

# Melatonin, vitamin C (LAA15%) and resveratrol the gold treatment for psoriasis and skin disease related to endothelin 1 and tumor necrosis factor alfa



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## SUMMARY

*The molecular complex consisting of Vitamin C and melatonin is effective in regulating cellular processes by regulating the expression of various cytokines as well as other cell modulators such as Et1 (Endothelin 1), PGE (prostaglandin E), TNF $\alpha$  (tumor necrosis factor alpha) and the proopiomelanocortin (POMC).*

*Starting from these premises, our study has highlighted how melatonin, resveratrol and Vitamin C, if combined, are able to perform their function at the cellular and biomolecular level by intervening in the processes of melanogenesis and tissue repair, thanks to greater stability as demonstrated from our studies at 55 and 125 days, so to guarantee the availability of these substances during topical treatment, unlike most cosmetics and medical products that use these substances individually.*

*In fact, it has been seen how the treatment of skin hyperpigmentation, psoriasis in different regions of the body and scarring processes, particularly in diabetics, respond in the vast majority of cases with excellent results by restoring normal tissue integrity and a normal and homogeneous skin pigmentation.*

*Furthermore, by promoting the restoration of normal cellular homeostasis, the use of this molecular complex offers promising results both in cosmetics but above all in dermatology, finding use in the prevention of inflammatory and hyperpigmentation processes by reducing damage to mitochondrial DNA and allowing normal expression of both TNF $\alpha$  and of endothelin 1 both involved in the aforementioned processes and being themselves the cause of alteration of tissue partition mechanisms.*

*The results of our study comprising 70 patients with hyperpigmentation, psoriasis and diabetic skin lesions, encourage the use of our molecular complex as more than others it has shown superiority in the treatment of skin lesions of different genesis compared to other topical products used in dermatology and cosmetics.*

## KEYWORDS

**skin melatonin, resveratrol, psoriasis, diabetic wounds, melatonin vitamin C LAA, melasma treatment endothelin1, tumor necrosis factor alpha and melatonin and LAA 15% and resveratrol, wound healing and L ascorbic acid 15, hyperpigmentation**

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## INTRODUCTION

With the development of biotechnologies and the in-depth knowledge of the biochemistry of the hyperpigmentation process, various regulatory strategies for the biogenesis of skin spots have been developed.

In particular, the melanin synthesis process has been studied and, above all, clarity has been given to the cellular biochemical mechanisms that regulate the protection of DNA [1,2].

It has been known for some time that the keratinocyte and melanocyte, two epidermal cells, interact with each other (Keratinocyte Melanocyte unit) and with the external environment. With the production of two proteins, keratin and melanin, they play a main role in the protection of cellular DNA; in particular they absorb both uVb (290-320 nm) and uVA (320-420 nm). Proof of this is the rare onset of skin tumours in black-skinned subjects where both melanin and keratin are abundantly represented.

Several photoprotective substances are now used in the prevention of skin spots and actinic keratoses and/or skin tumours, psoriasis certainly related to genetic mutations and the cellular inability to repair them [1,2,5].

Various substances have also been studied for the treatment of sunspots or other types of skin lesions, but many of these have been found to be toxic and therefore their use has been banned; for example, in some countries hydroquinone (a derivative of benzene) has been used, now prohibited in Europe, Asia, Australia and New Zealand because it has been found to be carcinogenic and correlated with various autoimmune diseases. Kojic acid has also recently been banned in Switzerland, Japan and Korea due to its skin toxicity and hepatotoxicity.

On the other hand, there are several non-toxic active ingredients that are equally effective in the prevention and treatment of many skin diseases; for example, various substances extracted from grapes have been studied which have shown excellent healing abilities without causing major complications. In particular we wanted to study the action of melatonin and resveratrol both extracted from grapes, whose biological action and effectiveness were of great interest. Melatonin has been found to be effective in regulating many cellular processes; in particular it effectively regulates the expression of cytokines, regulates the secretion of proopiomelanocortin (PoMC), precursor of MsH (Melanocyte

stimulating Hormone); it modulates the process of melanogenesis favouring or inhibiting the expression of  $\alpha$ MsH and regulating but above all inhibiting the production of Et1 (Endothelin 1), PGE (prostaglandin E) and TNF $\alpha$  (tumour necrosis factor alpha). It also plays an important role in paradoxical or post-inflammatory hyperpigmentation [12].

