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TANNING TEST

013729 PRELINE - RAPID BRONZ
LOTTO 259612

Placebo-controlled clinical-instrumental assessment of the efficacy of a tanning accelerator cosmetic product called

RAPID BRONZ

DESIGN OF THE TRIAL

1.1. Title
Placebo-controlled clinical-instrumental assessment of the efficacy of a tanning accelerator cosmetic product.

1.2. Nature and purpose of the trial
The trial aims to assess the efficacy of a tanning accelerator cosmetic product. For the purpose, a placebo-controlled clinical trial is carried out on 10 healthy female subjects. The site of application of both active and placebo products is randomized by the responsible of the trial.
The assessment of the efficacy of the product is performed after repeated application of the product and controlled UV exposure on a skin site. Another adjacent site has been treated with a placebo formulation. The efficacy of the product is assessed through one colorimetric probe that can record skin colour variations.

1.3. Product subject to trial
1.3.1. Information supplied by the Client
- Product name:
  013729 PRELINE RAPID BRONZ BATCH 259612 - Active
  013729 PRELINE RAPID BRONZ BATCH 259612 - Placebo
- The cosmetic products being studied were formulated without using substances whose use is forbidden in cosmetic and body hygiene products (EC laws), preservation agents and UV filters introduced in the formula of products are in the positive list published by the EC ad their concentration complies with the use provided for by this law. For all the substances for which there is a concentration limit, the thresholds and the warnings as per the relevant attachments to the 76/768 EEC Regulations are complied with.
- The safety of use of the cosmetic products being studied has been assessed on human volunteers (cosmetic dossier).
- Mode of employment: apply the product once a day with a soft circular massage until complete absorption. Subjects were ordered to use a quantity of products equal to about 2 mg/cm2. Prior to (about 30 minutes) UV radiation, 100 mg of active product or placebo were applied by the investigator in a 50 cm2 area within which the irradiation sites have been marked.

1.4. Ethical requirements
The trial was carried out in compliance with the following ethical requirements.
1.4.1. All the subjects taking part in the trial are healthy volunteers of at least 18 years of age.
1.4.2. All the subjects taking part in the trial were selected under the supervision of the dermatologist by applying the inclusion/non-inclusion criteria (see § 1.5.1.1.-2.).
1.4.3. The participation of the volunteers in the trial was totally free.
1.4.4. All the subjects taking part in the trial were volunteers informed of the purpose and nature of the trial.
1.4.5. All the subjects taking part in the trial were informed of the possible risk involved in carrying out the trial.
1.4.6. All the subjects taking part in the trial provided a written informed consent form signed prior to the beginning of the trial.
1.4.7. Before the volunteers were exposed to the product being analyzed all the relevant safety information on the product and on each component has been evaluated.
1.4.8. All the procedures of the trial were carried out in compliance with the ethical principles for medical research (ethical principles for medical research involving human subjects, adopted by the 18th AMM General Assembly of AMM in Helsinki, Finland, in June 1964 as amended).
1.4.9. All the precautions required to avoid adverse skin reactions must be adopted.
1.4.10. In case of adverse/unexpected reactions the medical investigator will judge their seriousness (by writing it in detail in the data collection form) and will adopt the treatment required.

1.5. Subjects taking part in the trial

1.5.1. Selection of subjects

Subjects taking part in the trial were selected by the dermatologist from a panel of healthy subjects, both females and males (over 18 years of age) by applying the inclusion and non-inclusion criteria set out below.

1.5.1.1. Inclusion criteria

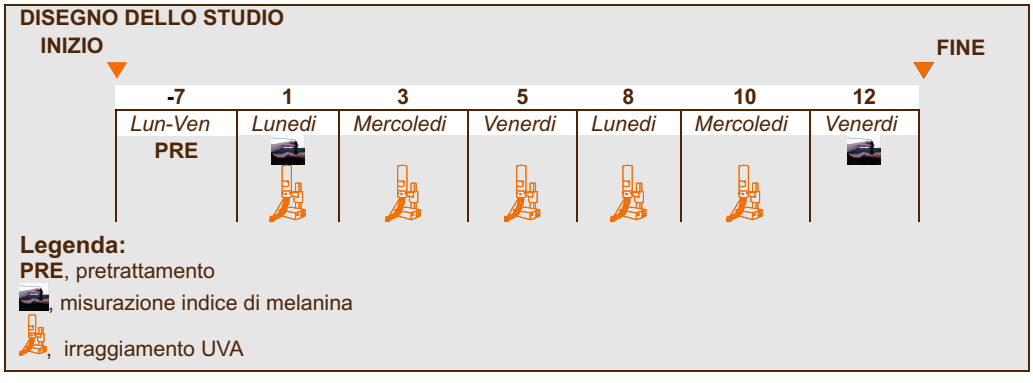
Healthy female and male subjects
Age: over 18
Race: caucasian
Subjects that have not taken part in similar studies for at least two months
Commitment not to use topical or systemic treatments whose activity could be comparable with that of the product being examined for the whole duration of the trial
Subjects that have not been exposed to sun radiation in the two months prior to the trial
Absence of sun erythema, tanning, skin dyschromia or active skin lesions in the areas chosen for the trial
Even colour of the area of trial: absence of nevi, stains or sun lentigo and hairless
Subjects informed on the procedures of the trial and that have signed an informed consent form
Absence of skin pathologies
Negative medical history for atopy
Commitment not to change normal daily routine
Subjects informed on the procedures of the trial and that have signed an informed consensus form

