Efficacy of a Soy Moisturizer in Photoaging: A Double-Blind, Vehicle-Controlled, 12-Week Study

Warren Wallo,a Judith Nebus,a James J. Leyden MD*b
b. University of Pennsylvania, Philadelphia, PA

Abstract
Serine protease inhibitors (soybean trypsin inhibitor [STI] and Bowman-Birk protease inhibitor [BBI]) found in soybeans have been shown to inhibit melanosome phagocytosis by keratinocytes via protease-activated receptor 2 (PAR-2). Preclinical studies have confirmed the skin lightening potential of these molecules. In this study, we investigated the efficacy of a novel soy moisturizer containing nondenatured STI and BBI for the improvement of skin tone, pigmentation, and other photoaging attributes. Sixty-five women, with moderate facial photodamage, were enrolled in the 12-week, parallel, vehicle-controlled study. Efficacy was monitored through clinical observation, self-assessment, colorimetric evaluations, and digital photography. The results showed that the novel soy moisturizer was significantly more efficacious than the vehicle in improving mottled pigmentation, blotchiness, dullness, fine lines, overall texture, overall skin tone, and overall appearance. Differences were significant from week 2 to week 12 for all above parameters (except dullness which started at week 4). In this study, we found that a moisturizer containing stabilized soy extracts is safe and effective, and can be used to ameliorate overall skin tone and texture attributes of photoaging.

Introduction
Wrinkles and hyperpigmentation are important concerns that drive consumers to purchase antiaging products. The total sale of antiaging facial care products in US department stores was $664 million in 2005, which is up 33% from 2001.1 There are several topical drugs, cosmeceuticals, and cosmetic procedures targeting hyperpigmentation. Among the most used drugs are hydroquinone, tretinoin, steroids, and azelaic acid.2 With limited options in depigmenting formulations, there is a need to find alternative safe and effective ingredients to achieve an even skin tone for the mass market consumer.

Soybeans are known to contain many components with biological activity in the skin, such as antioxidants (isoflavones)4-6 and small proteinase inhibitors.7-11 Among the latter are soybean trypsin inhibitor (STI) and Bowman-Birk protease inhibitor (BBI), which inhibit the keratinocyte protease-activated receptor 2 (PAR-2),12-14 that is involved in the regulation of pigmentation.12-15,17-18 Several in vitro and preclinical investigations have demonstrated that the modulation of PAR-2 activation by serine protease inhibitors affects melanosome transfer and produces reversible depigmentation.8,12 Serine protease inhibitors (STI in particular) are inactivated by heat19 and the processing of soybeans and soymilk can destroy their depigmenting activity. Recently, a soy formulation was developed, containing nondenatured STI and BBI.20,21 Preliminary in vivo human studies support the skin lightening effect of nondenatured soy extracts.20,22-24 In this article we discuss the use of a soy moisturizer as an alternative agent in evening skin tone and as an overall antiaging formulation in humans.

Materials and Methods
Study Population
A total of 68 female subjects, aged 30 to 61 with Fitzpatrick phototypes I to III, were enrolled in the study. To be included, subjects had to present with a moderate severity of the following facial parameters: tactile roughness, mottled hyperpigmentation, lentigines, blotchiness, and dullness. Subjects were excluded if they had 1) a facial dermatologic condition or dermatitis interfering with the study, 2) used systemic retinoids within 3 months prior to study, or 3) used topical retinoids for 3 months or alpha- or beta-hydroxy acid, retinol or soy for one month prior to study start.

Study Design
This was a 12-week, parallel, randomized, double blind, and vehicle controlled study, conducted during the fall in the Northeast region of the US. The study was approved by an institutional review board and informed consent was obtained from all patients prior to initiation of the investigation. The study compared an active moisturizer containing stabilized soy extracts (Aveeno® Positively Radiant™ Daily Moisturizer, Johnson & Johnson CCI, Skillman, NJ) against its vehicle. Both products contained SPF 30. The active soy moisturizer contained nondenatured serine protease inhibitors (STI and BBI), vitamins, and fatty acids.

Each subject was given 1 of the 2 test products (active or vehicle) and instructed to use it daily on her entire face, once in the morning and again in the evening, for a 12 week period. Thirty-one subjects received the active product, while 32 subjects received the vehicle. All subjects were required to avoid direct sun exposure for the duration of the study. After the baseline evaluation (week 0), subjects returned to the test site at weeks 1, 2, 4, 8, and 12.

