition to this, another type of self-medication may include reviewing their dietary life. Immunity is an important function for the human body to defend it from disease. Therefore it is highly desirable to keep it at a high level to lead healthy everyday lives³⁾. However, human face the decline of immunity since its peak at the age of 20, and this decline is caused by various factors such as advancing age, stress or inadequate lifestyle, which are all unavoidable matters in daily lives. The decline of immunity not only increases the risks of disease or infection, but also diminishes the countervailing power against stress and leads to the decline of QOL (Quality of Life). Therefore, in order to

live a healthy life, it is important to maintain immunity at high level.

“Jo-yaku” is one of the categories in traditional Chinese medicine, which is not curative medicine but characterized as a medication made for everyday consumption in order to prevent illness and stay healthy⁵⁾, and “Reishi” has been categorized as Jo-yaku since ancient times. Reishi is the Japanese name for the fruiting body of *Ganoderma lucidum*, a genus of fungi belonging to the family *Polyporaceae*, and is one of the most well-known herbal remedies in traditional Chinese medicine. This species of the *Polyporaceae* family of fungus is also commonly referred to as *Ling zhi*, or “God’s Herb”.

It is believed that *Ganoderma lucidum* has six color types (red, black, blue, white, yellow, and purple), and the contained ingredients are different depending on these types⁶⁾. There is a report that *Ganoderma lucidum* has a function of controlling immunity; it boosts immunity when it is weakening, and alleviates when excessing⁷⁾. Also, in addition to this function it reportedly owns a wide variety of other functions such as the control of blood glucose levels⁸⁾, lowering-blood-pressure⁹⁾, anti-allergy¹⁰⁾, and improving conditions of hyperlipidemia¹¹⁾ or type B hepatitis¹²⁾. As for *Ganoderma lucidum*, there have been many clinical studies carried out for examining its

effectiveness of controlling immunity function, but most of the study's goal were to review the alleviation of symptoms such as cancer and therefore there are few published reports that discuss whether it is effective in controlling immunity in healthy humans.

Therefore in this study, we examined how *Ganoderma lucidum* affects immunological vigor of healthy humans. We used the processed food of Reishi (*Ganoderma lucidum*) containing β -glucans as the test product, and applied a randomized, placebo-controlled, double-blind study as the test method.

2. METHODS

2.1. Trial design

A randomized, placebo-controlled, double-blind study was conducted with the aid of a fund from LINKS Inc. (Tokyo) at two centers (OZ clinic, Tokyo, and JACTA, Tokyo). The study period was 12 weeks, from January 22nd to April 15th, 2016.

This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Pharmaceutical Law Wisdoms (Tokyo). Written informed consent was obtained from all subjects.

The allocation of the test product to the subjects was carried out by the person in charge of allocation. The allocation list was sealed and strictly controlled in a safe deposit box of JACTA until the end of the study.

2.2. Subject

Healthy subjects participated in the present study. All of the subjects in this study were public volunteers who had enrolled in the monitor bank of CROee Inc. (Tokyo), recruited from December, 2015 through January, 2016.

2.2.1. Inclusion criteria

- (1) Healthy Japanese males and females aged between 30 and 59 years with daily tiredness and fatigue;
- (2) With relatively low self-examination of immunological vigor (SEIV)* ≥ 90 .

*: SEIV is a self-examination of immunological vigor with Likert scales. (Appendix 1)

2.2.2. Exclusion criteria

- (1) Previously suffered with malignant tumors, heart failure, or cardiac infarction;
- (2) Under the care of a doctor for the treatment of chronic diseases such as atrial fibrillation, uneven heartbeat, rheumatism, diabetes, high blood pressure, and diseases of the liver, kidney, cerebral system, circulatory system, and lipid metabolism;
- (3) Taking medicines, including herbal medicines;
- (4) Pregnant, nursing, or were likely to become pregnant during the trial;
- (5) Judged to be unsuitable to participate in the trial by the principle investigator.

2.3. Randomization

From all of 81 applicants, 54 were eliminated according to SEIV or age. The inclusion criteria was judged by the

Table 1 Nutritional content of the test samples per 100 g

Item	Reishi	Placebo
Moisture	2.5 g	4.0 g
Protein	4.4 g	less than 0.1 g
Lipid	3.3 g	2.2 g
Ash	3.5 g	1.9 g
Available carbohydrate	43.2 g	91.9 g
Dietary fiber	43.1 g	—
Energy	306 kcal	387 kcal
Salt equivalent	0.023 g	1.64 g

principle investigator. All subjects were sequentially allocated to Group A (n = 13, M; 6, F; 7) and Group B (n = 14, M; 8, F; 6) based on a random number table. In the process of subject assignment, background factors such as gender, age, and SEIV were taken into consideration to avoid biased distribution. Subjects in Group A ingested the placebo and subjects in Group B ingested the test sample for 12 weeks.

