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Effect of onion (*Allium cepa* L.) peel extract on natural killer cell and cytokines in a randomized, double-blind, placebo-controlled trial

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ABSTRACT

BACKGROUND/OBJECTIVES: Onion, particularly onion peel, is a quercetin-rich food with, anti-inflammatory and immunomodulatory effects. However, the effect of onion peel extract (OPE) in humans is unclear. Thus, the present study aimed to investigate whether OPE improves natural killer (NK) cell activity and cytokine concentration in a randomized double-blind placebo-controlled trial.
SUBJECTS/METHODS: Eighty participants aged 19–64 yrs old with a white blood cell count of 4,000–10,000 cells/μL, symptoms of upper respiratory infection at least once within the previous 12 mon, and perceived stress scale (PSS) over 14 were included. Participants were randomly assigned to take either 1,000 mg/day OPE or a placebo for 8 weeks.
RESULTS: Compliance were 87.4 ± 8.6% and 86.9 ± 79.0% in OPE and placebo groups. Compared to the placebo, OPE supplementation improved "Hoarseness" (P = 0.038) of the Wisconsin Upper Respiratory Symptom Survey (WURSS)-21 symptom, and stress scores (P = 0.001; 0.021) of PSS. Supplementation of OPE had no significant effect on NK cell activity and concentrations of cytokines such as interleukin (IL)-2, IL-6, IL-12, IL-1β, interferon-γ, and tumor necrosis factor-α. At baseline, the WURSS-21 symptom and PSS score (P = 0.024; 0.026) were higher in the OPE group than the placebo group. Among participants with higher than median WURSS-21 symptom score, OPE supplementation increased NK cell activity (P = 0.038). Supplementation of OPE had no significant effects on safety measurements and adverse events.
CONCLUSIONS: The present study suggested that OPE supplementation improves NK cell activity in participants with moderate upper respiratory symptoms without any significant adverse effects.

Trial Registration: ClinicalTrials.gov Identifier: NCT05666752

Keywords: Cytokine; natural killer cells; onion; perceived stress scale; respiratory symptom

INTRODUCTION

Onion, *Allium cepa* L., is one of the oldest vegetables and contains flavonoids, including flavonols, anthocyanins, and dihydroflavonols [1,2]. Major forms of flavonoids in onions are flavonols, particularly quercetin and its derivatives, which account for more than 95% [3]. Onion is a

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Conflict of Interest

The authors declare no potential conflicts of interests.

Author Contributions

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quercetin-rich food, whose content is higher in onion peels than in edible parts [4]. Particularly, 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone, oxidized quercetin, is present in onion peel, which has higher anti-inflammatory effect than quercetin [5].

Quercetin has been postulated to exert an immunomodulatory effect by inhibiting production of inflammatory enzymes and pro-inflammatory cascades [6]. Previous clinical trials have shown that quercetin supplementation significantly decreases the blood concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 in patients with post-myocardial infarction [7], rheumatoid arthritis [8], polycystic ovary syndrome [9], high-cardiovascular disease risk phenotype [10], type 2 diabetes [11], and pre-hypertension [12]. After changing dietary patterns to high-fiber, fruit, vegetable, and low-fat, quercetin intake was inversely associated with serum IL-6 concentration in participants with at least one histologically confirmed colorectal adenoma identified by complete colonoscopy within 6 mon before study entry [13]. Quercetin treatment also increased natural killer (NK) cell activity and the expression of NK cell-activating ligands as well as decreased the expression of immunosuppressive cytokines such as TNF-α and IL-10 in human erythroleukemic, NK, and breast cancer cells [14-16].

Onion extract supplementation with red wine decreased plasma Factor VII, an inflammatory marker in patients with hypocholesterolemia, compared to red wine supplementation only [17]. Onion extract supplementation also increased the counts of white blood cells (WBCs) and CD4 cells in the blood of Wistar rats [18] and beluga juveniles [19] as well as decreased the concentration of interferon (INF)-γ in bronchoalveolar lavage fluids of ovalbumin-sensitized asthma rats [20]. Additionally, onion extract treatment increased the NK cell count in peripheral blood mononuclear cells (PBMCs) of healthy human donors [21] and decreased the expression of TNF-α, IL-6, and IL-1β in lipopolysaccharide (LPS)-treated microglial cells [22].

Our previous study showed that supplementation of onion peel extract (OPE) increased NK cell activity in immunosuppressed mice induced by cyclophosphamide [23]. Consistently, OPE supplementation decreased the expressions of TNF-α, INF-γ, IL-6, and IL-8 in the liver of diabetic rats [24], liver of rat with nonalcoholic fatty liver disease [25], prostatic tissue of rats with atypical prostatic hyperplasia [26], adipose tissue of rats with high fat diet [27], and nasal mucosa of mice with allergic rhinitis [28]. OPE treatment also decreased the concentrations of IL-6, TNF-α, and IL-1β in LPS-treated macrophages [29]. Majority of previous studies suggested that quercetin, onion, and OPE modulate NK cells and cytokines. In contrast, OPE supplementation had no effect on the blood concentration of TNF-α in overweight and obese women [30], and patients with metabolic syndrome [31]. Aside from the effect of OPE on TNF-α, there has been no clinical trial studying how OPE modulates NK cells and cytokines. Therefore, the present study aimed to investigate whether OPE improves NK cell activity and cytokine concentrations in a randomized, double-blind, placebo-controlled trial.

