

An 8-week randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of red *Platycodon grandiflorus* root extract on enhancement of immune function

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ABSTRACT

Background: The immune-enhancing effects of red *Platycodon grandiflorus* root extract (RPGE) has been reported *in vitro* and *in vivo*, but there are few studies on humans. Therefore, this study aimed to investigate the efficacy and safety of RPGE in enhancing immune function in healthy subjects.

Subjects and methods: An 8-week randomized, double-blind, parallel, placebo-controlled clinical trial was conducted at the Gachon University Gil Medical Center, Incheon, South Korea. A total of 100 adults aged 20–75 years with white blood cell counts of 3000–10,000 cell/ μ L were randomly divided into two groups (RPGE group, 50 and placebo group, 50) using a computer-generated random list with a 1:1 allocation ratio. The subjects consumed RPGE (2 times/day, 2 tablets/time, 375 mg RPGE powder/tablet) or placebo for 8 weeks. All test foods for the human study were coded and administered under double-blind conditions. The primary outcome was a change in the NK cell activity after 8 weeks of treatment compared to the baseline.

Results: Among 100 subjects enrolled for the study, 87 completed the study. NK cell activity ($p = 0.005$) and IFN- γ level ($p = 0.003$) of the RPGE group ($n = 41$) were higher than those of the placebo group ($n = 46$). The findings of the safety assessment revealed absence of clinically significant changes in any test and serious adverse events throughout the study.

Conclusion: In conclusion, these results demonstrate the efficacy and safety of RPGE, suggesting it to be a beneficial agent for enhancing immune function in humans.

Trial registration: CRIS Registration Number KCT0005945, <https://cris.nih.go.kr>.

Introduction

The immune system is a defense system that protects the human body from various harmful substances, including infectious agents, allergens, and cancer cells, plays a crucial role in suppressing the occurrence of diseases (Parkin and Cohen, 2001). Therefore, it is important to strengthen your immune system that can help you stay healthy.

Cells that play a defensive role in the human body include complement, phagocytes, and natural killer cells (NK cells) (Gombart et al.,

2020). In particular, NK cells were originally defined as effector lymphocytes, and are regarded as an important part of the innate immune system that regulates microbial infection and tumor progression (Morvan and Lanier, 2016; Vivier et al., 2011). It has been frequently used as a biomarker for immune function in various studies (Lee et al., 2020; Lyu et al., 2020). NK cells are activated during their interaction with target cells or by cytokines in the microenvironment, and secrete cytokines and chemokines (Morvan and Lanier, 2016). Besides interferon gamma (IFN- γ), which has been identified as a cytokine produced by NK

Abbreviations: IFN- γ , interferon gamma; ILs, interleukins; ITT, intention-to-treat; NF-Kb, nuclear factor-kappa B; NK cells, natural killer cells; OD, optical density; PP, per-protocol; RPGE, red *Platycodon grandiflorus* root extract; TNF, tumor necrosis factor.

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cells, interleukins (ILs), tumor necrosis factor (TNF), growth factors such as granulocyte-macrophage colony-stimulating factor, and chemokines, including C-C motif chemokine ligands, are also secreted by NK cells (Morvan and Lanier, 2016; Vivier et al., 2011).

Platycodon grandiflorus has been widely used as an oriental phyto-medicine to treat various human diseases, such as asthma and inflammation (Nyakudya et al., 2014; Park et al., 2019). It has also been reported to reduce obesity and improve liver function (Ke et al., 2020; Kim et al., 2019; Leng et al., 2018). Accumulating evidence suggests the potential role of *Platycodon grandiflorus* root in immunomodulation *in vitro* and *in vivo* (Choi et al., 2019; Lee et al., 2020; Park et al., 2020; Park and Lee, 2020). We have reported that red *Platycodon grandiflorus* root extract (RPGE), which is made by steaming and drying *Platycodon grandiflorus* root several times, contains elevated level of components, such as saponins, including platycoside E and platycodin D (Lee et al., 2020; Park et al., 2020). RPGE has been reported to have potent immune-enhancing effects by modulating the nuclear factor kappa B (NF- κ B) signaling pathway in murine macrophage RAW 264.7 cells (Park et al., 2020). In addition, RPGE showed immune-stimulating effects in cyclophosphamide-induced immunosuppression model mice (Lee et al., 2020). Collectively, various *in vitro* and *in vivo* studies have shown the immune-enhancing effects of RPGE; however, its effects in humans remain unclear. Therefore, this study aimed to investigate the efficacy and safety of RPGE in enhancing immune function in healthy subjects.