2.4. Description of test foods and blinding

The test product "Genkatsu-Reishi" ("Reishi") was prepared by LINKS Inc., and is a tablet containing β -glucan. The amount of a daily intake is 12 tablets (1 tablet weighs 320 mg, therefore 12 tablets weigh 3.84 g). The Placebo does not contain β -glucan.

Table 1 shows the nutritional content of the sample. Both tablets were indistinguishable in shape, color or taste. Tablets were managed by the identification symbol. All involved were blinded.

2.5. Experimental procedures

2.5.1. Experimental protocol

Subjects consumed 12 tablets of the supplement with hot or cold water every day for 12 weeks. Subjects were instructed as follows: to take the assigned foods as indicated; to maintain their usual lifestyles and habits; to avoid excessive amounts of food, drink, or alcohol; to maintain a daily record of lifestyle factors such as what they ate and pedometer measurement during the test period; and to send the diary to the study coordinator.

2.5.2. Outcome

According to the schedule shown in **Table 2**, we measured parameters on efficacy and safety.

2.5.2.1 SIV

The objective of this study is to verify immunological vigor of ingesting food of Reishi (*Ganoderma lucidum*) containing β -glucans. Immunological vigor was set as the primary outcome. SIV was used to evaluate that. SIV is the scoring system that can combine seven immunological parameters, the number of T cells, naive T cells, CD8⁺CD28⁺ T cells, B cells, and NK cells, and ratios of CD4/CD8 T cell and naive/memory T cell, and express the immune status of individuals as a simple numeral. This system also shows T lymphocyte age. Due to this, we can compare the real age with the age

Table 2 Schedule for the study.

Item \ Term	Screening	Pretrial test	Test period (12 w)
SEIV	●		●
Informed consent	●		
Selection and/or allocation	●		
Immunological parameters		●	●
Blood pressure		●	●
Blood biochemical and urine analysis		●	●
Ingestion of test foods			↔
Log			↔

● : Implementation
 ↔ : Daily practice during the test period

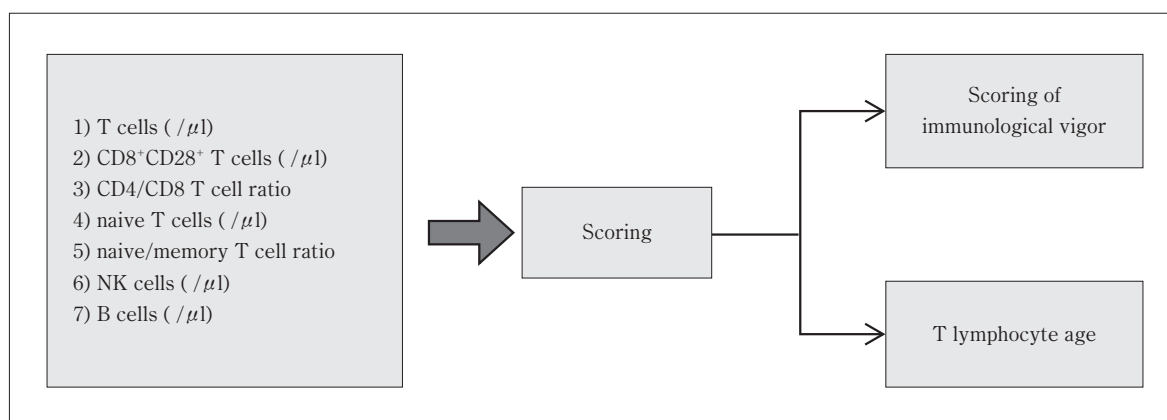


Fig. 1 SIV

evaluated from immunological vigor's point of view (**Fig. 1**). The SIV scoring system is an index system, which utilizes the database of test results for every particular item, which are accumulated by Hirokawa and Utsuyama¹³⁾, and by using the database, its index is calculated from several subsets. Therefore it enabled us to evaluate the condition of a variety of immune cells in a comprehensive and multilateral manner, and it has already been introduced in past research as the new testing method of immune strength¹⁴⁾.

2.5.2.2. Blood pressure

As the primary outcome, blood pressure was tested. Blood pressure was measured using an automated sphygmomanometer UDEX-i (Elquest Corporation, Japan). Measurements were performed three times successively. The medium values were used as an estimate of blood pressure.

2.5.2.3. Safety

As a secondary endpoint, blood biochemical and urine analysis, and inquiries were made to assess safety. Adverse events were collected by means of a written questionnaire during the study.

