Biological effects of rutin on skin aging

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Abstract. Rutin, a quercetin glycoside is a member of the bioflavonoid family which is known to possess antioxidant properties. In the present study, we aimed to confirm the anti-aging effects of rutin on human dermal fibroblasts (HDFs) and human skin. We examined the effects of rutin using a cell viability assay, senescence-associated-β-galactosidase assay, reverse transcription-quantitative polymerase chain reaction, and by measuring reactive oxygen species (ROS) scavenging activity *in vitro*. To examine the effects of rutin *in vivo*, rutin-containing cream was applied to human skin. A double-blind clinical study was conducted in 40 subjects aged between 30-50 years and divided into control and experimental groups. The test material was applied for 4 weeks. After 2 and 4 weeks, dermal density, skin elasticity, the length and area of crow's feet, and number of under-eye wrinkles following the application of either the control or the rutin-containing cream were analyzed. Rutin increased the mRNA expression of collagen, type I, alpha 1 (*COL1A1*) and decreased the mRNA expression of matrix metallopeptidase 1 (*MMP1*) in HDFs. We verified that ROS scavenging activity was stimulated by rutin in a dose-dependent manner and we identified that rutin exerted protective effects under conditions of oxidative stress. Furthermore, rutin increased skin elasticity and decreased the length, area and number of wrinkles. The consequences of human aging are primarily visible on the skin, such as increased wrinkling, sagging and decreased elasticity. Overall, this study demonstrated the biological effects of rutin on ROS-induced skin aging.

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Introduction

The clinical manifestations of aging are fine wrinkles, thin and transparent skin, loss of underlying fat leading to hollowed cheeks and eye sockets, dry and itchy skin, lack of sufficient perspiration, hair graying, hair loss or hirsutism, and thinning of the nail plates (1). It is widely accepted that intrinsic aging is primarily caused by accumulated damage due to free radicals and by reactive oxygen species (ROS)-induced damage to critical cellular macromolecules (2). Not only does ROS production increase with age but the ability of human skin cells to repair DNA damage steadily decreases over time (3).

ROS play an important role in skin aging. In the skin, about 1.5-5% of the consumed oxygen is converted into ROS by intrinsic processes (4). ROS are continuously produced as byproducts in the electron transport chain of aerobic metabolism in the mitochondria, and are regarded as the principal cause of intrinsic aging (5). Keratinocytes and fibroblasts are the main producers of mitochondrial ROS in the skin.

Rutin, a quercetin glycoside (6), is a member of the bioflavonoid family also referred to as vitamin P (7). Previous research has identified rutin as an antioxidant with anti-inflammatory, antiallergenic, antiviral, and anticarcinogenic properties, capable of scavenging superoxide radicals (8-13). It has also been demonstrated that rutin is capable of inhibiting human platelet aggregation stimulated by collagen (14), decreasing capillary fragility (15), prolonging activated partial thromboplastin time (16) as well as of exerting anti-thrombotic effects (17).

The present study confirms that rutin reduces skin aging by strengthening dermal density and elasticity through the regulation of enzymes in the extracellular matrix (ECM).

Materials and methods

Cell culture. Human dermal fibroblasts, (HDFs; Lonza, Basel, Switzerland) were cultured in Dulbecco's modified Eagle medium (Gibco/Life Technologies, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin/streptomycin (Gibco/Life Technologies) at 37˚C in an atmosphere of 5% CO_2. Rutin was purchased from Sigma-Aldrich and dissolved in dimethyl sulfoxide.

Cell viability assay. HDFs were seeded at a density of $3x10^3$ cells/well in a 96-well plate and incubated for 24 h. Rutin (0-200 μM) was later added to the cells for 24 h to measure the cytotoxicity of rutin. To determine the protective effects against oxidative stress, the cells were pre-treated with rutin (0-100 μM) for 3 h and then exposed to 0.2 mM H_2O_2 for 24 h. The cytotoxicity of rutin in HDFs was evaluated using a water-soluble tetrazolium salt (WST-1) assay (EZ-Cytox cell viability assay kit; Itsbio, Seoul, Korea). The WST-1 solution was added to cultured cells at a volume equal to 10% of the culture medium, and then the cells were incubated at 37˚C for 1 h. Cell viability was evaluated by measuring the absorbance at 450 nm using an iMark microplate reader (Bio-Rad, Hercules, CA, USA).

