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Research Article

Enhanced immunity effect of Korean Red Ginseng capsule: A randomized, double-blind and placebo-controlled clinical trial

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ABSTRACT

Background: As a physiological function of body, immunity can maintain health by identifying itself and excluding others. With economic development and increasingly fierce social competition, the number of sub-healthy population is gradually increasing, and the most basic problem exposed is human hypoimmunity. Hypoimmunity can be manifested as often feeling tired, catching colds, mental depression, etc. In order to enhance immunity, eating healthy foods with the effect of enhancing immunity may become an effective choice. KRG has pharmacological effects of enhancing immunity. Because the screening and evaluation method of immune population are not unified, there are relatively few KRG immunity tests for sub-health population. It is of great significance to study the effect of KRG on people with hypoimmunity to improve sub-health status.

Methods: This was a 180-day, randomized, double-blind, placebo-controlled clinical trial. According to the trial scheme design, 119 qualified subjects were included and randomly divided into the test group taking KRG and the placebo control group. Subjects need to check safety indicators (blood pressure and heart rate, blood routine, liver and kidney function, urine routine and stool routine) and efficacy indicators (main and secondary) inspection at baseline, efficacy indicators inspection during the mid-term of the test (90th days of administration), safety and efficacy indicators inspection after the test (180th days of administration).

Results: After the test, the safety indicators of placebo control group and KRG test group were basically within the normal range, and there is no significant difference in fireness score between the two groups. Through follow-up interviews, it was found that the subjects in the test group and the control group had no adverse reactions and allergic reactions such as nausea, flatulence, diarrhea, and abdominal pain during the test period. Self-comparison of the test group, the results of the main efficacy indicators: (1) immune related health scores were significantly improved in the mid-term and after the test ($P < 0.01$), (2) CD3 and CD4/CD8 increased significantly after the test ($P < 0.05$), (3) IgG, IgA, IgM and WBC increased significantly in the mid-term and after the test ($P < 0.01$); the results of the secondary efficacy indicators: (1) TNF-α decreased significantly in the mid-term ($P < 0.05$), IFN-γ decreased significantly in the mid-term ($P < 0.01$), (2) NK increased significantly in the mid-term and after the test ($P < 0.05$), (3) monocyte increased significantly in the mid-term and after the test ($P < 0.01$). Inter-group comparison of the test group and the control group, the results of the main efficacy indicators: (1) immune related health scores were higher than that of the control group in the mid-term and after the test ($P < 0.01$), (2) IgA of the test group was higher than that of the control group in the mid-term and after the test ($P < 0.05$); the results of the secondary efficacy indicators: (1) WBC of the test group was higher than that of the control group in the mid-term ($P < 0.05$); (2) monocytes of the test group were higher than that of the control group in the mid-term and after the test ($P < 0.05$), neutrophils of the test group were higher than that of the control group in the mid-term ($P < 0.05$).

Conclusion: Taking KRG has no adverse effects on the health of the subjects. According to the standard of clinical trial scheme, the immune related health scores and IgA in the main efficacy indicators were positive, which shows that KRG is helpful in enhancing human immunity.

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1. Introduction

Human immunity is an important defense mechanism for body to protect itself, which can identify and eliminate foreign invading objects [1]. With economic development and increasingly fierce social competition, hypoimmunity has become one of the health problems for modern people [2]. Hypoimmunity leads to the change of physical status from health to sub-health, and problems such as fatigue, sleep disorder, frequent colds, allergies, mental depression [3–5]. In order to enhance immunity, eating healthy foods with the effect of enhancing immunity may become an effective choice.

Red ginseng is a kind of ginseng product processed by steaming, which has a variety of pharmacological effects and has been widely used [6]. The Pharmacopoeia of China (2020 edition) records that red ginseng is warm in nature, sweet in taste and slightly bitter, and has the effects of tonifying vital energy, restoring pulse, strengthening the meridians and absorbing blood [7]. Modern research has found that red ginseng contains ginsenoside, arginine disaccharide, maltol, trace elements and other components, with enhanced immunity, anti-tumor, anti-oxidation, anti-aging, anti-fatigue, anti-diabetes and other pharmacological effects [8,9]. Korean Red Ginseng (KRG) is a kind of red ginseng that is produced in South Korea and widely used in East Asia [10]. Animal study shows that KRG has distinct immune enhancing effects by increasing the roles of T cells and NK cell in porcine [11].

