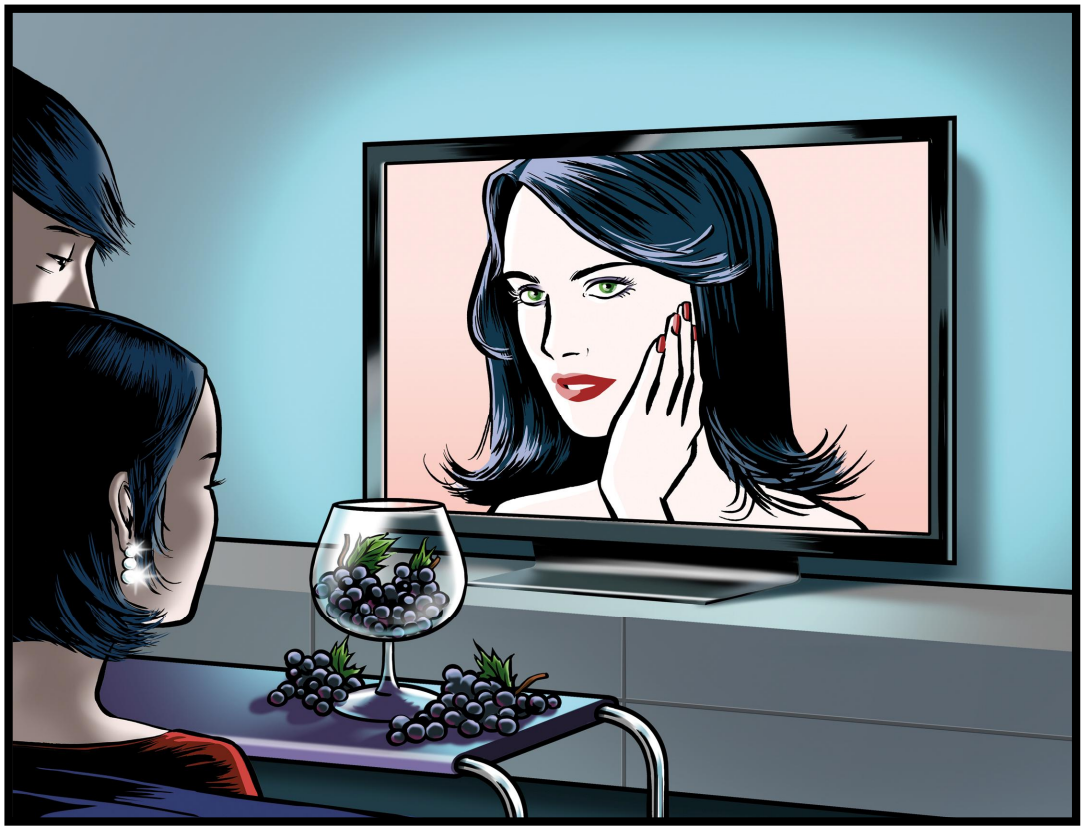
DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

In-vivo Efficacy Data

REGU®-FADE for Skin Care

A Safe, Effective and Efficient Skin Lightener



The most effective solution for noticeably brighter skin

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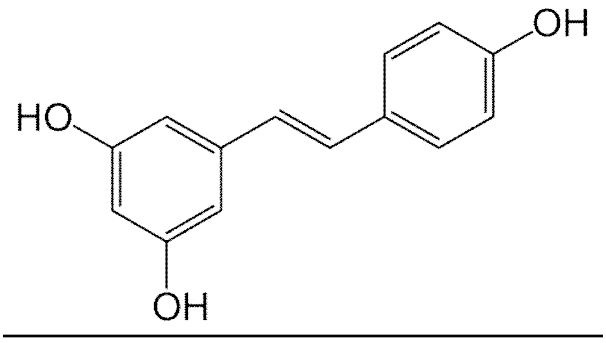
What is REGU®-FADE

REGU®-FADE is DSM's new skin brightener that brings unique properties "all in one": it is high performing, fast acting, and safe. REGU®-FADE is the brand name of a pure, nature-identical resveratrol that fulfils highest safety and quality standards.



