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Research article

A single-center, randomized, double-blind, placebo-controlled study on the efficacy and safety of "enzyme-treated red ginseng powder complex (BG11001)" for antiwrinkle and proelasticity in individuals with healthy skin

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ABSTRACT

Background: During the aging process, skin shows visible changes, characterized by a loss of elasticity and the appearance of wrinkles due to reduced collagen production and decreased elasticity of elastin fibers. *Panax ginseng* Meyer has been used as a traditional medicine for various diseases due to its wide range of biological activities including skin protective effects. Ginsenosides are the main components responsible for the biological activities of ginseng. However, the protective activities of an enzymatic preparation of red ginseng against human skin aging have not been investigated.
Methods: The efficacy of an enzyme-treated powder complex of red ginseng (BG11001) in preventing human skin aging was evaluated by oral administration to 78 randomized individuals. All patients were requested to take three daily capsules containing either 750 mg of BG11001 or a placebo vehicle for 24 wk; at the end of the testing period, skin roughness, elasticity, and skin water content were measured.
Results: BG11001 significantly reduced the average roughness of eye wrinkles and the Global Photo Damage Score compared with the placebo, although there were no significant differences in arithmetic roughness average between the groups. In addition, gross elasticity and net elasticity values increased, and transepidermal water loss level decreased, indicating improved skin elasticity and moisture content.
Conclusion: In conclusion, enzyme-treated red ginseng extract significantly improved eye wrinkle roughness, skin elasticity, and moisture content. Moreover, enzyme-treated red ginseng extract would be useful substance as a bio-health skin care product.

1. Introduction

Aging is a dynamic and highly complex process defined by multiple physiological changes over time. All bodily systems are subject to the aging process and the integumentary system is not an exception. The human skin covers the whole outer body, which makes the skin the largest organ of the integumentary system. The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments, and internal organs. During the process of aging, the skin changes show the most visible signs characterized by decreased elasticity, increased roughness, uneven skin tone with dark spots, and the formation of wrinkles [1]. Wrinkles on the face are the most dominantly recognized signs of skin aging [2]. Over time, the epidermis becomes thinner, even though the number of cell layers remains unchanged. Not only does the dermal layer thin, but also less collagen is produced, and the

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changes in the connective tissue reduce the skin's strength and elasticity [3,4]. These changes in the structure of the skin cause the skin to wrinkle and slacken. Facial skin sites like the corners of the eyes are especially susceptible to wrinkle formation, which is also popularly known as "crow's feet".

The maintenance of younger-looking skin is constantly desired by a large proportion of the world's population. Therefore, many institutes and cosmetic and pharmaceutical companies have been trying to develop functional cosmetic materials from natural substances such as herbs, roots, essential oils, and flowers. *Panax ginseng* has a history of medical use for over 5,000 yr. *Panax* derived from Greek word "*Panakos*" presents *Pan*—meaning "all" and akos meaning "cure". Ginseng is said to mean "wonder of the world." Like the literal meaning of its name, *Panax ginseng* Meyer has been used as a traditional medicine for various diseases with wide range of biological activities, including anti-inflammatory [5], antioxidant [6], antitumor [7], and antistress effects [8]. In recent studies, researchers investigated the protective effects of *Panax ginseng*, against the UVB-irradiation on epidermal keratinocytes and dermal fibroblasts. They found that ginseng recovered the UVB-induced decrease in antiapoptotic gene expression in the human keratinocytes and dermal fibroblast, indicating that ginseng can protect cells from apoptosis caused by strong UVB radiation [9]. Another study showed that ginseng extract induced type I collagen production in human dermal fibroblast cells by activation of Smad signaling, suggesting ginseng as a potential candidate as a wrinkle-reducing agent by topical application [10].

Ginsenosides are the pharmacologically active components and are responsible for the biological functions in ginseng. Among more than 50 isolated ginsenosides, major ones (Rb1, Rb2 , Rc, Rd, Re, Rg1, and Rf) constitute more than 80% of the total ginsenosides and the minor ginsenosides (F1, F2, Rg3, Rh1, Rh2 compound Y, compound Mc, and compound K) are present at low concentrations in ginseng [11]. Many studies show that the minor ginsenosides have pharmacologically active than major ones because absorption of major ginsenosides by the gastrointestinal tract is quite poor [12]. As a result, the minor ginsenosides have been demonstrated to be pharmaceutically active and excellent potential drug candidates [13]. However, hydrolysis of sugar moieties to convert major ginsenosides to minor forms by digestive enzymes in gastrointestinal tract are quite low even though the minor ginsenosides are more easily absorbed into the bloodstream [12]. Since it is possible to transform into minor ginsenosides by enzyme treatment, enzyme-treated red ginseng has been shown to have strong antiwrinkle activity and reduced toxicity in *in vitro* and animal studies [14]. Also, our previous studies demonstrated that enzyme-treated ginseng protected UVB-induced skin damage through the regulation of procollagen type I and matrix metalloproteinase (MMP)-1 expression in hairless mice [15,16]. However, the protective activity of the enzymatic preparation of red ginseng against human skin aging has not been investigated. In this study, we investigated whether enzyme-treated powder complex of red ginseng (BG11001) prevents human skin aging by reducing skin wrinkles and enhancing elasticity.

