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Fermenting Red Ginseng Enhances Its Safety and Efficacy as a Novel Skin Care Anti-Aging Ingredient: *In Vitro* and Animal Study

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ABSTRACT The objective of this study was to evaluate the anti-aging potential and skin safety of red ginseng (RG) and fermented red ginseng (FRG) using *Lactobacillus brevis* for use as cosmetic ingredients. Concentrations of uronic acid, polyphenols, and flavonoids, and antioxidant activities were greater in FRG compared to RG. The contents of total ginsenosides were not significantly different. However, the ginsenoside metabolite content was higher in FRG (14,914.3 μg/mL) compared to RG (5697.9 μg/mL). The tyrosinase inhibitory activity (IC_{50}) of FRG was 27.63 μg/mL, and more potent compared with RG (34.14 μg/mL), ($P < .05$). The elastase inhibitory activity (IC_{50}) of FRG was 117.07 μg/mL also higher compared with RG (157.90 μg/mL). In a primary skin irritation test, 10% RG and 10% FRG were classified as practically nonirritating materials. In a skin sensitization test, the RG group showed a sensitization rate of 100% and its mean evaluation score of irritation was 1.4, whereas the FRG group showed 20% and 0.2%, respectively. By fermentation of RG, FRG has increased contents of ginsenoside metabolites, such as Rg3, Rg5, Rk1, compound K, Rh1, F2, Rg2, and flavonoids content. Therefore, FRG offers increased anti-wrinkle efficacy, whitening efficacy, and reduced toxicological potency compared to RG.

KEY WORDS: • ***cosmeceutical*** • ***fermented red ginseng*** • ***red ginseng*** • ***skin safety***

INTRODUCTION

GINSENG IS ONE OF THE MOST COMMONLY USED traditional medicines in many Asian countries. Ginseng refers to the root of several species within the plant genus *Panax* (C.A. Meyer Araliaceae). Among them, *Panax ginseng* is the most widely used ginseng and is indigenous to Far Eastern countries.[1] Ginseng has a long history of cultivation. It is typically harvested after 3–6 years of growth, and then undergoes extensive cleaning and air drying (white ginseng [WG]) or steaming at 98°C–100°C for 2–3 h (red ginseng [RG]).[2] Interestingly, after these two treatment methods, the roots differ in their saponin contents.[3] Differences in the biological activities of these ginsengs may be the result of changes in chemical constituents that occur during steam treatment.[4] It has been reported that RG has more potent pharmacological activities than WG.[2,5] Ginseng saponins, referred to as ginsenosides, are believed to play important roles in the pharmacological action.[4]

The pharmacological actions of ginsenosides have suggested to be dependent upon biotransformation by human intestinal bacteria.[6,7] Orally administered ginsenosides are barely decomposed by either gastric juices or liver enzymes, and their absorption rates from the intestine are very low. Like other plant glycosides, ginsenosides are hydrolyzed by intestinal bacteria before absorption. Several investigators have reported new ginsenosides in RG that are not usually found in WG. These compounds are the ginsenosides Rg3, Rg5, Rg6, Rh2, Rh3, Rh4, Rs3, and F4.[2,8,9] These compounds are more concentrated in RG than in WG,[2,10] and Kim *et al.*[2] reported that ginsenosides F4, Rg3, and Rg5 were not present in WG, and are produced after steaming. Ginsenosides can induce antioxidant enzymes, such as superoxide dismutase and catalase, which are important for maintaining cell viability.[11] Many previous studies have reported that *P. ginseng* has anti-wrinkle effects,[12] protects against ultraviolet radiation-induced skin aging,[13,14] and has anti-melanogenesis effects.[15] Furthermore, it was reported that ginseng has immunostimulating activity.[16,17] Immune responses are known to play essential roles in host defense mechanisms, but may also cause general toxic, idiosyncratic, and allergic adverse reactions (which are sometimes associated with immunostimulating agents), thereby

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resulting in exacerbation rather than suppression of some disease processes.[18] It is possible that RG could occasionally cause skin safety problems, such as allergenic responses. However, skin problems, including irritation and sensitization, have not been reported for RG.