Structure of Resveratrol

Resveratrol, a naturally occurring molecule found in red grapes has been subject of intense research in recent years. Scientific reports are increasingly demonstrating the multi-functional benefits of resveratrol. Resveratrol is reported to be an extremely potent anti-oxidant, a modulator of genetic expression via signal transduction, an inhibitor of inflammatory mediators, to have phytohormonal benefits, and to inhibit tyrosinase activity. Furthermore, we have recently found that resveratrol (REGU®-FADE) attenuates melanogenesis by suppressing a number of regulatory factors in addition to the one of tyrosinase suppression. The efficacy of such mechanism of action tackling the melanin production process at various points was successfully demonstrated *in-vitro* by the ability of REGU®-FADE to reduce total melanin synthesis in normal human melanocytes. REGU®-FADE efficacy *in-vitro* was more than 10 times stronger than benchmark products. After the in-vitro efficacy, it is the primary objective of this report to demonstrate the skin lightening efficacy *in-vivo*. Hence the safety and efficacy of REGU®-FADE are further substantiated with the following clinical trials:

I. Safety Evaluation using Human Patch Test Technique

Patch test technique is a tool developed to determine the potential of specific substances to cause irritancy of the skin. Irritants are substances that damage the skin by direct toxic action. The damage will depend upon the nature of the irritant, its concentration, and duration of exposure. Irritation is manifested as inflammatory responses such as erythema (redness), oedema (swelling), or vesiculation. It may also lead to an intense suppurative reaction without the involvement of immune system. Patch test evaluates all these skin reactions and hence is an valuable tool in assessing safety of a product aiming at topical applications.

I.1 Protocol for Patch Test

<table>
<tr><td>Aim</td><td>To determine skin irritation potency or hypersensitivity to the tested product</td></tr>
<tr><td>Test subjects</td><td>25 volunteers from the Mumbai region in the age group of 18-65 years.</td></tr>
<tr><td>Method</td><td>This test has been adapted from IS 4011: 1997 Amendment 2, November 2007 guidelines using Draize scale. It has been carried out under dermatological control. Its design is mono-centric, randomized, controlled, and double blinded. The test involves the application of various test substances to the skin using IQ chambers that are then left in test site for 24 hours. The skin is then examined 24 hours after patch removal for any response.</td></tr>
<tr><td>Test site</td><td>Upper arms</td></tr>
<tr><td>Test substances</td><td>Test Product 1- Cream containing 1% REGU®-FADE, Batch 100250-082/003
Test Product 2- Placebo (Base cream without active), Batch 100250-099/002
Test Product 3- Cream containing 0.2% REGU®-FADE, Batch 100250-100/002
Test Product 4- Cream containing 2% Ascorbyl-Glucoside, Batch 100250-101/003
Test Product 5- Negative control – 0.9% Isotonic saline solution, Batch 759211225</td></tr>
<tr><td>Application and frequency</td><td>0.04 g of the test products and 0.04 ml of the negative control (0.9% isotonic saline solution) were filled in different wells of IQ chambers (via randomization) and were applied occlusively on any 1 upper arm (as per randomization) of each volunteer by C.L.A.I.M.S. technician at hour 0. All the volunteers were SLS responders from the C.L.A.I.M.S. database.</td></tr>
<tr><td>Duration</td><td>48 h</td></tr>
<tr><td>Evaluation</td><td>Evaluation of cutaneous tolerance by dermatological scoring of skin reactions with Draize scale. Scales are defined as follows:

<table>
<tr><th>Score for erythema/dryness/wrinkles (E)</th><th>Score for oedema (O)</th></tr>
<tr><td>0 = No reaction
1 = Very slight erythema/dryness with shiny appearance
2 = Slight erythema/dryness/wrinkles
3 = Moderate erythema/dryness/wrinkles
4 = Severe erythema/wrinkles/scales</td><td>0 = No reaction
1 = Very slight oedema
2 = Slight oedema
3 = Moderate oedema
4 = Severe oedema</td></tr>
</table>

Final scores for E and O were defined using the following equations:

$E = \frac{\text{Total score Erythema/glaze/wrinkles}}{\text{No. of Volunteers}}$ $O = \frac{\text{Total score Oedema}}{\text{No. of Volunteers}}$</td></tr>
<tr><td>Test Institute</td><td>C.L.A.I.M.S. Pvt. Ltd. Mumbai, India.</td></tr>
</table>

I.2 Results Human Patch Test

As shown in Table 1, the evaluation of cutaneous tolerance by dermatological scoring of skin reactions with Draize scale demonstrated that both dosages (0.2% and 1%) of REGU®-FADE are **non-irritant**. The same applies for the placebo and the cream formulation containing Ascorbyl glucoside, which are also found non-irritant.

Furthermore, none of the 25 volunteers reported or showed signs of any adverse effects.

