Research article

Antiaging effects of the mixture of Panax ginseng and Crataegus pinnatifida in human dermal fibroblasts and healthy human skin

Eunson Hwang¹,², Sang-Yong Park¹,², Chang Shik Yin², Hee-Taek Kim³, Yong Min Kim³, Tae Hoo Yi¹,*

¹Department of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University Global Campus, Giheung-gu, Yongin-si, Gyeonggi-do, Korea
²Acupuncture and Meridian Science Research Center, College of Korean Medicine, Kyung Hee University, Seoul, Korea
³College of Oriental Medicine, Semyung University, Jecheon, Chungbuk, Korea

Abstract

Background: Human skin undergoes distinct changes throughout the aging process, based on both intrinsic and extrinsic factors. In a process called photoaging, UVB irradiation leads to upregulation of matrix metalloproteinase-1, which then causes collagen degradation and premature aging. Mixtures of medicinal plants have traditionally been used as drugs in oriental medicine. Based on the previously reported antioxidant properties of Panax ginseng Meyer and Crataegus pinnatifida, we hypothesized that the mixture of P. ginseng Meyer and C. pinnatifida (GC) would have protective effects against skin aging.

Methods: Anti-aging activity was examined both in human dermal fibroblasts under UVB irradiation by using Western blot analysis and in healthy human skin by examining noninvasive measurements.

Results: In vitro studies showed that GC improved procollagen type I expression and diminished matrix metalloproteinase-1 secretion. Based on noninvasive measurements, skin roughness values, including total roughness (R1), maximum roughness (R2), smoothness depth and average roughness (R3), and global photodamage scores were improved by GC application. Moreover, GC ameliorated the high values of smoothness depth (R4), which means that GC reduced loss of skin moisture.

Conclusion: These results suggest that GC can prevent aging by inhibiting wrinkle formation and increasing moisture in the human skin.

Copyright © 2016, The Korean Society of Ginseng, Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Similar to all organ systems, the human skin undergoes physiological changes with advancing age. There are two distinct types of skin aging, intrinsic and extrinsic. Intrinsic aging (chronological aging), which is likely under the control of individual genetics, inevitably occurs as a natural consequence of physiological changes [1]. By contrast, ambient conditions such as exposure to UV radiation, chemical toxins, and smoking lead to extrinsic aging (photoaging) [2]. These two forms of aging are synergistically responsible for different skin changes that can generate lesions, often on the face. The representational clinical sign of both intrinsically and extrinsically aged skin is wrinkles [3]. Fine wrinkles, loss of underlying fat leading to hollowed cheeks and eye sockets, and dry and itchy skin are manifestations of intrinsic aging. The development of permanent facial lines results from the repetitive actions of facial musculature. In particular, eye wrinkles, generally called crow’s feet, are formed by contraction of the orbicularis oculi muscle [4]. Deep, coarse wrinkles and roughness are characteristics of photoaging [5].

Collagen alterations are considered to be a primary cause of skin aging and wrinkle formation. In the human dermis, collagen accounts for roughly 90% of the proteins. A subfamily of matrix metalloproteinases (MMPs), MMP-1, initiates the degradation of
collagen types I, II, and III in the skin [6]. Type I collagen is synthesized from procollagen type I, a soluble precursor [7]. Exposure to UV irradiation leads to upregulation of MMP-1 production, which disrupts collagen and the other extracellular matrix proteins [8].

Panax ginseng Meyer has been used as an oral herbal medicine for treatment of various diseases. The biological and pharmacological activities of ginseng, such as antiaging, antiinflammatory, and antioxidative effects, have been previously investigated [9–11]. The main components of ginseng are ginsenosides, which are steroidal saponins responsible for the biological activities of ginseng. The major ginsenosides (Rx1, Rx2, Rg, Rd, Re, and Rg1) account for about 80% of ginseng components, and the minor ginsenosides (F1, F2, Rg3, Rh1, Rh2, compound Y, compound Mc, and compound K) are absent or present at low concentrations in ginseng [12]. There are many studies about the pharmacological activities of ginseng extract and ginsenosides with regard to skin aging. The ginsenoside Rb1 exhibits antiaging activities in the skin that result from an increase of type I collagen production and the suppression of UV-induced apoptosis [13,14]. The topical application of compound K increased the amount of hyaluronan in the skin of hairless mice [15]. The ginsenoside F1 protected human HaCaT keratinocytes against UVB-induced apoptosis [16]. Furthermore, our previous studies showed that enzyme-modified ginseng extract and enzymatically processed Korean Red Ginseng have inhibitory effects against UVB-induced skin aging in human dermal fibroblasts and hairless mice [17,18].