Cellular senescence analysis. The expression of lysomal galactosidase as a marker for senescent HDFs was determined using the senescence-associated (SA)-β-galactosidase staining kit (BioVision, Milpitas, CA, USA) according to the manufacturer's instructions. HDFs were seeded at a density of $2x10^5$ cells/well in a 60-mm cell culture plate and incubated until they reached 90% confluence. Subsequently, the cells were treated with rutin and H_2O_2 for 24 h, the medium was removed, and the cells were washed once with phosphate-buffered saline (PBS). A fixing solution (0.5 ml; 4% formaldehyde and 0.5% glutaraldehyde in PBS buffer, pH 7.2) was added to the culture plate for 1 h in order to fix the cells, and 0.5 ml of a staining solution [staining solution 470 μl and 5 μl staining supplement, and 20 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in 25 μl dimethylformamide] for 24 h. The cells then were treated with 1 ml 70% glycerol, and images were captured using an Olympus IX51 microscope (Tokyo, Japan). The percentage of senescent cells was calculated as the senescence ratio [senescent cells (%) = stained cells/total cells x100].

Measurement of ROS scavenging activity. Intracellular ROS formation was measured by adding 2',7'-dichlorofluorescein diacetate (DCF-DA; Sigma-Aldrich). DCF-DA is non-fluorescent until it is hydrolyzed by intracellular esterases and oxidized into the highly fluorescent 2',7'-dichlorofluorescein (DCF) in the presence of ROS. The cells were seeded in 60-mm cell culture plates at a density of $2x10^5$ cells/well and incubated for 24 h. Following incubation, the cells were pre-treated with rutin (0-50 μM) for 3 h, and then exposed to 0.2 mM H_2O_2 for 24 h. After 24 h, 10 μM DCF-DA was added to the culture media for 30 min, and the medium containing DCF-DA was removed by washing with PBS. The cells were detached from the plates with 1% trypsin-EDTA and washed with PBS, and ROS was detected by flow cytometry (Becton-Dickinson, Franklin Lakes, NJ, USA).

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was isolated using TRIzol reagent (Life Technologies) according to the manufacturer's instructions. The purity and concentration of the RNA were evaluated using MaestroNano®, a microspectrophotometer (Maestrogen, Las Vegas, NV, USA). All cDNAs for sensitive and specific miRNA detection were synthesized using the miScript II RT kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The following primers were used: collagen, type I, alpha 1 (*COL1A1*) COL1A1 forward, 5'-AGGGCCAAGACGAAGACATC-3' and reverse, 5'-AGATCACGTCATCGCACAACA-3'; matrix metalloproteinase (*MMP)1* forward, 5'-TCTGACGTTGAT CCCAGAGAGCAG-3' and reverse, 5'-CAGGGTGACACCAG TGACTGCAC-3'. qPCR was performed using EvaGreen dye (Solis BioDyne, Tartu, Estonia) and LineGene K software (Bioer, Hangzhou, China). The CT value for each gene expressed was normalized to that of β-actin forward, 5'-GGATTCCTA TGTGGGCGACGA-3' and reverse, 5'-CGCTCGGTGA GGATCTTCATG-3'. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of each gene (6).

Subjects for clinical evaluation. The study protocols were approved by the Institutional Review Board of GeneCellPharm Incorporated (Cheongju-si, Korea). All subjects were informed of the objective of the study and provided informed consent and agreed to use skin care products during the study. Forty women, aged 30-50 years, were selected for a randomized and double-blind clinical trial (control group, 45.50±5.79 years; experimental group, 45.70±5.34 years). The selection of subjects was based on age, signs of skin aging, and being neither pregnant nor nursing. Subjects who presented with symptoms of itching or erythema, or those who hindered the evaluation process due to excessive drinking or smoking were excluded from the experiment. The subjects were divided into control and experimental groups, each containing 20 subjects. All conditions were the same except for the test material used on the experimental group. The study was conducted for 4 weeks. Biometric parameters were measured three times: prior to application, and 2 and 4 weeks after application. During this study, each subject performed a self-evaluation, and using a scale of severity (0, none; 1, mild; 2, severe; 3, very severe), completed a questionnaire to indicate to what extent they exhibited any skin disorders, such as erythema, itching, scaling, edema and tingling and burning sensations, at each visit. The subjects who presented with skin disorders withdrew from the clinical evaluation process.