Materials and methods

Ethics

The study protocol was approved by the Gachon University Gil Medical Center Institutional Review Board (IRB No. GDIRB2020-072) and complied with the ethical principles of the Declaration of Helsinki. It was registered at the Clinical Research Information Service (CRIS), a primary registry of the WHO International Clinical Trial Registry Platform (ICTRP) (registration number: KCT0005945). Written informed consent was signed from all study participants after giving detailed description of the research procedures and informing of that data of the study would be published in scientific articles without their identities.

Participants

The participants were recruited from the Gachon University Gil Medical Center (Incheon, Republic of Korea) by advertisements on hospital bulletin boards and banners. Participants who were aged between 20 and 75 years and agreed to provide written informed consent were screened for this study. Participants who met any of the following criteria were excluded: (1) WBC < 3000 cell/ μ l or > 10,000 cell/ μ l; (2) BMI < 18.5 kg/m² or > 35 kg/m²; (3) unable to perform an ongoing examination in this study; (4) presence of any of the symptoms/disease as below: (a) gastrointestinal diseases and surgery such as Crohn's disease affecting the absorption of test products, (b) patients with uncontrolled diabetes mellitus (fasting blood sugar (FBS) $\geq$ 180 mg/dL or started taking new diabetes medication within 3 months), (c) patients with uncontrolled hypertension (blood pressure $\geq$ 160/100 mm Hg or started taking new antihypertensive drug within 3 months), (d) severe acute cardiovascular diseases such as heart failure, myocardial infarction, or stroke, (e) severe liver or kidney damage, or immune system disease, (f) disease of thyroid or pituitary gland, (g) malignant tumor, lung disease, leukemia, collagenosis, multiple sclerosis, allergic skin disease, or autoimmune disease; (5) abnormal laboratory results (creatinine > 2.0 mg/dL, AST or ALT $\geq$ 2 times the upper limit of normal); (6) history of antipsychotic treatments within 2 months; (7) two week history of taking drug, herbal medicine, or health functional food that affects immune system; (8) Possibility of taking prohibited

medicine during the trial; (9) history of hypersensitivity to any ingredient of the test products; (10) participated in other clinical trial within 2 weeks; (11) consistent intake of raw materials or processed food containing test products within 2 weeks; (12) pregnant or nursing women; (13) women of childbearing age who refused to use contraception; or (14) any other reasons considered unsuitable for the trial by the principal investigator.

Study design

An 8-week, randomized, placebo-controlled, parallel, double-blind clinical trial was conducted from April 2020 to August 2020. During screening, participants were evaluated for suitability based on inclusion and exclusion criteria within 2 weeks before the first visit. The eligible participants were randomly assigned to either the RPGE group or the placebo group in a 1:1 ratio using a computer-generated block randomization list (block size of 2 or 4) at the first visit (0 week). The randomization code was prepared by investigator who had not participated in the trial, and was sealed in opaque envelope for allocation concealment. The test supplements were coded based on the randomization list and provided according to the visit sequence of enrollment. Participants were instructed to consume four tablets/day (two tablets each after breakfast and dinner) of RPGE or placebo for 8 weeks. All participants and investigators were blinded to the treatment allocation until the database was locked.

The participants were scheduled to visit 4 times (screening, 0 week, 4 weeks, and 8 weeks) during the trial. Baseline assessment data, including sex, age, and medical diagnosis history of the participants, were evaluated during screening. Participants were assessed for lifestyle factors, including smoking, alcohol consumption, degree of stress, and dietary intake before and after the intervention. To assess dietary intake, a 24 h recall questionnaire was administered by a trained investigator at baseline. In addition, all participants were instructed to record a detailed food diary for three days (2 weekdays and 1 weekend day) in the previous week of the next visit, which was reviewed by the investigator during the intervention. Moreover, the participants were asked to maintain habitual dietary intake and were prohibited from any medications or functional foods that could affect the immune system. The participants were provided with new RPGE or placebo tablets at each visit and returned unconsumed tablet at the next visit. Treatment compliance was assessed by a trained investigator based on counting the number of tablets returned.

