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Original Research Article

Evaluation of the effectiveness and safety of Korean red ginseng extract as immune-enhancer in Vietnamese adults: A randomized placebo-controlled trial

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Abstract

Purpose: *To investigate the safety and immune-enhancing effect of Korean red ginseng (KRG) extract in Vietnamese adults.*
Methods: *Participants in this randomized, placebo-controlled double-blinded study were administered either 960 mg of KRG extract (n = 51) or placebo capsules (n = 50) for 12 weeks at Hanoi Medical University, Vietnam. The KRG extract was standardized to contain 5.27 mg of 3 ginsenosides (Rg1, Rb1, and Rg3) per gram. Blood samples for assessment of treatment effectiveness were collected from the subjects on days 21 and 84. Immune cytokines were quantified using enzyme-linked immunosorbent assay (ELISA). Blood samples for safety assessment, hematological and biochemical variables, and urinalysis were performed at the beginning and end of the intervention (days 21 and 84, respectively).*
Results: *The KRG group showed a significant increase in lymph T cell % at the end of the 12-week intervention ($p < 0.001$). There were significant decreases in numbers of white blood cells (WBC) and natural killer (NK) cells in both groups. However, there were no significant differences in populations of WBC and NK cells between the 2 groups. Serum concentrations of cytokines, i.e., TNF-α, β, γ; IFN-α, γ; IL-1β and 4β were decreased in the KRG group when compared to baseline values. There were no probable or definite adverse events (AEs) related to KRG. A total of 9 participants (4 in KRG group and 5 in placebo group) tested positive for COVID-19 during the trial.*
Conclusion: *The KRG extract-induced increase in population of T-cells and reductions in cytokine levels following a 12-week exposure may be beneficial for COVID-19 patients.*

Keywords: *Panax ginseng, 6-year-old Korean Red Ginseng Extract, Ginsenoside, COVID-19, Cytokine, T cells*

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INTRODUCTION

In the wake of COVID-19 pandemic, substantial emphasis was placed on boosting the immune system [1]. A decrease in normal immune function facilitates the invasion of foreign pathogens, thereby increasing the risk of viral and bacterial infections and diseases. Therefore, improving immunity means enhancing the immune defense system which has been compromised by congenital and environmental factors [2]. Immune response to foreign substances is broadly categorized into innate and adaptive immune responses [3]. The innate immune response refers to an immune response that directly responds to and destroys foreign antigens without previous exposure to the specific antigens, and it is referred to as intrinsic immunity or non-specific immunity.

White blood cells such as neutrophils and macrophages are involved in the innate immune response through phagocytic function. Cytokines are proteins secreted by cells involved in innate and adaptive immunity which regulate many functions of immune cells. The cytokines involved in innate immunity are tumor necrosis factor-alpha (TNF-α), interferon (IFN)-α and IFN-β, and interleukin (IL)-1, IL-6, IL-10 and IL-12 [4]. In contrast, adaptive immunity memorizes a previous antigen to which it was exposed and produces an amplified immune response the next time the same antigen is encountered. Adaptive immunity is divided into humoral and cell-mediated immunity involving B lymphocytes and T lymphocytes, respectively. The cytokines involved in adaptive immunity are IL-2, IL-4, and IFN-γ [5].

Extant evidence shows that ginseng in general, and red ginseng in particular, improve immunity. The effectiveness of ginseng has led to its description as an adaptogen (a substance that enhances "non-specific resistance" during stress) that normalizes body function and maintains homeostasis. Korean red ginseng (KRG) is one of the functional ingredients recognized by the Korea Ministry of Food and Drug Safety (MFDS). Indeed, it has been claimed that KRG boosts immunity, reduces fatigue, improves circulation by preventing blood platelet aggregation, enhances memory, exerts antioxidant properties, and boosts female health in menopause [6]. With an annual growth rate of 15.9 %, the Korean functional food market reached $ 4.5 billion in 2022, and KRG accounted for 25 % of this market as the best-selling functional ingredient in Korea.

Before clinical trials, KRG extract was tested in toxicological studies on laboratory animals to ensure its safety. An acute toxicity study in mice revealed that the maximum tolerated dose of KRG was 25 g/kg. No Adverse event was observed in mice after oral administration for 90 days. This study was designed as a 12-week parallel, randomized placebo-controlled trial for investigating the safety and efficacy of KRG hard capsules in subjects with WBCs of 3000 – 10,000/μL who recovered from COVID-19 infection within the previous 12 months.

