Clinical Trial/Experimental Study

Medicine

OPEN

Efficacy of *Phellinus linteus* extract on immunity enhancement

A CONSORT-randomized, double-blind, placebo-controlled pilot trial

Yong Ho Ku, KMD, PhD[a,b,c], Jae Hui Kang, KMD, PhD[b,c,*]

Abstract

Background: Immunity is a major system that defends the human body from the outside. Recently, interest in foods related to immunity has been increasing.

Methods: The purpose of this clinical trial was to determine the safety and efficacy of Phellinus linteus (PL) extract in improving immune function. A total of 30 participants were randomly assigned to 3 groups: the PL1000 group (n = 10) took 1000 mg of PL extract and 1000 mg of dextrin per day; the PL2000 group (n = 10) took 2000 mg of PL extract per day; and the placebo group (n = 10) took 2000 mg of dextrin per day. All participants took 2 capsules twice a day for 8 weeks. We measured their natural killer cell activity and cytokine levels in blood before and after consuming the clinical trial food. Variables were also investigated to evaluate safety, such as adverse reactions, vital signs, and abnormal findings. Student t test or the Mann–Whitney U test, a paired t test or the Wilcoxon signed-rank test, a chi-square test, analysis of variance, and Kruskal–Wallis test were conducted according to the characteristics of the data to compare the differences between each group before and after participants ate the clinical trial food.

Results: The natural killer cell activity and interleukin-6 levels of the PL1000 group tended to improve compared to those of the placebo group. Immunoglobulin G1, immunoglobulin G2, and immunoglobulin M levels did not show significant changes, but tended to improve in the PL1000 and PL2000 groups compared to those of the placebo group. Both the Per Protocol and Intention to Treat populations had improved validation parameters. It is safe because no hazards were found in the safety assessment.

Conclusion: PL extract can help improve immunity. Evidences to conduct the main clinical trial is secured through this pilot study. A future large-scale main trial will be conducted based on this pilot study results.

Abbreviations: ALP = alkaline phosphatase, ALT = alanine transaminase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, Hct = hematocrit, IgG1 = immunoglobulin G1, IgG2 = immunoglobulin G2, IgM = immunoglobulin M, IL-1β = interleukin-1β, IL-12 = interleukin-12, IL-2 = interleukin-2, IL-6 = interleukin-6, LDH = lactate dehydrogenase, NK = natural killer, PL = *Phellinus linteus*, RBC = red blood cell, TNF-α = tumor necrosis factor-α, WBC = white blood cells.

Keywords: immune function, NK cell, *Phellinus linteus*, randomized controlled trial

1. Introduction

Immunity is a sophisticated defense mechanism against external antigens. Immunity is affected by a variety of factors that cause an increase or a decrease in its function, such as psychological stress, nutritional status, diet, and disease.[1,2] These factors reduce the activity of immune cells, IgG, IgM, and the complement cascade, which reduce phagocytosis and humoral immunity.[3]

Extensive studies have been conducted on immune stimulators that increase host resistance.[4] Basidiomycetes are used for food

This research was supported by the Ministry of Trade, Industry and Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Encouragement Program for the Industries of Economic Cooperation Region (no. P0006194).

The authors have no conflicts of interest to disclose.

The data used to support the findings of this study are included within the article. Raw data will be provided with the permission of the Principal Investigator upon request.

This clinical trial was approved by the IRB of the Daejeon University Cheonan Korean Medicine Hospital (DJUMC-2019-BM-10-1). The study protocol is registered at CRIS (Registration number: CRIS-KCT0004272).

[a] Department of Korean Medicine, The 20th Fighter Wing, Republic of Korea Air Force, Seosan, Republic of Korea, [b] Department of Acupuncture & Moxibustion Medicine, College of Korean Medicine Daejeon University, Daejeon, Republic of Korea, [c] Department of Acupuncture & Moxibustion Medicine, Cheonan Korean Medicine Hospital of Daejeon University, Cheonan, Republic of Korea.

**Correspondence: Jae Hui Kang, Department of Acupuncture & Moxibustion Medicine, College of Korean Medicine Daejeon University, 62, Daehak-ro, Dong-gu, Daejeon, Republic of Korea (e-mail: rkd12@hanmail.net).*

How to cite this article: Ku YH, Kang JH. Efficacy of Phellinus linteus extract on immunity enhancement: A CONSORT-randomized, double-blind, placebo-controlled pilot trial. Medicine 2022;101:40(e30829).

Received: 9 November 2021 / Received in final form: 29 August 2022 / Accepted: 30 August 2022

http://dx.doi.org/10.1097/MD.0000000000030829

and contain various physiological substances, including beta-glucan polysaccharide and protein-bound polysaccharide, which are the main physiological agents of mushrooms. *Phellinus linteus* (PL), a member of the Hymenochaetaceae family, has drawn attention due to its anticancer and immunostimulant effects.[5,6] PL mycelium has been reported to promote anticancer activity in gastrointestinal cancer[7] by inhibiting harmful enzymes from the microbiome.[8] Meshima, a prescription drug, is prescribed as an immunostimulant in the Republic of Korea.[9]

In Republic of Korea, the lactate dehydrogenase (LDH) cytotoxicity assay has been necessary to obtain approval for healthy functional supplements from the National Institute of Food and Drug Safety Evaluation of Korea. However, NK cell activity has been confirmed in Republic of Korea mainly using flow cytometry or enzyme-linked immunosorbent assay to measure immunity. Therefore, clinical trials using LDH cytotoxicity assay are required, but there are no clinical trials for safety and efficacy of PL using LDH cytotoxicity assay.

As the demand for health supplements continues to increase, the interest in enhancing immunity has also recently increased due to the current pandemic. PL has been widely studied for its anticancer and immune-boosting effects, and is likely to be a functional food ingredient. This clinical trial was implemented as a pilot trial for exploratory purposes in relation to dosage and effectiveness before implementation of the main clinical trial.

2. Materials and Methods

2.1. Study design

A randomized, double-blind, placebo-controlled pilot trial (registration number: CRIS-KCT0004272) was conducted to identify the efficacy and safety of PL extract in improving immunity. This pilot clinical trial was conducted at the Cheonan Korean Medicine Hospital of Daejeon University. The clinical trial protocol was designed in compliance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 checklist. It was approved by the Institutional Review Board (IRB) of the Daejeon University Cheonan Korean Medicine Hospital (DJUMC-2019-BM-10-1) and has been published.[10] Due to errors, the clinical trial protocol, case report form (CRF), and the instructions and informed consent form ver. 1.2 were approved on November 15, 2019. Due to the inclusion of vulnerable individuals, ver. 1.3 of the instructions and informed consent form was approved on November 26, 2019. The final approval of the CRF ver. 1.3 and instructions and informed consent form ver. 1.4 took place on February 14, 2020. Unimportant details have been changed; the changes were reported to the IRB and will be applied after approval.