Table 1. Mean/Average scores observed on Draize scale for test and control products. 25 volunteers participated in this study

Formulation	Mean Score Erythema/ Dryness	Mean Score Oedema	Total Score (E+O)	Irritancy Assessment
Placebo + REGU-FADE® 1%	0.200	0.000	0.200	Non - Irritant
Placebo	0.200	0.000	0.200	Non - Irritant
Placebo + REGU-FADE® 0.2%	0.240	0.000	0.240	Non - Irritant
Placebo + Ascorbyl Glucoside 2%	0.280	0.000	0.280	Non - Irritant
Neg. Control Isotonic Solution 0.9%	0.400	0.000	0.400	Non - Irritant

I.3 Conclusion

Based on the above results it can be concluded that REGU®-FADE is non-irritant, is safe for topical applications on human skin, and enjoys good skin compatibility.

II. Long term skin lightening clinical study

II.1 Protocol for skin lightening

Aim	1. To evaluate skin lightening efficacy of 2 test products versus initial state in women. 2. To evaluate skin lightening efficacy of 2 test products versus placebo in women. 3. To evaluate skin lightening efficacy of benchmark product versus placebo. 4. To evaluate the skin tolerance of all the 4 products tested.
Test subjects	52 women volunteers from the Mumbai region in the age group of 18-44 years. Skin type IV
Method	Test under dermatological control, mono-centric, randomized, double blinded, controlled, comparative, versus initial state, placebo, and benchmark. At Visit 1, the **test sites (volar forearms)** were cleaned with Cetaphil cleansing solution and volunteers were acclimatized under controlled conditions of temperature and humidity for 1 hour. Two test sites (3 cm x 3 cm) were marked on each volar forearm of volunteers. This was followed by clinical evaluation of test sites by the Dermatologist. Clinical grading of skin colour by experts was carried out using a shade card. Digital Photographs under standardized conditions were taken thereafter. Baseline measurements using spectrophotometer and SIAscopy were carried out. The 4 products were applied on the 4 test sites on volar forearms (1 patch per product) as per randomization. The Products were applied on test sites daily under supervision of trained personnel. The trained personnel went to houses of volunteers twice a day (once in the morning and once towards late evening) for application of product. The volunteers were asked to return to study centre for follow up visits at Day 15, Day 30, Day 60, and Day 90. For all visits clinical grading of skin colour for any change in skin colour was carried out. SIAscopy and spectrophotometric measurements and digital photographs under standardized conditions were taken at all visits.
Test site	Volar forearms
Test substances	**Test Product 1**- Cream containing 1% REGU®-FADE, Batch 100250-082/003 **Test Product 2**- Placebo (Base cream without active), Batch 100250-099/002 **Test Product 3**- Cream containing 0.2% REGU®-FADE, Batch 100250-100/002 **Test Product 4**- Cream containing 2% Ascorbyl-Glucoside, Batch 100250-101/003
Application and frequency	About 0.018g of a given product as per randomization, were applied on the four sites on volar forearms. Massage gently the areas applied with fingers in circular motion for 30 – 60 seconds.
Duration	90 Days
Evaluation	Skin spectrophotometer measurements for determining skin luminescence at Visit 1 (Day 0), Visit 2 (Day15), Visit 3 (Day 30), Visit 4 (Day 60) and Visit 5 (Day 90).These measurements were used to determine the individual typology angle (ITA°) and Δ ITA° by subtracting average ITA° of the treated site from that of the average baseline (First day of the study, Day 0). A Δ ITA° > 3 can be distinguished visually.

Definition of ITA°	ITA evaluates the skin pigmentation degree. Increase in ITA° indicates a decrease in skin pigmentation. $$ITA° = \frac{\left[ArcTangent\left(\frac{L^* - 50}{b^*} \right) \right] * 180}{\pi}$$ When Arc Tangent calculated in radians L* = Luminance (dark – light) b* = blue to yellow spectrum Lighter skin has a high ITA°, darker skin low or negative ITA° (Fig. 1)
Statistical Analysis	For this study SPSS version 10.0 statistical software was used for analysis. Continuous variables were summarized by treatment group using summary statistics (number of observations, mean, standard deviation, median, minimum and maximum). Categorical values were summarized by treatment group using frequencies and percentages. Additionally, the comparison of Test Product 2 (Code 099) versus Test Product 1 (Code 082), Test Product 3 (Code 100), and Test Product 4 (Code 101) was performed as follows: The area under the curve (AUC) was calculated per treatment per subject. The AUC was then normalized to the duration in days. Due to baseline-variability between the four locations on an arm, the baseline was subtracted. The resulting AUCs reflect individual changes over time versus baseline. A repeated measures ANOVA was used to compare the baseline-corrected AUCs between groups. The AUCs were calculated in R *v*ersion 2.92, and the repeated measures ANOVA was performed in SPSS *v*ersion 17.0.2. All p-values were reported based on two-sided significance test and all the statistical tests were interpreted at 5% level of significance.
Test Institute	C.L.A.I.M.S. Pvt. Ltd. Mumbai, India.