SUBJECTS AND METHODS

Study design

This randomized, double-blind, placebo-controlled, parallel-group trial was conducted from October 2022 to June 2023 in Korea according to the guidelines of the Declaration of Helsinki and registered with ClinicalTrials.gov (NCT05666752). All procedures involving human subjects were approved by the Hanyang University Institutional Review Board

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(HYUIRB-202210-001-1), and written informed consent was obtained from all participants before enrollment in the study.

There were 1 screening and 3 study visits at weeks 0 (baseline), 4, and 8. During the screening visit, information regarding age, vaccination, medical history, and medication was collected, and a pregnancy test and perceived stress scale (PSS) were conducted. Body mass index (BMI), WBC count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine in fasting blood samples were also measured during screening. Normal (≥ 18.5 and < 23 kg/m^2), overweight (≥ 23 and < 25 kg/m^2), and obese (≥ 25 kg/m^2) were categorized according to Asian-Pacific cutoff [32]. Within one week of the screening visit, eligible participants were randomly assigned to the OPE or placebo groups in a 1:1 ratio. OPE or placebo was administered at weeks 0 and 4. At weeks 0 and 8, NK cell activity, cytokine concentrations, complete blood count and blood chemistry tests in fasting blood samples, urinary measurements, blood pressure, pulse rate, body temperature, one-day dietary record, Wisconsin Upper Respiratory Symptom Survey (WURSS)-21, and Global Physical Activity Questionnaire (GPAQ) were measured. At weeks 4 and 8, patient global assessment (PGA), compliance, adverse events, and any changes in medical history were assessed.

Participants

The participants were recruited via poster advertisements from October 2022 to March 2023. After screening 147 participants, 80 were enrolled based on inclusion and exclusion criteria. Inclusion criteria were age between 19–64 yrs old, WBC count between 4,000–10,000 cells/μL, symptoms of upper respiratory infection at least once within the previous 12 mon, and PSS over 14. Exclusion criteria were pregnant, lactating, vaccination within the previous 2 mon, BMI < 18.5 kg/m^2 or ≥ 35 kg/m^2, serum creatinine level ≥ 2.0 mg/dL, and AST and ALT level ≥ 3 times the normal upper limit. Participants having uncontrolled acute or chronic diseases, allergic or hypersensitivity to onion, history of alcoholism or drug abuse, and taking antipsychotic medication, and any medication or supplements regularly which affect immune system within the previous 3 mon were also excluded.

Interventions

Participants were asked to take 4 capsules/day of OPE or a placebo (Jeonbuk Institute for Food-Bioindustry, Jeonju, Korea). One capsule of OPE contained 249.9 mg OPE powder, 49.2 mg cellulose, and 0.9 mg silicon dioxide. Meanwhile, one capsule of placebo contained 294.6 mg cellulose, 0.9 mg silicon dioxide, and 4.5 mg caramel color. OPE dose was obtained from our previous study, showing that OPE supplementation with 200 mg/kg body weight (equivalent to 1,053 mg with 65 kg human) significantly increased WBC count, NK cell activity, and levels of immunoglobulin G in blood of cyclophosphamide-immunosuppressed mice [23]. Participants were asked to not take any supplements or medications that could affect their immunity or change their usual lifestyle and diet during the study.

Onions (Muan, Korea) were washed and peeled, and the onion peels were collected and dried. Dried onion peels were stirred in 90°C purified water for 8 h, and cooled at 15°C with a 25 μL filter. OPE was concentrated on 600–700 mH vacuum at 65°C (EES-120; HS Tech, Seoul, Korea), freeze-dried (PVTFD 300R; Ilshin Wrap, Yangju, Korea), grounded (PM-700; HS Tech), and sieved through a 100-mesh screen. OPE yield was 11.9%, which was calculated as [Weight of OPE Powder/Weight of Dried Onion Peels] × 100. Quercetin content in OPE was calculated by high-performance liquid chromatography (Agilent 1260 Infinity Binary LC; Agilent Technology, Santa Clara, CA, USA) with Zorbax Eclipse Plus C18 UG 120 (4.6 × 250

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mm, 5 μm) column. For gradient elution using mobile phases A and B, mobile phase A was 5% formic acid and mobile phase B was methanol [23]. The UV length, flow rate, and column temperature were 360 nm, 0.8 mL/min, and 40°C, respectively. The quercetin content of OPE was 38 mg/g (**Supplementary Fig. 1**).

Primary outcome measure: NK cell activity

Peripheral PBMCs were isolated by density gradient separation, resuspended in phosphate-buffered saline, and quantified with trypan blue solution. PBMCs and effector cells were seeded with target cells (K562 cells; Korean Cell Line Bank, Seoul, Korea) in 96-well plates, and incubated for 4 h at 37°C. The ratio of effector to target cells was 12.5:1, and each assay was performed in duplicate. NK cell activity was measured using an iMark Tm microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at 490 nm with nonradioactive cytotoxicity assay kit (Promega Inc., Madison, WI, USA). NK cell activity was calculated using the following formula [33]: Cytotoxicity (%) = (Experimental − Effector Spontaneous − Target Spontaneous)/(Target Maximum − Target Spontaneous)] × 100.

