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TYROSINASE GENE EXPRESSION PROFILE EVALUATION AFTER TREATMENT WITH A COSMETIC PRODUCT ON CELL CULTURES

AUTHORS:
Dr. Clara Sinigaglia, Dr. Elena Bocchietto, Dr. Valentina Celada.

STUDY DESIGN

Purpose of the test

The test purpose is to point out and quantify the changing in the expression level of tyrosinase in human melanoma cells exposed to different concentrations of the tested substance respect to untreated cells.

Analysis is performed by RT-PCR since it is the most sensitive and reliable method for the detection and quantitation of nucleic acids levels. Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (ie, in real time) as opposed to the endpoint detection.

Assay procedures:

1. Cell model:

The experimental model here employed consists of a cell of human melanoma named MNT1. These are fibroblast-like cells able to produce melanin. Cells are cultured in MEM with 20% FBS, 10% AMV, glutamine, pyruvic acid and antibiotics.

2. Treatment and Exposure:

Cells are seeded in 6 wells plates for 24 hours. Fresh medium is added, supplemented with tested product. Sample was dissolved directly in the culture medium. Tested concentrations were decided after a preliminary cytotoxic test.

Untreated cells were used as negative control and cell treated with 5µg/ml of hydroquinone were used as positive control. Every sample has been tested in duplicate. After 72 hours of exposure, total RNA was purified from cells by RNA later protocol. After precipitation and centrifugation (30' 12000rpm, 4°C). RNA is resuspended in 20% steril water and its concentration determined spectrophotometrically. 300 ng of total RNA are retro-transcribed into cDNA using random primers at 37°C for 2h in a thermal cycler following manufacture's instruction (Applied Byosistems, Foster City, CA).

3. Analysis of gene expression profile by real time RT-PCR

Changes in gene expression profile are analyzed by "real time PCR" technology using a Syber-green based chemistry. Primers pair sequences for tyrosinase analysis were designed across intron –exón spanning regions and blasted against non redundant database (GENEBANK) to verify the unicity of amplified region across the genome. This signal increases in direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is posible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product correlates to the initial amount of target template.

EXPRESSION OF RESULTS

Modulation in gene expression profile due to the treatment with the sample were reported as a relative measure compared to the untreated sample. Data can be expressed according to two different criteria:

Modulation in gene expression profile due to the treatment with the sample were reported as a relative measure compared to the untreated sample. Data can be expressed according to two different criteria:

A) as fold changes compared to the control.
B) as a percentage compared to the control.

In any case results are expressed as a ratio and do not represent an absolute quantification. Before starting the data analysis, each value is normalized towards an housekeeping gene, as actin, whose expression is assumed to be unmodified by the treatment. For each sample, data are normalized according to the following formula:For each sample, data are normalized according to the following formula:

DCT = CT (target) - CT (normalized)

CT= thermal cycle

Therefore, you can obtain a CT normalized.

There are two different ways to express the results:
In the case a) data are expressed as fold change compared to the control, according to the following formula:

DDCT= DCT normalized treated- DCT normalized negative control.

This value is calculated for each sample to be quantitated; one of these samples should be chosen as the reference (control, untreated sample) for each comparison to be made. The comparative DDCT calculation involves finding the difference between each sample's DCT and the control's DCT. The last step in quantization is to transform these values to fold changes according to the formula:

Fold change = 2 - DDCt

In case b) data are reported as percentage of the control. In this situation, data should be transformed into normal scale according the following formula:

Fluorescence intensity arbitrary unit normalized = 2^-DCT and data are then expressed as percentage to the control.

Acceptance criteria of method

Per il controllo positivo(CP): fold change ≤ 0,5 o inibizione percentuale ≥ 50%
For positive control (CP): fold change ≤ 0,5 or percentage inhibition ≥ 50%

Results interpretation

A fold change ≤ 0.5 or percentage inhibition ≥ 50% compared to untreated cells is an index of a gene target modulation.
This value is compared to the untreated cell to give a judgement on the sample activity.

RESULTS AND CONCLUSIONS

Assay validity requirements

	Value	Limits	Result
CP: fold change **percentage reduction**	0,08 93,6%	≤ 0,5 ≥ 50%	Conforme Complies

Acceptance criteria of the assay (see § 2.3) comply, hence the assay is valid.

Results

Sample	72 hours % expression (DS)	72 hours Inhibition % compared with CN
Depigmenting Daily Serum Ref. ME0201 Lotto/Batch: K1 0,01 mg/ml	28,05 (± 4,51)	71,95
Depigmenting Daily Serum Ref. ME0201 Lotto/Batch: K1 0,002 mg/ml	72,89 (± 21,77)	27,10
Hydroquinone 5µg/ml	6,36 (± 0,99)	93,64
Negative control	100,00 (± 0,01)	0,00

Conclusions

The tested sample:

Depigmenting Daily Serum Ref. ME0201
Batch: K1

Is able to reduce (71.95%) the expression of messenger RNA codifying for the enzyme tyrosinase in melanoma cells culture, compared to untreated cell after 72 h exposure.

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IN VITRO EVALUATION OF MELANIN SYNTHESIS INHIBITION BY A COSMETIC PRODUCT ON CELL CULTURES

AUTHORS
Dr. Clara Sinigaglia, Dr. Elena Bocchietto, Dr. Valentina Celada.

STUDY DESIGN

Purpose of the test

The test purpose is to point out and quantify the inhibition in the melanin synthesis in human melanoma cells exposed for some days to different concentrations of the tested substance/product compared to untreated cells. The tested product is a topical product designed to inhibit the epidermis pigmentation and to retard the development of spots and lentigo. The cell model here used is a more complex model if compared to a simple biochemical assay on the tyrosinase enzymatic activity. It allows to investigate a inhibiting effect that may work at different cell metabolism levels beyond the enzyme synthesis, for example at the gene expression level, at the genetic transcription level or at the melanosome maturation level. Hydroquinone, a well-know depigmenting agent, has been used as a positive control.

Assay procedures:

Test systems:
The experimental model here employed consists of a cell line of human melanoma named MNT1.

Sample preparation
For the test, the sample was solubilized in water at 10mg/ml concentration and diluted in the culture medium MEM + 20% FBS with added glutamine, aminoacids and antibiotics (2000 UI/ml penicillin, 1000 UI/ml streptomycin, 2 µg/ml gentamycin). The positive control hydroquinone was tested at 5 µg/ml.

Treatment and exposure
Cells are seeded in 6 wells plates for 24 hours at 60000 cell/well. Fresh medium is added, supplemented with tested product. Untreated cells were used as negative control and cell treated
with 5 µg/ml of hydroquinone were used as positive control. Every sample has been tested in duplicate. After 72 hours of exposure, intracellular melanin and protein are quantified.

Melanin dosage
The cells are washed one time with phosphato buffer (PBS) and lysed by NaOH 0,1N. The cell lysate is heated at 60°C for 1 h for melanin solubilization. The quantification of intracellular melanin is made at 405nm with a colorimeter (tecan, Sunrise) equipped with a plate-reader. A standard curve with titrated melanin is set with a concentration range from 15 to 500 µg/ml.

Total protein assay
The protein dosage is tested according to Bradford method (Bradford M, 1975). Cells at the end of the treatment are washed twice with PBS at 4°C and lysated by treatment with 400 µl of purified
water at 4°C for 15'. Dye reagent are added to each sample. A standard curve with titrated albumin is set in the same conditions with 20, 40, 80 e 100 µg/m. 200 µl of each sample and of the standards and controls, each in three replica, are transferred to a 96 wells plate. Reading is made at 595 nm with a colorimeter (tecan, Sunrise) equipped with a plate-reader.

Data interpretation
To quantify proteins and melanin, a standard plot with albumin is designed and the simple concentrations are determined on the bases of their absorbance values using the interpolation plot formula. Mean values of every set of data are calculated, and for each sample the percentage of inhibition of melanin production is determined by comparison with the negative control:

$$\% \text{ inhibition of melanin} = 100 - \frac{\mu g/ml \text{ melanin} / \mu g/ml \text{ protein sample}}{\mu g/ml \text{ melanin}/ \mu g/ml \text{ protein negative control}} * 100$$

Documentation through images
At the end of the treatment, to point out the melanin granules inside the cells, images of the cells with a Leika phase contrast inverted microscope and a digital camera with a imaging software system ISCapture are collected.

Acceptance criteria of method
For positive control (CP): **% inhibition >20%**

RESULTS AND CONCLUSIONS

Assay validity requirements

	Value	Limits	Result
CP: inhibition:	38,87	>20%	Conforme/ Complies

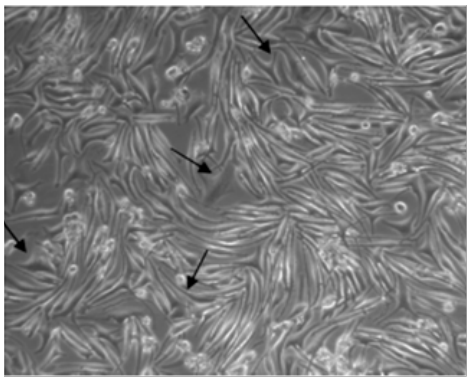
Acceptance criteria of the assay (see § 2.3) comply, hence the assay is valid.

Melanin dosage and images

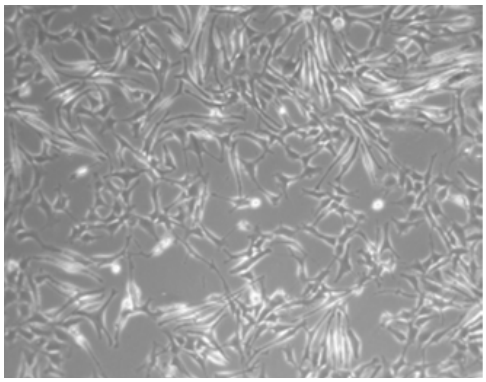
Sample	Melanin µ/ml	Protein µ/ml	µ melani / µ protein	% Melanin inhibition versus negative control
Depigmenting Daily Serum Ref. ME0201 Lotto/Batch: K1 0,01mg/ml	28,05 (± 4,51)	71,95	0,21	29,80
Depigmenting Daily Serum Ref. ME0201 Lotto/Batch: K1 0,002mg/ml	15,49	89,36	0,17	41,65
Hydroquinone 5µg/ml	10,31	56,75	0,18	38,87
Negative control	26,75	90,03	0,29	

Comment:
The sample is able to inhibit melanin synthesis at both tested concentrations.

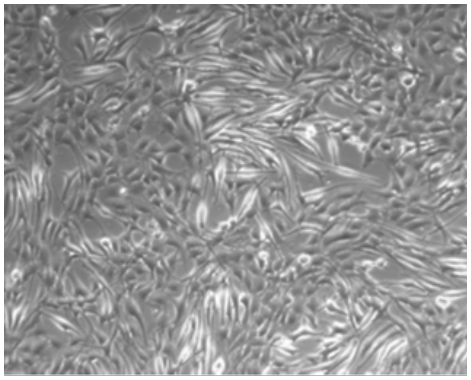
Microscopic images
Negative control: Untreated cells



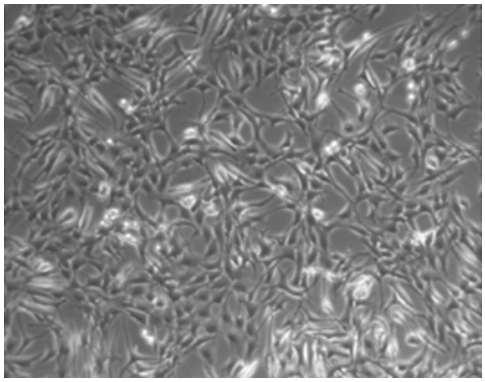
Melanin grains desposition is evident in the cytoplasm of the cell body and in the extensions. Positive control Hydroquinone



A evident reduction of the intracellular melanin grains is pointed out. Depigmenting Daily Serum Ref. ME0201 - Lotto/Batch: K1 0,01mg/ml



Depigmenting Daily Serum Ref. ME0201 - Lotto/Batch: K1 0,002mg/ml



Conclusions

The tested sample:

Depigmenting Daily Serum Ref. ME0201
Lotto/Batch: K1

Is effective in inhibiting melanin synthesis at both tested concentrations (-41.65%) compared to untreated cells.

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Analysis is performed by RT-PCR since it is the most sensitive and reliable method for the detection and quantitation of nucleic acids levels. Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (ie, in real time) as opposed to the endpoint detection.

Assay procedures:

1. Cell model:

The experimental model here employed consists of a cell of human melanoma named MNT1. These are fibroblast-like cells able to produce melanin. Cells are cultured in MEM with 20% FBS, 10% AMV, glutamine, pyruvic acid and antibiotics.

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Cells are seeded in 6 wells plates for 24 hours. Fresh medium is added, supplemented with tested product. Sample was dissolved directly in the culture medium. Tested concentrations were decided after a preliminary cytotoxic test.

Untreated cells were used as negative control and cell treated with 5µg/ml of hydroquinone were used as positive control. Every sample has been tested in duplicate. After 72 hours of exposure, total RNA was stabilized by RNA later protocol. After precipitation and centrifugation (30' 12000rpm, 4°C). RNA is suspended in 20%steril water and its concentration determined spectrophotometrically. 300 ng of total RNA are retro-transcribed into cDNA using random primers at 37°C for 2h in a termal cycler following manufacture's instruction (Applied Byosistems, Foster City, CA).

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EXPRESSION OF RESULTS

Modulation in gene expression profile due to the treatment with the sample were reported as a relative measure compared to the untreated sample. Data can be expressed according to two different criteria:

A) as fold changes compared to the control.
B) as a percentage compared to the control

In any case results are expressed as a ratio and do not represent an absolute quantification. Before starting the data analysis, each value is normalized towards an housekeeping gene, as actin, whose expression is assumed to be unmodified by the treatment.

For each sample, data are normalized according to the following formula:

DCT = CT (target) - CT (normalized)

CT= thermal cycle

Therefore, you can obtain a CT normalized.

There are two different ways to express the results:
In the case a) data are expressed as fold change compared to the control, according to the following formula:

DDCT= DCT normalized treated- DCT normalized negative control.

This value is calculated for each sample to be quantitated; one of these samples should be chosenas the reference (control, untreated sample) for each comparison to be made. The comparative DDCT calculation involves finding the difference between each sample's DCT and the control's DCT. The last step in quantization is to transform these values to fold changes according to the formula:

Fold change = 2 - DDCt

In case b) data are reported as percentage of the control. In this situation, data should be transformed into normal scale according the following formula:

Fluorescence intensity arbitrary unit normalized = 2^-DCT and data are then expressed as percentage to the control.

Acceptance criteria of method
For positive control (CP): fold change ≤ 0,5 or percentage inhibition ≥ 50%

Results interpretation
A fold change ≤ 0.5 or percentage inhibition ≥ 50% compared to untreated cells is an index of a gene target modulation.
This value is compared to the untreated cell to give a judgement on the sample activity.

RESULTS AND CONCLUSIONS

Assay validity requirements

	Value	Limits	Result
CP: fold change **percentage reduction**	0,08 93,6%	≤ 0,5 ≥ 50%	Conforme Complies

Acceptance criteria of the assay (see § 2.3) comply, hence the assay is valid.

Results

Sample	72 hours **% expression (DS)**	72 hours **Inhibition % compared with CN**
Depigmenting Night Cream Ref. ME0204 Lotto/Batch: K1 0,001 mg/ml	55,18 (± 18,03)	44,82
Depigmenting Night Cream Ref. ME0204 Lotto/Batch: K1 0,005 mg/ml	10,37 (± 4,22)	89,63
Hydroquinone 5µg/ml	6,36 (± 0,99)	93,64
Negative control	100,00 (± 0,01)	0,00

Conclusions

The tested sample:

Depigmenting Night Cream Ref. ME0204
Lotto/Batch: K1

Is able to reduce (89.6%) the expression of messenger RNA codifying for the enzyme tyrosinase in melanoma cells culture, compared to untreated cell after 72 h exposure.

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INNOVATION IN THE CONCEPT OF CONTROLLED CHEMICAL DERMABRASION: **MELINE® CAUCASIAN SKIN**, WITH DEPIGMENTING, REJUVENATING AND RESTORATIVE ACTIONS FOR PHOTOTYPES I-IV.

AUTHORS:
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SUMMARY

In the field of Cosmetic Dermatology and Esthetic Medicine, we have numerous therapeutic options at our disposal. The controlled chemical dermabrasion technique represents a major breakthrough, particularly for cases of severe hyperpigmentation, be it melanotic or hemato-melanotic, seen in the dermatology or esthetic medicine clinic. Here, we describe a new anti-age spot treatment with other associated therapeutic actions, including an innovation incorporating a transdermal therapy associated with the topical treatment.

MATERIALS AND METHODS

The controlled chemical dermabrasion treatment is formed from a combination of the products **MELINE® 00 PREP** and **MELINE® 01 CAUCASIAN SKIN** in association with **MELINE® 01 ID**, and is made up of a series of active ingredients at high concentrations, applied in the form of an occlusive emulsion over a time-frame ranging from 30 minutes to 1 hour. Various active ingredients are added to its basic composition in line with the predominant lesion to be treated. The innovation consists in the incorporation of **MELINE® 01 ID**, a product with active ingredients potentiating the treatment's effectiveness on mixed melasma, thanks to their transdermal application. For maintenance, **MELINE® 02 CAUCASIAN SKIN DAY** and NIGHT CREAMS were used. 98 cases of melasma-type acquired hyperpigmentation were treated, of which three left the study. Results were evaluated via visual inspection, measurement of the MASI index and before/after photographs. The study was carried out on patients with phototypes I-IV on the Fitzpatrick scale.

RESULTS

The evaluation evidenced an improvement in melasma. 4 weeks after the first application of **MELINE® CAUCASIAN SKIN**, 30.5% of patients were scored at 0. After 12 weeks, after the second application, 57.9% were scored at the same level, with a complete disappearance of the hyperpigmentation in cases with a MASI index of 3. Similarly, we were able to demonstrate a clear improvement in the signs of skin aging evaluated using the Glogau scale.

CONCLUSION

The **MELINE® CAUCASIAN SKIN** treatment represents a major innovation in the field of Dermo-cosmetics thanks to its results, producing significantly significant improvements in the treatment of cutaneous melasma.

KEYWORDS

Controlled Chemical Dermabrasion / **Dermoabrasión Química Controlada**

Pigmentation / **Pigmentación**

Rejuvenation / **Rejuvenecimiento**

Melasma / **Melasma**

INTRODUCTION

Cutaneous aging represents a set of changes and deteriorations affecting skin due to the passage of time. Dryness, fragility and epidermal atrophy appear, alongside a progressive increase in wrinkles. Skin photo-aging is due to an excess of sun exposure throughout life, starting in childhood. One of the most important signs of photo-aging is hyperpigmentation, associated with increased melanin within the skin due to melanocyte hyperactivity.

Melasma is more common in women: male cases represent around 10%. It is generally accepted that it is more common in photo-exposed areas and that it reduces during periods of time where the patient avoids ultra-violet rays. Acute exposure to ultra-violet radiation provokes one of the skin's most important adaptation mechanisms: the process of inflammation followed by tanning, as well as the induction of pigmentation in damage-exposed areas.

In melasma, chronic multi-factorial mechanisms trigger the progressive accumulation of pigment in photo-exposed areas. Exposure to ultra-violet radiation provokes progressive DNA damage, which is invisible to the naked eye. α-MSH is induced via the local production of proopiomelanocortin by keratinocytes in damaged skin, which also occurs following stimulation by ACTH (1,2). Exposure to ultra-violet radiation B (UVB, of wavelength 290-320nm) induces thymidine breakages in DNA leading to the formation of cyclobutane dimers, which, alongside complex molecular mechanisms activating p53, p21 and PCNA (proliferating cell nuclear antigen), can trigger pigment production. It has also been demonstrated that the chronic stimulation of α-MSH activates a mitogenic protein-kinase called p38 and this, alongside continuous chronic stimulation, as is the case in melasma, can trigger a chain reaction including endothelin-1, fibroblast growth factor β, nitric oxide and stem cell factor (SCF) (2, 3).

It is possible to determine that the epithelial damage caused by ultra-violet radiation provokes the paracrine secretion of endothelin 1, fibroblast growth factor, stem cell factor and alpha melanocyte stimulating hormone by keratinocytes. This, alongside the secretion of other fibroblast products, stimulates dendrite proliferation and cell division, as well as increased melanin activity and transfer, which normally occurs in the context of acute tanning, but at a slower rate (3, 4). The melanocyte's melanocortin 1 receptor (MC1R) is the binding site for alpha melanocyte stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH). It has been demonstrated that irradiation with UV light provokes DNA damage and increased α-MSH and ACTH synthesis, hence activation of the receptor leads to the stimulation of eumelanin synthesis, increased eumelanosomes and eumelanosome transfer into keratinocytes (2, 3, 5).

In melasma, various studies on histopathological findings have identified a genuine increase in the number of melanocytes in the basal layer of the epidermis, as well as an increase in the number of dendrites and pigment transfer into keratinocytes. A simultaneous melanocyte metabolism increase has also been identified (increased number and size of mitochondria, Golgi apparatus, rough endoplasmic reticulum and ribosomes) (6, 7).

The product **MELINE® CAUCASIAN SKIN** represents a new therapeutic option for the treatment of melasma, providing boosted synergistic activity thanks to controlled chemical dermo-abrasion combined with the power of intradermal therapy. The technique's innovation lies in its application as an occlusive emulsion, its composition of different depigmenting active ingredients acting at different stages of the pigmentation cascade, and its application time, as well as its ability to act on deeper melanin deposits for more satisfactory results. The combination of active ingredients in **MELINE® 01 ID** also offers a potent battery of anti-oxidants with depigmenting effects, thus completing the treatment by boosting its efficacy and significantly improving pigmented lesions with a dermal component. Its demonstrated ability to diffuse through the basal membrane guarantees free flow, thus providing a suitable therapeutic approach to both epidermal and dermal lesions.
The objective of this study is to evaluate the evolution, benefits and safety of **MELINE® CAUCASIAN SKIN** in the treatment of melasma on skin phototypes I-IV on the Fitzpatrick scale, assessing the integration of the mask application and the innovative ID product, this demonstrating the therapy's efficacy and safety.

This topical treatment is essentially based on the use of depigmenting agents and controlled chemical dermabrasion, offering beneficial results based on pre-determined protocols. The active ingredients operate via various mechanisms: tyrosinase activity inhibition, melanin production inhibition, selective melanocyte toxicity, non-selective melanogenesis inhibition and DNA synthesis inhibition in hyperactive melanocytes, free radical control, mineral chelation and reduction of epidermal melanin content.

