EXOPOLYSACCHARIDE C3: ANTIOXIDANT ACTIVITY AND UV PROTECTION FOR SKIN CARE FORMULATIONS

ABSTRACT

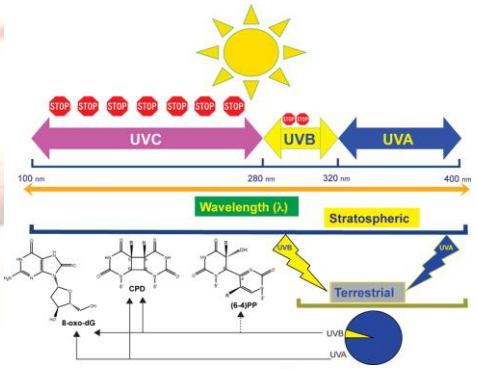
Exposure of skin to ultraviolet light has been shown to have a number of deleterious effects including photoaging, photoinduced DNA damage and photo-immunosuppression, which can lead to the development of skin cancer [*Maini et al., 2015*]. These deleterious effects could be prevented with the use of UV protective functional ingredients. More and more skin care products are incorporating these ingredients into their formulas and our studies demonstrated that **Exopolysaccharide EPS C3 (P0236) protects against DNA lesions induced by UVB radiation** and has a **powerful antioxidant activity**, scavenging and detoxifying the cell from free radicals through the metabolic pathways of glutathione and 8-hydroxy-2'deoxyguanosine.

INTRODUCTION

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA.

Evolution has provided the human organism with protection mechanisms against the harmful effects of free radicals, based on a complex defense system formed by antioxidant agents. These can be enzymatic mechanisms termed endogenous antioxidant agents (including enzymes SOD, catalase, glutathione peroxidase, coenzyme Q) or **exogenous antioxidant agents**, which we are responsible for making them available to our cells. Thus, it is important that the industry provides appropriate antioxidant delivery products cosmetic or alimentary that can fulfill our need for exogenous antioxidant agents.

On the other hand, exposure of skin to UV light has been shown to have a number of deleterious effects. While most UV-C is absorbed by the ozone layer and does not reach the earth, both UV-A and UV-B radiations have been implicated as carcinogens (cancer causing agents), though their methods of action are distinct [*Wölfle et al., 2007*]. The extent of their effect also varies, with UV-B being described as the most carcinogenic among all types of solar radiation, which main deleterious effect is DNA damage caused by its direct interaction with the DNA molecule.



EXTRACELLULAR POLYSACCHARIDES (EPS)

Extracellular polymeric substances (EPS) are high-molecular weight compounds secreted by microorganisms into their environment. EPS are mostly composed of polysaccharides (exopolysaccharides) and proteins, but include other macro-molecules such as DNA, lipids and humic substances. Exopolysaccharides generally consist of monosaccharides and some non-carbohydrate substituents (such as phosphate, acetate, succinate and pyruvate) and they have found diverse applications in cosmetic, food and pharmaceutical industries.

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EFFICACY STUDIES

Inkemia IUCT and Bionos Biotech S.L. have assessed the efficacy of Exopolysaccharide EPS C3 (P0236) from Inkemia IUCT.

The effect upon the global oxidative cell state of **human keratinocytes** has been evaluated through a validated metabolomics analytic technique, according to the Food and Drug Administration (FDA) guidelines. 10 cellular metabolites (reduced and oxidized forms) involved in the cell antioxidant protection system, have been quantified through **HPLC/MS-MS**; allowing the integrative analysis of the antioxidant global capability with improved accuracy than widely used in vitro antioxidant chemical reactions (TAC, ORAC…). As shown in Figure 1, **EPS C3 (P0236)** at the concentration of 10 µg/ml significantly reduced the oxidized form of glutathione, **GSSG** (Glutathione disulphide), in a **87.25 ± 15.87 %**, without decreasing the reduced form, GSH (Glutathione). Thus, ratio GSH/GSSG (Figure 2), biomarker of oxidative stress, is increased in a **6.25 ± 0.67 fold**. P0236 also showed activity upon the **8OHdG** (8-hydroxy-2'deoxyguanosine), a critical biomarker of oxidative stress and carcinogenesis. P0231 significantly reduced this metabolite in **65.17 ± 19.72** and **76.21 ± 17.36**, when added to keratinocytes at concentrations of 1 µg/ml and 10 µg/ml respectively (Figure 3).

In parallel, it has been assessed the **capacity to protect against UVB-induced DNA lesions**, after treating human keratinocytes with the product during 24 hours and irradiated them with UV-B (aprox. dose = 0,5 J/cm^2). Thymine-dimers formation was quantified using Flow Cytometry immediately after irradiation. Results showed that treatment with Exopolysaccharide C3 P0236, applied at 10 µg/ml before irradiation, significantly protected from UV-B induced-thymine dimers formation. The percentage of cells (t=0) presenting **thymine dimers** was **56.6 ± 11.5 % lower** in keratinocytes previously treated with the product than in untreated group (Fig. 4). When the same experiment was performed on medaka eleutheroembryos (*in vitro*), results showed that **EPS C3 protected from UVB-induced DNA** lesions by **38.7 ± 12.3 %, 42.1 ± 5.9 % and 59 ± 6.2 %,** when applied at **0,01 mg/ml, 0,1 mg/ml and 1 mg/ml**, respectively.