Evaluation
Efficacy was measured by clinical evaluation, colorimetry, digital photography, and self-assessment. Improvement of skin tone was clinically defined as a reduction in mottled hyperpigmentation, lentigines, and blotchiness, with an increase in...
skin brightnes (ie, reflection of light from skin's surface). Improvement of skin texture was defined as a reduction in the surface roughness and/or an improvement in fine lines and wrinkling. Safety was measured by the frequency and incidence of both cutaneous irritation parameters and adverse events.

Clinical evaluations were performed by the dermatologist on weeks 0, 2, 4, 8, and 12. On evaluation days, the moisturizer was applied only after the visit was completed. Variables were scored on a 0 (none) to 9 (severe) scale and included roughness (tactile), mottled hyperpigmentation, lentigines, blotchiness, dullness (lack of brightness/clarity), and fine lines (global assessment including under eye and crow's feet areas). The dermatologist also conducted an overall investigator global assessment using a 0 (very good) to 9 (very poor) scale on the following parameters: overall skin texture, overall skin tone, and overall skin appearance. Using the same 0 (none) to 9 (severe) point scale, cutaneous reactions for safety were recorded, which included erythema, dryness, itching, and burning/stinging.

The subjects' self-evaluations were conducted at weeks 0, 1, 2, 4, 8, and 12, and consisted of rating a list of attributes as they pertained to the appearance and feel of their skin while looking in a mirror, using a 1 (very unfavorable) to 10 (very favorable) scale.

Digital images were taken using a Nikon camera at weeks 0, 4, 8, and 12. Camera set-up, lighting conditions, and the subjects' positions were standardized during the study.

Colorimetry
Chroma Meter (Minolta, Mahwah, NJ) was used to quantify skin brightness and color at weeks 0, 4, 8, and 12. The instrument translates color values into L* (luminosity; 0=black to 100=white), a' (positive=red to negative=green), and b' (positive=yellow to negative=blue) coordinates. Subjects were acclimatized for 30 minutes in a controlled environment prior to the evaluations. Three readings were obtained from either the left or right cheek, from an area located by dropping a line from the corner of the eye and across from the base of the nose.

Statistical Analyses
A paired t test was used to compare the individual scores from the dermatologist, self-assessment, and instrumental data at each time point relative to their respective baseline within each treatment group. A Student t test was used to compare the differences between the test products at each time point for the dermatologist, self-assessment, and instrumental data. A Student t test was also employed to compare the net change from baseline scores between the test products at each time point for the dermatologist and instrumental data. Additionally, in the dermatologist's assessments, the scores for each attribute were converted to the number of panelists who showed "improvement," "stayed the same," or "worsened." In the case of self-assessments, the scores for each attribute were shown as a distribution and converted to the number of panelists who showed "improvement," "stayed the same," or "worsened." A chi-square test was performed on the frequency of panelists who selected the top 2 responses (+2 and +1 combined) versus those who selected the bottom 2 responses (-1 and -2 combined). A 2-sided P value of less than or equal to 0.05 was considered statistically significant.

Results
A total of 68 female subjects were enrolled in the study. During the study, 5 subjects were dropped for noncompliance or withdrew for personal reasons, and 63 subjects completed the 12-week treatment.

Efficacy
At the end of the study, both groups showed significant improvement from baseline in almost all efficacy parameters. Blotchiness, however, improved only with the active formulation. When the 2 test products were compared, the active soy moisturizer was statistically better than the vehicle (P≤0.05) in the improvement of mottled pigmentation, blotchiness, dullness, fine lines, overall texture, overall skin tone, and overall appearance (Figure 1). The superiority of the active formulation was observed starting at week 2 (with the exception of dullness which started at week 4) and continued until the end of the study (Figure 2).

It is noteworthy that after 12 weeks of using the active soy formulation, the vast majority of subjects showed at least 1 grade of improvement in mottled hyperpigmentation (28 of 31) and fine lines (27 of 31) compared to 17 out of 32 subjects for mottled hyperpigmentation and 9 out of 32 for fine lines in the vehicle group. At 12 weeks, 9 subjects in the active group showed 2 grades of improvement, while only 1 subject in the vehicle treatment group showed this level of improvement. Additionally, all subjects (100%) on the active soy moisturizer showed decreased skin roughness (overall texture), increased skin clarity (less dullness), and all but 5 had improved skin tone (Table 1 and Figure 3).