2.2. Inclusion and exclusion criteria

Inclusion criteria: over 20 and under 65 years of age; peripheral white blood cell (WBC) count between 3×10^3/μL and 8×10^3/μL; past history of upper respiratory infection (URI) and common cold twice before the year prior to the clinical trial; and signing of the consent form to participate voluntarily. Exclusion criteria: currently being treated with acute or chronic cardiovascular, immune, respiratory, hepatobiliary, renal and urinary, nervous, musculoskeletal, psychological, or infectious, and neoplastic disease; uncontrolled hypertension; non-controlled diabetes; vaccination within 3 months prior to screening; Aspartate Aminotransferase (AST), also called Glutamic Oxaloacetic Transaminase (GOT) or Alanine Aminotransferase (ALT), also called Glutamic Pyruvic Transaminase (GPT) over 120 IU/L; creatinine level over 2.4 mg/dL for men and 1.8 mg/dL for women; ingesting immunity-related health foods within 2 weeks prior to screening; severe gastrointestinal symptoms; pregnant, lactating, or planning to be pregnant during this trial; sensitivity or allergy to the test food; planning to participate in other studies during this trial; and taking other trial medications within 4 weeks of the trial.

2.3. Randomization and blinding

Participants were assigned 1:1:1 to the PL1000, PL2000, and placebo groups using the block randomization method. Random allocation tables were generated by statisticians using nQuery Advisor 7.0, SAS 9.0, or SPSS 21.0. This was communicated to the manufacturer and sealed by a statistician in a nontransparent envelope for each participant. Statisticians randomly set the block size to make it impossible to guess the random allocation. The number of random allocations was generated at 120% of the target recruitment. Participants were given a three-digit randomization number in the order of their screening by the investigator, and they received the clinical trial food according to this randomization number. The assigned numbers were not reused even if participants dropped out. This pilot clinical trial was designed to be double-blind; both participants and investigators were blinded to group allocation. The test food and placebo food had the same appearance and the same label. The assignment details of each participant were sealed and managed by the principal investigator, and were not released until the end of the clinical trial. If a serious adverse effect occurred, only that participant's blind envelope could be viewed to verify the details of the random assignment.

2.4. Objective and subjects

We targeted 30 people who met the inclusion and exclusion criteria as well as the Intention to Treat (ITT) criteria. The purpose of this pilot clinical trial was to increase the chances of success in the main clinical trial through preliminary exploration. Therefore, we wanted to identify the basis for calculating group number, setting the appropriate intake amount, identifying the efficacy, and exploring validation variables, adverse reactions, and problems in the clinical trial process. The optimal empirical sample size for pilot studies and parallel designs is ten people per group, considering a medium effect size.[11] For this reason, 30 people who met the selection criteria were recruited and the pilot clinical trial was carried out. Screening was conducted on 32 volunteers interested in participating in this clinical trial. Among them, 30 were registered and randomly assigned to each group. The study procedures were fully explained and participants signed the consent form themselves. There was no significant difference between the Per Protocol (PP) groups in other baseline characteristic.

2.5. Clinical trial food and placebo

The clinical trial food capsules, or the test food and the placebo, were similar in appearance and weight. The test food 1 capsule consisted of 500 mg of PL extract, and the placebo 1 capsule consisted of 500 mg of dextrin. The clinical trial food was produced at Hankookshinyak Pharmaceutical Co. (Nonsan,

Table 1

The number of participants.

Category	Total
Screening	32
Enrolled	30
Completed	26
Dropout	4
Withdrawal of consent	2
Nonconformity of criteria	1
Other (self-isolation for COVID-19)	1
Not enrolled	2
Inclusion criteria violation	2

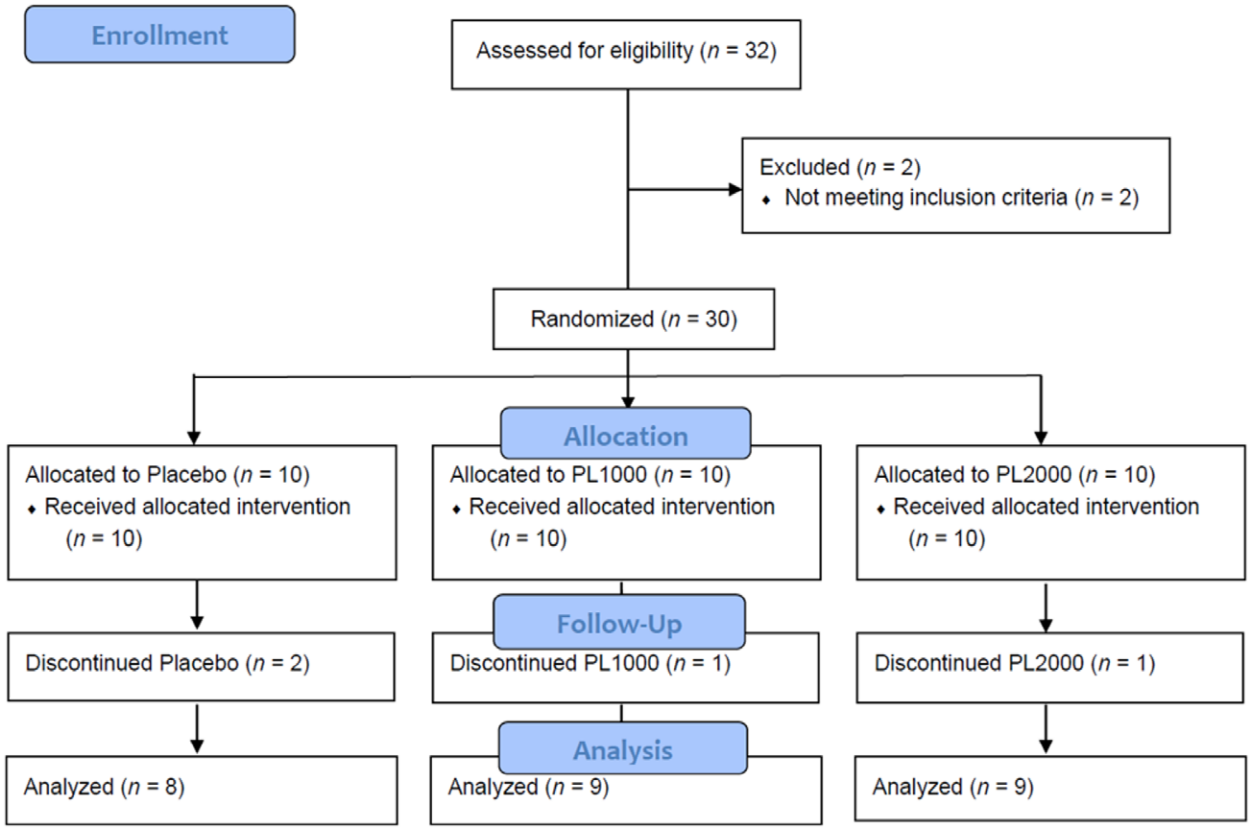

Figure 1. CONSORT 2010 flow chart. CONSORT = CONsolidated Standards of Reporting Trials.