METHODS

Study design

This single-center study was approved and conducted at the Centre of Clinical Pharmacology, Hanoi Medical University, Hanoi, Vietnam (approval no. IRB-VN01001). The study was conducted in strict compliance with the Declarations of Helsinki [7]. The primary objective of the study was to investigate the safety of KRG extract and its immunological effect on Vietnamese subjects at the end of the 12-week study period. The trial was registered at the clinical trial registry (no. NCT05480774). Subjects visited the hospital for screening (visit 1 on day 1), randomization (visit 2, day 21), midterm assessment (visit 3 on day 28 and visit 4, day 56); and end-of-study or early termination assessment (visit 5 on day 84). Assessment through the telephone was performed for a safety check on day 98.

Inclusion criteria

The subjects were male and female individuals aged 20 - 65 years. The main inclusion criteria were peripheral WBCs ranging from 3000 to 10,000 count/μL, and recovery from previous COVID-19 infection within 12 months before the trial, in line with the diagnostic criteria of the Ministry of Health.

Exclusion criteria

Subjects were excluded if they had acute or chronic cardiovascular, respiratory, renal, infectious, immunological, hepatobiliary, neurological, psychiatric, hematologic, musculoskeletal, or oncologic diseases. In addition, subjects with uncontrolled hypertension (systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg) or uncontrolled diabetes (fasting blood sugar > 126 mg/dL; subjects who started taking antidiabetic drugs within the previous 3 months); those who had more than 3 times the normal blood range of

AST or AST, or blood creatinine levels more than 2.4 mg/dL (for males) or 1.8 mg/dL (for female), were excluded from the trial. The subjects were instructed not to use immunity-impacting dietary supplements within 2 weeks before the screening visit. Subjects who experienced severe gastrointestinal symptoms such as heartburn and indigestion, were excluded. Moreover, pregnant or lactating women, women intending to become pregnant during the study period, and subjects with a history of allergy/irritation to foods containing ingredients similar to the components of KRG, were excluded.

Preparation of KRG

The KRG hard capsule was manufactured by the Daedong Korea Ginseng Co. Ltd. The main ingredient of KRG was Korean red ginseng (*Panax ginseng* C. A. Meyer). The process involved in the production of KRG was as follows: 6-year-old fresh ginseng roots were washed, steamed at 90 – 1000 °C for 80 - 100 min, and then dried under a stream of hot air at a temperature range of 45 – 550 °C and humidity < 15.5 %. The dried red ginseng was extracted with water at a raw material to solvent ratio of 1:10 (g: mL) at 850 °C for 12 h.

Randomization and drug administration

The randomization code was generated using Excel by an independent statistician. To maintain double blindness, the hospital assigned one pharmacist (or qualified personnel) who was not blinded to the treatment groups to distribute the tested products. The subjects were assigned equally to the KRG group and the placebo group. The calculation of sample size was performed as per the international recommendations for the sample size of phase I studies and the guidelines of the Ministry of Health for clinical trials on development of plant-derived products as medications.

Subjects in KRG group (n = 51) received KRG capsules (2 capsules/day), while those in placebo group (n = 50) were given placebo hard capsules. Both treatments were given orally for 12 weeks. Each capsule was taken 2 h after breakfast and 2 h after lunch. A 500-mg capsule contained 480 mg of KRG powder (test product). The test drug product was distributed once a month to the participants during each follow-up visit. The participants were instructed to record (in their diaries) each time they took test products. Apart from taking the product in the right dose and in strict compliance with the study timeline, participants were also encouraged not to use any medication during the 12-week study period. If participants had a disease that required treatment, the medication administered was recorded.

Evaluation of parameters/indices

Treatment effectiveness

Blood samples for assessment of treatment effectiveness were collected from the subjects on the first day of intervention (day 1) and at the end of intervention (day 84). All blood samples were collected after an 8-hour fast. Samples were analyzed on the BD FACSCanto™ II flow cytometer (Becton, Dickinson and Company, BD Biosciences).

ELISA

The immune cytokines were quantified using enzyme-linked immunosorbent assay (ELISA). Blood samples for safety assessment were collected during the screening visit (day 21) and at the end of intervention (day 84).

Hematological and biochemical variables

Hematological and biochemical variables were analyzed using the ADVIA® 2120i Hematology System (Siemens Healthcare Diagnostics Manufacturing Ltd.).

Urinalysis

Urine samples were collected during the screening visit and at the end of intervention (day 21 and day 84, respectively).

Statistical analysis

Data were analyzed using Microsoft Excel 2010 and SPSS Windows 22.0. Values are presented as mean ± standard deviation (SD). The normal distribution of each group was evaluated with the Kolmogorov-Smirnov test. Variables with normally distributed data were compared using parametric methods, while non-parametric tests were used for the comparison of non-normally distributed variables. A *p-value* less than 0.05 was considered indicative of statistically significant difference.