Azelaic acid has selective effects on hyperactive melanocytes and abnormal melanocytes in skin and minimal effects on normal pigmentation. It is a week reversible competitive tyrosinase inhibitor and regulates melanocyte stimulation by free radicals (8). Conversely, kojic acid acts to inhibit the enzyme by chelating copper at its active site (9, 10), thus reducing the conversion of tyrosine to dopa and its subsequent conversion to dopaquinone.

Various retinoids have been successfully used in the treatment of melasma. Their mechanism of action is thought to involve the stimulation of keratinocyte turnover, reducing melanosome transfer and allowing for superior penetration of other active ingredients. They inhibit tyrosine transcription, interrupt melanin synthesis, inhibit proteins 1 and 2 (TRP-1 and TRP-2) associated with tyrosine and have been shown to reduce post-transcription levels of tyrosine and TRP-1 after exposure to UVB light UVB (11).

The action mechanism of arbutin is via the inhibition of tyrosine activity, 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and

melanosome maturation (12, 13). Tranexamic acid inhibits the binding of plasminogen to keratinocytes, which, in turn, reduces arachidonic acid formation and leads to reduced levels of prostaglandins and leukotrienes. This reduction in inflammatory mediators has an inhibitory effect on the tyrosine enzyme, which regulates the formation of melanin pigment. Plasmin is known to participate in the release of fibroblast growth factor (FGF), which, in turn, is an effective stimulator of melanocyte growth. Histological examination of improved cases shows reduced epidermal pigmentation and fewer mastocytes in the clarified skin (14).
Another fundamental aspect of melasma treatment is the control of free radicals, which contribute to the stimulation of melanin synthesis and increased pigment deposits, hence the regulation of these factors represents a positive contribution to effective therapies. A number of active ingredients participate in the control of free radicals, such as lipoic acid, copper tripeptide, glutathione and phytic acid. The latter ingredient also offers a significant enzyme-inhibiting effect since it also participates in copper chelation.

Glutathione is one of the compounds that inhibits melanin production. One mechanism is the inhibition of tyrosinase activity. This is a widely recognized function of thiol compounds in general (15, 16). Another mechanism in terms of its skin whitening effect is the activation of the pheomelanin pathway (16, 17, 18). Thirdly, it offers anti-oxidant activity (19). Glutathione is able to eliminate ROS generated in epidermal cells following exposure to radiation (20).

MATERIALS AND METHODS

This was a prospective randomized study lasting 12 weeks. Patients with a clinical diagnosis of melasma attended the clinic and were selected and recruited for the study. Pregnant or lactating women, patients with known hypersensitivity to the chemical active ingredients constituting the product, patients having been treated with isotretinoin over the last 6 months, patients with skin surface disorders, active infection or recent tanning were excluded.

The treatment process and its possible complications were explained to all patients. The informed consent form was authorized and signed by all patients prior to the application of any products. A detailed history was recorded, featuring data such as the appearance, duration and extent of the disease, family history, previous treatments and aggravating factors. A clinical examination was performed including visualization under natural light, with a magnifying glass, with a Wood lamp, photography and calculation of the severity index (MASI). The MASI score was calculated by a single specialist medical practitioner in terms of the darkness (D) and homogeneity (H) of the skin's appearance and the percentage surface area of the face affected (A), before using the following formula.

MASI = 0.3 (DF + HF) AF + 0.3 (DMR + HMR) AMR + 0.3 (DML + HML) AML + 0.1 (DC + HC) AC

Darkness was classed from 0 to 4, homogeneity from 0 to 4 and surface area from 0 to 6. The lesions were photographed with and without flash with a standard 20-pixel digital camera at 30cm distance with a resolution of approximately 2 Mb.

Taking into account everything mentioned previously, the diagnosis and follow-up were determined in line with the following clinical-pathological correlation:

Type	Normal Light	Wood Lamp
Epidermal	Light brown	Enhances color contrast
Dermal	Bluish gray	Does not enhance color contrast
Mixed	Dark brown	Enhances color contrast in some areas but not in others
Indeterminate	Bluish gray	Irregular results

Similarly, the Glogau scale was used to evaluate most signs of aging in the population studied.

Type	Description	Characteristics
Type 1 (mild)	No wrinkles	Slight pigmentation irregularities No keratoses Expression lines
Type 2 (moderate)	Dynamic wrinkles	Visible early lentigos Palpable but non-visible keratoses Early vertical wrinkles Somewhat fatigued appearance
Type 3 (advanced)	Static wrinkles	Dyschromias and telangiectasias Visible keratoses Static wrinkles present Fatigued appearance
Type 4 (severe)	Wrinkles, significant laxity	Grayish yellow skin Pre-malignant and malignant lesions Wrinkles and slack skin

Treatment protocol:
The study was run on 98 patients, 86 of them women and 12 of them men. Of the total 98 patients studied, 38 had mild melasma, 47 moderate melasma and 13 severe melasma, in terms of their MASI scores. Treatment was performed with **MELINE® 01 CAUCASIAN SKIN**, a semi-occlusive mask applied in a compact, uniform manner over the surface of the facial skin. Its baseline composition consists in variable concentrations of tranexamic acid, mandelic acid and kojic acid, as well as vitamin A derivatives, arbutin, histamine, mimetic peptides and chelating agents. After taking off the semi-occlusive mask, **MELINE® 01 ID** was applied directly to the lesions via mesotherapy using the point by point technique.

Mandelic acid offers favorable desquamating and melanin synthesis-inhibiting activity, and produces very little irritation. Kojic acid is a potent non-cytotoxic depigmenting agent, which inhibits tyrosinase via copper-chelating ions at the active sites for the inhibition of the tautomerization of the enzyme 5,6-dihydroxyindole-2-carboxylic acid, as well as inhibiting the conversion of o-quinones, norepinephrine and dopamine into their corresponding forms of melanin. The retinoids act via exfoliation and dispersal of melanin granules within keratinocytes, which promotes their elimination via an increased turnover of epidermal cells. Arbutin inhibits tyrosinase via 5,6-dihydroxyindole-2-carboxylic acid (DHICA) polymerase, as well as inhibiting the maturation of the melanosome.

For maintenance, **MELINE® 02 CAUCASIAN SKIN DAY** and **NIGHT** were used. Application was started from the fifth day after application of **MELINE® 01 CAUCASIAN SKIN** and **MELINE® 01 ID**, with the day cream used over the whole skin surface followed by sun protection, whereas the night cream was only indicated for application onto the pigmented lesions. The active ingredients in the maintenance products include tranexamic acid, mandelic acid, glutathione, chelating agents, phytic acid, vitamin A derivatives and oligopeptides.

The study consisted in achieving results after the application of the **MELINE® 01 CAUCASIAN SKIN** controlled chemical dermabrasion product over 2 sessions, combinied with the **MELINE® 01 ID**, within a month apart, where **MELINE® 02 CAUCASIAN SKIN** was applied, with results observed after both the first and the second session.

Contraindications for the technique
Active skin infections (particularly herpes simplex)
Known allergy to any of the active ingredients in **MELINE®**
Presence of purpuric lesions
Recent sun exposure
Photosensitivity and auto-immune disease
Imminent social commitments

Application technique for MELINE® product:
First, **MELINE® 00 PREP** is applied as an exfoliant and vehicle solution prior to treatment with **MELINE® 01 CAUCASIAN SKIN**, with a view to achieving a superior bioavailability of the various active ingredients.

The steps are as follows:
Skin preparation
1. Cleanse and de-grease the skin in-depth so as to eliminate any traces of make-up or sebum with the potential to interfere with the peel's results, before drying the skin.
2. Pour the contents of the **MELINE® 00 PREP** ampoule into a suitable container and apply an even layer over the whole face using a cotton swab and leave on to act for 3 minutes, depending on the professional's judgment and the skin's tolerance and pigmenta tion level.
3. Remove the product using plenty of water and dry.

Application of Controlled Chemical Dermabrasion Mask
1. Apply a medium thickness layer of **MELINE® 01 CAUCASIAN SKIN** as evenly as possible.
2. 15 minutes later, apply a second layer over the areas considered to have the most lesions, be this more marked hyperpigmenta tion or a higher concentration of fine expression lines.
3. Application time is as follows:
    a) First session: 30-45 minutes total.
    b) Second session: 45-60 minutes total (30 days after the first session)
4. Once the leave-on time is up, clean off the cream mask with a gentle lipid-rich soap.

After completely cleaning the mask off skin's surface, **MELINE® 01 ID** is applied in line with the protocol described below:
1. Disinfection of the area to be treated.
2. Point by point mesotherapy application to the areas with hypermelanosis, with 1 centimeter between points, injecting 0.02 milliliters of product into the dermis at each point.
3. Disinfect the skin to finish.

Post-treatment:
After 24 hours, pruritus and erythema appear, accompanied by a sensation of pulling skin. In cases where application time has been longer, mild edema may appear, lasting 1-3 days. After 48 hours, a light desquamation begins, lasting 3-4 days. During and beyond this period, patients should use **MELINE® 03 MOIST**, a highly concentrated hyaluronic acid serum to repair and hydrate skin, promoting its recovery. Similarly, with a view to protecting skin against ultra-violet radiation, the treatment should be accompanied by **MELINE® 04 B.B.**

From the 4th day following application of the controlled dermabrasion mask, application of **MELINE® 02 CAUCASIAN SKIN DAY** and **NIGHT** should be started at home, once a day.

Evaluation
This treatment was continued on all patients for 12 weeks, comprising two sessions of controlled dermo-abrasion 30 days apart, carried out on day 0 and day 30 respectively.

Initial result
During the study period, the MASI score was calculated at the beginning of the study (week 0), after 4 weeks and after 12 weeks. Photographs were taken at the beginning and end of the study. Detailed inspection and analysis were performed at week 0, week 4 and week 12. Initial results were evaluated in the fourth week.

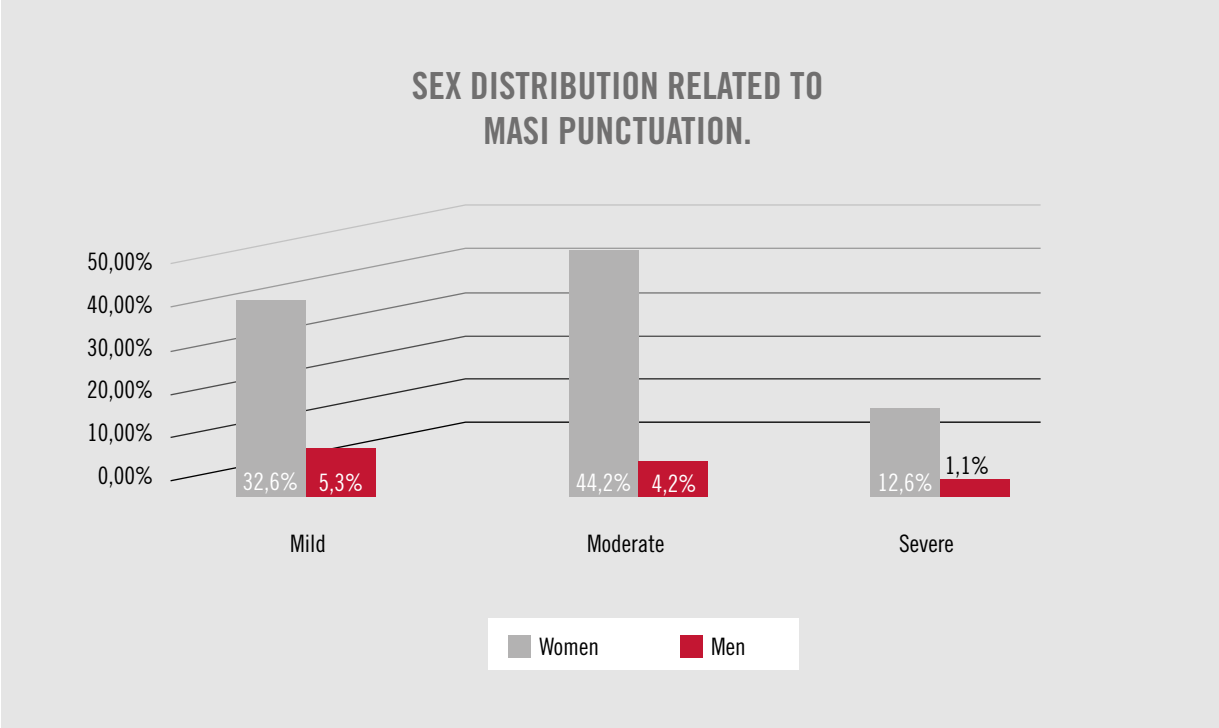
Secondary result
These results were evaluated after 12 weeks.

RESULTS

Of the total number of patients starting the treatment, 3 abandoned the therapy and did not return for assessments. Two of these were men and one was a woman. In line with the evaluation carried out on all patients in week 0, the total patients completing the study (total 95 patients) and undergoing the first **MELINE® 01 CAUCASIAN SKIN**, were distributed as follows:

MASI	Mild (1)	Moderate (2)	Severe (3)
Female	31 (32,6%)	42 (44,2%)	12 (12,6%)
Male	5 (5,3%)	4 (4,2%)	1 (1,1%)
Total	36 (37,9%)	46 (48,4%)	13 (13,7%)

Table 1. Gender distribution of MASI scores.

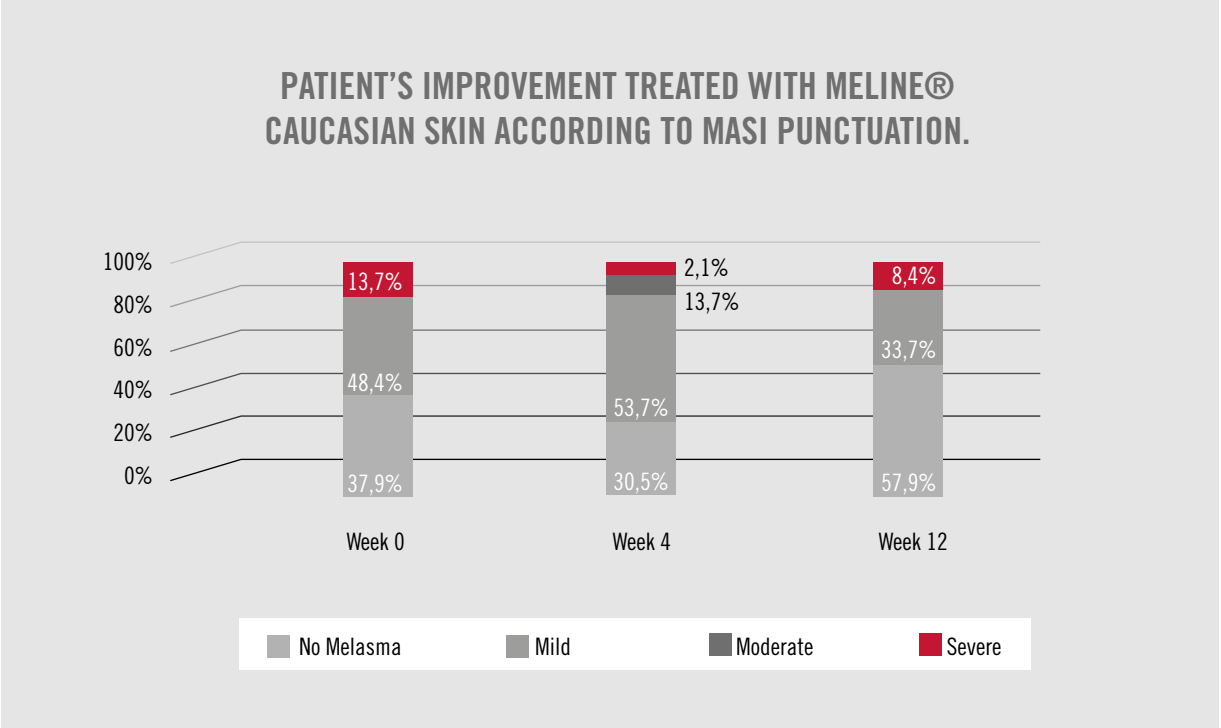


Patients were assessed at week 4 and 12, after the two applications of **MELINE® 01 CAUCASIAN SKIN**.

The MASI score results were distributed as follows:

MASI	Week 0	Week 4	Week 12
No Melasma (0)	0 (0%)	29 (30,5%)	55 (57,9%)
Mild (1)	36 (37,9%)	51 (53,7%)	32 (33,7%)
Moderate (2)	46 (48,4%)	13 (13,7%)	8 (8,4%)
Severe (3)	13 (13,7%)	2 (2,1%)	0 (0%)

Table 2. Improvement in patients treated with **MELINE® CAUCASIAN SKIN** in terms of MASI scores.



The results achieved demonstrate the improvement in a significant percentage of patients at week 4, evidenced by a disappearance of melasma in 30.5% of people treated, rising to more than half of the patients treated (57.9%) after 12 weeks.

Another important aspect to be taken into account is the fact that, at week 12, all cases of severe hyperpigmentation according to the MASI index had disappeared. Similarly, the percentage of moderate melasma fell from 48.4% of patients to just 8.4%. Although this was not the primary purpose of the study, signs of aging were also assessed at the beginning and end of the treatment. For this purpose, the Glogau scale was used and the following results were achieved:

GLOGAU	Week 0	Week 4	Week 12
Tipo I	0 (0%)	32 (33,7%)	57 (60%)
Tipo II	48 (50,5%)	45 (47,4%)	34 (35,8%)
Tipo III	41 (43,2%)	16 (16,8%)	4 (4,2%)
Tipo VI	6 (6,3%)	2 (2,1%)	0 (0%)

Table 3. Evaluation of improvement in signs of skin aging according to Glogau scale with **MELINE® 01 CAUCASIAN SKIN**.

The results achieved on skin aging signs with **MELINE® CAUCASIAN SKIN** show an excellent improvement, in which, at the beginning of the treatment, there were no patients classed as Type I, 50.5% were classed as Type II and 43.2% as Type III. After 12 weeks and completion of the treatment, 60% were classed as Type I on the Glogau scale and 35.8% as Type II, demonstrating that a large percentage of the patients gained significant improvements.

In terms of treatment tolerance, no unexpected effects or noteworthy complications were observed. The aforementioned erythema and desquamation were easily controlled using the planned protocol.

Analysis

The many existing techniques in the field of Dermo-cosmetics (chemical peels, dermabrasion, laser, intense pulsed light, etc.) have evolved rapidly in recent years, thanks to both an increasing level of interest on the part of dermatologists and the pharmaceuticals industry, and demand on the part of patients, who wish to improve their physical appearance.

All of the existing techniques offer excellent results if their indications are respected and patients are selected appropriately.

Within the category of peels, there are many options available on today's market. Traditionally, they tend to be divided into superficial, medium and deep. The most frequently used peels are superficial, based on alpha-hydroxy acids (50-70%) and salicylic acid (20-30%). Depending on its concentration, trichloroacetic acid can be applied as superficial (10-20%), medium (20-35%) or deep (35-50%).

Controlled chemical dermabrasion acting at medium depth is very effective in expert hands at the dermatology clinic.

The new controlled chemical dermabrasion product **MELINE® CAUCASIAN SKIN** studied here offers the innovation of combining different active ingredients at different and variable concentrations. It is applied as a compact, uniform, semi-occlusive emulsion. It offers profound effects on the epidermis and papillary dermis. The result of this product's combined active ingredient is a lightening of pigmentation, a reduction in the size of the pilo-sebaceous orifices, an attenuation of fine expression lines and an improvement in the texture and plumpness of the skin. The depigmenting agents block the enzyme tyrosinase, inhibiting the conversion of tyrosine into melanin. This mechanism is promoted by the facilitation of the agents' penetration thanks to the exfoliant action of the active ingredients in the formula. The exfoliant agents reduce the skin's pH and the proteases break bonds between the proteins, causing corneocytes to detach and the epidermis to desquamate. The semi-occlusion allows for superior penetration of the active substances. The results achieved allow us to confirm this treatment's polyvalence, both for pigmented lesions (particularly melasma, a condition resistant to known treatments to date), and for the treatment of skin aging.

The depigmenting effect achieved with **MELINE®** on melasma in two sessions is very difficult to achieve with other products available on the market up until now, which almost always cause a very significant rebound pigmentation effect. Although pigmentation disorders are known to have a high recurrence rate, result maintenance is ensured through the use of **MELINE® 02 CAUCASIAN SKIN** to inhibit every stage of melanin production, with no rebound effect.

Very promising results were achieved on skin aging, particularly considering the technique's ease of application and minimal risk. This excellent safety profile sets it apart from other medium-depth peels or laser resurfacing. In order to maintain the rejuvenating effects, an annual maintenance treatment is recommended.

This study has allowed us to confirm the favorable tolerance of **MELINE® CAUCASIAN SKIN** and its excellent safety profile on phototypes I-IV, with no complications seen in the 95 patients who completed the trial. In order to achieve these results, it is essential to determine the product's application time. Even after one week, clinical follow-up allowed us to observe a clear improvement. On the fourth day, patients can start applying **MELINE® 02 CAUCASIAN SKIN DAY** and **NIGHT**, adjusting application frequency in line with tolerance. After a month, the complete results can be seen, the patient's usual cosmetics can be re-introduced and the treatment can be re-applied.

Another advantage offered by **MELINE® CAUCASIAN SKIN** is the possibility of treating non-facial areas such as the neck and pre-sternal area. In the case of melano-hematic pigmentation, the product was applied to various areas with no complications. In order to confirm its efficacy on these areas, more cases and further studies will be necessary. Based on the results achieved using the various agents on various pathologies, we look for a common pattern indicated for all of them, with each of the active ingredients considered to be essential at ideal concentrations to achieve optimal therapeutic results.

VISIBLE RESULTS

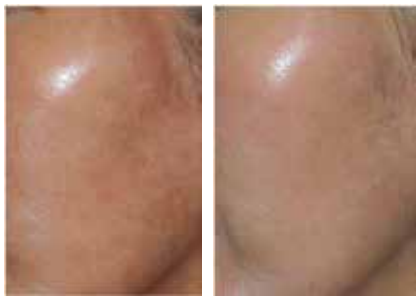


Figure 1.
Patient with melasma-type hyperpigmentation.

Figure 2.
Visible results achieved after 60 days' treatment.

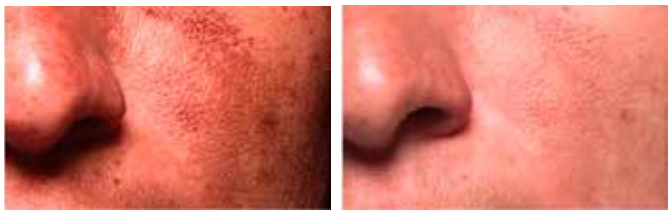


Figure 3.
Patient with melasma affecting the face.

Figure 4.
Visible results achieved after 60 days' treatment.

Figure 5.
Patient with pronounced skin aging, showing pigmented lesions and expression lines.

Figure 6.
Same patient treated with **MELINE® 01 PREP** and **MELINE® 02 PIGMENT HOME MASK** products after 60 days' treatment.