Republic of Korea) according to the random allocation table. One clinical trial food box included 66 doses, including doses for 4 weeks and a spare 5 days, depending on the dosing schedule. The PL1000 group took a test food capsule and a placebo capsule, the PL2000 group took two capsules of the test food, and the placebo group took two capsules of the placebo.

2.6. Protocol and outcome measures

Randomly assigned individuals were distributed the test food, which was taken twice a day. Inspection of validation and safety evaluation variables was conducted at 0, 4, and 8 weeks. At screening, demographic data, such as age, gender, date of birth, alcohol intake, smoking habit, weight, and height, were recorded. A physical examination and an examination of vital signs were conducted at every visit. The laboratory tests were conducted after at each visit after participants fasted for 6 hours. AST, ALT, Alkaline phosphatase (ALP), total cholesterol, FBS, total bilirubin, blood urea nitrogen (BUN), creatinine, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), WBC, RBC, Hgb, hematocrit (Hct), platelet, triglyceride, HDL, LDL, Na, K, and Cl levels were assessed during screening. NK cell activity, TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-12, IgG1, IgG2, and IgM levels were assessed during the first visit; AST, ALT, ALP, total cholesterol, FBS, total bilirubin, BUN, creatinine, CRP, ESR, WBC, RBC, Hgb, Hct, and platelet levels were

Table 2

Participant baseline characteristics (PP population).

	Variable	Placebo (n = 8)	PL1000 (n = 9)	PL2000 (n = 9)	*P* value
Sex	Male	0 (0.0%)	5 (56.6%)	1 (11.1%)	.014*
	Female	8 (100.0%)	4 (44.4%)	8 (88.9%)	
Drinking	No drinking	8 (100.0%)	6 (66.7%)	8 (88.9%)	.368*
	Moderate drinking	0 (0.0%)	1 (11.1%)	0 (0.0%)	
	Heavy drinking	0 (0.0%)	2 (22.2%)	1 (11.1%)	
Smoking	No smoking	7 (87.5%)	5 (55.6%)	8 (88.9%)	.051*
	Past smoking	0 (0.0%)	4 (44.4%)	0 (0.0%)	
	Smoking	1 (12.5%)	0 (0.0%)	1 (11.1%)	
Age		44.38 ± 10.43	51.00 ± 9.63	53.11 ± 8.82	.176‡
Weight (kg)		70.63 ± 12.49	66.23 ± 14.87	65.63 ± 12.28	.709†
Height (cm)		159.49 ± 6.71	162.17 ± 8.77	161.79 ± 9.50	.786†

PP = Per Protocol.

**P* values were derived using a chi-square test.

†*P* values were derived from ANOVA.

‡*P* values were derived from Kruskal–Wallis test.

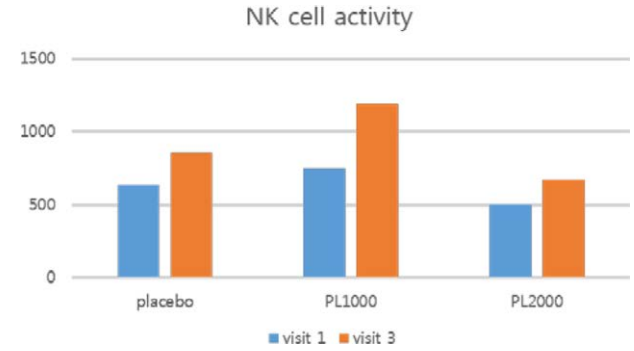

Figure 2. Primary outcome. NK, natural killer.

assessed during the second visit; and AST, ALT, ALP, total cholesterol, FBS, total bilirubin, BUN, creatinine, CRP, ESR, WBC, RBC, Hgb, Hct, platelet, triglyceride, HDL, LDL, Na, K, Cl, NK cell activity, TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-12, IgG1, IgG2, and IgM levels were assessed during the third visit. Symptoms related to URI and safety were measured at every visit. The primary outcome was NK cell activity; secondary outcomes were peripheral WBC, TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-12, IgG1, IgG2, and IgM levels. NK cell activity indicated immunity improvement. Secondary outcomes were indicators of immunity and allergic reaction.

2.7. Data analysis

Statistical analysis was adopted to analyze the PP population data. An analysis of the ITT population data was conducted as a reference. PP analysis was performed on the individuals deemed suitable based on the eligibility criteria and having ingested 80% or more of the clinical trial food. All participants with at least one outcome were included in the ITT analysis. The paired continuous data analysis was conducted through either a paired *t*-test or Wilcoxon signed-rank test. Categorical data analysis was conducted through either a chi-square. Depending on normality, either paired *t* test or Wilcoxon signed-rank test was performed. The normality test of the continuous variable was done using the Shapiro–Wilk test. The significance level was set to 5%, and 95% confidence intervals were calculated where necessary.

Table 3

Outcome comparison between and within each group (PP population).