RESULTS

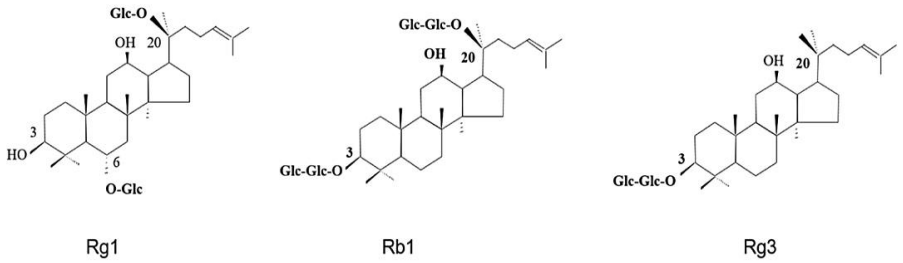
Standardization of KRG

The KRG was analyzed using high-performance liquid chromatography (HPLC) and 203 nm UV-VIS reader. As shown in Figure 1, KRG contained a total of 3 main ginsenosides (Rg1,

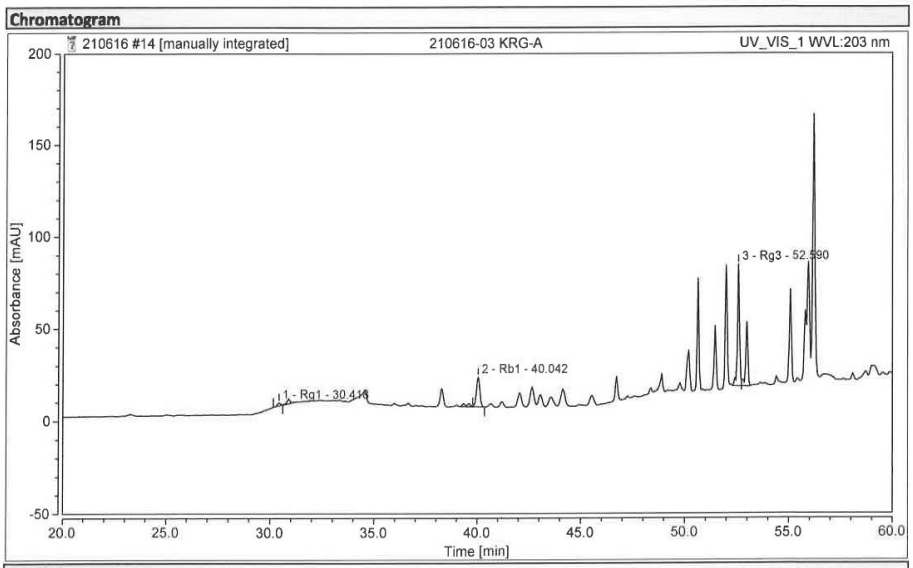
Rb1 and Rg3) amounting to 5.27 mg/g. The contents of Rg1, Rb1, and Rg3 were 0.1259 mg/g (2.93 %), 1.7914 mg/g (29.85 %), and 3.3542 mg/g (78.72 %), respectively.

Baseline demographic data of participants

As summarized in Figure 2, 144 individuals were screened, out of which 101 subjects were selected and randomly assigned to two groups given either KRG or placebo capsules. Ten (10) of the participants withdrew consent, while one reported pregnancy during the study period. Thus, 91 subjects completed the trial (44 in KRG group and 46 in placebo group). Baseline characteristics such as age, gender, body weight, BMI, pulse rate, and systolic and diastolic blood pressures were comparable in the two groups. These data are shown in Table 1.



Rg1 Rb1 Rg3



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount mg/g
1	Rg1	30.418	0.305	1.652	2.93	1.97	0.1259
2	Rb1	40.042	3.110	16.203	29.85	19.31	1.7914
3	Rg3	52.590	7.004	66.068	67.22	78.72	3.3542
Total:			**10.419**	**83.923**	**100.00**	**100.00**	**5.272**

Figure 1: Ginsenosides (Rg1, Rb1 and Rg3) and HPLC chromatogram of KRG

Table 1: Baseline characteristics of KRG and placebo groups

Parameter	KRG (n=51)	Placebo (n=50)	Total (n=101)
Age (years)	26.69±6.71	25.15±5.51	25.93±6.16
Gender (n (%))	15 males (29.4) 36 females (70.6)	15 males (30.0) 35 females (70.0)	30 males (29.7) 71 females (70.3)
Body weight (kg)	54.09±9.22	52.85±7.71	
Body mass index (kg/m^2)	21.19±2.34	20.79±1.84	
Pulse rate	82.63±12.66	87.06±11.90	84.82±12.43
Systolic blood pressure (mmHg)	110.59±9.83	111.38±11.00	110.98±10.38
Diastolic blood pressure (mmHg)	73.88±7.11	74.86±7.12	74.37±7.09

Note: Analysis of immunological parameters of the 2 groups at the 12th week. Values are presented as mean ± SD, or as numbers (n) and percentages of participants (%)