Ginseng is typically administered orally, after which the ingredients are exposed to gastric juices or liver enzymes and digestive and bacterial enzymes. Intact ginsenosides are absorbed only from the intestines (the absorption rate is as low as 0.1–3.7%), but their intestinal bacterial metabolites are also absorbed into the blood. The pharmacological actions of these ginsenosides have been attributed to their biotransformation by human intestinal bacteria.[19–21] To improve the oral absorption and bioavailability of these compounds, many different strategies have been used. Several studies have shown that the transformation of ginsenosides into deglycosylated ginsenosides (metabolites) is required for them to provide more effective *in vivo* physiological action.[7] In a previous study, we fermented RG to improve ginsenoside bioavailability and the physiological activities in comparison with nonfermented RG. Fermented red ginseng (FRG) had dramatically improved bioavailability compared to RG, as indicated by skin permeation, intestinal permeability, and ginsenoside levels in the blood.[22]

As a cosmeceutical, RG is applied topically and not biotransformed by human intestinal bacteria; therefore, dermal absorption is an important factor for cosmetic ingredients. In this study, we investigated the skin safety and potential as cosmeceutical ingredients *in vitro* and in guinea pig skin by spreading of FRG in cojunction with RG.

MATERIALS AND METHODS

Materials

Six-year-old RG extract (60 brix) was purchased locally (ginseng market, Geumsan, Korea). Standard ginsenosides, including the compounds Rg1, Re, Rf, Rh1, Rg2, Rb1, Rc, Rb2, Rd, Rg3, F2, CK, Rk1, Rg5, and Rh2, were purchased from Ambo Laboratory (Daejeon, Korea). 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent, mushroom tyrosinase (167 U), N-succinyl-$(Ala)_3$-p-nitroanilide, dinitrochlorobenzene (DNCB) were purchased from Sigma Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from local suppliers.

Preparation of fermented RG

The RG extract was diluted with water (1:2, vol/vol) and adjusted to a pH of 6.0. Next, 50 mL of each diluted ginseng extract was poured into a 250-mL Δ-flask and sterilized at 121°C for 15 min. *Lactobacillus brevis* M2 (KCTC11390BP) (10^9 colony-forming units [CFU]/mL) was isolated from the ginseng extract, which was then inoculated into a 1.0-mL suspension. Finally, the diluted extracts were incubated at 37°C for 2 days with mild shaking. The strains were precultured in an MRS broth (Difco, Detroit, MI, USA) containing 0.05% L-cysteine·HCl under mild aerobic conditions for 24 h at 37°C, and then subcultured in the MRS broth and used to ferment the ginseng extract.

Analysis of total sugars, uronic acid, polyphenols, flavonoids, and antioxidant activities

Total sugar and uronic acid contents were determined by the phenol–sulfuric acid method[23] and m-hydroxydiphenyl method,[24] respectively, using galactose and galacturonic acid as the respective standards. The total polyphenol content was measured by the Folin–Cocialteu method,[25] and the total polyphenol concentration was calculated from a calibration curve using gallic acid as the standard. The total flavonoid content was measured using a colorimetric assay[26] using catechins as the standard.

The antioxidant activities of the samples were evaluated according to DPPH-scavenging activity and ABTS radical–scavenging activity. The DPPH-scavenging activity was measured using the method described by Quang *et al.*[27] and the ABTS radical–scavenging activity was determined as previously described.[28]

High-performance liquid chromatography analysis of ginsenosides

The solid-phase extraction sample (2 mL) was assayed according to the method described by Lou *et al.*[29] using a C18 ODS cartridge (Waters Associates, Milford, MA, USA). The levels of 16 major ginsenosides were analyzed using a high-performance liquid chromatography (HPLC)-based technique developed by Lee *et al.*[22] with a Varian Prostar 200 HPLC system (Varian, Inc., Palo Alto, CA, USA) equipped with a quaternary solvent delivery system, an autosampler, and UV detector, with measurements at 203 nm. The column was a IMtakt Cadenza CD-C18 (4.6 mm × 75 mm; Imtakt Corporation, Kyoto, Japan).

Tyrosinase and elastase inhibition

L-Tyrosine oxidation by tyrosinase was spectrophotometrically determined as described previously.[30] The percentage of inhibition was calculated as follows:

$$\% \text{ inhibition} = [(A - B) - (C - D)]/(A - B) \times 100$$

where A is OD at 492 nm with tyrosinase, but without the test substance; B is OD at 492 nm without the test substance and tyrosinase; C is OD at 492 nm with the test substance and tyrosinase; and D is OD at 492 nm with the test substance, but without tyrosinase.

The inhibition of elastase activity was evaluated spectrophotometrically by the method of Kraunsoe *et al.*[31] The inhibition rate was calculated as follows:

$$\% \text{ inhibition} = (A - B)/A \times 100$$

where A is with elastase, but without sample, and B is with sample and elastase.

