Introduction

Facial skin and hair conditions are indicative of overall nutritional status and blood flow. Furthermore, skin condition is often the key feature observed in the boshin (feature observation) of kampo medicine. The skin is considered healthy when it is bright and moist. Some diseases can result in dryness, hyperkeratosis, pigmentation, and subcutaneous bleeding.

According to the triad of “ki”, “ketsu” and “sui” in kampo medicine, “ketsu” is a red fluid that circulates throughout the body. Although controlled by ki, ketsu circulates autonomously throughout the body, providing nutrients to 2) and eliminating wastes from all tissues. Health is maintained when ketsu, along with ki and sui, is in neither excess nor deficit 3). However, when this dynamic equilibrium is disrupted, ketsu becomes static and declines 2). Diseases in this state are called ketsuo, a condition in which ketsu becomes static or the effects of ketsu become excessive in localized areas.

We have previously discussed the effects of kamishoyosangoshimotsuto, a kampo formula originating from Tsuda Gensen’s Treatment Experience Records that eliminates ketsuo and supplements ketsu, on improving skin condition and peripheral blood flow in facial skin following 8 weeks of treatment. The present clinical study was conducted to evaluate, through quantitative measurement using instruments, the effect of 24-week treatment with kamishoyosangoshimotsuto on skin condition and hair properties in women with skin and hair problems (e.g., skin dryness, pigmented spots, roughness, thinning hair, and hair loss) and symptoms of ketsuo and kekkyo. Facial photographs were taken before and after treatment to analyze changes in appearance.

Original Article
KGS1 Kamishoyosangoshimotsuto Extract Tablets Improved Skin Condition and Hair Properties

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Abstract

We conducted a 24-week clinical study in 20 women with skin and scalp hair problems (e.g., skin dryness, pigmented spots, roughness, thinning hair, and hair loss) and symptoms of ketsuo or kekkyo to investigate the effects of kamishoyosangoshimotsuto extract (KGS1) on skin condition and hair properties. Skin evaluation revealed significant improvements in stratum corneum moisture content, skin texture score, and spot brightness. Hair evaluation showed significant increases in hair thickness and hair breakage strength. Furthermore, we visually analyzed the effects of KGS1 on skin condition using facial photographs taken before and after test product treatment. In almost half of subjects, facial color and the skin color of spots were visibly brightened, skin dullness was improved, and skin luster was increased.

KEY WORDS: kamishoyosangoshimotsuto, ketsuo, kekkyo, skin, hair
Methods

1. Subjects

This study was performed in compliance with the ethical principles of the “Declaration of Helsinki” and the Personal Information Protection Law. The protocol of this study was approved by the institutional review board of Shinkohkai Medical Corporation, and informed consent was obtained from each subject. Females (aged 20-59 years) with skin problems (e.g., dryness, spots, roughness) were selected by a doctor for the present study to evaluate the efficacy of KGS1 based on an evaluation for ketsuo, kekkyo, kikyo, kan’utsu, kanka, hiikyo, nessho and other features. Subjects were asked to use sunscreen (SPF≥20, PA++ or greater) daily. For the hair evaluation, 8 subjects with hair problems (e.g., hair thinness, hair loss), who had not used dye, bleach, or perm for at least 6 months before the study were enrolled. Subjects who had alopecia areata or used topical minoxidil solution were excluded from the study.

2. Restrictions

During the study, subjects were not allowed to engage in activities involving exposure to intense ultraviolet radiation and were required to use sunscreen during outdoor activities. Subjects were asked to use the same sunscreen they used before the start of the study. In addition, subjects were asked not to use any new topical product (cosmetic, quasi-drug product, or drug product) during the study and to use the same products which were used before the study.

Subjects for the hair study were asked not to use any new hair product (e.g., shampoo, conditioner, hair treatment, hair dressing product) during the study and were asked to continue using products used before the start of the study. Moreover, the subjects were not allowed to dye, bleach, or perm their hair or do anything else that could affect the properties of their hair during the study.

3. Test Product and Dosage

KGS1, a tablet containing 3.9 g of kamishoyosangoshi motsuto extract (in a daily dose of 18 tablets; Kobayashi Pharmaceutical, Osaka-city, Osaka) was used as the test product. Six tablets were taken with plain cold or hot water before or between meals 3 times daily.

