Efficacy of a cosmetic formulation containing a blend of nanoencapsulated antioxidants by in vitro, ex-vivo and in vivo studies

Fonseca, A. P. [1], Dal Pizzol, C 1[1], Eberlin, S[2], Vanzo, A. C.[1], Campos, P. M.[3]

1 Sallve Comércio de Cosméticos, São Paulo, Brazil
2 Kosmoscience, Clinical Research Organization, Valinhos, Brazil
3 Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, Brazil

Corresponding author: Ana Paula Pereira da Fonseca, Rua Cônego Eugênio Leite, 767, Pinheiros, São Paulo – SP – Brazil. E-mail: anapaula.fonseca@sallve.com. Telephone: +55 21 965325955

IFSCC presenter: Prof. Dr. Patricia Maia Campos

Abstract

Application of antioxidants in cosmetic products contributes to collagen preservation and synthesis, acting in melanogenesis and overall skin health and appearance improving. Therefore, the objective of this study was to evaluate the efficacy and safety of a cosmetic formulation containing a blend of ingredients, by ex-vivo and clinical evaluations. In the ex-vivo study, the treated human skin presented a statistically ($p<0.001$) decreased melanin pigmentation by 81.53% and increased type 1 procollagen by 260,40%. The clinical study results showed a significant ($p<0.001$) decrease on skin superficial oiliness after 1 and 4 hours of product application and a significant ($p<0.001$) increase in the dielectric constant after 1, 4, 8, 12 and 24h of product. There was no adverse event, physical sign nor reported sign, showing that the studied cosmetic product presents a high tolerance profile on normal skin. The acne lesions count presented a significant decrease in non-inflammatory lesions ($p= 0,0135$) and non-significant reduction in inflammatory lesions ($p=0,6641$). The Vectra XT imaging analysis showed that the cosmetic product promoted a significant ($p<0.05$) reduction in the swelling around the eyes in 95% of subjects after 1h of application. Finally, 120 subjects answered the self-assessment questionnaire, and the product was well appreciated by them: 91% reported their skin softer and 83% noticed an improvement in the skin overall appearance. The proposed cosmetic product was safe and effective in the improvement of skin conditions and appearance by increasing collagen synthesis, resulting in firmer, less rough, softer and smoother skin.

Keywords: nanoencapsulated antioxidants; cosmetic formulations; collagen; efficacy; instrumental measurements

Introduction.

Chronic exposure to extrinsic factors produces changes in skin structure which have a profound impact on the functional mechanisms and appearance of the skin. Among them is skin pigmentation. The pigmentation process (melanin synthesis) depends both on the increase in the number of melanocytes and on the activity of melanogenic enzymes and after exposure of the skin to ultraviolet radiation, melanogenesis is initiated. Melanin plays an important role in protecting the skin against the harmful effects of solar radiation, but the excessive accumulation of this pigment can become an unsightly problem [1].

Regarding skin structure, despite the important role of extracellular matrix proteases (ECM) in dermal remodeling, it is well established that stress factors, such as ultraviolet and infrared A radiation, lead to an excessive increase in collagen degradation [2].

The ECM is a complex and dynamic structure composed of collagen fibers, elastin and proteoglycans, such as hyaluronic acid. In this sense, the components of the ECM are responsible for maintaining the skin's firmness, elasticity, and support, in addition to assisting in the processes of adhesion and cell signaling [3,4]. Collagen represents about 90% of the proteins in the human dermis and changes in its production are considered a primary cause of wrinkle formation and an aged appearance of the skin. In addition, collagen is essential during connective tissue remodeling and the tissue repair and regeneration process [5].

The natural barrier, formed by the stratum corneum, depends critically on its composition, represented by proteins (75-80%), lipids (5-15%) and other constituents (5-10%). Of the total proteins present in the EC, approximately 5% correspond to a structural portion called the epidermal cornified envelope (ECE), which is formed by a set of highly insoluble and resistant proteins, which externally surround the keratinocytes and play a fundamental role in the structuring of the keratinocytes. and organization of intracellular lipids [6]. Many constituents of ECE have been identified, among them the proteins profilagrin and filaggrin [7-9].

In this way, the measurement of the mentioned parameters can be a useful tool for evaluating the effectiveness of cosmetic products and actives with tissue support appeal, anti-aging and anti-wrinkle protection.

Thus, the objective of this study was to evaluate the efficacy and safety of a cosmetic formulation containing a blend of ingredients, such as nano ascorbyl palmitate, nano resveratrol, tocopherol, nano caffeine, carnosine and niacinamide by ex-vivo and clinical evaluations.

Materials and Methods.

In vitro study

Human fibroblasts were incubated with 3 non-cytotoxic concentrations of the evaluated product for further exposure to infrared-A (IR-A) radiation and measurement of the fluorescence intensity generated by mitochondrial ROS synthesis (ROSmit – MitoSOX probe). Then, human fibroblasts (FBH) were seeded in 75 cm² bottles (Corning, USA), cultured and expanded in an incubator at 37°C in the presence of 5% CO2, using specific culture medium. Upon reaching confluence, the cells were seeded in 96 and 6-well plates (Corning, USA) for determination of non-cytotoxic concentrations of the evaluated product and subsequent quantification of the proposed mediator. Cell viability was determined by a colorimetric method using MTT dye (Sigma Chemical, St. Louis, Mo.). To perform the assay, the product was prepared in culture medium and added to the 96-well plate in a serial dilution in the range of 100.00 to 0.03 mg/mL using a dilution factor of 3.164. The culture was incubated for a period of 48 hours. MTT was then added to the culture at a concentration of 5 mg/mL (30 µL/well) and incubated for an additional 4 hours. The well content was removed and 100µL of isopropanol was added in order to solubilize the formed formazan crystals. The absorbance of each well was determined at 570 nm in a Multiskan GO monochromator (Thermo Scientific, Finland).