2.6. Data analysis

A full analysis set was adopted in the present study and no sample size was used. All statistics were expressed as

mean ± standard deviation (SD). For SIV, immunological parameters of blood, T lymphocyte age, blood pressure, blood biochemical and urine, changes from the baseline in the same group were assessed using the paired t-test. Student's-t test was used for intergroup comparisons of changes from the baseline. Student's-t test was used to compare subject's backgrounds between groups.

Multiplicity according to the occasions was not adjusted. Any subjects with missing values were eliminated from the analysis. Statistical analyses were performed using Statcel 3 (Yanai, 2011). The results were considered significant at a <5% level in the two-sided test.

3. RESULTS

3.1. Participant demographics

The 27 subjects were randomly assigned to intervention groups and made a start with ingestion. 6 were withdrawn due to being unwell or unexpected business, and the remaining 21 subjects completed the study. Thus, data obtained with 21 subjects (Reishi; 13 < M; 7, F; 6 > , Placebo; 8 < M; 3, F; 5 >) was used for the analysis of efficacy (**Fig. 2**). There were no significant differences in the mean age, gender ratio or SEIV between groups (**Table 3**).

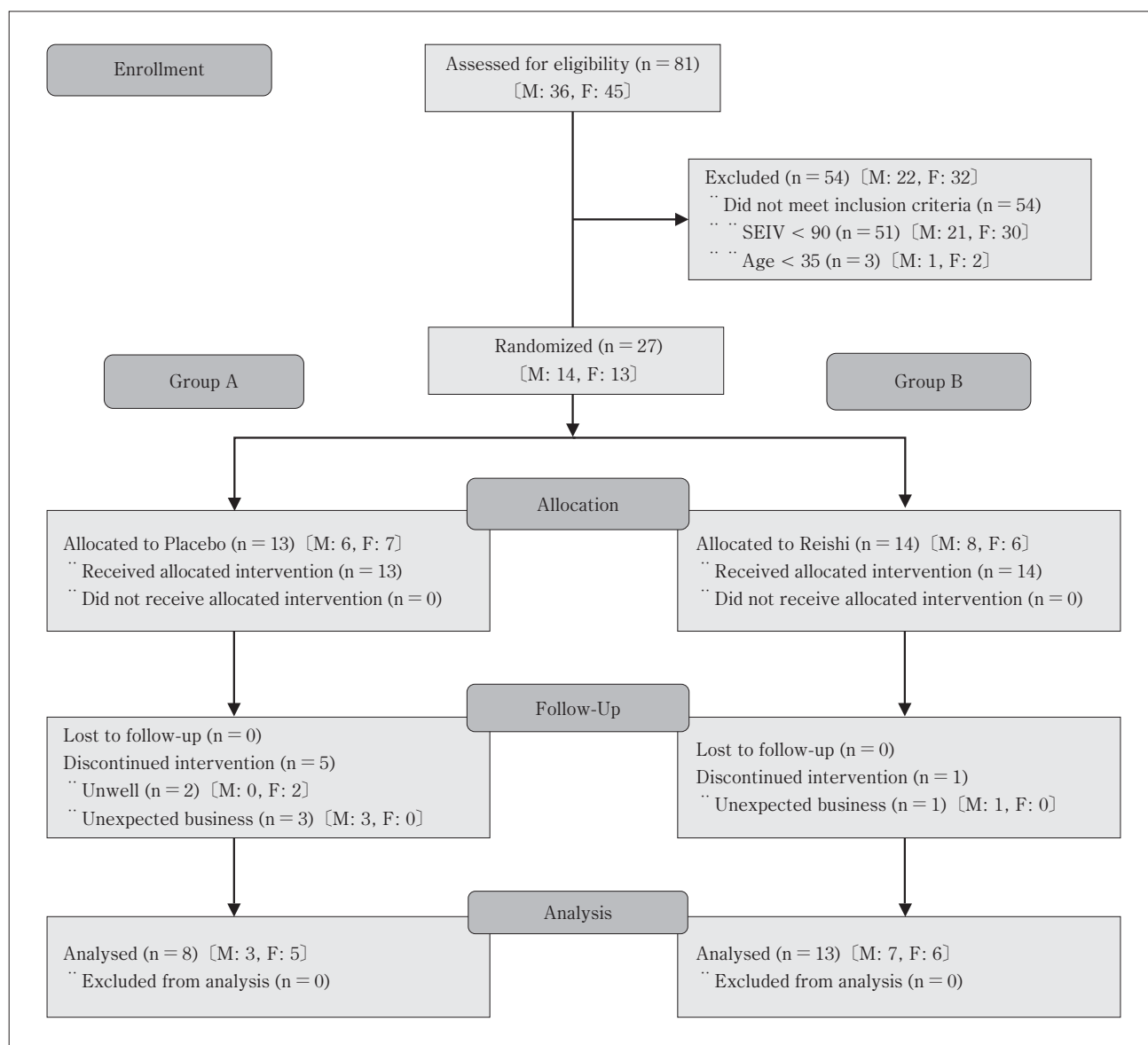


Fig. 2 Flow diagram of subject disposition

3.2. SIV

The results of the statistical analysis of SIV, other immunological parameters and T lymphocyte age are shown in **Table 4**. Significant differences of T cells, CD8⁺CD28⁺ T cells, and T lymphocyte age were observed between two groups after 12 weeks of ingestion.

3.3. Blood pressure

Table 5 shows changes in blood pressure. Δ 0–12 w of SBP between two groups illustrated a significant difference.

3.4. Blood and urine test

Table 6 and **7** shows blood biochemical and urine parameters. With respect to the blood test, a significant difference was observed in Sodium and Potassium levels in Reishi after 12 weeks of ingestion. The same difference was found in Uric acid levels in Reishi (female) after 12 weeks of ingestion. In either case, since the difference