	Observed value				Change from baseline						
Variable	Placebo (n = 8)	PL1000 (n = 9)	PL2000 (n = 9)	*P* value†	Placebo (n = 8)	*P* value*	PL1000 (n = 9)	*P* value*	PL2000 (n = 9)	*P* value*	*P* value†
NK cell activity											
Visit 1	639.47 ± 271.51	746.9 ± 592.86	502.21 ± 324.53	.654§							
Visit 3	857.5 ± 575.37	1187.8 ± 698.44	674.07 ± 531.79	.401‡	218.03 ± 427.53	.267	440.9 ± 569.72	.219	171.86 ± 666.31	.520	.746‡
WBC											
Screening	5.94 ± 1.08	6.13 ± 1.16	5.73 ± 1.46	.802‡							
Visit 2	5.84 ± 1.12	5.81 ± 1.63	5.00 ± 1.12	.332‡	−0.11 ± 0.55	.605¶	−0.31 ± 1.10	.419¶	−0.74 ± 0.93	.044¶	.351‡
Visit 3	5.58 ± 1.53	5.10 ± 0.95	5.22 ± 1.44	.748‡	−0.37 ± 0.68	.174¶	−1.03 ± 1.10	.023¶	−0.51 ± 1.25	.257¶	.398‡
TNF-α											
Visit 1	9.62 ± 3.69	11.53 ± 3.83	8.85 ± 1.92	.219‡							
Visit 3	9.06 ± 4.36	11.39 ± 3.46	9.15 ± 1.41	.258‡	−0.56 ± 2.72	.580¶	−0.14 ± 1.21	0.731¶	0.31 ± 1.06	.410¶	.610‡
IL-1β											
Visit 1	1.48 ± 0.51	1.69 ± 0.83	1.68 ± 0.91	.969‖							
Visit 3	1.19 ± 0.14	1.64 ± 1.26	1.44 ± 0.45	.599‖	−0.30 ± 0.47	.091#	−0.06 ± 0.98	.600#	−0.24 ± 0.77	.398#	.893‖
IL-2											
Visit 1	2.75 ± 1.47	2.70 ± 2.22	4.83 ± 5.39	.638‖							
Visit 3	2.84 ± 1.91	1.80 ± 1.25	2.15 ± 1.40	.312‖	0.09 ± 2.51	.923¶	−0.90 ± 1.36	.080#	−2.67 ± 5.73	.176#	.814‖
IL-6											
Visit 1	3.23 ± 1.25	8.78 ± 17.81	3.06 ± 1.97	.826‖							
Visit 3	3.21 ± 1.47	10.50 ± 25.35	2.85 ± 1.37	.381‖	−0.02 ± 0.72	.937¶	1.71 ± 7.67	.173#	−0.21 ± 1.01	.552¶	.531‖
IL-12											
Visit 1	7.36 ± 1.94	5.11 ± 3.07	6.69 ± 3.70	.304‡							
Visit 3	6.63 ± 1.45	4.91 ± 2.05	6.67 ± 2.64	.161‡	−0.73 ± 0.98	.075¶	−0.20 ± 2.27	.797¶	−0.02 ± 2.18	.979¶	.744‡
IgG1											
Visit 1	683.61 ± 110.88	731.57 ± 135.98	714.44 ± 127.50	.734‡							
Visit 3	633.53 ± 114.39	688.68 ± 135.46	681.78 ± 154.99	.674‡	−50.09 ± 77.95	.112¶	−42.89 ± 104.35	.253¶	−32.67 ± 70.26	.201¶	.915‡
IgG2											
Visit 1	494.09 ± 136.19	464.44 ± 141.90	476.19 ± 141.29	.909‡							
Visit 3	465.23 ± 149.39	441.17 ± 137.39	461.48 ± 140.88	.931‡	−28.86 ± 45.84	.118¶	−23.28 ± 39.58	.116¶	−14.71 ± 65.87	.522¶	.852‡
IgM											
Visit 1	114.25 ± 20.56	93.41 ± 45.68	97.56 ± 45.73	.536‡							
Visit 3	107.82 ± 23.63	89.46 ± 43.07	94.78 ± 36.96	.569‡	−6.44 ± 8.56	.071¶	−3.95 ± 6.94	.126¶	−2.78 ± 10.96	.468¶	.701‡

IgG1 = immunoglobulin G1, IgG2 = immunoglobulin G2, IgM = immunoglobulin M., IL-12 = interleukin-12, IL-1β = interleukin-1β, IL-2 = interleukin-2, IL-6 = interleukin-6, NK = natural killer, PP = Per Protocol, TNF-α = tumor necrosis factor-α, WBC = white blood cells.

**P* values were compared within each group from baseline.

†*P* values were compared between groups.

‡*P* values were derived from ANOVA.

§*P* values were derived from Welch's ANOVA.

‖*P* values were derived from the Kruskal–Wallis test.

¶*P* values were derived from a paired *t* test.

#*P* values were derived from the Wilcoxon signed-rank test.

