HIGH GLOBAL ANTI-OXIDANT PROTECTION (RMS) AND STIMULATION OF THE COLLAGEN SYNTHESIS OF NEW ANTI-AGE PRODUCT CONTAINING AN OPTIMIZED ACTIVE-MIX

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INTRODUCTION

The skin aging exposome consists of external and internal factors and their interactions, affecting a human individual from conception to death, as well as the response of the human body to these factors that lead to biological and clinical signs of skin aging[1]. Oxidative stress in skin plays a major role in the aging process. This is true for intrinsic aging and even more for extrinsic aging [2-3]. Indeed, homeostasis of the skin can be negatively influenced by the presence of reactive molecules ("Reactive Molecular Species", RMS), including reactive oxygen species (ROS), which are the most abundant, nitrogen (RNS)) and carbon (RCS), which contribute very significantly to the aging process [4].

The action that marks the starting point in the skin alteration process is an increase in ROS that induces oxidative stress, responsible for damage to membranes such as lipid peroxidation (especially relevant at the mitochondrial level), changes in the structure and function of proteins (protein glycosylation and/or activation of enzymes that degrade the proteins of the Extra-Cellular Matrix -MEC-), alterations in DNA and / or modifications in the expression of genes [5].

On the other hand, two complementary processes are involved in the formation of wrinkles: degradation and the *novo* synthesis of matrix proteins, among which collagen stands out.
The use of cosmetics helps to protect the skin against external factors and maintain adequate physiological conditions to combat cell damage. Therefore, research is essential for the study and development of effective products in the protection against oxidation, the maintenance of the natural balance and the functions of the skin, as well as the improvement of the signs of aging.
An anti-aging formula has been developed containing peptides, a high concentration of vitamin C (10%), hyaluronic acid, and Vichy volcanic water (Peptide-C). It is formulated in daily-dose amber glass ampoules at optimal pH for maximum bioavailability of vitamin C and with a minimalist formula that requires no preservatives due to the low pH.

AIM

The aim of this study was to assess the efficacy of a new product with a mix of actives contained in an ampoule format (10% vitamin C + peptides + hyaluronic acid + mineralizing water) on the different factors involved in skin ageing, by evaluating the following aspects: 1. Potential antioxidant and derived effects. 2. Protection against global oxidative stress. 3. Effect on collagen synthesis.

RESULTS

1. Very high direct antioxidant capacity and efficacy on derived effects: lipid peroxidation, protein glycosylation, inhibition of hyaluronidase, elastase and collagenase activities.