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Evaluation of Melasma reduction using the **MELINE®** protocol

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INTRODUCTION

The pigmentation system of normal skin is confined to the epidermis. The only cell capable of synthesizing melanin is the melanocyte. It is found in the basal layer of the epidermis and is neuroectodermal in origin. It constitutes up to 5% of epidermal cells. Keratinocytes obtain melanin from melanocytes, as well as providing the necessary micro-environment for their survival, proliferation, differentiation and migration through the production of various ligands that interact with melanocyte receptors. By way of its dendrites, each melanocyte interacts with around 36 keratinocytes in what is known as the "epidermal melanin unit."

Melasma or chloasma is a circumscribed, patchy and symmetrical form of acquired hypermelanosis. It affects photo-exposed areas. The various studies into the histopathological findings in melasma have demonstrated a genuine increase in the number of melanocytes in the basal layer, as well as the number of dendrites and the degree of pigment transfer into keratinocytes. These studies have also identified increased melanocyte metabolism (increased number and size of mitochondria, Golgi apparatus, rough endoplasmic reticulum and ribosomes). (Sánchez NP, Pathak MA, Sato S, Fitzpatrick TB, Sánchez JL, Mihm MC. "Melasma: A clinical, light microscopic, ultrastructural and immunofluorescence study". J Am Acad Dermatol 1981; 4: 698-710. Kang WH, Yoon KH, Lee ES, Kim J, Lee KB, Yim H y cols. "Melasma: Histopathological characteristics in 56 Korean patients". J Am Acad Dermatol 2002; 146: 228-237.).

Clinically speaking, the condition manifests itself in the form of irregular brown macules of varying degrees of darkness, over the forehead, temples, top lip and cheeks. It is more common in women, particularly those of Hispanic (Victor FC, Gelber J, Rao B. Melasma: a review. J Cutan Med Surg. 2004;8:97-102.) and Oriental ethnicity, living in regions with strong sun exposure.

i. Objective.
A clinical study was planned to demonstrate the effectiveness of a topical treatment based on depigmenting active ingredients, in conjunction with the co-adjuvant use of a semi-occlusive mask peel in patients with Melasma to assess their improvement and possible side effects.

ii. Sample.
22 volunteers were recruited consecutively between 01-02-2016 and 15-02-2016. A melasma frequency of 1% was assigned to the Spanish population (p1%, q99%, z1.96), minimum n = 14.

Exclusion criteria:
- Diagnosis of systemic pathology (auto-immune diseases)
- Chronic use of medication or treatments
- Pregnancy/breastfeeding
- Minors
- Specific contraindications regarding product to be applied (e.g. allergy)
- Contraindications regarding the procedure, e.g.:
· Active skin infections (especially herpes simplex)
· Presence of lesions in application area (e.g. purpura)
· Recent sun exposure
· Photosensitivity
· Immediate social engagements

-MASI score less than 16 or greater than 31
-High carotene intake

Inclusion criteria:
- Presence of non exclusion criteria
- Agreement not to undergo any other aesthetic treatments (from 30 days prior to the first session until the end of follow-up).
- Agreement not to make any dietary changes.
- Signature of informed consent form.

MATERIAL AND METHODS

i. Application Protocol

Application technique for topical products

The use of two topically applied products is indicated: **MELINE® CAUCASIAN SKIN DAY**, which is a solution to be applied in the morning to the pigmented facial areas only; and **MELINE® CAUCASIAN SKIN NIGHT**, which is a cream to be applied in the evening to the whole face.

MELINE® CAUCASIAN SKIN DAY contains ingredients that regulate and control melanocyte activity, working to block melanin synthesis. This is achieved through the combined actions of kojic acid, phytic acid and tranexamic acid, which inhibit the activity of the enzyme tyrosinase, as well as working to control free radical activity, thus reducing a powerful stimulus for pigment synthesis. The product also contains lactobionic acid, which helps maintain cutaneous hydration levels and boosts the skin's barrier function.

MELINE® CAUCASIAN SKIN NIGHT reinforces the treatment, firstly by inhibiting melanin synthesis and deposition thanks to its depigmenting active ingredients, and secondly through its continuous controlled exfoliation activity, with a view to boosting epidermal turnover and thus reducing the number of affected keratinocytes, to promote general skin tone uniformity over the entire skin area treated.

Semi-occlusive mask peel application technique
First of all, a pre-peel solution (**MELINE® 00 PREP**) is applied, whose primary active ingredients are lactobionic acid (10%), lactic acid (10%) and ferulic acid (10%), as a vehicle solution and exfoliant agent, with a view to achieving superior penetration of the mask's main active ingredients.

1. Procedure
· Cleanse and de-grease skin in-depth with a view to eliminating any make-up residues or sebum with the potential to interfere with the peel's final result.
· Dry the skin.
· Pour the contents of the solution ampoule into a suitable container and apply a uniform layer over the whole face using a brush.
· Leave on to act for 3 minutes.

2. Application of Controlled Chemical Dermo-abrasion Mask
· Apply a uniform medium thickness layer – as even as possible – using a brush.
· Wait for 15 minutes.
· Apply a second layer to areas of skin with more lesions. The first session lasts 30-45 minutes in total.

3. Cleanse and eliminate the cream mask using a gentle lipid-rich soap.

Post-treatment care
24 hours after the treatment, pruritus, erythema and a pulling sensation appear. In cases where the product has been left on for longer, slight swelling may appear, lasting 1-3 days. 48 hours after the treatment, fine desquamation appears, lasting 3-4 days. During and after this time-frame, a moisturizing product (**MELINE® 03 MOIST**) should be used with a view to repairing and hydrating skin, aiding in its recovery. Similarly, and with a view to protecting skin against ultraviolet radiation, the treatment should be accompanied by SPF 30+ sun protection, **MELINE® 04 B.B.**

From day 4 following application of the controlled dermabrasion product, the patient starts to apply the at-home treatment products, which are used once a day.

iii. Measurements

MELASMA AREA AND SEVERITY INDEX (MASI)		
Forehead	F	30%
Right malar	RM	30%
Left malar	LM	30%
Chin	C	10%

% area affected (A), score from 0 to 6
Pigment darkness (D), score from 0 to 4
Color homogeneity (H), score from 0 to 4

MASI Score = 0,3 (DF + HF) AF
\+ 0,3 (DMR + HMR) AMR
\+ 0,3 (DML + HML) AML
\+ 0,1 (DC + HC) AC

A:
0: not involved
1: <10%
2: 10-29%
3: 30-49%
4: 50-69%
5: 70-89%
6: 90-100%

D y H:
0: absent
1: mild
2: moderate
3: marked
4: maximum

Range: 0-48

Numerical value	0	1	2	3	4	5	6
Pigment darkness (D) scale from 0 to 4	Absent	Mild	Moderate	Marked	Very marked		
Pigment homogeneity (H) scale from 0 to 4	No pigment	Specks	Patches less than 2 cm	Patches greater than 2 cm	Homogeneous		
Area involved	None	Less than 10%	11-29%	30-49%	50-69%	70-89%	90-100%

Severity	Mild	Moderate	Severe
Total score	less than or = 15	between 16 and 31	greater than or = 32

The Melasma Area and Severity Index (MASI) was created by Kimbrough and Green in 2004, in an attempt to standardize the subjective assessment of melasma. It is calculated by dividing the face into four areas: forehead (f), right malar region (rm), left malar region (lm) and chin (c).

Kimbrough-Green CK, Griffiths CEM, Finkel LJ, Hamilton TA, Bulengo-Ransby SM, Ellis CN, et al. Topical retinoic acid (tretinoin) for melasma in black patients. Arch Dermatol 2004;130:727-33.

Skin scanner (VisioScan or similar)
This type of technology provides parameters, which, in conjunction with standard inspection of the patient, form the basis of the subjective assessment of the condition. The scanner provides: standard photographs, polarized light photographs and colorimetric photographs (melanin and hemoglobin).

Colorimetry
Due to skin's structure, composed of several translucent layers, measurement of its color is completely different from color measurements of other materials. Skin modifies light. Environmental light and the light emitted by measurement devices penetrate skin to different depths and are not absorbed or reflected in the same way. The primary components influencing skin's color are: melanin (pigmentation), which appears grey or brown in the superficial layers of skin, and hemoglobin (the red component of blood), which appears red or sometimes blue and is found in skin's deep layers. If the light emitted by the shadow penetrates skin deeply, the red component of skin's color will be overestimated. These physical properties of skin make measuring its color a very complex enterprise. The different light sources of the measurement devices, the different pressures on skin's surface and the different measurement areas, in addition to skin's unique properties make it impossible to determine skin's true color using traditional measurement techniques and their results are not fully comparable.

To measure skin's color, we will use the Skin-Colorimeter® Probe CL 400, Courage -Khazaka, GmBH. The raw data from the probe are corrected using a special color matrix, bringing them even closer to normal values. The measured skin color is expressed as an x-y-z value, and can be converted to a related value (L*a*b).

L* regards the white-black axis, shine, and a* and b* are the coordinates in the color space. a* expresses erythema values on the red-green axis and b* shows the skin's position on the blue-yellow axis. The L* value (shine) is inversely proportional to pigmentation. L* is related to pigmentation. The a* value is proportional to redness (erythema/microcirculation). In addition, the "Individual Typology Angle" ITA is automatically calculated (the classification of an individual's skin color). The ITA° formula is: [Arctangent ((L*-50)/b*)]180/∏. Using the ITA° data, it is possible to classify skin into different types:

ITA° >55°: "very fair"
55° >ITA°> 41°: "fair"
41° >ITA°> 28°: "intermediate"
28° >ITA°> 10°: "dark"

Colorimeter technical details
Skin-Colorimeter® CL 400
Dimensions: 13 cm
Measurement area nucleus: Ø 5
Area illuminated: approx. 17 mm Ø
Cable length: approx. 1.3 m
Weight: 85 g
Measurement technique: reflection
Light: 8 white LEDs in circular arrangement
Wavelength range of light emitted: 440-670 nm
Units: xyz
RGB, L*a*b* index values (due to skin's unique structure and the special light source, the values do not correspond exactly to ISO standards and are thus expressed as index values). Calibration to skin colors using a special correction matrix.
Relative error: ± 5 %

Subjective patient scale
This scale is to be used by the PATIENT to describe the IMPROVEMENT seen on the face: it comprises 5 categories.

Score	Guide
5	Spectacular improvement
4	Significant improvement
3	Moderate improvement
2	Slight improvement
1	No improvement

Assessor's subjective scale

The subjective scale used by the ASSESSOR to describe the patient's skin's condition will comprise 5 categories (although intermediate scores such a 2-3 or 4-5 may also be used if considered appropriate by the assessor):

Score	Guide
5	Very good
4	Good
3	Fair
2	Poor
1	Very poor

Statistical processing

- A single database will be generated, in which the first row corresponds to the number of variables and each subsequent row repre sents the data for each patient in the study.
- The final database will be revised using SPSS software or similar.
- In line with traditional practice, for continuous quantitative variables, we have used the mean as the central tendency index and the standard deviation as the dispersion index.
- Statistical significance (p): 0.05
- CI: 95%
- Mean comparison using Student Test: paired samples, 2-tailed test.

RESULTS

Initial sample: 22 subjects
Lost to follow-up: 1. Not convenient to attend follow-up. No adverse effects.
Final total sample (n): 21

1. MASI scale variable

	Before MASI scores	**After MASI scores**
Mean	25.50	12.79
Std. Dev. (SD)	7.65	6.17
Std. Err. of Mn. (SEM)	2.05	1.65
P:	<0.0001	
CI95 Diff.:	12.71 (11.00 - 14.43).	
T: 16.0047		
Df: 13		
Std. err. diff: 0.794		

2. Colorimetry variables

3. Variable "L*"

P value. Two-tailed P value: 0.0012
Confidence interval: mean "Group One" – "Group Two" = -356.57 (CI95% -544.32 to -168.82)

	Group One	Group Two
Mean	6030.71	6387.29
SD	251.15	236.59
SEM	67.12	63.23

Variable "a"
P value. Two-tailed P value: 0.2898.
Confidence interval: mean of "Group One" – "Group Two" = 65.07 (CI95% confidence interval of this difference -62.31 to 192.46

	Group One	Group Two
Mean	1513.29	1448.21
SD	220.47	130.93
SEM	58.92	34.99

Variable "ItA".
Individual Typology Angle (ITA), for the classification of an individual's skin color. The ITA° formula is: [Arctangent ((L*-50)/b*)]180/∏.

P value. Two-tailed P value = 0.0011.
Confidence interval: mean "Group One" - "Group Two" = -11.00 (CI95% -16.70 to -5.30)

	Group One	Group Two
Mean	38.00	49.00
SD	7.95	6.25
SEM	2.13	1.67

DISCUSSION

The evaluation of the results of any treatment aimed at offering a solution to a condition or pathology that is primarily aesthetic in nature must take into account both statistical significance and clinical significance. That is, a good esthetic treatment must provide results that are significant, measurable and repeatable, as well as allowing the patient to perceive these differences. If there are no objectively measurable differences before and after the treatment, or if the patient does not perceive these changes, the treatment in question is bound to fail.

The severity of melasma can be clinically determined according to the skin surface affected, its color, the homogeneity of the lesions and the time over which it has developed. It is classified as mild, moderate or severe. For the quantitative assessment of melasma severity, the Melasma Area and Severity Index (MASI) is used, a clinimetric method allowing for greater precision in the determination of the disorder's severity in a more systematic manner.

In melasma treatment, the general objective is to lighten the intensity of the hyperpigmentation and reduce the affected area. The specific objectives are: reducing hyperpigmentation to the patient's satisfaction, both in terms of its severity and its spread; avoiding recurrence; improving quality of life; educating the patient to avoid risk factors and carrying out a more in-depth assessment of each patient, looking for endogenous factors causing the recurrence of the lesions with the potential to be modified. One of the main factors in achieving a satisfactory response to treatment is compliance with same. In this vein, it is possible to achieve compliance rates close to 90% when there is a favorable doctor-patient relationship and effective therapy is given leading to a high degree of satisfaction. Patient education is the key to achieving this. During the process of educating the patient, it is important to emphasize certain aspects, such as the chronic nature of the condition, the need for lifestyle and occupational changes, modifications to clothing and the need to avoid sun exposure, even on cloudy days. If the doctor does not manage to convince the patient to modify his/her habits mentioned above, the patient will not fulfil the necessary preventive measures, leading to a failure of therapy, chronic persistence of the condition, lesion recurrence, the seeking of multiple treatments and, by consequence, forms of melasma that are difficult to treat.

When evaluating the results of a melasma treatment, there are 3 areas or variables that must be carefully considered: melanin load, load of other pigments and clinical impact. In this paper, these three areas are represented by the variables: "MASI", "L*", "ITA" and "a".

First of all, it is very important to consider the MASI variable.

This is the main variable used in this paper. It is a validated, globally accepted scale, which is agreed upon by the entire medical community.

It takes into account the extent of the lesions, the heterogeneity of the affected areas and the pigment load. It generates very high statistical significance (<0.0001), with a difference between the means of 12.71 points. Given that the MASI variable is made up of a 48-point scale, this difference could be interpreted as an improvement well above 20%.
Secondly, we have the results for the L* variable, which are consistent with those for the MASI variable. The greater the L* value, the less pigmented the skin. The pre-treatment L* value is 6030.71 while the post-treatment value is 6387.29. Once again, the difference is very significant. P value: 0.0012.

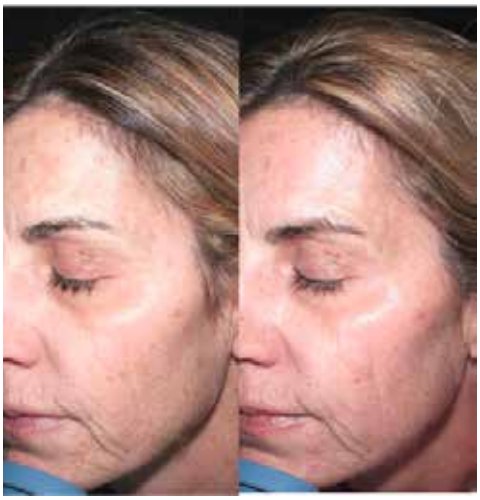
Lastly, the results for the "ITA" variable are in concordance with the MASI and L* values, confirming the correlation between the different variables measured. The greater the ITA value, the lighter the skin. We move from a pre-treatment ITA value of 48.00 to a post-treatment ITA value of 49.00. According to the ITA classification, this represents a shift from "intermediate" skin to "fair" skin. Once again, this difference is very significant. P value: 0.0011.

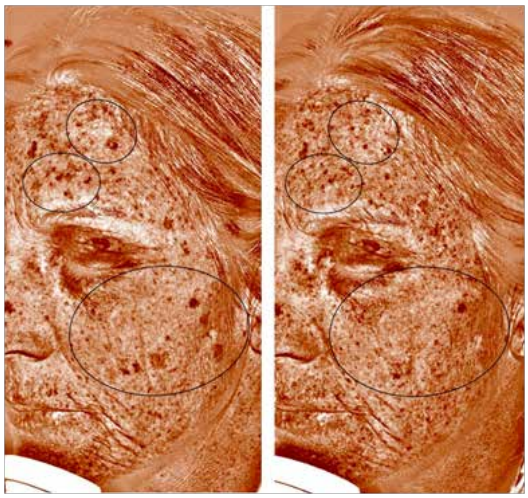
On the other hand, it is very important to take into account variations in skin's other pigments, which may occur concomitantly and mask the results. Traditionally, human skin color is attributed to 4 pigments: melanin, oxyhemoglobin, deoxyhemoglobin and carotenoids. In this paper, the study design takes into account any excess of or change in carotene intake (inclusion and exclusion criteria). Nonetheless, hemoglobin was not accounted for. That is why variable "a" is so important, which measures changes in the red spectrum. Should there be significant changes in this variable, the changes observed con be attributable to optical effects or changes in pigments other than melanin. However, variable "a" is stable. There is no significant variation in the p value, as expected.

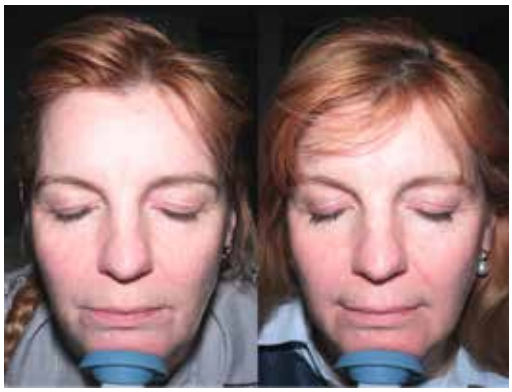
The combined analysis of these 4 variables reveals positive, significant and coherent changes in all 3 variables related to the reduction in the melanin load, in the absence of changes in the hemoglobin load. The results are highly consistent. With regard to the analysis of the different ingredients used in the treatment of this condition, it is important to consider the fact that there are different ways of classifying depigmenting agents. They can be grouped by chemical origin into: phenolic and non-phenolic agents; by their action mechanism into: tyrosinase inhibitors (hydroquinone, mequinol, kojic acid, azelaic acid, vitamin B6, licorice, arbutin); melanin synthesis inhibitors (ascorbic acid, glutathione); non-selective melanogenesis inhibitors (indomethacin, corticosteroids), melanocyte selective toxicity inducers (acetyl-cisteaminylphenol, N-acetylcysteine, isopropylcatechol, mercurial agents), agents promoting the absorption of the depigmenting ingredients (retinoic acid, alpha hydroxy acids). In line with their galenic formulation, topical depigmenting agents are found as monotherapies (single agents) or combination therapies (two or three depigmenting agents combined), with a view to boosting the depigmenting effect while minimizing adverse events.

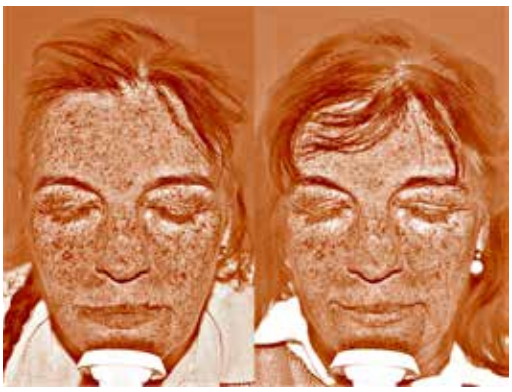
Generally speaking and in line with the medical consensus, melasma treatment is divided into two phases: the intensive phase and the maintenance phase. The intensive phase is thought to achieve satisfactory subjective and objective results within eight weeks, achieving a 50% reduction on the MASI scale with respect to the patient's baseline, followed by a six-month maintenance phase during which it is hoped that further reduction will be achieved. This paper specifically evaluates the intensive therapy phase. The activity of the ingredients applied daily represents a specific treatment for melasma control and must always be accompanied by a broad spectrum sun protection product with an SPF of at least 30 plus a synthetic, fragrance-free and non-abrasive skin cleanser.

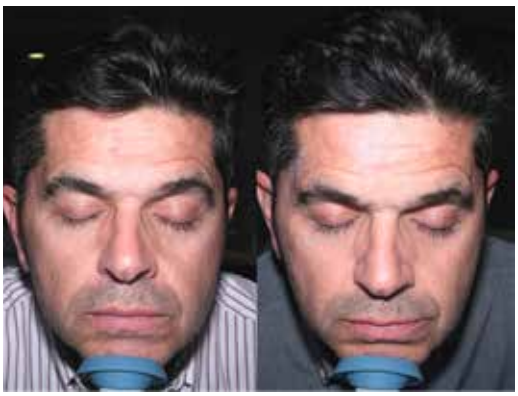
VISIBLE RESULTS

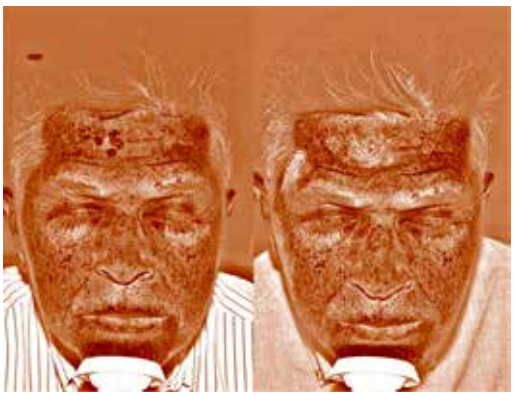


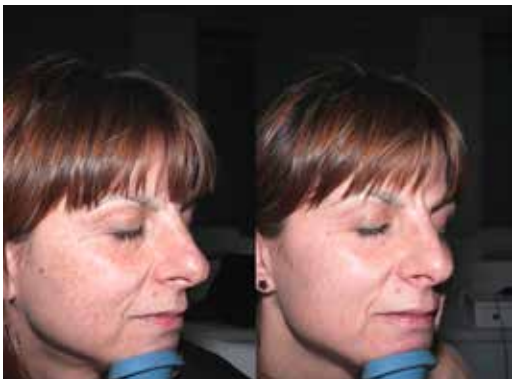












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AN EVALUATION OF THE DEPIGMENTING EFFICACY OF **MELINE® 01 CAUCASIAN SKIN.**

AUTHORS:
Dr. Cisneros Vela, José Luís.