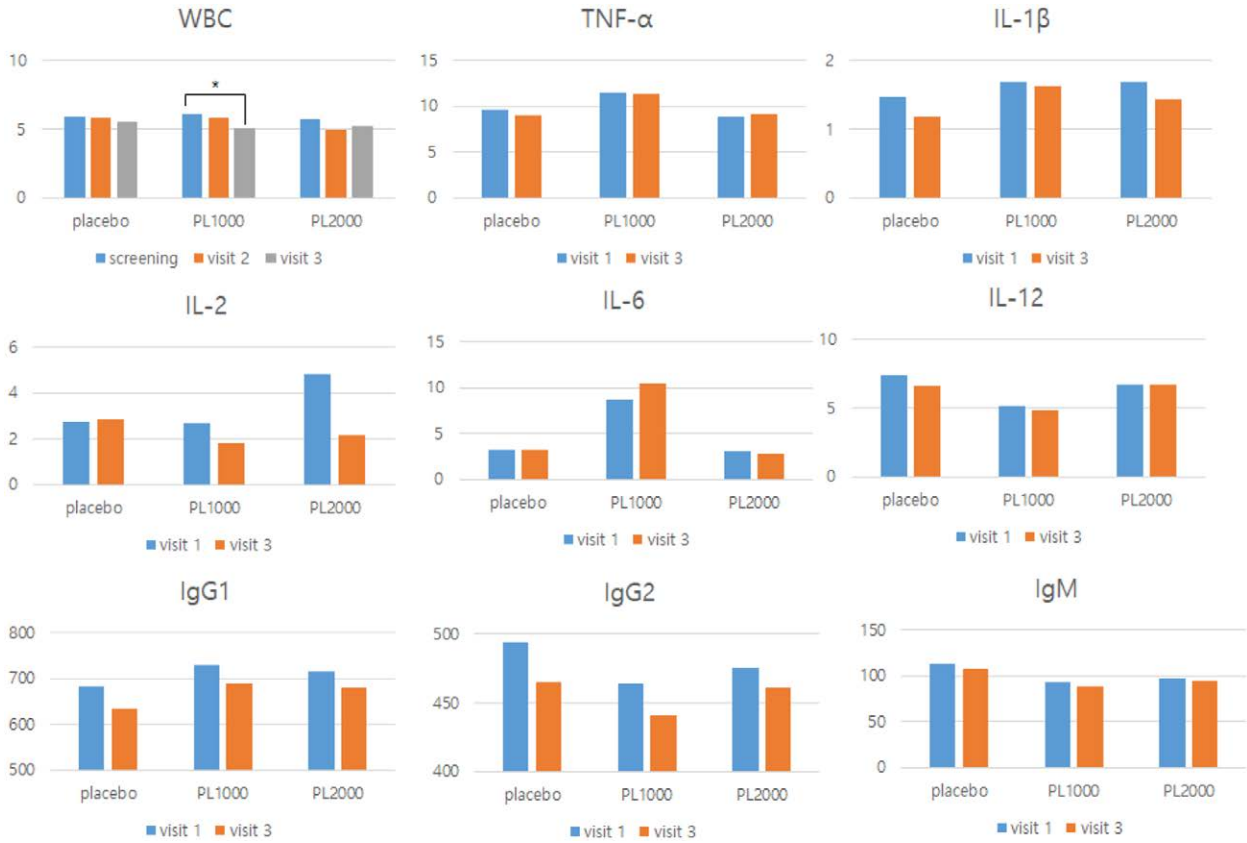

Figure 3. Secondary outcomes. **P* values were derived from a paired *t* test. IgG1 = immunoglobulin G1, IgG2 = immunoglobulin G2, IgM = immunoglobulin M, IL-12 = interleukin-12, IL-1β = interleukin-1β, IL-2 = interleukin-2, IL-6 = interleukin-6, TNF-α = tumor necrosis factor-α, WBC = white blood cells.

2.8. Withdrawal and dropout

Participants who were given the test food for 56 days (±5 days) were deemed to have completed this clinical trial. Participants could drop out or be dropped out for the following reasons: violation of inclusion or exclusion criteria during screening; request to withdraw participation; experience of a serious adverse event; taking prohibited drugs that could affect the validation variables; need to take medications that may affect the clinical trial result; pregnancy during the trial; and the investigator deciding to remove the participant for safety. In all cases of dropout, the cause is detailed in the CRF. Lab tests were conducted as much as possible for the safety assessment of the participants who dropped out. In the case of an adverse event, follow-up observations were made until the cause of the abnormal reaction was identified, and the results were reported. If a serious adverse event occurred and the participant dropped out, the IRB would have been notified immediately.

3. Results

From December 12, 2019, to July 13, 2020, 32 individuals volunteered to participate in this clinical trial, 30 of whom were recruited and assigned 1:1:1 to one of three groups. One individual from the PL1000 group dropped out, as well as one from the PL2000 group and two from the placebo group. As a result, 26 people completed the clinical trial schedule (Table 1; Fig. 1). The PP group comprised 26 participants (PL1000: 9; PL2000: 9; Placebo: 8) and the ITT group comprised 30 participants (PL1000: 10; PL2000: 10; Placebo: 10). Two people dropped out from withdrawal of consent due to personal reasons. One participant was dropped from nonconformity of criteria due to violation of the inclusion criteria (WBC count below $8 \times 10^3/\mu L$). The other participant dropped out because they had difficulty visiting due to self-isolation caused by COVID-19. None of these were directly related to the safety assessments. No statistically significant differences in age, weight, or height were observed between PP populations (Table 2). The two groups were homogeneous, and random allocation was statistically appropriate.

The primary outcome, NK cell activity, was compared at week 0 (visit 1) and after administration (visit 3, week 8). Variance was not significant, but NK cell activity tended to increase in the PL1000 group (440.9 ± 569.72) compared to the placebo group (218.03 ± 427.53; Fig. 2; Table 3).

In terms of secondary outcomes, WBC count in the PL1000 group was reduced by 1.03 ± 1.10 ($P = .023$) after 8 weeks of the clinical trial food intake. Variance between the groups was not significant. The change in IL-6 level was not significant, but tended to increase in the PL1000 group compared to that in the placebo group. Changes in IgG1, IgG2, and IgM levels showed no statistical significance, but they tended to decrease less in the PL1000 and PL2000 groups than in the placebo group (Fig. 3; Table 3).

Four cases of adverse events were reported (14.4%): three cases were common colds and one was constipation. All adverse events were settled; there were no subsequent dropouts and blinding was maintained. Adverse events were not related to the clinical trial food. There was no significant changes between groups in a clinical laboratory examination (Table 4). From the results of a safety evaluation via a clinical laboratory

Table 4

Clinical laboratory examination (ITT population).