Traditionally, Crataegus pinnatifida, commonly named Hawthorn, has been used in oriental medicine as a peptic agent and a blood circulation enhancer [19]. Several recent studies have analyzed the dermatological effects of C. pinnatifida. Kao et al [20] reported that polyphenols from fruits of C. pinnatifida have antitransformation and antioxidant effects on TPA-induced skin tumors in ICR mice. Huang et al [21] reported that neoliogand glycosides from seeds of C. pinnatifida have antioxidant and tyrosinase inhibitory effects. Furthermore, our earlier study demonstrated hair growth activity in C57BL/6 mice treated with the fruits of C. pinnatifida [22].

These results have led us to hypothesize that the mixture of Korean P. ginseng and C. pinnatifida (GC) inhibits wrinkle formation in the aging of human skin. To test this hypothesis, markers of skin photoaging, MMP-1 and procollagen type 1, were analyzed using Western blot with normal human dermal fibroblasts (NHDFs). Moreover, a greater understanding of the effect of GC was established by conducting a clinical trial. These results suggest that GC works effectively in cosmetic applications through control of collagen metabolism.

2. Materials and methods

2.1. Part 1. In vitro study

2.1.1. Preparation of the GC mixture

The GC mixture contains a 1:1 ratio of each ingredient and was produced using a patented protocol (Korea Patent, no. 1013848730000; composition comprising extracts of P. ginseng and Crataegus fructus for improving skin beauty). Six-year-old Korean P. ginseng was provided by Dr. Byung-Goo Cho (R&D Headquarters, Korea Ginseng Corporation, Daejeon, Korea) and was extracted using a patent protocol (Korea Patent, No. 1014540660000). Dried C. pinnatifida fruits were purchased from Kumkang Pharm Inc. (Ansung, Korea) and were extracted as previously described. In addition, the compositions of P. ginseng and C. pinnatifida have been analyzed and previously characterized by our research group [17,22]. Based on the results of our patent, we established an optimal extraction ratio to produce a 1:1 mixture of P. ginseng and C. pinnatifida by weight.

2.1.2. Cell culture

NHDFs (MCTT, Seoul, Korea) were plated in 100-mm tissue culture dishes and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin–streptomycin (Gibco-BRL, Gaithersburg, MD, USA) at 37°C in a humidified atmosphere containing 5% CO2. All experiments were performed using only those cells between passages 6 and 10.

2.1.3. UV irradiation and sample treatment

UVB irradiation and sample treatment were performed according to the method previously reported by Hwang et al [23]. NHDFs were irradiated with UVB (144 mJ/cm²) using the UVB irradiation machine (Bio-Link BLX-312; Vilber Lourmat GmbH, France). The treatment concentration of the extracts of Korean P. ginseng, C. pinnatifida, and GC was 100 μg/mL. Control cells were subjected to the same culture conditions without UVB exposure. For Western blot analysis, the cells were harvested 48 h after UVB irradiation.

2.1.4. Cell viability

After various treatments, the culture medium was removed and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.1 mg/mL) was added, followed by incubation at 37°C for 2 h in a CO2 incubator. After the formazan crystals were completely dissolved by dimethyl sulfoxide, absorbance was measured at 570 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.1.5. Western blot analysis

For Western blot analysis, the cells were lysed with lysis buffer [50mM Tris-Cl, pH 8.0, 0.1% sodium dodecyl sulfate (SDS), 150mM NaCl, 1% NP-40, 0.02% sodium azide, 0.5% sodium deoxycholate, 100 μg/mL phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, and phosphatase inhibitor] and centrifuged at 12,000 g for 7 min. The cells were then homogenized in an equivalent amount of protein, and the protein concentration was measured using Bradford reagent (Bio-Rad, Hercules, CA, USA). Homogenized proteins were electrophoresed on 8% or 10% SDS-polyacrylamide gels (SDS-PAGE) and were transferred from SDS-PAGE to a nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). Nonspecific binding was then blocked with 5% nonfat milk in TBST (Tris-buff ered saline with Tween 20; 50 mM Tris–HCl, pH 7.5, 150 mM/NaCl and 0.1% Tween 20) for 1 h at room temperature, and the primary antibody was applied overnight at 4°C. The membrane was then washed three times with TBST buffer and incubated with secondary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) for 1 h at room temperature. Finally, the protein level was determined using chemiluminescent detection ECL reagents (Fuji-film, LAS-4000, Tokyo, Japan) and ImageMaster 172D Elite software, version 3.1 (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.2. Part 2. Clinical trial

2.2.1. Preparation of GC cream

GC and placebo formulations were identical in color, fragrance, and packaging. The composition of formulations is presented in Table S1.