In order to determine whether the rutin-containing cream had any adverse effects, the subjects were asked individually about the condition of their skin, and a visual evaluation of skin reactions, such as erythema, itching, scaling, tingling, tightness and prickling or burning sensations was performed at each visit. No adverse effects were reported based on either a visual evaluation or the questionnaire (Table I). To examine dermal density, skin elasticity as well as any improvement in wrinkles around the eye, the subjects were instructed to apply 2 g of test material to the face, including the eye rim, every morning and night for 4 weeks. The subjects and investigators were blinded to the test and control treatments. At each visit, all subjects washed with the cleanser provided and remained quietly in a room with constant temperature (22±1˚C) and humidity (45±5%) so that all subjects would be evaluated under the same conditions.

The cream was prepared by incorporating the ingredients in the 3 phases (A, B, C). The ingredients in the A phase (distilled water, glycerin, 1,3-butylene glycol) were combined and heated until all the components were melted, and the ingredients in the B phase (distilled water, dipotassium phosphate, sodium hydroxylate, rutin) were combined and heated to the same temperature, to ensure homogeneity. The A and B phase ingredients were combined and emulsified using a homo

Table I. Abnormal skin reactions reported by subjects.

Abnormal reaction	Severity	Abnormal reaction	Severity
Erythema	0	Tingling	0
Swelling (edema)	0	Burning	0
Scaling (epidermis)	0	Tightness	0
Itching	0	Prickling	0

Scale of severity, 0, none; 1, mild; 2, severe; 3, very severe.

mixer (Tokushu Kika Kogyo Co., Ltd., Japan) at 5,000 rpm for 10 min. The mixture was cooled to 60˚C and blended with the homogenized phase C (emulium delta, sepipuls 400) at 5,000 rpm for 10 min. By then, the temperature of the mixture had dropped to 45˚C. The mixture was combined and homogenized, while maintaining the pH at 6.2. The cream provided to the experimental group contained 2% (wt %) rutin and the cream provided to the control group was prepared using the same volume of water in place of rutin.

Measurement of skin elasticity. To evaluate the improvement in skin elasticity, a DermaLab USB elasticity probe (Cortex Technology ApS, Hadsund, Denmark) was applied and the results were analyzed using the associated application software (version 1.09). Measurements were obtained using a fixed elasticity probe on the left cheek of each subject. To analyze the elasticity measurements, Young's modulus (E) was calculated as a dose-dependent representation of skin elasticity. The measurements of elasticity were taken three times: prior to application, and 2 and 4 weeks after application. The measurement unit is MPa.

Measurement of dermal density. To evaluate dermal density, a DUB SkinScanner (tpm taberna pro medicum GmbH, Lüeneburg, Germany) was used. Dermal density was measured 3 cm from the left eye, applying a couplant for ultrasonic examination. The analysis range was limited to the area between the dermis and upper panniculus. The measurements were taken three times: prior to application, and 2 and 4 weeks after application.

Measurement of the length and area of crow's feet as well as under-eye wrinkles. To evaluate the extent of improvement in wrinkles, particularly at the eye rim, a Robo skin analyzer CS50 (Inforward Inc., Tokyo, Japan) was used. The facial images were captured from each subject placed in identical positions with equal lighting: on the front, left and right sides of the face. To evaluate improvement, measurements were taken three times: prior to application, and 2 and 4 weeks after application. We analyzed the captured images, matching the facial feature points accurately. The measurement unit of crow's feet length is mm and area is mm^2.