Because the screening and evaluation method of immune population are not unified, there are relatively few KRG immunity tests for sub-health population. In this study, people with hypoimmunity were taken KRG or placebo for 180 days. Through the randomized controlled trial design, the differences of various immune indicators within and between groups before and after the test were compared. To study the effect of KRG on hypoimmunity people and provide theoretical basis and technical support for improving sub-health status.

2. Materials and methods

2.1. Study population

This randomized, double-blind and placebo-controlled clinical trial study was approved by medical ethics committee of the Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine (2022-011-01). This study was registered with Chinese Clinical Trail Registry (ChiCTR2200059381). The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects have always been observed. Sub-healthy population with hypoimmunity, who signed a consent form for the human study, were assessed based on test criteria. The selected subjects were randomly assigned to the KRG or placebo groups based on their registration order. The inclusion criteria were as follows [12,13]: (1) people aged 20–65 (80 % between 40 and 65 years old), no gender limitation, no nationality limitation; (2) the comprehensive evaluation score of the immune related health score scale was $\leq$60; (3) people with weak constitution and vulnerability to diseases (eligible for one of them): ① colds $\geq$3 times per year, ② bronchitis or pneumonia $\geq$2 times per year, ③ prone to acute diarrhea (except lactose, wheat gum and other food intolerance) $\geq$ 3 times per year, ④ urinary tract infection $\geq$3 times per year; (4) sign the informed consent form. Meanwhile, the exclusion criteria were: (1) subjects who were unable to take the test article orally or as prescribed; (2) subjects over the age of 65, pregnant or lactating women, people who are intolerant or allergic to the test product; (3) those with unclear chief complaint; (4) severe patients with serious occupational diseases; (5) combined with heart, brain, liver, kidney and hematopoietic system and other serious diseases and mental illness; (6) subjects suffer from immune related diseases such as systemic lupus erythematosus, rheumatoid arthritis, systemic vasculitis, scleroderma, pemphigus, dermatomyositis, mixed connective tissue disease, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, Hashimoto thyroiditis, primary myxedema, hyperthyroidism, ulcerative colitis, etc.

2.2. Study design

This study was designed as a randomized, double-blind, placebo-controlled human study. A study drug was JungKwanJang Red ginseng capsule manufactured by the Korean Society of Ginseng, sample appearance: hard capsule, contents are yellowish-brown, product materials: G1899(P) Korean Ginseng Powder (6). Study drug specification: 0.465 g/granule $\times$ 90 granules/bottle, total saponin content 3.5-4.8 g/100 g. The placebo capsule, also provided by the same organization, was identical to the test product in dosage form, taste, appearance, and packaging, except for its efficacy. The ingredients of placebo capsule contain lactose, microcrystalline cellulose, silica, magnesium stearate, tartrazine, allura red, brilliant blue, food flavoring essence. The sample provider is solely responsible for any safety problems caused by the test product. Each subject was randomized in a 1:1 ratio to the KRG or placebo group according to randomized block design. All investigators, participants, and their caregivers were blinded to group assignment throughout the study period. Subjects assigned to the KRG and placebo group received the JungKwanJang Korean Red Ginseng capsule or placebo capsule for 180 days, taking 3 capsules orally twice a day. Subjects need to check safety and efficacy indicators inspection at baseline, efficacy indicators inspection during the mid-term of the test (90th days of administration), safety and efficacy indicators inspection after the test (180th days of administration).

2.3. Observation indicators

2.3.1. Safety indicators

To evaluate the health status, allergies and adverse reactions of the subjects before and after taking KRG or placebo, safety indicators were checked before and after the test as follows: (1) Blood pressure and heart rate. (2) Blood, urine and stool routines. (3) Liver and kidney function. (4) ECG, abdominal B-ultrasound examination and chest X-ray (checked once before the test). (5) Fireness score scale [14]: Inquire and investigate the occurrence of fireness symptoms among participants during the trial, and calculate the number and incidence of fireness symptoms for both groups.

2.3.2. Main efficacy indicators

To evaluate the changes in immunity of the subjects before and after taking KRG or placebo, main efficacy indicators were checked before, mid-term and after the test as follows: (1) Health survey questionnaire: Inquire about the health status and cold occurrence of the subjects in detail, calculate the cold/incidence rate and refer to it when determining efficacy indicators. (2) Immune related health score scale [15]: This questionnaire is divided into four aspects, individual perception (allergies/susceptibility to illness/lethargy, etc.), physiological feelings (happy mood/anxiety/tension/poor memory/forgetful memory, etc.), psychological sensation (appetite/stomach/face/physical strength/-breathing, etc.) and comprehensive score. When scoring, it is necessary to consider the scores of each aspect and the whole questionnaire. The evaluation method of combining the scores of each aspect and the overall score is used to evaluate the effectiveness of the test sample before and after the trial. Compared with self-comparison before and after test, two or more of the four indicators of individual perception, physiological feelings, psychological sensation and comprehensive score increased, and the statistical difference was significant ($P < 0.05$). There was no statistically significant difference ($P > 0.05$) between the placebo control group and the self-comparison group before and after the test. At the same time, at least one of the four indicators in the comparison between the test group and the placebo control group after the trial increased and the statistical difference was significant ($P < 0.05$). The result was that the immune related health scores were positive. (3)