	Observed value				Change from baseline						
Variable	Placebo (n = 10)	PL1000 (n = 10)	PL2000 (n = 10)	*P* value†	Placebo (n = 10)	*P* value*	PL1000 (n = 10)	*P* value*	PL2000 (n = 10)	*P* value*	*P* value†
RBC											
Screening	4.20 ± 0.26	4.50 ± 0.21	4.40 ± 0.45	0.130‡							
Visit 2	4.25 ± 0.23	4.48 ± 0.28	4.31 ± 0.36	0.197‡	0.05 ± 0.16	0.385¶	−0.02 ± 0.13	0.644¶	−0.09 ± 0.14	0.060¶	0.109‡
Visit 3	4.19 ± 0.25	4.49 ± 0.34	4.35 ± 0.37	0.148‡	−0.01 ± 0.15	0.834¶	−0.02 ± 0.16	0.762¶	−0.05 ± 0.17	0.405¶	0.853‡
Hb											
Screening	13.28 ± 0.99	13.79 ± 2.32	13.82 ± 1.18	0.138‖							
Visit 2	13.36 ± 0.79	13.77 ± 2.50	13.47 ± 0.90	0.256‖	0.08 ± 0.53	0.623#	−0.02 ± 0.40	0.905#	−0.35 ± 0.42	0.028¶	0.105‡
Visit 3	13.16 ± 0.96	13.80 ± 2.51	13.65 ± 0.98	0.074‖	−0.12 ± 0.49	0.460¶	0.01 ± 0.39	1.000#	−0.17 ± 0.50	0.307¶	0.671‡
Hct											
Screening	38.83 ± 3.05	39.98 ± 5.25	40.20 ± 3.24	0.210‖							
Visit 2	39.03 ± 2.48	40.00 ± 5.81	39.51 ± 2.29	0.245‖	0.20 ± 1.49	0.682¶	0.02 ± 1.30	0.476#	−0.69 ± 1.38	0.149¶	0.439‖
Visit 3	38.69 ± 2.74	40.05 ± 5.96	39.77 ± 2.36	0.183‖	−0.14 ± 1.49	0.774¶	0.07 ± 1.18	0.838#	−0.43 ± 1.68	0.439¶	0.748‡
Platelet											
Screening	262.30 ± 67.11	244.90 ± 61.28	247.40 ± 25.57	0.423‖							
Visit 2	259.50 ± 64.00	243.90 ± 51.62	243.30 ± 30.13	0.628‖	−2.80 ± 16.26	0.599¶	−1.00 ± 24.88	0.721#	−4.10 ± 11.34	0.282¶	0.931‡
Visit 3	265.30 ± 65.46	235.20 ± 65.77	248.30 ± 25.61	0.195‖	3.00 ± 23.48	0.696¶	−9.70 ± 27.72	0.221#	0.90 ± 25.84	0.915¶	0.506‡
Na											
Screening	139.30 ± 1.64	139.90 ± 1.91	140.30 ± 1.83	0.466‡							
Visit 3	138.90 ± 1.91	138.40 ± 2.17	139.90 ± 1.66	0.226‡	−0.40 ± 2.07	0.555¶	−1.50 ± 1.72	0.022¶	−0.40 ± 2.12	0.565¶	0.369‡
K											
Screening	4.36 ± 0.25	4.37 ± 0.31	4.72 ± 0.42	0.073‖							
Visit 3	4.43 ± 0.13	4.28 ± 0.36	4.62 ± 0.31	0.023‖	0.07 ± 0.21	0.323#	−0.09 ± 0.40	0.574#	−0.10 ± 0.52	0.562¶	0.574‡
Cl											
Screening	102.80 ± 1.55	102.70 ± 1.34	103.30 ± 1.49	0.622‡							
Visit 3	104.30 ± 2.31	102.80 ± 1.14	104.30 ± 1.16	0.077‡	1.50 ± 2.32	0.071¶	0.10 ± 0.99	0.758¶	1.00 ± 1.94	0.138¶	0.174§
Triglyceride											
Screening	115.00 ± 48.94	130.10 ± 76.48	169.90 ± 108.41	0.315‡							
Visit 3	110.50 ± 73.95	161.80 ± 100.83	150.30 ± 103.37	0.440‖	−4.50 ± 45.02	0.401#	31.70 ± 86.35	0.276¶	−19.60 ± 71.86	0.411¶	0.528‖
HDL											
Screening	69.50 ± 14.35	64.00 ± 15.41	58.40 ± 11.92	0.284‖							
Visit 3	67.00 ± 15.03	61.80 ± 15.87	60.70 ± 12.80	0.642‖	−2.50 ± 8.62	0.440#	−2.20 ± 6.49	0.312¶	2.30 ± 7.29	0.344¶	0.486‖
LDL											
Screening	122.38 ± 42.18	102.27 ± 24.76	110.14 ± 20.98	0.538‖							
Visit 3	121.74 ± 35.16	106.51 ± 27.54	127.64 ± 31.59	0.319‡	−0.64 ± 22.61	0.889#	4.24 ± 17.73	0.469¶	17.50 ± 21.33	0.029¶	0.182‖
T. cholesterol											
Screening	220.90 ± 60.55	193.50 ± 28.69	198.90 ± 25.18	0.572‖							
Visit 2	217.30 ± 47.47	188.00 ± 20.95	210.20 ± 54.45	0.306‡	−3.60 ± 23.07	0.401#	−5.50 ± 26.82	0.533¶	11.30 ± 36.00	0.347¶	0.576‖
Visit 3	211.70 ± 47.27	193.60 ± 26.48	216.80 ± 45.19	0.420‡	−9.20 ± 28.58	0.327#	0.10 ± 21.53	0.989¶	17.90 ± 27.12	0.067¶	0.077‡
AST											
Screening	23.40 ± 6.28	31.10 ± 12.08	23.30 ± 5.70	0.188‖							
Visit 2	19.60 ± 2.95	32.50 ± 18.64	21.70 ± 5.58	0.091‖	−3.80 ± 6.49	0.058#	1.40 ± 10.07	1.000#	−1.60 ± 2.22	0.049¶	0.511‖
Visit 3	20.40 ± 3.78	40.00 ± 25.37	22.50 ± 6.50	0.078§	−3.00 ± 6.86	0.205#	8.90 ± 20.11	0.195¶	−0.80 ± 1.93	0.223¶	0.143‖
ALT											
Screening	21.60 ± 10.13	31.40 ± 21.47	20.70 ± 10.97	0.525‖							
Visit 2	16.10 ± 5.20	30.70 ± 19.93	21.40 ± 14.35	0.245‖	−5.50 ± 8.97	0.085¶	−0.70 ± 12.67	0.865¶	0.70 ± 4.95	0.673#	0.078‖
Visit 3	16.60 ± 4.99	37.50 ± 23.36	21.30 ± 14.83	0.088‖	−5.00 ± 9.81	0.141¶	6.10 ± 18.29	0.319¶	0.60 ± 5.64	0.858#	0.059‖
ALP											
Screening	71.70 ± 28.81	63.80 ± 17.30	77.00 ± 29.22	0.366‖							
Visit 2	68.70 ± 25.37	65.10 ± 18.72	70.10 ± 23.14	0.754‖	−3.00 ± 4.03	0.020#	1.30 ± 7.21	0.583¶	−6.90 ± 7.96	0.023¶	0.076‖
Visit 3	67.80 ± 25.35	67.00 ± 18.34	70.50 ± 25.09	0.765‖	−3.90 ± 6.28	0.075#	3.20 ± 7.77a	0.225¶	−6.50 ± 8.20b	0.033¶	0.020‡
T. bilirubin											
Screening	0.54 ± 0.15	0.77 ± 0.28	0.56 ± 0.11	0.025‡							
Visit 2	0.57 ± 0.12	0.73 ± 0.17	0.61 ± 0.21	0.118‡	0.03 ± 0.18	0.605¶	−0.04 ± 0.21	0.594¶	0.05 ± 0.18	0.404¶	0.447‖
Visit 3	0.56 ± 0.22	0.73 ± 0.25	0.63 ± 0.18	0.239‡	0.02 ± 0.26	0.815¶	−0.04 ± 0.15	0.466¶	0.07 ± 0.20	0.313¶	0.265‖
CRP											
Screening	1.25 ± 1.42	0.82 ± 1.12	1.71 ± 1.78	0.166‖							
Visit 2	1.16 ± 1.15	0.73 ± 0.61	1.33 ± 1.76	0.586‖	−0.09 ± 0.44	0.441#	−0.09 ± 0.93	0.683#	−0.38 ± 0.45	0.037#	0.155‖
Visit 3	1.24 ± 1.09	0.55 ± 0.51	1.78 ± 2.70	0.128‖	−0.01 ± 0.89	0.917#	−0.27 ± 0.89	0.241#	0.07 ± 1.16	0.575#	0.702‖
Glucose											
Screening	98.50 ± 7.99	95.90 ± 10.31	102.70 ± 7.72	0.233‡							
Visit 2	99.20 ± 7.45	100.80 ± 6.39	101.50 ± 7.28	0.758‡	0.70 ± 4.60	0.642¶	4.90 ± 7.62	0.073¶	−1.20 ± 7.16	0.609¶	0.100‖
Visit 3	96.50 ± 7.15	96.80 ± 6.18	103.60 ± 6.52	0.049‖	−2.00 ± 5.68	0.294¶	0.90 ± 9.83	0.779#	0.90 ± 4.70	0.560¶	0.579‡
BUN											
Screening	13.61 ± 5.12	14.40 ± 5.00	13.62 ± 2.37	0.668‖							