2.2.2. Participants and study design

In this randomized, double-blind study, 21 healthy Korean women aged 30–65 yr presenting with crow’s feet wrinkles were enrolled, 20 of whom were recruited as participants. One of the initial 21 participants was excluded from subsequent analysis owing to agreement withdrawal/treatment refusal (n = 1). The study was approved by the institutional review board at the National Medical Center, Seoul, Korea. All participants gave written, informed consent before participating in the study.
participants were clinically diagnosed with a global photo damage score of 2–6 according to the Jung score for photoaging of facial skin. Participants were requested to (1) avoid exposure to sunlight during the trial; (2) refrain from using lotions, creams, or other products on the face; (3) refrain from smoking or drinking alcohol in excess; and (4) refrain from using makeup less than 12 h prior to assessment. Participants were excluded if they reported: (1) use of topical antiaging products in the past 3 mo; (2) use of tretinoin or retinoic acid in excess; and (4) refrain from using makeup less than 12 h prior to assessment. Participants were not allowed to use any other cosmetic products during the trial period. The study was conducted in accordance with the guidelines of the Korea Food and Drug Administration. The trial was approved by the Ethics Committee (Oriental Hospital of Se-Myung University, Jecheon, South Korea) in August 2011. This study was carried out in accordance with the Declaration of Helsinki (1964), which was subsequently modified in Tokyo (2004) and Seoul (2008).

2.2.3. Blinding

Only the study coordinator knew the group allocation and prepared the creams 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 case. Group allocation information was opened to the participant at the end of the trial period.

2.2.4. Treatment

All volunteers were instructed on application of the formulation, and crow’s feet wrinkles were selected as the testing area. The steps were performed according to the method previously reported by Hwang et al [24]. Active formulation (GC) was applied on the left or right foot manifesting signs of crow’s feet, and the placebo (vehicle formulation) was applied on the other foot, twice daily (morning and night) for 12 wks. Prior to application, participants were asked to wash their skin using the same mild facial nickel-tested cleanser. Participants were not allowed to use any other cosmetic products during the treatment period.

2.2.5. Visual assessment

Patients were assessed at the investigating center at baseline (Week [Wk] 0) and at Wk 4, Wk 8, and Wk 12. The primary efficacy endpoint was the investigator’s assessment of the global photodamage score (GPDS) at Wk 12. GPDS ranged from 0 to 7 as follows: 0, none; 1, none/mild; 2, mild; 3, mild/moderate; 4, moderate; 5, moderate/severe; 6, severe; and 7, very severe [25].

2.2.6. Photography

Photographs of the patients’ faces lateral of the right and left eyes were taken under standardized conditions at Wk 0, Wk 4, Wk 8, and Wk 12. All photographs were taken with participants looking straight ahead under constant lighting conditions using the same camera, camera settings, and camera placement. A Nikon D200 camera (Nikon, Tokyo, Japan) was positioned on an SVK 35D tripod (Slik, Saitama, Japan) to the right of the participant’s face and perpendicular to the area to be photographed. Simultaneously, an Olympus twin T28 Macroflash unit (Olympus, Shinjuku, Japan) was positioned with one flash head directly above the lens pointed at the participant and the other directed away. Exposure was controlled by adjusting the lens aperture with the flash set on manual at full output.

2.2.7. 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 50 ± 10%. An acclimatization time of at least 30 min was allowed before measurements were taken. To obtain skin replicas, 1 cm of light-bodied silicone (SilfloR, Flexico, Colchester, UK) was prepared from two components mixed at a 1:1 ratio under decompression (2 drops each, catalyst and thinner). The mixture was maintained using an opaque case. Group allocation information was opened to the participant at the end of the trial period.
was then applied to the skin surface. After drying and hardening, the replicas were subjected to further analysis (Fig. 1). Image analysis was performed using the Skin Viscometer SV 600 program (Courage & Khazaka, Cologne, Germany). Arbitrary units (R1) were analyzed using the Skin Viscometer SV 600 program (Courage & Khazaka, Cologne, Germany). Arbitrary units (R1) were assigned to each sample based on furrow depth, which was determined from shadow size and brightness due to interference under illumination. Roughness parameters included R1 (skin roughness), R3 (average roughness), and R5 (arithmetic average roughness). Skin roughness (R1) was defined as the difference between the highest crest and lowest furrow. Maximum roughness (R2) was the largest value recorded. Depth of smoothness (R4) was also recorded.

2.2.8. Questionnaire study

In clinical research, questionnaires are routinely used to assess the reliability and validity of the study [26]. At the completion of the 12-wk study period, all participants answered the following questions presented in a questionnaire: “Has your skin anti-wrinkling improved?”, “Have you noticed an improvement in your skin elasticity?”, “Have you noticed an improvement in your skin moisture?”, and “Have you noticed an improvement in the shining of your skin?”. The answer choices were as follows: A, excellent; B, good; C, moderate; D, poor; and F, bad.