2. Materials and methods

2.1. Preparation of enzyme-treated powder complex of red ginseng (BG11001)

Enzyme-treated extract of red ginseng was prepared following a patented protocol [Korea patent no. 10-2011-0091287 (in private), in press] [15]. Red ginseng powder with 10 times volume of distilled water was mixed for 2 h using a homo-mixer and then enzyme treated for 24 h at 55°C. Enzyme-treated red ginseng was heated up to 90°C, cooled down to 10–15°C, and then centrifuged. Supernatant was concentrated, added 10 times volume of 50% ethanol, and extracted for 40 min at 85°C. Finally, the same amount of malt dextrin was added, spray dried and used as raw material of BG11001.

For thin layer chromatography analysis of the ginsenoside compositions, the total ginsenosides were spotted together with the standard 20(S)-protopanaxdiol (PPD) or 20(S)-protopanaxtriol samples on thin layer chromatography plate (silica gel 60 F254, Merck) containing $CHCl_3$-MeOH-H_2O (65:35:10, v/v), thereafter stained by spraying with 30% H_2SO^4, followed by heating at 105°C.

2.2. Study design

This study was designed as a randomized, double-blind study to assess the effects of 250 mg of oral BG11001 given thrice a day for 24 wk of the trial period in patients with cutaneous photoaging. The compositions of the BG11001 and placebo tablets are shown in Table 1.

2.3. Global photodamage score

Patients' periorbital wrinkles were evaluated based on a global photodamage score (0, none; 1, none/mild; 2, mild; 3, mild/moderate; 4, moderate; 5, moderate/severe; 6, severe; 7, very severe) at Wk 0 (baseline). If the investigators' evaluations differed, low-grade efficacy and high-grade adverse effect were selected. The patients' periorbital wrinkles were classified into eight grades.

2.4. Participants

Ninety-eight healthy Asian women, aged between 40 and 60 yr, clinically diagnosed with a global photodamage score of 2–6 according to the Jung score of photoaging of facial skin were recruited and included in this study after written informed consent. Those who experienced any esthetic procedure like peeling, laser, intense pulsed light, dermabrasive therapies, or have used any antiaging cream or nutritional supplement within the past 3 mo could not participate in the study. Participants were requested not to expose themselves to sunlight during the trial. They were also requested not to use lotions, creams, or other products on the face and forearms. Participants agreed to follow these instructions during the

Table 1
Compositions of the BG11001 and placebo tablets

Ingredient	Tablet (mg)	Percentage (%)
BG11001 tablet		
BG11001	250	41.7
Microcrystalline cellulose	88.4	14.7
Dextrin	150	25
Maltitol syrup powder	96	16
Magnesium stearate	9	1.5
Silicon dioxide	3	0.5
HPMC	3.3	0.55
Glycerol fatty acid ester	0.3	0.05
Total	600	100
Placebo tablet		
Microcrystalline cellulose	210	35
Lactose powder	331.5	55.25
Caramel coloring	1.32	0.22
Gardenia yellow pigment	0.78	0.13
Maltitol syrup powder	43.5	7.25
Magnesium stearate	9.3	1.55
HPMC	3.3	0.55
Glycerol fatty acid ester	0.3	0.05
Total	600	100

HPMC, hydroxypropylmethylcellulose.

trial period. On completion of the abovementioned enrollment process of the participants in the study, the study coordinator allocated them into each group.

2.5. Groups

Participants were randomly allocated into two groups which were of the same size and matched on the basis of global photodamage score and age. A computer-generated table of random numbers was prepared in advance and was later used to randomly allocate participants into one of the two groups. Participants in the BG11001 experimental group were supplemented with "enzyme-treated powder complex of red ginseng (BG11001)" capsules and those in the placebo control group with placebo capsules. Those who used any nutritional supplement other than the test material during the trial period were dropped from the trial.