INTRODUCTION

Recent years have seen rapid developments in the dermocosmetics sector, moving from so-called invasive techniques towards non-invasive techniques, which have become the established choice for both doctors and patients.

The conventional view on peelings is that they involve chemical substances, which enable variable levels of exfoliation once applied to a patient's skin, whilst also stimulating the dermis. Another use involves complementing other procedures in the form of daily skin treatments or adjuvant therapy for some diseases, as well as pre-malignant or benign lesions, such as keratosis among others.

They now represent a basic treatment for improving photoageing, acne, and hyperpigmentations. Using home use maintenance cream to complement the treatment always helps achieve definitive results.

DERMATOPATHOLOGICAL ASPECTS

Skin pigmentation changes are one of the most common reasons for a dermatological consultation, given that they represent a significant aesthetic problem, even though they are usually symptom-free and unimportant from a medical standpoint. Along with haemoglobin in blood vessels and carotenoids in the connective tissue, skin colour mainly derives from its pigment, melanin.

Melanin pigment synthesises from tyrosine inside melanocyte cells found in the basal layer of the epidermis. After formation, the melanocyte secretes the melanin as it migrates towards the surface, which progressively oxidises, taking on a darker tone.

Melanin pigmentations comprise a series of skin complaints that occur in circumscribed, regional, or generalised form, characterised by more intense skin colour. The most common forms of hyperchromia treated with cosmetic products are:

· Melasma
· Chloasma
· Solar lentigenes
· Sun-induced pigmentation
· Postinflammatory hyperpigmentation

The first two types feature melanocyte hyperactivity, but without an increase in numbers. The others feature increased melanocyte numbers in the basal layer of the epidermis.

Some interesting facts about depigmenting products:

In Europe:
Over 90% of Caucasian people over the age of 50 years old have solar lentigos, considered the third skin problem, after wrinkles and flaccidity. Dermatologists estimate that 63% of lentigos are sun-induced, whereas 13% originate through ageing. Depigmenting products have almost 20% of market share.

In Asia:
Depigmenting products comprise 35% of the facial care products market, with use mainly in Japan and South Korea. Traditional Asian culture has always revered whiter skin. Asian skin is thicker and quickly loses its luminosity, even taking on an olive tone.

MATERIALS:

The controlled chemical peeling cream included in this study, **MELINE® 01 CAUCASIAN SKIN**, is a mix of vitamin A derivatives and other depigmenting active ingredients. Comprised of vitamin A and its synthetic analogues, retinoids are a group of molecules that have played a very important role in dermatosis treatment over recent decades.

We classify Vitamin A derivatives as:

1st Generation:
· Retinol, Retinaldehyde or Vitamin A acid or Tretinoin
· Isotretinoin

2nd Generation:
· Etretinate
· Acitretin

3rd Generation:
· Adapaleno
· Tazaroteno
· Bexaroteno

The first generation derivatives are more specific to treating acne, hyperpigmentations, and skin ageing, whilst the second and third derivatives are more specific to treating acne, psoriasis, and other skin diseases. This dermabrasion mask includes vitamin A derivatives due to their proven pigmentation treatment properties.

Hyperkeratinisation and vitamin A derivatives

Vitamin A acts in the stratum corneum and has similar effects to AHAs on corneocyte cohesion. Corneocytes bind together through covalent bonds (disulphide bridges) and non-covalent bonds. The latter include hydrogen bridges, which are most common. Certain agents such as lithium bromide, urea, and alkalis can break those bonds. Any water present weakens the bonds by interacting and forming new hydrogen bridges.

Ionic bonds establish between negatively charged groups (such as phosphates, sulphates, carboxyl...) and positive groups (amine groups). Three factors affect the bonds between corneocytes:

· The distance between the positive and negative groups
· The medium between them
· The density of the groups

The influence of water and vitamin A derivatives on corneocyte cohesion mainly derives from the effect on those ionic bonds. Vitamin A and derivatives specifically reduce corneocyte cohesion by inducing or activating specific enzymes, such as sulphatase or phosphatase. The action of those enzymes reduces the number of sulphate and phosphate groups in the corneocyte walls, leading to the weakening of the cohesive forces.

Absorption

Once applied, vitamin A derivatives and other depigmenting active ingredients do not penetrate into the deepest part of the dermis, meaning no systemic absorption occurs. This explains the lack of systemic toxicity from vitamin A derivatives when applied locally.

Effects

Histolological

Tretinoin penetrates cell cytoplasm using a specific transporter found in the epithelial and endothelial cells, which explains why it does not pass through the dermis and into the system. Once inside the cell nucleus, it binds to DNA, affecting cytoskeletal protein synthesis in the cell (collagen, fibronectin, keratin...), the enzymes, and cell turnover. This controlled chemical dermabrasion also reduces skin thickness, leading to healing by secondary intention.

The following processes occur after applying the exfoliation cream:

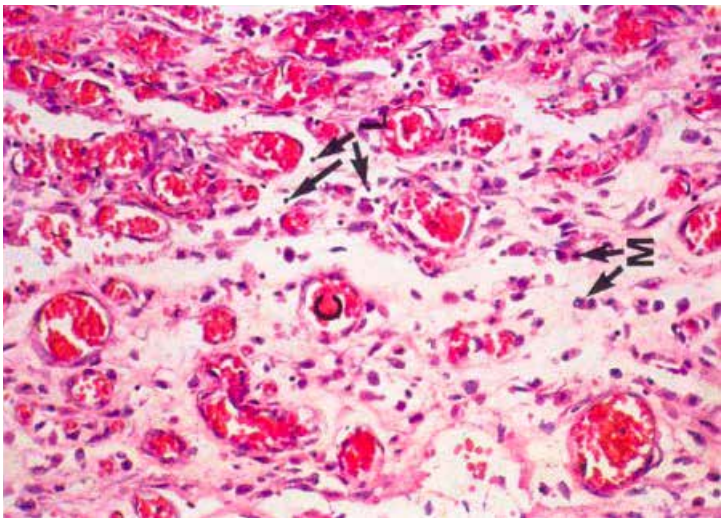
· Protein coagulation and inflammation
· Inflammation mediators activate, neutrophils remain for 3-5 days, macrophages remain for 10 days, and lymphocytes arrive 6 days after the lesion occurred.

Re-Epithelialisation:

Keratinocytes migrate from the periphery and the annexes. This process occurs within the first 14 hours. Keratinocytes need a fibronectin matrix in order to migrate, which enables collagen and fibrin adhesion too.

Granulation tissue formation

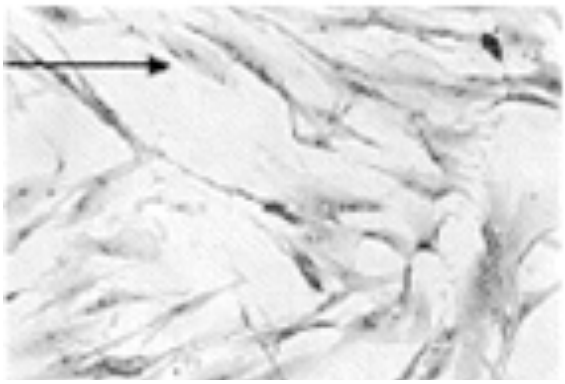
Occurs on the second or third day and involves an accumulation of cell compounds (fibronectin, fibroblasts, glycosaminoglycans, and cholesterol).



GRANULATION TISSUE AND ANGIOGENESIS

Collagen remodelling

It is responsible for the skin's texture after applying the exfoliation cream. The process involves fibroblast migration, which promotes new collagen fibre formation.



FIBROBLAST MIGRATION

We can act on different levels using controlled chemical dermabrasion cream:

1) On the skin

1.1 Epidermis
The peeling has several effects:
· Cell renewal acceleration.
· Acceleration of cell desquamation from the stratum corneum cells.
· Stratum granulosum thickening.
· The homogeneous redistribution of melanosomes.
· Reduced melanin content

1.2 The dermoepidermal junction
· An increase in the number of collagen-comprised anchoring fibrils.
· An increase in collagen synthesis
· Restoration of the dermoepidermal junction bond

1.3 The dermis
· Fibroblast activity stimulation
· Collagen degradation inhibition and synthesis stimulation.
· Melanin deposit dispersion.
· Fibronectin synthesis stimulation.

These actions affect skin diseases in the following way:

Pathologically

Melanin pigmentations:
· Reduced basal layer pigmentation
· Keratinocyte pigment granules dispersal.
· Pigmentation elimination promotion on accelerating epidermis turnover.
· Melanin degradation stimulation.

Photoageing:
· Expression line reduction.
· Medium and surface wrinkle reduction
· Improvement of skin luminosity, texture, and tone.

THE EFFICACY STUDY

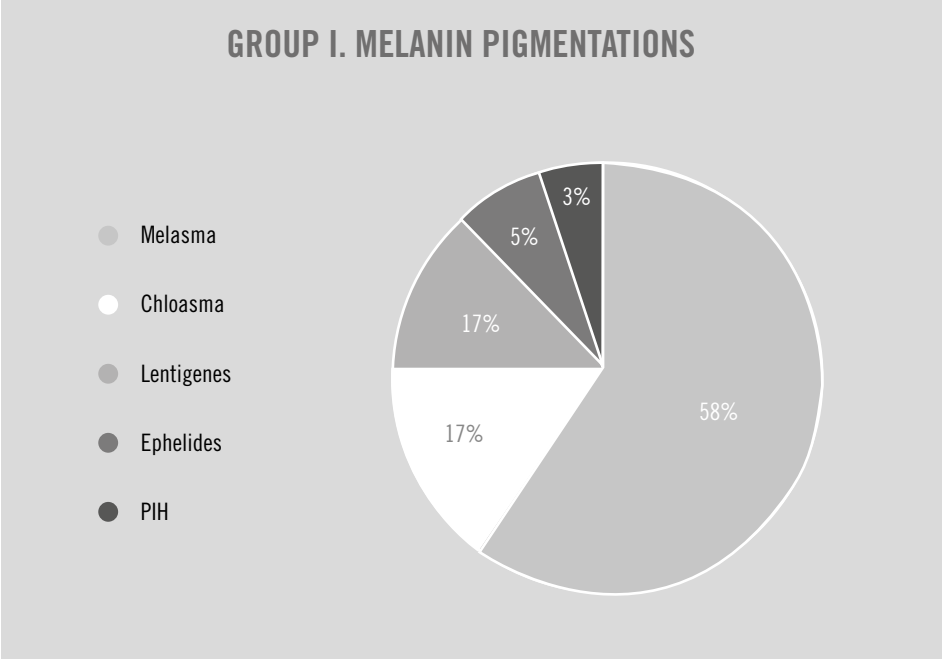
The controlled chemical dermabrasion in this study involves an exfoliation cream that produces a semi-occlusive covering comprised of depigmenting active ingredients, which are a good option for the disease in question. This temporary semi-occlusion enables the active ingredients to penetrate further.

This study aims to show this exfoliating cream's efficacy compared to other basic cosmetic active ingredients:

-Melanin hyperpigmentations in the epidermis: as it clears them.
-Elastic fibre regeneration in the dermis: involving attenuating fine lines and reducing pilosebaceous orifices.
-Its high level of safety when used on patients.

MATERIALS AND METHOD

60 patients with a range of melanin pigmentations participated in the study, represented as follows:



Includes phototypes I to IV.

Patients applied a COLIPA method, factor 50+ sunscreen prior to applying the exfoliation cream.

Application protocol
Before applying.

· The healthcare professional responsible for applying the dermabrasion cream should establish:
· The patient's skin phototype
· The problem requiring treatment
· The thickness of the patient's stratum corneum
· The area of affected skin

The application
Preparing the skin

1. Thoroughly clean with a degreasing solution.
2. Use an occlusive product to apply to areas not requiring abrasive action.
3. Put the contents of the **MELINE® 00 PREP** ampoule into a container and use a brush to apply an even layer across the entire face, allowing it to act for 3 minutes.
4. Apply **MELINE® 01 CAUCASIAN SKIN** dermabrasion cream.
5. Leaving the **MELINE® 00 PREP** solution on, evenly apply a thin layer of the **MELINE® 01 CAUCASIAN SKIN** dermabrasion cream, leaving it to act for a period between 30 minutes and 1 hour, depending on the patient's sensitivity.
6. 15 minutes after applying the first layer, apply a second layer of **MELINE® 01 CAUCASIAN SKIN** to the areas where changes are more extensive.
7. After leaving on for the appropriate time, remove the dermabrasion cream with a gentle lipid-rich soap.
8. Apply a thin layer of **MELINE® 03 MOIST** to the clean skin, and once absorbed, apply sunscreen with **MELINE® 04 B.B. CREAM**.
9. Home treatment:

· The patient is to apply **MELINE® 03 Moist** at least 2 times a day for the next 4 or 5 days, as required, as well as **MELINE® B.B. CREAM**.

· After the fifth day, they are to start using **MELINE® 02 CAUCASIAN SKIN DAY**, applied by dabbing the hyperpigmented areas alone, followed by the sunscreen once it has absorbed. In addition, they are to apply **MELINE® 02 CAUCASIAN SKIN NIGHT** in small amounts, spreading it across the entire skin surface.

Technique contraindications

· Active skin infections
· A known allergy to vitamin A or any of the formula's active ingredients.
· Any purpuric lesions.
· Recent sun exposure
· Photosensitivity and autoimmune diseases.
· Imminent social commitments.
· Avoid use during pregnancy.

INSTRUMENTAL EVALUATION OF THE DEPIGMENTING EFFECT

Evaluated objectively and quantitatively using melanin index scores before and after the treatment. The experimental area represents a skin blemish selected by the investigator. This blemish (≥ 5mm) requires recording in a specific location in the investigator's journal, along with pre and post treatment measurements for the same blemish.

The measuring device is a Mexameter® MX 16 (Courage & Khazaka), which has a probe with a 5 mm diameter measuring surface. Melanin and haemoglobin (erythema) are mainly responsible for skin colour. The probe emits light at two wavelengths (red and near-infrared), which melanin absorbs. Haemoglobin pigment absorption is minimal, thus avoiding interference from the skin's blood vessels when assessing the melanin content. The amount of light the skin absorbs derives from the amount of light emitted by the instrument and reflected by the skin, giving rise to the melanin index (M parameter):

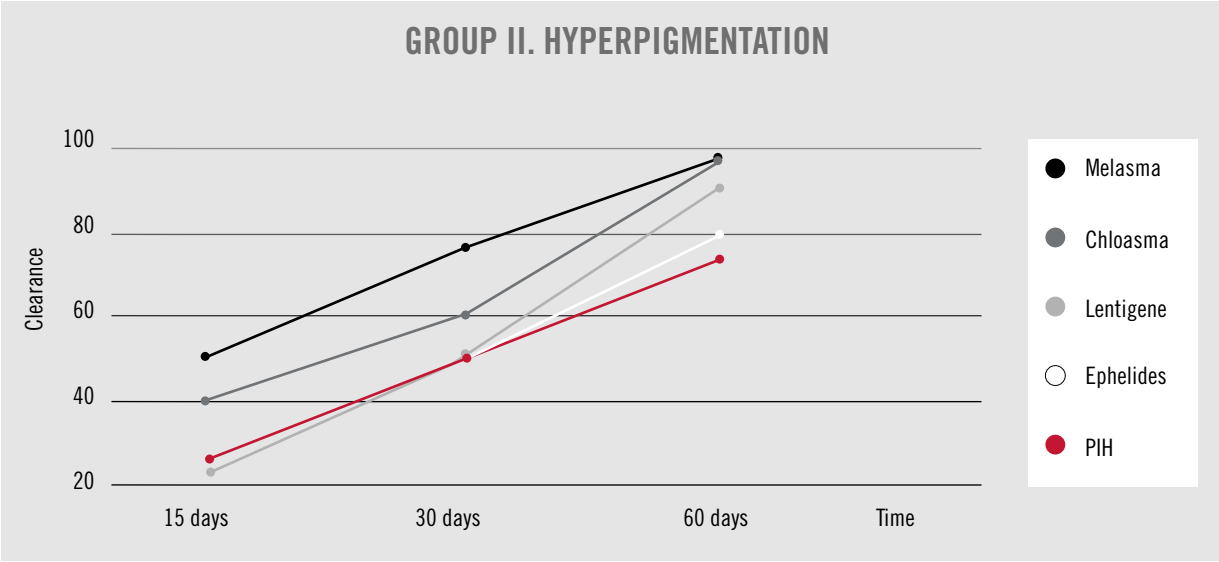
M= (500/Log 5) x {Log (Infrared – Reflection/Red – Reflection) + Log 5}

Arbitrary units between 0 and 1000 express the melanin content (melanin index). The larger the M value, the greater the amount of melanin detected. Three melanin index measurements were taken for each blemish at each experimental time period, i.e. M1, M2, M3, as well as a non-pigmented skin area surrounding the blemish, i.e. M1', M2', M3'. The melanin index was measured at D0 and D28.

RESULTS

Efficacy as a depigmenting agent

The study group showed excellent results for 90% of cases, good for 7 % of cases, and moderate for 3% of cases. Melasma and chloasma clearance stands out, with 75% clearance observed after a single session. Lentigo and ephelide type hyperpigmentations improve, but require two applications. There is anecdotal evidence of cases showing significant improvement in bags under the eyes. Figure II shows the results in graph form.



Measured at D0 and D28, ΔM indicates the melanin index. There was a notable depigmenting effect in a percentage of active volunteers, with measurements at least 10% smaller at D28, compared to D0. The following tables show the average values and standard deviations obtained for the M parameter at each experimental time period for the control and treated areas. In addition, there are ΔM values for each experimental time period and the % variation at D28 compared to D0.

THE MELANIN INDEX							
	D0			D28			
Volunteer	Control area	Treated area	ΔM D0 (Tr-Co)	Control area	Treated area	ΔM D28 (Tr-Co)	% variation ΔM(D28-D0)
Mean	486,1	511,5	25,4	486,9	506,0	19,1	-32%
Standard deviation	10,5	14,2	12,3	10,9	15,5	13,1	21%

Table I. Confirmation of depigmenting efficacy on melasma
There was a mean melanin index reduction of 32% in the group of volunteers with melasma.

THE MELANIN INDEX							
	D0			D28			
Volunteer	Control area	Treated area	ΔM D0 (Tr-Co)	Control area	Treated area	ΔM D28 (Tr-Co)	% variation ΔM(D28-D0)
Mean	475,3	547,1	71,8	475,5	529,3	53,8	-27%
Standard deviation	10,8	13,8	12,3	12,4	12,4	11,9	17%

Table II. Confirmation of depigmenting efficacy on chloasma
There was a mean melanin index reduction of 27% in the group of volunteers with chloasma.

THE MELANIN INDEX							
	D0			D28			
Volunteer	Control area	Treated area	ΔM D0 (Tr-Co)	Control area	Treated area	ΔM D28 (Tr-Co)	% variation ΔM(D28-D0)
Mean	501,2	536,4	35,2	502,8	516,8	14,0	-17%
Standard deviation	11,8	14,6	13,2	12,4	16,2	14,3	16%

Table III. Confirmation of depigmenting efficacy on lentigenes
There was a mean melanin index reduction of 17% in the group of volunteers with lentigenes.

THE MELANIN INDEX							
	D0			D28			
Volunteer	Control area	Treated area	ΔM D0 (Tr-Co)	Control area	Treated area	ΔM D28 (Tr-Co)	% variation ΔM(D28-D0)
Mean	478,4	517,3	38,9	480,4	501,7	21,3	28%
Standard deviation	12,8	13,7	13,2	14,4	16,7	15,5	11%

Table IV. Confirmation of depigmenting efficacy on ephelides
There was a mean melanin index reduction of 28% in the group of volunteers with ephelides.

THE MELANIN INDEX							
	D0			D28			
Volunteer	Control area	Treated area	ΔM D0 (Tr-Co)	Control area	Treated area	ΔM D28 (Tr-Co)	% variation ΔM(D28-D0)
Mean	467,1	537,8	70,7	466,8	499,6	32,8	-29%
Standard deviation	11,4	16,7	14,0	14,8	12,4	13,6	13%

Table V. Confirmation of depigmenting efficacy on PIH
There was a mean melanin index reduction of 29% in the group of volunteers with PIH.

CONCLUSIONS

These results lead to the conclusion that the **MELINE® CAUCASIAN SKIN** product, containing vitamin A derivatives and other depigmenting ingredients, presents good clinical efficacy. The results are particularly good when treating melanin pigmentations, with excellent results obtained in 90% of the cases studied, without showing any adverse effects for the patient.

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THE EFFICACY OF **MELINE® ETHNIC SKIN** FOR TREATING MELASMA: A RANDOMISED, DOUBLE BLIND, PLACEBO-CONTROLLED STUDY.

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INTRODUCTION

Numerous studies now document the increase in frequency of disorders characterised by hyperpigmentation in darker racial ethnicity groups (1, 2). Melanocytes in dark-skinned individuals show labile and exaggerated responses to skin lesions. Pigmentation disorders were the third most common motive for seeking out skin treatment in a study that evaluated the frequency of common dermatoses (excluding vitiligo) occurring in 2000 black patients. In addition, pigmentation disorders were the third most common reason for seeking out treatment among a group of Latin American patients (3). A study undertaken by Alexis et al (2) on 1412 patient visits to a dermatology department in an urban centre reported that dyschromias were the most common diagnoses among black race patients. In fact, dyschromias are clearly important issues for concern among non-white skinned people.

Skin colour classification is the cornerstone of clinical practice and used to decide skin involvement treatment. The Fitzpatrick skin classification system from 1975 arose out of a need for better prediction of a patient's response to UVA radiation when used to treat psoriasis (4). Patients with a similar visual tone reacted differently to the same dose of UVA. The classification was expanded to include the response to environmental stress, i.e. ultraviolet radiation in general. Therefore, the response to ultraviolet radiation serves as an element to predict reactions to other stresses, such as laser energy and chemical peelings, for example. The skin's inherent colour, known as constitutive pigmentation, and reactivity to ultraviolet radiation or facultative pigmentation, gave rise to 6 skin phototypes (5).

Latin Americans live in region stretching from Mexico and the Caribbean islands down to the southern cone. The inhabitants of Latin American countries have different origins and those with a mix of two or more races of origin are called mixed race. Consolidating Latin Americans through their language is common, but there are a wide range of phototypes and skin colours among the heterogeneous population.

Skin disease forms a significant part of primary healthcare providers' workload in Latin American communities, where involvement can occur in up to 40% of a community.