Table 3 Subject demographics

Item	Unit	Reishi	Placebo
Subjects	numbers	13	8
Male: female *	numbers	7:6	3:5
Age *	years	48.5 ± 7.4	49.4 ± 5.2
SEIV *	score	115.5 ± 16.3	106.9 ± 9.6

mean ± SD

* No significant difference

was minor one, the investigator judged it as the range of physiological variation (or clinically safe).

3.5. Safety

No adverse effects associated with the test product were observed in the course of the reporting.

Table 4 SIV, other immunological parameters and T lymphocyte age

Item	Unit	Time points	Scores/ Values		P-value ²⁾
			Reishi (n = 13) ¹⁾	Placebo (n = 8) ¹⁾	
SIV	score	Baseline	16.6 ± 2.0	17.1 ± 1.4	0.963
		12-week	16.2 ± 3.0	16.6 ± 1.2	
		Δ 0-12 w	- 0.5 ± 1.9	- 0.5 ± 1.6	
T cells	numbers/ μl	Baseline	1339.3 ± 336.6	1554.4 ± 522.6	0.047 [#]
		12-week	1364.0 ± 397.8	1291.0 ± 371.6	
		Δ 0-12 w	24.7 ± 141.3	- 263.4 ± 462.4	
Naive T cells	numbers/ μl	Baseline	384.9 ± 146.8	365.8 ± 133.3	0.306
		12-week	363.4 ± 150.3	305.5 ± 114.9 [*]	
		Δ 0-12 w	- 21.5 ± 87.2	- 60.3 ± 72.1	
CD8 ⁺ CD28 ⁺ T cells	numbers/ μl	Baseline	196.1 ± 108.6	274.5 ± 153.4	0.031 [#]
		12-week	218.5 ± 119.4	221.6 ± 77.1	
		Δ 0-12 w	22.4 ± 52.7	- 52.9 ± 96.2	
B cells	numbers/ μl	Baseline	328.5 ± 159.3	279.0 ± 131.2	0.363
		12-week	338.6 ± 169.0	253.3 ± 117.2	
		Δ 0-12 w	10.2 ± 89.0	- 25.8 ± 79.6	
NK cells	numbers/ μl	Baseline	282.2 ± 133.3	238.8 ± 84.7	0.380
		12-week	317.6 ± 179.0	217.6 ± 102.5	
		Δ 0-12 w	35.5 ± 127.4	- 21.1 ± 159.6	
CD4/CD8 T cell ratio	ratio	Baseline	3.76 ± 2.35	2.87 ± 1.69	0.499
		12-week	3.22 ± 1.30	2.73 ± 1.65 [†]	
		Δ 0-12 w	- 0.54 ± 1.64	- 0.13 ± 0.19	
Naive/memory T cell ratio	ratio	Baseline	0.87 ± 0.57	0.77 ± 0.46	0.383
		12-week	0.75 ± 0.40	0.75 ± 0.45	
		Δ 0-12 w	- 0.12 ± 0.25	- 0.03 ± 0.19	
T lymphocyte age	year	Baseline	54.4 ± 11.0	50.1 ± 12.4	0.031 [#]
		12-week	52.8 ± 10.8 [†]	53.5 ± 7.5	
		Δ 0-12 w	- 1.6 ± 3.2	3.4 ± 6.7	

Scores and Values are expressed as the mean ± SD.

1) [†] p < 0.1, * p < 0.05, ** p < 0.01 against baseline.

2) [#] p < 0.05 between-group differences in change from baseline.