The trial started in the summer of 2012 and was completed in the winter of 2013. The protocol has been reviewed and approved by the Ethics Committee (Oriental Hospital of Se-Myung University, Jecheon, South Korea) and was approved in June 2012. The study was carried out in accordance to the Declaration of Helsinki (1964) changed in Tokyo (2004) and Seoul (2008).

2.6. Treatment

All patients were requested to take three capsules daily for 24 wk. The capsules were prepared to contain either 250 mg of BG11001 or the excipients of lactose powder, microcrystalline cellulose, and powered maltitol syrup. Participants were instructed to take one capsule in the morning, another in the afternoon, and the other in the evening with a glass of water. Placebo and BG11001 capsules were identical in color, taste, odor, and packaging and their content was blinded to the participants and investigator.

2.7. Blinding

Only the study coordinator knew the group allocation and prepared the capsules for both groups accordingly. Both the participants and the investigator were blinded to the information on the group allocation until the end of the study. Allocation concealment was maintained using an opaque envelope. Group allocation information was opened to the participant at the end of the trial period.

2.8. Compliance

Those participants whose compliance was under 80% were dropped from the study.

2.9. Serology

All parameters were measured at baseline and after 24 wk of supplementation. Blood samples were collected from fasting participants at baseline and after a 24-wk supplementation. To evaluate the safety of oral administration of BG11001, creatinine, albumin, total protein, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, gamma-glutamyltransferase of serum were determined. Other parameters analyzed were white blood cells, red blood cells, mean corpuscular volume, hemoglobin, hematocrit, and platelet count. Specific gravity, pH, protein, glucose, blood (red blood cells), human chorionic gonadotropin of urine were also investigated.

2.10. Noninvasive measurements of the skin

All measurements were performed under standardized conditions, i.e., room temperature of 22 ± 2°C and a relative humidity level of 45 ± 5%. An acclimatization time of at least 30 min was respected before measurements started.

Microrelief (roughness) of the skin was measured with a skin visiometer SV 600 (Courage-Khazaka, Cologne, Germany). Investigated roughness parameters were R3 (average roughness) and R5 (arithmetic average roughness). Elastic properties of the skin were measured with a Cutometer (Courage-Khazaka, Cologne, Germany). Investigated elastic parameters were R2 (gross elasticity) and R5 (net elasticity). Hydration, transepidermal water loss (TEWL), elastic properties, and microrelief of the skin were evaluated on the crow's feet area at baseline and after 24 wk of supplementation with the following noninvasive methods. Hydration level and TEWL of the skin surface was measured with the Corneometer CM 825 (Courage-Khazaka, Cologne, Germany) and Tewameter TM300 (Courage-Khazaka, Cologne, Germany). Elastic properties of the skin were measured with the Cutometer (Courage-Khazaka, Cologne, Germany).

2.11. Statistical analysis

Results were determined using PRISM Statistical Analysis System (Version 5, GraphPad, La Jolla, CA, USA). Differences between groups were evaluated with Student *t* test and differences within groups were analyzed with analysis of covariance. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Ginsenoside composition changes of red ginseng powder by enzyme treatment

When red ginseng powder was enzyme treated, ginsenoside compositions were changed which showed significantly increased F2, Rd+Re, F1+Rh1, and C-K (Fig. 1 and Table 2). Even if enzyme-treated red ginseng contained relatively high amounts of ginsenosides-Rd and Re, ginsenosides Re as a 20(S)-protopanaxtriol type saponin [17] was almost unhydrolyzed by the enzyme. Ginsenoside-Rd is an intermediate product hydrolyzed from PPD type saponin (e.g., ginsenosides-Rb1, -Rb2, and Rc), thus, it has a

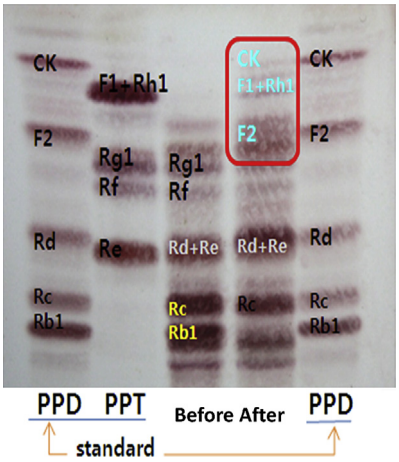

Fig. 1. Thin liquid chromatography profiles of crude red ginseng ginsenosides before and after treatment of enzyme. CK, compound K; PPD, 20(S)-protopanaxdiol (PPD); PPT, 20(S)-protopanaxtriol.