The regulatory function of melatonin is mainly exercised in clinical use, which means that if there is depigmentation, melatonin and resveratrol favour the return to colour normality. In the event of over exposure to the sun, on the other hand, melatonin would favour the release of  $\alpha$ MsH by modulating the production of melanin and reducing the overproduction of Et1 endothelin, TNF $\alpha$  which represent the triggering cause of the hyperpigmentation process [3,4].

## Resveratrol, Melatonin and vitamin C

Resveratrol is a very powerful natural antioxidant found in grapes and protects the grape from solar radiation. This protective action is also carried out on the skin, favouring the vascularization of the vessels and tissue oxygenation. Resveratrol protects and stabilizes the cell membrane of the melanocyte and also stimulates cell proliferation and collagen production, thus producing an overall anti-aging effect. In the hair, it promotes the neovascularization of the bulb and improves its oxygenation. Identified by Harvard School of Medicine as the best anti-aging molecule, resveratrol is the active ingredient that

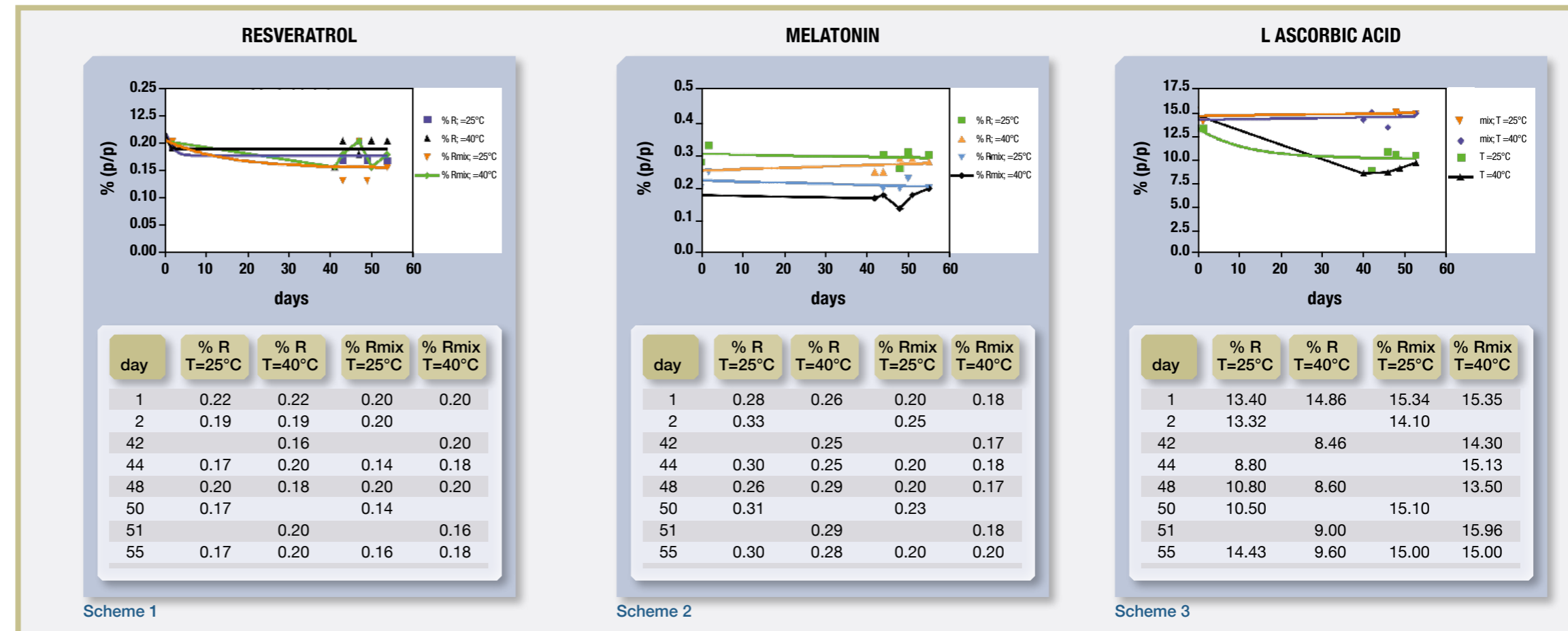
allows in time to have a skin reidentified, firmed and younger looking. Melatonin is another important antioxidant that performs a regulatory function towards the skin and defence of the immune system from external attacks; it also reduces the damage that UV rays produce on DNA and enhances biological functions and has a role in vasodilation phenomena. Finally, it effectively counteracts the weakening of the scalp and of the skin tissue in general [5,6,7,8].