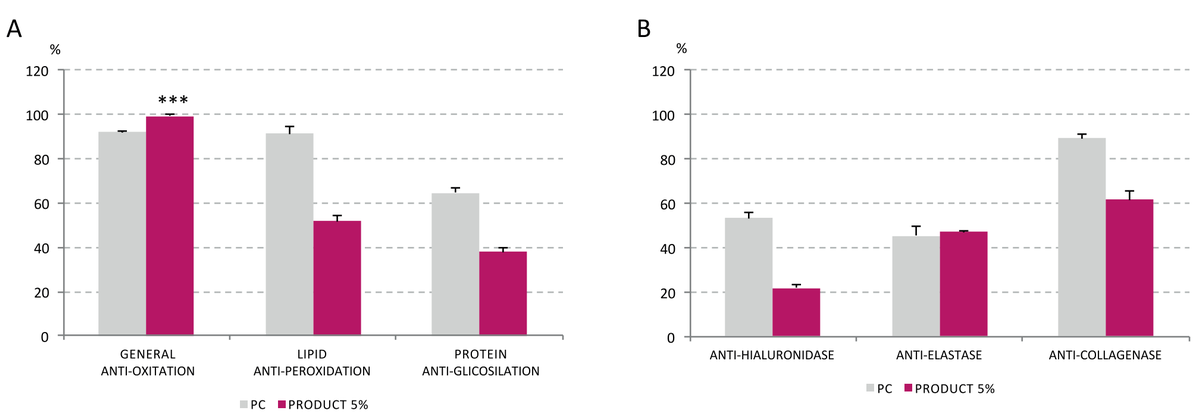

FIGURE 1. Results from *in-tube* assays. A, General anti-oxidation, lipid anti-peroxidation and protein anti-glycosylation effectiveness of the product (5%); B, Inhibition of hyaluronidase, elastase and collagenase activities by the action of product (5%). PC, positive control. ***, p<0.001.
This *in-tube* assays were performed using INVITOOLS kit, following supplier instructions. As positive controls were used: Vitamin E analogue at final concentration of 31.25 µg/mL for General Anti-oxidation and of 250 µg/mL for Lipid Peroxidation; Aminoguanidine at a final concentration of 690 µg/mL for Protein Glycosilation); EDTA at final concentration of 1.25 mg/mL for Hyaluronidase assay and of 5 mg/mL for Collagenase assay, and PMSF at final concentration of 130µg/mL for Elastase assay.
The product shows a protective effect against the different processes in all cases (statistically significant differences with respect to the negative control). It highlights its general anti-oxidant power over positive control.

2. Protective effect against damage by global oxidative stress (RMS).

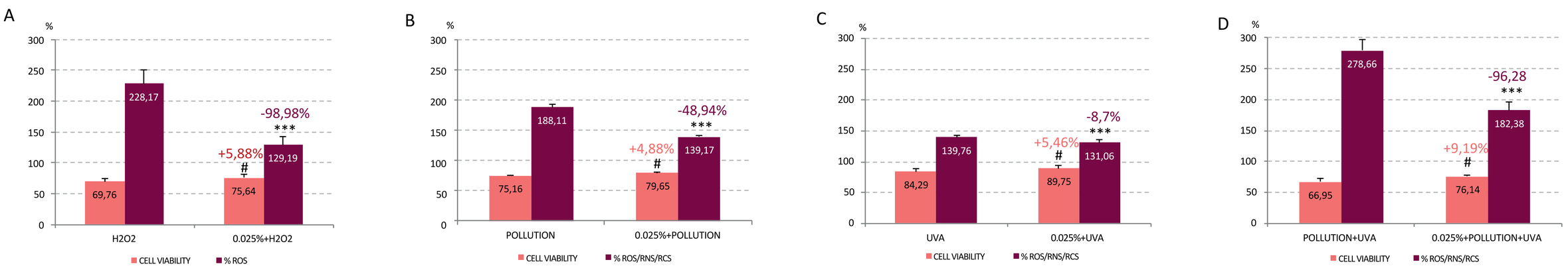

FIGURE 2. Results from cellular assays (human keratinocytes) treated with Active-Mix (0.025%). A, Oxidative stress by ROS (H2O2, hydrogen peroxide 150 µM, 2h**; 0.025+H2O2,** cells treated with active Mix and H2O2 150µM 2h)**; B, Oxidative stress (RMS) by pollution (POLLUTION**, cells treated with diesel particles 700 µM, 24h; **0.025 + POLLUTION**, cells treated with Mix and diesel particles 700 µM, 24h); **C, Oxidative stress (RMS) by UVA radiation (UVA 3 J/cm²); 0.025+UVA,** cells treated with Mix and UVA 3 J/cm²)**; D, Oxidative stress (RMS) by combined factors (POLLUTION + UVA; 0.025+POLLUTION+UVA) Cell viability (grey bar) and Reactive Species (purple bar). #/ *, p<0.05; ##/ **, p<0.01; ###/ ***, p<0.001**.
The exposure of the keratinocyte culture to the different factors causes decreased cell viability (MMT assay) and increased free radical levels (2',7'-dichlorodihydrofluorescein diacetate (H2DCF-DA) fluorescent probe (Molecular Probes, Invitrogen, Europe), statistically significant compared to standard culture conditions (100%). The oxiadtive stress levels from combined factor is remarkable.
In the presence of the product (0.025%), there is an increase in cell viability and a decrease in free radical levels, both statistically significant, in all cases. These results are indicative of a clear protective effect against damage caused by the increase in oxidative stress by ROS and RMS (ROS, RNS and RCS) from isolated and combined factors (pollution and UVA radiation).