Cell culture treatment and exposure protocol:

Cell cultures were incubated with 3 non-cytotoxic concentrations of the evaluated product determined according to item 3.3. The concentrations of the Investigational Product (IP) product evaluated in this study were 10.01; 3.17 and 1.00 mg/ml. The fibroblast culture was treated with the evaluated product for 48 hours, exposed to a dose of 360 J/cm² of IV-A

radiation using the Hydrosun 750 and HBM1 devices (Hydrosun Medizintechnik GmbH, Müllheim, Germany) and maintained in culture conditions. for an additional 24 hours. Then, cells were incubated with MitoSOXTM, for further semi-quantitation of mitochondrial ROS synthesis.

Semi-quantitation of mitochondrial ROS synthesis:

After treatment and exposure to IR-A radiation, the culture medium containing the evaluated product was removed and the cells were incubated with MitoSOXTM Red (ThermoFisher Scientific). The fluorescence intensity resulting from the oxidation of MitoSOXTM Red by the superoxide anion of mitochondrial origin (ROSmit) was analyzed using a Fluorescence Microscope (OLYMPUS BX53, Japan) using the cellSens Standard software (©2010 OLYMPUS CORPORATION). After obtaining the images, the fluorescence intensity was quantified using the ImageJ software (Arbitrary Units - U.A.).

Statistical analysis:

In the statistical evaluation, the ANOVA test was used, which made it possible to measure the variation in the results, comparing the data between the groups. Then, the Bonferroni post-test was applied, which reinforced and made the result presented in the ANOVA test even more accurate. A significance level of 5% (GraphPad Prism v6) was used.

Ex-vivo study

To measure melanin and pro-collagen I, human skin fragments obtained from elective plastic surgery were subjected to UV radiation before histological evaluation with Fontana-Masson staining (semi-quantification of melanin) or measurement of the fluorescence intensity generated by the probe. procollagen I fluorescence. In addition, skin fragments were subjected to barrier disruption with 5% sodium lauryl sulfate and treated with the evaluated product for 48 hours for filaggrin semi-quantitation using the immunofluorescence technique.

Human skin culture:

The ex vivo skin fragments used in this study came from one (01) healthy individual, female, phototype II, 44 years old, who underwent elective plastic surgery in the abdomen region (abdominoplasty). After the surgical procedure, the skin fragments were collected in plastic bottles containing 0.9% saline solution and kept under refrigeration for up to 24 hours.

This project did not include the storage and storage of biological material for future use, and the spare fragments were properly disposed of as infectious waste. The use of human skin fragments from elective surgeries to carry out this study was submitted to the Research Ethics Committee of Universidade São Francisco – SP, CAAE 82685618.9.0000.5514, under opinion 2.493.285.

Stress treatment and simulation protocol:

Ex vivo skin fragments were fractionated into pieces of approximately 1.5 cm^2, placed in culture plates (Corning, USA) with appropriate culture medium, treated with the Investigational Product (IP) in the proportion of 25-30 mg/cm^2 and kept in an incubator at 37°C in the presence of 5% CO2 for 7 days. During this period, the fragments were exposed 4 times to 10 J/cm^2 of UV radiation using the UVA Cube 400, SOL 500 H1 filter and UV Meter devices (Honle UV America Inc., MA, USA). Then, 24 hours after the last exposure to UV radiation and retreatment with the evaluated product, the fragments were collected and submitted to histological procedures to measure the proposed mediators (melanin and pro-collagen I). Another group of fragments was subjected to barrier disruption with sodium lauryl sulfate (LSS 5%), treated with the product evaluated at the proportion of 25-30 mg/cm^2 and kept in an incubator at 37°C in the presence of 5% CO2 for a period of 48 hours. After this period, they were submitted to the immunofluorescence assay of the proposed marker (filagrin).

Semi-quantitation of melanin (Fontana-Masson):

After the treatment period, skin fragments were embedded in Tissue-Tek® O.C.T.™ and then serial 12 micrometer histological sections were collected directly onto cryostat silanized slides (Leica , GER – CRYOCUT 1800). The sections were washed with 0.1 M phosphate buffer (PB) and stained using the Fontana-Masson technique. Slides were mounted on specific mounting media and analyzed under a microscope (OLYMPUS BX53) with Standard cellSens software (© 2010 OLYMPUS CORPORATION). The parameter evaluated was the percentage of melanin present per corresponding area of the basal layer and thorny layer in the epidermis. The percentage values were obtained using the ImageJ Software (version 1.47) through the conversion of the images obtained into binary images followed by the quantification of the black pixels present in the basal layer and area of the spinous layer.