Statistical analysis. In cellular efficacy tests, all results are presented as the mean percentages ± standard deviation (SD) of three independent experiments. A P-value <0.05 as determined by the Student's t-test, was considered statistically

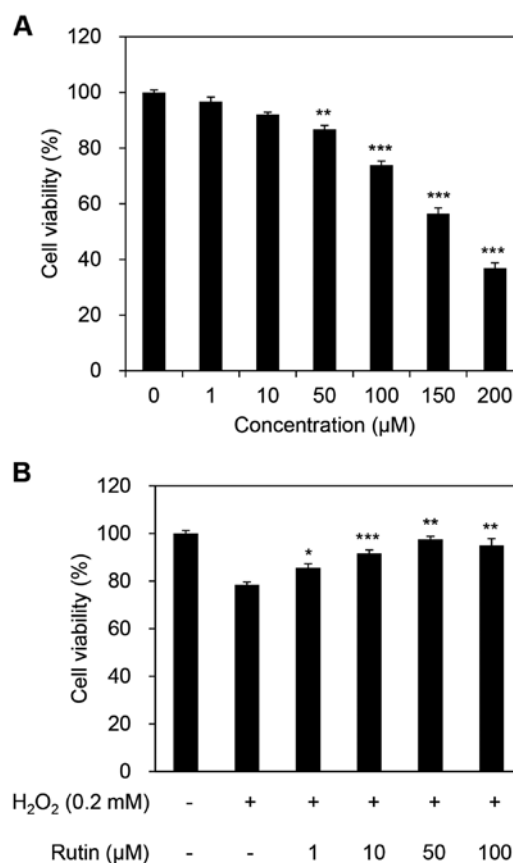Figure 1. Effects of rutin on cell viability in rutin- and H_2O_2-treated human dermal fibroblasts (HDFs). (A) Measurement of cell cytotoxicity of rutin in HDFs. (B) Inhibitory effects of rutin on H_2O_2-induced cellular damage response. *P<0.05, **P<0.01 and ***P<0.001 as determined by the Student's t-test.

significant. In clinical efficacy tests, statistical analyses were conducted using SPSS software (version 17.0 for Windows; IBM, Armonk, NY, USA). Paired t-tests were performed in cases of repeated measurements on the same subject. To analyze subject questionnaires, mean values, SDs, and percentages were used. The formula used to measure the percentage change for each skin parameter was percentage change = [(A-B)/B] x100, where A is defined as the individual value of any parameter at the 2 and 4 week visits, and B represents the zero hour of the assessed parameter.

Results

Cytotoxicity of rutin in HDFs. To determine whether rutin affects the viability of HDFs, cultured HDFs were exposed to concentrations of rutin ranging from 0-200 μM for 24 h. Rutin reduced the cell viability of HDFs by 13.23% at a concentration of 50 μM and by 26.05% at 100 μM (Fig. 1A). The cytotoxicity of rutin increased at concentrations >100 μM, thus, we used 100 μM as the maximum concentration in subsequent experiments (data not shown). To examine the ability of rutin to inhibit cellular damage, we examined cell viability, which increased in a dose-dependent manner, in 0.2 mM H_2O_2-exposed HDFs (Fig. 1B). H_2O_2 reduced cell viability to 78.48%; however, following the addition of rutin to the H_2O_2-exposed cells, the viability of HDFs increased to 85.53, 91.67, 97.50 and 95.04% with the addition of 1, 10, 50 and 100 μM rutin, respectively.
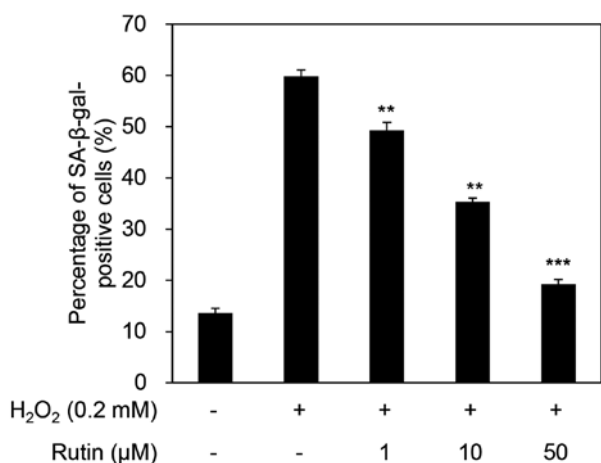
Figure 2. Inhibitory effects of rutin on H_2O_2-induced cellular senescence of human dermal fibroblasts (HDFs). **P<0.01 and ***P<0.001 as determined by the Student's t-test. SA-β-gal, SA-β-galactosidase.
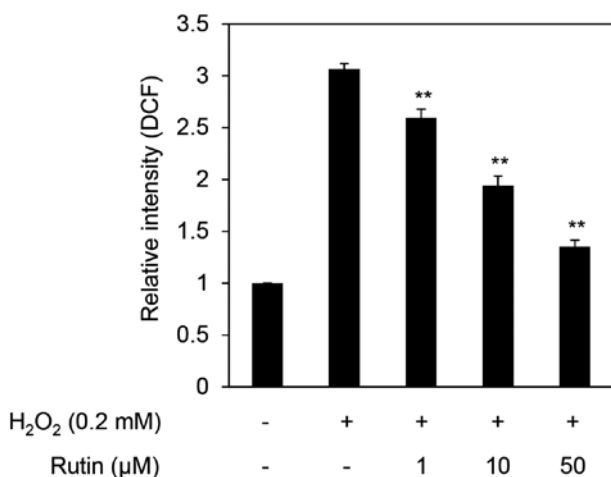