4. DISCUSSION

We conducted a randomized, placebo-controlled, double-blind study to verify how the ingestion of the processed food Reishi (*Ganoderma lucidum*) containing β-glucans affects immunological vigor of human. As the primary outcome, the study showed significant differences in several items of SIV and other immunological parameters. The blood pressure also showed a significant improvement in SBP (systolic blood pressure). In addition, as the secondary outcome, it proved that no abnormal change was triggered by the ingestion of the test product.

Main Findings

This study examined how the ingestion of the test product affects the immunity system of humans. In this

study, we used SIV (scoring of immunological vigor) by Hirokawa, et al. as a comprehensive assessment method of immunological vigor. Among seven parameters defined by this assessment method, the study result showed the significant improvement in “T lymphocyte age”, “T cells”, and “CD8⁺CD28⁺ T cell” after the ingestion of the test product for 12 weeks. CD8⁺CD28⁺ T cell is a cell population which differentiate into the killer T cell that targets a virus and a cancer cell. The number of CD28⁺ T cells decrease with age, and as a result the score of CD8⁺CD28⁺ T cell is lowered. Along with this, the T-lymphocyte age calculated from its numerical value also rose¹⁵⁾. Since this study used healthy Japanese males and females aged between 35 and 59 years with daily tiredness and fatigue as the test subject, it is possible for immunity to decline during the 12-week test period

Table 5 Blood pressure

Item	Unit	Time points	Values		P-value ²⁾
			Reishi (n = 13) ¹⁾	Placebo (n = 8) ¹⁾	
SBP <systolic blood pressure>	mmHg	Baseline	119.3 ± 12.1	109.1 ± 7.8	< 0.001 ^{##}
		12-week	113.2 ± 11.2 ^{**}	115.6 ± 8.8 ^{**}	
		Δ 0-12 w	- 6.1 ± 5.3	6.5 ± 4.4	
DBP <diastolic blood pressure>	mmHg	Baseline	74.5 ± 13.4	72.4 ± 13.4	0.826
		12-week	73.3 ± 12.2	70.4 ± 9.8	
		Δ 0-12 w	- 1.2 ± 7.8	- 2.0 ± 9.4	

Values are expressed as the mean ± SD

1) ** p < 0.01 against baseline.

2) ## p < 0.01 between-group differences in change from baseline.

without any action. According to the evidence, the data of the test group showed an improvement tendency by the significant decrease in the T-lymphocyte age, while the placebo group showed a tendency of rising in age. On the other hand, we could not find such tendency in the scores of B cells or NK cells. We also could not observe the changes in SIV which is regarded as the total score of immunity. Based upon these results, it is inferred that the ingestion of the test product was effective for the maintenance of immunity; in particular the anti-inflammatory power related to the T-cell system such as CD28⁺ T cells. However, it is also inferred that the ingestion did not show any influence in B cells or NK cells and, as a result, the 12-week test period could not raise the score of SIV.

We measured the blood pressure which can assess body condition and is reported to have relationship with immunity¹⁶⁾, and observed a significant improvement in systolic blood pressure. There is also a report that the ingestion of *Ganoderma lucidum* by hypertensive rat resulted in the decline of blood pressure¹⁷⁾. Although it is generally said that the systolic blood pressure rises with advancing age¹⁸⁾, the result of this study illustrated that the ingestion of the test product (Reishi) contributes to curbing the rise of systolic blood pressure.

The test product mainly consists of "Reishi" (*Ganoderma lucidum*), a type of fungus which has been regarded as the most valuable Chinese herb since ancient times. *Ganoderma lucidum* consists of amino acids, β -D-glucan and plenty of minerals such as calcium, phosphorus, magnesium, potassium, sodium, iron, copper, zinc, and manganese¹⁹⁾. β -D-glucan is a type of polysaccharides and has many variations. Some variations of β -D-glucan are considered to be effective against cancer and are currently being used medically in the form of medicines such as Krestin or Lentinan^{20,21)}. Although the β -glucan contained in *Ganoderma lucidum* is now under research and the detail remains unclear, it is believed that it has β -1,3-D-glucan as its main chain and β -1, 6-D-glucan as a side chain^{22,23)}, β -1,3/1,6-D-glucan

is an ingredient which is plentifully contained in fungus, and the researchers have been continuously studying its function of improving immunity and the protective / remedial effect against cancer, since its discovery. Focusing on its functional mechanism, it is said that the surface of immune cells called macrophages or lymphocyte has the receptor molecules β -1,3/1,6-D-glucans link together with, and this linkage activates these immune cells²⁴⁾.