2.2.9. Clinical assessment

Assessments were performed at Wk 0, Wk 4, Wk 8, and Wk 12 by the same two professional investigators who were blinded to previous assessments. Right and left eyes were graded separately. Adverse effects included erythema, edema, scaling, itching, stinging, burning, tightness, and pricking, and were graded as none, mild, severe, or very severe. Evaluation was performed in the same location at each visit and with the same lighting.

2.3. Statistical analysis

Statistical analysis was performed for those who completed the trial. SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Differences between groups were evaluated with Student t test, and differences within groups were analyzed with analysis of covariance. A p value < 0.05 was considered statistically significant. Descriptive statistical analysis was performed using the questionnaire assessment.

3. Results

3.1. Part 1. In vitro study

3.1.1. Effects of GC on cell viability and expression of MMP-1 and procollagen type I in UVB-irradiated NHDFs

An MTT assay was conducted to measure the effects of P. ginseng, C. pinnatifida, and GC on the cell viability of NHDFs. The cell viability was increased by 112%, 127%, and 132% with 100 μg/mL of P. ginseng, C. pinnatifida, and GC, respectively, compared with that of the UVB-irradiated control (Fig. 1). MMP-1 and procollagen type I protein levels were measured in UVB-irradiated NHDFs, and those treated with P. ginseng, C. pinnatifida, and GC. Western blot data showed that UVB irradiation elevated MMP-1 protein levels and inhibited expression of procollagen type I within 24 h (Fig. 2A). To confirm the comparative effects of P. ginseng, C. pinnatifida, and GC, NHDFs were treated with 100 μg/mL of each substance. The P. ginseng treatment decreased the expression of procollagen type I, whereas C. pinnatifida increased it. As shown in Fig. 2B, GC treatment strongly improved the procollagen type I expression that was reduced by UVB exposure (up to 249%). By contrast, MMP-1 expression decreased by 17% and 40% after treatment with 100 μg/mL of P. ginseng and C. pinnatifida, respectively. Moreover, Western blot data showed that GC-treated cells had the highest reduced UVB-induced protein accumulation of MMP-1 (down to 54%; Fig. 2C). These results suggest that GC may more effectively inhibit photaging in NHDF cells than either P. ginseng or C. pinnatifida alone.

3.2. Part 2. Clinical trial

3.2.1. Participant demographics

Twenty-one individuals (female) with an average age of 47.00 ± 7.97 yr entered the study (Fig. 3). Owing to failure to adhere to the protocol, one person withdrew. As shown in Table 1, eye wrinkles on the right and left sides of the face were assessed by GPDS prior to treatment with GC. Average GPDS values were 4.29 ± 1.45 and 4.19 ± 1.36 for the right and left sides of the face, respectively. Prior to the topical application of GC and placebo, 57% and 43% of the participants reported dry skin with normal elasticity. Additionally, 62% had thin crow’s feet, and 38% had thick, deep crow’s feet.

3.2.2. Analysis of GC effects on eye wrinkles in human skin

As shown in Table 2, eye wrinkle comparisons were made by measuring GPDS prior to and after 4 wks, 8 wks, and 12 wks of...
treatment. Prior to the treatment with GC and placebo, baseline GPDSs were 4.25 ± 1.48 and 4.15 ± 1.40, respectively. At 8 wks of treatment, no difference was observed in the GC group; however, the GPDS value decreased after 12 wks of GC treatment (3.95 ± 1.76). A small change was seen in the placebo group (4.20 ± 1.40 at 4 wks and 8 wks, 4.15 ± 1.46 at 12 wks).

At Wk 0, Wk 4, Wk 8, and Wk 12, comparisons of the assessments at each eye wrinkle site (right and left) were measured using an SV600 visiometer. Prior to the treatment with GC and placebo, values of total skin roughness (R1) were 0.14 ± 0.03 arbitrary unit (AU) and 0.14 ± 0.05 AU, respectively. At Wk 4, Wk 8, and Wk 12, R1 values for the GC group were significantly decreased by 0.013 ± 0.03 AU, 0.12 ± 0.03 AU, and 0.11 ± 0.03 AU, respectively. Baseline maximum roughness (R2) was 0.09 ± 0.02 AU and 0.08 ± 0.03 AU in the GC and placebo groups, respectively. GC treatment decreased R2 values by 0.08 ± 0.02 AU, 0.07 ± 0.02 AU, and 0.07 ± 0.01 AU at Wk 4, Wk 8, and Wk 12, respectively. However, no significant difference in R1 or R2 value was found at Wk 4, Wk 8, or Wk 12 in the placebo group. Prior to the treatment with GC and placebo, values of average roughness (R3) were 0.06 ± 0.02 AU and 0.06 ± 0.01 AU, respectively. After 12 wks, R3 values were 0.05 ± 0.01 AU in both groups. After 12 wks, the GC group demonstrated more effectively decreased R1, R2, and R3 roughness values than the placebo group.