4. Study Design

In the present study, a single and open label method was applied. The study was an unblinded open-label study conducted at one center. The study was conducted from September 18, 2009 to March 15, 2010, and the subjects took the test product over a 24-week period during the study. Skin conditions were observed before the start of treatment (week 0) and in weeks 4, 8, 12, 16, 20, and 24 during the study. Hair properties were evaluated before the start of treatment (week 0) and at weeks 12 and 24.

5. Measurements and Evaluations

Skin measurements and hair sampling were conducted by the Ebisu Skin Research Center of Inforward (Shibuya-ku, Tokyo). Hair properties were evaluated by Kato Tech (Kyoto-city, Kyoto).

Skin measurements were conducted at a constant temperature of 22°C and humidity of 45% after adjustment to these conditions for 15 min. Hair properties were measured at a constant temperature of 20°C and humidity of 60% using hair samples that had acclimatized to these conditions for 2 h.

(1) Skin analyses and evaluation

1) Stratum corneum moisture content

The moisture content of the stratum corneum of facial cheek skin was measured using a CM825 Corneometer (Courage + Khazaka Electronic, Köln, German).

2) Skin Texture Score

The Robo Skin Analyzer (Inforward) was used to measure the skin texture score as follows. In facial cheek photographs at ×40 magnification, the darkness of the sulci cutis and crista cutis was alternatively discriminated as black or white on a binary scale. Resemblance to a skin texture model (a model of aligned equilateral triangles with sides measuring 0.4 mm) was numerically converted to a skin texture score on a scale of 1 to 100.

3) Spots

i) Number and area of spots

Spots were defined as areas of pigmentation measuring ≥0.6 mm² that were detected as slightly darker or darker than the surrounding area in full facial photographs taken with the Robo Skin Analyzer. Numbers and areas of skin spots were determined.

ii) Spot brightness (L* value)

Spot brightness was determined using the largest of the pigmentation detections as dark spots in each subject’s full facial photographs taken with the Robo Skin Analyzer. Adobe Photoshop CS3 (Adobe Systems Inc., Shinagawa-ku, Tokyo) was used to extract the area of pigmentation in question from each full facial photograph and calculate the brightness (L* value) from RGB values of the area.

4) Full facial photographs

Full facial photographs were taken to allow the comparison of facial color and skin condition before and after treatment. Photographs were taken under identical lighting conditions, with the same camera, subject clothing, and background, and from the same distance and angle. The full facial photographs taken at week 0 and at weeks 12 and 24 were visually compared.

(2) Hair measurements and evaluation

The region 8-18 mm from the scalp was measured. Human hair generally grows 8-12 mm/month and was assumed to grow 10 mm/month for the purposes of this clinical study. The region analyzed at week 12 therefore corresponded to the region growing from weeks 3 to 8, and the region analyzed at week 24 corresponded to the region growing from weeks 15 to 20.

1) Hair diameter

Hair diameter was measured using the KES-2000 hair diameter-measuring device (Kato Tech). Five hair samples were measured per subject. The four locations 10 mm, 12 mm, 14 mm, and 16 mm from the scalp were measured in the measurement region.

2) Hair breakage strength

Using the KES-G1-SH high-sensitivity hair stretching test device (Kato Tech), hairs were stretched lengthwise at 0.1 mm/s, and the load applied at the time of breakage (breakage strength load, gf) was determined. Five hair samples were measured per subject.

6. Statistical Analysis

Differences between week 0 measurements and measurements at observation days in weeks 4, 8, 12, 16, 20, and 24 from all 20 subjects were statistically analyzed using Bonferroni’s
multiple comparison test, and values of $p<0.05$ were taken to constitute statistical significance. Data variance was evaluated with standard error of the mean (SEM). Differences between individual subject values at week 0 and at observation days in weeks 4, 8, 12, 16, 20, and 24 were statistically analyzed using significant difference testing with regression analysis, and values of $p<0.05$ were considered statistically significant.

**Results**

1. **Subject Characteristics**
   
   Twenty women aged from 24-58 years fulfilling the subject criteria were enrolled. Mean age of the subjects was 39.8 years. 8 subjects were included as subjects for hair evaluation. Mean age of these 8 subjects was 48.3 years. No subjects dropped out of the study. Skin condition data for 20 subjects and hair data for 8 subjects were analyzed.