Experimental animals

The experimental protocol was reviewed and approved by the Korea University Animal Care Committee (KUACUC-2010-106). Hartley strain guinea pigs weighing 300–400 g were obtained from Nara Biotech (Daejon, Korea). They were individually housed in plastic cages with grated stainless steel floors. The colony room was maintained at 24°C ± 1°C with 60% atmospheric humidity, and a 12-h light/12-h dark cycle. The animals were provided food and water ad libitum. The period of acclimation to this environment was 1 week.

Primary skin irritation test

A primary skin irritation test was conducted according to the method of Draize *et al.*[32] About 0.1 mL of RG or FRG (10%, vehicle: saline) was spread onto cotton lint (1.5 cm × 1.5 cm), and then the lint was applied to two sites (one site intact, and the other abraded with a sterilized syringe needle). Approximately 24, 48, and 72 h after application, the animals were examined for signs of irritation and any skin reactions were evaluated according to the Draize method:[32]

1. *Erythema and eschar formation:*

   Score 0, no erythema
   Score 1, very slight erythema
   Score 2, well-defined erythema
   Score 3, moderate to severe erythema
   Score 4, severe erythema and slight eschar formation

2. *Edema formation:*

   Score 0, no edema
   Score 1, very slight edema
   Score 2, slight edema
   Score 3, moderate edema
   Score 4, severe edema

The primary irritation index (P.I.I.), which is the sum of scored reactions (both for erythema/eschar and edema formations) for all animals at 72 h divided by number of animals, was calculated following test completion. Body weight was measured on the day before administration and at test termination for all animals.

Skin sensitization test

Skin sensitization was evaluated according to a maximization test method, as described by Magnusson and Kligman.[33] One day before the first stage of induction, the animals were separated into the following four groups ($n = 5$ for each): (1) treated with vehicle (saline) (negative control), (2) treated with DNCB (positive control), (3) treated with RG, and (4) treated with FRG. Skin reactions were evaluated visually 24 and 48 h after patch removal according to the following well-defined sensitization scoring system:[34] Score 0, no visible changes; Score 1, discrete or patchy erythema; Score 2, moderate and confluent erythema; Score 3, intense erythema and swelling.

Cytokine measurements

All blood samples were drawn into tubes containing ethylenediaminetetraacetic acid and immediately centrifuged. Plasma and tissue were stored at −80°C until analyzed. Plasma levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were determined by enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Oxford, United Kingdom). The same ELISA kits were used to determine the treated tissue samples after the homogenization of 200 mg of frozen tissue in 400 μL of a buffer (pH 7.4) containing 10 mM/L Tris-HCl, 250 mM/L sucrose, and a cocktail of protease inhibitors (Complete, Roche Molecular Biochemicals, Burlington, NC, USA), as previously reported.[35]

Statistical analysis

Data are expressed as means ± standard deviations (SD). Statistical analyses were performed using the SPSS v10.0 (SPSS, Inc., Chicago, IL). Significant differences between RG and FRG were determined by paired Student's *t*-tests. Comparisons of cytokine concentrations of all groups were performed using one-way ANOVA and Duncan's multiple range tests at $P < .05$.

RESULTS

Chemical compositions, antioxidant activities, and ginsenoside contents

Table 1 shows the chemical compositions and antioxidant activities of RG and FRG. Total sugar content was not significantly different, but uronic acid of FRG significantly higher than in RG ($P < .01$). The polyphenols are the active components of ginseng; therefore, we also measured polyphenols and flavonoids and compared their activities with that of the compounds from ginseng. Compared with RG, there were significant differences in polyphenols and flavonoid contents between FRG and RG ($P < .01$ and $P < .001$, respectively).

After fermentation, the IC_{50} values on ABTS• or DPPH• radicals were 2.8 and 4.4 mg/mL, respectively. After

Table 1. Chemical Composition and Antioxidant Activities of Red Ginseng and Fermented Red Ginseng

	RG	*FRG*
Total sugar (mg/mL)	359.9 ± 25.5	354.7 ± 23.2
Uronic acid (mg/mL)	5.9 ± 1.4	48.4 ± 6.0**
Polyphenol (mg/mL)	10.1 ± 0.4	14.3 ± 0.6*
Flavonoid (μg/mL)	9.7 ± 0.7	133.2 ± 6.1**
ABTS (IC_{50}, mg/mL)	4.1 ± 0.0	2.8 ± 0.0**
DPPH (IC_{50}, mg/mL)	5.7 ± 0.2	4.4 ± 0.1*

The experiment was independently repeated three times ($n = 3$) and data expressed as a mean ± SD.

