

RESEARCH LETTER

## Anti-Spot, Lightening Effect and Cutaneous Acceptability of a Stable Anhydrous Ecobiological Formulation of 10% L-Ascorbic Acid

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Vitamin C or L-ascorbic acid (AA), its most biologically active form, is the most abundant antioxidant in healthy skin. Vitamin C is well-known for its anti-aging skin effects, like the inhibition of skin damage by free radicals, stimulation of collagen biosynthesis, inhibition of melanogenesis, and improvement of various inflammatory skin disorders. Thus, vitamin C is widely used in skin care products. However, vitamin C is highly unstable in aqueous solution, in the presence of oxygen and metal ions. Its oxidation is associated with a yellowish coloration of the formulation and loss of biochemical properties. AA stability in cosmetics remains a challenge despite several strategies, like the reduction of water content, the addition of antioxidants, the encapsulation in micro or nanoparticles, the use of more stable derivatives of vitamin C or multiple phase emulsions.<sup>2</sup>

To address this problem, an anhydrous formulation was developed, consisting of pure AA (10%) dissolved in two natural polyols compounds, propanediol and glycerin - a naturally occurring molecule of the skin. The anhydrous formulation stored in glass vials and white bottles remained colorless with a perfect stability of AA for 24 months at room temperature protected from light.

Few clinical studies reported the efficacy of topical application of vitamin C alone or its derivatives on hyperpigmentation disorders caused by exposure to UV radiations (photoaging).<sup>3,4</sup> AA decreases UVA-induced melanogenesis through its antioxidant properties<sup>5</sup> and may interfere with tyrosinase action, the main enzyme involved in melanin synthesis.<sup>1</sup>

A clinical study was conducted to assess the anti-spot/lightening effect and the acceptability of the new product. This clinical study complied with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local laws and regulations. The European Directive 2001/20/EC and regulations issued by the Minister of Health (Order of the Minister of Health of May 2, 2012 regarding Good Clinical Practice, Dz.U. 2012, item 491) was not applicable. Therefore, this study was considered as non-interventional and did not require the Ethics Committee Approval and the Competent Authority Authorization. All the subjects provided written informed consent prior to their participation in the study. Thirty-three healthy women, aged between 33 and 64 years, with sensitive skin, phototype I to III, and at least 1 pigmentary spot (lentigo > 3 mm) on the face were enrolled at the end of October 2020. Thirty-one subjects completed the study. They applied twice daily for 2 months 1 drop of the product on each cheek and on the forehead, then massaged until completely absorbed. Two zones were defined on the face, one with pigmentary spot and one with normal pigmentation, for color measurements using a Konica Minolta CM700-d spectrophotometer equipped with a 3 mm diameter head. The parameters L\* (luminance) and b\* (cutaneous melanin yellow color) were recorded on day 0 (baseline) (D0), then 28 (D28) and 56 days (D56) after product application. The Individual Typological Angle (ITA°) was calculated from the L\* and b\* parameters, ITA° being inversely related to pigmentation. The cutaneous acceptability of the product was assessed through an evaluation questionnaire on D56. L\* and ITA° mean values increased on D28 and

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D56 on the pigmented area, reaching values close to those recorded on the "normal" skin area, where both parameters remained stable (Figure 1). The increase from baseline of both parameters was significantly higher on D28 and D56 on the pigmented skin area than on the "normal" skin. Lighter (L\* parameter) and less pigmented spots (ITA° parameter) were obtained on D56 in 93% and 90% of subjects, respectively. The product was very well tolerated (only one subject presented mild burning sensation after the product application from D8 to D32 judged as not relevant by the investigator), assessed as pleasant by 84% of women, with a visible improvement of the skin (Figure 2).

This new formulation composed of biomimetic ingredients, like vitamin C, showed a great stability and a significant anti-spot/lightening effect. This study confirmed that the use of pure ingredients, rightly dosed, and naturally assimilated can help the skin restore its balance and adapt to its environment. This is consistent with an ecobiological approach, which considers the skin in relation to its environment, as an ever-evolving ecosystem, for which the natural resources and mechanisms must be preserved<sup>6,7</sup> and can be applied to develop specifically tailored skincare products.

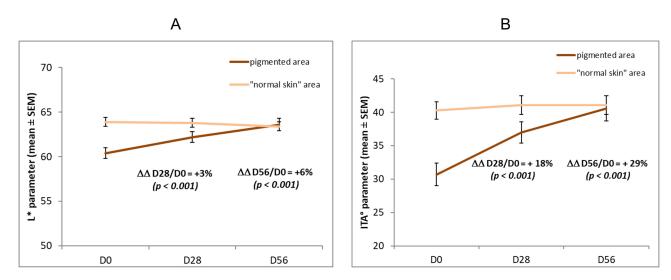


Figure 1 Evolution of the L\* (skin lightness) (**A**) and ITA° (skin pigmentation degree) (**B**) parameters. Mean values  $\pm$  SEM of the L\* (**A**) and ITA° (**B**) parameters on the pigmented and normal skin zones were recorded after twice daily application of the product for 28 (D28) and 56 days (D56). Three measurements were made using a spectrophotometer per zone and time-point. ITA°= [Arc tan ((L\*-50)/b\*)]  $\times$  180/ $\pi$ . For the L\* and ITA° parameters, the following changes ( $\Delta$ ) from baseline (T0) were calculated at each time-point (Ti) for the pigmented zone (PZ) and normal skin zone (NZ):  $\Delta$ PZ= (PZ Ti – PZ T0),  $\Delta$ NZ= (NZ Ti – NZ T0) and  $\Delta\Delta = \Delta$ PZ –  $\Delta$ NZ. An expected effect of the product corresponds to a  $\Delta\Delta$  on D56 compared to baseline  $\geq$  0.1 for the L\* parameter and  $\geq$  1 for the ITA° parameter.  $\Delta$  from D28 to D0, and from D56 to D0 were compared between zones using paired Student's t-test.

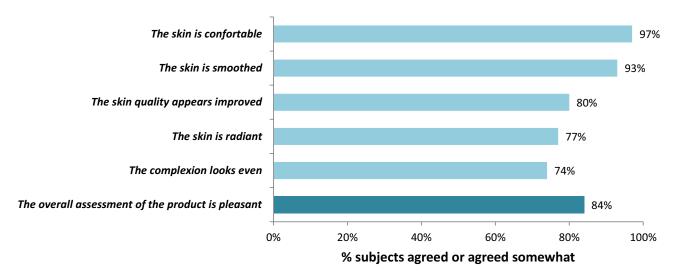


Figure 2 Skin acceptability of the product after 2 months of application.

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## **Disclosure**

All authors are employees of NAOS Ecobiology Company. The authors report no other conflicts of interest in this work.

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