Figure 3. Reactive oxygen species (ROS) scavenging effects of rutin on H_2O_2-treated human dermal fibroblasts (HDFs). **P<0.01 as determined by the Student's t-test.
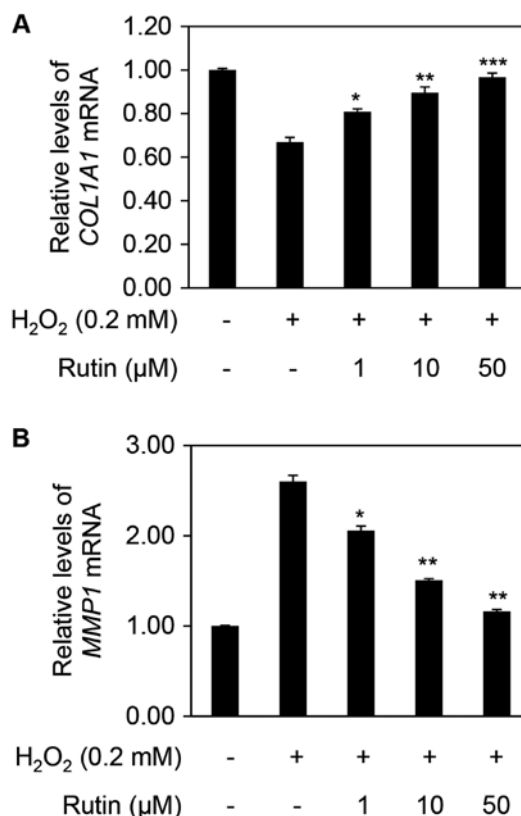

Figure 4. Effects of rutin on the mRNA expression of collagen, type I, alpha 1 (*COL1A1*) and matrix metalloproteinase 1 (*MMP1*) in human dermal fibroblasts (HDFs). Relative mRNA expression levels of (A) *COL1A1* and (B) *MMP1* in H_2O_2- and rutin-treated HDFs. The results are representative of three independent experiments (means ± SD). *P<0.05, **P<0.01 and ***P<0.001 as determined by the Student's t-test.

Senescent cell detection assay. Next, we examined the inhibition of senescence by rutin using the SA-β-galactosidase assay. The percentage of senescent cells was 59.80% in the cells exposed to H_2O_2 only. This ratio decreased in a dose-dependent manner to 49.28, 35.30 and 19.26% with the administration of 1, 10 and 50 μM rutin (Fig. 2). These results indicate that H_2O_2 acts as a stimulator of senescence and that rutin inhibits H_2O_2-induced cellular senescence.

Detection of ROS scavenging activity. To determine cellular ROS levels, we performed the DCF-DA assay in H_2O_2- exposed HDFs. The exposure of HDFs to 0.2 mM H_2O_2 increased ROS levels >three times that of the control cells. The cells exposed to 0.2 mM H_2O_2 and treated with rutin exhibited decreased ROS levels in a dose-dependent manner to a relative intensity of 2.6-, 1.9- and 1.6-fold following treatment with 1, 10 and 50 μM rutin (Fig. 3). These results demonstrate that rutin decreases cellular levels of ROS.