2. **Skin Condition Evaluation**
   
   Ages of the subjects, skin condition measurements at week 0 and weeks 12 and 24, and the results of significant difference testing with regression analysis are shown in Table 1. Significant differences in individual subjects were noted in the stratum corneum moisture content of 10 subjects, skin texture scores, spot numbers, and spot areas of 1 subject each, and spot brightness of 5 subjects.

| Table 1 Skin measurements of subjects |

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Stratum corneum moisture content (score)</th>
<th>Skin texture score</th>
<th>Number of spots</th>
<th>Area of spots (mm²)</th>
<th>Spot brightness (L* value)</th>
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Significant difference testing with regression analysis * $p < 0.05$, ** $p < 0.01$

(1) **Stratum corneum moisture content**

Data on moisture content in the corneum layer of the epidermis are shown in Fig. 1. Stratum corneum moisture content increased substantially from weeks 4 to 8 and increased gradually from week 8 onward. Moisture content was significantly higher at and after week 20 than at week 0.

(2) **Skin texture scores**

Skin texture score data are shown in Fig. 2. The highest possible skin texture score was 100. Higher scores represent better texture. Skin texture scores increased according to the use duration and were significantly higher at week 20 than at week 0. Scores at week 24, however, was slightly lower than at week 20.

(3) **Spots**

1) Number and area of spots

Spots number data are shown in Fig. 3, and spots area data are presented in Fig. 4. Numbers of spots before and after treatment did not differ significantly. Spot area was significantly smaller at week 16 than at week 0, but no overall trends in spot area were observed.

2) Spot brightness (L* value)

Spot brightness data are shown in Fig. 5. Spot brightness increased with the duration of use and at week 16 was significantly higher than at week 0. Spot brightness was maintained at and after week 16.

(4) **Full facial photographs**

Typical facial photographs are shown in Fig. 6. Skin in Subject No. 5 (29 years old) was less dull after treatment.
Effects of Kamishoyosangoshimotsuto on Skin Condition

**Fig. 1.** Stratum corneum moisture content

Stratum corneum moisture content measured using the CM825 Corneometer. Moisture content is significantly higher at and after week 8 than at week 0. Mean±SE, n=20
Bonferroni’s multiple comparison: **p<0.01, ***p<0.001

**Fig. 2.** Skin texture scores

Skin texture scores determined using the Robo Skin Analyzer. Skin texture scores increased with the duration of use and at week 20 was significantly higher than at week 0. Mean±SE, n=20
Bonferroni’s multiple comparison: *p<0.05

**Fig. 3.** Number of spots

Number of spots determined using the Robo Skin Analyzer. Numbers before and after treatment did not differ. Mean±SE, n=20
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Although blotch area was significantly lower at Week 16, no overall trends were observed. Means±SE, n=20
Bonferroni’s multiple comparison: *p<0.05

Spot area determined using the Robo Skin Analyzer

Fig. 4. Area of spots

Spot brightness increased with duration of use and at week 16 was significantly higher than at week 0. Means±SE, n=20
Bonferroni’s multiple comparison: **p<0.01

Fig. 5. Spot brightness (L* value)

3. Hair Properties Evaluation

(1) Hair diameter

Hair diameter data are shown in Fig. 7. Hair diameter was significantly higher following treatment than at the start of treatment. The increase in diameter stabilized at week 12 and was maintained to week 24.

(2) Hair breakage strength

Hair breakage strength data are shown in Fig. 8. Hair breakage strength was higher after treatment than at the start of treatment and at week 24 was significantly higher than at week 0.

compared with the start of treatment, and facial color was brightened. Pigmentation on the upper right cheek was improved. The skin in Subject No. 15 (45 years old) was less dull after treatment than at the start of treatment, facial color was brightened, and skin luster was increased.

These marked changes in appearance were evident in about half of the subjects. No changes distinct enough to be recognizable in the full facial photographs were seen in the other subjects.

No changes distinct enough to be recognizable in the full facial photographs were seen in the other subjects.
Fig. 6. Full facial photographs taken before and after treatment
Case 1: Dullness has improved, facial color is brighter, and pigmentation of the upper right cheek has improved.
Case 2: Dullness has improved, facial color is brighter, and skin luster has increased.