Table 2
Changes in ginsenoside composition

Ginsenoside	Content (%)		Changes (%)	
	Before	After		
C-K	—	6.41	↑	6.41
F1+Rh1	—	7.26	↑	7.26
F2	—	40.78	↑ ↑	40.78
Rg1	20.22	—	↓ ↓	20.22
Rf	12.60	—	↓ ↓	12.60
Rd+Re	14.51	27.17	↑ ↑	12.66
Rc	25.27	18.38	↓	6.89
Rb1	25.27	—	↓ ↓	25.27

C-K, compound K.

high possibility to switch to other saponins. Ginsenoside C-K and F1+Rh1 were also increased in enzyme-treated red ginseng, but the portion of the total amount from those ginsenosides was very small. In the case of ginsenosides-F2, it is also hydrolyzed from a PPD-based major saponin, but the F2 content of the enzyme-treated red ginseng was relatively high and with content variation (coefficient of variation) of each batch was quite low (11% in lab scale and 5.7% in scale-up). In addition, our previous studies demonstrated that enzyme-treated ginseng (index material: ginsenosides F2) protected UVB-induced skin damage through the regulation of procollagen type I and MMP-1 expression in hairless mice [15,16]. Therefore, ginsenoside-F2 is considered as an index material of enzyme-treated red ginseng (BG11001).

3.2. Demographics of participants

In total, 98 women were enrolled and randomized into two groups—placebo and BG11001. Twenty out of the initial 98 enrolled women were excluded from subsequent analysis for the following reasons: (1) agreement withdrawal/treatment refusal ($n = 6$); (2) noncompliance ($n = 5$); (3) intake of prohibited medication ($n = 4$); (4) exclusion criteria violation ($n = 2$); (5) side effects ($n = 2$; right anterior cruciate ligament rupture and finger fracture); and (6) agreement acquisition violation ($n = 1$). Thus, the number for final data analysis was 78 (BG11001: $n = 39$, placebo: $n = 39$) (Fig. 2). In the treatment group ($n = 39$), the average age was 51.16 ± 4.41 yr, and the average weight was 56.71 ± 7.07 kg; in the placebo group ($n = 39$), the average age was 51.14 ± 3.83 yr, and average weight was 59.26 ± 7.91 kg. The demographic data of the participants are summarized in Table 3. The Student t test revealed no significant difference in initial age or weight. Laboratory evaluations revealed no significant abnormalities in complete blood count and chemistry (Table 4).

3.3. Analysis of BG110001 effect on eye wrinkles in human skin

Comparisons of eye wrinkles before and after 8 wk, 16 wk, and 24 wk of BG11001 consumption between the treatment group and the placebo group, as measured with the SV600 visiometer and Global Photo Damage Score (GPDS) are shown in Table 5–7. At 8 wk, value of average roughness (R3) for eye wrinkles was reduced 0.007 ± 0.009 AU in the treatment group and 0.003 ± 0.011 AU in the placebo group. Those reductions were similarly maintained after 16 wk (0.005 AU in the treatment group and 0.003 AU in the placebo group) and 24 wk (0.006 AU in the treatment group and 0.004 AU in the placebo group), respectively (Table 5). Those R3 values were significantly decreased within the treatment group, but not in the placebo group. Therefore, each improvement of R3 values in the treatment group at each time point showed statistical significance after adjustment with baseline. However, the value of arithmetic roughness average (R5) did not show improvement by BG11001. When the R5 values were compared within the group,