Secondary outcome measures: cytokines concentration, WURSS-21 symptom score, PGA, and PSS

Serum concentration of cytokines, including IL-2, IL-6, IL-12, IL-1β, INF-γ, and TNF-α were measured using the spectrophotometer (Multiscan GO; Thermo Fisher Scientific, Waltham, MA, USA) with enzyme-linked immunosorbent assay kit (Abcam, Cambridge, UK) according to the manufacturer's protocol in triplicate. WURSS-21 symptoms (range 0–70) were assessed using 10 items on 8 scales [34], and the PSS (range 0–40) was assessed using 10 items on 5 scales [35]. Modified PGA contained the question "How much did your immunity improved compared to before supplementation of OPE or placebo?", with 5 scales including "strongly better," "better," "the same," "worse," and "strongly worse" [36].

GPAQ, compliance, safety assessment, and adverse events

GPAQ consists of 16 questions to measure physical activity, which is calculated as 4 or 8 metabolic equivalents for moderate or vigorous physical activity per hour, respectively [37]. Compliance was calculated based on the number of remaining capsules. Complete blood count, blood chemistry tests, and urine analysis were conducted using an XN-10 analyzer (Sysmex, Kobe, Japan), an AU 5800 automated analyzer (Beckman Coulter Inc., Brea, CA, USA), and a Cobas 6500 automated urine chemistry analyzer (Roche Diagnostic, Mannheim, Germany), respectively. Blood pressure and pulse rate were measured using an Omron HEM-7051 device (Omron Healthcare, Kyoto, Japan) and body temperature was measured using an infrared thermometer (Thermoscan IRT-4020; Braun Corporation, Kronberg, Germany). Adverse events were defined as signs or symptoms that the participants complained of after initiating OPE supplementation or placebo. The trial can be stopped if there is any significant adverse event related to intervention.

Sample size and random assignment

Sample size of 32 participants in each group was calculated based on findings of Lee *et al.* [38], with a power of 80% and an α level (2-tailed) of 5%. Considering a predicted dropout rate of 20% during the study period, 40 participants were enrolled in each group. An independent external researcher conducted computer-generated randomization using SAS software (version 9.3; SAS, Inc., Cary, NC, USA) and assigned subject identity codes to each group. The researcher assigned a subject identity code to each participant and all other study personnel and participants were blinded to the identity code throughout the course of

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the study. Identity code assignment in each group was concealed in sequentially numbered opaque envelopes, managed by the study investigators, and monitored by clinical research associates (HC & Management, Inc., Jeonju, Korea).

Statistical analysis

Intention-to-treat (ITT) analysis and per-protocol (PP) analysis were performed, and in the ITT analysis, missing data were imputed using mean imputation. Continuous variables were presented as means and SDs, and the differences between the OPE and placebo groups were compared using an independent *t*-test. Categorical variables were presented as numbers (percentages), and the differences between the OPE and placebo groups were compared using the χ^2 test. Changes from baseline to week 8 were compared between the OPE and placebo groups using analysis of covariance after adjusting for WURSS-21 symptom and PSS score. Statistical analyses were conducted using SPSS (version 27.0; SPSS Inc., Chicago, IL, USA), and statistical significance was set at $P < 0.05$.

RESULTS

Participant characteristics

Compliance was not significantly different between the OPE and placebo groups (87.4 ± 8.6% vs. 86.9 ± 9.0%; $P = 0.781$). WURSS-21 symptom ($P = 0.024$) and PSS score ($P = 0.026$) were higher in the OPE group than in the placebo group; however, there were no significant differences in other baseline characteristics (**Table 1**). Two participants in the OPE and 5 in the placebo groups withdrew their study consent (**Fig. 1**). Additionally, one participant with OPE and one participant with placebo were excluded for taking prohibited medications during the study, and one participant with placebo was excluded because the z-score of NK cell activity and cytokine concentrations was > 6 or < −6 (outlier), resulting 37 participants in OPE and 33 participants in placebo were included in PP analysis.

NK cell activity and cytokines concentration between the OPE and placebo groups

OPE had no significant effect on NK cell activity and concentrations of IL-2, IL-6, IL-12, IL-1β, INF-γ, and TNF-α as compared with placebo after adjusting for WURSS-21 symptom score in

Table 1. Baseline characteristics of participants with OPE and placebo

Characteristics	OPE (n = 40)	Placebo (n = 40)	*P*-value[1)]
Age (yrs)	27.08 ± 10.76	24.50 ± 5.25	0.179
Male	22 (55.0)	25 (62.5)	0.496
BMI (kg/m^2)	23.12 ± 2.69	23.10 ± 2.40	0.976
Normal	22 (55.0)	20 (50.0)	0.461
Overweight	7 (17.5)	13 (32.5)	
Obese	11 (27.5)	7 (17.5)	
Smoking	4 (10)	5 (12.5)	0.723
Drinking	25 (62.5)	24 (60.0)	0.818
WBC (10^3/μL)	6.19 ± 1.40	6.22 ± 1.42	0.938
PSS (score)	20.95 ± 4.66	18.70 ± 4.20	0.026
GPAQ (MET-min/week)	3,152.0 ± 2,765.5	4,236.0 ± 5,168.9	0.246
WURSS-21 symptom (score)	21.53 ± 16.96	13.85 ± 12.54	0.024

Values are means ± SDs or number of participants (percentage distribution).
OPE, onion peel extract; BMI, body mass index; WBC, white blood cell; PSS, perceived stress scale; GPAQ, Global Physical Activity Questionnaire; MET, metabolic equivalents; WURSS-21, Wisconsin Upper Respiratory Symptom Survey-21.
[1)]*P*-values were determined by the independent *t*-test or χ^2 test between OPE and placebo groups.