Immunofluorescence for semi-quantitation of Pro-Collagen I and Filaggrin:

After the stress exposure treatments, the ex vivo skin fragments were cryoprotected in a formulation of glycols and water-soluble resins (Tissue-Tek ® OCT™). Then, serial sections of 10 μm were collected directly on silanized slides with the aid of a Cryostat (Leica – CRYOCUT 1800). After making the sections, they were washed with 0.1M phosphate buffer saline (PBS) and incubated overnight with anti-filagrin and anti-procollagen I primary antibodies. Subsequently, the sections were washed again. with 0.1M PBS and incubated for 1 hour with secondary antibody Alexa Flour 488 – Goat anti Rabbit (1:500). Immediately after the completion of the steps described above, a new incubation was carried out (1 minute) with DAPI (4'-6-Diamidino-2-Phenylindole; DNA marker) followed by 3 10-minute washes with 0.1 M PBS.

The slides were mounted in a specific mounting medium and analyzed using a Fluorescence Microscope (OLYMPUS BX53) using cellSens Standard software (©2010 OLYMPUS CORPORATION). The parameter evaluated was the intensity of fluorescence emitted by labeling with a specific antibody. After obtaining the images, the fluorescence intensity was quantified using the ImageJ software (Arbitrary Units - A.U.).

Statistical analysis:

For the statistical evaluation, the ANOVA test was used, which made it possible to measure the variation in the results, comparing the data between the groups. Then, the Bonferroni post-test was applied, which reinforced and made the result presented in the ANOVA test even more accurate. A significance level of 5% (GraphPad Prism v6) was used.

Clinical trial

The study was previously submitted for evaluation by the Research Ethics Committee (CEP), as provided for by the current law, in particular Resolution No. 466 of December 12, 2012, of the National Health Council. The research protocol, under number 45818921.6.0000.5514, was approved by the CEP according to opinion 4,789,328.

On the first day of the study, the research participants who attended the laboratory received the Informed Consent Form (ICF), in two copies.

The research participants were informed by the main researcher about the procedures of the study, ethical and legal aspects, risks and benefits of the study procedure, medical

support, forms of reimbursement of the costs of participation and requested to sign the ICF in case of agreement and after full clarification of doubts.

The research participants received a copy of the informed consent, signed by themselves and by the researcher at the research institute, and the duplicate was filed in the study documentation,

The research participants were submitted to the clinical examination for the evaluation of the Criteria of Inclusion and Exclusion of the study.

The evaluation of the performance of use of each research participant was carried out by weighing the vials of investigational products at baseline and after home use, for each of the products, the in order to assess its correct use.

Research participants were instructed to discontinue use of any topical products on the face 48 hours before the start of the study. They also received instructions on timetables, filling in the use, types of assessments that were carried out, duration of the study and requirements required during the study period, such as:

1. Do not apply any other product in the experimental region;
2. Do not change cosmetic habits, including hygiene;
3. Do not perform exfoliation or other facial aesthetic treatments;
4. Do not expose yourself to prolonged intense sunlight and do not submit to artificial tanning beds;
5. Do not change diet habits;
6. Do not change hormonal treatment;
7. Do not change the medicated contraceptive method;
8. Not using medication described in the Exclusion Criteria;

Evaluation of the comedogenic and acnegenic potential in use

The evaluation of the comedogenic and acnegenic potential was carried out through a clinical evaluation at the beginning of the study and after 28 days of home use, whose objective was to verify the comedogenic and acnegenic potential, that is, the ability of the investigational product to develop or potentiate the appearance of blackheads (comedones) and lesions acne (papules, pustules and nodules).

The evaluation was carried out in the frontal region (forehead), malar (cheeks) and chin (chin) of the participants of the survey, by counting comedones (open and closed), papules, pustules and nodules using white fluorescent. To perform the count, the research participant was accommodated on a stretcher, and in the following regions were read: Frontal (right and left), malar (right and left) and chin of each region research participant.

From the lesion count, total comedones (open+closed) and acne values were obtained total (papules + pustules + nodules).

Suborbital swelling

Obtaining the images

The images were obtained, under controlled conditions, by the researcher at the beginning of the study, after 1 hour of application of the investigational product, after 14 and 28 days of home use.

The images were obtained using Canfield's VECTRA XT equipment, whose advanced technology system uses 6 digital cameras to capture and build a 3D model of the research participant's face.

According to the morphology of the face of each research participant, size and location of the region with suborbital swelling (bags), a region will be determined for analysis, determining the relative volume, defined as swelling intensity (SI).

Collagen Synthesis

Fluorescence spectroscopy

In this technique, the light from a xenon lamp passes through a monochromator, where the wavelength is selected, and with the aid of a bifurcated optical fiber it is directed to the skin. This light comes into contact with the skin and is reflected through the other guide of the optical fiber that passes through another monochromator and is finally directed to the photomultiplier cell.

Obtaining the spectra

The spectra were acquired on the hemiface (right or left) of the research participants at the beginning of the study and after 28 days of use of the investigational product. For this evaluation, excitation spectra were obtained under the following conditions:

a) Maximum excitation of tryptophan at 295 nm with emission at 345 nm;

b) Pepsin digestible collagen peak excitation at 340 nm with emission at 420 nm;

Through these spectra, the fluorescence intensity of each fluorophore (tryptophan and collagen) for each research participant was obtained.