(Continued)

Table4

(Continued)

Variable	Observed value				Change from baseline						
	Placebo (n = 10)	PL1000 (n = 10)	PL2000 (n = 10)	*P* value†	Placebo (n = 10)	*P* value*	PL1000 (n = 10)	*P* value*	PL2000 (n = 10)	*P* value*	*P* value†
Visit 2	14.32 ± 4.49	12.99 ± 2.20	16.08 ± 3.82	0.136‖	0.71 ± 2.55	0.484#	−1.41 ± 3.49	0.233¶	2.46 ± 3.88	0.076¶	0.051‡
Visit 3	13.79 ± 4.72	12.60 ± 2.14	16.54 ± 3.92	0.052‖	0.18 ± 1.97	0.944#	−1.80 ± 3.79	0.168¶	2.92 ± 4.37	0.064¶	0.060‖
Creatinine											
Screening	0.69 ± 0.12	0.82 ± 0.12	0.71 ± 0.11	0.055‖							
Visit 2	0.64 ± 0.14	0.78 ± 0.19	0.64 ± 0.08	0.156‖	−0.05 ± 0.08	0.106#	−0.04 ± 0.11	0.355¶	−0.07 ± 0.05	0.001¶	0.487‖
Visit 3	0.64 ± 0.15	0.72 ± 0.15	0.67 ± 0.09	0.282‖	−0.06 ± 0.07	0.042#	−0.09 ± 0.11	0.027¶	−0.04 ± 0.06	0.041¶	0.470§
ESR											
Screening	18.30 ± 13.98	7.80 ± 7.04	14.20 ± 11.4	0.195‖							
Visit 2	17.00 ± 13.17	7.60 ± 6.33	12.90 ± 7.89	0.165‖	−1.30 ± 4.85	0.419¶	−0.20 ± 3.39	0.765#	−1.30 ± 6.07	0.516¶	0.846‡
Visit 3	18.30 ± 14.23	7.10 ± 8.90	15.80 ± 8.98	0.036‖	0.00 ± 4.57	1.000¶	−0.70 ± 3.77	0.591#	1.60 ± 6.82	0.477¶	0.606‡

ALP = Alkaline phosphatase, ALT = alanine transaminase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, Hb = hemoglobin, Hct = hematocrit, ITT = Intention-to-treat, PL = *Phellinus linteus*, RBC = red blood cell.

**P* values were compared within each group from baseline.

†*P* values were compared between groups.

‡*P* values were derived from ANOVA (post hoc: Scheffe test).

§*P* values were derived from Welch-ANOVA.

‖*P* values were derived from Kruskal–Wallis test. (post hoc: Bonferroni test).

¶*P* values were derived from paired *t* test.

#*P* values were derived from Wilcoxon's signed rank test.

examination and physical examination, there was no reason to judge PL extract as clinically harmful.

4. Discussion

Mushrooms have been used as food for thousands of years, and include nutrients and healthy ingredients. PL is a mushroom native to China, Cambodia, and Japan.[12] PL has been reported to have efficacy in promoting anticancer and antimetastatic activity and B cell stimulation.[13,14] Kim et al[15] reported that PL extract could affect NK cell activity similarly to IL-2 in mouse spleen cells. The spread of infectious diseases has recently led to an increased interest in health, which increases the demand for food supplements. In particular, food supplements associated with immunity remain in high demand. We wanted to determine the value of PL extract, which has previously been used as an immunostimulant for anticancer antimetastatic activity, as a food ingredient.

Blood cells differentiate and mature in stem cells. WBCs, which affect immunity, circulate in blood systems. Macrophages detect and treat foreign substances that have entered the body. WBCs participate in various immune reactions in the body to protect it from the outside. Polysaccharides isolated from PL have been reported to activate various immune responses, such as by promoting mixed lymphocyte reactions and the differentiation of T cells. PL is also involved in humoral immunity through activating B cells along with nonspecific immune responses by NK cells.[15] Several cytokines are involved in immune responses: IL-1 is a cytokine that acts on a variety of cells, such as B cells, T cells, and monocytes, and is involved in the production of TNF-α; IL-6 is an extensively multifunctional lymphokine that is produced in a variety of cells, including T cells, B cells, mononucleocytes, and fibroblasts, and is involved in humoral immunity, such as B cell differentiation, C-reactive protein production, T cell differentiation, and promotion of monocyte maturity; and TNF-α is produced in mononuclear cells, macrophages, mast cells, lymphocytes, and NK cells. NK cells destroy certain cancer cells or virus-infected cells. They play an important role in tumor prevention, host defense, humoral immunity, and hematopoietic action.[16] TNF-α amplifies lymphocytes and activates NK cells, increasing lymphocyte MHC expression and antigen presentation ability. IL-2 and IFN play an important role in immunity by increasing TNF-α production.[17] Immunoglobulin is a serum protein produced by antigens, and is a type of glycoprotein present in lymphatic fluid. IgG is present in extracellular fluids and makes up 80% of all immunoglobulins. IgG is produced later in the immune response and acts as a major antibody, activating the complement system against bacteria and viruses. IgM is mainly present in blood vessels and makes up 5-10% of all globulins. IgM is the first immunoglobulin released into the blood by plasma cells. IgM binds strongly to antigens while circulating in the body.[18,19]

In this clinical trial, 32 people volunteered to participate. After being screened, 30 were registered and randomly assigned to one of three groups. One person in the PL1000 group, one in the PL2000 group, and two in the placebo group dropped out, leaving a total of 26 participants in the clinical trial. Consuming 80% of the test food was considered compliant. The PP population for statistical analysis comprised participants who ingested at least 80% of the test food, and the ITT population comprised all participants with at least one validation variable. The compliance of the PP population (n = 26) was 100%. The compliance of the ITT population (n = 30) was 86.7%. Participants' baseline characteristics showed no differences between all groups (Table 2).