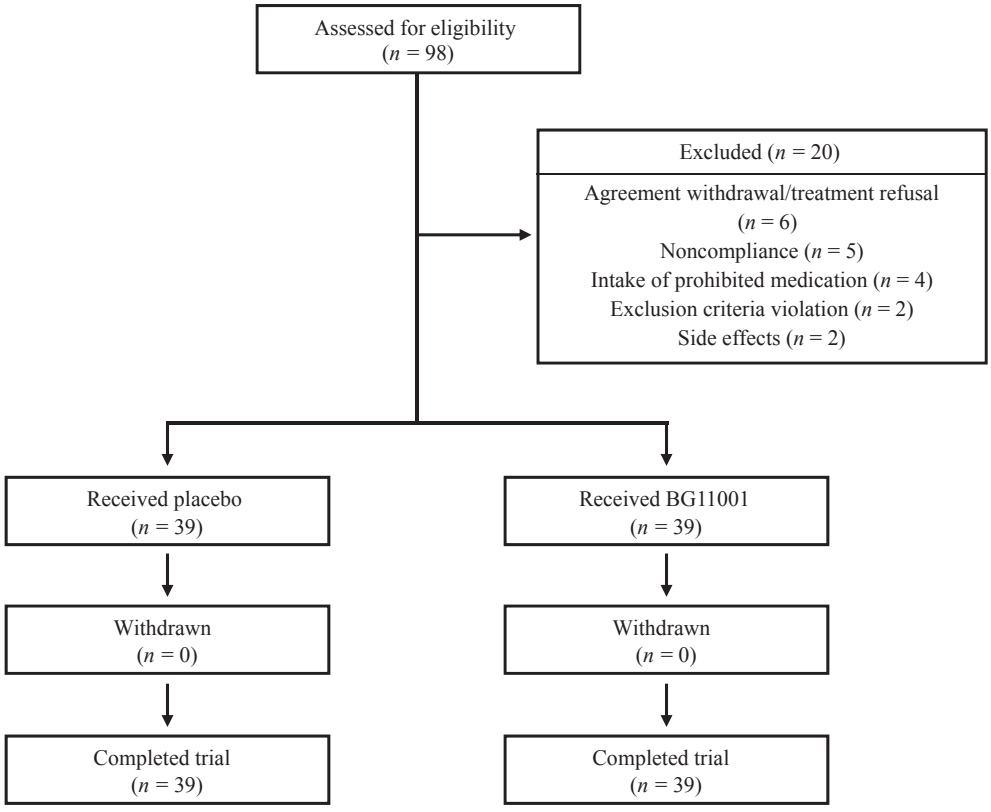

Fig. 2. Study flowchart of the participants describing trial progress.

Table 3
Demographics of the participants in each group

	BG11001	Placebo	*p*
No. enrolled	49	49	
No. completed	39	39	
Average age (yr)	51.16 ± 7.07	51.15 ± 3.83	0.981
Average weight (kg)	56.71 ± 7.07	59.26 ± 7.91	0.095

Data are presented as mean ± SD.

there were meaningful decreases by statistics. But, after adjustment with baseline, we could not find any statistical difference (Table 6).

Global photodamage was scored using a randomized and double-blinded method. Even if GPDS might be judged subjectively, only BG11001 significantly decreased the scores. Decreased scores were observed from 8 wk of treatment and tended to decline until 24 wk (Table 7).

3.4. Observation of improved skin elasticity

The values of gross elasticity (R2) and net elasticity (R5) were measured with a Cutometer (Tables 8 and 9). After BG11001 treatment, R2 values were increased in each time point, from 0.89 ± 0.05 AU at baseline to 0.91 ± 0.04 AU at 24 wk, but not in the placebo group. Within the treatment group, R2 values were statistically different after 16 wk and 24 wk. Also, when we compared R2 values between groups using analysis of covariance, after adjustment with baseline, the gross skin elasticity was statistically improved by enzyme-treated red ginseng extract (Table 8). Then, net elasticity (R5) was measured and showed that R5 values were significantly increased within the treatment group. In addition, statistics of comparison between the treatment and placebo group showed that BG11001 improved net skin elasticity (Table 9).

3.5. Changes of the moisture content of the stratum corneum and TEWL

The hydration level of the stratum corneum was measured using a corneometer (Table 10). During the treatment of BG11001 for 24 wk, hydration level was decreased from 8 wk treatment. However, decreased hydration level was maintained until 24 wk in the treatment group. In addition, the degrees of decrease were much smaller than those in the placebo group. Then, the measurement of TEWL was performed with a Tewameter (Table 11). After 24 wk of BG11001 consumption, TEWL values were decreased in the treatment group, even at 8 wk of treatment; however, TEWL values were increased in the placebo group in each time point for 24 wk. Also, the statistics of comparison between groups at the 24 wk time point, showed that BG11001 significantly prevented TEWL.

4. Discussion

The present study demonstrated that 24-wk oral administration of enzyme-treated powder complex of red ginseng (BG11001) has substantial benefits over the placebo in the improvement of eye wrinkle roughness, elasticity, and skin moisture content. The administration of BG11001 significantly reduced average roughness (R3) of eye wrinkles and GPDS score, compared with a placebo; although, there was no significant difference in arithmetic roughness average (R5) between both groups. In addition, gross elasticity (R2) and net elasticity (R5) values were increased and TEWL level was decreased, indicating improved skin elasticity and moisture content. However, hydration levels of the stratum corneum were decreased even in the treatment group. To interpret this result, we needed to consider the weather conditions during the study. When the study was started at early July, the weather was humid (average humidity: 78.5%) and warm (average temperature: 23.4°C) (Korea Meteorological Administration). However, as the treatment proceeded, the weather became drier (average humidity: 69.4% at the 24 wk time point) and colder (average temperature: −5.2°C at the 24 wk time point), meaning that skin could have lost moisture and elasticity more than the baseline time point [18]. Therefore, with considering the weather conditions, results should be reinterpreted, as eye wrinkle roughness was remarkably improved by showing the average roughness (R3) value and GPDS score. Even though values of arithmetic roughness average (R5) were not decreased enough to show any statistical difference, BG11001 prevented worsening of