Sebum measurement

In the frontal region (forehead) of the research participant, two areas measuring 2.5 cm x 4.0 cm were demarcated, called sites. The determination of the control site (without application of any products) and the site of application of the investigational product was randomized among the demarcated sites, as recorded in the correlation worksheet (Annex IV).

After acclimatization, baseline measurements (prior to the application of the investigational product) of skin oiliness were obtained at the demarcated sites. Then, the sites were cleaned with hydrophilic cotton moistened with 1 mL of water. The cotton was passed over the sites 3 times in the same direction, keeping the pressure and speed constant. Afterwards, 20mL of the investigational product were applied, massaging it homogeneously over the site with the aid of a disposable finger. The cleaning procedure was also performed at the control site, but without the application of products.

The research participants remained in the laboratory to perform the oiliness measurements after 1, 4, 8 and 12 hours of application of the investigational product.

Acquisition of measures

The measurement was performed by positioning the Sebumeter SM 810 Cassette over the center of the site, maintaining constant pressure for 30 seconds. Afterwards, the amount of sebaceous secretion collected from the skin is analyzed by the reading unit attached to the MPA 5-Sebumeter® 815 equipment (CKeletronics, Germany).

The working principle of the MPA 5 - Sebumeter 815® equipment is based on the direct photometric measurement of sebaceous secretion, not being influenced by humidity. The sebum secretion is collected on the translucent plastic tape of the Sebumeter SM 810 Cassette which becomes transparent, and the result is obtained by measuring the difference in light transmittance value across the tape, before and after fat impregnation. The amount of sebum secretion is given in mg/cm2.

Results and Discussion

Semi-quantification of melanin (Fontana-Masson)

Figure 1 represents the histological evaluation of melanic pigmentation by the Fontana-Masson technique in human skin fragments obtained from elective plastic surgery and incubated with the product evaluated.

As we can see, the skin fragment subjected to UV radiation (Figure 1, D-F) has a melanin density 1.51 times greater than the control fragment (Figure 1, A-C; $P<0.001$). However, it appears that the skin fragment incubated with the evaluated product and irradiated (Figure 1, G-I) presents a reduction of the melanic pigmentation located in the basal and thorny layer, when compared to the UV group (reduction of 81.53%; $P<0.001$ - Figure 2).