Table 1
Comparison of the balance between the two groups before the test ($\bar{x}$ ±SD).

Name	Test group (n = 52)	Control group (n = 52)
Male/Female	18/34	14/38
Age (years old)	54.37 ± 9.41	54.33 ± 12.65
Immune comprehensive evaluation score	52.69 ± 5.73	53.85 ± 5.25
CD3 (%)	67.35 ± 9.48	69.78 ± 9.16
CD4 (%)	37.91 ± 8.11	40.96 ± 9.12
CD8 (%)	22.92 ± 7.22	23.18 ± 8.01
CD4/CD8	1.81 ± 0.66	2.10 ± 1.16
CD19 (%)	12.18 ± 4.21	12.17 ± 3.19
IgA (g/L)	2.35 ± 0.90	2.30 ± 0.98
IgG (g/L)	13.22 ± 2.04	12.90 ± 2.95
IgM (g/L)	0.93 ± 0.47	1.11 ± 0.58
WBC ($\times 10^9$/L)	6.02 ± 1.50	6.03 ± 1.52

Inter-group comparison $P > 0.05$.

Lymphocyte subsets: CD3, CD4, CD8, CD4/CD8 and CD19 tested by immunofluorescence. (4) Immunoglobulins: IgG, IgA and IgM tested by turbidimetric inhibition immuno assay. (5) White blood cell count (WBC) tested by blood routines on an empty stomach.

2.3.3. Secondary efficacy indicators

To study the possible changes in immunity of the subjects before and after taking KRG or placebo, secondary efficacy indicators were checked before, mid-term and after the test as follows: (1) Cytokines: TNF-α, IFN-γ, IL-2 and IL-4 tested by flow cytometry. (2) NK tested by immunofluorescence. (3) Monocytes and neutrophils tested by blood routines on an empty stomach.

2.4. Data statistical analysis and result judgment

2.4.1. Data statistical analysis

The test data is measurement data, which can be analyzed by *t*-test. Where self-control data can use paired *t*-test, the comparison of two groups of means uses grouped *t*-test, and the latter needs homogeneity test of variance. For data with non-normal distribution or uneven variance, appropriate variable conversion should be performed. After the normal variance is satisfied, *t*-test is performed with the converted data. If the converted data still cannot meet the requirement of normal homogeneity of variance, t'-test or the rank sum test shall be used, but rank sum test shall be applied if the coefficient of variation is too large (such as CV>50 %).

2.4.2. Result judgment

At least one of the main efficacy indicators is evaluated as positive if there is a significant change in self-comparison between before and after the test of the test group and inter-group comparison with the control group after the test, then the study drug has the effect of enhancing immunity.

3. Clinical trial results

3.1. Recruiting and screening of the subjects

After hospital screening, 119 subjects met the inclusion and exclusion criteria in the human feeding trial plan and signed informed consent forms. 119 subjects were included and randomly divided into a test group and a control group. During the experiment, there were 8 cases in the test group and 7 cases in the control group who did not recheck on time at the specified time or did not take the test article according to regulations, which affected the efficacy or safety judgment. Meet the exclusion criteria, shedding criteria of subjects. There were 52 valid subjects in the test group and 52 cases in the control group. The test group and the control group had deletion rates of 13.3 % and 11.9 % respectively.

3.2. Observation results of safety indicators

Before the test, the grouping of the subjects is shown in Table 1. The two groups were comparable in age, gender, immune comprehensive evaluation score, CD3, CD4, CD8, CD4/CD8, CD19, IgG, IgA, IgM and WBC without significant differences before the test. The abdominal B-ultrasound, ECG and chest X-ray in the test group and the control group were all within the normal range.

Table 2 shows that the blood pressure and heart rate of the subjects were basically within the normal range before and after the test; the blood routine (except for WBC, monocytes and neutrophils), urine routine, stool routine, liver and kidney function indicators of the test group and the control group before and after the test are basically within the normal range; there is no significant difference in fireness score between the test group and the control group in both self-comparison and inter-group comparison.