Test supplement

The test supplement, RPGE, and placebo were provided by Hyundai Bioland (Ansan, Gyeonggi, Korea). RPGE powder was prepared using a previously described method (Lee et al., 2020) and RPGE powder contained approximately 0.6 mg/g of platycodin D and platycoside E, separately. Each RPGE tablet contained 375 mg of RPGE powder while the placebo tablets comprised only the excipients and were identical in size, shape, color to the RPGE tablets. Both the RPGE and placebo tablets were manufactured under good manufacturing practice (GMP)-certified facility, and packed in 140 numbers per bottle.

Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated for NK cell activity test immediately after receiving heparinized whole blood. Ficoll-Hypaque (GE Healthcare, Little Chalfont, UK) is dispensed in a 15 ml tube in an amount equal to twice that of whole blood. Whole blood was diluted in a 1:1 ratio with phosphate buffered saline (PBS) and dispense it in layers on top of Ficoll-Hypaque, then centrifuged at 2000 rpm for 15 min at 20 °C. After separate the PBMCs gathered in a thin white layer, the cells were washed with PBS twice. Washed cells were suspended using RPMI1640 media (GIBCO, Gaithersburg, MD, USA).

Cell culture

K562 cells were purchased from the Korean Cell Line Bank (Seoul, Korea) and maintained in RPMI1640 supplemented with 10% fetal bovine serum (FBS) in 5% CO₂ at 37 °C. All cell culture reagents were obtained from GIBCO (Gaithersburg, MD, USA).

Primary outcome measure

The primary efficacy endpoint was the change in NK cell activity from baseline to after 8 weeks of intervention. The cytotoxic activity of NK cells was measured against the NK-sensitive K562 cell line. The effector cells (PBMcs) were co-incubated with the target cells (K562) in different effector/target ratios of 2:1 and 10:1 for 24 h. Cytotoxic activity was assessed by MTT assay using a cell proliferation kit (sigma-aldrich, Saint Louis, MO, USA) following the manufacturer's protocol. NK cell activity (%) was calculated as follows:

$$\left[1 - \left(\text{OD}_{\text{Effector+Target}} - \text{OD}_{\text{Effector}} \right) / \text{OD}_{\text{Target}} \right] \times 100.$$

Secondary outcome measure

The secondary efficacy endpoints were changes in the levels of IL-6, IL-12, IFN- γ , and TNF- α before and after the intervention. Plasma IL-6 and IL-12 levels were measured using human IL-6 and human IL12p40/p70 ELISA kit (Diacclone, Besancon, France). Plasma IFN- γ and TNF- α were assessed using human TNF alpha and IFN gamma human ELISA Kit (Abcam, Cambridge, UK). To measure efficacy, venous blood (5 ml) was collected from the subjects after 12 h fasting into heparin tubes. The tube was centrifuged at 2000 rpm at 20 °C for 15 min to isolate the plasma and stored at -70 °C until analysis.

Safety assessment

Safety outcomes were assessed based on reports of adverse events, physical examination, vital signs, and laboratory tests. All participants were asked to report any adverse events during the intervention. Before and after 8 weeks, weight, height, and BMI (kg/m²) of the participants were measured. The vital signs of systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, and body temperature were evaluated at each visit using the same device. Laboratory tests, including complete blood count (WBC, RBC, hemoglobin, hematocrit, platelets, Seg. Neu, lymphocytes, monocytes, eosinophils, and basophils), blood chemistry (aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -GTP, alkaline phosphatase (ALP), bilirubin total, total protein, albumin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), creatinine, uric acid, glucose, C-reactive protein (CRP)), and urinalysis (pH, protein, glucose, WBC, RBC, and specific gravity) were measured at baseline and 8 weeks. Additionally, women of childbearing age were tested for urine HCG to check for pregnancy. Analysis of laboratory parameters was performed on 12 h-fasted venous blood samples from the clinical pathology department of the hospital.

Statistical analysis

Based on a previous study that used NK cell activity as the primary outcome, the sample size was calculated using the estimated effect size of 1.41, and the pooled standard deviation. Therefore, a total of 100 participants (50 participants per group) were selected with a statistical power of 80% and at the significance level of 5% (two-sided test), allowing a dropout ratio of 25%.