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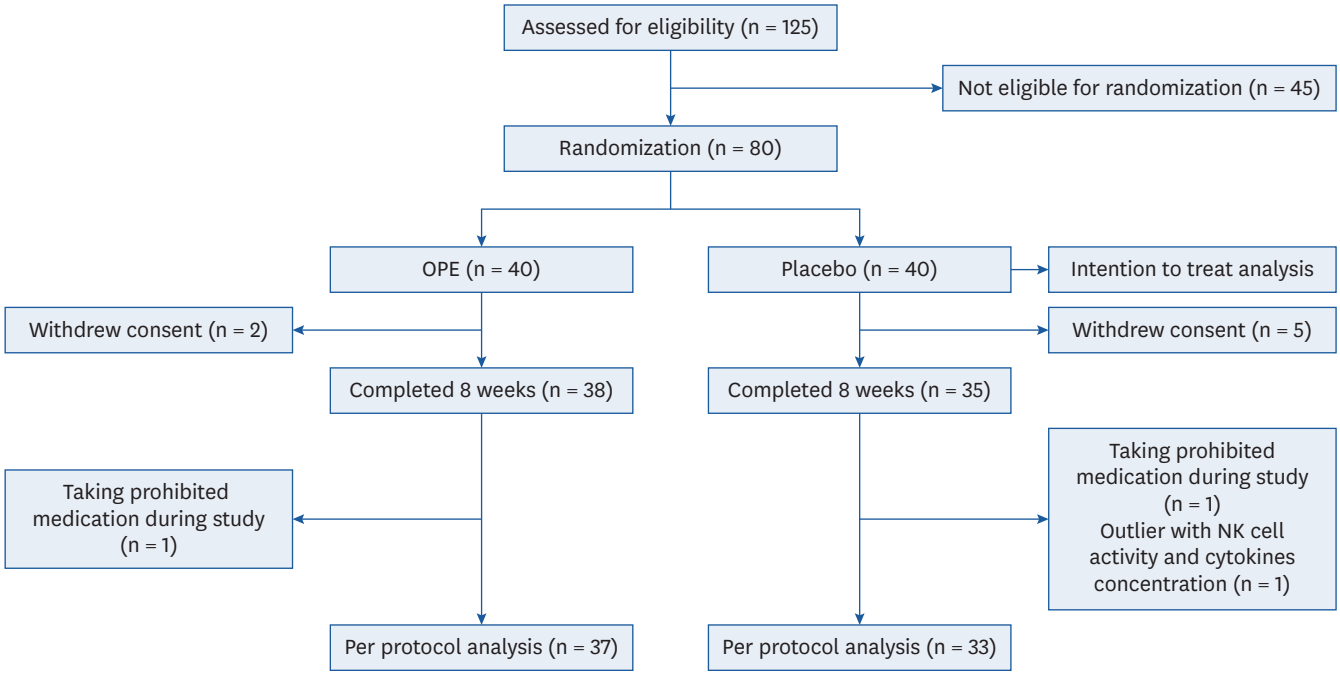

Fig. 1. Schematic diagram of the study design.
NK, natural killer; OPE, onion peel extract.

ITT analysis (**Table 2**). Due to the higher WURSS-21 symptom and PSS score in OPE group, effects of OPE on NK cell activity were analyzed in participants with higher and lower than median WURSS-21 symptom and PSS scores, separately, in PP analysis. Supplementation of OPE increased ($P = 0.038$) NK cell activity compared to the placebo in only participants with higher than median WURSS-21 symptom scores in PP analysis, but had no effect on NK cell activity in participants with higher or lower than median PSS score (**Fig. 2**).

WURSS-21, PSS, GPAQ, and PGA between the OPE and placebo groups

Supplementation of OPE had no significant effect on WURSS-21 symptom score, PSS, and GPAQ as compared to placebo in ITT analysis (**Tables 3** and **4**). There was no significant difference in the PGA at weeks 4 and 8 between the OPE and placebo groups (**Table 5**). However, OPE supplementation improved the symptom of "Hoarseness" ($P = 0.038$) compared to placebo (**Table 3**). Additionally, OPE supplementation decreased the stress scale

Table 2. NK cell activity and cytokines concentration of participants with OPE and placebo for 8 weeks