3. Collagen neosynthesis from fibroblast stimulated by keratinocytes.

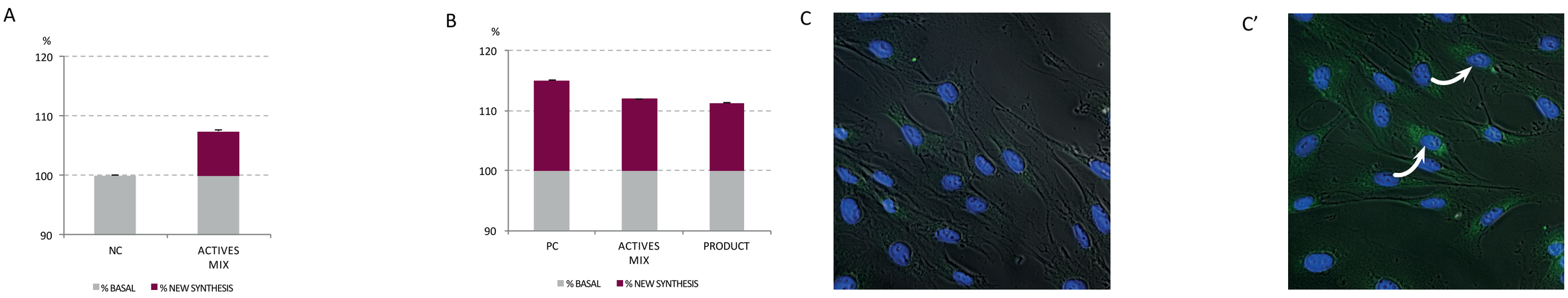

FIGURE 3. Quantification (%) of collagen pool. A, human keratinocytes and fibroblasts co-culture treated with Mix of actives (0.01%) compare with negative control; NC, Negative Control. **B, Human fibroblasts treated with supernatants from RHE (Reconstructed Human Epidermis) incubated with Mix of actives (0.01%) or treated with the product in normal use conditions. PC**, Positive Control (Sodium ascorbate, 0.01%). **C-C'. Expression of Collagen VII in human keratinocytes and fibroblasts co-culture treated with active Mix (0.01%). C, Control, basal expression of Collagen VII in co-culture of human keratinocytes and fibroblasts; C', Mix 0.01%, increase the expression of collagen VII** (arrows) **by fibroblasts**.
The amount of collagen was quantified using INVITOOLS kit, following supplier instructions. For the immunolocation cells were fixed using standard protocols and then cells were labeled for Collagen VII (C6805, SIGMA-ALDRICH. MO, USA).
The mix of actives contained in the product stimulates a dialogue between keratinocytes and fibroblasts to **increase the neosynthesis of Collagen**, compared to standard culture conditions (100%, figure 3.A).

CONCLUSIONS

1. The *"screening in-tubo"* results show that the product (5%) presents: A. High antioxidant level, blocking oxidation 99.04%. B. Protection against damage caused by oxidative stress processes: - decreases lipid peroxidation by 51.8% and protein glycosylation by 37.8%. - inhibits the action of hyaluronidase by 21.9%, the action of elastase by 47.1% and collagenase by 61.8%.
2. The cellular human keratinocytes culture results demonstrate that the active mix at 0.025%: C. Protects against cell damage by reducing oxidative stress by ROS in 98.98% D. Protects against damage by reducing global oxidative stress: - induced from pollution by 48.94%. - induced from UVA radiation by 8,7%. - induced from the factors combination (pollution + UVA radiation) by 96.28%.
3. The reconstituted human epidermis (RHE) and human fibroblasts culture experiments demonstrate that product and the active-mix (0.01%) induce new collagen throught keratinocyte stimulation.

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