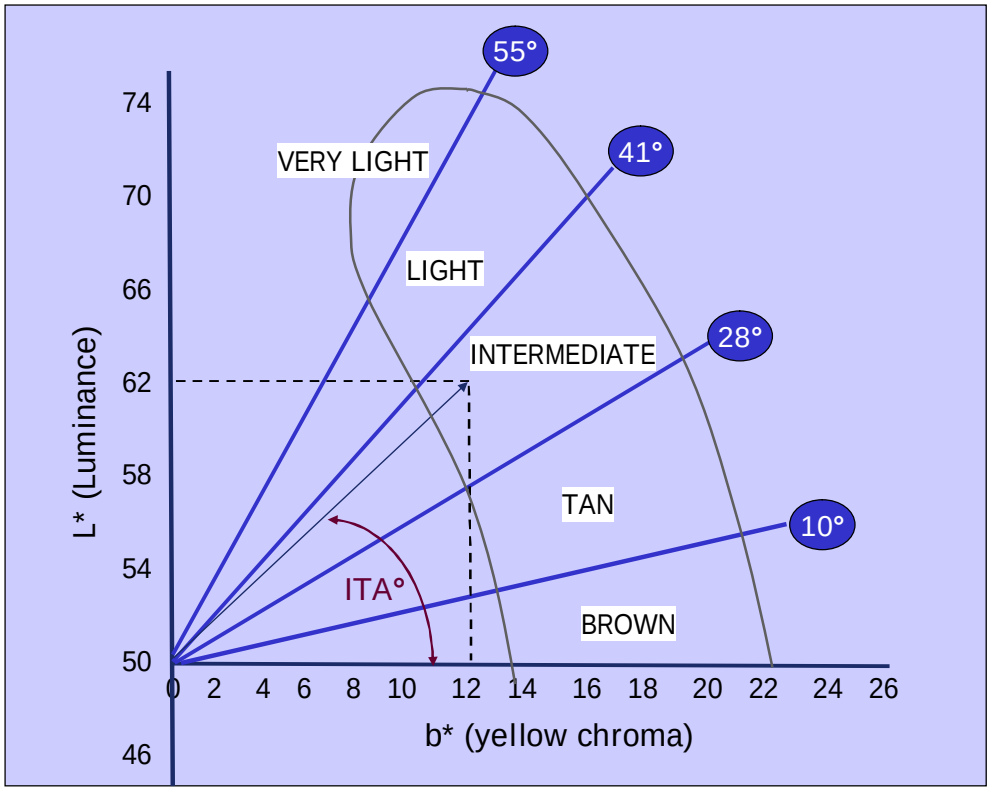


Figure 1. Individual Typology Angle (ITA°)

II.2 Results

In this study run with 52 female volunteers in India (skin type IV), REGU®-FADE (1%) induced an increase of individual typological angle (ITA°) from 14.90 to 18.13 (mean values). This change in ITA° (Δ ITA°) was about 50% ($p < 0.05$) as compared to placebo (vehicle containing UV-filters, SPF 10). Ascorbyl-glucoside showed an increase in ITA° values from 14.44 to 16.98 (mean values). This change in ITA° was marginal as compared to the vehicle (~15%), Fig. 2. Moreover, the time-course profiles of Δ ITA° indicated that REGU®-FADE (even at low concentrations) tends to be at least 50% faster than ascorbyl-glucoside in influencing changes in skin pigmentation (Fig. 3).