Characteristics	OPE (n = 40)		Placebo (n = 40)		*P*-value[1]	*P*-value[2]
	Baseline	Week 8	Baseline	Week 8		
NK cell activity (%)	12.02 ± 9.30	14.61 ± 12.39	16.33 ± 12.78	18.38 ± 12.21	0.237	0.928
IL-2 (pg/mL)	15.34 ± 2.88	17.99 ± 3.89	14.74 ± 1.42	18.99 ± 3.96	0.392	0.133
IL-6 (pg/mL)	2.24 ± 1.47	2.59 ± 1.08	2.72 ± 2.82	3.04 ± 2.09	0.392	0.867
IL-12 (pg/mL)	27.49 ± 27.80	52.78 ± 87.94	27.47 ± 39.61	47.33 ± 77.45	0.884	0.604
IL-1β (pg/mL)	17.87 ± 4.38	19.77 ± 2.09	19.40 ± 6.96	20.69 ± 3.52	0.943	0.743
INF-γ (pg/mL)	0.74 ± 0.55	0.95 ± 0.77	0.84 ± 0.36	0.99 ± 0.56	0.827	0.487
TNF-α (pg/mL)	11.24 ± 5.43	27.01 ± 15.54	41.41 ± 143.60	64.18 ± 176.46	0.705	0.490

Values are means ± SDs.
NK, natural killer; OPE, onion peel extract; IL, interleukin; INF, interferon; TNF, tumor necrosis factor; WURSS-21, Wisconsin Upper Respiratory Symptom Survey-21.
[1]*P*-values for differences between OPE and placebo groups at baseline were determined by the independent *t*-test.
[2]*P*-values for changes from baseline to week 8 between OPE and placebo groups were determined by the analysis of covariance after adjusting for WURSS-21 symptom score and perceived stress scale score.

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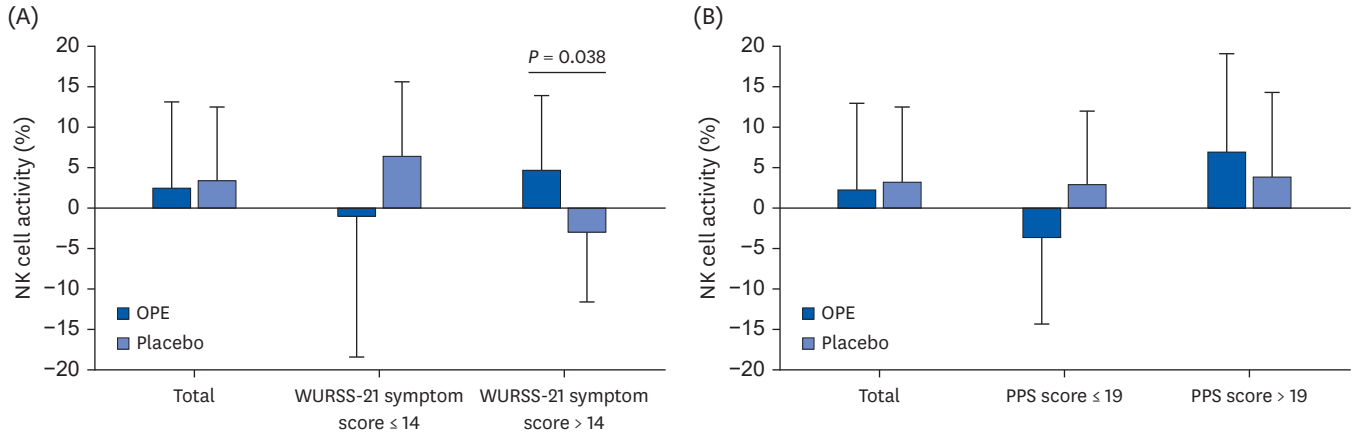

Fig. 2. NK cell activity according to the WURSS-21 symptom score between OPE and placebo for 8 weeks. *P*-values were determined by the independent *t*-test or χ^2 test between OPE and placebo groups.
NK, natural killer; OPE, onion peel extract; WURSS-21, Wisconsin Upper Respiratory Symptom Survey-21.

in the question "In the last month, how often have you felt you were unable to control the important things in your life?" ($P = 0.001$) and "In the last month, how often have you felt nervous and stressed?" ($P = 0.021$) compared to placebo (**Table 4**).

Safety assessment, adverse events, and dietary intake

There were no significant differences in vital signs, complete blood counts, or blood chemistry test results between the OPE and placebo groups at weeks 0 and 8, suggesting that OPE had no safety issues (**Table 6**). Eight participants with OPE and 11 participants treated with placebo reported 11 and 13 cases of adverse events, respectively; however, any adverse events were not found to be unrelated to supplementation (**Supplementary Table 1**). There were no significant differences in urine analysis or food and nutrient intake between the OPE and placebo groups at baseline and week 8 (**Supplementary Tables 2-4**).

DISCUSSION

This 8-week, randomized, double-blind, placebo-controlled trial showed that OPE supplementation had no significant effect on NK cell activity and serum concentrations