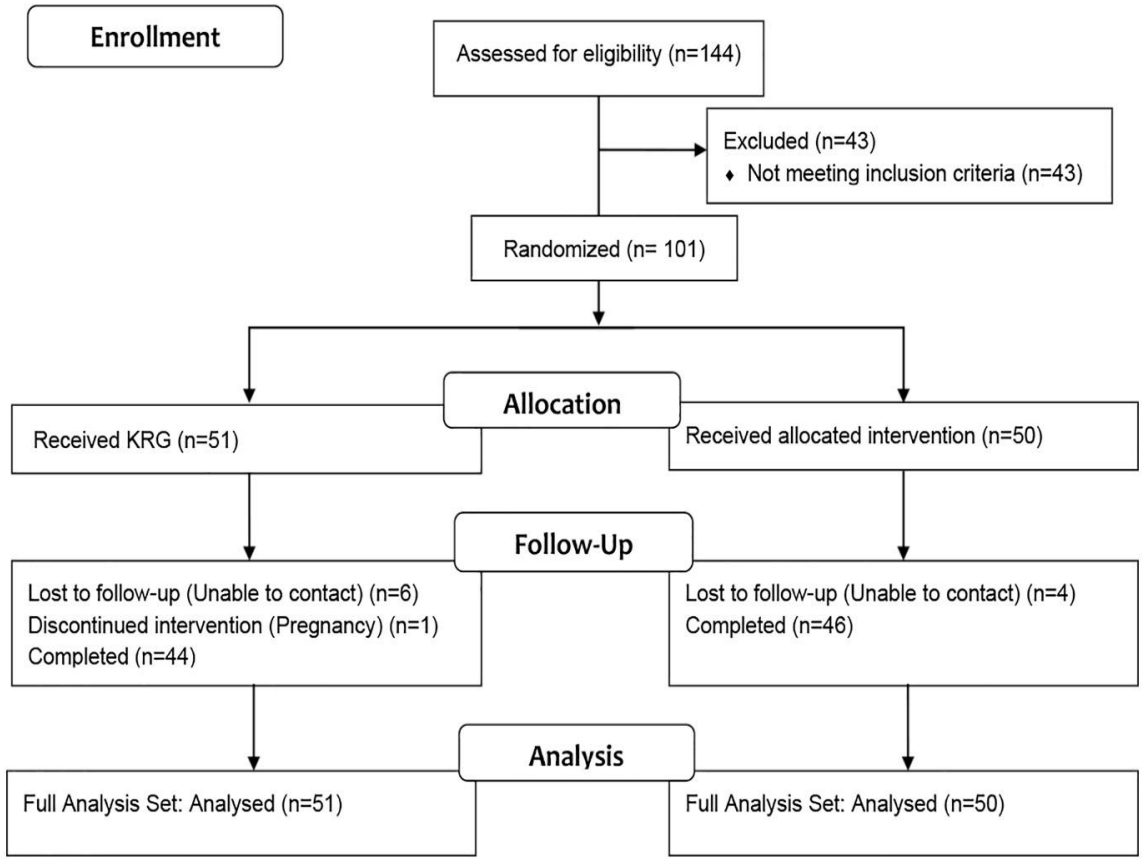


Figure 2: Schematic diagram depicting screening, grouping and analysis of subjects

The within-group comparison of peripheral blood lymphocyte subsets showed that the KRG group had a significant increase in the number of lymphocyte T cells (%) at the end of the 12-week intervention (baseline and week 12 values were 70.6 ± 5.9 and 72.6 ± 6.4, respectively; Table 2). In both groups, there were decreases in the numbers of WBC and NK cells after 12 weeks, when compared with the corresponding baseline values. However, there were no significant differences in the levels of these parameters between the 2 groups.

Table 2: Levels of immunological parameters in participants in both groups at baseline and 12 weeks