Values are significantly different (*$P < .01$, **$P < .001$, Student's *t*-test).

RG, red ginseng; FRG, fermented red ginseng; ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 1,1-diphenyl-2-picrylhydrazyl.

fermentation, the ABTS and DPPH radical-scavenging activities of the FRG were about 1.4-fold and 1.3-fold higher compared with RG, respectively (Table 1). The above results showed that fermentation significantly enhanced the overall radical-scavenging activities of ginseng, which could be explained by the increased amount of antioxidant compounds, especially polyphenol compounds.

The total ginsenoside contents of RG and FRG were 35,715.2 μg/mL and 34,822.9 μg/mL, respectively, but their levels were not significantly different. Rb1 and Rg1 are the two main ginsenosides contained in RG. RG had a higher sum of Rb1 and Rg1 (9096.5 μg/mL) than FRG (5562.0 μg/mL). RFG had a higher level of ginsenoside metabolites (sum of Rg3, Rg5, Rk1, CK, Rh1, F2, and Rg2) (14,914.3 μg/mL) compared to FRG (5697.9 μg/mL). Especially, the increase of Rk1 and Rg5 were about 325.0% and 341.0% higher than those of RG, respectively. Otherwise, Rg1 and Rb1 content (751.8 and 4810.2 μg/mL, respectively) in RFG showed lower than those (1272.1 and 7824.4 μg/mL, respectively) of RG (Table 2).

Inhibitiory activities against tyrosinase and elastase

In animals, L-tyrosine is converted to the red-brown dopaquinone via l-3,4-dihydroxyphenylalanine by tyrosinase-catalyzed oxidation; dopaquinone is further oxidized to yield the mostly brown to black colored polymeric melanins, which are responsible for the different colors of skin and hair of mammals.[36] During the melanogenesis, the key enzyme is tyrosinase, which contains a binuclear copper cluster in the common mushroom (*Agaricus bisporus*) and in human malignant melanoma.[37] The tyrosinase inhibitory activities of RG and FRC are shown in Figure 1. The tyrosinase-inhibitory activity (IC_{50}) of FRG was 27.63 μg/mL, which is more potent compared with RG (34.14 μg/mL), ($P < .05$).

Table 2. Ginsenoside Contents of Red Ginseng and Fermented Red Ginseng

	Concentration (μg/mL)		
Ginsenoside	*RG*	*FRG*	*FRG/RG (%)*
Rg1	1272.1 ± 126.1	751.8 ± 93.0	59.1
Re	3367.2 ± 351.1	1882.4 ± 222.6	55.9
Rf	1223.6 ± 113.2	1121.7 ± 130.7	91.7
Rh1 + Rg2(s)	1621.8 ± 104.2	2095.3 ± 204.4	129.2
Rg2(r)	568.9 ± 247.7	994.1 ± 75.4	174.7
Rb1	7824.4 ± 854.6	4810.2 ± 537.0	61.5
Rc	6199.6 ± 662.3	3807.5 ± 413.5	61.4
Rb2	5394.8 ± 521.9	3424.9 ± 408.3	63.5
Rd	4166.7 ± 425.4	3116.0 ± 234.3	74.8
Rg3	1671.7 ± 150.2	4903.4 ± 584.0	293.3
F2	18.3 ± 0.7	47.1 ± 2.1	257.4
CK	34.7 ± 2.4	49.8 ± 11.5	143.5
Rk1	1244.3 ± 121.7	4043.6 ± 478.1	325.0
Rg5	1107.1 ± 114.6	3775.0 ± 454.1	341.0
Rh2	ND	ND	ND
Total	35,715.2 ± 3235.5	34,822.9 ± 3650.2	97.5
Rg1 + Rb1	9096.5 ± 980.4	5562.0 ± 629.8	61.1
Metabolites[a]	5697.9 ± 394.9	14,914.3 ± 1566.0	261.8

The experiment was independently repeated three times. Data are expressed as a mean ± SD.

[a]Sum of Rg3, Rg5, Rk1, CK, Rh1, F2, and Rg2.

CK, compound K; ND, not detected.