Analysis of mRNA expression levels of COL1A1 and MMP1. ROS are known to be inducers of MMPs, which degrade intracellular substances (18,19). MMPs are divided into three types: collagenase (MMP1 and MMP8), gelatinase (MMP2 and MMP9), and stromelysin (MMP3); additionally, MMP14 and MMP15 have transmembrane domains which determines their substrate specificity (20-24). MMP1, generated in fibroblasts, is highly present in senescent cells and ultimately decreases collagen degradation (25-33). Collagen, the most abundant protein in the dermis, provides structural support; >90% of collagen in the body is type I collagen (34). *COL1A1* encodes the major component of type I collagen and its expression level decreased to 0.67 following treatment with H_2O_2 compared with no treatment. The relative expression of *COL1A1* increased to 0.81, 0.90 and 0.97 in a dose-dependent manner with the addition of 1, 10 and 50 μM rutin, respectively, compared with H_2O_2 alone (Fig. 4A). On the other hand, the expression of *MMP1* in the cells exposed to H_2O_2 increased to 2.6 compared with that in the untreated cells. However, the relative *MMP1* expression decreased to 2.06, 1.50 and 1.16 following treatment with 1, 10 and 50 μM rutin, respectively (Fig. 4B).

Evaluation of dermal density. As aging occurs, the expression of MMPs is increased which results in the degradation of skin substrate proteins (35). Dermal thickness and density were found to decrease with increasing concentrations of MMPs and degradation of albuminoids and the collagen layer (35). To
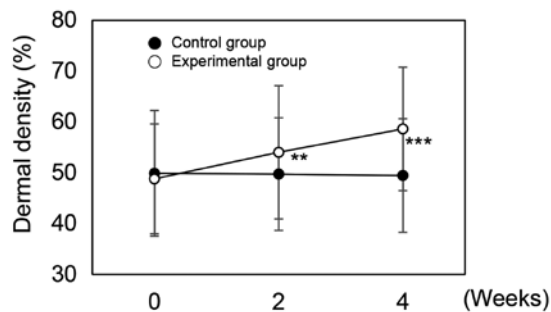
Figure 5. Dermal density of skin to which rutin-containing or control cream was applied. **P<0.05 and ***P<0.001 versus the control group at the same time point as determined by the Student's t-test.

evaluate the clinical efficacy of rutin on aging skin, we demonstrated the effect of cream containing rutin on dermal density. The measurements obtained for the control cream were dermal densities of 49.88% prior to use, and 49.72% after 2 weeks and 49.45% after 4 weeks of use (Fig. 5). The subjects using rutin-containing cream were found to have a dermal density of 48.79% prior to use, 54.02% after 2 weeks and 58.62% after 4 weeks of application (Fig. 5). Dermal density measurements, represented as percentages, are proportional to density. These experimental data were statistically significant (P<0.05 and P<0.001). To determine the extent of any improvement in skin density, we calculated the improvement as a percentage, based on the value prior to application. Notably, the dermal density improvement rate was -0.30% after 2 weeks and -0.85% after 4 weeks in the control group. On the other hand, in the experimental group, the improvement rate of dermal density was 10.73% after 2 weeks and 20.16% after 4 weeks of use. These results indicate that rutin increases dermal thickness.

Evaluation of length and area of crow's feet. Wrinkles, a representative aging phenomenon, occur in response to structural alterations of cells and tissues. Wrinkles arising due to intrinsic aging are formed by decreases in collagen and elasticity, denaturalization of elastic fibers and the stratum corneum, and a loss of skin moisture (36). Wrinkles arising from extrinsic aging are formed by ROS, which damage lipids and proteins in the skin, through the production of inflammatory cytokines (27,37). Previous research has found that ROS reduce collagen and elastin synthesis and are a major cause of wrinkle formation as a result of increased protease activities in various cellular signal transduction systems (38). Thus, we examined whether rutin may potentially be used as an anti-wrinkle ingredient in creams through clinical efficacy experiments.