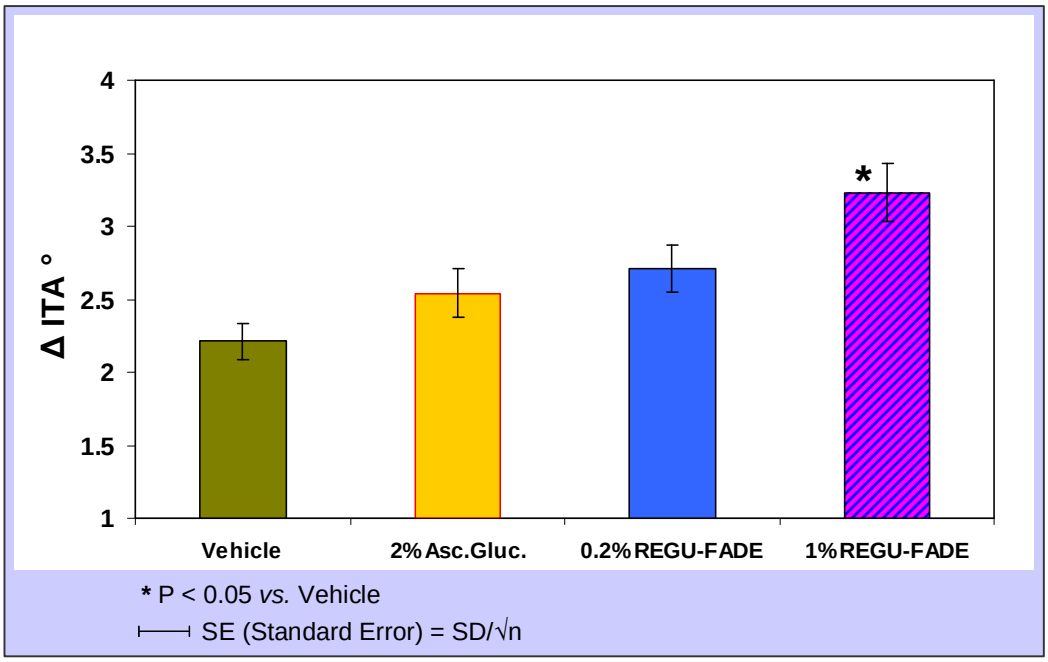


Figure 2. Comparative skin lightening results with REGU®-FADE, the benchmark Ascorbyl-glucoside, and placebo (vehicle) at the end of 90 days treatment.

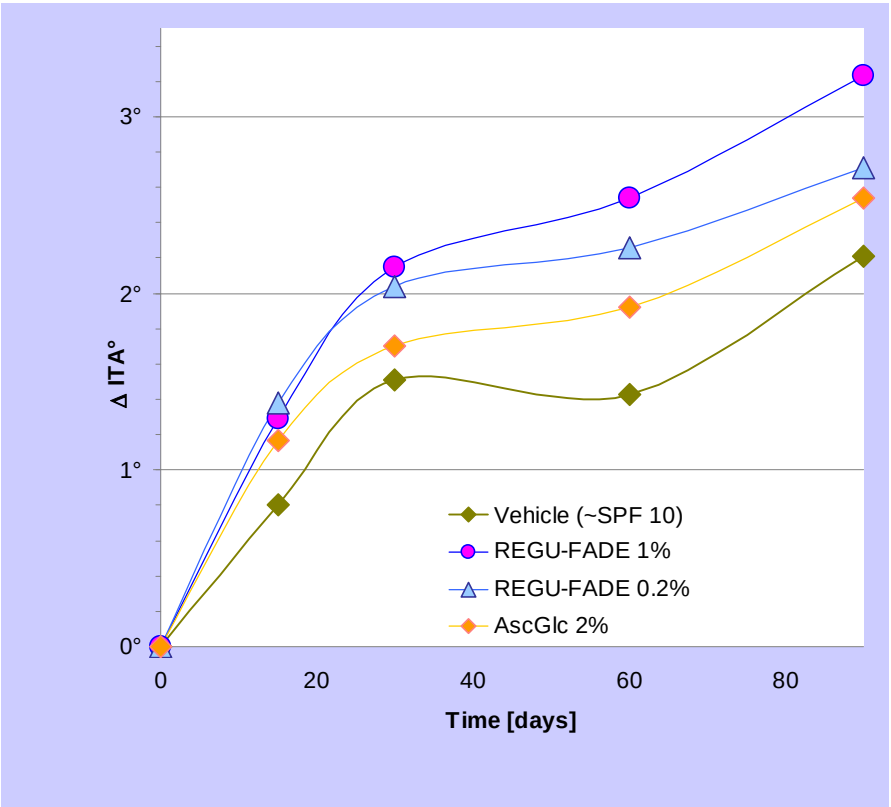


Figure 3. Time-course profiles of the skin lightening properties of REGU®-FADE as compared to Ascorbyl glucoside and placebo (vehicle)