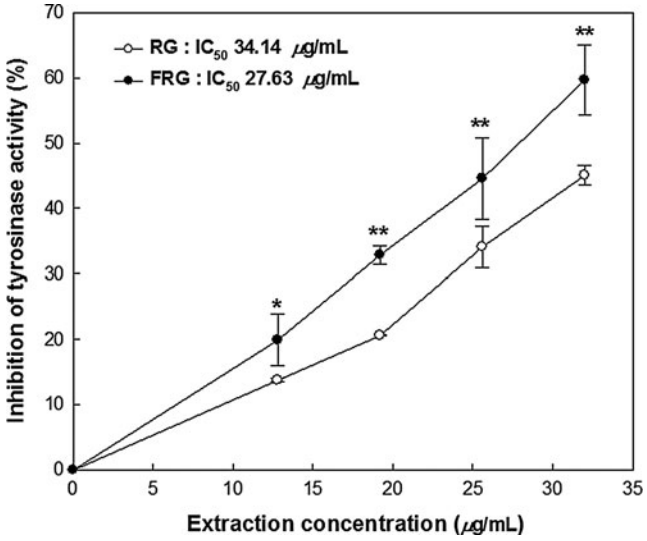

FIG. 1. Tyrosinase inhibitory activities of red ginseng (RG) and fermented red ginseng (FRG). Inhibition $(\%) = [(A-B)-(C-D)]/(A-B) \times 100$, where A is with tyrosinase, but without sample; B is without sample and tyrosinase; C is with sample and tyrosinase; and D is with sample, but without tyrosinase. Data are expressed as mean ± SD ($n = 5$). Values are significantly different at same concentration (*$P < .01$, **$P < .001$, Student's t-test). IC_{50}; the amount of tested extract required for a 50% decrease in activity of tyrosinase.

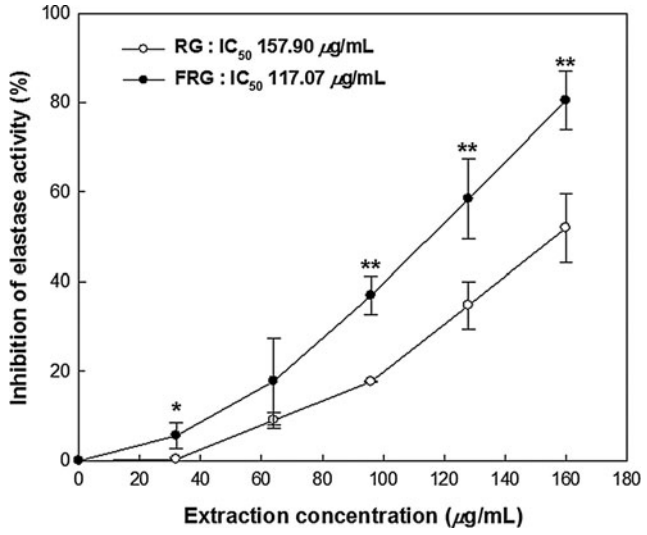

FIG. 2. Elastase inhibitory activities of RG and FRG. Inhibition $(\%) = (A-B)/A \times 100$, where A is with elastase, but without sample, and B is with sample and elastase. Data are expressed as mean ± SD ($n = 5$). Values are significantly different at the same concentration (*$P < .01$, **$P < .001$, Student's t-test). IC_{50}; the amount of tested extract required for a 50% decrease in activity of elastase.

Biologically, the elastase activity significantly increases with age and this results in decreased skin elasticity, and subsequently in the appearance of wrinkles or stretch marks.[38] The elastic fiber network plays an important role in sustaining skin elasticity, the decrease of which is an essential factor in the formation of wrinkles. Their elastase-inhibitory activities (IC_{50}) are shown in Figure 2, and FRG had a higher activity (117.07 μg/mL) than RG (157.90 μg/mL) ($P < .05$).

Primary irritation testing and skin sensitization testing

There were no overt clinical signs or unscheduled deaths occurring from the RG or FRG treatments. All animals survived until the study termination. For the skin irritation test, the guinea pigs had dermal patches of 10% RG or FRG (10-fold of the prospective amount for cosmetic ingredients) attached for 24 h (Table 3). Neither RG nor FRG induced adverse reactions, such as erythema or edema, on intact skin sites. However, on abraded skin sites, some guinea pigs treated with RG showed very slight erythema. The P.I.I. score of RG was 0.05, and it was classified as a practically nonirritating material (P.I.I. 0–0.5). FRG was also classified as a practically nonirritating material based on its P.I.I. score of 0.

Figure 3 and Table 4 show the results of the skin sensitization tests. In the negative control group (saline), the positive rate after evocation was 0% and the mean evaluation score was 0. In the RG group, the positive rate 72 h after evocation was 100%, and the mean evaluation score was 1.4. However, in the FRG group, the positive rate 72 h after evocation was 20% and the mean evaluation score was 0.2. In the positive control DNCB group, the positive rate was 100% and the mean evaluation score was 2.2.