The β -glucans used in the medical settings are administered into the body by injection. The orally-ingested β -glucans, on the other hand, are not digested by the digestive organs due to their long side chains, and arrive in the intestines in an unchanged form. There are intraepithelial lymphocytes (IEL) between the epithelial cells in the mucosa of intestines. These lymphocytes form many lymphocyte-mechanisms in the intestinal system, and because of this fact it is said that the gut immunity is the largest immune tissue in the human body. There is a report that β -glucan is ingested by the macrophage existing inside the intestinal canal as an activity of the gut immunity²⁵⁾. This ingestion is thought to stimulate the activity of T cells, NK cells and B cells of macrophage white blood cells in the gut immunity, and stimulate the generation of interleukin²⁶⁾. Based on the above functional mechanism, it is thought that the immunity scores mainly about T cells in the result of SIV parameter have improved. In addition, since β -glucan has a characteristic of not being digested, it has a function of trapping cholesterol and carrying it out of the body. As a result, it lowers the blood-cholesterol levels and contributes to the prevention of arterial sclerosis²⁷⁾. Thanks to this function, it can be expected to lower the systolic blood pressure⁹⁾.

Secondary Findings

In this study we examined the safety of the test product by blood and urine tests. Although both groups showed significant differences in the levels of sodium, potassium or uric acid after ingestion, the differences were minor and the investigator judged them as the range of physiological variation (or clinically safe).

Table 6 biochemical blood test

Item	Unit	Std. Value	Gender	Time points	Values		
					Reishi (n = 13) [M:7, F:6]	Placebo (n = 8) [M:3, F:5]	
Total Bilirubin	mg/dL	0.2-1.2	M/F	Baseline	0.63 ± 0.28	0.53 ± 0.12	
				12-week	0.60 ± 0.24	0.51 ± 0.14	
				Δ 0-12 w	- 0.03 ± 0.13	- 0.01 ± 0.12	
Total Protein	g/dL	6.3-8.3	M/F	Baseline	7.4 ± 0.3	7.6 ± 0.6	
				12-week	7.5 ± 0.3	7.7 ± 0.7	
				Δ 0-12 w	0.1 ± 0.3	0.1 ± 0.2	
Albumen	g/dL	3.8-5.3	M/F	Baseline	4.5 ± 0.3	4.4 ± 0.4	
				12-week	4.5 ± 0.3	4.4 ± 0.5	
				Δ 0-12 w	0.0 ± 0.1	0.0 ± 0.2	
AST (GOT)	U/L	8-38	M/F	Baseline	20.2 ± 6.2	21.5 ± 5.6	
				12-week	17.1 ± 2.4 †	20.5 ± 5.5	
				Δ 0-12 w	- 3.2 ± 5.2	- 1.0 ± 5.7	
ALT (GPT)	U/L	4-43	M/F	Baseline	21.3 ± 12.4	17.6 ± 6.0	
				12-week	16.0 ± 5.1 †	15.6 ± 3.6	
				Δ 0-12 w	- 5.3 ± 9.9	- 2.0 ± 4.5	
ALP	U/L	110-354	M/F	Baseline	238.2 ± 83.1	220.5 ± 110.7	
				12-week	245.5 ± 96.7	213.6 ± 83.2	
				Δ 0-12 w	7.3 ± 35.5	- 6.9 ± 32.7	
LD (LDH)	U/L	121-245	M/F	Baseline	173.6 ± 39.4	170.3 ± 31.3	
				12-week	172.5 ± 32.3	169.5 ± 31.3	
				Δ 0-12 w	- 1.2 ± 14.7	- 0.8 ± 18.1	
γ-GT (γGTP)	U/L	86 and under	M	Baseline	40.0 ± 32.7	42.3 ± 20.6	
				12-week	30.1 ± 14.0	34.3 ± 14.2	
		48 and under	F	Baseline	16.2 ± 4.9	20.8 ± 10.5	
				12-week	17.2 ± 4.3	24.4 ± 12.9 *	
Δ 0-12 w				1.0 ± 2.6	3.6 ± 2.6		
	CK (CPK)	38-196	M	Baseline	170.1 ± 156.4	210.7 ± 114.0	
12-week				121.7 ± 60.1	143.0 ± 103.4		
Δ 0-12 w					- 48.4 ± 120.9	- 67.7 ± 95.6	
		30-172	F	Baseline	83.8 ± 26.4	71.8 ± 22.4	
12-week	93.3 ± 33.3			137.8 ± 162.0			
Δ 0-12 w				9.5 ± 25.1	66.0 ± 141.6		
	Total Cholesterol	mg/dL	130-219	M/F	Baseline	201.7 ± 33.4	222.3 ± 81.9
12-week					207.3 ± 24.2	220.3 ± 105.2	
Δ 0-12 w					5.6 ± 23.3	- 2.0 ± 28.7	
Neutral Fat (TG)	mg/dL	30-149	M/F	Baseline	166.7 ± 95.3	89.3 ± 53.2	
				12-week	158.7 ± 122.4	108.5 ± 74.6	
				Δ 0-12 w	- 8.0 ± 84.0	19.3 ± 33.6	
Sodium	mEq/L	135-150	M/F	Baseline	144.6 ± 2.1	144.3 ± 1.3	
				12-week	142.5 ± 1.8 **	141.3 ± 1.8 **	
				Δ 0-12 w	- 2.2 ± 1.9	- 3.0 ± 1.5	
Chloride	mEq/L	98-110	M/F	Baseline	105.0 ± 1.7	105.3 ± 1.7	
				12-week	104.8 ± 2.2	104.1 ± 2.3 †	
				Δ 0-12 w	- 0.2 ± 2.3	- 1.1 ± 1.6	
Potassium	mEq/L	3.5-5.3	M/F	Baseline	4.5 ± 0.5	4.7 ± 0.5	
				12-week	4.0 ± 0.4 **	4.3 ± 0.5 *	
				Δ 0-12 w	- 0.5 ± 0.3	- 0.3 ± 0.3	
Calcium	mg/dL	8.4-10.2	M/F	Baseline	9.8 ± 0.3	9.7 ± 0.2	
				12-week	9.6 ± 0.4	9.5 ± 0.3 *	
				Δ 0-12 w	- 0.2 ± 0.4	- 0.2 ± 0.2	
Inorganic Phosphorus	mg/dL	2.5-4.5	M/F	Baseline	3.6 ± 0.7	3.5 ± 0.4	
				12-week	3.3 ± 0.6 †	3.7 ± 0.5	
				Δ 0-12 w	- 0.2 ± 0.4	0.3 ± 0.6 #	
Urea Nitrogen	mg/dL	8.0-20.0	M/F	Baseline	15.9 ± 4.6	13.5 ± 4.0	
				12-week	15.0 ± 2.4	13.4 ± 3.5	
				Δ 0-12 w	- 0.9 ± 4.1	- 0.1 ± 2.9	
Creatinine	mg/dL	0.61-1.04	M	Baseline	0.90 ± 0.12	0.87 ± 0.05	
				12-week	0.83 ± 0.17	0.84 ± 0.12	
		Δ 0-12 w				- 0.06 ± 0.11	- 0.03 ± 0.08
			0.47-0.79	F	Baseline	0.62 ± 0.12	0.63 ± 0.04
12-week	0.62 ± 0.05	0.61 ± 0.06					
Δ 0-12 w				0.01 ± 0.11	- 0.02 ± 0.03		
	Blood Sugar (Serum)	mg/dL	60-109	M/F	Baseline	74.0 ± 18.8	75.6 ± 16.5
12-week					69.4 ± 14.9	77.9 ± 25.7	
Δ 0-12 w					- 4.6 ± 11.4	2.3 ± 15.3	