Self-assessment showed that subjects in the active group noticed a significant improvement (P≤0.05) in skin imperfections as early as week 1 compared to week 12 in the control group. Both groups began to perceive significant improvement in dullness, radiance, discoloration, and fine lines within 4 weeks of treatment.

Colorimetry
Both test formulations improved significantly (both, P≤0.05) skin brightness (increased L* value) at weeks 8 and 12 of treatment and decreased the yellowness in skin color (decreased b' value) at all time points measured. No statistical differences were noted between the 2 products.

Safety
Both formulations were well-tolerated. Erythema, dryness, itching, and burning/stinging did not change throughout the study, although one subject, on the vehicle formulation, developed a mild contact dermatitis on her face. Patch testing was negative for an allergic reaction and the subject was able to continue the use of the product.
Figure 1. Clinical efficacy parameters: comparison between active and vehicle at the end of the study.

Figure 2. Clinical efficacy parameters: progression of improvement from week 2 to week 12 using the active formulation.

At week 12, the active group showed significant improvement ($P \leq 0.05$) against vehicle (*) in all the above parameters.

All parameters above showed significant improvement ($P \leq 0.05$) against baseline at all timepoints. All improvements, with the exception of dullness at week 2, were significant against vehicle (*).
Discussion

Many plants contain important compounds with potential dermatologic benefits. With improvement in chemical extraction and manufacturing processing, many natural ingredients are now incorporated in antiaging formulations. Soybeans are known to contain antioxidants as well as small proteinase inhibitors. These proteinase inhibitors have only recently been investigated for their depigmenting benefits. These molecules include the STI and BBI, which inhibit the keratinocyte protease-activated receptor 2 (PAR-2). PAR-2 is expressed on keratinocytes and has been shown to increase keratinocyte phagocytosis. It has been suggested that PAR-2 regulates pigmentation through keratinocyte-melanocyte contact, by facilitating phagocytosis and the transfer of

Table 1. Dermatologist evaluations. Comparison between active and placebo in percentage of subjects showing improvement from baseline. Data shown in percentages.

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Figure 3. Black-and-white (blue-channel) cross-polarized photography. Subject using the active soy moisturizer at baseline a) and at the end of the study b). After 12 weeks b), there is a visible decrease in pigmentation and blotchiness, with improved skin tone and brightness.
melanosomes into the keratinocytes. In vitro and preclinical investigations have demonstrated that STI and BBI produce reversible depigmentation by inhibiting PAR-2 regulated melanosome transfer.

In this study, we tested a moisturizer containing soy extracts against its vehicle for the improvement of various photodamage parameters. Both products contained SPF 30, minimizing further skin pigmentation during the investigation. The novelty of the active moisturizer consisted in the development of a stabilized formulation containing nondenatured STI and BBI. This is important since serine protease inhibitors are inactivated by heat during the processing of soybeans and soymilk.

This study was conducted in the fall in the Northeast region of the US. This time of year helps to limit sun exposure during the study, but also represents a period of natural reversal of sun damage from the summer UV exposure. This explains why subjects in both active and vehicle groups showed improvement from baseline in many photodamage parameters. The comparison between the 2 groups, however, demonstrated that the active soy formulation was significantly superior to the vehicle and was safe and effective in providing improvement in facial skin tone, clarity, mottled hyperpigmentation, blotchiness, and fine lines as well as overall texture and appearance. Significant improvement versus vehicle was detected after only 2 weeks of use and continued throughout the study. The skin lightening activity was in accordance with previous studies using nondenatured soy extracts.

Colorimetry confirmed the improvement in skin tone and skin lightening. The $L^*$ and $b^*$ values have been used extensively in the literature to quantify melanin-derived color. Our results showed an increase in skin brightness, $L^*$ with a significant decrease in $b^*$, although yellowish tone differences between groups were nonsignificant. The lack of colorimetric discrimination between the 2 groups is in contrast with the clinical evaluation results, which detected a significant superiority of the soy formulation compared to vehicle. The reason for the difference may be that colorimetry captures data from a limited skin area, while the clinical evaluation assesses and averages the appearance of a much larger site.

In summary, this investigation shows that a novel soy topical formulation containing nondenatured serine protease inhibitors is safe and effective in improving mottled pigmentation, as well as various skin tone and texture parameters when tested in a double-blind clinical study with a dermatologist.

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References

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