Parameter	KRG (n=51)		Placebo (n=50)	
	Baseline	***Week 12***	***Baseline***	***Week 12***
WBC count (x1000/µL)	6.4±1.8	5.8±1.3	6.6±1.3	6.3±1.5
Lymphocytes (%)	35.8±7.7	36.8±6.9	34.3±6.3	34.9±6.3
Lymphocyte T cells (%)	70.6±5.9	72.6±6.4[b]	69.2±5.6	70.9±5.6
Lymphocyte T CD8 (%)	29.3±5.7	30.1±6.7	28.6±4.8	28.7±5.2
Lymphocyte T CD4 (%)	35.4±6.5	36.0±7.3	35.3±5.7	36.1±5.8
Lymphocyte B cells (%)	13.7±3.7	13.0±4.2	13.6±3.4	13.4±3.6
NK cells (cells/µL)	320.5±121.8	284.0±140.1[a]	367.8±179.3	330.0±176.8
TNF-α (pg/mL)	14464.6±27362.5	8694.7±24004.6	14206.7±30634.9	19083.0±44871.6
TNF-β (pg/mL)	10503.0±37673.2	4855.8±12171.6	30104.7±141795.1	4941.0±10910.6
TNF-γ (pg/mL)	3.1±14.1	1.8±2.5	3.7±17.3	1.4±1.0
IFN-α (pg/mL)	29.4±60.4	21.3±30.8	76.7±220.5	36.6±54.0
IFN-β (pg/mL)	31.5±18.3	33.6±75.4[b]	63.3±231.3	38.4±101.5[b]
IFN-γ (pg/mL)	3020.9±5765.5	1476.7±2273.5	4299.7±8678.6	1849.4±2395.7
IL-1β (pg/mL)	14.9±54.8	6.7±24.0[a]	9.2±40.2	7.7±23.1
IL-4β (pg/mL)	13.6±20.4	9.9±16.8[b]	16.0±15.2	15.3±17.3[a]
IL-6β (pg/mL)	146.7±568.4	196.9±716.1	248.1±504.9	150.9±326.2

Note: [a]$P < 0.01$; [b]$p < 0.001$ vs baseline. (WBC = white blood cell; NK cell: natural killer cell; TNF: tumor necrosis factor; IFN: interferon; IL: interleukin)

The serum concentrations of immune cytokines, i.e., TNF-α, TNF-β, and TNF-γ were decreased in the KRG and placebo groups. However, within-group and between-group comparisons revealed that the differences between the two groups were not statistically significant. The levels of IFN-α and IFN-γ were significantly decreased in both groups when compared to baseline. At the end of the 12-week ingestion, IFN-β was decreased. The level of IL-1β was decreased in both groups, and the decrease in the KRG group was significant when compared to the corresponding baseline value. The level of IL-4β was significantly decreased, relative to baseline value. In both groups, the level of IL-6β tended to decrease after 12 weeks of intervention. These results are presented in Figure 3.

Levels of safety parameters in the KRG group and placebo group

The levels of hematological parameters and biochemical variables in the KRG placebo groups are shown in Table 3. At baseline, there were no differences in hematology and biochemistry between the 2 groups. Although the HDL-C concentration of the KRG group was significantly lower than that of the placebo group, and gamma-glutamyl transferase activity in the KRG participants was significantly higher than that of the placebo group, the levels of these parameters were within the reference ranges. Therefore, they were not classified as AEs by the investigators.