Values are expressed as the mean ± SD.

† p < 0.1, ** p < 0.01 against baseline.

p < 0.05 between-group differences in change from baseline.

Table 7 Transition of Urinalysis

Item	Unit	Std. Value	Gender	Time points	Values	
					Reishi (n = 13) [M:7, F:6]	Placebo (n = 8) [M:3, F:5]
Uric acid	mg/dL	3.6-7.0	M	Baseline	6.4 ± 1.5	5.6 ± 1.1
				12-week	6.4 ± 1.2	5.2 ± 0.1
				Δ 0-12 w	0.1 ± 0.6	- 0.4 ± 1.1
		2.3-7.0	F	Baseline	5.1 ± 1.0	4.6 ± 0.6
				12-week	5.4 ± 0.9 *	4.2 ± 0.4
				Δ 0-12 w	0.3 ± 0.2	- 0.3 ± 0.4 **
Specific Gravity	-	1.010-1.025	M/F	Baseline	1.022 ± 0.007	1.020 ± 0.007
				12-week	1.020 ± 0.007	1.013 ± 0.007 †
				Δ 0-12 w	- 0.002 ± 0.008	- 0.007 ± 0.010
pH	-	4.5-8.0	M/F	Baseline	6.3 ± 0.9	6.1 ± 0.9
				12-week	5.9 ± 0.7	5.9 ± 0.4
				Δ 0-12 w	- 0.4 ± 0.9	- 0.2 ± 0.8

Values are expressed as the mean ± SD.

† < 0.1, * p < 0.05 against baseline.