All statistical analyses were performed using the SAS®; version 5.1 (SAS Institute, Cary, NC, USA) and R version 4.0.3. Data are presented as the mean $\pm$ standard deviation (SD), or frequency (n). Baseline characteristics were assessed with an intention-to-treat (ITT) analysis,

including all randomized subjects. The efficacy outcomes were based on a per-protocol (PP) set analysis of the participants who complied with the study protocol. Safety analyses were performed on a safety set, which was defined as the set of participants who took at least one dose of the test products. The differences in the groups were analyzed using an independent *t*-test, and the changes before and after the intervention within groups were analyzed using paired *t*-tests. Categorical variables were compared between the groups using the chi-square test or Fisher's exact test. Statistical significance was set at *p* < 0.05.

Results

Study participants and baseline characteristics

A total of 101 screened subjects provided written informed consent; 100 subjects enrolled for the study were randomly divided into two groups (RPGE group and placebo group, *n* = 50 for each group). Nine subjects in the RPGE group and four subjects in the placebo group dropped out from the test, and 87 subjects completed the test (Fig. 1). Nine dropouts in the RPGE group withdrew consent because of personal reasons (*n* = 4), adverse events (*n* = 3), or violation of the study protocol (*n* = 2). Four dropouts in the placebo group withdrew consent because of personal reasons (*n* = 2), steroid injections therapy (*n* = 1), or intake of prohibited dietary supplements (*n* = 1). All subjects who consumed the test food were included in the ITT set, and 87 subjects were included in the PP set. The subjects who consumed the test food more than once were included in the safety set. The demographic and baseline characteristics, including age, sex, height, weight, BMI, alcohol use, smoking status, regular exercise, and self-reported stress scales, did not differ between the RPGE and placebo groups (Table 1). No significant differences were observed between the two groups with respect to dietary intake based on the 24 h recall questionnaire and food diary during the test.

Primary outcome

NK cell activity was compared between the RPGE and placebo groups and significant difference was observed between the two groups at 8 weeks (Table 2). RPGE group showed a significant increase in NK cell activity at a concentration of Effect/Target cell (E/T) ratio = 2:1 compared to the placebo group (at 8 weeks, *p* = 0.003). NK cell activity increased 30.34 $\pm$ 29.79% after 8 weeks from baseline in the RPGE group as compared to 14.58 $\pm$ 21.01% after 8 weeks in the placebo group (*p* = 0.005). At a concentration of E/T ratio = 10:1, the RPGE group showed a significantly increased NK cell activity compared to the placebo group at 8 weeks (*p* = 0.04). There was no significant difference, but there was a tendency between groups in the changes at the 8-week point compared to the baseline between the RPGE group and the placebo group (*p* = 0.09).

Secondary outcome

As shown in Table 3, among the secondary outcomes, IFN- γ was significantly increased in the RPGE group compared to the placebo group (changes, *p* = 0.003). Moreover, in intra-group comparison, IL-12 and IFN- γ increased significantly at 8 weeks in the RPGE group compared to the baseline (*p* < 0.001). However, there was no significant difference between the RPGE and placebo groups regarding IL-6 and TNF- α .

Safety assessment

Safety assessment included adverse effects, pregnancy test, complete blood count, blood chemistry, and urinalysis. The results showed absence of clinically significant changes in any test (Tables 4 and 5). Among 100 participants in safety set, adverse events were reported; 4