Through follow-up interviews, the test group (n = 52) and the control group (n = 52) had no adverse reactions and allergic reactions such as nausea, flatulence, diarrhea, and abdominal pain during the test period.

Table 2
Changes of safety indicators before and after the test ($\bar{x}$ ±SD).

	Test group (n = 52)		Control group (n = 52)	
	Before test	After test	Before test	After test
Systolic pressure (mm/Hg)	134.21 ± 16.91	132.54 ± 15.04	131.44 ± 20.73	130.67 ± 19.35
Diastolic pressure (mm/Hg)	83.96 ± 11.31	83.13 ± 10.56	81.65 ± 9.69	81.19 ± 8.83
Heart rate (times/minute))	69.25 ± 11.44	69.13 ± 10.98	66.73 ± 9.28	67.08 ± 9.04
RBC ($\times 10^{12}$/L)	4.58 ± 0.34	4.54 ± 0.40	4.55 ± 0.43	4.47 ± 0.38
PLT ($\times 10^9$/L)	249.85 ± 69.00	265.46 ± 69.40	250.04 ± 58.44	265.46 ± 60.86
HGB (g/L)	138.65 ± 12.04	137.08 ± 12.84	137.50 ± 13.34	134.52 ± 12.26
TP (g/L)	77.73 ± 3.83	76.73 ± 3.92	76.58 ± 4.53	75.20 ± 4.43
Alb (g/L)	46.81 ± 1.83	46.43 ± 2.76	46.55 ± 2.32	46.53 ± 2.06
ALT (U/L)	16.54 ± 6.95	19.46 ± 10.55	15.85 ± 7.42	19.23 ± 11.12
AST (U/L)	18.00 ± 3.50	20.58 ± 9.91	17.62 ± 4.63	18.38 ± 10.52
Urea (mmol/L)	5.73 ± 1.63	5.30 ± 1.41	5.42 ± 1.28	5.03 ± 1.14
Cre (μmol/L)	68.09 ± 13.46	69.18 ± 13.59	66.01 ± 12.81	66.71 ± 12.23
FPG (mmol/L)	5.70 ± 1.06	5.68 ± 1.12	5.65 ± 1.04	5.62 ± 1.18
Urine routine	Normal	Normal	Normal	Normal
Stool routine	Normal	Normal	Normal	Normal
fireness score	42.38 ± 14.38	42.04 ± 13.56	41.10 ± 14.42	40.96 ± 13.17

Self-comparison and inter-group comparison $P > 0.05$.

Table 3
Comparison of general conditions in the two groups before and after the test.

Item	Result	Test group (n = 52) N (%)			Control group (n = 52) N (%)		
		Before test	Mid-term	After test	Before test	Mid-term	After test
Whether allergic constitution	Yes	11 (21.2)	/	/	8 (15.4)	/	/
	No	41 (78.8)	/	/	44 (84.6)	/	/
Had a cold in nearly a month	Yes	37 (71.2)	26 (50.0)	40 (76.9)	39 (75.0)	38 (73.1)	47 (90.4)
	No	15 (28.8)	26 (50.0)	12 (23.1)	13 (25.0)	14 (26.9)	5 (9.6)
Number of visits to hospital due to illness in nearly a month	0	33 (63.5)	39 (75.0)	42 (80.8)	31 (59.6)	32 (61.5)	40 (76.9)
	1	15 (28.8)	12 (23.1)	10 (19.2)	16 (30.8)	15 (28.8)	12 (23.1)
	2	4 (7.7)	1 (1.9)	0 (0.0)	5 (9.6)	5 (9.6)	0 (0.0)
Had nucleic acid testing done in nearly two months	Yes (Negative)	52 (100.0)	52 (100.0)	6 (11.5)	52 (100.0)	52 (100.0)	3 (5.8)
	Yes (Positive)	0 (0.0)	0 (0.0)	46 (88.5)	0 (0.0)	0 (0.0)	49 (94.2)
	No	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Whether to take medicine at this stage	Yes	14 (26.9)	12 (23.1)	46 (88.5)	13 (25.0)	12 (23.1)	49 (94.2)
	No	38 (73.1)	40 (76.9)	6 (11.5)	39 (75.0)	40 (76.9)	3 (5.8)
Whether take other health foods during the test	Yes	/	0 (0.0)	0 (0.0)	/	0 (0.0)	0 (0.0)
	No	/	52 (100.0)	52 (100.0)	/	52 (100.0)	52 (100.0)
Number of colds during the test (times)	0	/	12 (23.1)	4 (7.7)	/	4 (7.7)	0 (0.0)
	1	/	28 (53.8)	38 (73.1)	/	29 (55.8)	40 (76.9)
	2	/	11 (21.2)	5 (9.6)	/	16 (30.8)	8 (15.4)
	3	/	1 (1.9)	4 (7.7)	/	2 (3.8)	4 (7.7)
	3+	/	0 (0.0)	1 (1.9)	/	1 (1.9)	0 (0.0)
Cure mode	self-healing	/	21 (52.5)	3 (6.3)	/	24 (50.0)	1 (1.9)
	take medicine	/	19 (47.5)	45 (93.7)	/	24 (50.0)	51 (98.1)
Cure Time (Days)		/	7.06 ± 4.61	8.23 ± 3.34#	/	8.08 ± 3.87	9.54 ± 2.72*
Incidence of cold during the test		/	76.9 %	92.3 %	/	92.3 %	100 %