Races have the same number of melanocytes, with rather the variation in the number, size, and aggregation of melanosomes inside the keratinocyte explaining skin colour variations (6). Different groups of melanosomes have been shown to correlate with how dark or light an individual's skin colour is. Toda et al. (7) showed the basal layer in black individuals with darker skin contains more melanosomes compared to those with lighter skin, with approximately 340 melanosomes per basal cell compared to 120 melanosomes per basal cell respectively. Goldschmidt and Raymond (8) showed this same trend when comparing Asians with darker and lighter skin colour too. Unaggregated large melanosomes occur in black subjects with dark skin in contrast to black subjects with light skin, who have aggregated and unaggregated large melanosomes. Total melanin content has also been shown to be higher in individuals with skin phototype VI compared to skin phototypes I and II (9). Melanosome distribution appears to correlate with skin colour too. Melanosomes appear throughout the epidermis in black skin (10) in contrast to their absence in the upper layers of light, white, or unexposed skin (11).

Pigmentation disorders, such as melasma and postinflammatory hyperpigmentation, can cause psychological and emotional distress, and may have a significant negative impact on a person's quality of life in terms of their health (12,
13). In a prospective cohort study on the prevalence of pigmentation disorders and their impact on quality of life, 47.3% of patients admitted being aware of their skin to a certain extent, 21.8% considered others focused on their skin, 32.7% reported feeling unattractive due to their skin, 32.7% made efforts to hide their pigmentation changes, and 23.6 % thought that their skin affected their activities (12, 13).

Melasma is an acquired symmetrical hypermelanosis characterised by irregular light brown to brownish-greyish coloured patches that affect the face, and particularly the cheeks, nose, forehead, chin, and upper lip. Lesions can also appear on other areas

exposed to the sun, such as the back, chest, and arms. It mainly affects women, with men comprising 10% of all cases. The disease affects all racial groups, but it occurs most commonly in darker skinned individuals, classified as Fitzpatrick IV-VI skin types, such as those from Latin American, eastern Asians, southeast Asians, and blacks that live in areas with intense ultraviolet radiation. The exact cause of melasma is unknown, but factors affecting its appearance include genetic influences, exposure to UV radiation, pregnancy, the use of oral contraceptives, hormone replacement treatments for menopause, thyroid dysfunction, the use of cosmetics, as well as the use of phototoxic contraceptives and anticonvulsant drugs. Genetic influences and exposure to UV radiation are believed to be the most important factors. Several studies have reported the presence of abnormal levels of circulating hormones in patients with melasma (14, 15). The data suggests that the increase in stem cell factor expression in the dermis and its c-kit receptor in the epidermis play an important role in the hyperpigmentation mechanism in melasma (16, 17). Biopsies of involved and uncompromised patient skin suggest that melanocyte amounts do not significantly increase in hyperpigmented areas. Conversely, melanocytes in the affected area are larger, with prominent dendrites compared to normal skin (18).

Skin treatments, such as peelings, laser energy, and using skin whitening agents to treat dark patches, can cause inflammation. The potential adverse effects of the treatment are the lesions and subsequent inflammation. Therefore, the impact of ultraviolet exposure to skin colour, i.e. the pigmentation response, is a useful model when it comes to considering the potential effects of other skin treatments. Ethnicity / skin colour has a clinically significant influence when deciding which treatment type to apply. Hyperpigmentation can increase when the inflammatory source acts on prostaglandins (PGE2, PGF2a), thromboxanes, and leukotrienes to stimulate tyrosinase, causing increased melanin synthesis and its transfer to keratinocytes and macrophages (19). These findings lead to the belief that the more the skin damage, the larger the inflammatory effect, and therefore, the more likely a different response will occur when it comes to pigmentation. This leads to the idea that significant factors need considering, particularly in the case of melasma in ethnic skin, given it is much more reactive.

Postinflammatory hyperpigmentation (PIH) is characterised by an acquired increase in pigmentation after an inflammatory process. Excess pigment is deposited in the epidermis or in both the epidermis and dermis. The common causes of PIH in darker skin types include acne vulgaris, atopic dermatitis, allergic and/or irritating contact dermatitis, trauma, psoriasis, lichen planus, and drug-induced rashes. Postinflammatory hyperpigmentation is caused by an increase in melanin production or abnormal pigment distribution. After skin trauma or inflammation, melanocytes can react by inducing normal, increased, or reduced melanin production. Although the precise pathogenesis is unknown, hyperpigmentation is believed to derive from cytokines, inflammatory markers, and reactive oxygen species. In vitro studies have shown that LTC4, LTD4, PGE2, and TxB2 stimulate the increase of human melanocytes and dendrocyte proliferation. LTC4 increases tyrosinase activity and mitotic activity in cultivated melanocytes significantly too (20). Studies also suggest that direct melanocyte stimulation by inflammatory markers, such as IL\ alpha, stem cells factor, and endothelin 1 can cause hyperpigmentation. Additionally, reactive oxygen species, nitric oxide, and superoxide in damaged skin or inflammatory cells stimulate hyperpigmentation (21).

For decades, hydroquinone has been the gold standard for treating hyperpigmentation disorders such as melasma, lentigos, and postinflammatory hyperpigmentation. However, there are many options for treating dyschromias, including a range of topical agents, orally administered drugs, chemical peelings, or lasers. More recent studies show that several non-hydroquinone agents, such as arbutin, kojic acid, azelaic acid, liquorice, vitamin C, and retinoic acid, among others, can also play an important role in hyperpigmentation therapy.
This study's main aim is to determine whether the active ingredients in **MELINE® ETHNIC SKIN** are effective and safe in treating skin melasma in patients with ethnic skin. The following is a description of the properties of each main active ingredient in the MELINE® Ethnic Skin formulas and their modes of action for treating melasma-type skin hyperpigmentations.

MODE OF ACTION

Firstly retinoids, which are structural and functional analogues of vitamin A, and effective either when used alone or in combination with other agents for treating melasma and postinflammatory hyperpigmentation in ethnic patients. Vitamin A derivatives act by inhibiting tyrosinase transcription and inhibiting the spreading of pigment granules in keratinocytes. They also induce desquamation, boost epidermal call renewal, and reduce the length of contact between keratinocytes and melanocytes, therefore promoting a quick loss of pigment via epidermopoiesis (21). Retinoids have been used to treat melasma in black race patients. Evaluations over 40 weeks showed a statistically significant reduction on the severity index of the area of melasma among the group using retinoids, with side effects reported in 67% of patients, which were mild in most cases. Equally, another study used 0.1% Adapalene during an open, 12-week period on 65 African patients who had postinflammatory hyperpigmentation. At the start of the study, 20% of the patients had severe PIH, with a significant improvement observed in the grade of PIH at weeks 4, 8, and 12 compared to baseline ($P < 0.01$). Fewer than 5% of patients reported moderate or severe skin irritation during treatment (22).

Ascorbic acid is an antioxidant that affects melanogenesis by reducing o-dopaquinone to DOPA and shows activity that biochemically reduces melanin from black to a lighter colour. Ascorbic acid is highly unstable and quickly oxidises in an aqueous solution. Some ascorbate esters, such as magnesium ascorbil-2-phosphate, were synthesised to prevent such drawbacks (23). Ascorbic acid generally combines with other active ingredients and darker racial ethnic groups tolerate it well. It interacts with copper ions at the tyrosinase activity site and reduces the oxidised dopaquinone (24, 25). Vitamin C makes the skin glow and provides other benefits, including antioxidant, anti-inflammatory, and photoprotective effects (26,
27). There is proof that this active ingredient and its derivatives are safe and somewhat effective in some racial / ethnic population groups, including Latin and Asian patients (28, 29).

It is worth mentioning another active ingredient, niacinamide, the physiologically active form of niacin. It is a precursor for NADH and NADPH. Niacinamide inhibits melanosome transfer to epidermal keratinocytes. It has been shown to improve photodamage in Asian subjects. In a study of 18 Asian subjects with hyperpigmentation, a moisturiser with 5% niacinamide content led to a significant reduction in facial hyperpigmentation (30). One of niacinamide's benefits is that it remains stable under the effects of light, humidity, acids, alkalis, and oxidants (24).

Used topically with a 2 to 5% content, this active ingredient has been shown to be somewhat effective when used alone or in combination with N-acetylglucosamine for treating UV-induced melasma and hyperpigmentation in patients with Asian skin (30, 31).
Arbutin is another of the products' ingredients, which is extracted from the dry leaves of the bearberry, pear, or blackcurrant bush. It is a hydroquinone derivative, but without the melanotoxic effects (32, 33). It causes a decrease in tyrosinase activity, without affecting RNA expression, whilst also inhibiting melanosome maturation (24).

Melanostatine 5 is a biomimetic peptide that inhibits α-MSH induced melanin synthesis and prevents hyperproduction, providing improved control over skin tone. It does not interfere with melanocyte functions, meaning it is a skin-whitening agent, inhibiting melanogenesis without cytotoxicity.

When it comes to glutathione, the melanin index has been used to evaluate the effects of its use as an objective outcome indicator, alongside subjective evaluations, showing positive effects on whitening patches and skin colorimetric tone uniformity (34). In vitro studies have looked at the mode of action behind glutathione inhibiting melanogenesis. One mode of action involves tyrosinase activity inhibition (35, 36). Another mode of action involves activating the pheomelanin pathway, which starts with L-dopaquinone conjugation formed from L-tyrosine with cysteine. This reaction produces the pheomelanin precursor, cysteinyldopa. Glutathione can also conjugate L-dopaquinone in the presence of glutathione S-transferase and produce the cysteinyldopa precursor, glutathione-dopa. This results in the induction of cysteinyldopa synthesis, which leads to increased pheomelanin production, which is a red-yellow colour (37, 38). Lastly, glutathione is an active antioxidant (39).
Tranexamic acid has been evaluated on different occasions for topical application when treating melasma. A prospective study (40) showed that after 12 weeks of applying 2% tranexamic acid, the MASI scores reduced significantly from baseline. Two studies compared its topical application to more standard methods of treating melasma (41). A split-faced prospective study compared the effects of 5% tranexamic acid topical liposome to a 4% hydroquinone topical cream over 12 weeks. There was a significant clinical improvement in the melasma compared to baseline. Another study (42) investigated a 3% TA topical suspension applied to one side of the face and a 3% hydroquinone, 2% vitamin C, and 0.01% dexamethasone suspension applied to the other side for 12 weeks. Both treatment groups showed a significant improvement in MASI scores from baseline to follow up, but again, the difference between both groups was insignificant.

Those results indicate that tranexamic acid applied topically is as effective as topical hydroquinone for treating melasma.

Lastly, cysteamine hydrochloride (β-mer-captoethylamine hydrochloride) is the most stable simple aminothiol that has been shown to have potent depigmentation capabilities. This molecule occurs naturally in the human body and is a product of L-cysteine amino acid degradation. Under high concentrations of L-cysteine, melanocytes exhibit lower tyrosine activity and produce notably higher concentrations of pheomelanin.

Previous cell culture studies have confirmed that cysteamine acts by inhibiting melanogenesis instead of melanocytotoxicity. Several studies have successfully shown the efficacy of cysteamine cream in reducing and eliminating melasma in humans (43). As can be observed, the active ingredients in the different **MELINE® ETHNIC SKIN** products aim to improve control over melanin synthesis and deposition, inhibiting cytotoxic or inflammatory activity that can trigger unwanted effects in ethnic group skin types identified as phototypes IV to VI.

METHOD

A randomised, double blind, placebo-controlled study in the Skin Academy research centre. On completing recruitment and collecting data from clinical records, a diagnostic analysis was performed using a Dermacatch®, a Mexameter®, and the Melasma Area and Severity Index (MASI) to evaluate skin pigmentation.

Written informed consent was obtained from all patients prior to registration. The study started in October 2016 and ended in April 2017. The treatment period was four months. The patients were aged between 38 and 50 years old, and classified as Fitzpatrick type IV, V, and VI. The inclusion criteria were a melasma diagnosis, confirmed using the Wood lamp exam, and a minimum disease evolution of one year. None of the patients received any treatments for a minimum of two months prior to starting the study. The exclusion criteria were the use of oral contraceptives at the time of the study or during the three-month period prior to its start, the use of topical or oral corticosteroids, any other pigmentation disorders, a history of endocrine disorders, pregnancy, and breastfeeding.

Clinical evaluation: the melasma severity scores were calculated at the start and end of the study, i.e. after 4 months of follow up. The MASI scores were calculated at the start of the study and after 2 and 4 months of follow up. The MASI was calculated applying the formula from Kimbrough-Green et al. (44), where D means darkness, H is homogeneity, A represents area, F is forehead, RM means right malar, LM left malar, C refers to the chin, and the 0.3, 0.3, 0.3, and 0.1 values are the respective percentages of the entire facial area. The MASI scores range between 0 and 48.

An average was calculated from the MASI scores obtained by each investigator. Dermacatch® (Colorix, Neuchatel, Switzerland) is a Swiss-made, highly advanced visible light spectrum reflectivity colorimeter. It was specifically developed to instantly show measurements, including melanin and erythema values, and uses the reflectivity of the entire visible light spectrum. The measured area involves a 5.5 mm diameter circle, i.e. 24 mm (45). Statistical analysis was used to evaluate the differences in pigment content (melanin) between lesions and a normal area of skin.

The topical products application technique

Two topically applied products were indicated. **MELINE® 02 ETHNIC SKIN DAY**, comprising a serum applied every morning on the pigmented facial areas only and **MELINE® 02 ETHNIC SKIN NIGHT**, comprising a cream for night time application over the entire facial skin area.

The peeling application technique in controlled dermoabrasion cream.

1. Procedure

· Clean and degrease the skin deeply with a view to removing any makeup residue or sebum that may interfere with the outcome of the peeling.
· Dry the skin.
· Pour the contents of the **MELINE® 00 PREP** solution ampoule into a suitable container and apply a uniform layer over the entire face using a brush.
· Leave on to act for 3 minutes.

2. Application of the Controlled Chemical Dermoabrasion Mask

· It involves using a brush to apply a uniform medium thickness layer as evenly as possible.

· Wait for 15 minutes.
· Apply another layer to the areas with the most skin differences – the first session lasts 30 minutes.
3. Clean and remove the cream mask using a mild, lipid-rich soap.

POST TREATMENT CARE

At 24 hours and in cases with longer application times, pruritus, erythema, and a taut skin sensation appears. At 48 hours, mild desquamation occurs, lasting 3-4 days. A moisturising product, **MELINE® 03 MOIST**, should be used during and after the period, with the aim of appropriately rehydrating and repairing the skin. Similarly and with a view to protecting against ultraviolet radiation, complement the treatment using **MELINE® 04 B.B.** sunscreen.

Home treatment product application began from day 4 of the applied controlled dermoabrasion. They were used every other day for the first two weeks and if no skin reddening or burning sensations occurred, the application regimen was changed to once daily.

RESULTS

5 of the potential 45 patients based on the inclusion criteria were not included in the study, given they stated it was impractical as they lived in other cities. The remaining 40 patients were divided into two groups, with 20 treated using the **MELINE® ETHNIC SKIN** protocol and 20 with placebo, all of which completed the four-month treatment protocol and attended all the follow up visits.

Variable	MELINE® Ethnic Skin (n=20)	Placebo (n=20)
Age (years)		
Sex (%) Male Female	4 (20) 16 (80	2 (10) 18 (90)
Fototype (%) IV V VI	4 (20) 10 (50) 6 (30)	6 (30) 12 (60) 2 (10)
MASI Scores	18.1 ± 8.1	13,2 ±7,4
Mexameter	93.6± 42.4	72.3 ± 27.8
Dermacatch	65.4 ± 22.6	52.9 ± 16.4

Of the 40 patients, 6 (15%) were men and 34 (85%) were women. The mean disease duration was 5.6 ± 4.4 years. The melasma severity evaluations indicated that 9 patients (22.5%) had mild melasma, 27 (67.5%) had moderate melasma, and 4 (10%) had severe melasma, as shown in figure 1. Statistically significant differences were observed between placebo and the use of **MELINE® ETHNIC SKIN** at 4 months of follow up (p = 0.01).

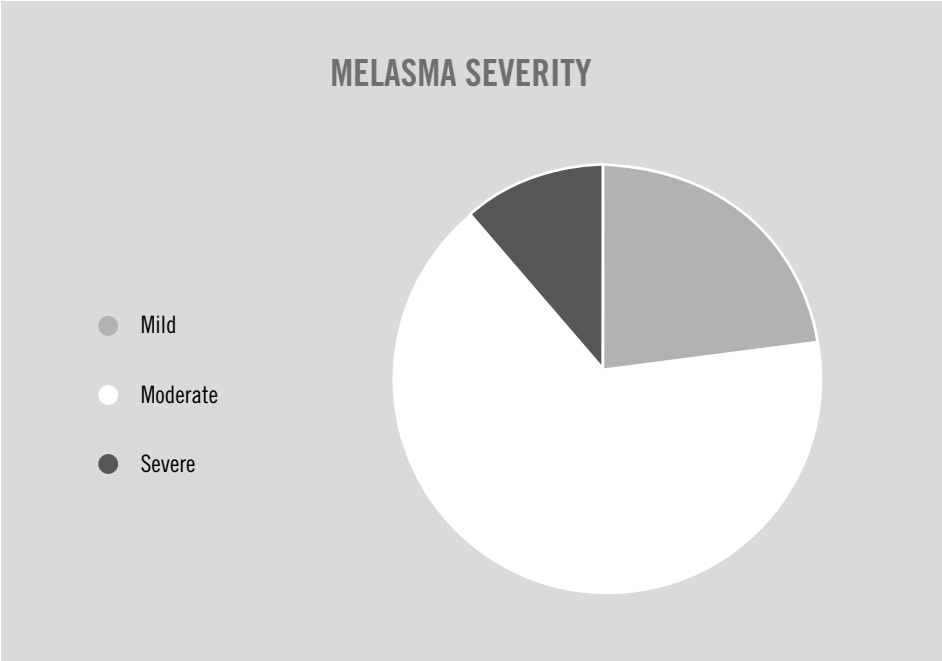


Figure I. Study group distribution based on melasma severity.

Table 2 shows the mean MASI scores for the placebo and **MELINE® ETHNIC SKIN** groups prior to treatment, after 2 months of treatment, and at 4 months of follow up. Statistically significant differences between the placebo and MELINE® product groups were only observed at the end of the four-month treatment period. Statistically significant differences between the placebo and **MELINE® ETHNIC SKIN** groups were found at 2 and 4 months follow up using both measurement methods (see Table 2).

Table 2 shows the MASI index, the differences in the amount of pigment contained in skin with lesions and normal skin, using the Mexameter® and Dermacatch® prior to, at 2 months, and at the end of the study or the 4th month.

	MASI Scores			Mexameter			Dermacatch		
	Before T	After 2 M	After 4 M	Before T	After 2 M	After 4 M	Before T	After 2 M	After 4 M
Placebo	13,2 ±7,4	12.5 ± 7.4	12.2 ± 7.4	72.3 ± 27.8	68 ± 2 6.2	64.9 ± 25.3	52.9 ± 16.4	51.2 ± 16.8	50 ± 18
MELINE® Ethnic Skin	18.1 ± 8.1	11.2 ± 6.2	8.03 ± 5.2	93.6± 42.4	49.9 ± 19	38.1 ± 15.3	65.4 ± 22.6	33.5 ± 16.1	23.8 ± 12.9
P value	0.66	0.55	0.04	0.07	0.01	0.0001	0.055	0.002	0.0001

The correlation between Dermacatch® and Mexameter® prior to treatment, at 2 and 4 months, was 0.82, 0.74, and 0.76 respectively (Pearson's correlation). These results indicate a good correlation between the Dermacatch® and Mexameter® scores.

Seven patients from the **MELINE® ETHNIC SKIN** group reported some adverse effects. The effects were very mild and tolerable in those patients, and resolved as the treatment continued. No patients from the placebo group complained of any adverse events.

DISCUSSION

Controlled chemical dermoabrasions may be an effective method for treating skin hyperpigmentations, for both white and coloured skin people. Patients with darker skin often undergo surface exfoliations, medium depth exfoliations to a lesser extent, but always avoid using deep chemical exfoliations given the post-treatment complication risks, such as depigmentation, cheloid formation, and hypertrophic scarring. In general, the use of surface, controlled chemical dermoabrasions with a combination of agents derives in appropriate epidermal regeneration and replacement with the ability to produce dermal stimulation (46).

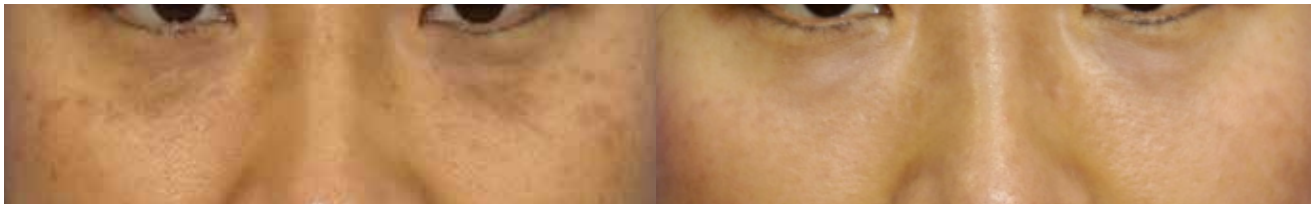
Despite the photoprotective effects provided by the higher quantity of skin eumelanin in darker skinned groups, studies have shown that some racial groups, particularly Asians and Latin Americans, will develop more pigmentation changes from ultraviolet radiation than Caucasians (47, 48).

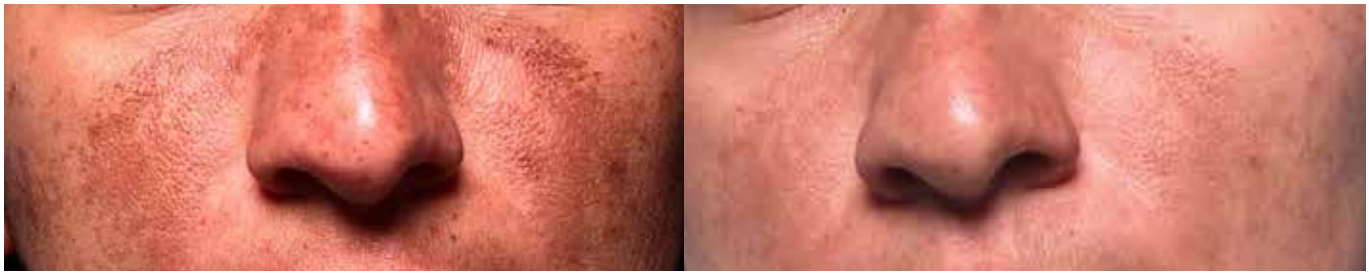
Variations are also possible within specific racial and ethnic populations. For example, a Hillebrand and collaborators (49) study showed how geographical location produced differences in the skin ageing rate in Japanese women that live in northern and southern regions, with the latter receiving more UVB radiation given they are closer to the equator. The study showed that facial hyperpigmentation occurred 16 years earlier in women that lived in the southern region compared to those living in the northern region. There may be differences in the response to chemical peelings too, even within the skin colour spectrum. Postinflammatory hyperpigmentation induced by chemical peelings may be the most common complication in deeply pigmented skin (46, 50). It is important to consider the following points: 1) given the labile response of skin melanocytes (46) in deeply pigmented skin (Phototypes V and VI), those patients can develop pigmentation changes after chemical peelings more commonly than those with less pigment, meaning more care is required; 2) more superficial chemical peelings are safe, even for phototypes V and VI (46, 50, 51, 52); and 3) photodamage tends to occur less frequently and less severely in patients with deeply pigmented skin (53, 54). This all supports the fact that the **MELINE® ETHNIC SKIN** protocol includes controlled chemical dermoabrasion with minimal exposure time, thus avoiding any possible changes deriving from the procedures, which could cause unwanted effects.