Table 4
Serology data

	BG11001		*p*	Placebo		*p*
	Before	After 24 wk		Before	After 24 wk	
	Average ± SD			Average ± SD		
Hematology						
WBC	5.92 ± 1.37	5.90 ± 1.33	0.940	5.79 ± 1.39	5.87 ± 1.45	0.564
RBC	4.31 ± 0.28	4.39 ± 0.25	0.002**	4.31 ± 0.27	4.33 ± 0.26	0.271
Hemoglobin	13.26 ± 0.85	13.57 ± 0.84	0.002**	13.22 ± 0.90	13.36 ± 0.89	0.048*
Hematocrit	39.99 ± 2.31	40.82 ± 2.23	0.001**	40.02 ± 2.31	40.26 ± 2.12	0.204
Platelets	239.08 ± 46.76	247.98 ± 48.90	0.041*	247.51 ± 60.29	246.41 ± 65.58	0.793
Blood chemistry						
ALP	225.18 ± 68.63	251.12 ± 72.89	0.000**	215.14 ± 53.37	240.96 ± 65.18	0.000**
AST	23.04 ± 5.31	26.31 ± 13.42	0.066	23.76 ± 8.31	24.45 ± 8.43	0.430
ALT	20.00 ± 8.14	24.80 ± 16.11	0.011*	22.24 ± 14.52	23.33 ± 14.61	0.375
γ-GTP	22.49 ± 18.34	25.12 ± 22.09	0.086	21.06 ± 22.45	18.90 ± 19.84	0.033*
Total protein	7.16 ± 0.31	7.16 ± 0.34	0.885	7.24 ± 0.31	7.15 ± 0.32	0.042*
Albumin	4.45 ± 0.17	4.49 ± 0.22	0.077	4.49 ± 0.20	4.46 ± 0.18	0.261
Creatinine	0.85 ± 0.12	0.80 ± 0.11	0.004**	0.82 ± 0.08	0.81 ± 0.13	0.425
MCV	92.84 ± 3.31	92.91 ± 3.17	0.682	92.95 ± 3.50	92.98 ± 3.54	0.858
Glucose	85.94 ± 9.05	91.55 ± 18.37	0.029*	87.69 ± 11.48	91.53 ± 19.29	0.048*
Total cholesterol	206.14 ± 37.72	203.43 ± 35.72	0.471	195.06 ± 24.11	192.49 ± 24.63	0.031*
HDL	61.57 ± 15.55	61.29 ± 15.89	0.806	61.98 ± 13.90	61.39 ± 14.84	0.686
LDL	127.20 ± 34.03	129.31 ± 34.68	0.490	116.14 ± 26.03	113.86 ± 29.75	0.402
Triglycerid	150.39 ± 93.85	134.53 ± 77.14	0.219	124.49 ± 73.69	115.27 ± 58.02	0.349

*$p < 0.05$, **$p < 0.01$ by Student's *t*-test for comparison between before and 24 wk treated group.
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GTP, guanosine-5′-triphosphate; HDL, high density lipoprotein; LDL, low density lipoprotein; MCV, mean corpuscular volume; RBC, red blood cells; Std., standard deviation; WBC, white blood cells.

Table 5
Average roughness (R3) value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
SV600 (R3)	Baseline	0.037 ± 0.012	0.040 ± 0.013	0.280	
	After 8 wk	0.030 ± 0.008	0.037 ± 0.012	0.004	0.004**
	Difference[3)]	−0.007 ± 0.009	−0.003 ± 0.011	0.095	
	$P^{4)}$	0.000**	0.057		
	After 16 wk	0.032 ± 0.008	0.037 ± 0.011	0.008	0.014*
	Difference[5)]	−0.005 ± 0.011	−0.003 ± 0.010	0.226	
	$P^{4)}$	0.003**	0.106		
	After 24 wk	0.031 ± 0.007	0.036 ± 0.010	0.010	0.017*
	Difference[6)]	−0.006 ± 0.011	−0.004 ± 0.009	0.382	
	$P^{4)}$	0.004**	0.018*		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p-value by Student t-test.
2) Compared between groups: p-value by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p-value by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

the eye wrinkle formation despite the cold and dry weather conditions. With the same point of view, skin elasticity (R2 and R5) and moisture content (TEWL) after treatment was substantially improved, and decreased skin hydration was prevented by BG11001, even with poor weather conditions for skin.