Self-comparison *$P < 0.05$; inter-group comparison #$P < 0.05$.

Table 4
Changes of immune related health score scale ($\overline{x}$ ±SD).

	Test group (n = 52)			Control group (n = 52)		
	Before test	Mid-term	After test	Before test	Mid-term	After test
Comprehensive score	52.69 ± 5.73	59.62 ± 5.44**##	60.27 ± 4.79**##	53.85 ± 5.25	54.67 ± 4.81	53.33 ± 4.13
Individual perception	16.40 ± 2.06	18.52 ± 1.81**##	18.73 ± 1.59**##	16.54 ± 1.90	16.79 ± 1.67	16.48 ± 1.46
Psychological feelings	18.50 ± 2.17	20.79 ± 2.00**##	20.92 ± 1.78**##	19.08 ± 2.19	19.33 ± 2.06	18.79 ± 1.74
Physiological sensation	17.79 ± 1.83	20.31 ± 1.90**##	20.62 ± 1.69**##	18.23 ± 1.86	18.56 ± 1.64	18.06 ± 1.38

Self-comparison **$P < 0.01$; inter-group comparison ##$P < 0.01$.

3.3. Observation results of efficacy indicators

3.3.1. The result of health survey questionnaire and cold incidence questionnaire

See Table 3 for the result of health survey questionnaire. Self-comparison of the control group in the mid-term and after the test, cold cure time increased significantly ($P < 0.05$). Inter-group comparison of the test group and the control group after the test, cold cure time of the test group was lower than that of the control group ($P < 0.05$).

3.3.2. The result of immune related health score scale

See Table 4 for the result of immune related health score scale. Self-comparison of the test group in before and after the test, comprehensive score, individual perception, psychological feelings and physiological sensation increased significantly ($P < 0.01$). Inter-group comparison of the test group and the control group after test, comprehensive score, individual perception, psychological feelings and physiological sensation of the test group was higher than that of the control group ($P < 0.01$).

3.3.3. The result of immune indicators

See Table 5 for the result of lymphocyte subsets, immunoglobulins, cytokines and other indicators. Self-comparison of the test group in before and after the test, CD3 and CD4/CD8 increased significantly ($P < 0.05$). Self-comparison of the test group in before and after the test, IgG, IgA, IgM and WBC increased significantly in the mid-term and after the test ($P < 0.01$). Inter-group comparison of the test group and the control group in the mid-term and after the test, IgA of the test group was higher than that of the control group ($P < 0.05$). Inter-group comparison of the test group and the control group in the mid-term, WBC of the test group was higher than that of the control group ($P < 0.05$). Self-comparison of the control group in before and after the test, IgG increased significantly after the test ($P < 0.05$). Self-comparison of the test group in before and after the test: TNF-α decreased significantly in the mid-term ($P < 0.05$), IFN-γ decreased significantly in the mid-term ($P < 0.01$), NK increased significantly in the mid-term and after the test ($P < 0.05$), monocytes increased significantly in the mid-term and after the test ($P < 0.01$). Inter-group comparison of the test group and the control group in the mid-term, neutrophils of the test group were higher than that of the control group ($P < 0.05$). Inter-group comparison of the test group and the control group in the mid-term and after the test, monocytes of the test group were higher than that of the control group ($P < 0.05$).

4. Conclusion

119 subjects were enrolled, and the final number of effective cases was 104, which 52 cases were in the test group and 52 cases in the control group. The drop-out rates of subjects in the test group and control group were 13.3 % and 11.9 % respectively. The balance test was performed before the feeding test, and the two groups were comparable in age, gender, immune comprehensive evaluation score, CD3, CD4, CD8, CD4/CD8, CD19, IgG, IgA, IgM and WBC without significant differences.