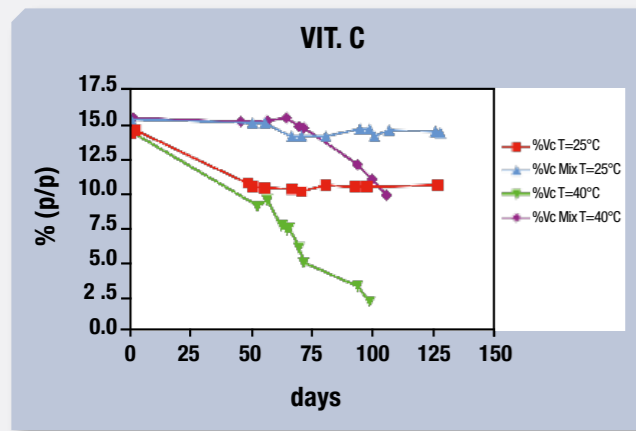
Vitamin C has anti-inflammatory and healing properties, as well as being a strong antioxidant substance. It acts against free radicals and stimulates the production of collagen. These substances that we have used and apply in the skin are not associated

with hydroquinone or with parabens or other kinds of preservatives being naturally derivative and acting in synergy, surprisingly reducing skin imperfections and improving its quality, regulating cellular mechanisms, repairing skin damage caused to DNA and accelerating the tissue healing process [9,10,11].

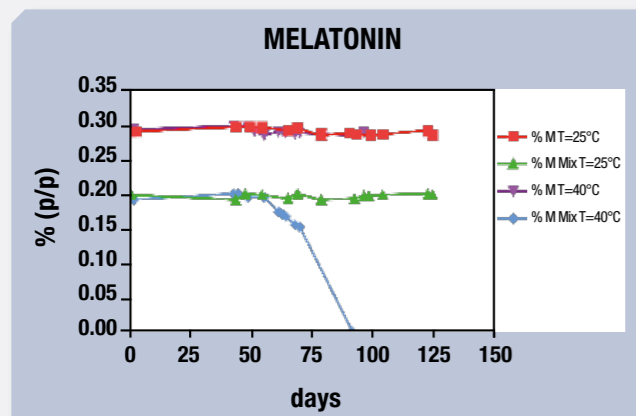
## Stability studies of vitamin C with melatonin and resveratrol

The stability of the complex used in the work was previously assessed through specific studies conducted

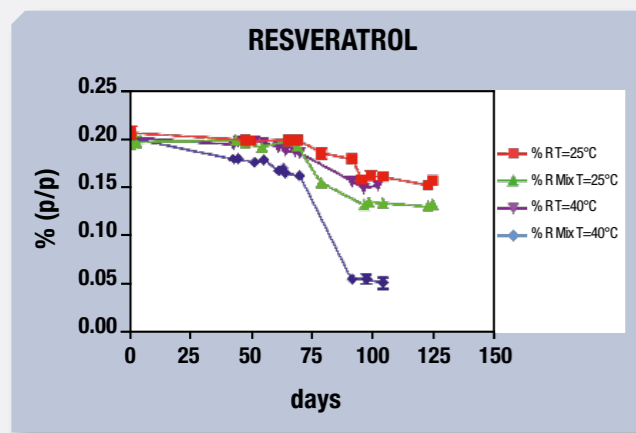




Scheme 4



Scheme 5



Scheme 6

for this purpose.

The results of these studies at 55 and 125 days are shown in the graphs shown in schemes 1 to 6, where the percentages found (p/p%) are plotted with respect to the days of storage of the formulations at the two temperatures 25 °C and 40 °C. In particular, the percentages were determined by comparison with the standard. By examining the graph shown in **scheme 1**, it can be seen that the concentration of R (Resveratrol) 0.20% in the formulation does not change significantly compared to the Mix, that is, in the presence of the other two compounds M (Melatonin) and AA (Ascorbic Acid), and there is no significant difference as the storage temperature varies over the course of 55 days. both in the Mix and in the single formulation at both temperatures there is a variation of 10%.