The primary outcome was NK cell activity and the secondary outcomes were WBC, TNF-α, IL-1β, IL-2, IL-6, IL-12, IgG1, IgG2, and IgM levels. Validation was evaluated through statistically significant differences by analyzing and comparing the changes in primary and secondary outcomes. LDH cytotoxicity assay reacts NK cells with K562 cells (chronic myelogenous leukemia cells) and measures LDH concentration released after cell dissolution. NK cell activity can be evaluated by measuring the concentration of the released LDH.[20] NK cell activity in the PL1000 group did not show significant changes, but tended to increase compared with that in the placebo group in the PP populations. In terms of secondary outcomes, WBC count in the PL1000 group significantly decreased in the PP ($P = .023$) populations. However, group comparisons did not show significant results. IL-6 levels showed no significant changes before and after administration in the PP populations, but tended to increase in the PL1000 group. IgG1, IgG2, and IgM levels showed no significant changes in the PP populations, but decreased less in the PL1000 and PL2000 groups. TNF-α, IL-1β, IL-2, and IL-12 levels showed no significant changes. It was confirmed that the

PL1000 and PL2000 groups had improved immunity compared to the placebo group through NK cell activity, IL-6, IgG1, IgG2, and IgM levels. This trend was confirmed in the PP populations, which confirms that PL extract is effective in improving immunity. However, this result is from a pilot clinical trial, so the statistical significance of the results cannot be confirmed due to the small number of participants. If the clinical trial is carried out and the number of participants is higher, the significance of the results may be confirmed.

Safety was evaluated for the ITT population using adverse events and the results of laboratory tests. In this clinical trial, four adverse events were reported in four individuals (13.3%). Three were common colds and one was constipation. These were all mild adverse events that were not related to the clinical trial. Safety assessment variables did not differ significantly between groups; there were no abnormalities in the results of individual laboratory tests. In addition, there were no adverse events or abnormalities in the participants' vital signs and physical examinations. It is clinically safe according to the results of the safety assessment, which was conducted by the principal investigator. It can thus be deemed safe for adults to consume PL extract at a dose of 1000 and 2000 mg/d.

5. Conclusion

This clinical trial evaluated the efficacy and safety of PL extract on immunity enhancement. NK cell activity in the PL1000 group tended to be increased compared with that in placebo group. Significant changes could not be observed in the secondary outcomes. No adverse effects have been reported in the clinical trial. There were also no side effects related to PL in clinical laboratory tests, vital signs, and physical examination. Detailed data were obtained for a future large-scale main trial. This pilot trial will be a useful reference for the main clinical trial.

Author contributions

All authors participated in the study design and reviewed the manuscript. YHK acquired the clinical trial data, performed the analysis, and wrote the article. JHK supervised, reviewed, and edited the article.
Conceptualization: Jae Hui Kang.
Data curation: Yong Ho Ku.
Investigation: Yong Ho Ku.
Project administration: Jae Hui Kang.
Software: Jae Hui Kang.
Supervision: Jae Hui Kang.
Visualization: Yong Ho Ku.
Writing – original draft: Yong Ho Ku.
Writing – review & editing: Jae Hui Kang.

References

[1] Sierra S, Lara-Villoslada F, Olivares M, et al. Increased immune response in mice consuming rice bran oil. Eur J Nutr. 2005;44:509–16.
[2] Gonzalez-Rey E, Chorny A, Delgado M. Regulation of immune tolerance by anti-inflammatory neuropeptides. Nat Rev Immunol. 2007;7:52–63.
[3] Kim JS. Mechanism of aging and prevention. Immune Netw. 2001;1:104–8.
[4] Franz G. Polysaccharides in pharmacy: current applications and future concepts. Planta Med. 1989;55:493–7.
[5] Kim YS, Lee BE, Cho KB, et al. Antitumor and immunomodulatory activities of mushroom (Phellinus linteus) cultured on oak and mulberry. Kor J Immunol. 2000;22:165–71.
[6] Lee BE, Ryu SY, Kim EH, et al. Immunostimulating effect of mycelium extract of Phellinus linteus. Kor J Pharmacogn. 2012;43:157–62.
[7] Chung KS, Kim SS, Kim HS, et al. Antitumor activity of Kp, a protein-polysaccharide from the mycelial culture of Phellinus linteus. Yakhak Hoeji. 1994;38:158–65.
[8] Kim DH, Choi HJ, Bae EA, et al. Effect of artificially cultured Phellinus linteus on harmful intestinal bacterial enzymes and rat intestinal α-glucosidases. J Fd Hyg Safety. 1998;13:20–3.
[9] Han MW, Ko KS, Chung KS. inventors; Hankookshinyak Pharmaceutical Co.; assignee. Liquid cultivation of Phellinus linteus mycelium and preparation of antitumor and immunostimulating substance. Korea Patent 1001248530000. September 30, 1997.
[10] Jung GH, Kang JH. Efficacy of Phellinus linteus (sanghuang) extract for improving immune functions: study protocol for a randomized, double-blinded, placebo-controlled pilot trial. Medicine (Baltimore) 2020;99:e18829.
[11] Whitehead AL, Julious SA, Cooper CL, et al. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. Stat Methods Med Res. 2015;25:1057–73.
[12] Park WH, Lee HD. Korean Medicine Mushroom Book. Seoul, Korea: Kyohak Co; 1999:558.
[13] Han SB, Lee CW, Jeon YJ, et al. The inhibitory effect of poltsaccharides isolated from Phelinus linteus on tumor growth and metastasis. Immunopharmacology. 1999;41:157–64.
[14] Lee JH, Cho SM, Song KS, et al. Characterization of carbohydrate-peptide linkage of acidic heteroglycopeptide with immuno-stimulating activity from mycelium of Phellinus linteus. Chem Pharm Bull. 1996;44:1093–5.
[15] Kim HM, Han SB, Oh GT, et al. Stimulation of humoral and cell mediated immunity by polysaccharide from mushroom Phellinus linteus. Int J Immunopharmacol. 1996;18:295–303.
[16] Orange JS, Ballas ZK. Natural killer cells in human health and disease. Clin Immunol. 2006;118:1–10.
[17] Maeng EH, Lee YT, Cho KB, et al. Immunomodulating and antitumor activities of exo-secretion from Phellinus linteus. Immune Network. 2002;2:115–24.
[18] Widmaier EP, Raff H, Strang KT. Vander's Human Physiology. 10th ed. (Kang SS, Ahn TI, et al. Translator) Seoul, Korea: Kyo Bo Book Centre Co; 2009:730–3. (Original work published in 2006).
[19] Thomas JK, Richard AG, Barbara AO. Kuby Immunology. 6th ed. (The Korean Society for Microbiology Translator) Seoul, Korea: E*PUBLIC Co; 2008:100–5. (Original work published in 2006).
[20] Bae DS, Lee JK. Development of NK cell expansion methods using feeder cells from human myelogenous leukemia cell line. Blood Res. 2014;49:154–61.