High smoothness depth (R4) values mean that the skin loses moisture, which results in rough surfaces. Baseline R4 values in the GC and placebo groups were 0.07 ± 0.02 AU. At 12 wks, the R4 value was improved only in the GC group (0.06 ± 0.02 AU). GC ameliorated the high values of smoothness depth (R4), which means that GC improved loss of skin moisture. No differences in arithmetic roughness average (R5) value were seen in either group (Table 3). The values of R1, R2, R3, and R4 decreased in a statistically significant manner in the GC group. Photographs of clinical results are shown in Figs. 1–3. Representative pictures of clinical results are shown in Fig. 4. Crow’s feet wrinkles in the GC treatment group appeared thinner and dimmer in photographs compared with placebo.

### 3.2.3. Self-satisfaction assessment after the treatment

Responses to the questionnaire are summarized in Fig. 5. The respondents also noted that GC was more effective for skin elasticity, moisturizing, and shining than placebo. However, almost all participants reported no difference in antiaging effect between the two products.

### Table 2

| GPDS values in Week 0 | GC         | Placebo    | p<sup>1</sup> | p<sup>2</sup> |
|-----------------------|------------|------------|--------------|--------------|
|                       | Arbitrary units (average ± SD) |
| GPDS Baseline         | 4.25 ± 1.48 | 4.15 ± 1.39 | 0.041        | 0.330        |
| After 4 wks           | 4.25 ± 1.48 | 4.20 ± 1.40 | 0.050*       | 0.000        |
| Difference<sup>1</sup> | 0.00 ± 0.00 | 0.05 ± 0.22 | 0.000         | 0.050*       |
| p<sup>2</sup>          | 0.000       | 0.050*     |              |              |
| After 8 wks           | 4.25 ± 1.48 | 4.20 ± 1.40 | 0.041        | 0.330        |
| Difference<sup>1</sup> | 0.00 ± 0.00 | 0.05 ± 0.22 | 0.000         | 0.050*       |
| p<sup>2</sup>          | 0.000       | 0.050*     |              |              |
| After 12 wks          | 3.95 ± 1.76 | 4.15 ± 1.46 | 0.000        | 0.025*       |
| Difference<sup>1</sup> | -0.30 ± 0.47 | 0.00 ± 0.32 | 0.128        |              |
| p<sup>2</sup>          | 0.105       | 0.073      |              |              |

<sup>1</sup>p < 0.05, by Student t test for comparison with placebo group.

<sup>2</sup>GC, mixture of Panax ginseng and Crataegus pinnatifida; GPDS, global photodamage score; SD, standard deviation.

<sup>1</sup>Compared between groups: p value by Student t test.

<sup>2</sup>Compared between groups: p value by analysis of covariance (adjustment with baseline).

<sup>3</sup>After 4 wks – baseline.

<sup>4</sup>Compared within groups: p value by paired t test.

<sup>5</sup>After 8 wks – baseline.

<sup>6</sup>After 12 wks – baseline.
studies have reported that complement and enhance the effects of one another [28]. Many drugs because compounds in the mixtures have the potential to growth induced by the fruits of

3.2.4. Safety results

An analysis of skin replicas

Table 3

| Smoothness depth (R4) | Arithmetic average (R5) |
|----------------------|-------------------------|
| Baseline             | GC                      | Placebo | p<sup>1</sup> | p<sup>2</sup> |
|                      | 0.07 ± 0.02             | 0.07 ± 0.02 | 0.574 | 0.359 |
| After 4 wks          | 0.06 ± 0.02             | 0.06 ± 0.02 | 0.006** | 0.000** |
| p<sup>1</sup>        | 0.005**                 | 0.000** |
| After 8 wks          | 0.06 ± 0.02             | 0.06 ± 0.03 | 0.279 | 1.000 |
| Difference<sup>1</sup> | 0.01 ± 0.02            | 0.01 ± 0.02 | 0.006** | 0.000** |
| p<sup>2</sup>        | 0.003**                 | 0.005* |
| After 12 wks         | 0.06 ± 0.02             | 0.07 ± 0.03 | 0.955 | 0.034* |
| Difference<sup>2</sup> | 0.01 ± 0.02            | 0.00 ± 0.02 | 0.007** | 0.000** |
| p<sup>3</sup>        | 0.005**                 | 0.005** |

* p < 0.05, ** 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 4 wks — baseline.
4) After 8 wks — baseline.
5) After 12 wks — baseline.
6) After 24 wks — baseline.