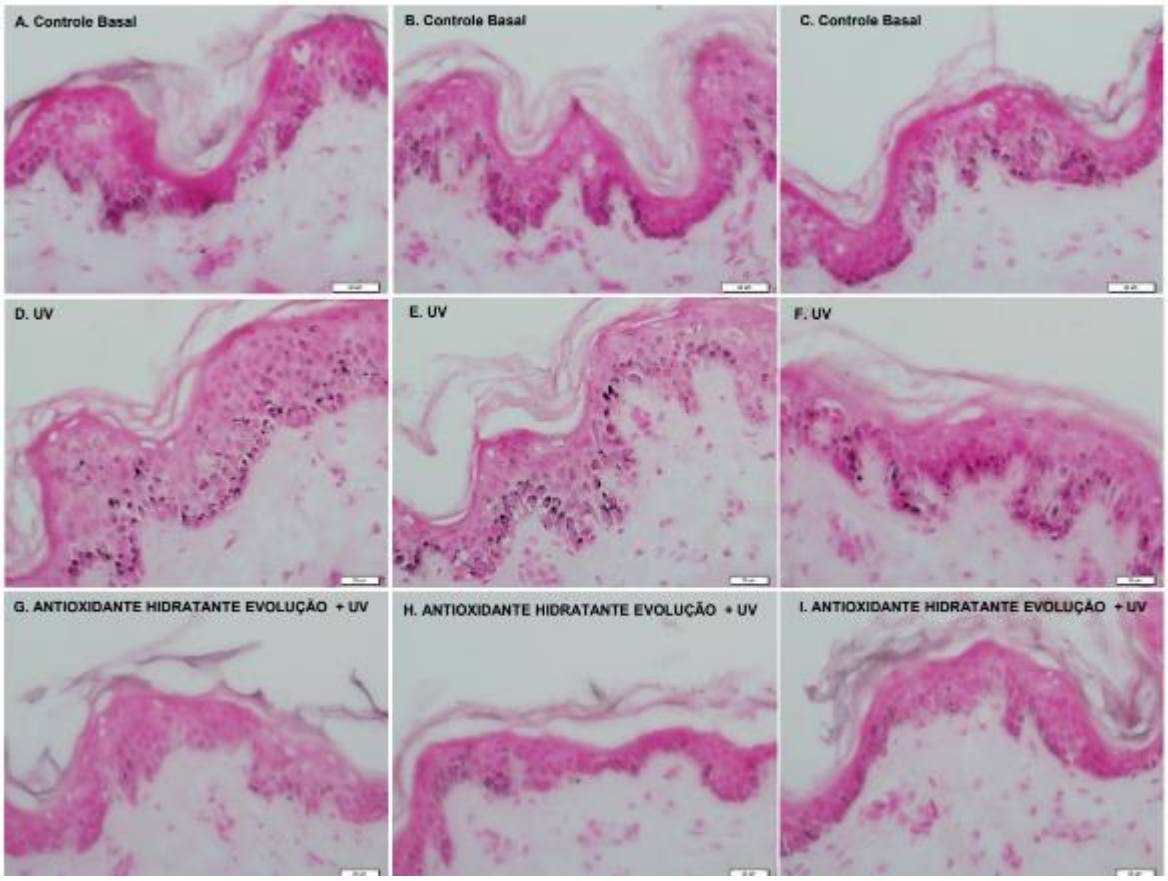
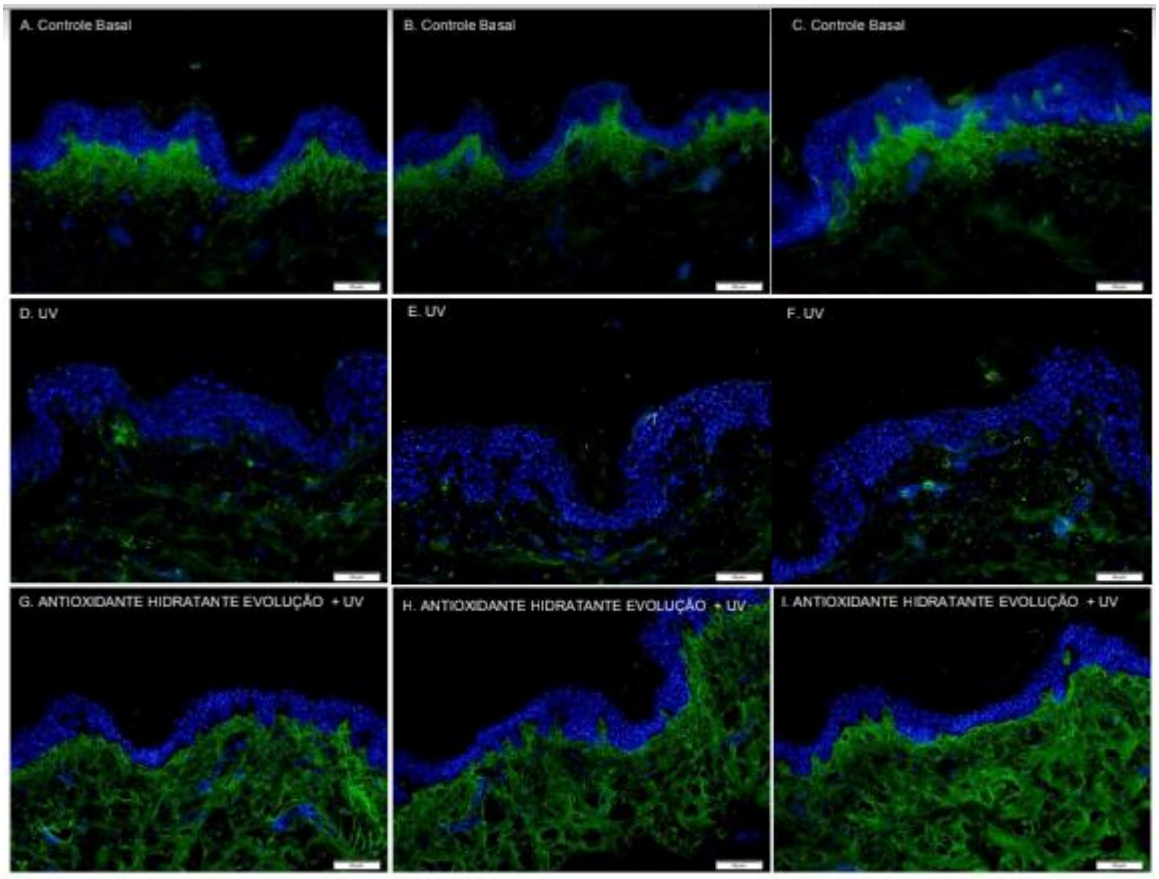


Figure 1. Histological evaluation of melanic pigmentation in ex vivo skin fragments treated with the evaluated product and subjected to UV radiation. (B.C). Basal Control. (D-F). UV; (G-I). Ex vivo skin fragments treated with the investigational product and subjected to UV radiation. Reference bar corresponds to 50 μm.