The primary cause for eye wrinkles, such as crow's feet, is exposure to sunlight. Sunlight ages the skin, and it also encourages people to squint, wrinkling the skin around their faces. In UV-damaged skin, increased MMP-1 triggers collagen degradation, especially collagen type I which is a source of structural support in the dermis [19]. Also, UV irradiation increased the expression of activator protein-1, which in turn interferes with the synthesis of collagen type I by blocking transforming growth factor-β1 and procollagen type I [20,21]. In a previous study, *Panax ginseng* restored decreased expression of transforming growth factor-β1 and TIMP-1 (Tissue Inhibitor of Metalloproteinase-1) and increased expression of MMP-1. *Panax ginseng* also markedly attenuated MMP-1 expression. Those regulations finally increased collagen type I (or procollagen type I) expression [15,16,22]. Also, other studies observed that *Panax ginseng* induces collagen type I synthesis through activation of Smad or peroxisome proliferator-activated receptor-delta signaling [10,23]. In addition, enzyme-treated extract of red ginseng reversed the inhibitory effects of UV irradiation on filaggrin and profilaggrin production in both the epidermis and dermis in mice [15]. These results are important because filaggrin in stratum corneum are incorporated into lipid envelopes and release free amino acids to assist in water retention [24–26]. Another previous study reported that dietary supplementation of red ginseng protected skin from UV-induced dryness with an accumulation of ceramides via elevated expression of serine palmitoyltransferase, a key enzyme involved in *de novo* ceramide synthesis [27]. With all these previous results, we could suggest that BG11001 improved human eye wrinkle roughness by the regulation of collagen type I expression, and BG11001 was also able to increase skin elasticity and maintain moisture content by inhibition of filaggrin expression and elevation of serine palmitoyltransferase expression.

Table 6
Arithmetic roughness average (R5) value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
SV600 (R5)	Baseline	0.017 ± 0.008	0.022 ± 0.009	0.039	
	After 8 wk	0.014 ± 0.006	0.016 ± 0.007	0.074	0.927
	Difference[3)]	−0.004 ± 0.006	−0.005 ± 0.008	0.407	
	$p^{4)}$	0.000**	0.000**		
	After 16 wk	0.015 ± 0.006	0.017 ± 0.008	0.079	0.722
	Difference[5)]	−0.003 ± 0.008	−0.004 ± 0.010	0.530	
	$p^{4)}$	0.026*	0.016*		
	After 24 wk	0.015 ± 0.006	0.016 ± 0.007	0.305	0.259
	Difference[6)]	−0.003 ± 0.007	−0.005 ± 0.008	0.139	
	$p^{4)}$	0.020*	0.000**		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p-value by Student t-test.
2) Compared between groups: p-value by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p-value by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

Table 7
Global photodamage score value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
GPDS	Baseline	3.31 ± 1.15	3.44 ± 1.25	0.639	
	After 8 wk	3.15 ± 1.04	3.41 ± 1.21	0.318	0.262
	Difference[3)]	−0.15 ± 0.75	−0.03 ± 0.54	0.386	
	$p^{4)}$	0.205	0.767		
	After 16 wk	3.13 ± 1.01	3.33 ± 1.13	0.400	0.397
	Difference[5)]	−0.18 ± 0.64	−0.10 ± 0.60	0.586	
	$p^{4)}$	0.026*	0.016*		
	After 24 wk	3.05 ± 1.03	3.38 ± 1.14	0.178	0.062
	Difference[6)]	−0.26 ± 0.60	−0.05 ± 0.61	0.135	
	$p^{4)}$	0.010*	0.599		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p by Student t-test.
2) Compared between groups: p by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

Table 8
Skin elasticity (R2) value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
Cutometer (R2)	Baseline	0.89 ± 0.05	0.86 ± 0.07	0.185	
	After 8 wk	0.90 ± 0.04	0.86 ± 0.05	0.000	0.000**
	Difference[3)]	0.01 ± 0.05	−0.01 ± 0.08	0.118	
	$p^{4)}$	0.058	0.485		
	After 16 wk	0.91 ± 0.05	0.87 ± 0.07	0.002	0.003**
	Difference[5)]	0.02 ± 0.04	−0.00 ± 0.50	0.040*	
	$p^{4)}$	0.006**	0.732		
	After 24 wk	0.91 ± 0.04	0.87 ± 0.07	0.003	0.006**
	Difference[6)]	0.02 ± 0.05	−0.00 ± 0.07	0.108	
	$p^{4)}$	0.011*	0.946		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p by Student t-test.
2) Compared between groups: p by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