Effects against UVB-induced skin aging [23,29,31,32]. There have been many studies elucidating the antioxidant activities of P. ginseng and C. pinnatifida. P. ginseng was shown to abrogate oxidative stress in hepatoma cells and brain cells, as well as in human clinical trials [33–35]. Additional studies have detailed the antioxidant activities of C. pinnatifida in mouse lymphocytes, rat pups with selenite-induced cataract, and in the livers of high-fat fed mice [36–38]. Therefore, we hypothesized that GC has protective effects against UVB-induced skin aging. Fig. 2 shows that the cells treated with GC exhibited suppressed MMP-1 and increased procollagen type 1 levels under UVB-irradiated conditions, as expected. Additionally, GC promoted proliferation of NHDFs. These effects were weaker when cells were treated with only P. ginseng or C. pinnatifida. However, we did not investigate alternative mechanisms to explain these synergic effects in NHDFs. We will examine the mechanism of GC effects in future studies.

Life expectancy in the United States and other industrialized countries continues to increase and is expected to reach 100 yr by about 2025 [39]. In modern society, there is a strong desire to find methods to maintain a youthful appearance. Because the skin is the most visible indicator of age, reducing the appearance of facial wrinkles is effective in creating a younger visage [40]. In aged skin, reductions in moisture and fat emulsion are observed [41]. Changes in the amino acid composition of aged skin may reduce the amount of cutaneous natural moisturizing factor that supports skin rejuvenation, thereby decreasing its capacity for water binding [42]. Therefore, hydration is an important antiaging factor for the skin. Although our clinical trial was conducted in dry, cold conditions (average humidity, 62.3%; average temperature, 6.9°C; Korea Meteorological Administration), when GC was topically applied at the site of eye wrinkles, skin moisture was improved (R4). Furthermore, the volunteers in the GC group responded in

3.2.4. Safety results

No participants reported adverse reactions to treatment, such as erythema, edema, scaling, itching, stinging, burning, tightness, or pricking.

4. Discussion

Previous studies have shown that chemical-based ingredients can cause allergic reactions or irritation. Because of these negative effects, many studies have focused on developing new products using herbal plants or their derived compounds, which are considered to be less toxic and free of adverse effects [27]. Moreover, mixtures of medicinal plants have been used as traditional drugs because compounds in the mixtures have the potential to complement and enhance the effects of one another [28]. Many studies have reported that P. ginseng is a powerful skin antiaging agent [11,14,16–18]. Also, our earlier study demonstrated hair growth induced by the fruits of C. pinnatifida in a C57BL/6 mouse model [22]. Therefore, we investigated GC, the mixture of P. ginseng and C. pinnatifida, in UVB-induced NHDFs and healthy human skin.

The mechanisms of the skin that lead to photoaging are induced mainly by the repetitive absorption of UV radiation, which can generate reactive oxygen species and stimulate the expression of extracellular matrix proteases via AP-1 signaling [29]. AP-1, made of Jun and Fos family proteins, is a transcription factor that inhibits collagen production and upregulates collagen breakdown by activating enzymes called MMPs [30]. Because the skin’s natural antioxidant defenses are disrupted by high doses of UV light, reactive oxygen species are considered to be key factors involved in the initiation of the skin aging process [23]. Our previous studies demonstrated that Aloe vera, coriander, azuki beans, and Galla chinensis, all of which have antioxidant activity, have protective
Fig. 4. Representative pictures of prior to and after the treatment.

Fig. 5. Questionnaires following a 12-wk treatment with GC and placebo. The answer choices were as follows: A, excellent; B, good; C, moderate; D, poor; and F, bad. GC, mixture of *Panax ginseng* and *Crataegus pinnatifida*. 
questionnaires that the treatment had a more moisturizing effect than did those in the placebo group. Noninvasive devices permit evaluation of the correlation between hydration and roughness; thus, many investigators use such devices to assess antiwrinkle efficacy [43]. In the present study, we used the SV600 visiometer. Based on this device, the decreased skin roughness values (R1, R2, and R3) observed after GC application suggest that the appearance of wrinkles was more effectively improved compared to the placebo. Moreover, statistical differences in the results were significant. The participants in the GC group showed decreased GPDSs after 12 wks. In photographs, the GC treatment group appeared to have faint and thin crow's feet. However, all antiaging phenomena resulting from GC application could not be explained by our findings. Therefore, we suggest that additional studies be conducted to assess the histological changes and precise mechanisms of GC treatment in the skin.

In conclusion, this study demonstrated that GC can limit the skin aging process. First, GC has a protective effect against UVB-exposed photoaging of skin by regulating procollagen type I and MMP-1 expression in NHDs. Second, after 12 wks, participants in the GC group showed decreased GPDSs and skin roughness, a precursor to wrinkle formation. Third, in spite of low humidity in the environment, the moisturizing effects of GC were remarkable. These findings suggest that GC as a natural ingredient may be powerful in regulating skin aging.