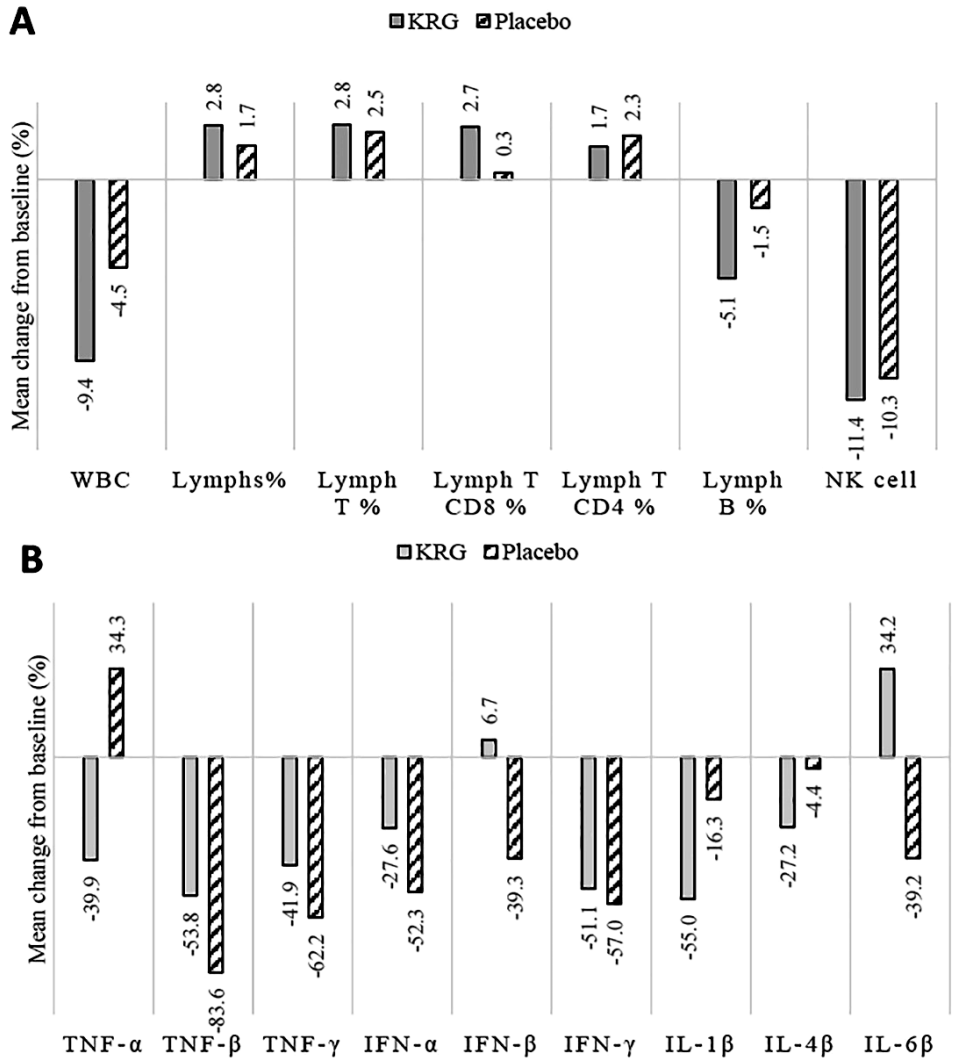


Figure 3: Mean changes in levels of peripheral blood lymphocyte subsets and serum concentrations of immune cytokines between day 84 and baseline in participants who took KRG extract and those who took placebo capsules. (A) Peripheral blood lymphocyte subsets; (B) serum concentrations of immune cytokines (TNF-α, TNF-β, TNF-γ, IFN-α, IFN-β, IFN-γ, IL-1β, IL-4β, and IL-6β)

The intake of KRG capsules for 12 weeks led to significant decreases in red blood cells, neutrophils, triglycerides, and alkaline phosphatase, and significant increases in mean corpuscular volume, lymphocytes, creatinine, HDL-C, direct bilirubin, and aspartate transferase. However, these parameters were still within the reference ranges and were not classified as AEs.

In the placebo group, there were also significant changes in hematocrit, mean corpuscular volume, and erythrocyte sedimentation rates in the first hour and the second hour, in addition to changes in levels of aspartate transferase, alanine transaminase, gamma-glutamyl transferase, and alkaline phosphatase. However, these values were within the reference ranges and were not classified as AEs.

Results from complete urinalysis showed that most participants in both groups had normal urine. Urine pH values of the KRG group after 12 weeks of treatment were decreased significantly, when compared to pre-treatment values. However, there were no significant differences in urine pH between the KRG group and placebo group (data not shown).

Mild AEs were reported by 24 participants in the KRG group (47.1 %; 41 cases) and by 24 participants in the placebo group (48.0 %; 24 cases). Moderate AEs were reported by 5 participants in the KRG group (9.8 %; 12 cases) and by 3 participants in the placebo groups (6.0 %; 8 cases). However, there were no severe AEs, and there were no drug-related AEs. The high-prevalence AEs (≥ 6.0 %) reported in both groups were cold/flu, COVID-19, headache, and difficulty in falling asleep/insomnia (Table 4).

DISCUSSION

This study is a randomized, double-blinded, placebo-controlled, phase 1 clinical trial aimed at assessing the safety of KRG extract and its effects on immunological indices in Vietnamese subjects. The safety endpoints centered on the prevalence and characteristics of AEs associated with the use of the KRG hard capsules after 12 weeks of treatment. Treatment effectiveness was assessed based on changes in WBC, NK cell count, and serum concentrations of several immune cytokines, i.e., TNF-α, TNF-β, TNF-γ, IFN-α, IFN-β, IFN-γ, IL-1β, IL-4β, and IL-6β, after 12 weeks of treatment.