The blood pressure, heart rate, blood routine, urine routine, stool routine, liver and kidney function of the test group and the control group were all within the normal range before and after the test. Before the

Table 5
Changes of immune indexes ($\overline{x}$ ±SD).

	Test group (n = 52)			Control group (n = 52)		
	Before test	Mid-term	After test	Before test	Mid-term	After test
CD3 (%)	67.35 ± 9.48	66.88 ± 9.78	69.54 ± 9.04*	69.78 ± 9.16	68.05 ± 10.36*	68.73 ± 9.49
CD4 (%)	37.91 ± 8.11	38.94 ± 7.43	39.33 ± 6.56	40.96 ± 9.12	40.34 ± 8.86	41.13 ± 8.24
CD8 (%)	22.92 ± 7.22	21.73 ± 7.33	21.72 ± 6.84	23.18 ± 8.01	21.95 ± 7.38	22.51 ± 7.56
CD4/CD8	1.81 ± 0.66	1.96 ± 0.62	1.97 ± 0.64*	2.10 ± 1.16	2.13 ± 1.05	2.13 ± 1.04
CD19 (%)	12.18 ± 4.21	11.94 ± 3.68	11.90 ± 3.73	12.17 ± 3.19	11.44 ± 4.07	11.14 ± 3.53*
IgA (g/L)	2.35 ± 0.90	2.84 ± 1.02**#	2.76 ± 0.86**#	2.30 ± 0.98	2.43 ± 0.93	2.39 ± 0.97
IgG (g/L)	13.22 ± 2.04	13.92 ± 2.67*	13.90 ± 2.06*	12.90 ± 2.95	13.37 ± 3.54	13.47 ± 3.04*
IgM (g/L)	0.93 ± 0.47	1.20 ± 0.53**	1.06 ± 0.46*	1.11 ± 0.58	1.15 ± 0.56	1.00 ± 0.50*
WBC ($\times 10^9$/L)	6.02 ± 1.50	6.47 ± 1.46*#	6.47 ± 1.51*	6.03 ± 1.52	5.97 ± 1.10	6.21 ± 1.54
TNF-α (pg/ml)	2.63 ± 0.77	2.23 ± 0.90*	2.90 ± 0.88	2.86 ± 1.08	2.42 ± 1.41	3.07 ± 0.99
INF-γ (pg/ml)	2.10 ± 0.73	1.54 ± 1.28**	2.36 ± 0.71	2.19 ± 0.64	1.46 ± 1.10**	2.62 ± 1.01*
IL-2 (pg/ml)	1.81 ± 0.83	1.72 ± 1.47	1.56 ± 0.70	1.91 ± 0.61	1.77 ± 1.53	1.41 ± 0.51**
IL-4 (pg/ml)	2.81 ± 0.83	2.50 ± 1.28	2.71 ± 1.53	3.04 ± 1.03	2.72 ± 1.40*	2.68 ± 0.90*
NK (%)	18.95 ± 9.72	20.63 ± 10.01*	21.26 ± 9.57*	17.18 ± 8.34	19.93 ± 9.48**	19.18 ± 8.48*
Monocytes ($\times 10^9$/L)	0.41 ± 0.14	0.49 ± 0.18**#	0.50 ± 0.17**#	0.41 ± 0.15	0.42 ± 0.13	0.43 ± 0.16
Neutrophils ($\times 10^9$/L)	3.55 ± 1.15	3.82 ± 1.06#	3.75 ± 1.15	3.33 ± 1.12	3.36 ± 0.94	3.47 ± 1.05

Self-comparison $^{**}P < 0.01$ $^{*}P < 0.05$; inter-group comparison $\#P < 0.05$.

Table 6
Comparison of COVID-19 symptom questionnaire and scores.