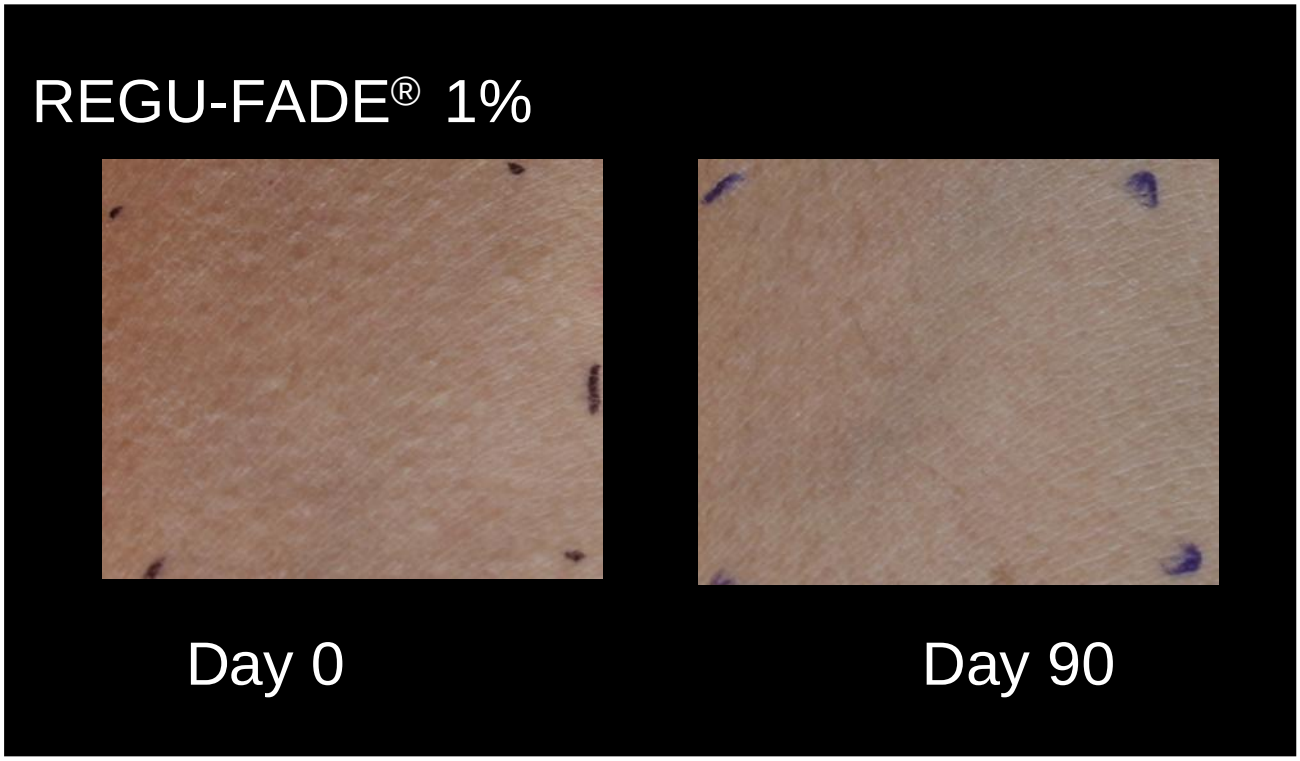


Figure 4. Volar forearm of Asian volunteer (Mumbai region) before (Day 0) and after (Day 90) treatment with REGU®-FADE.

II.3 Conclusion

Skin of Asian volunteers treated with 1% REGU®-FADE showed a significant increase in the ITA° values, indicating a visible lightening of the skin after 3 Months of treatment. REGU®-FADE was well tolerated by all the volunteers. None of them reported adverse effects.

III. Short term skin lightening clinical study

III.1 Protocol for skin lightening

<table>
<tr><td>Aim</td><td>To evaluate the skin lightening efficacy of a cosmetic test product.</td></tr>
<tr><td>Test subjects</td><td>12 (2 male, 10 women) from the Hamburg region in the age group of
39.0 ± 13.8 years (mean ± standard deviation)</td></tr>
<tr><td>Test Design</td><td>Randomized, open for negative control and positive control, blind for test products, blind observer for subjective evaluation, intra-individual comparison.</td></tr>
<tr><td>Method</td><td>
<table>
<tr><th>Day</th><th>-8</th><th>0 (Baseline)</th><th>14</th><th>28</th></tr>
<tr><td>Screening, Inclusion</td><td>X</td><td></td><td></td><td></td></tr>
<tr><td>Chromametric Measurement</td><td></td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Application of Test Products</td><td></td><td>X*</td><td>X*</td><td></td></tr>
</table>
* From day 8 to day 28 test products were applied by the subjects themselves at home, three times per day (including application at the study site on days 1 and 14).