Table 3. WURSS-21 of participants with OPE and placebo for 8 weeks

Characteristics	OPE (n = 40)		Placebo (n = 40)		*P*-value[1)]
	Baseline	Week 8	Baseline	Week 8	
WURSS-21 symptom (score)	21.84 ± 17.39	14.16 ± 13.10	13.18 ± 11.28	13.12 ± 15.21	0.164
Runny nose	2.89 ± 2.50	2.49 ± 2.28	1.70 ± 2.01	1.55 ± 1.95	0.679
Plugged nose	2.78 ± 2.55	1.92 ± 2.20	1.76 ± 1.97	1.55 ± 2.06	0.449
Sneezing	2.14 ± 2.39	1.51 ± 1.73	1.48 ± 1.97	1.24 ± 1.68	0.640
Sore throat	2.03 ± 2.47	0.95 ± 1.62	1.06 ± 1.71	1.00 ± 1.94	0.179
Scratchy throat	1.86 ± 2.56	0.95 ± 1.70	0.67 ± 1.24	1.21 ± 2.03	0.059
Cough	1.86 ± 2.41	1.11 ± 1.58	0.91 ± 1.61	1.27 ± 2.16	0.215
Hoarseness	1.35 ± 1.99	0.73 ± 1.39	0.24 ± 0.66	0.70 ± 1.59	0.038
Head congestion	2.38 ± 2.20	1.70 ± 1.91	1.76 ± 1.92	1.64 ± 1.98	0.245
Chest congestion	0.86 ± 1.75	0.59 ± 1.09	0.76 ± 1.17	0.67 ± 1.27	0.896
Feeling tired	3.68 ± 2.08	2.22 ± 2.10	2.85 ± 2.18	2.30 ± 2.17	0.184

Values are means ± SDs.
WURSS-21, Wisconsin Upper Respiratory Symptom Survey-21; OPE, onion peel extract.
[1)]*P*-values for changes from baseline to week 8 between OPE and placebo groups were determined by the analysis of covariance after adjusting for perceived stress scale score.

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Table 4. GPAQ and PSS of participants with OPE and placebo for 8 weeks

Characteristics	OPE (n = 40)		Placebo (n = 40)		*P*-value
	Baseline	Week 8	Baseline	Week 8	
GPAQ (MET-min/week)	3,152.0 ± 2,765.5	3,121.5 ± 2,586.1	4,236.0 ± 5,168.9	3,578.8 ± 2,671.8	0.597[1)]
PSS (score)	21.03 ± 4.68	14.92 ± 5.31	19.06 ± 4.37	21.03 ± 4.68	0.164[2)]
In the last month, how often have you been upset because of something that happened unexpectedly?	2.32 ± 0.75	1.54 ± 0.80	2.00 ± 0.90	1.58 ± 0.94	0.122[2)]
In the last month, how often have you felt you were unable to control the important things in your life?	2.27 ± 0.93	1.32 ± 0.85	1.48 ± 0.67	1.36 ± 1.03	0.001[2)]
In the last month, how often have you felt nervous and stressed?	2.84 ± 0.76	1.78 ± 0.92	2.27 ± 0.88	1.73 ± 1.01	0.021[2)]
In the last month, how often have you felt confident about your ability to handle your personal problems?[3)]	2.08 ± 0.86	2.57 ± 0.65	2.03 ± 0.73	2.33 ± 0.99	0.440[2)]
In the last month, how often have you felt that things were going your way?[3)]	1.73 ± 0.90	2.32 ± 0.71	1.94 ± 0.75	2.39 ± 0.90	0.609[2)]
In the last month, how often have you found that you could not cope with all the things that you had to do?	1.81 ± 0.76	1.24 ± 0.86	1.82 ± 0.95	1.55 ± 1.09	0.971[2)]
In the last month, how often have you been able to control irritations in your life?[3)]	2.22 ± 0.85	2.59 ± 0.93	2.30 ± 0.85	2.82 ± 0.85	0.729[2)]
In the last month, how often have you felt that you were on top of things?[3)]	1.67 ± 0.96	2.14 ± 0.95	1.67 ± 0.96	2.06 ± 1.09	0.554[2)]
In the last month, how often have you been angered because of things that happened that were outside of your control?	1.73 ± 0.99	1.41 ± 1.01	1.79 ± 0.93	1.06 ± 0.93	0.286[2)]
In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	1.65 ± 1.06	1.24 ± 1.01	1.64 ± 0.82	1.24 ± 1.09	0.999[2)]

Values are means ± SDs.
GPAQ, Global Physical Activity Questionnaire; PSS, perceived stress scale; OPE, onion peel extract; MET, metabolic equivalent; WURSS-21, Wisconsin Upper Respiratory Symptom Survey-21.
[1)]*P*-values for changes from baseline to week 8 between OPE and placebo groups were determined by analysis of covariance after adjusting for WURSS-21 symptom score and PSS score.
[2)]*P*-values for changes from baseline to week 8 between OPE and placebo groups were determined by analysis of covariance after adjusting for WURSS-21 symptom score.
[3)]Scores for questions were calculated reversely.

Table 5. PGA of participants with OPE and placebo for 8 weeks

Characteristics	OPE (n = 40)		Placebo (n = 40)		*P*-value
	Week 4	Week 8	Week 4	Week 8	
PGA					0.591[1)]; 0.346[2)]
Strongly better	0 (0)	0 (0)	1 (2.5)	2 (5)	
Better	10 (25)	19 (47.5)	9 (22.5)	15 (37.5)	
The same	30 (75)	21 (52.5)	30 (75)	21 (52.5)	
Worse	0 (0)	0 (0)	0 (0)	1 (2.5)	
Strongly worse	0 (0)	0 (0)	0 (0)	1 (2.5)	

Values are number of participants (percentage distribution).
PGA, patient global assessment; OPE, onion peel extract.
[1)]*P*-values for difference between OPE and placebo groups at week 4 were determined by χ^2 test.
[2)]*P*-values for difference between OPE and placebo groups at week 8 were determined by χ^2 test.