Item	Level	Test group (n = 46)	Control group (n = 49)	P
Age (years old)		54.52 ± 9.04	54.53 ± 12.03	0.997
Gender	male	17 (37.0 %)	13 (26.5 %)	0.275
	female	29 (63.0 %)	36 (73.5 %)	
Number of vaccinations (times)		2.98 ± 0.15	2.98 ± 0.14	0.964
Number of colds in daily life		3.15 ± 0.70	3.29 ± 0.76	0.377
Vaccine companies	SINOVAC BIOTECH CO., LTD.	20 (43.5 %)	19 (38.8 %)	0.641
	BEIJING INSTITUTE OF BIOLOGICAL PRODUCTS CO., LTD	26 (56.5 %)	30 (61.2 %)	
Severity of COVID-19		6.11 ± 0.61	6.55 ± 0.61	0.001
Symptom	/	**Test group (n = 46)**	**Control group (n = 49)**	**P**
Fever		5.89 ± 1.49	6.45 ± 1.32	0.057
Cough		6.02 ± 2.06	6.57 ± 1.63	0.155
Sore throat		6.46 ± 1.60	6.47 ± 1.42	0.967
Muscle soreness		5.98 ± 1.88	6.08 ± 2.00	0.796
Dizzy		5.72 ± 1.52	6.57 ± 1.63	0.010
Loss of taste		5.89 ± 1.68	6.02 ± 1.90	0.727
Fatigue (lack of strength)		5.93 ± 1.63	6.71 ± 1.47	0.016
Memory disorder		5.39 ± 1.82	5.39 ± 2.02	0.993
Insomnia		4.83 ± 1.82	4.82 ± 1.82	0.979

test, the subjects' chest X-ray, ECG, and abdominal B-ultrasound were all within the normal range. There was no significant difference in fireness score between the test group and the control group compared before and after the test, as well as between the groups. There was no allergy or adverse reaction during the test, indicating that this product had no adverse effects on the health of the subjects.

Self-comparison of the test group in before and after the test, comprehensive score, individual perception, psychological feelings and physiological sensation increased significantly in the mid-term and after the test ($P < 0.01$); CD3 and CD4/CD8 increased significantly after the test ($P < 0.05$); IgG, IgA, IgM and WBC increased significantly in the mid-term and after the test ($P < 0.01$); TNF-α decreased significantly in the mid-term ($P < 0.05$), IFN-γ decreased significantly in the mid-term ($P < 0.01$), NK increased significantly in the mid-term and after the test ($P < 0.05$), monocytes increased significantly in the mid-term and after the test ($P < 0.01$).

Inter-group comparison of the test group and the control group, comprehensive score, individual perception, psychological feelings and physiological sensation of the test group was higher than that of the control group in the mid-term and after the test ($P < 0.01$); IgA of the test group was higher than that of the control group in the mid-term and after the test ($P < 0.05$); WBC of the test group was higher than that of the control group in the mid-term ($P < 0.05$); neutrophils of the test group were higher than that of the control group in the mid-term ($P < 0.05$); monocytes of the test group were higher than that of the control group in the mid-term and after the test ($P < 0.05$).

According to the standard of clinical trial scheme, immune related health scores and IgA in main efficacy indicators are positive, it can be shown that KRG has reached the positive result of human food test to help enhance human immunity.

5. Discussion

A study has proved that KRG can enhance the immune system by increasing T cells, B cells and WBC by analyzing the intake of oral KRG tablets for 8 weeks in healthy adults [16]. The immune system is the basis for human body to resist pathogenic microorganisms, which can be divided into innate immune system and acquired immune system [17]. However, immune responses will gradually weaken with advancing age, and immunocompetence may be impaired as early as 35–40 years old [18]. Therefore, the people aged between 40 and 65 years old in this study accounts for 80 % of the included population. Then the people with hypoimmunity are screened out by the comprehensive evaluation score of the immune related health score scale and the standard of people with weak constitution and vulnerability to diseases. Among the main efficacy indicators designed in this study as the basis for judging the efficacy of KRG, lymphocyte subsets and immunoglobulins belong to the acquired immune indicators, and WBC belongs to the innate immune

indicators. At the same time, the secondary efficacy indicators were designed to study more effects of KRG. Among them, NK, monocytes and neutrophils belong to the innate immune indicators, cytokines are proteins that can interact with both innate immune system and acquired immune system.

This study examined the effect of KRG in enhancing immunity, immune related health scores, IgA and monocytes achieved positive results. Through the results of immune-related health score, it is found that the subjects have improved in comprehensive score, individual perception, psychological feeling and physiological sensation. IgA has an important immune barrier function, which can prevent pathogens from invading the body [19]. KRG and KRG-P (KRG polysaccharide) can up-regulate the IgA level in the intestine of mice [20]. The results of this study further show that KRG up-regulates IgA to help enhance immunity. An essential function of monocytes is to seed tissues with sufficient macrophages to replace loss from infection and tissue damage [21]. Therefore, people often study more macrophages that can play an important role in immune response. For example, red ginseng acidic polysaccharide has been proved to increase the phagocytic activity and quantity of macrophages in cyclophosphamide immunosuppressed mice [22]. At present, there are few reports on the effects of KRG on monocytes in immunity. Our research results indicate that KRG may enhance immunity by increasing the number of monocytes in blood, but the mechanism needs further exploration.