Production of type I procollagen

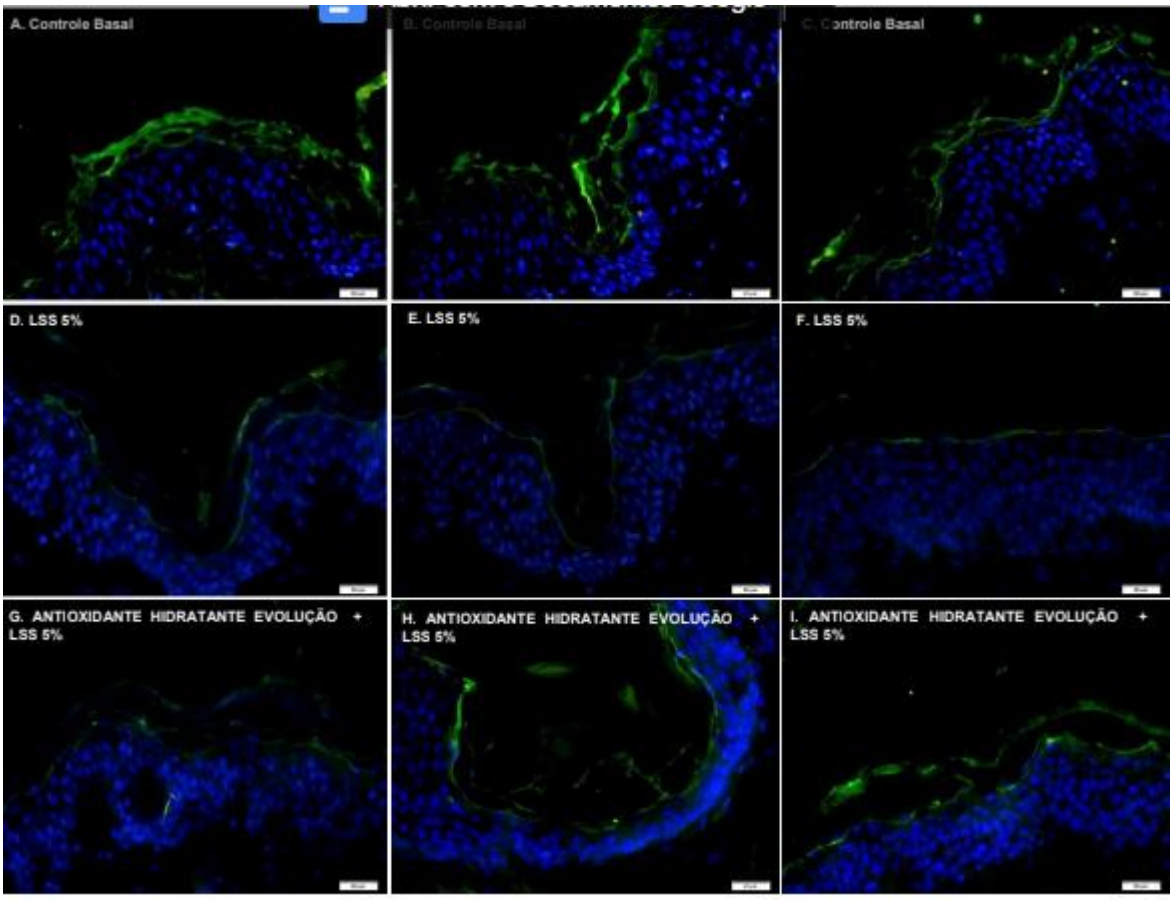
Figures 2 present the immunofluorescence images of the protein synthesis of type I pro-collagen. As we can see, through the semi-quantification of the images obtained (Figure 3), the exposure to UV radiation decreased by 49.64% to type I procollagen concentration compared to baseline control (P<0.001). On the other hand, treatment with the evaluated product was able to significantly increase the production of type I procollagen by 260.40% (P<0.001), when compared to the UV group.



Filaggrin production

Figures 3 show immunofluorescence images of filaggrin protein synthesis. As we can see, through the semi-quantification of the images obtained (Figure 3), breaking the barrier with LSS 5% decreased the production of filaggrin by 28.28% in relation to the basal control (P<0.001).

On the other hand, treatment with the evaluated product was able to significantly increase the production of filaggrin by 23.12% (P<0.05), when compared to the LSS 5% group.



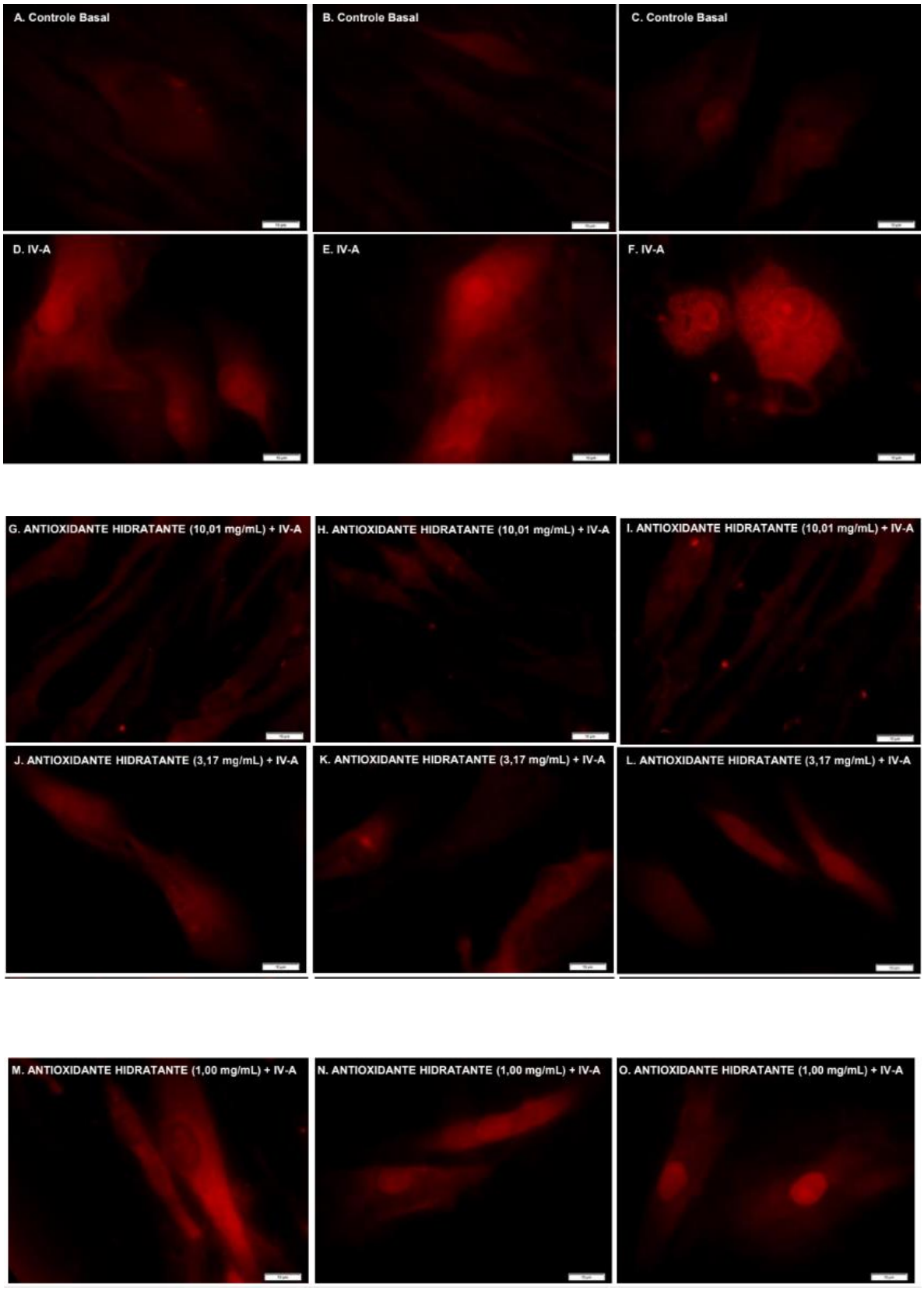
Semi-quantitation of mitochondrial ROS synthesis