1.5.1.2. Non-inclusion criteria

Subjects that to do not meet inclusion criteria
Pregnant or breastfeeding women
Prior history of allergy, photoallergy, phototoxicity or other abnormal responses to sun exposure
Prior history of allergy or sensitivity to cosmetic, toiletry, sun products and/or topical medications
Subjects with skin problems in the test area
Subjects that have used self-tanning products on the skin treated in the month preceding the date of the trial
Frequent users of UVA sunbeds
Subjects being treated, upon recruitment or in the preceding month, with medications with a photosensitizing potential, medicines and/or dietary supplements that can provide for skin colour, corticoids
Subjects being treated upon recruitment or in the week preceding the trial with sedative antihistamines or anti-inflammatory drugs

1.6. Performance of the trial

After recruitment, the informed subjects were given the product so as to proceed with a pre-treatment period of one week. After the first pre-treatment period the subjects came to our office, where both the region treated with the active product and the region treated with the placebo product were exposed to a UVA radiation dose (10 J/cm2, single dose). The areas destined to radiation were marked for the whole duration of the trial with a dermographic pen. Prior to each ensuing exposure both skin areas were measured through a colorimetric probe (MEXAMETER MX 18 (Courage + Khazaka electronic GmbH). The following exposures/measurements were performed 2 days after one another, except for the weekend (after 3 days). Below you can see a summary of the planning of the trial.



1.7. MATERIALS AND METHODS

In the paragraphs below you can find a detailed description of the materials, methods and procedures of the trial.

1.7.1. UV radiation source and UV radiation control

The UV radiation source employed in the trial was the solar simulator MultiportTM Output 601 - 300 Watts produced by Solar Light Co. Inc., PA. Said tool is fitted with a UV radiation source (represented by a Xenon short-arc lamp) and by suitable filters (Schott WG345 and UG11) to create a spectral quality meeting the acceptability limits set out by the JCIA Persistent Pigmentation Darkening (PPD).

In compliance with such method the solar simulator used:

- emits a total energy < 150 mW/cm2,
- has 92%-100% of UVA component with respect to total radiation
- has a UVB/UVA ratio < 0.1%
- has a UVAII/UVA ratio between 8.0 and 20.0%
- produces a steady and uniform outgoing UV radiation along the whole UV ray.

The UV radiation dose applied to each exit of the lamp was adjusted with a PMA2100 radiometre (Solar Light Co. Inc., PA) fitted with a UVA PMA 2113 UVA detector (Solar Light Co. Inc., PA).

Both the MultiportTM Output 601 - 300 Watts solar simulator and the PMA2100 radiometre with the relevant UVA PMA 2113 detector were subject to a yearly check and calibration control performed at the parent company's headquarters, as well as to regular internal checks.

1.7.2. Skin colour assessment

Pigmentation intensity is assessed through a colorimetric MEXAMETER MX 18 probe (Courage + Khazaka electronic GmbH). The assessment is performed within two sites of 1.0 cm2 marked for the whole duration of the trial with a dermographic pen.

The MEXAMETER MX 18 tool measures specifically the melanin content of the skin. The measure is based on the principle of adsorption. The tool proble emits light in three different wavelengths. A receiver measures the light reflected by the skin. The position of the emitter and the receiver ensures that only diffused and spread light is measured. Since the quantity of light emitted is defined, it si possible to calculate the quantity adsorbed by the skin. In order to measure the melanin index two different wavelengths are used that correspond to the peaks of melanin absorption. The tool calculates the parametre known as melanin index based on absorption data.