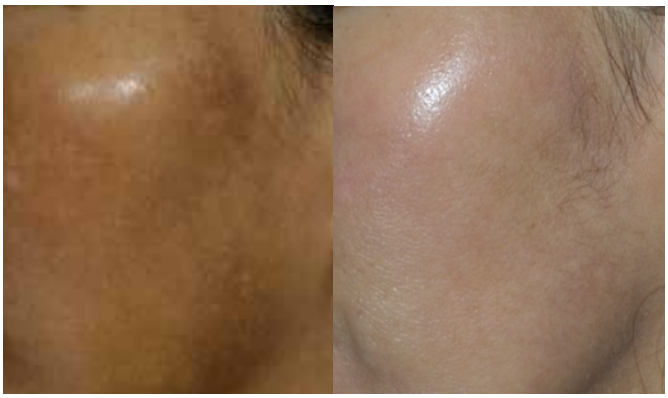
Treating melasma in Latin American patients is viewed no differently from treating other ethnic groups. It is worth noting, however, that melasma incidence is higher in those ethnic groups, and that Latin American patients with darker skin can suffer the effects of irritation and sensitivity. This is why postinflammatory hyperpigmentation must be considered a post-treatment risk. Published melasma treatment recommendations for the general population already exist (55). The consensus for treating melasma in Latin American patients involves a first line treatment of effective topical treatments, mainly with a combination of active ingredients that do not cause cytotoxicity, irritation, or inflammation. The idea, therefore, is that combined treatments have a good safety and efficacy profile, and considered better than using the components separately. This occurs with **MELINE® ETHNIC SKIN**, which has shown statistically significant data when used on melasma conditions and contains a mix of active ingredients that conserve those characteristics both separately and through the doses used, which is an equally important factor to consider.

In accordance with values obtained from applying **MELINE® ETHNIC SKIN**, there is evidence that it is a safe and effective tool for treating melasma in patients with skin phototypes IV, V, and VI. It must be noted, however, that exposure to the sun often reverses the treatment results for hyperpigmentation disorders, compromising the process of prolonged treatment. As such, the first line of treatment against hyperpigmentation is using a broad-spectrum sunscreen and closely monitoring the risk factors.

VISIBLE RESULTS







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MELINE® SPOTS: A SPECIFIC METHOD FOR TREATING SOLAR LENTIGOS.

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ABSTRACT

Introduction: In recent years there has been an increase in skin lesions on sun-exposed areas as a result of the deterioration of the ozone layer, among them is the solar lentigines, that although it is a benign lesion, represents a challenge therapeutic from the cosmetic point of view.
Objectives: To determine the effectiveness of the **MELINE® SPOTS** treatment in the aesthetic improvement of solar lentigos on the face and back of the hand and forearms.
Methods: An open, controlled and comparative study of 25 patients with skin types between II and IV, using the **MELINE® SPOTS** treatment in lesions located on the left side compared to only the placement of sunscreen on the right side. Measurement by observation, iconographic study and Mexameter were performed.
Results: A statistically significant decrease in the mean melanin score recorded using Mexameter ($p < 0.02$) could be demonstrated. Mean values of erythema also showed a significant reduction. In the clinical evaluation in the patients they showed an improvement of 40% excellent, 50% good, 10% sufficient, whereas for the observers it was 30% excellent, 45% good and 25% sufficient.
Conclusions: The use of **MELINE® SPOTS** represents a good therapeutic alternative for the treatment of solar lentigines.

KEYWORDS

Solar Lentigines / Lentigo Solar

Skin Phototype Skin / Fototipo Cutáneo

INTRODUCTION

A solar lentigo is a pigmented, uniform, rounded macule located in sun-damaged areas of skin, often appearing in clusters. This form of hyperpigmentation presents as small, brown patches on the skin. Their lens shape gave rise to the name and they have even edges and colour. They appear most prominently on the face, neckline, and back of the hands, because of intense, acute sun exposure or chronic sun exposure (1). From a histological point of view, these lesions have lengthened rete ridges, similar in shape to a hockey stick. Over time, they can grow deeply into the dermis in the form of keratinocyte finger-like extensions that can merge, taking on a grid-type histological appearance, as occurs with seborrheic keratosis. Basal hyperpigmentation, sometimes very prominent, commonly occurs alongside papillomatosis. Papillomatosis is often less intense or even absent in facial lesions (1, 2).

They occur in older people, on areas exposed to the sun (the face, hands, and forearms), being generally associated with other photoageing skin changes. They tend to come in clusters, growing slowly, and often merging. Using a Wood lamp enables observation when they are no longer visible to naked eye. Solar lentigos are common benign signs of sun damage in over 90% of white people over 50 years old (3). They have a potentially negative social impact, given that the lesions appear on the visible areas of the body and face (4). They range from a few millimetres to over one centimetre in diameter, with their sizes tending to increase gradually. The main pathogenic factors are genetic predisposition and accumulated chronic solar radiation exposure (5). The precise mechanisms behind pigmentation in solar lentigos remain unresolved. Increasing evidence points to the essential role played by interactions between mesenchymal and epithelial cells through fibroblast-led growth factors release (6).
Any diagnosis needs to differentiate between ephelides, the simple lentigo, seborrheic keratosis, and the malignant lentigo Lentigos and ephelides appear more prominently when observed under a Wood lamp. However, lentigos are both larger and darker, generally appearing after the age of 40 years old. Ephelides, on the other hand, appear from infancy and have a strong tendency to merge, darkening due to exposure to the sun, with limited melanocytic hyperplasia occurring. Seborrhoeic keratoses, on the other hand, are somewhat flaky macules with a cribriform surface. A malignant lentigo is usually at least 5 mm in diameter, with marked pigment variation. These lesions contain dysplastic or atypical melanocytes in a radial horizontal pattern throughout the dermoepidermal junction (2, 7).
Solar lentigos are completely benign lesions. Their extirpation or removal using a range of therapeutic methods only occurs for aesthetic reasons. The ideal treatment for solar lentigos should be inexpensive, provide quick results, ensure social activities remained unaffected, and be exempt of any risks deriving from complications. Given the lack of a specific treatment to date and numerous options being available, choice depends on the patient and their willingness to comply with the indications (8, 9).

Current treatment strategies include using sunscreens, topical pigmentation lightening treatment, cryotherapy, chemical peelings, and light therapy devices (10, 11, 12).

Frequently used physical therapies offer excellent clinical success rates, but they often come with side effects and recurrence rates. The ideal treatment for solar lentigos should be effective, radical, and without side effects. A range of topical treatments for solar lentigos currently exist (12, 13, 14, 15).
The **MELINE® SPOTS** treatment involves controlled chemical peeling, giving rise to a treatment protocol effective at clearing lesions, which is highly likely to bring aesthetic satisfaction to the patient.

MATERIAL AND METHODS

The protocol was open, controlled, and comparative to photoprotection use, with the participation of 25 patients, 21 women and 4 men, with lesions on their face, backs of hands, and forearms. The randomised study ran from January to March 2016. Each patient provided informed consent.

The patients had Fitzpatrick II-IV type skin (table 1). The target lesions had to be at least 5 mm long and surrounded by skin with normal pigmentation. All patients had used chemical sunscreens during the previous summer. The inclusion criteria were:

1. The presence of solar lentigos.
2. Voluntary participation.
3. Previous continuous pigmentation lightening therapies at least 12 months prior to starting the study.
4. An agreement not to use other treatments during the study, as well as an agreement to apply sunscreens every day.

The exclusion criteria were:

1. Pregnancy.
2. Active skin diseases.
3. Atopy, allergic contact dermatitis.
4. Retinoid therapy, hormonal therapy.
5. Currently participating in another clinical study, any pigmentation lightening treatment in the last 12 months, exposure to ultraviolet light, and an unwillingness to cooperate.

The established protocol for applying **MELINE® 01 SPOTS** on lesions was as

1. Clean thoroughly with a cleaning agent. Rinse with abundant water and dry. Remove any grease from the skin.
2. Apply a fine uniform layer of **MELINE® 01 SPOT Step 1** directly onto the lentigos as evenly as possible by dabbing. Once the frosting process starts, move to the next step.
3. Apply a layer of **MELINE® 01 SPOTS Step 2** to cover the lentigos using a cotton bud, allowing it to act for 1 hour.
4. Subsequently remove the covering and wash with abundant water.

Post treatment:

Use **MELINE® MOIST** and **MELINE® B.B. Cream** every day for 7 days until the desquamation process ends. On completion, apply **MELINE® 02 SPOTS** on the lesions every night for 30 days. Simply use sunscreen three times a day on the opposite side

Each patient was subjected to a clinical dermatological exam beforehand (T0), after 30 days (T1), and after 60 days (T2) to evaluate the solar lentigos. Digital and ultraviolet photos taken during each visit using a QUANTIFICARE 3D LifeViz® mini device enabled accurate digital image analysis of skin colour. A Mexameter® (MX18, Courage + Khazaka, Cologne, Germany) was also used to evaluate the solar lentigos to calculate and compare the numerical erythema and melanin scores recorded before treatment, and then 30 and 60 days after treatment ended.

The Mexameter has a fixed 5 mm diameter aperture and was only used on solar lentigos ≥ 5 mm. Analysis of the normal skin colour surrounding the lentigo acted as a control for each patient. The investigator evaluated the treatment's clinical effect, as did the patient, using a four-point scale: excellent, good, sufficient, and insufficient.

RESULTS

All patients completed the study. There was no evidence of erythema, itching, scarring, or postinflammatory hyperpigmentation at T1 and T2. The results were excellent and uniform with almost complete removal of the lesions and a good cosmetic appearance. The application is simple, requiring a single session, without the use of anaesthetics.

There was evidence of clinical success in a larger proportion of patients treated with **MELINE® SPOTS** compared to the opposite side, which only received sunscreen.

Image analysis showed that the hyperpigmentation significantly reduced at T2 compared to T0.

Photo 1. Day 1.

Photo 2. Day 4.

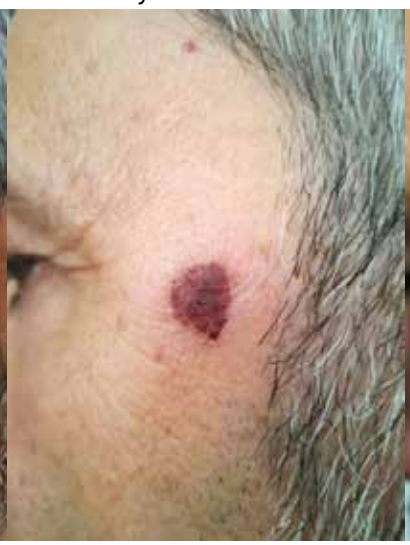


Photo 3. Day 6.

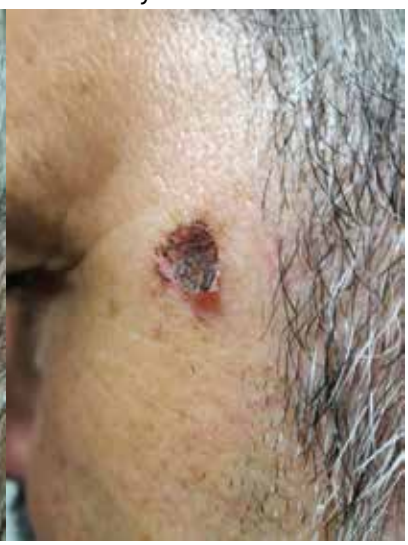
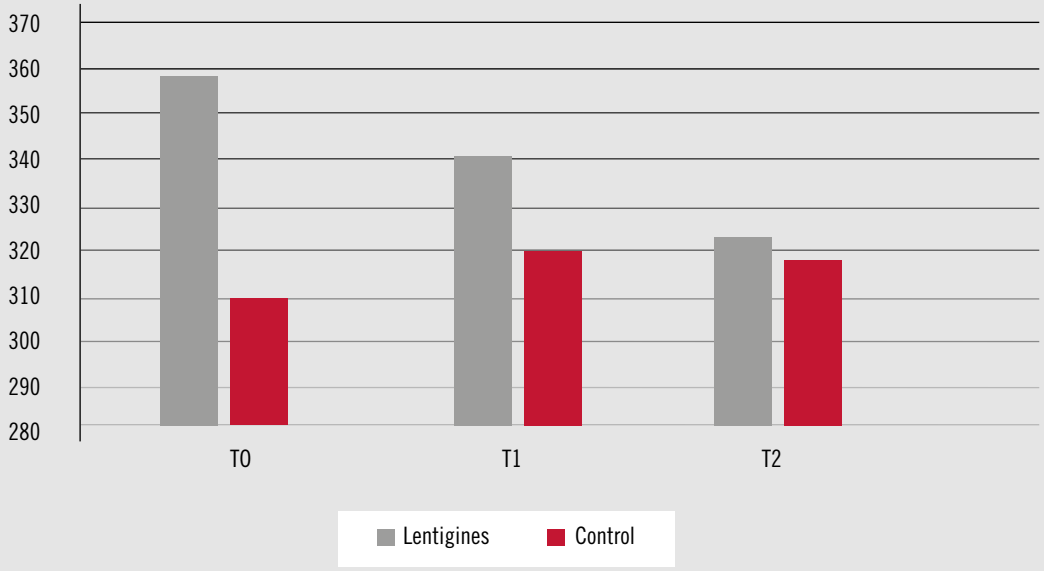


Photo 4. Day 16.

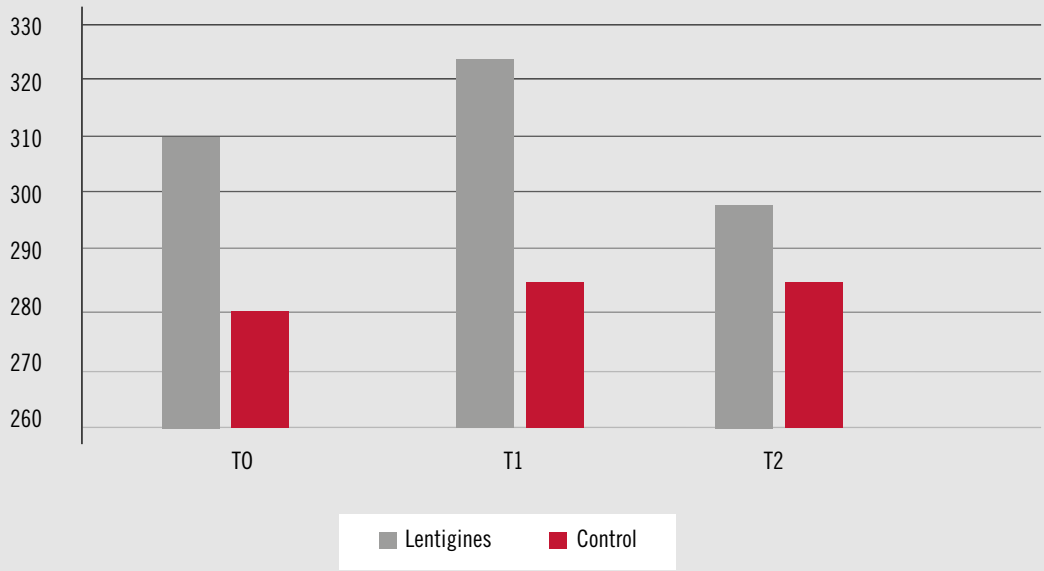
Photo 6. Final Outcome

The results were confirmed by a statistically significant reduction in the mean melanin score recorded using a Mexameter in T2 (322.967) compared to T0 (358.6125) and in the normal surrounding skin at T0 (300.95) and T2 (304.65) ($p < 0.02$). There was a significant reduction in mean erythema score at T2 too (299.425) compared to T0 (310.625) and in the normal surrounding skin at T0 (280.75) and T2 (283.25) ($p < 0.02$).

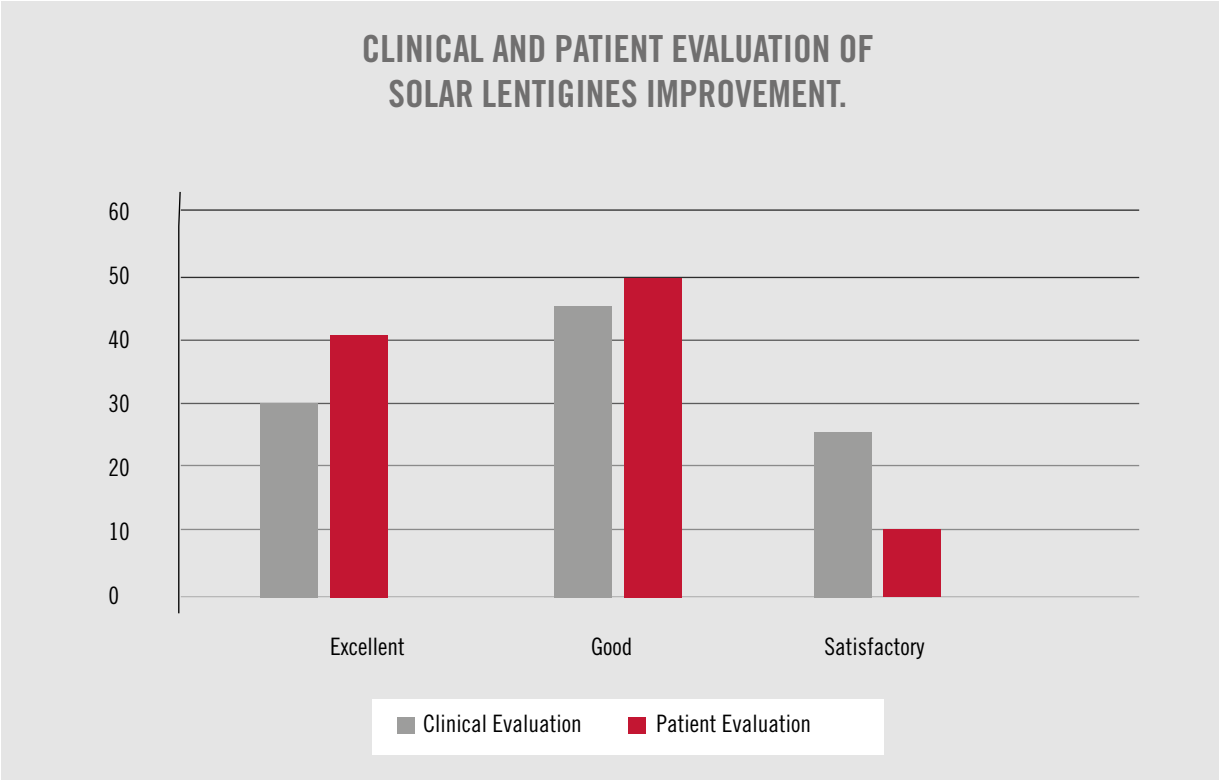
MELANIN SCORES RECORDED BY MEXAMETER AT BASELINE (T0), 30 DAYS AFTER TREATMENT (T1) AND 60 DAYS (T2) ON LENTIGINES AND SURROUNDING NORMAL SKIN.



ERYTHEMA SCORES RECORDED BY MEXAMETER AT BASELINE (T0), 30 DAYS (T1) AND 60 DAYS AFTER TREATMENT (T2) ON LENTIGINES AND SURROUNDING NORMAL SKIN.



The patient's lentigos showed good improvement in terms of pigmentation and size during the T2 clinical evaluation, with values of 40% excellent, 50% good, 10% sufficient, whilst for the observers it was 30% excellent, 45% good, and 25% sufficient.



DISCUSSION

Solar lentigos are a very common reason for consultation. After ruling out any possible malignancies in a lesion, several personalised treatment options are available for treating and removing the benign lesion. One of the main outcomes from treating pigmented lesions is finding a topical agent that induces significant clearance whilst also avoiding adverse reactions.

Reports indicate that retinoids have good depigmentation activity (16). Specifically RAL, a precursor to retinoic acid (RA), acts as a skin-clearing agent in several models (17), with reports stating it has both antioxidant activity and depigmentation properties (18).

Retinol is the main circulating retinoid, with two oxidative phases converting it into its active form, retinoic acid. The rate-determining step of this conversion is the reversible oxidation in retinaldehyde, a transitory retinoid, which quickly and irreversibly oxidises into retinoic acid. This in turn reduces tyrosinase ultraviolet B radiation and in vitro melanogenesis induced activity by binding to the nuclear receptors (19). There is also evidence that it stimulates epidermal exchange, along with pigmented cellular elements (20). The retinaldehyde molecule itself, as well as being a transitory form of the biologically active forms of vitamin A, exercises a specific non-melanocytotoxic activity to clear the skin. Equally, the epidermal hyperplasia induced by this active ingredient leads to the dissipation of melanin deposits into a larger tissue volume, which lowers its concentration (17).

4-(1-phenylethyl)-resorcinol, a phenolic compound, also shows antioxidant and pigmentation lightening properties [8]. In vitro studies show that phenylethyl resorcinol inhibits tyrosinase. A study undertaken on a pigmented 3D epidermis model with a daily topical application of a 0.1% phenylethyl resorcinol solution produces clear depigmentation activity after seven days of treatment. There is evidence for almost complete melanin synthesis suppression after 19 days. In vivo studies undertaken on Asian subjects showed that phenylethyl resorcinol clears human skin effectively at a 0.5% concentration. (21, 22).

Tranexamic acid, on the other hand, acts by inhibiting UV-induced plasmin activity in keratinocytes, through a process that stops plasminogen from binding to keratinocytes, as such reducing free arachidonic acid and prostaglandin production, with latter known to be tyrosinase activity stimulators (23). Many studies supports its use for treating ultraviolet radiation damage-induced pigmentation, and it has been implemented as a treatment using oral administration, topical administration, and with microneedles as direct intradermal therapy (24, 25, 26, 27). Piruvic acid is an alpha-keto acid with excellent keratolytic, antimicrobial, and sebostatic properties, as well as a great capacity for stimulating collagen and elastic fibre formation (28).

The peeling agent employed was a chemical medium, with concentrations ranging from 40 to 70%. Its capacity for treating several skin complaints such as wrinkles, pigmentation disorders, acne, and acne scarring (29, 30) is a recent discovery in the clinical evidence base. It has proven to be effective and safe, combining better results with fewer side effects (30).