The results of health survey questionnaire showed that 46 people in the test group and 49 people in the control group had been diagnosed as positive for COVID-19. This was due to the widespread spread of COVID-19 in China around the 155th day of the test, the occurrence of this incident may have affected cold cure time and incidence of cold. A study revealed the production of COVID-19 patients produce IgM and IgG within 3 weeks after symptoms appear, and humoral immune response developed within 3–7 weeks after infection, with a stepwise increase of IgG and decreasing of IgM [23]. The results of this study are consistent with its trend, IgG in the control group increased significantly after the test than before, the possible reason is that almost all the subjects in the control group are in the period of COVID-19 infection or recovery after the test, and there is an inflammatory reaction in the body. The results of cytokines showed that TNF-α in the test group decreased significantly in the mid-term and increased after the test than before, IFN-γ in the test group decreased significantly in the mid-term and increased after the test than before, IFN-γ in control group decreased significantly in the mid-term and increased significantly after the test than before, the possible reason is that the subjects included in this study are immunocompromised people who are prone to allergies, colds, urinary tract infections and other symptoms, the level of inflammatory factors in the subjects relatively high before the test. The inflammatory reaction in the subjects may be relieved in the mid-term, resulting in a corresponding decrease in helper T cell-mediated type 1 (TH1) response, thus reducing the production of proinflammatory cytokine TNF-α and IFN-γ. After test, almost all the subjects in the control group and the test group were in the period of COVID-19 infection or recovery, and there was an inflammatory reaction in the body. According to a study, ginseng may correct the immune dysfunction in COVID-19 patients by regulating the balance of pro-inflammatory and anti-inflammatory factors, antiviral and regulating immune cells, so as to prevent or alleviate cytokine storms and reduce the occurrence of lung injury [24]. In this study, the test group took KRG capsule, which may help to increase the release of TNF-α and IFN-γ, thus activating macrophages, enhancing their ability to kill phagocytosed pathogens, and promoting the generation of IgG. WBC include neutrophils, lymphocytes, monocytes, eosinophils and basophils [25]. Among them, neutrophils account for 50%-70 %, lymphocytes account for 20%-40 %, monocytes account for 3%-8%, eosinophils account for 0.5%-5%, and basophils account for 0%-1% [26]. Because the number of monocytes, eosinophils and basophils is relatively small in WBC, from the results of this study, we guess that the significant changes in the comparison of WBC in the test group may mainly come from the changes in the number of lymphocytes. The reason why there was no significant difference between two groups of WBC after the test may be that the number of WBC in the control group increased due to COVID-19. However, the results of lymphocyte subsets, IL-2, IL-4 and NK cannot analyze the obvious trend changes, possibly be caused by problems such as small sample size or short administration time. It is necessary to adjust the method and conduct in-depth research in the future.

In addition, we added survey statistics to this study for subjects who were judged to be COVID-19 positive in the experiment. Some of them went to the hospital for throat swab sampling and were diagnosed as positive by fluorescence quantitative PCR; some people took throat swab samples in the nucleic acid pavilion (the nucleic acid pavilion is the place where every resident community conducts professional nucleic acid testing in China) and found that they were positive by fluorescence quantitative PCR detection; and the other part used COVID-19 antigen detection kit produced by Cofoe, Winner, Andon and other companies to collect nasal swab samples, and the judgment results were T-ray positive by colloidal gold method. See Table 6 for the results of COVID-19 symptom questionnaire and scores. The two groups were comparable in age, gender, number of vaccinations, number of colds in daily life and vaccine companies without significant differences ($P > 0.05$). Inter-group comparison of test group and control group, severity of COVID-19 of test group were lower than that of control group ($P < 0.01$). The COVID-19 symptom scores survey was scored by the subjects themselves, with a standard definition of 5 points for cold symptoms, ranging from less than 5 points for mild symptoms to more than 5 points for severe symptoms. Inter-group comparison of test group and control group, dizzy and fatigue (lack of strength) of test group were lower than that of control group ($P < 0.05$). Other COVID-19 symptom scores such as fever, cough, muscle soreness and loss of taste were lower than those in the control group, but the comparison between groups was not significant. It is suggested that KRG may be helpful to reduce the risk of severity or improve the symptoms of COVID-19, but further specific research is needed on this effect.

Declaration of competing interest

All authors have no conflicts of interest to declare.

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