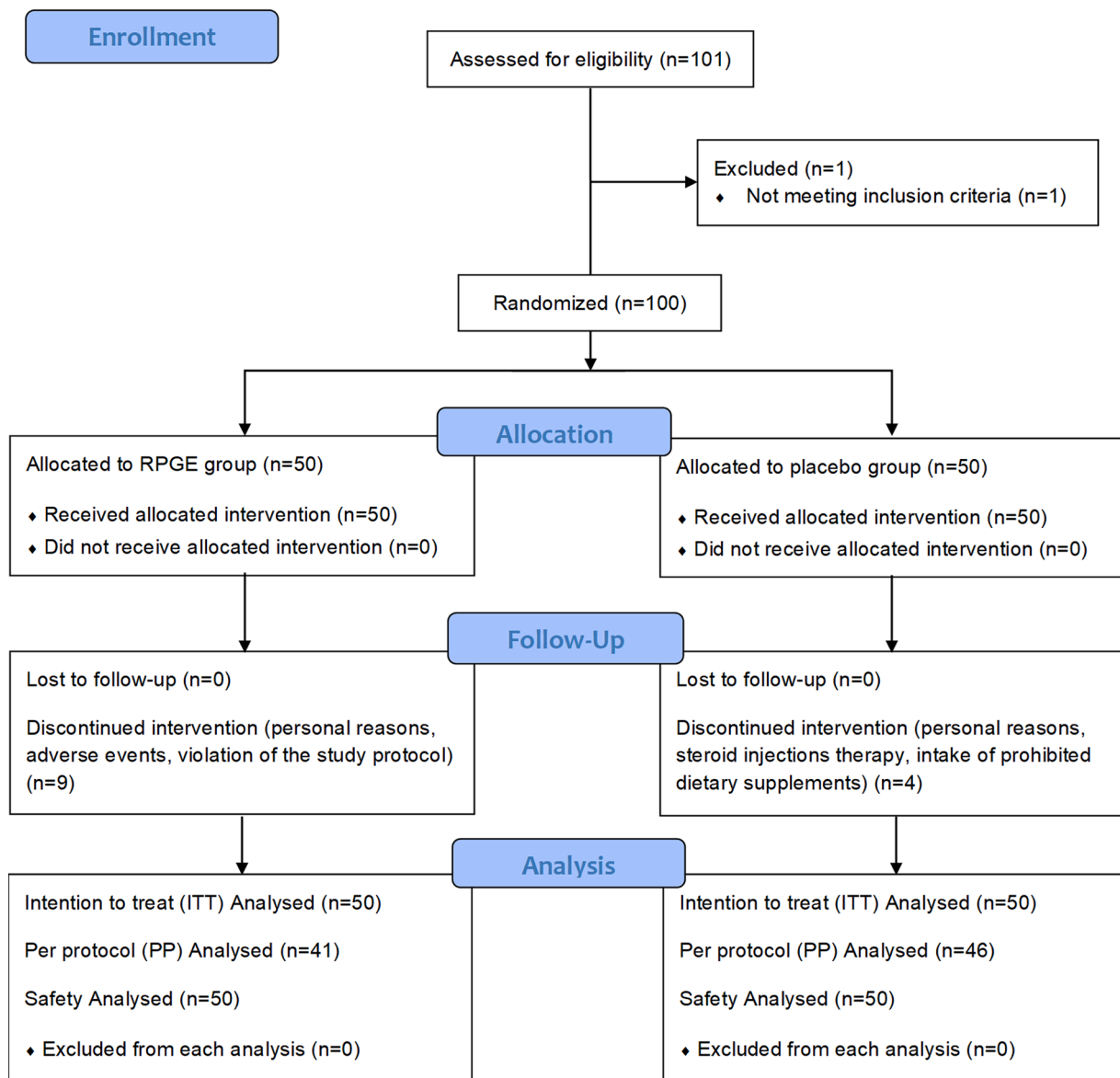


Fig. 1. CONSORT flow diagram.
RPGE, red *Platycodon grandiflorus* root extract.

subjects (8%, 5 cases) were in the RPGE group and 6 subjects (12%, 6 cases) in the placebo group. Adverse effects of skin and subcutaneous tissue disorders showed the highest incidence rate of five to six cases. However, no serious adverse reactions were observed, and there was no significant difference between the RPGE group and the placebo group with respect to the occurrence of adverse effects.

Discussion

The human body is exposed to various pathogenic risk factors, such as viruses and environmental pollutants (Childs et al., 2019; Manisalidis et al., 2020), and several studies are focused on immune-enhancing effects to prevent such diseases. In addition, interest in products that improve immune function through food is increasing. In particular, pertaining to the recent pandemic situation caused by COVID-19, plant-based prevention or therapeutic approaches are being proposed to combat these infectious diseases (Khanna et al., 2021).