The stability of M (Melatonin) is shown in the graph in **scheme 2**. There is no significant change in the level of M in all the samples in this time interval. It should be noted that the percentage of M at time zero in the Mix was found to be lower (0.20%) than in the relative single-component formulation (0.28%). The same percentages were unchanged after 55 days, after storage at 25 °C and 40 °C.

A significant percentage decrease occurred in the case of AA (Ascorbic Acid) (**scheme 3**) in the single component formulation, where there was a decrease of 22% at 25 °C and of 36% at 40 °C. On the other hand, the formulation containing all three ingredients was stable and the percentage of AA (14.5%) did not undergo a significant decrease.

These data show that, in the time period examined, the levels of AA in the Mix are maintained by the simultaneous presence of M (Melato-

nin) and R (Resveratrol).

Encouraged by the stability data at 55 days, the stress test was extended up to 125 days (4 months) at both ambient temperatures. From the data that emerged from the stress test conducted for 4 months it can be observed that in the mixture containing melatonin, resveratrol and vitamin C (LAA) at 25 °C, melatonin and ascorbic acid L remained stable, while resveratrol underwent a drop of about 20%. instead in the mix at 40 °C there is a degradation of all three components: melatonin goes to zero, vitamin C is reduced by 30% (from 14.5 to 10%) and resveratrol loses 75% (**schemes 4, 5 and 6**).

The results of the stability tests confirm that when the three substances are mixed together, there is an increase of the stability of the ascorbic acid. In the thermal stress tests, the first 55 days are significant when vitamin C maintains its stability together with melatonin and resveratrol, even with temperatures reaching 40 °C.

The results are even more encouraging after 125 days when at a temperature of 25 °C melatonin and vitamin C maintain their stability while resveratrol has a drop of only 20%, this playing a key role in maintaining stability of the other two components during the four months of the test.

The antioxidant action of the association of melatonin and resveratrol in the stabilization of L-form vitamin C is proven by the fact that in the stress test at 40 °C for 4 months, melatonin oxidizes until it is reduced to zero, resveratrol oxidizes, losing 75%, while vitamin C is reduced by 30%, remaining in the serum at a concentration of 10%.

The clinical application of this serum has shown that melatonin not only has an action in stabilizing

L-scorbic acid, together with resveratrol, but the serum itself has an important biological efficacy in clinical application both for action of melatonin, both for the effects of vitamin C and resveratrol. surprising is the fact that ascorbic acid with melatonin seems to have a synergistic action not only in promoting the re-epithelialization and production of collagen, but even more surprising is the regulation of skin colour.

## Materials and methods

- 35 patients between the ages of 30 and 50 with psoriasis in different parts of the body such as cervicocephalic region, elbow, sacral region and lower limbs (**figg. 1-5**).
- 20 patients affected by skin spots of any origin and phototype have been treated since January 2019 (**figg. 6-7**).
- 15 patients between age 58 to 85 affected by diabetic skin lesion and skin wound (**figg. 8-11**).

The topical cream system includes:

1. A cleansing solution which reduces the pH of the skin; this cleanser is made based on polyphenols melatonin and resveratrol extracts.
2. A vitamin C LAA serum solubilized in *Vitis vinifera* and made stable in the form of aqueous serum with melatonin and resveratrol.
3. A restructuring cream that serves to rebalance the pH of the skin containing melatonin and resveratrol of natural biological origin.

The patients were selected after a dermatological visit and each was given the natural creams products with precise instructions for use to be performed in the morning and in the evening. Each patient was in-

structed to apply the creams twice a day.

The first phase of the skin care action involves cleansing the skin with the liquid cleanser. The second skin action involves the application of about 10 drops of vitamin C serum with an energetic massage all over the face, for the patients affected from cervicofacial psoriasis skin spots. Each patient was instructed in an extra application of serum on the spots and in any skin lesion.

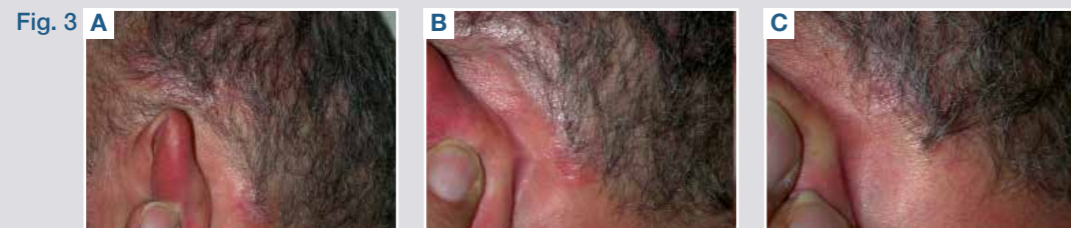
In Patients with psoriasis the advise was also to live down the skin to the cleanser exposure in order to be able with a gentle scrub to remove the part of keratotic tissue in order to facilitate skin absorption.