Salicylic acid, on the other hand, has strong lipophilic properties, meaning it easily penetrates epidermal intercellular spaces, helping to dissolve fats, leading to cellular exfoliation. Additionally, an anti-inflammatory effect is likely given that salicylic acid inhibits arachidonic acid metabolism during inflammatory reactions.

As expected, better results were obtained with more recent lentigos located on the face with limited evolution compared to lentigos on the backs of hands with the same evolution and lentigos that appeared earlier with longer evolution

Histological studies have shown that hyperpigmentation occurs in the basal layer along with dermal papilla lengthening, with more epidermal melanocytes in the epidermal basal layer (31). Current investigation focuses on using non-invasive clinical tools to characterise and monitor skin diseases. The significant reduction in pigmentation detected during treatment follow up indicated the treatment's efficacy.

This study involved a clinical analysis of the depigmentation activity of **MELINE® SPOTS**, with the treatment showing good tolerability, as well a significant instrumental and clinical improvement in solar lentigo depigmentation, making it a safe, tolerable, and effective tool for treating solar lentigenes.

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CONTROLLED CHEMICAL PEELING: MELINE® IN THE THERAPEUTIC APPROACH FOR FEMALE INTIMATE ZONES.

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ABSTRACT

Introduction: Treatment of external genital area requires a comprehensive protocol that includes medical therapy prior to the procedure and subsequent therapy that will maintain the results and prevent future damage. It is important to recognize the importance for the female population therapeutic approach in this area and hence the basis for studies to corroborate the results and prove the existence of significant side effects.

Objectives: To determine the effectiveness of the **MELINE® INTIMATE** treatment in the aesthetic improvement of the hyperpigmentations of the genital area.
Methods: Controlled study, performed in 65 patients with cutaneous types between III and V, by application of the **MELINE® INTIMATE** treatment in vulva and groin. Measurement by observation and iconographic study, as well as patient satisfaction survey.
Results: We observed a 70% improvement in the texture of the skin treated and 63% in achieving uniformity of color with clearance of the area, significantly improving pigmentation. There were no major complications reported.
Conclusions: The use of **MELINE® INTIMATE** represents a good therapeutic alternative for the treatment of genital to improve its texture and pigmentation areas.

KEYWORDS

Vulva / Vulva

Área genital / Intimate Zone

Foto tipo / Phototype

Textura / Texture

INTRODUCTION

If we performed a web search for information about aesthetic procedures for the gynaecological area, we would definitely find over 300,000 entries from around the world. Aesthetic gynaecological procedures are undoubtedly an increasing cause for concern among patients, who visit the doctor with queries about this increasingly publicised area. The authors of this work have used this assumption as the basis for a study on the potential activity of MELINE® Intimate to improve the general external appearance of intimate areas of the body.

Surgical treatment procedures on the female genital area have been undertaken since the early nineteen seventies, but it was Hodgkinson who first published an article on cosmetic procedures in 1983 (1). There have been many proposals and improvements in treatment techniques since (2, 3), leading to the development of the application of a series of procedures in less than 5 years, which had mainly only been used to treat skin ageing before.
These are now being extrapolated to gynaecological cosmetics, where technological approaches have also led to interesting research in the area, and providing very interesting results in patients.

Differences in skin colouration in intimate areas are a significant factor of concern for patients, and particularly hyperchromias, which lead to an unappealing aesthetic appearance (4). There is a lack of uniformity of colour in these cases, with tonal differences, whereby some areas appearing with normal pigmentations and others with different intensity tones (5). Several factors affect melanin production, such as solar radiation, the melanocyte stimulating hormone, endothelin-1, fibroblast growth factor, and the underlying tyrosinase stabilising protein and enzyme activity (6).

Hyperchromia treatment involves using several substances, whether as a single treatment or in combination. The results completely depend on the problem requiring treatment and its depth (7). Chemical peeling is a skin treatment that aims to substantially improve tissue structure (7). The process leads to improvements in skin texture, colour, and surface imperfections (8).

Surface peelings induce desquamation, which subsequently speeds up the cell cycle. Those solutions can remove the surface layer of the corneal stratum, generating a softer skin texture, with more homogeneous pigmentation (9). Chemical peeling applications help to eliminate the effects of skin ageing, allow cells to proliferate with increased metabolic activity, and affect skin lipids, which all contributes to keeping the skin in good condition (10).

Acid exfoliations on the skin activate specific mechanisms, such as peptide bond hydrolysis and biochemical reactions, represented by an inflammatory response in the tissue. The chemical mode of action involves eliminating the existing epidermal cell structures and replacing them with new ones, after first releasing cytokines and mediators via keratinocyte stimulation. Interleukin-1 induces the release of interleukin-6, which stimulates fibroblasts to increase the production of matrix metalloproteinases (MMPs), which are involved in connective tissue remodelling processes. This in turn promotes an increase hyaluronic acid synthesis and deposition and the production of new collagen fibres, which stimulate the natural regeneration of the dermal tissue, improving its physiological properties (10, 11).

Vitamin A derivatives are fat-soluble substances, which have been determined to require a specific protein (CRABP) for transportation, with much higher levels found in the epidermis than the dermis (4). The mode of action of retinoids involves an increase in epidermal cell replacement and increased exfoliation of the stratum corneum, which leads to less melanin production (7). These compounds have successfully treated melasma and post-inflammatory hyperpigmentations in different concentrations (8). Another ingredient, salicylic acid, is used widely in dermatology due to its keratolytic action, with its potency based on the percentage use. Keratolytic agents do not act on the proliferation, they only act to remove the corneum layer, generally reducing the corneocyte cohesion and inducing epidermal cell rejection (12). Current thinking is that the exfoliating action of salicylic acid would cause de-cementation (the dissolving of the intercellular cement) and inhibit cholesterol sulfotransferase activity. This means salicylic acid reduces the pH of the corneum, increasing skin hydration and promoting keratolytic activity (13). The ingredient, lactobionic acid, characterised by the presence of d-galactose, a natural sugar required to synthesise glycosaminoglycans, is a potent moisturiser and has a significant antioxidant effect. The large size of the molecule means that it enters the skin slowly and safely, with a much milder action than alpha hydroxy acids, meaning it is not considered an irritant (14, 15). Recent studies undertaken using products containing lactobionic acid have shown a significant improvement in all photoageing parameters, with a significant increase in skin thickness, without signs of intolerability (16). Another of the formula's active ingredients, mandelic acid, a long chain alpha hydroxy acid, penetrates the skin slowly and in a controlled manner thanks to its size and is considered a safe surface-peeling agent (17).

Tranexamic acid is a relatively new active ingredient in hyperpigmentation treatment, with Nijo Sadako first describing it in this context in 1979 (18). It is a synthetic lysine derivative and acts by inhibiting ultraviolet radiation-induced plasmin activity on keratinocytes. It achieves this by blocking the bond between the plasminogen and the keratinocytes, reducing free arachidonic acid levels, which leads to prostaglandin production, known as tyrosinase activity stimulators (19). A number of studies have evaluated its activity on hyperpigmentations (20, 21, 22, 23) and shown significant decreases in MASI scores, without significant side effects, even showing evidence of good clinical activity when compared to standard depigmenting active ingredients such as hydroquinone.

Phytic acid, used in concentrations between 2% and 4%, has proven effective in treating epidermal melasma, particularly when used in combination with alpha hydroxy acids or retinoids. The typical burning sensation with exfoliations using alpha hydroxy acids do not occur with exfoliations containing phytic acid (24), and in general,studies have shown very interesting results when this active ingredient is present in hyperpigmentation treatments (25, 26).

Other important active ingredients worth mentioning include kojic acid, discovered by Saito in 1907, which has a chelation action on transition metal ions such as Cu2+ and Fe3+, being an effective remover of free radicals (27), and inhibiting both tyrosinase cresolase and catecholase activity. Another is arbutin, another hydroquinone derivative, but without the toxic effects of melanin (28), and it inhibits both tyrosinase activity and melanosome maturation (29).

In order to undertake this study, 228 patients attending the gynaecological office were surveyed to collect data to support the application of cosmetic methods. With respect to the results, 65.78% stated they knew about simple, mildly invasive aesthetic procedures to improve the external appearance of the genital area with a quick recovery, and 49.12% stated they were willing to undergo any of those procedures.

MATERIAL AND METHODS

This study involved including 65 patients that referred to hyperpigmentation issues in the genital area. Follow up occurred over a 120-day period. None of the patients had a significant medical history.

Patients were classified ranging from Fitzpatrick phototypes III to V. All patients signed the informed consent form and they received an explanation on the procedure and the possible side effects. A controlled protocol was put in place:

Inclusion Criteria:
· Female patients aged between 20 and 40 years old.
· Body mass index under 24.9 kg/m2.
· Patients without associated endocrinological diseases.
· Patients who had not received treatments for the genital area within the last 3 months.

Pre-session:
· The patient had to have shaved within the two days prior to the peeling application in the office.
· The procedure did not go ahead in the presence of any active gynaecological infection.
· Patients with genital herpes received antibiotic prophylaxis.
· The treatment did not take place in the event of any allergic reaction, wound, or erythema in the genital area.

The session:
· The patient had a photo taken in the lithotomy position beforehand.
· Alcohol and a gauze were used to clean the genital area of grease.
· An insulin syringed was used to extract approximately 1 cc **MELINE® 01 INTIMATE** peeling.
· Drips were placed on the surface of the vulva.
· It was spread homogenously across the darkened genital area with an applicator.
· The patient was asked for the level of burning or discomfort on a 1 to 10 scale (with 1 meaning the least discomfort and 10 the most discomfort).
· The product was left on the genital area for 8 minutes.
· Gauzes and fresh water were used to remove the product.
· Paper towels were used to dry the treated area.

Post-Peeling treatment:
· Initiate **MELINE® 02 INTIMATE** 48 hours after the genital area treatment, applying it at night and removing it the following morning each day.
· Suspend the use of **MELINE® 02 INTIMATE** 48 hours before visiting the office again for the next session with **MELINE® 01 INTIMATE**.
· Avoid using very tight fitting clothes.
· Avoid sun exposure.
· Do not undergo mechanical peelings, such as microdermoabrasion, etc.
· Do not manually remove any desquamation that may occur.
· Avoid hair waxing during the treatment.

The process duration:

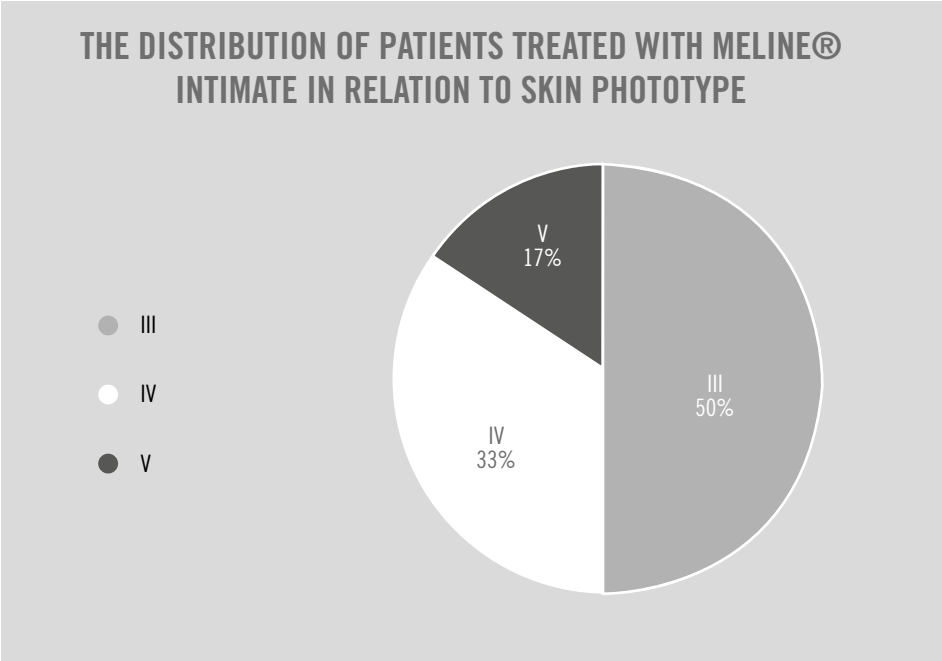
· 3 office sessions with **MELINE® 01** Intimate peeling, with 15 day intervals, during which the patient uses **MELINE® 02 INTIMATE** every day at home. Photos are taken before and after to compare the post-treatment results. Photographic and satisfaction follow up a month after completing the treatment protocol. **MELINE® 02 Intimate** is used at home either weekly or for two-week periods to maintain the results.

· The following aspects are taken as contraindications when undertaking the technique:

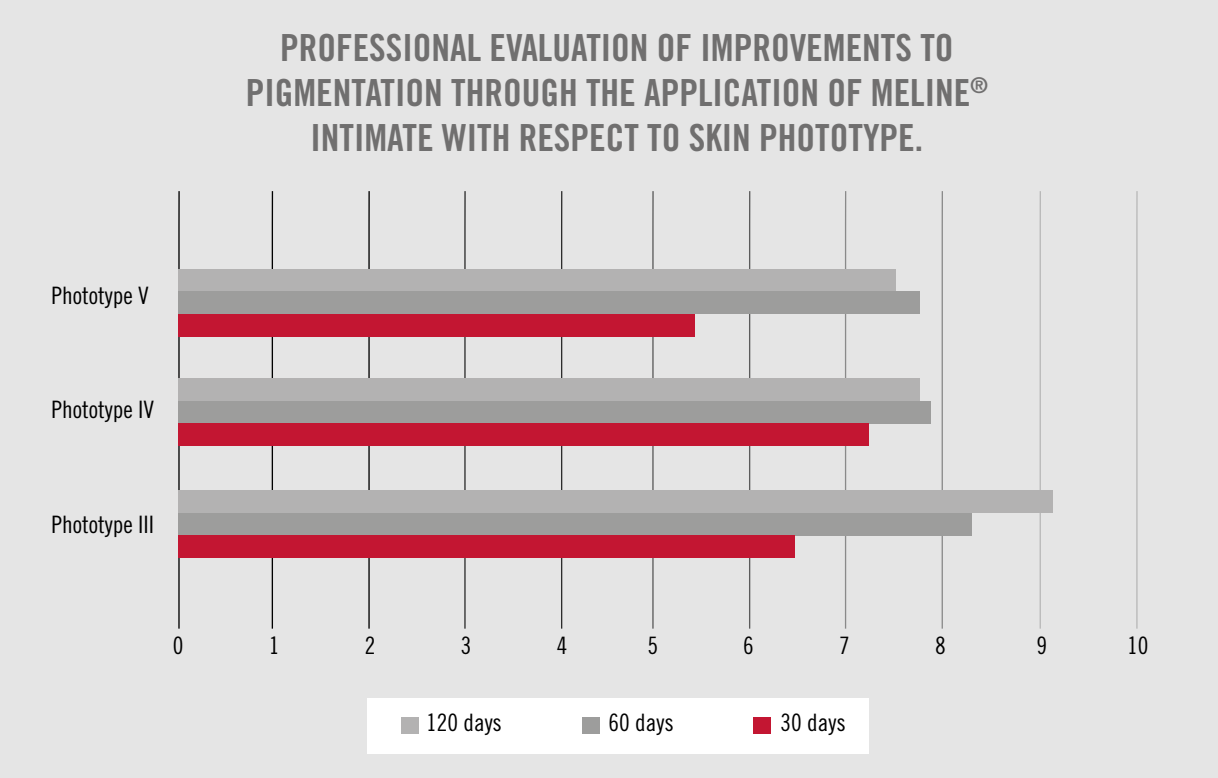
1. A known allergy to any of the active ingredients in **MELINE® INTIMATE**.
2. Recent sun exposure.
3. Photosensitivity and autoimmune diseases.
4. Imminent social commitments.
5. Any changes to the genital area.

RESULTS

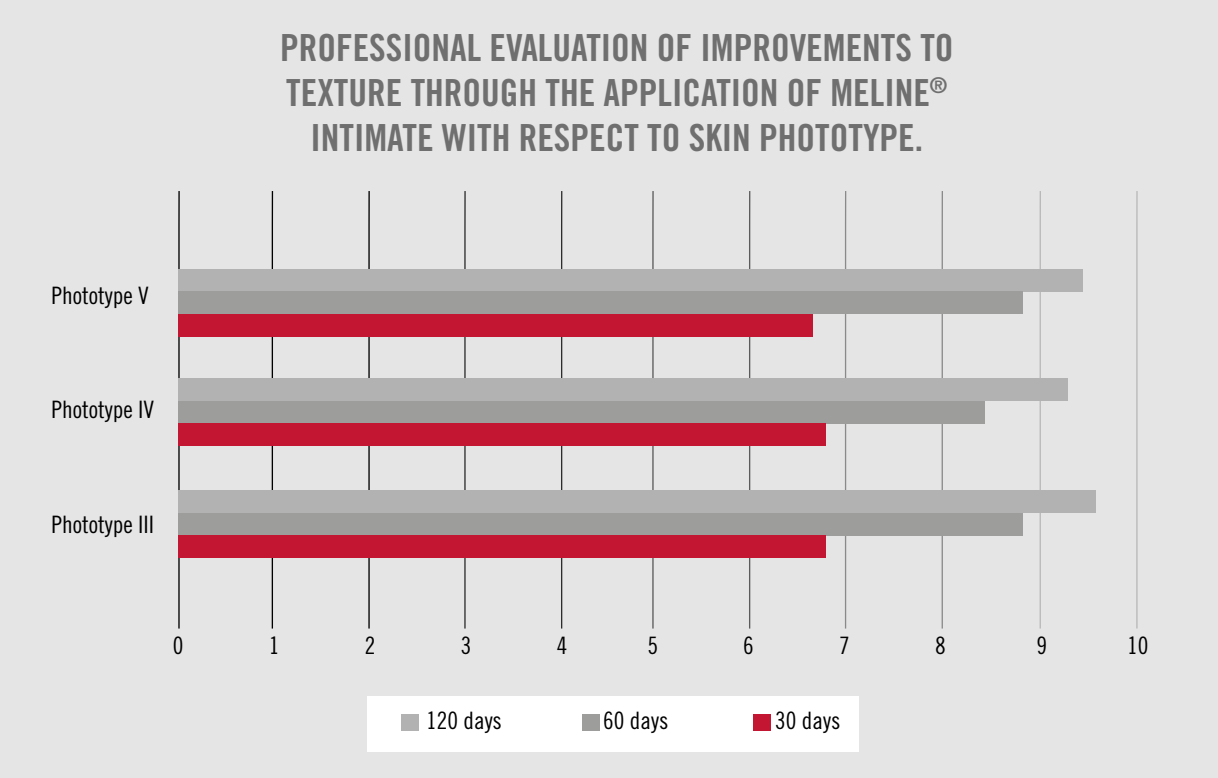
Patients subjected to treatment with **MELINE® 01 Intimate** were evaluated using the Fitzpatrick scale, establishing that 50 % of them had skin phototype III, 33% had skin phototype IV, and 17% had skin phototype V.



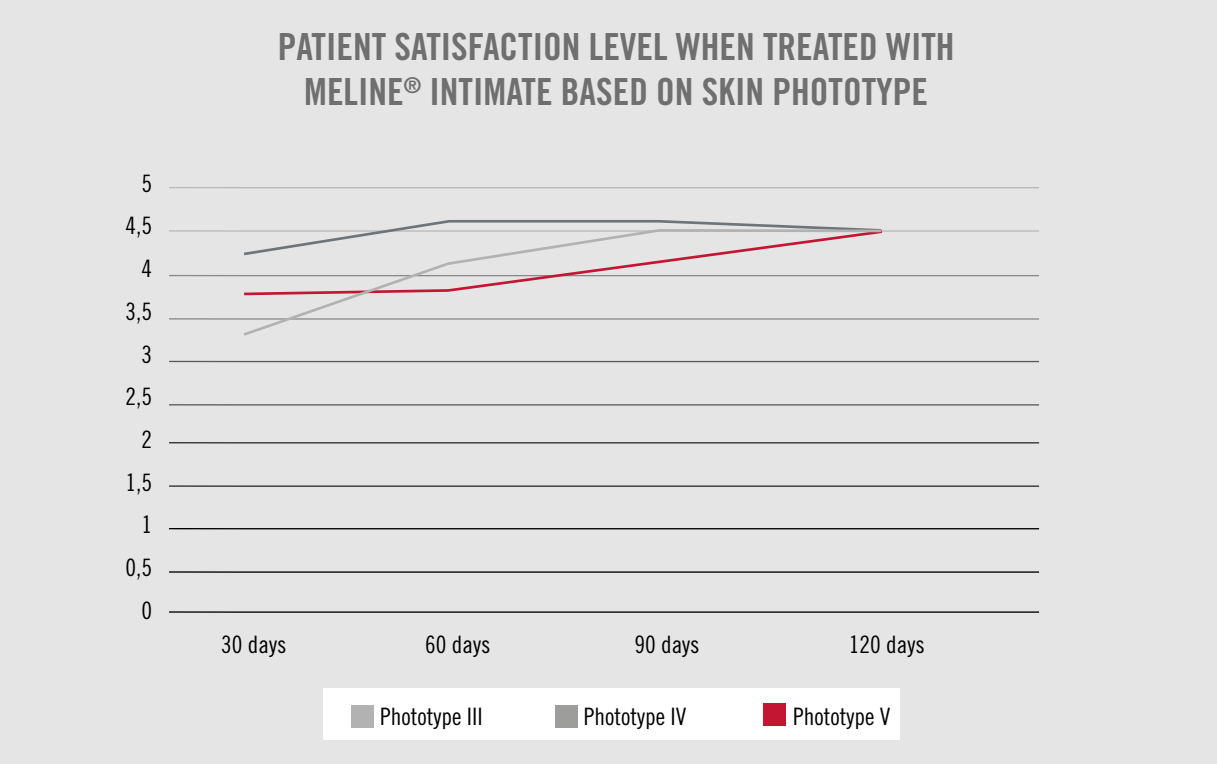
The results were measured on the satisfaction scale, with observations scrutinised by 3 different gynaecologists, who evaluated improvements in conditions using a 0 to 10 scale, where 0 represents no improvement and 10 represents extreme improvement.