The length of crow's feet were 63.50 mm prior to application, 64.25 mm after 2 weeks, and 64.95 mm after 4 weeks in the control group (Fig. 6A). In the experimental group, lengths were 68.40 mm prior to application, and 59.20 mm after 2 weeks and 52.05 mm after 4 weeks of application (Fig. 6A). The measured values of the experimental group were statistically significant (P<0.05 and P<0.001). To compare improvements in the length of crow's feet between the control and experimental groups, we analyzed the data over time. In the control group, improvement rates were -1.18% after 2 weeks and -2.28% after 4 weeks application. In the experimental group, improvement rates were 13.45% after 2 weeks and 23.90% after 4 weeks of
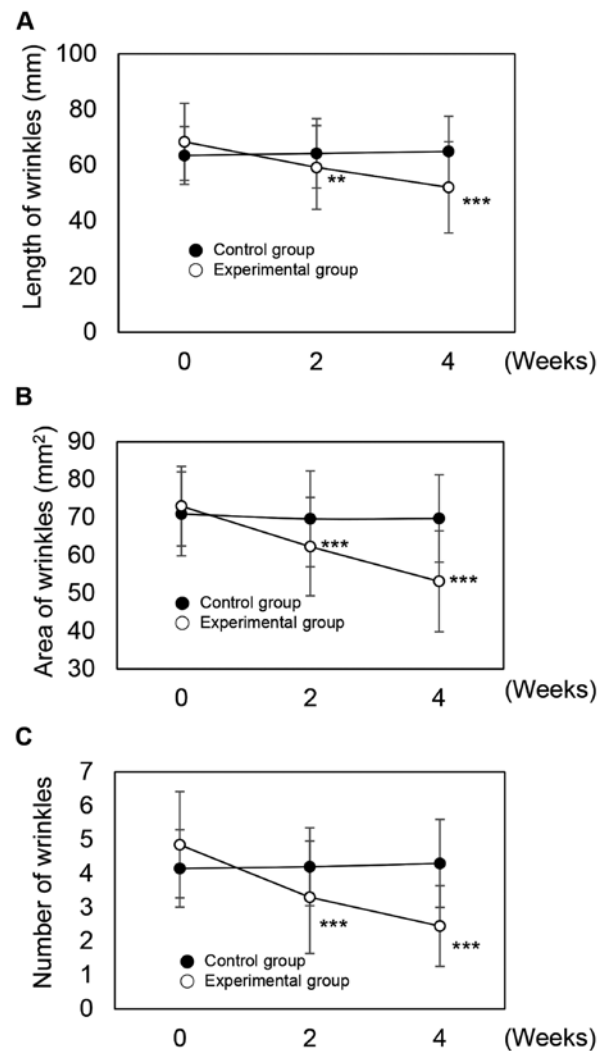

Figure 6. Beneficial effects of rutin-containing cream on eye wrinkles. The measurements of the (A) length and (B) area of crow's feet. (C) Measurements of wrinkle number under the eye rim. **P<0.05 and ***P<0.001 versus the control group at the same time point as determined by the Student's t-test.

use. From these results, we confirmed the effects of rutin on wrinkle improvement.

In the control group, the area affected by crow's feet was 70.95 mm² prior to application, and 69.65 mm² after 2 weeks and 69.75 mm² after 4 weeks of use (Fig. 6B). In the experimental group, the area affected by crow's feet was 73.00 mm² before application, and 62.30 mm² after 2 weeks and 53.15 mm² after 4 weeks of application (Fig. 6B). The experimental group results were statistically significant (P<0.001).

The use of rutin-containing cream significantly reduced the areas affected by crow's feet over time. In the control group, the improvement rates were -1.83% after 2 weeks and 1.69% after 4 weeks. The improvement rates in the experimental group were 14.66% after 2 weeks and 27.19% after 4 weeks. From these results, we again confirmed the effects of rutin on wrinkle improvement.

Evaluation of under-eye wrinkles. In the control group, the average number of under-eye wrinkles was 4.15 prior to application, 4.20 after 2 weeks, and 4.30 after 4 weeks (Fig. 6C). In the experimental group, the number of under-eye wrinkles was
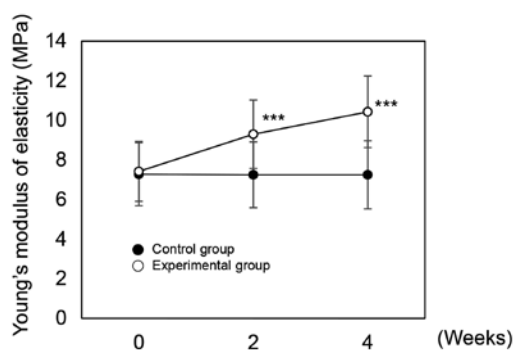
Figure 7. Effects on Young's modulus of skin elasticity of rutin-containing cream applied over time. ***P<0.001 versus the control group at the same time point as determined by the Student's t-test.