Conflicts of interest

All authors have no conflicts of interest to declare.

Acknowledgments

This research was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (iPET, 810006-03-3-SB110), Republic of Korea.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2016.01.001.

References

[1] Gilchrest BA, Yaar M. Ageing and photoaging of the skin: observations at the cellular and molecular level. Br J Dermatol 1992;127:25–30.
[2] Fisher GJ, Wang Z, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med 1997;337:1419–28.
[3] Rittie L, Fisher GJ. Natural and sun-induced aging of human skin. Cold Spring Harb Perspect Med 2015;5:a015370.
[4] Poljak B, Dalmane R, Godic A. Intrinsic skin aging: the role of oxidative stress. Acta Dermato-Venereolog Alp Panonica Adriat 2012;21:33–6.
[5] Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, Voorhees JJ. Mechanisms of photoaging and chronological skin aging. Arch Dermatol 2002;138:1462–70.
[6] Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ, Fisher GJ. Matrix-degrading metalloproteinases in photoaging. J Invest Derm Symp Proc 2009;14:20–4.
[7] Rittie L, Fisher GJ. UV-light-induced signal cascades and skin aging. Aging Res Rev 2002;1:705–20.
[8] Fisher GJ, Quan T, Purohit T, Shao Y, Cho MK, He T, Varani J, Kang S, Voorhees JJ. Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. Am J Pathol 2009;174:101–14.
[9] Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH. Ginseng in traditional herbal pre-scriptions. J Ginseng Res 2012;36:225–41.
[10] Hong CE, Lyou SY. Anti-inflammatory and antioxidative effects of Korean red ginseng extract in human keratinocytes. Immune Netw 2011;11:42–9.
[11] Kang TH, Park HM, Kim YB, Kim H, Kim N, Do JH, Kang C, Cho Y, Kim SY. Effects of red ginseng extract on UVR irradiation-induced skin aging in hairless mice. J Ethnopharmacol 2009;123:446–51.
[12] Lu JM, Yao Q, Chen C. Ginseng compounds: an update on their molecular mechanisms and medical applications. Current Vascular Pharmacol 2009;7:293–300.
[13] Cai BX, Jin SL, Luo D, Lin XF, Gao J. Ginsenoside Rd1 suppresses ultraviolet radiation induced apoptosis by inducing DNA repair. Biol Pharm Bull 2009;32:437–41.
[14] Kwon OW, Kim SY, et al. Enzyme-processed Korean Red Ginseng extracts inhibit UVB irradiation in hairless mice. J Ginseng Res 2013;37:425–34.
[15] Chang Q, Zuo Z, Harrison F, Chow MS, Hawthorn. J Clin Pharmacol 2002;42:87–92.
[16] Kao ES, Wang CJ, Lin WL, Chu CY, Tseng TH. Effects of polyphenols derived from fruit of Crataegus pinnatifida on cell transformation, dermal edema and skin tumor formation by phorbol ester application. Food Chem Toxicol 2007;45:1795–804.
[17] Huang XX, Liu QR, Wu J, Yu LH, Cong Q, Zhang Y, Lou LL, Liu Z, Song SJ. Antioxidant and tyrosinase inhibitory effects of neooligan glycosides from Crataegus pinnatifida seeds. Planta Med 2014;80:1732–8.
[18] Shin HS, Lee JM, Park SY, Yang JE, Kim JH, Yi TH. Hair growth activity of Crataegus pinnatifida on C57BL/6 mouse model. Phytother Res 2013;27:1352–7.
[19] Hwang E, Kim SH, Lee S, Cho SG, Kim J, Kim SY. A comparative study of baby immature and adult shoots of Aloe vera on UVB-induced skin photoaging in vitro. Phytother Res 2013;27:1874–82.
[20] Hwang E, Park SY, Jo H, Lee DG, Kim HT, Kim YM, Yin CS, Yi TH. Efficacy and safety of enzyme-modified Panax ginseng for anti-wrinkle therapy in healthy skin: a single-center, randomized, double-blind, placebo-controlled study. Rejuvenation Res 2015;18:449–57.
[21] Chung JH, Lee SH, Youn CS, Park BJ, Kim KH, Park KC, Cho KH, Eun HC. Cutaneous photodamage in Koreans: influence of sex, sun exposure, smoking, and skin color. Arch Dermatol 2001;137:1043–51.
[22] Edwards P. Questionnaires in clinical trials: guidelines for optimal design and administration. Trials 2010;11:2.
[23] Aliq F, Mukhtar H. Photo chemo prevention by botanical antioxidants. Skin Pharmacol Appl Skin Physiol 2002;15:297–306.