Cytokine levels

Cytokines are mediators with multiple functions, including the initiation or influence of numerous biological processes, such as inflammation, sepsis, and wound healing.

Table 3. Evaluation of Skin Irritation for Red Ginseng and Fermented Red Ginseng

		Score of skin irritation (number of animals)											
Skin reaction	*Hours*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Mean score*	*P.I.I.*
Control (saline)													0.00
Intact skin													
Erythema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Edema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Abraded skin													
Erythema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Edema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
RG													0.05
Intact skin													
Erythema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Edema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Abraded skin													
Erythema	24	0	0	1	0	0	0	0	0	2	0	0.3	
	72	0	0	1	0	0	0	0	0	1	0	0.2	
Edema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
FRG													0.00
Intact skin													
Erythema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Edema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Abraded skin													
Erythema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	
Edema	24	0	0	0	0	0	0	0	0	0	0	0	
	72	0	0	0	0	0	0	0	0	0	0	0	

Mean score = total score/number of animals ($n = 10$).

Primary irritation index (P.I.I.) = Σtotal score/(number of animals × 4).

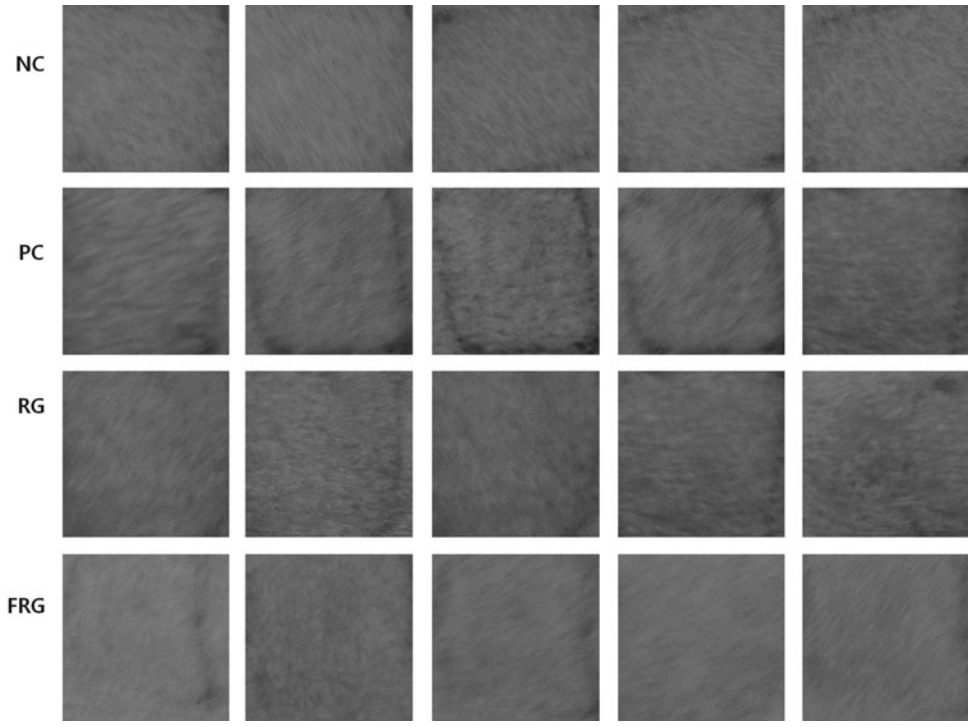

FIG. 3. Photomicrograph of skin treated with test sample for sensitization using a guinea pig maximization test. Negative control (NC), treated saline; positive control (PC), treated 1% dinitrochlorobenzene (DNCB); RG, treated 10% of red ginseng; FRG, treated 10% fermented red ginseng.

The proinflammatory cytokines, IL-6 and TNF-α, play key roles within the cytokine network.[39]

TNF-α levels were decreased in the plasma and tissue samples of guinea pigs treated with FRG as compared to RG, but the groups did not differ significantly (Fig. 4). IL-6 was significantly decreased in the animals treated with FRG as compared to RG. Epidermal keratinocytes are a primary producer of IL-6 within the skin, while macrophages, Langerhans cells, and fibroblasts in the dermis represent other sources of the cytokine. Increased levels of IL-6 have been associated with a number of skin pathologies, such as psoriasis, scleroderma, and systemic lupus erythematosus.[40] These data suggest that FRG has less cytotoxicity or skin allergenic than RG. Therefore, FRG is safe for functional cosmetics.