Fig. 7. Hair diameter
Hair diameter measured using the KES-2000 hair diameter-measuring device
Hair diameter is significantly higher than at week 0 after treatment.
Means±SE, n=8
Bonferroni’s multiple comparison: **p<0.01

Fig. 8. Hair breakage strength
Hair breakage strength measured using the KES-G1-SH high-sensitivity hair stretching test device
Hair breakage strength increased after treatment and at week 24 was significantly higher than at week 0.
Means±SE, n=8
Bonferroni’s multiple comparison: *p<0.05
**Discussion**

According to the triad of “ki”, “ketsu” and “sui” in kampo medicine, diseases of the **ketsu** manifest as skin and hair symptoms. Similarly, as **ketsu-related** disease conditions are improved, skin and hair symptoms are improved. This relationship of **ketsu** to skin and hair symptoms is routinely observed in the clinic and widely known. In the present study, we tried to evaluate changes in patient appearance objectively using dermatological analytical instruments. More specifically, we first in the world demonstrated simultaneously both improvements in clinical observation of patient appearance and improvements in numerical data with dermatological analytical instruments using kamishoyosangoshimotsuto, a kampo formula with ketsu-eliminating and ketsu-supplementing effect, as a test product. Hair properties were also evaluated objectively and quantitatively using instruments to demonstrate the effect of the test product, kamishoyosangoshimotsuto, on hair properties.

Objective evaluation of skin condition included stratum corneum moisture content, skin texture score, and spots (number, area, brightness). Significant improvements in stratum corneum moisture content, skin texture score, and spot brightness following treatment were obtained. Improvements in these indices of skin condition increased over time beginning at the start of treatment, generally stabilizing by weeks 16-20, and were subsequently maintained. Although significant improvement in spot brightness was observed, numbers and areas of spots did not change significantly. Spots in this clinical study were areas of pigmentation measuring ≥0.6 mm² that were detected as slightly darker or darker than the surrounding area according to the analytical definition of the Robo Skin Analyzer facial photographing device. Regions of spots were detected by comparing their brightness with that of the surrounding region. The lack of any significant difference in spot numbers or areas despite significant improvement in spot brightness is possibly attributable to the brightness of regions surrounding the spots, which increased concurrently with the brightness of the spots. Brightness of the regions around spots also increased to the level comparable to that of the spots. This increase was statistically significant at week 16 (data not shown), suggesting that the test product does not locally improve the brightness of spot alone, but instead improves the overall brightness of the skin.

An evaluation of hair diameters and breakage strength revealed a significant increase at week 12 as compared to week 0. This suggests that KGS1 treatment thickens individual hairs and makes hair more resistant to breakage. Measurements at weeks 12 and 24 did not differ substantially, suggesting that improvement stabilizes at week 12 measurement and is subsequently maintained by continuing treatment.

We previously reported on improvements in peripheral skin blood flow and skin condition following 8 weeks of treatment with the test product and on the relationship between the increase in peripheral skin blood flow and improvements of skin condition (stratum corneum moisture content, transepidermal water loss, skin texture score, elasticity). A number of studies by other research groups have also indicated that increased skin blood flow contributes to improvement in skin condition and, in contrast, decreased blood flow accelerates skin aging and exacerbates skin condition. Together with other reports, our results indicate that the improvement in skin condition following test product use is attributable to better skin blood flow due to the formulation’s ketsu-eliminating and ketsu-supplementing effects of the kampo formula, and to increased metabolic activity in the skin, and higher turnover of epidermis. This hypothesis regarding the mechanisms of the test product would explain the improvement in spot brightness stated earlier. In case blood flow is promoted by the test product, metabolic activity and turnover would be promoted not only in spots, but also in regions of skin surrounding spots leading to improvement of the overall brightness of skin.

Improved skin condition was maintained over time during the 24 weeks of long-term treatment in this clinical study, indicating that improvements in skin blood flow and metabolic activity were also maintained. The improvements seen in hair properties may be partially due to activation of hair matrix cells and cells in the inner root sheath and outer root sheath as a result of improvements in the microcirculation around the bulb of a hair.

Full facial photographs of the subjects before and after treatment were visually compared in this clinical study to support the objective and quantitative data obtained with the analytical instruments. In half of subjects, facial skin color including spots had brightened, skin dullness improved, and skin luster increased after the start of treatment. In the present study, these clinical improvements together with the objective data suggest that the test product improves skin and hair conditions.

Kamishoyosangoshimotsuto, a kampo formula for alleviating ketsu-related undesirable skin troubles, improved them and maintained the improvements through long-term treatment (24 weeks) and could be an effective Anti-Aging drug for skin and hair.
Effects of Kamishoyosangoshimotsuto on Skin Condition

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