Day - 8 (A week previous to the treatment): Subjects came to the Study Site. They were informed about the study and gave their written consent.
Day 0 (Baseline): Subjects came to the Study Site. Subjective evaluation was performed. Chromametric measurements were performed. Test products were applied by the subjects themselves under supervision of the technician. Test products were allowed to soak in for 5 minutes.
Day 0 to Day 28: Test products were applied by the subjects themselves at home, three times per day (including application at the study site on days 0 and 14). Test products were allowed to soak in for 5 minutes.
Day 14: Subjects came to the Study Site. Chromametric measurements were performed. Test products were applied by the subjects themselves under supervision of the technician. Test products were allowed to soak in for 5 minutes.
Day 28: Subjects came to the Study Site. Chromametric measurements were performed. Test products were returned to the study personnel.
A deviation of ± 1 day of the study days 14 and 28 was accepted, since no substantial influence on the outcome of the study was expected.

</td></tr>
<tr><td>Test site</td><td>Volar forearms.
Tests areas of 4x4 were delineated on the volar forearms, which were re-delineated by the volunteers over the use period.</td></tr>
</table>

Test substances	**A-** Untreated area (Negative control) **B- 100250-185** - Cream containing 2% Ascorbyl-Glucoside (Positive Control) **C- 100250-187** - Cream containing 1% REGU®-FADE (Test Product)
Application and frequency	A pea-sized amount of the product was applied by the volunteers themselves at home, three times per day. The amount of product used was determined by weighing the test products before and after the use period.
Duration	2 to 4 Weeks
Evaluation	The individual typology angle (ITA°) value for every subject gives estimation about the actual sensitivity to light at the moment. Further, the parameter is used as primary parameter to assess skin tan. It was calculated by L* and b* values using the equation provided in next section. L* and b* values were obtained from direct skin chromameter measurements for determining skin luminescence at baseline (Day 0), and after 14 days and 28 days of treatment. These measurements were used to determine the individual typology angle (ITA°) and Δ ITA° by subtracting average ITA° of the treated site from that of the average baseline (Day 0). A Δ ITA° > 3 can be distinguished visually.
Definition of ITA°	ITA evaluates the skin pigmentation degree. Increase in ITA° indicates a decrease in skin pigmentation. $ITA° = \frac{\left[ArcTangent\left(\frac{L*-50}{b*} \right) \right] * 180}{\pi}$ When Arc Tangent calculated in radians L* = Luminance (dark – light) b* = blue to yellow spectrum Lighter skin has a high ITA°, darker skin low or negative ITA° (Fig. 1)
Statistical Analysis	Computation of the statistical data has been carried out using a commercially available statistics program (SPSS for windows). All raw data of all valid subjects as well as calculated values were listed. N, means, standard deviations and 95% confidence limits of all valid subjects were calculated. Statistical analysis of chromametric measurements were based on ITA° values. Pair-wise comparisons of treatment C to A and B were performed with paired t-test. A significance level of 0.05 (alpha) was chosen for statistical analysis.
Test Institute	proDERM Institute for Applied Dermatological Research, Schenefeld/Hamburg, Germany

III.2 Results

Table 2 depicts the mean values of ITA° measured on day 0 (baseline, before product application), and after 14 days of treatment.

Table 2: Mean Values of ITA°; N = 12

Day	Product	ITA°
0	**A (Negative Control)**	44.68
	B (Positive Control) – Ascorbyl-Glucoside 2%	43.18
	C (REGU®-FADE 1%)	43.50
14	**A (Negative Control)**	46.31
	B (Positive Control) – Ascorbyl-Glucoside 2%	45.67
	C (REGU®-FADE 1%)	48.32

Table 3 shows the mean values of differences to day 0 (baseline) of ITA° for the products tested after 14 days of treatment. Furthermore, the table shows results of pair-wise t-test for the comparison of treatments A (negative control), B (positive control), and C (REGU®-FADE 1%) on differences to day 0 (baseline).

As compared to the baseline (Day 0), significantly lower difference in ITA° was found on the untreated area A (negative control) than on the area treated with test product C (REGU®-FADE 1%). The comparison of the positive control (Ascorbyl glucoside) to the negative control did not show significant differences after 14 days of treatment. It needed at least 28 treatment days to become significant (data not shown). This implies that between baseline and day 14 the skin color became significantly lighter on the test areas treated with test product C (REGU®-FADE 1%) than on the untreated test area (A).

Table 3. Mean Values of Differences to Baseline of ITA° and p-Values for Comparison of Treatments on Differences to Day 0 (Paired t-Test, N = 10-12). * significant, $p \leq 0.05$, n.s.: not significant

Day	Product	Diff. to Day 0	p-Value (t-Test): Comparison of Treatments	
			A	B
14	**A** (Negative Control)	1.63	--	--
	B (Positive Control) Ascorbyl-glucoside 2%	3.42	0.358 n.s.	--
	C (REGU®-FADE 1%)	5.53	0.030 *	0.351 n.s.