DISCUSSION

Ginseng is one of the most commonly used traditional medicines for treating various diseases. In addition, RG contains many bioactive constituents, including various ginsenosides, which are believed to have antioxidant, immunostimulatory, and anti-aging activities. Recently, RG was studied for cosmetic applications and as a cosmeceutical ingredient.[13–15,41,42] Some researchers have studied the fermentation of RG as a way to improve its biological

Table 4. Sensitization Scores and Rates by Challenge Reaction 72 h After Topical Application of Red Ginseng and Fermented Red Ginseng

Skin reaction				*Sensitization score*					*Mean response*	*Sensitization rate (%)*	*Evaluation grade (class)*
Group	*Induction*	*Challenge*		*1*	*2*	*3*	*4*	*5*			
Negative control	Saline	Saline	24	0	0	0	0	0	0	0	I (weak)
			48	0	0	0	0	0	0		
			72	0	0	0	0	0	0		
Positive control	1% DNCB	0.1% DNCB	24	2	3	2	2	1	2.0	100	V (extreme)
			48	3	2	3	3	2	2.4		
			72	1	3	3	2	2	2.2		
RG	10% RG	1% RG	24	2	2	2	1	2	1.8	100	V (extreme)
			48	1	1	3	1	1	1.4		
			72	1	1	3	1	1	1.4		
FRG	10% FRG	1% FRG	24	0	0	1	0	1	0.5	20	II (mild)
			48	0	0	0	0	1	0.2		
			72	0	0	0	0	1	0.2		

DNCB, dinitrochlorobenzene.

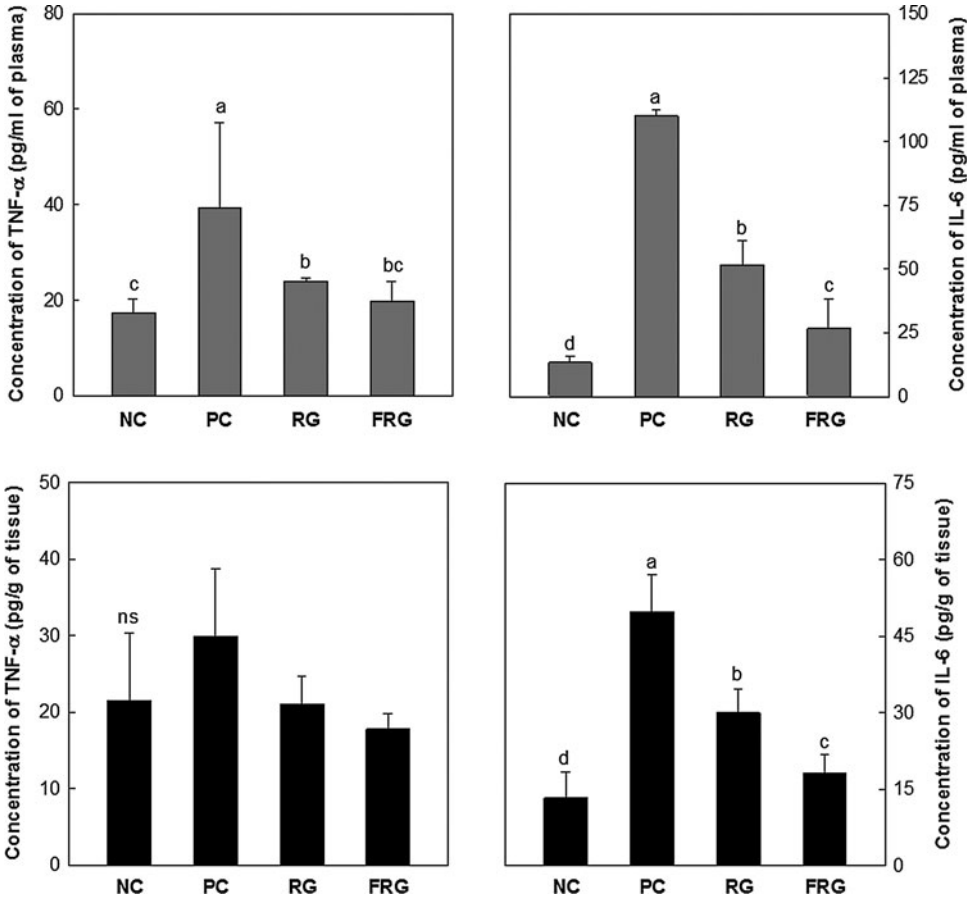

FIG. 4. Cytokine levels of guinea pigs treated with red ginseng and fermented red ginseng. Data are expressed as mean ± SD ($n = 5$). [abcd]The data were evaluated for statistical significance ($P < .05$) with one-way ANOVA followed by Duncan's multiple range test. Means with the same letter are not significantly different. NC, treated saline; PC, treated 1% DNCB; RG, treated red ginseng; FRG, treated fermented red ginseng; ns, not significant.