Figures 4 represent the antioxidant effects of the evaluated product in human fibroblast culture exposed to the oxidative effects of IR-A radiation.

As expected, exposure to IV-A radiation produced a 3.54-fold increase ($P<0.001$) in mitochondrial ROS labeling/production (ROSmit; mitochondrial superoxide anion) when compared to the unexposed control, thus contributing to the process of installation of oxidative stress.

On the other hand, treatment with the evaluated product demonstrated a protective effect of cell cultures when compared to group IV-A, preventing the production of superoxide anion at all concentrations evaluated (10.01; 3.17 and 1.00 mg/mL).

As we can see, the treatment with the product evaluated at concentrations of 10.01; 3.17 and 1.00 mg/mL promoted reductions of 73.13%; 64.58% and 59.88%, respectively, in the production of mitochondrial superoxide anion (ROSmit), when compared to group IV-A ($P<0.001$).


A. Controle Basal
B. Controle Basal
C. Controle Basal
D. IV-A
E. IV-A
F. IV-A
G. ANTIOXIDANTE HIDRATANTE (10,01 mg/mL) + IV-A
H. ANTIOXIDANTE HIDRATANTE (10,01 mg/mL) + IV-A
I. ANTIOXIDANTE HIDRATANTE (10,01 mg/mL) + IV-A
J. ANTIOXIDANTE HIDRATANTE (3,17 mg/mL) + IV-A
K. ANTIOXIDANTE HIDRATANTE (3,17 mg/mL) + IV-A
L. ANTIOXIDANTE HIDRATANTE (3,17 mg/mL) + IV-A
M. ANTIOXIDANTE HIDRATANTE (1,00 mg/mL) + IV-A
N. ANTIOXIDANTE HIDRATANTE (1,00 mg/mL) + IV-A
O. ANTIOXIDANTE HIDRATANTE (1,00 mg/mL) + IV-A

Clinical trial

Corneometer for hydration measurement

The increase in skin hydration conferred by the investigational product was significantly higher ($P < 0.05$) after 1, 4, 8, 12 and 24 hours of application, when compared to the control.

It was also observed that 100% of research participants showed improvement in skin hydration after 1, 4 and 8 hours of application and 95% of research participants showed improvement in skin hydration after 12 and 24 hours of application.

Sebumeter for sebum measurement

In the control site, there was a significant reduction ($P < 0.05$) in the values of sebaceous secretion after 1 hour, indicating that cleaning the skin with hydrophilic cotton moistened with water reduced the skin oiliness for up to 1 hour, in relation to the initial condition. After 4 and 8 hours, there were no significant variations ($P > 0.05$) in the values of sebaceous secretion, indicating the maintenance of skin oiliness, in relation to the baseline condition. After 12 hours, there was a significant increase ($P < 0.05$) in the values of sebaceous secretion, indicating that there was a significant increase in skin oiliness, in relation to the initial condition.

At the site of application of the investigational product, a significant reduction ($P < 0.05$) was observed in the values of sebaceous secretion after 1 and 4 hours, indicating that the application of the investigational product reduced skin oiliness for up to 4 hours, compared to to the initial condition. After 8 and 12 hours of application of the investigational product, no significant variations ($P > 0.05$) in the values of sebaceous secretion were observed, indicating the maintenance of skin oiliness, in relation to the initial condition.

The investigational product provided a significant reduction in facial skin oiliness of 39.5% after 1 hour and 28.5% after 4 hours of application, in relation to the baseline condition. It was possible to observe that 100% and 95% of the research participants showed a reduction in skin oiliness after 1 and 4 hours of application, respectively.