4.85 before application, and 3.30 after 2 weeks and 2.45 after 4 weeks application (Fig. 6C). The measured values of the experimental group were statistically significant (P<0.001).

We then assessed the improvement in under-eye wrinkles over time. In the control group, improvements were -1.20% after 2 weeks and -3.61% after 4 weeks application. Improvement rates in the experimental group were 31.96% after 2 weeks and 49.48% after 4 weeks. From these results, we confirmed the effects of rutin on wrinkle improvement.

Evaluation of skin elasticity. The dermis is composed of ECM that contains fibrous proteins such as collagen and elastin. Dermal fibroblasts regulate skin elasticity (39). Factors, such as ROS, ultraviolet (UV) rays or age, cause skin damage, wrinkle formation, and decreased skin elasticity by degrading collagen and elastin (39,40). In this study, we examined the effects of rutin on skin elasticity. In the control group, elasticity was 7.28 before application, and 7.25 after 2 weeks and 7.25 after 4 weeks application. In the experimental group, elasticity was 7.42 before application, and 9.30 after 2 weeks and 10.43 after 4 weeks of use (Fig. 7). The experimental group results were statistically significant (P<0.001). To compare the improvement in skin elasticity between the control and experimental groups, we analyzed the measured data over time. In the control group, improvements were -0.41% after 2 weeks and -0.48% after 4 weeks application. Improvement rates in the experimental group were 25.34% after 2 weeks and 40.50% after 4 weeks. From these results, we confirmed the beneficial effects of rutin on elasticity. All subjects had no adverse effects following a self-evaluation to determine the presence of any skin disorders (Table I).

Discussion

The present study examined the antioxidant and anti-aging effects as well as the beneficial effects of rutin on skin barrier function through cellular and clinical efficacy experiments. The results of cellular experiments on HDFs indicated the antioxidant and anti-aging effects of rutin. In HDFs, rutin protects cells against damage induced by H_2O_2. Furthermore, we identified a ROS scavenging effect of rutin by DCF-DA assay and reduced H_2O_2-induced cellular senescence. After analyzing the expression of *MMP1* and *COL1A1*, we verified that rutin reduces *MMP1* expression and increases *COL1A1* expression. Notably,

cutaneous elasticity was reported to be the composite result of the amount of elastic fibers and ECM-filling materials (41,42). Additionally, MMPs are known to degrade all components of the ECM, such collagen, elastin, fibronectin, proteoglycans and laminin (34,43,44). Thus, MMP expression not only impairs procollagen synthesis and degrades the ECM, but leads to a loss of elasticity and skin dryness (45,46). Thus, we conducted experiments to determine the clinical efficacy of rutin.

We first manufactured rutin-containing cream. The clinical efficacy tests were conducted with 40 subjects, women between 30-50 years of age, selected for a randomized and double-blind study. The subjects were divided into two groups, experimental and control, and 1% rutin-containing or basic cream were applied, respectively. Dermal density improved 10.73% after 2 weeks and 20.16% after 4 weeks of application of the rutin-containing cream in the experimental group. We also showed that rutin-containing cream improved skin elasticity 25.34% after 2 weeks and 40.50% after 4 weeks of application. Reduced skin elasticity contributes to damaged skin structure and aging, particularly wrinkle formation (47-50). Thus, we investigated the length, area and number of wrinkles around the eyes of the rutin-treated subjects.

The length of crow's feet improved by 13.45% after 2 weeks and 23.90% after 4 weeks. Additionally, the area of crow's feet improved by 14.66% after 2 weeks and 27.19% after 4 weeks. After analyzing the average number of under-eye wrinkles, we demonstrated the effects of rutin on facial wrinkles. The number of under-eye wrinkles improved by 31.96% after 2 weeks and 49.48% after 4 weeks. These results indicate that rutin improves skin dermal density, reduces fine winkles, and enhances elasticity. We suggest that rutin may be used as a major ingredient in anti-aging cosmetics in order to improve skin elasticity and reduce wrinkles.

Acknowledgements

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