Several studies have demonstrated the immunomodulatory effects of *Platycodon grandiflorus* root extract prepared after treatment with various procedures such as fermentation and aging, however, most of

the studies have been conducted *in vitro* or *in vivo* (Choi et al., 2019; Lee et al., 2020; Noh et al., 2019; Park et al., 2020; Park and Lee, 2020). In the previous study, we have demonstrated that RPGE, made by steaming and drying process repeated four times, has immune-enhancing effects in murine macrophage RAW 264.7 cells and cyclophosphamide-induced immunosuppression model mice. RPGE treatment increased phagocytic activity in addition to increase in nitric oxide and cytokine production by modulating the NF- κ B signaling pathways in RAW 264.7 cells (Park et al., 2020). Moreover, splenocytes proliferation rate, NK cell activity, and cytokine (IL-1 β , IL-6, IFN- γ) expression levels were improved by RPGE supplementation compared to that of the control group in cyclophosphamide-induced immunosuppression model mice (Lee et al., 2020). Despite the presence of sufficient evidence for improvement of immune function by RPGE, no studies have been conducted in humans. To the best of our knowledge, this is the first study to demonstrate the immune-enhancing effects of RPGE in humans.

NK cells are lymphocytes that constitute the major component of an innate immune system and play a crucial role in antimicrobial and antitumor activity (Vivier et al., 2011), and therefore, NK cell activity has been used as an important clinical outcome. NK cells are activated

Table 1
Baseline characteristics of the study participants.

Characteristics	RPGE group	Placebo group	p-value
Age (years)	40.40 ± 9.93	42.30 ± 10.48	0.344 ^a
Sex (n)			
Male	9	13	0.334 ^b
Female	41	37	
Height (cm)	161.73 ± 8.20	164.27 ± 9.17	0.149 ^a
Weight (kg)	60.78 ± 9.68	62.22 ± 13.40	0.538 ^a
BMI (kg/m ²)	23.17 ± 2.75	22.84 ± 3.00	0.563 ^a
Alcohol use			
Drinker	33	30	0.534 ^b
Non-drinker	17	20	
Smoking status			
Smoker	4	6	0.869 ^b
Ex-smoker	1	1	
Nonsmoker	45	43	
Regular exercise			
Never	20	23	0.827 ^b
< 3 days/week or < 30 min/day	14	13	
≥ 3 days/week or > 30 min/day	16	14	
Self-reported stress scales			
Never	13	10	0.384 ^b
Sometimes	26	32	
Fairly often	9	5	
Very often	2	1	
Always	0	2	

Values are presented as mean ± standard deviation (SD) or frequency (n)

^a : p-value by Independent t-test.

^b : p-value by chi-square test or Fisher's exact test.

by interferons or macrophage-derived cytokines such as IL-12, and activated NK cells secrete cytokines, including IFN- γ , to defend against infection and various diseases, such as cancer (De Maria et al., 2011; Morvan and Lanier, 2016; Vivier et al., 2011). IFN- γ has been identified as a marker for NK cell activity, and a diagnostic kit to indirectly measure NK cell activity by measuring IFN- γ level has also been developed and widely used. The findings of the present study showed increase in IFN- γ level ($p = 0.003$) along with increase in NK cell activity ($p = 0.005$) of the RPGE group compared to the placebo group (Tables 2 and 3), and are suggestive of immune-enhancing effects of RPGE.

In this study, although significant differences were observed between the RPGE and placebo groups with respect to NK cell activity, however,

the placebo group also showed increase in NK cell activity at 8 weeks compared to the baseline. This could be explained by factors, such as placebo effect, improved stress management, and increased amount of exercise (data not shown). On the contrary, the RPGE group showed increase in IFN- γ level, whereas no change was observed in the placebo group (Table 3); the results indicate that the increased NK cell activity of the RPGE group is related to IFN- γ .

The ratio of female subjects (77%) is a limitation of our study, as is the lack of ethnic diversity (100% of the subjects were Asian). A larger number of subjects is required to have power for each subgroup analysis, such as gender.

Platycodin D and platycoside E are triterpenoid saponins that mainly constituent RPGE (Lee et al., 2020; Park et al., 2020). Platycodin D is the most well-known *Platycodon grandiflorus* saponin and has been reported to increase splenic and peritoneal NK cell activity in tumor-bearing mice (Park et al., 2014), supporting our results of immune enhancing effect of RPGE. Platycoside E is also known as the main ingredient of *Platycodon grandiflorus*, although its activity is less known than that of Platycodin D. However, several studies have shown that it can be converted into active Platycodin D by enzymes such as β -Glucosidase (Shin et al., 2020; Su et al., 2021). Moreover, *Platycodon grandiflorus* contains various immune-related active ingredients in addition to saponins such as acacetin and luteolin (Park et al., 2018), therefore it is thought to enhance immunity overall. Further studies are needed to investigate the supposed active ingredients really reach to target organs to increase NK cells.