of cytokines, such as IL-2, IL-6, IL-12, IL-1β, INF-γ, and TNF-α. Consistent with the present study, supplementation with OPE containing 100 mg and 162 mg quercetin, the bioactive compound of onion, had no significant effect on blood concentration of TNF-α in overweight and obese women [30] or pre-hypertension adults [31]. In the previous trials, participants were relatively healthy individuals with very low levels of inflammation, which could limit further reduction of blood cytokine concentration by OPE [30,31]. Additionally, supplementation with 1,000 mg quercetin had no effect on blood concentrations of IL-2, IL-6, TNF-α, and IL-1β in runners [39-41] and cyclists [42-44], who had also low basal cytokine concentrations. Chronic exercise is known to decrease basal blood level of cytokines such as C-reactive protein, IL-6, and TNF-α in healthy participants [45]. The World Health Organization defines regular physical activity as at least 150–300 min of moderate-intensity aerobic activity, 75–150 min of vigorous-intensity aerobic activity, or a combination of both throughout the week [46]. In the present study, 73% of participants exercised regularly and

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Table 6. Safety assessments of participants with OPE and placebo for 8 weeks

Characteristics	OPE (n = 40)		Placebo (n = 40)		*P*-value[1)]	*P*-value[2)]
	Baseline	Week 8	Baseline	Week 8		
Weight (kg)	65.72 ± 10.42	66.20 ± 10.32	67.21 ± 10.17	67.36 ± 9.00	0.715	0.946
Vital signs						
SBP (mmHg)	124.75 ± 14.95	122.78 ± 14.45	124.98 ± 14.04	121.43 ± 11.51	0.579	0.507
DBP (mmHg)	76.55 ± 9.90	75.13 ± 9.52	75.33 ± 9.22	74.10 ± 7.73	0.922	0.952
Pulse rate (beats/min)	77.68 ± 11.06	77.55 ± 8.89	76.55 ± 12.33	75.00 ± 10.21	0.580	0.366
Body temperature (°C)	36.56 ± 0.44	36.26 ± 0.35	36.62 ± 0.35	36.33 ± 0.29	0.914	0.840
Blood						
RBC (10^6/μL)	4.86 ± 0.50	4.81 ± 0.49	4.82 ± 0.49	4.82 ± 0.43	0.438	0.435
WBC (10^3/μL)	6.19 ± 1.40	5.86 ± 1.74	6.22 ± 1.42	5.90 ± 1.44	0.987	0.588
Hemoglobin (g/dL)	14.49 ± 1.31	14.33 ± 1.39	14.51 ± 1.62	14.40 ± 1.45	0.741	0.631
Hematocrit (%)	44.16 ± 3.52	43.69 ± 3.67	44.00 ± 4.42	43.97 ± 3.99	0.427	0.413
Platelet (10^3/μL)	271.08 ± 50.20	265.00 ± 52.06	275.28 ± 48.28	274.40 ± 46.59	0.441	0.663
Lymphocyte (%)	34.55 ± 7.01	35.93 ± 8.87	35.33 ± 7.55	35.65 ± 7.27	0.568	0.814
Monocyte (%)	7.03 ± 1.85	7.60 ± 2.19	7.65 ± 1.75	7.88 ± 2.23	0.456	0.542
Segmented neutrophil (%)	54.25 ± 7.96	52.20 ± 11.48	52.85 ± 7.91	52.55 ± 8.73	0.418	0.644
Eosinophil (%)	3.10 ± 2.31	3.38 ± 2.74	2.95 ± 1.78	2.85 ± 1.66	0.293	0.406
Basophil (%)	0.80 ± 0.52	0.90 ± 0.55	0.98 ± 0.53	1.08 ± 0.57	1.000	0.983
AST (U/L)	25.48 ± 10.43	23.03 ± 4.66	24.13 ± 7.26	23.83 ± 10.62	0.341	0.477
ALT (U/L)	18.38 ± 8.04	18.98 ± 9.09	18.00 ± 8.01	19.90 ± 11.49	0.560	0.560
Total protein (g/dL)	7.58 ± 0.35	7.48 ± 0.41	7.59 ± 0.36	7.56 ± 0.43	0.322	0.333
Glucose (mg/dL)	91.60 ± 17.32	91.05 ± 5.53	90.00 ± 7.61	90.80 ± 6.52	0.660	0.316
Total cholesterol (mg/dL)	186.18 ± 30.12	187.43 ± 27.20	188.23 ± 31.61	192.70 ± 30.15	0.515	0.342
BUN (mg/dL)	12.88 ± 3.50	12.99 ± 2.77	13.38 ± 3.7	13.66 ± 2.92	0.826	0.353
Creatinine (mg/dL)	0.84 ± 0.19	0.80 ± 0.22	0.89 ± 0.17	0.86 ± 0.16	0.423	0.476
Uric acid (mg/dL)	5.61 ± 1.36	5.86 ± 1.47	5.51 ± 1.19	5.60 ± 1.07	0.397	0.513
Calcium (mg/dL)	9.88 ± 0.30	9.80 ± 0.33	9.91 ± 0.29	9.85 ± 0.25	0.606	0.642
Phosphorus (mg/dL)	4.13 ± 0.53	4.11 ± 0.56	3.95 ± 0.52	3.99 ± 0.48	0.696	0.997

Values are means ± SDs.
OPE, onion peel extract; SBP, systolic blood pressure; DBP, diastolic blood pressure; RBC, red blood cell; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.
[1)]*P*-values for differences between OPE and placebo groups at baseline were determined by the independent *t*-test.
[2)]*P*-values for changes from baseline to week 8 between OPE and placebo groups were determined by the analysis of covariance after adjusting for Wisconsin Upper Respiratory Symptom Survey-21 symptom score.

had also low levels of inflammation. While the present study was the first study investigating the effect of OPE on NK cell activity in human model, supplementation with 500–1,000 mg quercetin had no significant effect on NK cell activity in healthy female adults [47].