Table 3: Levels of safety parameters in participants in the 2 groups at week 12

Parameter	KRG		Placebo		Reference range
	Baseline	***Week 12***	***Baseline***	***Week 12***	
Red Blood Cell count (10^{12}/L)	4.77±0.48	4.66±0.46[a]	4.74±0.50	4.74±0.49	4.0-5.9
Hemoglobin (g/L)	135.88±12.34	135.80±11.81	137.06±11.13	138.72±11.58	125-175
Hematocrit (L/L)	0.41±0.03	0.41±0.03	0.41±0.03	0.42±0.03[b]	0.4-0.53
Mean corpuscular volume (fL)	87.16±5.48	89.02±6.38[c]	87.58±4.02	89.13±5.05[c]	80-100
White blood cells (g/L)	6.03±1.46	5.90±1.33	6.21±1.20	6.36±1.60	4-10
Neutrophils (%)	56.08±7.68	53.20±9.46[a]	57.09±7.24	55.74±7.79	45-75
Lymphocytes (%)	33.27±6.73	36.70±9.23[b]	31.71±5.72	33.65±6.83	20-45
Platelets (g/L)	247.20±49.45	243.50±57.70	269.58±45.73	264.76±49.48	150-450
Erythrocyte sedimentation rate in the first hour (mm)	15.27±12.18	14.23±10.08	16.96±13.17	13.07±9.79[b]	<15
Erythrocyte sedimentation rate in the second hour (mm)	28.84±17.97	28.55±15.44	30.80±17.04	25.54±15.42[c]	<20
Urea (mmol/L)	4.11±0.92	4.23±1.04	4.20±0.96	4.08±1.00	2.76-8.07
Creatinine (µmol/L)	63.33±14.71	66.25±13.06[a]	62.56±12.87	65.96±12.80[c]	62-106
Cholesterol (mmol/L)	4.26±0.61	4.16±0.64	4.30±0.66	4.33±0.70	<5.2
Triglycerides (mmol/L)	1.24±0.54	1.06±0.44[a]	1.33±0.90	1.09±0.59[a]	<1.7
HDL-C (mmol/L)	1.30±0.26	1.32±0.28[a]	1.35±0.31	1.46±0.31[c]	>0.9
LDL-C (mmol/L)	2.56±0.54	2.50±0.57	2.55±0.55	2.54±0.63	<3.34
Total Bilirubin (µmol/L)	8.37±4.59	8.55±3.16	8.94±6.22	9.15±4.08	<24
Direct Bilirubin (µmol/L)	2.52±1.17	2.89±0.88[b]	2.71±1.32	2.97±1.10[a]	<3.4
Aspartate Transferase (U/L)	17.14±4.01	22.55±40.44[a]	17.60±3.76	14.72±3.98[c]	<40
Alanine transaminase (U/L)	15.49±11.42	20.45±37.25	15.26±9.35	12.67±6.53[a]	<41
Gamma-glutamyl transferase (U/L)	17.43±9.71	20.70±16.61	15.66±8.35	13.57±6.45[b]	8-61
Lactate Dehydrogenase (U/L)	185.47±66.06	187.59±72.90	176.80±37.80	176.09±54.92	135-225
Alkaline phosphatase (U/L)	64.57±16.53	60.48±18.33[c]	70.62±18.19	63.63±15.05[c]	40-129
High-sensitivity C-reactive protein (mg/dL)	<0.5	<0.5	<0.5	<0.5	<0.5

Note: [a]$P < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ vs baseline (within-group comparisons with the paired *t*-test or Wilcoxon signed-rank test)

Table 4: Adverse events in participants treated with KRG extract or placebo capsules for 12 weeks

Adverse event		KRG (n=51)	Placebo (n=50)
Infections and infestations	Sore throat	3 (5.9)	1 (2.0)
	Cold/Flu	4 (7.8)	4 (8.0)
	COVID-19	4 (7.8)	5 (10.0)
	Shingles	1 (2.0)	-
	Dengue	1 (2.0)	-
Nervous system disorders	Headache	5 (9.8)	3 (6.0)
	Difficulty falling asleep (insomnia)	4 (7.8)	4 (8.0)
	Dizziness	-	1 (2.0)
Ear and labyrinth disorders	Otitis externa	1 (2.0)	-
Respiratory, thoracic and mediastinal disorders	Chest pain	1 (2.0)	-
	Coughing	1 (2.0)	-
	Allergic rhinitis	-	1 (2.0)
Cardiovascular disorders	Tachycardia	2 (3.9)	-
	Increased heart rate	1 (2.0)	-
	Prehypertension, hypertension	2 (3.9)	-
	Hypotension	-	1 (2.0)
Gastrointestinal disorders	Gastrointestinal infection	1 (2.0)	1 (2.0)
	Epigastric pain, abdominal pain	1 (2.0)	4 (8.0)
	Wisdom tooth pain	1 (2.0)	-
	Bloating, burping, indigestion	2 (3.9)	1 (2.0)
	Gastritis	1 (2.0)	1 (2.0)
	Aphthous ulcers	1 (2.0)	-
	Gingivitis	1 (2.0)	-
	Constipation	2 (3.9)	1 (2.0)
	Loose stools	-	1 (2.0)
	Diarrhea	-	2 (4.0)
Renal and urinary disorders	Urinary tract infection	-	2 (4.0)
Skin and subcutaneous tissue disorders	Contact dermatitis	1 (2.0)	-
	Atopic dermatitis	-	1 (2.0)
Musculoskeletal disorders	Knee pain	1 (2.0)	-
	Hip joint pain	-	1 (2.0)
Reproductive disorders	Menstrual disorder	1 (2.0)	1 (2.0)
	Menorrhagia	1 (2.0)	-
	Gynecological infection	-	1 (2.0)
	Benign fibrocystic breast	-	1 (2.0)
General disorders	Feeling hot	1 (2.0)	-
	Fever	3 (5.9)	5 (10.0)
	Sore throat	1 (2.0)	-
	Armpit abscess	-	1 (2.0)

Most participants adhered to the assigned doses of the test products. The safety of KRG hard capsules was demonstrated by the similarity in the number of participants who experienced at least one AE in the 2 study groups: 54.9 % in the KRG group and 54.0 % in the placebo group. The doctors/investigators did not record any AEs that were probably related to KRG. Most of the AEs reported were mild. Indeed, there was no serious AE, life-threatening event, or death during the study period.