After cleaning, vit. C Serum at 15% LAA stabilized with melatonin and resveratrol and polyphenols was apply with gentle massage to enhance the complete penetration of the products. After Bit. C serum 15% absorption, a Melatonin cream containing Resveratrol, vit. C and poliphenols was apply with strong massage to facilitate uniform distribution and penetration. The third phase of the procedure consisted in the application of the restructuring cream applied with a energy massage until complete absorption was achieved.

For the patients affected from skin spots the third skin action consists in the application of the skin colour rebalancing cream based on melatonin, vitamin C and resveratrol. Each patient was instructed to apply an amount of cream corresponding in length to the third phalanx of the second finger of the hand, with a thickness of about 3 mm.

It was recommended to apply the cream all over the face with an energetic massage, doubling the application on the area of the spots, always



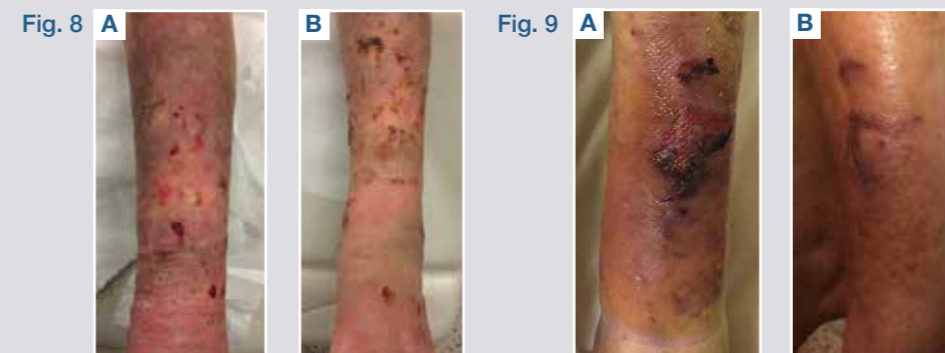
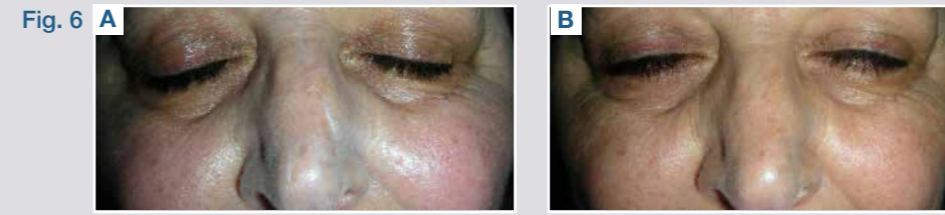


**Figure 1.**  
Patient with bilateral elbow psoriasis: photo pre treatment (A), control after 30 days (B) and 6 months of therapy (C).

**Figure 2.**  
Patient affected by psoriasis extended in the feet area: before (A) and after 6 months of topical treatment (B).

**Figures 3-4.**  
Patient affected from Cervico Facial Psoriasis: pre treatment (A) and results after 1 week (B) and one month of daily application of the creams.

**Figure 5.**  
Patient affected from facial psoriasis: pre (A) and after one month of application of the products (B).



**Figure 6.**  
Eye hyperpigmentation: before (A) and after (B).

**Figure 7.** Hyperpigmentation and facial skin spots: photos before (A) and after 4 months of topical treatment (B). Note the uniform tan colour due to the action of melatonin, resveratrol, vitamin C and polyphenols contained in the *Vitis vinifera*.

**Figure 8.**  
Patient with marked leg diabetic wound lesion, presence of senile keratoses: photo pre treatment (A) and control after 20 days of therapy (B).