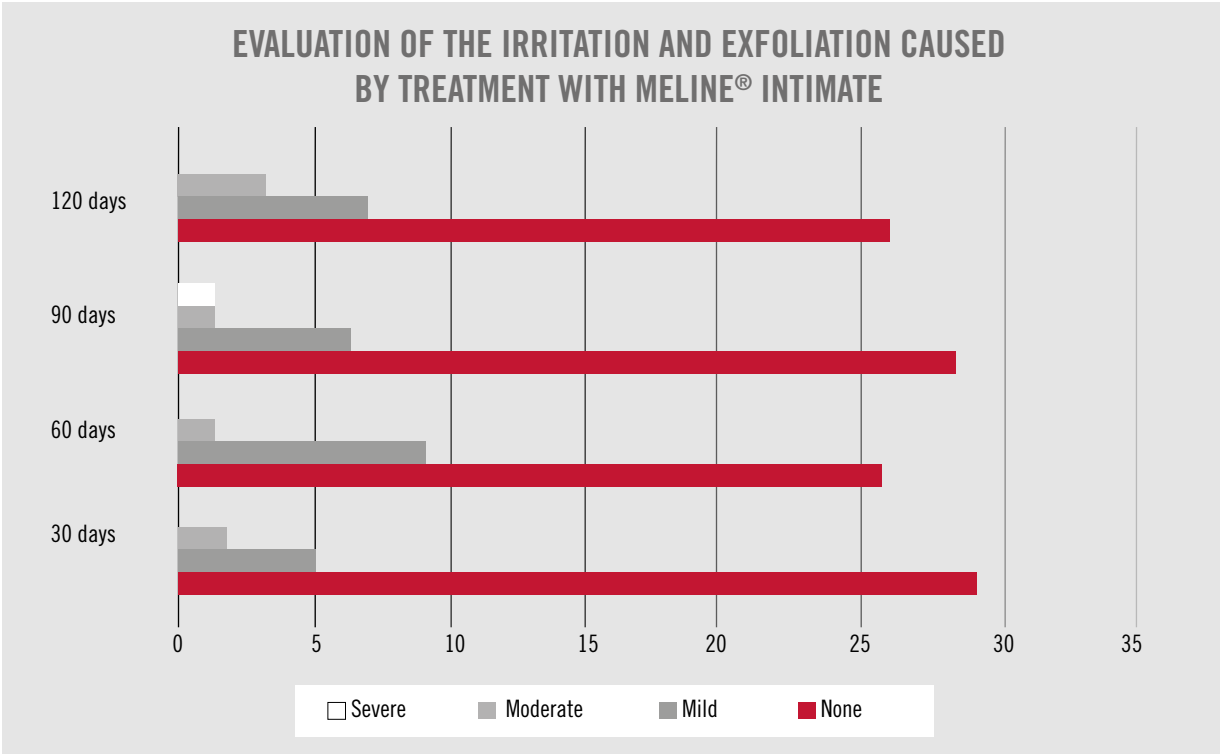
Based on the professional criteria, medium improvements to skin pigmentations were observed after 30 days, with patients with skin phototype IV showing the best change. After 60 days, the three groups showed very similar change, with better depigmentation results in the genital-anal area. However, there was a marked improvement in pigmentation in patients with skin phototype III at the final evaluation after 120 days of treatment, which was not the case for skin phototypes IV and V.



Improvements to skin appearance were also evaluated based on texture changes. All the study cases showed a higher than 50% improvement for this characteristic, which was much higher as the evaluation period progressed, with excellent improvements at the end of the study.



Patients also gave their opinion about the treatment using a 0 to 5 scale, where 0 represented no change and 5 outstanding changes. After 30 days on treatment with **MELINE® INTIMATE**, the study patient group's satisfaction level was over 50%, with a lower level of satisfaction during the evaluation period among those with phototype IV as expected. However, after 60 and 90 days, the satisfaction level for all phototypes was higher, with little change from that moment onwards. For dark phototypes, the evaluation increased from 30 days onwards until it levelled off, whilst it was significant from the first evaluation for skin phototype III.



With respect to adverse effects, a higher percentage of patients referred to no significant changes occurring during the treatment evaluation period. 8% of patients referred to mild irritation with light desquamation after the second treatment application in office.

DISCUSSION

Improving the appearance of the genital and surrounding areas is currently a significant motive for consultation. Pigmentation is one of the improvement aspects requiring improvement. The proposed treatment option led to the recovery of skin softness with a uniform tone and clearance. The combination of the **MELINE® INTIMATE** active ingredients provides a very beneficial effect and is useful on dark skin phototypes.

As the results show, the treatment leads to very quick and effective improvement, which was over 50% for the three skin phototypes according to evaluation by gynaecologists, with a tendency to increase over time on exposure to **MELINE® INTIMATE** products.

The combined actions of the product in office with the treatment at home led to the synergy of the active ingredients' activity. This enabled safe and very effective overall improvement to the skin's condition in the area, both in terms of texture, pigmentation reduction, and colour uniformity.

With respect to the data obtained in the study, we can conclude it is a safe treatment. However, any type of sensitivity in the area for treatment must be considered when it comes to using treatment protocols. All the same, the evaluation of adverse effects such as active exfoliation, irritation, and erythema revealed that they appeared in a very small percentage of patients, but without causing major discomfort.

CONCLUSION

MELINE® INTIMATE can be considered an effective and safe product for treating aesthetic hyperpigmentations in the genital-anal area for results in the short term that remain for at least 120 days.

VISIBLE RESULTS



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A STUDY OF THE EFFICACY AND SAFETY OF **MELINE® DARK CIRCLES** THERAPEUTIC PLAN FOR TREATING PERIORBITAL HYPERPIGMENTATION.

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INTRODUCTION

Periorbital hyperpigmentation, also known as periocular hyperpigmentation, periorbital melanosis, dark circles, infraorbital darkening, infraorbital discolouration, or idiopathic cutaneous hyperchromia, is a common condition encountered in aesthetic medicine practice, and can affect an individual's emotional well-being and influence their quality of life (1). This type of periocular skin change affects individuals of many different ages, both genders, and every race. Additionally, it worsens with the ageing process whereby skin flaccidity and changes to the subcutaneous fat distribution play an important role.

There is a scarcity of scientific data available about the clinical profile and pathogenesis, with several exogenic and endogenic factors potentially involved. The causative factors include genetic or heredity (2), excessive pigmentation (3), postinflammatory hyperpigmentation secondary to atopic and allergic contact dermatitis (4), periorbital oedema, excessive vascularity (5), and shadowing due to skin laxity and tear trough associated with ageing (6).

Excessive pigmentation is also present with conditions such as dermal melanocytosis. In some cases, pigmentation can be exacerbated by the swelling of the lower eyelids due to the pseudo-afference of orbital fat (7). Swollen lower eyelids add a shadowing effect and worsen appearance. Environmental causes of dermal melanocytosis include excessive solar exposure and drug ingestion. Infraorbital dark circles in patients with atopic or allergic contact dermatitis present as postinflammatory hyperpigmentation through rubbing or scratching the periorbital area.

Dark circles can also derive from thin and translucent lower eyelid skin covering the orbicularis oculi muscle of the eyes, making the subcutaneous venous plexus or vasculature inside the muscle visible. This condition generally involves the entire lower eyelid, appearing a violet colour.

Huang el al (8) carried out a clinical analysis and proposed a classification based on the clinical pattern of pigmentation and vasculature. Periorbital hyperpigmentation is classified as pigmented (brown colour), vascular (blue / pink / purple colour), structural (skin colour), and a mixed type, based on the doctor's evaluation of its clinical appearance. The mixed type included the following four subtypes: vascular pigmented (VP), structural pigmented (SP), structural vascular (SV), and a combination of the three.

The pigmented (P) type appears as a infraorbital brown tone. The vascular (V) type appears as infraorbital blue, pink, or purple colour, with or without a periorbital oedema. The structural (S) type appears as a structural shadowing formed by surface anatomical facial contours, and can involve infraorbital palpebral dark circles, blepharoptosis, and a loss of fat with bone prominence. The mixed (M) type combines two or three of the previous types. This classification can help to introduce the therapeutic modes based on the diagnosed type, given that they respond to different treatment types.

In general, different approaches to treating this change have been proposed, one of which involves using products with a topical action and chemical microexfoliations. Topical use products are designed with depigmenting agents with modes of action that inhibit tyrosinase activity, inhibit DNA synthesis of hyperactive melanocytes, reduce melanin content in the epidermis, and thicken the epidermis.

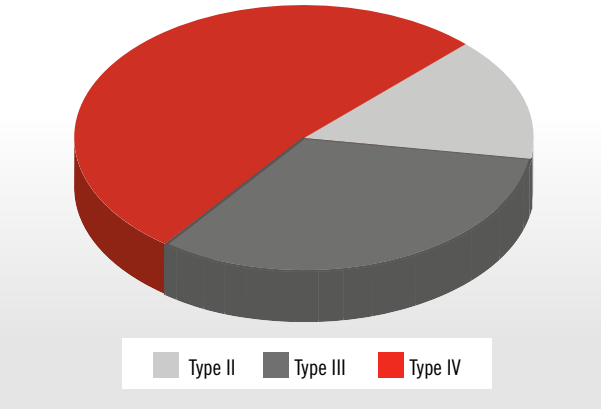
Retinoids are another key active ingredient in those treatments, which reduce pigmentation by inhibiting tyrosinase gene transcription, also deriving in significant thickening of the granular layer and the epidermis. Other compounds used as depigmenting agents include azelaic acid, kojic acid, tranexamic acid, ascorbic acid, which aim to increase the efficacy and limit the side effects when treating a range of hyperpigmentation disorders.

This study evaluates the efficacy and safety of a new treatment involving controlled chemical microexfoliation and topical product application (MELINE® Dark Circle, Laboratorio Innoaesthetics SL, Barcelona, Spain). This exfoliation combination improves the potency of the action without having to use high active ingredient concentrations, meaning that they do not cause any potential adverse effects when healing and permanent depigmentation.

MATERIAL AND METHODS

The study included 30 female and 10 male patients ranging in age from 25 to 45 years old (average age, 35 years old) with infraorbital hyperpigmentation. The patients were classified as Fitzpatrick skin phototypes II to IV. Figure 1 shows the distribution. Twenty two patients reported a family history of dark circles (55%). Each patient gave consent after being informed about the procedure. A detailed history was taken, and in some cases, clinical and blood tests were undertaken.

FIGURE 1. Distribution of the study population based on Fitzpatrick Skin Phototype



Inclusion criteria:

- Voluntary nature.
- Adults aged 25 to 45 years old with a diagnosis of dark circles.
- Not treated for dark circles in the last 6 months.
- Not using any topical or cosmetic treatment on the periocular area.
- No signs of anatomical changes through ageing, such as dark circles through accumulated fat or a deep tear trough.

Exclusion criteria:

- Congenital anomalies of the eyelids or any other relevant anatomical involvement.
- Corneal diseases.
- A history of exuberant scarring and allergic processes.
- Mental disorders that impede the procedure.
- Any involuntary eyelid movements.
- Dermatological diseases.
- Pregnancy or breastfeeding.
- Hypersensitivity to any of the components of the products used during the treatment.

Digital photos were taken of each patient both before and after the treatment. Each patient was scored on the severity index prior to treatment. According to the classification provided by Huang et al, 45% of patients have a pigmented type and 55% have a mixed vascular - pigmented type. As indicated in the exclusion criteria, patients with structural dark circles that agreed to participate in the study were excluded. The periorbital hyperpigmentation intensity was evaluated as mild, moderate, or severe, based on the colour of the orbital region compared to the patient's natural colour using Fitzpatrick's scale. Table 1 shows the diagnostic values.The data in table 1 therefore indicates that 52.5% of patients were diagnosed with mild dark circles, 32.5% with moderate dark circles, and 15% with severe dark circles.

The microexfoliation procedure (01 MELINE®® Dark Circles, Laboratorio Innoaesthetics, Barcelona – Spain) involved 2 steps. The step 1 components are acetic acid derivatives and lactic acid, and the step 2 components are vitamin A, ascorbic acid, and phytic acid. Both products are in the form of a solution. The application procedure was as follows:

1. Mark out the treatment area.
2. Clean and remove any greasiness from the area.
3. Apply three layers (3) using cotton buds, waiting 30 seconds between each application for step 1. The patients were also instructed to keep their eyes closed during the application.
4. Then apply two layers of solution 2 using cotton buds and leave each layer to act for 15 minutes.

DARK CIRCLE SEVERITY DIAGNOSIS BY SKIN PHOTOTYPE

		Mild	Moderate	Severe
Number of patients	SKIN PHOTOTYPE II	4	2	0
	SKIN PHOTOTYPE III	9	7	5
	SKIN PHOTOTYPE IV	8	4	1
%	SKIN PHOTOTYPE II	10	5	0
	SKIN PHOTOTYPE III	22,5	17,5	12,5
	SKIN PHOTOTYPE IV	20	10	2,5

Table 1. Distribution of patients by dark circle severity based on Fitzpatrick Skin Phototype.

5. Then clean the area with fresh water and apply sunscreen.
6. The patients received the procedure every two weeks, with a total of four treatments.

Every patient was instructed to avoid direct sunlight, to apply a sunscreen (SPF 50+) before going out into sunlight, and to use sunglasses. Home treatment (02 MELINE® Dark Circles, Laboratorio Innoaesthetics, Barcelona – Spain) was also indicated. This consisted of a gel containing active ingredients to improve the circulatory condition, such as ruscus aesculeatus, meliloti, flavonoids, and procyanidins, and depigmenting active ingredients like alpha arbutin, tranexamic acid, and kojic acid. This product is applied to the treatment area at night two days after the procedure in the office and then stopped 24 hours before returning for the next procedure in the office.

An improvement scale was created to observe the patients being studied, as shown in Table 2.

Evaluation	Description
Worse	Stronger colour, post-inflammatory hyperpigmentation.
No change	No apparent improvement
Deficient	Up to 25 % improvement.
Fair	26 to 50 % improvement.
Good	51 to 75 % improvement.
Excellent	76 % improvement or above.

Table 2. Improvement evaluation scale after treatment for dark circles.

Patients participating in the study also created their own evaluation scale as shown in Table 3:

Evaluation	Description
Very satisfied	Highly pleased with the treatment.
Mildly satisfied	Satisfied, but expected a more significant outcome.
Dissatisfied	Did not note any change with the treatment.

Table 3. Patient satisfaction scale relating to treatment with the MELINE®® Dark Circles plan.

RESULTS

The skin was observed to lighten in most patients, improving as the weeks of treatment completed. Patient observation and photographic evaluation was able to show that none of them could be classified as "worse" after the treatment.

Evaluation of the results

DARK CIRCLE SEVERITY DIAGNOSIS BY SKIN PHOTOTYPE

Evaluation at	Worse	No change	Deficient	Fair	Good	Excellent
SKIN PHOTOTYPE II						
30 days	0	2 (05.00%)	1 (02.50%)	3 (07.50%)	0	0
60 days	0	0	0	3 (07.50%)	1 (02.50%)	2 (05.00%)
90 days	0	0	0	1 (02.50%)	3 (07.50%)	2 (05.00%)
120 days	0	0	0	1 (02.50%)	3 (07.50%)	2 (05.00%)
SKIN PHOTOTYPE III						
30 days	0	4 (10.00%)	9 (22.50%)	8 (20.00%)	0	0
60 days	0	0	6 (15.00%)	7 (17.50%)	6 (15.00%)	2 (05.00%)
90 days	0	0	2 (05.00%)	7 (17.50%)	9 (22.50%)	3 (07.50%)
120 days	0	0	2 (05.00%)	7 (17.50%)	9 (22.50%)	3 (07.50%)
SKIN PHOTOTYPE IV						
30 days	0	4 (10.00%)	7 (17.50%)	2 (05.00%)	0	0
60 days	0	2 (05.00%)	6 (15.00%)	3 (07.50%)	2 (05.00%)	0
90 days	0	1 (02.50%)	3 (07.50%)	5 (12.50%)	4 (10.00%)	0
120 days	0	1 (02.50%)	0	0	4 (10.00%)	0

Table 4. Evaluation of the results of applying the MELINE® Dark Circles plan to patients with dark circles.

The evaluation of the treatment program during the first 30 days provided values for the first three skin phototypes that improved from deficient (42.5% of all patients in the study) to fair (32.5% of all patients in the study). However, on completion of the treatment cycle and evaluation at 90 days, 42.5% of patients showed a great improvement, with 40% of all patients in the study showing a good improvement, and 12.5% of all patients in the study showing an excellent improvement. This is in addition to 32.5% of patients showing a fair improvement with only one treatment cycle. During the final evaluation, only 1 patient (2.5%) with phototype IV remained unchanged.

Most patients (77.5%) were very satisfied with the outcome. They were interviewed about their level of satisfaction with the clinical results after the treatment. Patients were requested to assess the clinical results and select one of the three categories, with the results shown in Diagram 2.

The safety was evaluated by assessing the tolerability and adverse events. Most patients described good to excellent tolerability, with only some mild discomforts, such as a prickly feeling or mild itching during treatment. They did not present significant erythema, rashes, or swelling. They reported that the itchy feeling occurred temporarily while applying the product, but that it notably subsided after a few minutes. The exfoliation was more pronounced 24-48 hours after treatment when it presented.

Figure 2. Results from applying the patient satisfaction scale relating to treatment using the MELINE® Dark Circles plan.

DISCUSSION

There is no ideal treatment for hyperpigmentation in the periocular zone at present. Very little has been published in medical literature and the most mentioned treatment types specifically target tissue replenishment and not pigmentation treatment. In this study, 40 healthy individuals with periorbital hyperpigmentation (dark circles) under the eyes were treated using a therapeutic plan consisting of controlled microexfoliation using low concentration active ingredients and home use topical treatment. The study results show an effective improvement in pigmentation reduction and the general appearance of the treated skin.

Chemical peels have been a key tool in the therapeutic armamentarium of aesthetic medicine doctors over the last twenty years, given that they can be used to treat some skin disorders and provide a very good cosmetic benefit. This study presents a treatment that combines controlled microexfoliation, whereby the use of its active ingredients was conceived to be gentle on the treatment area and obtain better results with periocular hyperpigmentation. Thoroughly understanding the active ingredients and their modes of action is important in order to be able to apply the necessary care and to avoid possible undesirable effects.

The carboxylic acids include acetic acid derivatives and it is possible to obtain different formulations to replace some of the methyl group hydrogen atoms with chlorine atoms. Many acetic acid derivatives have been used in medicine for exfoliation purposes, like trichloroacetic acid, a versatile and safe component, dichloroacetic acid, and other acetic acid derivatives in order to produce aesthetic treatments (9), and chloroacetic (monochloroacetic) acid that has been used more as a component for treating warts.

The chemical effect of chlorinated carboxylic acids is protein denaturation. Chlorinated carboxylic acids applied to the skin produce changes in the epidermis and melanin dispersion, improving hyperpigmentations. Similarly, they derive in epidermis and dermis regeneration through new collagen deposition. Acetic acid derivatives have also been used to treat epidermal and mixed melasma without any significant side effects occurring, with the benefit of increasing the efficacy of other topical active ingredients when used in combination, such as alpha-hydroxy acids, promoting skin regeneration (10, 11).

One alpha-hydroxyacid, lactic acid, produces very superficial exfoliation and forms part of the natural moisturising factor, meaning it helps to replenish and maintain the skin barrier and rehydrate (10). Lactic acid reduces cohesion between corneocytes, deriving in dead cell elimination and stimulation of the growth of new cells in the basal layer (12).

Retinoids are vitamin A derivatives that affect multiple pathways to reduce the appearance of dark circles in the infraorbital region. Firstly, they promote collagen synthesis and collagen bundle reorganisation to improve the skin's turgor and quality. They also reduce melanin content and the size of the Golgi apparatus and the endoplasmic reticulum in melanocytes. Vitamin A derivatives can increase type I procollagen gene expression mediated by inhibited ultraviolet induction of c-Jun protein (13), leading to the inhibition of dermal collagen degradation, inhibiting metalloproteinase transcription factor activation (14, 15). Retinoids also improve dyschromia on inhibiting tyrosinase activity. This derives in reduced melanin synthesis, less melanosome transfer, and increased keratinocyte elimination (16, 17).

Topical use vitamin C (ascorbic acid) has been shown to reduce erythema induced by ultraviolet radiations and the appearance of wrinkles (18, 19). Vitamin C plays an important role in producing collagen and has been shown to stimulate its production when it is added to human skin fibroblast cultures (20). Vitamin C also restores the antioxidant capacity of vitamin E (21, 22), a much more potent inhibitor of lipid peroxidation.

The active ingredients contained in the home use product are very important and essentially look to improve the factors that lead to melanin pigmentation and to benefit the vasculature.

We mentioned the compound aimed at controlling melanin production earlier. Topical use niacinamide, the biologically active form of vitamin B3, both presents antioxidant and anti-inflammatory properties (23) and can improve hyperpigmentation on reducing melanosome transfer to keratinocytes. The effects of topical use niacinamide include improved skin texture and tone, along with a reduction in fine lines and hyperpigmentation (24).

Kojic acid is derived from fungi of natural origin produced by the species, Aspergillus and Penicillium. It acts by inhibiting tyrosinase in limiting steps (25). In a study undertaken by Lim et al. (26), adding kojic acid to a gel containing 10% glycolic acid and 2% hydroquinone was found to further improve melasma pigmentation. Despite the absence of studies, kojic acid has been tested anecdotally to treat periorbital hyperpigmentation and found to be effective.

When it comes to arbutin, we can say that it inhibits tyrosinase activity, while also inhibiting melanosome maturation. A randomised open trial undertaken by Ertam et al (27) found that a gel containing topical use arbutin was effective in reducing pigmentation in patients with melasma.

Lastly, tranexamic acid, a synthetic derivative of the amino acid, lysine, acts by inhibiting ultraviolet radiation-induced plasmin activity in keratinocytes, through a process that stops plasminogen from binding to keratinocytes, as such reducing arachidonic acid and prostaglandin production, with latter known to be stimulators of tyrosinase activity (28). Additionally, plasmin is believed to convert ECM-bound vascular endothelial growth factor (VEGF) into freely diffusible forms, deriving in angiogenesis. Kim et al. (29) discovered an increase in the gauge and count of dermal vasculature in melasma lesions along with an increase in VEGF expression compared to non-involved areas in the same patients. Therefore, when

treating pigmentations, tranexamic acid can have a dual effect, reducing promelanogenic factor production and reducing erythema and vasculature.

The ingredients chosen to act on the vasculature, Ruscus Aculeatus, Ginkgo Biloba, Troxerutin, and Melilotus Officinalis, combine to act to improve lymphatic circulation, strengthening capillaries through improving vascular integrity and reducing capillary permeability. Furthermore, other studies have shown additional activities from these ingredients that contribute to the treated periorbital change. Of those, Melilotus Officinalis, which has been shown to have anti-inflammatory effects, reducing the activation of circulating phagocytes and reducing citrulline production (30), and Ginko Biloba, which may have directly protective effects on mitochondria, contribute to its antioxidant effects because the mitochondrial respiratory chain is the main target and the source of oxygen reactive species (31, 32).

We can therefore assume that the skin lightening process observed during the studied treatment plan is due to the activity against melanin pigmentation, vascular effects, and the changes that occurred in the skin due to its restructuring. No significant complications were observed, meaning the products provide good safety when used. The treatment's effects lasted at least 4-6 months in most patients in combination with the use of suitable sunscreen.

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