[24] Chen Q. Evaluate the effectiveness of the natural cosmetic product compared to chemical-based products. Int J Chem 2009;1:57–9.
[25] Hwang E, Lee DG, Park SH, Oh MS, Kim SY. Coriander leaf extract exerts antioxidative activity and protects against UVB-induced photoaging of skin by regulation of procollagen type I and MMP-1 expression. J Med Food 2014;17:985–95.
[26] Quan T, He T, Voorhees JJ, Fisher GJ. Ultraviolet irradiation induces Smad7 via induction of transcription factor AP-1 in human skin fibroblasts. J Biol Chem 2005;280:8079–85.
[27] Hwang E, Park SY, Lee JH, Sun ZW, Lee TY, Song HG, Shin HS, Yi TH. Vigna angularis water extracts protect against ultraviolet B-exposed skin aging in vitro and in vivo. J Med Food 2014;17:1339–49.
[28] Sun ZW, Hwang E, Lee JH, Lee TY, Song HG, Park SY, Shin HS, Lee DG, Yi TH. Effects of Caulis chinensis extracts on UVB-irradiated MMP-1 production in human dermal fibroblasts. J Med Food 2015;18:224–32.
[29] Lee M, Sorn S, Baek S, Jang S, Kim S. Antioxidant and apoptotic effects of Korean white ginseng extracted with the same ratio of propanaxadiol and propanoxapinol saponins in human hepatoma HepG2 cell. Ann NY Acad Sci 2007;1117:217–27.
[30] Tu LH, Ma J, Liu HP, Wang RR, Luo J. The neuroprotective effects of ginsenosides on calcineurin activity and tau phosphorylation in SY5Y cells. Int J Mol Med 2009;24:45–50.
[31] Kao ES, Wang CJ, Lin WL, Chu CY, Tseng TH. Effects of polyphenols derived from baby immature and adult shoots of Aloe vera on UVB-induced skin photoaging in vitro. Phytother Res 2013;27:1078–82.
[32] Hwang E, Park SY, Jo H, Lee DG, Kim HT, Kim YM, Yin CS, Yi TH. Efficacy and safety of enzyme-modified Panax ginseng for anti-wrinkle therapy in healthy skin: a single-center, randomized, double-blind, placebo-controlled study. Rejuvenation Res 2015;18:449–57.
[33] Chung JH, Lee SH, Youn CS, Park BJ, Kim KH, Park KC, Cho KH, Eun HC. Cutaneous photodamage in Koreans: influence of sex, sun exposure, smoking, and skin color. Arch Dermatol 2001;137:1043–51.
[34] Li TP, Zhu RG, Dong YP, Liu YH, Li SH, Chen G. Effects of pectin pentaoligosaccharides on UVB irradiation in hairless mice. J Ginseng Res 2012;36:225–31.
[35] Cheng N, Wang Y, Gao H, Yuan J, Feng F, Cao W, Zheng J. Protective effect of Panax ginseng C.A. Meyer: a randomised, double-blind, placebo-controlled trial. PLoS One 2013;8:e51271.
[36] Cheng N, Wang Y, Gao H, Yuan J, Feng F, Cao W, Zheng J. Protective effect of extract of Crataegus pinnatifida polyol on DNA damage response to oxidative stress. Food Chem Toxicol 2013;59:799–14.
[37] Wang T, Zhang P, Zhao C, Zhang Y, Liu H, Lu G, Xiao Z. Preventive effect in semen-induced cataract in vivo and antioxidative effects in vitro of Crataegus pinnatifida leaves. Biogas Elem Res 2011;14:106–15.
[38] Li TP, Zhu RG, Dong YP, Liu YH, Li SH, Chen G. Effects of pectin pentaoligosaccharides from Hawthorn (Crataegus pinnatifida Bunge. var. Major) on the activity and mRNA levels of enzymes involved in fatty acid oxidation in the liver of mice fed a high-fat diet. J Agric Food Chem 2013;61:7599–605.
[39] Christensen K, Dobrhammer G, Rau R, Vaupel JW. Ageing populations: the challenges ahead. Lancet 2009;374:1196–208.

[40] Ramos-e-Silva M, Carneiro SCS. Cosmetics for the elderly. Clin Dermatol 2001;19:413–23.

[41] Fiers SA. Breaking the cycle: the etiology of incontinence dermatitis and evaluating and using skin care products. Ostomy Wound Manag 1996;42. 32–34, 36, 38–40.

[42] Jackson SM, Williams ML, Feingold KR, Elias PM. Pathobiology of the stratum corneum. West J Med 1993;158:279–85.

[43] Pena Ferreira MR, Costa PC, Bahia FM. Efficacy of anti-wrinkle products in skin surface appearance: a comparative study using non-invasive methods. Skin Res Technol 2010;2:189–94.