Table 9
Skin elasticity (R5) value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
Cutometer (R5)	Baseline	0.68 ± 0.18	0.62 ± 0.14	0.127	
	After 8 wk	0.75 ± 0.19	0.66 ± 0.15	0.021	0.071
	Difference[3)]	0.07 ± 0.12	0.04 ± 0.08	0.160	
	$p^{4)}$	0.001**	0.006**		
	After 16 wk	0.79 ± 0.21	0.67 ± 0.17	0.009	0.033*
	Difference[5)]	0.11 ± 0.19	0.05 ± 0.16	0.138	
	$p^{4)}$	0.001**	0.045*		
	After 24 wk	0.78 ± 0.22	0.65 ± 0.16	0.004	0.014*
	Difference[6)]	0.10 ± 0.16	0.03 ± 0.16	0.043	
	$p^{4)}$	0.000**	0.246		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p by Student t-test.
2) Compared between groups: p by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

Table 10
Skin hydration value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
Corneo-meter	Baseline	72.23 ± 10.20	70.90 ± 11.77	0.692	
	After 8 wk	67.79 ± 9.57	65.46 ± 9.67	0.222	0.083
	Difference[3)]	−4.44 ± 8.46	−5.44 ± 8.54	0.149	
	$p^{4)}$	0.058	0.485		
	After 16 wk	65.67 ± 10.34	63.43 ± 9.29	0.074	0.008**
	Difference[5)]	−6.56 ± 8.65	−7.46 ± 11.68	0.036	
	$p^{4)}$	0.282	0.071		
	After 24 wk	67.49 ± 10.49	63.89 ± 10.87	0.090	0.005**
	Difference[6)]	−4.74 ± 9.19	−7.01 ± 12.96	0.005	
	$p^{4)}$	0.172	0.013*		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p by Student t-test.
2) Compared between groups: p-value by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p-value by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

Table 11
Transepidermal water loss value changes

		BG11001	Placebo	$p^{1)}$	$p^{2)}$
		Arbitrary units (Average ± SD)			
Tewa-meter	Baseline	14.29 ± 2.60	14.94 ± 3.25	0.332	
	After 8 wk	13.67 ± 2.47	15.16 ± 3.98	0.051	0.062
	Difference[3)]	−0.62 ± 1.79	0.22 ± 2.47	0.091	
	$p^{4)}$	0.039	0.580		
	After 16 wk	13.68 ± 2.62	14.96 ± 3.73	0.083	0.109
	Difference[5)]	−0.61 ± 0.96	0.02 ± 2.56	0.154	
	$p^{4)}$	0.000**	0.958		
	After 24 wk	13.75 ± 2.92	15.30 ± 3.12	0.027	0.030*
	Difference[6)]	−0.53 ± 1.81	0.36 ± 2.57	0.081	
	$p^{4)}$	0.073	0.391		

* $p < 0.05$ by Student t-test for comparison with placebo group.
** $p < 0.01$ by Student t-test for comparison with placebo group.
1) Compared between groups: p by Student t-test.
2) Compared between groups: p-value by analysis of covariance (adjustment with baseline).
3) After 8 wk − baseline.
4) Compared within groups: p-value by paired t-test.
5) After 16 wk − baseline.
6) After 24 wk − baseline.

In this study, we used oral administration of BG11001 despite the possibility of oral intake delaying the effect on skin because the substance should be absorbed and metabolized. However, there are more benefits than topical application. Firstly, the aged do not absorb topical substances more rapidly than the young, probably less [28]. Secondly, when *Panax ginseng* is administrated orally, skin care effects would be better with its wide range of biological activities. For example, it promotes skin cell regeneration by a wound healing effect [29] and also has the effect of boosting blood circulation as well as detoxifying the blood [30,31]. Thirdly, with oral intake we did not need to be worried about topical skin irritation. Furthermore, there was no clinically significant adverse effects of BG11001 during the study.

In conclusion, enzyme-treated red ginseng extract significantly improved eye wrinkle roughness, skin elasticity, and moisture content. Moreover, enzyme-treated red ginseng extract would be useful substance as a bio-health skin care product.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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