Non-inflammatory and inflammatory lesions counting analysis

The total comedone count values obtained after 28 days of home use of the investigational product were compared to the values obtained in the initial evaluation, applying the paired Student t-test method, considering a confidence interval of 95%.

According to the result of the statistical analysis, listed in Annex VI, it was possible to verify that there was a significant reduction ($P= 0.0135$) in the number of total comedones after 28 days of home use, indicating that the investigational product can be clinically classified as “anti-comedogenic”, reduces lesions of comedones (open and closed).

The total acne count values obtained after 28 days of home use of the investigational product were compared to the values obtained in the initial evaluation, applying the paired Student t-test method, considering a 95% confidence interval.

According to the result of the statistical analysis, listed in Annex VI, it was possible to verify that there was no significant change (P=0.6641) in the total number of acne after 28 days of home use, indicating that the investigational product can be clinically classified as “non-acnegenic”, it does not induce or potentiate the appearance of acne (papules, pustules and nodules).

Suborbital swelling

The suborbital swelling intensity values, obtained at the beginning of the study, were statistically compared to the values obtained after 1 hour of application and after 14 and 28 days of use of the investigational product, using the t-Student, bimodal, paired test, considering a 95% confidence interval.

According to the results obtained, after 1 hour of application of the investigational product, there was a significant reduction ($P < 0.05$) in the intensity of suborbital swelling of 1.4%, reaching 1.8%, with 95% of the participants of the research showed a reduction in the intensity of suborbital swelling.

After 14 days of home use of the investigational product, there was a significant reduction ($P < 0.05$) in the intensity of suborbital swelling of 2.5%, reaching 3.0%, with 100% of the research participants showing a reduction in the intensity of the suborbital swelling.

After 28 days of home use of the investigational product, there was a significant reduction ($P < 0.05$) in the intensity of suborbital swelling from 2.7%, reaching 3.2%, with 100% of the research participants showing a reduction in the intensity of the suborbital swelling.

Collagen synthesis

The significance of the investigational product's effect on the increase in collagen synthesis was evaluated by applying the paired bimodal Student's t-test method, considering a 95% confidence interval, to the I420/I295 data obtained after 28, 56 and 84 days of treatment, in relation to the values obtained at the beginning of the study.

According to the results obtained in the statistical analysis, it was possible to observe that:

There was no significant increase ($P > 0.05$) in I340 / I295 values after 28 days of treatment, indicating that there was no significant increase in collagen synthesis in the facial skin.

There was a significant increase ($P < 0.05$) in the I340 / I295 values after 56 and 84 days of treatment, indicating that there was a significant increase in collagen synthesis in the facial skin.

The study was based on the hypothesis that the application of the investigational product, according to the way of use suggested, could increase collagen synthesis in the facial skin and evaluate the improvement of general aspects of the skin.

After 84 days of treatment:

93% of survey participants noticed a reduction in fine lines and wrinkles.

79% of survey participants noticed that their dark circles were less dark.

86% of survey participants noticed that the skin became more even (dark spots became smoother).

100% of the survey participants noticed that the skin became firmer and more strengthened.

Conclusion.

The proposed cosmetic product was safe and effective in the improvement of skin conditions and appearance by increasing collagen synthesis, resulting in firmer, less rough, softer and smoother skin.

The reduction of melanin and increased of filaggrin suggest a blemishes reduction and a more hydrated skin, which was shown by Corneometer® measurements.

In addition, the product was effective for application on the oily skin since it reduced the superficial sebum amount and can act in the control of skin hydrolipid balance. In the clinical evaluation the study participants also perceived the improvement of skin hydration and smoothness observed in the instrumental measurements.

Finally, the present study has an important contribution since it showed the benefits of a cosmetic product based on nanoencapsulated antioxidants using different and innovative evaluation methods.

Conflict of Interest Statement. None

References.

Examples:

1. Solano F, Briganti S, Picardo M, Ghanem G. Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. Pigment Cell Res 2006; 19: 550-571.
2. Hibbert SA, Watson RE, Gibbs NK, Costello P, Baldock C, Weiss AS, Griffiths CE, Sherratt MJ. A potential role for endogenous proteins as sacrificial sunscreens and antioxidants in human tissues. Redox Biol. 2015; 5:101-13.
3. Sklar LR, Almutawa F, Lim HW, Hamzavi I. Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: a review. Photochem Photobiol Sci. 2013; 12:54–64.
4. Baumann L. Skin ageing and its treatment. J Pathol. 2007; 211:241–51.
5. Langton AK, Sherratt MJ, Griffiths CEM, Watson REB. A new wrinkle on old skin: The role of elastic fibres in skin ageing. Vol. 32, International Journal of Cosmetic Science. Blackwell Publishing Ltd; 2010. p. 330–9.
6. Kalinin A, Marekov LN, Steinert PM. Assembly of the epidermal cornified cell envelope. Journal of Cell Science 2001; 114: 3069-70.
7. Ishida-Yamamoto A, Iizuka H. Structural organization of cornified cell envelopes and alterations in inherited skin disorders. Exp Dermatol 1998; 7:1-10.
8. Marekov LN, Steinert PM. Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope. J Biol Chem 1998; 273(28): 17763-70.