1.8. Results and statistics

1.8.1. Results

The results are written in the relevant measure units in charts.

1) Average values were calculated as: [1]

$$m = \frac{\sum_{1}^{10} p}{10}$$

where:

p is the value of the parametre being analyzed.

2) The standard error (SEM) of the data is calculated as: [2]

$$SEM = \frac{\sqrt{\frac{\sum_{1}^{n}(p_i^2) - \frac{\sum_{1}^{n} p_i^2}{n}}{(n-1)}}}{\sqrt{n}}$$

Tutti i calcoli sono stati fatti utilizzando un foglio di calcolo di Microsoft® Excel.

1.8.2. Analisi statistica

I dati sono stati sottoposti a test t di Student per dati appaiati.

*, $p<0.05$, significativo
**, $p<0.01$, moderatamente significativo
***, $p<0.001$ altamente significativo

RESULTS

CHART 1 – In the chart below are the data obtained in the course of the trial for both the treated and the untreated areas. Data are expressed as a ± SEM average.

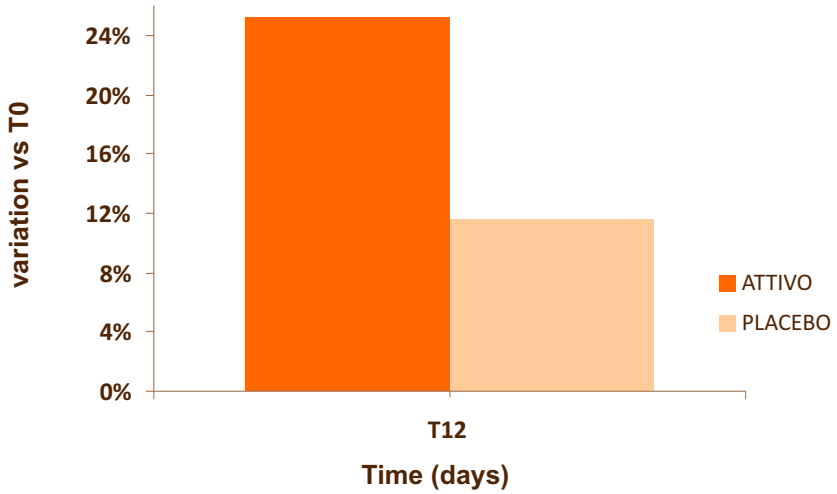
ATTIVO

n	Vol ID	T0	T12	Variazione vs. T0 (%) T12vsT0
01	VOL.1	93	121	30,1%
02	VOL.2	114	136	19,3%
03	VOL.3	99	131	32,3%
04	VOL.4	130	150	15,4%
05	VOL.5	118	149	26,3%
06	VOL.6	93	122	31,2%
07	VOL.7	123	152	23,6%
08	VOL.8	101	120	18,8%
09	VOL.9	131	161	22,9%
10	VOL.10	116	153	31,9%
	Media/Mean	111,8	139,5	25,2%
	SEM*	4,6	4,8	
	t-test vs T0		0,000	

PLACEBO

n	Vol ID	T0	T12	Variazione vs. T0 (%) T12vsT0
01	VOL.1	91	105	15,4%
02	VOL.2	116	130	12,1%
03	VOL.3	95	108	13,7%
04	VOL.4	131	147	12,2%
05	VOL.5	119	130	9,2%
06	VOL.6	95	107	12,6%
07	VOL.7	130	147	13,1%
08	VOL.8	106	117	10,4%
09	VOL.9	130	142	9,2%
10	VOL.10	121	130	7,4%
	Media/Mean	113,4	126,3	11,5%
	SEM*	4,9	5,2	
	t-test vs T0		0,000	

GRAPH 1 - In the chart below are the data obtained in the course of the trial for both the treated and the untreated areas (data source: chart 1).



Comment

The radiation doses used has determined an increase in skin pigmentation in the area treated with the placebo product that is instrumentally recordable.

In the area treated with the active product, the effect of the UVA radiation on skin pigmentation is greater than what was recorded for the area treated with placebo. Such data show the efficacy of the product in accelerating the physiological process of photoinduced pigmentation.

Conclusions

The data obtained show how skin application of product 13729 PRELINE RAPID BRONZ BATCH 259612 as well as repeated exposure to UVA rays can determine a statistically significant variation of skin pigmentation with respect to that obtained on the area treated with the placebo product.

In the experimental conditions adopted in the trial the 013729 PRELINE RAPID BRONZ BATCH 259612 product enhances natural skin pigmentation after UV exposition.