activity.[43,44] Bae *et al.*[43] reported that orally administered RG extract did not significantly protect against ischemica-reperfusion brain injury, whereas fermented RG did. These results suggest that ginsenoside Rh2 and compound K, which are found at higher contents in FRG than in RG, may improve ischemic brain injury. Trinh *et al.*[44] reported the inhibitory effects of FRG and its main constituents against hyperlipidemia and hyperglycemia in mice, which were more potent than the effects of RG. According to the results of both groups, ginsenoside Rg3 content increases during the fermentation of RG.

According to our results, contents of Rg5 (341.0%), Rk1 (325.0%), Rg3 (293.3%), and F2 (257.4%) were increased an average of 293.3% in FRG when compared to RG (100%). The inhibitory activities of FRG against tyrosinase and elastinase were also increased. Kim *et al.*[42] reported that the ginsenoside Rg3 stimulates type I collagen and fibronectin synthesis through changes in TGF-1 and AP-1 expressions in fibroblasts; thus, it is effective for suppressing wrinkle formation. We confirmed that RG has a cosmeceutical potential, and predicted that FRG would have even greater effects. It should be mentioned that the ginsenoside metabolites are effective anti-oxidants, for tyrosinase and elastinase inhibition. Therefore, the enhanced bio-activities resulting from the fermentation were primarily due to the hydrolysis of the glycoside polyphenols into aglycone polyphenols. The bio-active ingredients of plants existing in the form of glycosides are hydrophilic and soluble in water due to the glycosyl group. However, these properties of glycosides make them disadvantageous ingredients for skin cosmetics due to their low skin permeability. Meanwhile, aglycone ingredients are hydrophobic and can permeate human skin.[45] Thus, the hydrolysis of glycoside ingredients into aglycones has attracted attention as an effective way of enhancing the bio-activity of extracts.[46] The major effects of ginseng are thought to be due to enhanced skin nutrition as a result of stimulation and increased blood circulation and cell proliferation, resulting in increased metabolism which leads to an ''anti-aging'' effect.[47] Many studies indicate that the anti-aging activity is due to the free radical scavenging action of the ginseng ginsenosides and inhibition of lipoperoxidation.[48]

Ginseng has potential side effects. The American Herbal Products Association (AHPA) classifies ginseng as a Class 2d herb, which means that its use is subject to restrictions. Most side effects of ginseng have been reported in individuals who took high doses or who took ginseng continually for a long period of time. One reported case was of an individual who developed anaphylaxis-like symptoms shortly after ingesting a small amount of ginseng syrup.[49] Ginseng may cause a very serious skin reaction called Stevens–Johnson Syndrome (SJS). SJS is a life-threatening

condition affecting the skin, in which cell death causes the epidermis to separate from the dermis. The syndrome is thought to be a hypersensitivity complex that affects the skin and the mucous membranes.[50] From our results, the 10% RG caused skin sensitization in the guinea pig maximization test, but 10% FRG had less sensitization effects. Following the contact of skin with an allergenic compound, such as DNCB, skin cells are activated resulting in the rapid production of a whole array of inflammatory cytokines (including IL-1β, IL-6, and TNF-α).[51] Tissue and plasma levels of IL-6 were lower in the FRG group than in the RG group. Choo *et al.*[52] reported that CK has an anti-allergic activity from its cell membrane stabilizing activity. Flavonoids are recognized as possessing anti-inflammatory, antimutagenic, antiallergenic, and antioxidant activities.[53] Therefore, we presumed that the skin sensitization depression of FRG was caused by increases in compound K and flavonoids. It has been widely documented that ginseng can activate skin metabolism, reduce keratinisation, moisturise and soften, alleviate wrinkling, and increase dermal blood circulation. Cell proliferation resulting from increased metabolism leads to anti-aging effects that are very valuable for cosmetics. RG extracts are commonly used in anti-aging, anti-wrinkle, and after-sun products. The safety of cosmetic ingredients is more important than their efficacy. RG is a good cosmeceutical ingredient, but its safety has remained unclear. In our study, a high dosage of RG (10%) was used showing toxicological potency. However, FRG showed an increased anti-wrinkle efficacy and a reduced toxicological potency.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

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