** p < 0.01 between-group differences in change from baseline.

During the test period six (6) test subjects discontinued the test. The reasons for discontinuance were personal ones such as illness (catching a cold) or business-related matter, and had nothing to do with the ingestion of the test product. Therefore, based upon the blood/urine test and the medical interview, we observed no harmful influence against biochemical and/or physiological matters of the subjects, and this result indicated the safety of the ingestion of the test product for 12 weeks of test period.

General Information

It is generally said that immunity weakens with advancing age. Up until now, the functionality of β -glucan had been studied in order to clarify its effects for cancer patients or hypertension patients. On the other hand, this study indicated that the supplement containing β -glucan is also effective for the immunity of healthy people. Contemporary Japan is often described as a "stressful society", and this describes the irregular working hours and other problems that lead to regular fatigue. The supplement possibly helps these people to live healthier life by normalizing their immunological response. The everyday ingestion of tablet-type foods as a part of self-medication may lower the risk of illness such as cold, and eventually contribute to the increase in healthy population, the improvement of QOL and/or the reduction of medical expenditure.

Limitations

In this study we used the processed food *Ganoderma lucidum* containing β -glucan, as its test product. Although β -glucan has been under investigations and research mainly in the medical field, the research is still

ongoing as there are a wide variety of its original species. Also, as for *Ganoderma lucidum* itself, since it has a large variety such as red, black, blue, white, yellow and purple, therefore its contents and functionality inside the living organism is a matter of speculation.

In addition, while there are reports that the fungus-derived β -glucan increases the activity of NK cells or B cells, this study did not show an improvement of these cells. This result may have been produced because the change of immunity among healthy people tends to be seen gradually over a long period of time and therefore a 12-week test period was too short to obtain any tangible results. Or, it is also possible that the test product used for this study is specific only to T cells and not effective for NK cells or B cells. In any case, there are still uncertainties that remains, and it may be necessary to conduct the study for more than a 12-week period to further scrutinize this matter.

Regarding SBP, the difference of the salt equivalent between Reishi and Placebo may affect the result. That is a matter of future study.

5. CONCLUSION

In conclusion, we found out that the ingestion of the processed food Reishi (*Ganoderma lucidum*) containing β -glucans for 12 weeks contributed to improving the immunological vigor of human and the systolic blood pressure. In addition, no safety-related matter occurred during the 12-week test period.

CONFLICT OF INTEREST

All parts of this study were funded by LINKS Inc. Hiroyuki Sasaki is the principal. All authors state that the study was conducted in the absence of any other relationships that could be interpreted as a conflict of interest.

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Appendix 1. Question of SEIV

Scale of 1 to 5, with lower scores indicating a better result.

- #1 : Enjoyment of meal
- #2 : Meal is often 3 times per day.
- #3 : Nutritional balance of the meal is a considerable point.
- #4 : Eat more meat than fish.
- #5 : Meal is low in salt.
- #6 : Eat moderately in quantity.
- #7 : Eat mostly vegetables.
- #8 : Limit intake of animal fat.
- #9 : No food before sleep
- #10 : No alcohol
- #11 : Amount of drinking less than the standard quantity, i.e. beer < 500 ml, wine < 180 ml, sake < 180 ml, or whiskey < 50 ml
- #12 : Wake up energetically without fatigue from the previous night.
- #13 : Feeling of fatigue is reduced by resting on weekends.
- #14 : Going to bed before 12 pm.
- #15 : Getting sufficient quantity of sleep.
- #16 : No smoking
- #17 : Less frequency of stiff shoulder and lower back pain
- #18 : Catch a cold regularly.
- #19 : Less symptoms of gastro-intestinal problem

- #20 : Less symptoms of stomatitis
- #21 : No record of the following diseases: diabetes mellitus, liver disease, kidney disease, hypertension, hyperlipidemia, cancer, heart disease, autoimmune disease, depression.
- #22 : BMI < 25 BMI = BW{kg}/(BL{m} x BL{m})
- #23 : Normal bowel movement
- #24 : Try to take the stairs.
- #25 : Try to walk instead of vehicles.
- #26 : Fast pace walk
- #27 : Indifference to walking
- #28 : Indifference to standing in the train
- #29 : Using pedometer
- #30 : Indifference to running when needed
- #31 : Have a hobby containing physical exercise.
- #32 : Less distress / worry than usual
- #33 : Ability to forget problems / worries and move forward
- #34 : Enjoy talking with family and friends.
- #35 : Have a friend listen to your negative feelings / thoughts.
- #36 : Satisfied with your daily job.
- #37 : Have optimism for the future.
- #38 : Want to be helpful to people and society.
- #39 : Have a hobby, not related with your job.
- #40 : Daily activities except job decided by yourself.