The numbers of WBC and NK cells were decreased in both groups after 12 weeks of treatment. In healthy adults, the reference ranges for white blood cell count and NK cell count are 4 - 10 g/L and 51 - 652/μL, respectively [8]. After 12 weeks of treatment, the numbers of WBC and NK cells in both groups were still within the normal ranges.

Analysis of post-treatment changes in leukocyte sub-populations showed that the KRG group had increased percentages of lymphocytes, CD8 T, and CD4 T and a statistically significant increase in lymphocyte T cell population when compared to baseline values. However, there were no significant differences in the levels of these parameters between the 2 groups. It has been demonstrated that healthy adults who ingested KRG for 8 weeks had significant increases in the numbers of T cells and lymphocyte sub-populations such as CD4 and CD8 [9]. There were significant decreases in serum concentrations of the immune cytokines, i.e., TNF-α, TNF-β, TNF-γ, IFN-α, IFN-β, IFN-γ, IL-1β, IL-4β, and IL-6β after the 12-week treatment period, relative to values at baseline.

Clinical and non-clinical studies have revealed that ginseng and ginseng-derived ginsenosides down-regulate the levels of proinflammatory

cytokines under inflammatory conditions. Ginsenosides, the major component of KRG extract, has been reported to suppress inflammations and abnormal cell growth. Intestinal microflora metabolizes ginsenoside Rg3 to Rh2 which is the dominant metabolite. It has been reported that ginsenoside Rh2 exerts anti-allergic effect through stabilization of the cell membrane and suppression of inflammation through inhibition of nitric oxide and prostaglandin E2 [10]. One of the major metabolites resulting from intestinal bacterial metabolism of ginseng is compound K. This metabolite decreased the levels of TNF-α and IL-1β in a dose-dependent manner in lipopolysaccharide-induced inflammation [11].

In a study involving animal organ transplantation, ginsenoside Rd produced immunosuppressive effects through down-regulation of cytokines IL-2, IL-12, TNF-α, and IFN-γ [12]. In LPS-activated microglial cells, elevated levels of TNF-α were suppressed by ginsenosides Rd, Rb2, Rg1 and Re [13]. Moreover, ginsenoside Rb1 inhibited LPS-induced production of proinflammatory cytokines such as TNF-α and IL-6 in murine macrophage cell lines [14]. To evaluate the neuroprotective effect of KRG, rats with focal cerebral ischemia/reperfusion injury were administered KRG for 7 days.

It was observed that infarct volumes and neurological deficits were ameliorated through suppression of TNF-α, IL-1β and IL-6 which were elevated after transient middle cerebral artery occlusion operation [15]. Consumption of KRG at a dose of 0.25 g/kg or 0.50 g/kg for 4 weeks resulted in decreases in the expression levels of vascular endothelial growth factor A (VEGFA), IL-6, IL-1β, TNF-α, and cytochrome c oxidase subunit II (COX2) in male rats with chronic non-bacterial prostatitis [16].

A study showed that KRG extract suppressed allergic inflammation, e.g., atopic dermatitis, through the inhibition of the MAPK and NF-kB pathways [17]. The immunosuppressive effect of KRG has been reported in clinical applications. A case was reported of a 42-year-old man who had a liver transplantation which led to refractory acute graft-versus-host disease. The patient was administered KRG decoction (30 g slices/day), along with the regular antibiotic treatment. After 11 days of KRG administration, there were increased levels of WBC and decreased levels of TNF-α, IFN-γ, and IL-10. [18]. In children patients who underwent chemotherapy or stem cell transplantation, administration of KRG extract (60 mg/kg/day) for one year led to significant down-regulation of the inflammatory cytokines IL-2, 10, 12, TNF-α, and IFN-γ [19].

Limitations of this study

Seventy percent of participants in this study were females with a mean age of 25.93 ± 6.16 years. Therefore, there may be sex- and age-related bias. In addition, bias factors known to affect immune indicators, such as season and individual stress, were not adjusted.

CONCLUSION

In this trial, KRG extract has shown a tendency to increase T-cell population and decrease the expressions of pro-inflammatory cytokines in Vietnamese adults who recovered from COVID-19 in the previous 12 months. Therefore, KRG extract has potentials to be beneficial for managing COVID-19 patients by suppression of pro-inflammatory cytokines.

DECLARATIONS

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Ethical approval

This study was approved by the Center of Clinical Pharmacology, Hanoi Medical University, Hanoi, Vietnam (IRB-VN01001).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by them.

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