**Figure 9.**  
Diabetic wound right foot: photos before (A) and after 12 days of treatment (B).

**Figure 10.**  
Diabetic wound of the right lower leg: before (A, B) and after 12 days of treatment (C).

**Figure 11.**  
Diabetic wound of the first toe left foot: before (A) and after 4 days (B) and 8 days (C) of treatment with L Ascorbic Acid serum stabilized with melatonin and resveratrol.



to be penetrated with an energetic massage until completely absorbed. The same directions were given to the patients affected from psoriasis and diabetic skin lesions in the body.

Before sun exposure, patients with spots and psoriasis were instructed to apply extra melatonin and resveratrol cream, after sun exposure patients were advised to cleanse their face or part of the affected body and repeat the treatment with vitamin C serum and cream again.

In cases where hyperpigmentation was present, an extra application of serum and cream was recommended on the darker area of the skin. Solar exposure was not forbidden in any case. It has been suggested to use vitamin C serum stabilized with melatonin and resveratrol to prevent skin damage after sun exposure.

## Results

All patients found the program easy to perform and effective.

There have been no cases of post-inflammatory hyperpigmentation, nor major complications or intolerances to the active ingredients as these are of a biological nature.

The skin colour obtained was homogeneous in 100% of cases even in the diabetic skin lesions. subjects affected by dermal melasma obtained significant results at the end of the treatment.

A significant thing to note is that patients who have exposed themselves to the sun have reported a transition to a tanned complexion without the intermediate stage of redness. They also reported that for the first time they had a uniform and darker tanned complexion than in previous periods when this system was not used for sun exposure and therefore melatonin and resveratrol had not been used.

## Discussion

The biological and clinical effects of melatonin and the protective antioxidant action of resveratrol allow the regulation of the skin colour, favouring a good protective action already in the days immediately following the treatment. these effects appear to be mediated by the reversible inhibition of excess tyrosinase and by the regulation of Et1 and  $\alpha$ MSH production.

Any stimulus, external or internal, that causes membrane oxidation or a banal accumulation of free radicals that can be perceived as the cause of any cell damage causes the keratinocyte to release a series of cytokines. For example, in the case of a trauma, the Et1 endothelin is released from the first moment which in turn, also determining the release of tNF $\alpha$ , represents one of the main causes of the onset of post-inflammatory hyperpigmentation, fibrosis and reduction of the wound healing process [13,14,15,16].

The exaggerated production of ET1 by keratinocytes seems to be the pathogenetic mechanism of solar lentigos and hyper-pigmentations, also due to solar insult. the keratinocyte also secretes NGF (Nerve Growth Factor), bFGF (basic Fibroblast Growth Factor), HGF (Hepatocyte Growth Factor), GM-CSF (Granulocyte-Macrophage Colony-stimulating Factor) and LiF (Leukemia inhibitory Factor), all cytokines involved in melanocyte proliferation or differentiation. The differentiation of the melanocyte is mainly determined by  $\alpha$ MSH (20) and ACTH (Adrenocorticotrophic Hormone) which, through specific membrane receptors (MCR), stimulate the production of tyrosinase with a consequent increase in melanin. The latter plays a protective role because it absorbs the toxic effect of Ros (Reactive oxygen species),

preventing the oxidation of membrane lipids. Endothelin 1 and Tumor necrosis factor alfa, both play a key role in psoriasis and in the process of delay wound healing and fibrosis [12,13,14,15,16]. Melatonin and Resveratrol in combination of L Ascorbic Acid, not only are necessary for the stabilization of the LAA at 15% of concentration, but also carry out an important function in the processes of wound healing and psoriasis treatment by modulating the function of the Endothelin 1 and TNF $\alpha$  [5,9,15,16].

## Conclusions

This new system to achieve the restoration of skin homeostasis can be used both in dermatology and in cosmetics as prevention and protection.

The topical use of melatonin, vitamin C and resveratrol has been found to be effective in regulating the cytokines that induce the overproduction of tyrosinase as well as having in itself a regulating action on the production of pigment in cases where greater natural effective protection is required. DNA that can be guaranteed by a normal distribution and overproduction of melanin.

Melatonin, resveratrol and vitamin C find efficacy as a wound healing process in diabetic patients. In patient affected by psoriasis the action of the melatonin - LAA and resveratrol complex play an important role in regulating TNF Alfa and endothelin 1 promoting healing and reducing inflammation process. These ingredients may play a key role in the treatment of several diseases related to aging and in the treatment of various diseases such as melasma, hyperpigmentation, paradoxical and post-inflammatory hyperpigmentation, Psoriasis and diabetic wounds.

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