On the other hand, the present study showed that OPE supplementation significantly increased NK cell activity only in participants with higher than median WURSS-21 symptom scores in PP analysis, but not in those with lower than median scores. In PP analysis, sample size of 33 participants had the power of 82%, with an α level (2-tailed) of 5%, suggesting that there was enough power to detect the effect of OPE on NK cell activity. Symptom score of WURSS-21 can be interpreted as the severity of acute respiratory infection [48], which can impair the innate immune system, including changes in NK cell activity [49]. In the present study, NK cell activity (%) was lower in participants with higher than median WURSS-21 symptom score as compared with those with lower than median WURSS-21 symptom score (10.69 ± 6.60 vs. 14.98 ± 12.55) in OPE. Previous studies showed that supplementation of 160–2,000 mg quercetin decreased TNF-α, IL-1β, or IL-6 concentration in patients with post-myocardial infarction [7], rheumatoid arthritis [8], polycystic ovary syndrome [9], high-cardiovascular disease risk phenotype [10], type 2 diabetes [11], pre-hypertension [12], and sarcoidosis [50]. On the other hand, the present study showed that OPE supplementation had no effect on concentration of blood cytokines in participants with higher or equal to or lower than median WURSS-21 symptom scores. Phytochemicals activate NK cells by increasing

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the expression of NK cell-activating ligands and cytokines, such as IL-2 and IL-15 [51]. There are 2 subsets of NK cells in humans, $CD56^{bright}$ and $CD56^{dim}$; the major subset is $CD56^{dim}$, which is more cytotoxic and produces much lower amounts of cytokines than $CD56^{bright}$ [52]. Thus, changes in NK cell activity may not be accompanied by changes in cytokine levels in participants with higher than median WURSS-21 symptom score in the present study. The present study and previous studies consistently suggested that supplementation of OPE and quercetin could affect only in participants with increased inflammation or impaired immnune function.

Furthermore, supplementation of onion extract containing 2.5 mg quercetin per kg body weight (equivalent to 13 mg in human) had no effect on the bronchoalveolar lavage cytokines in asthmatic mice [53]. Rivera *et al.* [54] also showed that supplementation of 10 mg quercetin per kg body weight (equivalent to 21 mg in human) had no effect on adipose tissue cytokines in obese rat. However, OPE supplementation containing 4–18 mg quercetin per kg body weight (equivalent to 38–190 mg in human) significantly increased NK cell activity, and decreased the expression of TNF-α, IL-6, and IL-8 in immunosuppressed mice induced by cyclophosphamide [23] and prostatic tissue of rats with atypical prostatic hyperplasia [26]. Additionally, Oliveira *et al.* [53] showed that supplementation with onion extract containing 25 mg quercetin per kg body weight (equivalent to 132 mg in humans) decreased the concentration of IL-4, IL-5, and IL-13 in the bronchoalveolar lavage of asthmatic mice. Rivera *et al.* [54] showed that supplementation of 10 mg quercetin per kg body weight (equivalent to 105 mg in humans), decreased the TNF-α concentration in adipose tissue of obese rat. Thus, previous studies suggested that equivalent to 38–190 mg of quercetin could improve NK cell activity and cytokines in human, but equivalent to 13–21 mg of quercetin did not.

To the best of our knowledge, this is the first randomized, double-blind, placebo-controlled, parallel-group study to investigate the effect of OPE on NK cell activity in adults. However, the present study had several limitations. Symptom score of WURSS-21 and PSS score could be a confounding factor in the immune response, but baseline WURSS-21 symptom and PSS score were significantly different between the OPE and placebo groups in the present study. To minimize selection bias, WURSS-21 symptom and PSS score were adjusted for statistical analysis; however, a residual effect may exist. Second, the blood concentration of quercetin was not measured in the present study. Finally, the time to take quercetin capsules was not designated, and all biomarkers were measured in the fasting state with the last quercetin intake in the evening, 8–12 h before blood sampling, or in the morning, 24 h before blood sampling. However, it is unclear whether quercetin exerts acute effects.

In conclusion, the present clinical study suggests that supplementation with OPE increases NK cell activity in participants with moderate upper respiratory symptoms. Further studies are warranted to confirm whether OPE supplementation has anti-inflammatory and immunomodulatory effects in patients with inflammatory diseases or immunosuppression.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1

Adverse events of participants with OPE and placebo during 8 weeks

Supplementary Table 2

Urine analysis of participants with OPE and placebo

Supplementary Table 3

Food intake of participants with OPE and placebo during 8 weeks

Supplementary Table 4

Nutrient intake of participants with OPE and placebo during 8 weeks

Supplementary Fig. 1

High-performance liquid chromatography chromatogram of quercetin extracted from onion peel extract.

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