Conclusion

In conclusion, RPGE supplementation for 8 weeks in healthy adults aged 20–75 years show immune-enhancing effects by increasing NK cell activity and IFN- γ expression. Safety evaluation results showed no clinically significant changes in any of the tests; moreover, there was no difference between the groups with reference to occurrence of adverse events. Collectively, these results demonstrate the efficacy and safety of RPGE, suggesting it to be a beneficial agent for enhancing immune function in humans.

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Table 2
Change in NK cell activity between baseline and 8 weeks.

	RPGE group	8 weeks	Change	p ^a	Placebo group	8 weeks	Change	p ^a	p ^b
	Baseline				Baseline				
NK cell activity 2:1 (%)	74.28 ± 18.44	104.62 ± 18.67	30.34 ± 29.79	0.000***	76.01 ± 15.12	90.59 ± 22.81	14.58 ± 21.01	0.000***	0.005**
NK cell activity 10:1 (%)	75.34 ± 20.20	109.12 ± 19.01	33.78 ± 29.70	0.000***	76.41 ± 17.99	99.75 ± 22.56	23.35 ± 26.98	0.000***	0.09

Values are presented as mean ± standard deviation (SD) ($p < .05$ *, $p < .01$ **, $p < .001$ ***).

^a : p-value by paired t-test for comparison within groups.

^b : p-value by independent t-test for comparison between groups with respect to the measures of change from baseline.

Table 3
Change in cytokine level between baseline and 8 weeks.

	RPGE group	8 weeks	Change	p ^a	Placebo group	8 weeks	Change	p ^a	p ^b
	Baseline				Baseline				
IL-6 (pg/mL)	5.13 ± 5.72	5.54 ± 6.83	0.42 ± 4.13	0.523	5.05 ± 5.63	5.49 ± 6.36	0.44 ± 5.58	0.595	0.981
IL-12 (pg/mL)	109.42 ± 62.50	167.58 ± 112.99	58.16 ± 93.10	0.000***	104.74 ± 93.06	139.59 ± 106.96	34.85 ± 75.66	0.003**	0.201
IFN- γ (pg/mL)	2.06 ± 1.57	3.64 ± 3.27	1.58 ± 2.80	0.001**	2.07 ± 1.17	2.18 ± 1.24	0.11 ± 1.23	0.547	0.003**
TNF- α (pg/mL)	5.52 ± 7.25	6.17 ± 7.85	0.65 ± 6.07	0.495	5.62 ± 7.56	6.43 ± 10.21	0.81 ± 8.57	0.522	0.92

Values are presented as mean ± standard deviation (SD) ($p < .05$ *, $p < .01$ **, $p < .001$ ***).

^a : p-value by paired t-test for comparison within groups.

^b : p-value by independent t-test for comparison between groups with respect to the measures of change from baseline.

Table 4

Comparison of laboratory tests between baseline and 8 weeks.