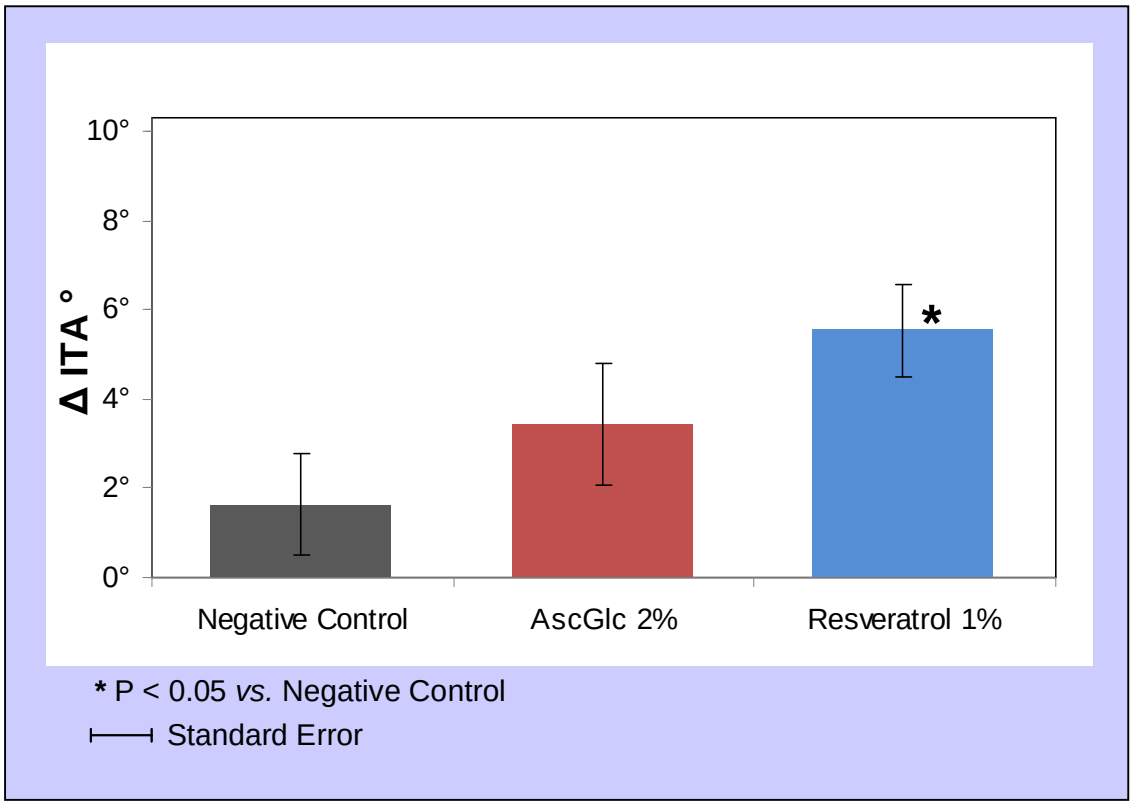


Figure 5. Comparative skin lightening results with REGU®-FADE and the benchmark Ascorbyl-glucoside (positive control) as compared to the negative control after 14 days of treatment.

III.3 Conclusion

This study was accomplished to assess the skin whitening efficacy of a cosmetic product, **100250-187** (REGU®-FADE 1%), a positive control (Ascorbyl Glucoside 2 %) **100250-185** (B), and on an untreated negative control (A). Chromameter measurements were performed on the skin of four test areas. Subsequently, a pea-sized amount of the test products was applied per test area (4x4 cm), three times per day, for 4 weeks. Further Chromameter measurements of the treated skin were performed after 2 weeks (day 14) and after 4 weeks (day 28). Significant skin whitening effects were found after 2 and 4 weeks of product application for calculated Chromameter values (ITA°):

- After 2 weeks as well as after 4 weeks of treatment with test products **100250-187** (REGU®-FADE 1%), the skin became significantly lighter than the untreated area (negative control).
- Only after 4 weeks of treatment with the positive control **100250-185** (Ascorbyl-glucoside 2%), the skin became significantly lighter than the untreated area (negative control).

A significant whitening efficacy can be attributed to test product 100250-187 (REGU®-FADE 1%). The skin became significantly lighter after 2 weeks as well as after 4 weeks of treatment with this test product, whereas this effect was only documented after 4 weeks for the positive control 100250-185 (Ascorbyl Glucoside 2%).