	RPGE group			Placebo group			p^b
	Baseline	8 weeks	p^a	Baseline	8 weeks	p^a	
AST (U/L)	21.18 ± 8.40	20.32 ± 7.62	0.457	21.30 ± 7.89	25.76 ± 35.21	0.400	0.335
ALT (U/L)	19.54 ± 15.16	17.73 ± 13.46	0.34	19.38 ± 12.86	20.72 ± 18.38	0.627	0.395
γ-GTP (U/L)	22.46 ± 18.53	21.54 ± 19.99	0.716	24.13 ± 25.23	26.33 ± 28.25	0.269	0.369
ALP (U/L)	58.38 ± 14.45	58.88 ± 15.56	0.104	55.98 ± 12.72	55.72 ± 11.74	0.645	0.285
Bilirubin total (mg/dL)	0.80 ± 0.38	0.87 ± 0.45	0.388	0.80 ± 0.30	0.84 ± 0.32	0.341	0.760
Total Protein (g/dL)	6.87 ± 0.37	6.90 ± 0.31	0.336	6.87 ± 0.39	6.88 ± 0.36	0.554	0.791
Albumin (g/dL)	4.51 ± 0.26	4.38 ± 0.19	0.006**	4.51 ± 0.26	4.42 ± 0.24	0.029*	0.399
TC (mg/dL)	193.28 ± 34.28	181.05 ± 31.90	0.002**	201.32 ± 32.95	194.61 ± 28.20	0.058	0.038*
TG (mg/dL)	100.16 ± 72.47	102.56 ± 79.06	0.227	134.38 ± 152.83	140.33 ± 283.19	0.957	0.412
HDL-C (mg/dL)	60.80 ± 11.90	58.61 ± 13.12	0.016*	62.62 ± 14.44	60.09 ± 13.64	0.012*	0.609
LDL-C (mg/dL)	120.26 ± 35.49	115.78 ± 32.57	0.432	119.86 ± 34.14	119.98 ± 33.71	0.593	0.557
BUN (mg/dL)	12.69 ± 2.57	12.15 ± 3.01	0.121	13.62 ± 2.84	14.11 ± 3.26	0.187	0.004**
Creatinine (mg/dL)	0.67 ± 0.11	0.70 ± 0.12	0.007**	0.70 ± 0.15	0.73 ± 0.15	0.06	0.323
Uric acid (mg/dL)	4.74 ± 1.34	4.61 ± 1.32	0.923	4.90 ± 1.50	5.06 ± 1.40	0.139	0.129
Glucose (mg/dL)	84.06 ± 7.24	82.56 ± 9.46	0.482	87.38 ± 12.02	83.72 ± 11.19	0.001**	0.606
CRP (mg/dL)	0.06 ± 0.08	0.10 ± 0.23	0.130	0.11 ± 0.22	0.09 ± 0.16	0.605	0.777
WBC ($\times 10^3/\mu\text{L}$)	5.38 ± 1.32	5.48 ± 1.19	0.395	5.46 ± 1.25	5.68 ± 1.35	0.22	0.467
RBC ($\times 10^6/\mu\text{L}$)	4.47 ± 0.38	4.41 ± 0.35	0.335	4.61 ± 0.45	4.60 ± 0.41	0.51	0.024*
Hemoglobin (g/dL)	13.53 ± 1.48	13.31 ± 1.47	0.093	14.10 ± 1.64	13.98 ± 1.54	0.077	0.042*
Hematocrit (%)	40.33 ± 3.49	39.73 ± 3.64	0.145	41.91 ± 4.22	41.80 ± 3.86	0.516	0.012*
Platelet ($\times 10^3/\mu\text{L}$)	268.24 ± 51.15	268.39 ± 51.12	0.732	253.82 ± 57.45	264.17 ± 53.72	0.085	0.709
Urine pH	5.850 ± 0.79	5.756 ± 0.73	0.266	5.770 ± 0.64	5.826 ± 0.71	0.855	0.651
Urine specific gravity	1.021 ± 0.01	1.020 ± 0.01	0.151	1.021 ± 0.01	1.019 ± 0.01	0.114	0.706

Values are presented as mean ± standard deviation (SD) ($p < .05$ *, $p < .01$ **, $p < .001$ ***).^a : p -value by paired t -test for comparison within groups.^b : p -value by independent t -test for differences between groups at 8 weeks.**Table 5**

Comparison of vital sign between baseline and 8 weeks

		RPGE group			Placebo group			p^b
		Baseline	8 weeks	p^a	Baseline	8 weeks	p^a	
Blood pressure (mmHg)	Systolic	118.16 ± 14.31	116.02 ± 14.55	0.362	116.58 ± 13.88	114.96 ± 12.76	0.382	0.716
	Diastolic	74.46 ± 10.44	71.32 ± 11.76	0.081	74.96 ± 10.58	71.67 ± 10.83	0.013*	0.883
Pulse (pulse/min)		78.24 ± 11.58	79.07 ± 10.09	0.264	77.82 ± 9.63	77.78 ± 10.96	0.847	0.571
Body temperature (°C)		36.47 ± 0.27	36.45 ± 0.19	0.683	36.44 ± 0.28	36.35 ± 0.18	0.061	0.015*

Values are presented as mean ± standard deviation (SD) ($p < .05$ *, $p < .01$ **, $p < .001$ ***).^a : p -value by paired t -test for comparison within groups.^b : p -value by independent t -test for differences between groups at 8 weeks.

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CRedit authorship contribution statement

Eun-Jung Park: Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Ah Jin Jung:** Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Sung-Hyen Lee:** Conceptualization, Methodology, Writing – original draft. **Seong-Kyu Kang:** Methodology, Supervision, Writing – review & editing. **Hae-Jeung Lee:** Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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