SAFETY PROFILE

- **Patch Test and HRIPT**: Non-irritating and very good cutaneous compatibility.
- **HetCam**: No ocular irritating effects.
- **Ames Test**: Non-mutagenic nor promutagenic.
- **Phototoxicity**: *Ongoing results. Expected at the end of 2016.*

DISCUSSION AND CONCLUSION

Antioxidant compounds such as Vit-C, Vit-E and retinol among others, have been proposed in cosmetic industry over the last decade, as functional ingredients for antiaging formulations and to prevent and modulate oxidative skin damages. At the same time, adequate protection of skin is essential to avoid DNA damage, oxidative stress and inflammatory processes induced by UV exposure [*Shin et al., 2013*].

These ingredients are usually incorporated in skin care formulations and our studies demonstrated that **Exopolysaccharide C3 (P0236) counteracts the malignant effects of UVB irradiation** in cells (HaCaT) and eleutheroembryos. It also shows a **powerful antioxidant activity**, scavenging and detoxifying the cell from free radicals through the metabolic pathways of glutathione and 8-hydroxy-2'deoxyguanosine. For these reasons, we proposed its use as ingredient in skin care formulations, showing several advantages over other compounds.

ADVANTAGES & APPLICATIONS

- **Powerful antioxidant activity** demonstrated according to FDA guidelines.
- **Oxidative stress pathways** through which it is exerting a **functional** benefit are **identified.**
- **Non-toxic** and biodegradable polymers.
- **UVB Protection capacity** demonstrated by reducing thymine dimers formation after UVB exposure in cells (HaCaT) and eleutheroembryos *in vitro*.
- Dermatologically tested**: Non-irritating** and **very good cutaneous compatibility.**
- **Easy to produce** in single strain systems and **low cost** cultivation conditions (short fermentation processes).

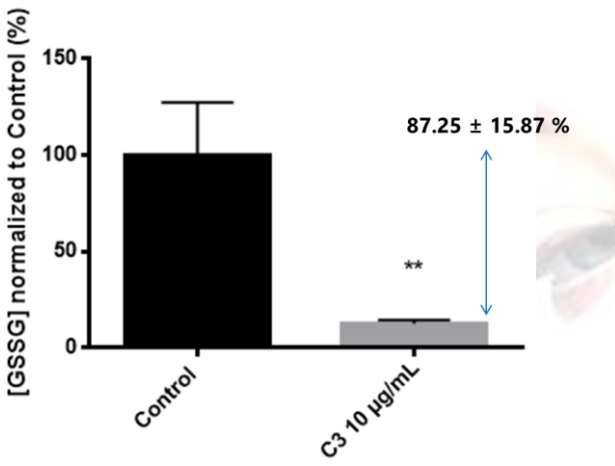

Figure 1: Graphical representation of the concentration of glutathione disulphide (GSSG), after treating cells with EPS C3 P.0236 at 10 µg/ml.

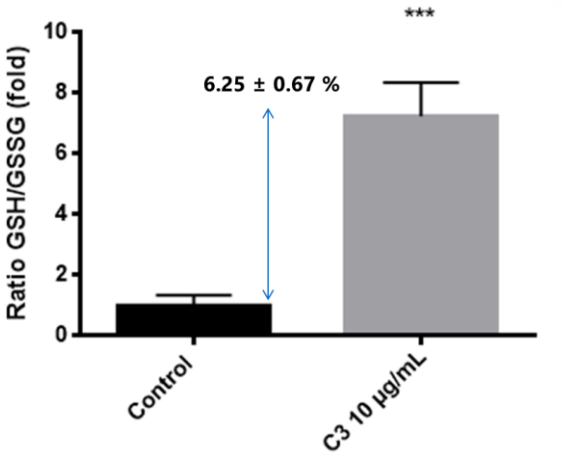

Figure 2: Graphical representation of the ratio GSH/GSSG, after treating cells with EPS C3 P.0236 at 10 µg/ml.

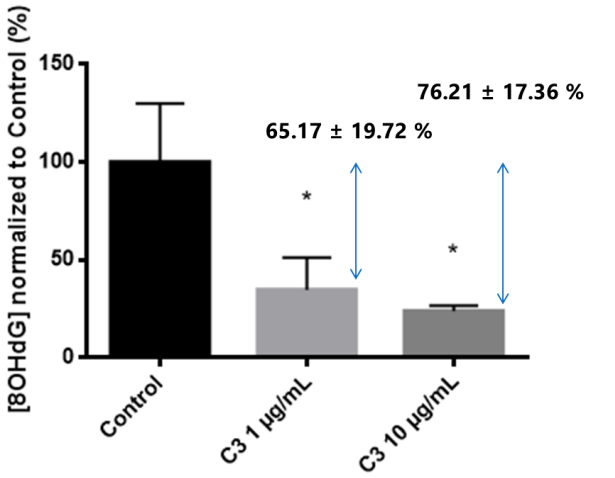

Figure 3: Graphical representation of the concentration of 8OHdG, after treating cells with EPS C3 P.0236 at 1 µg/ml and 10 µg/ml.

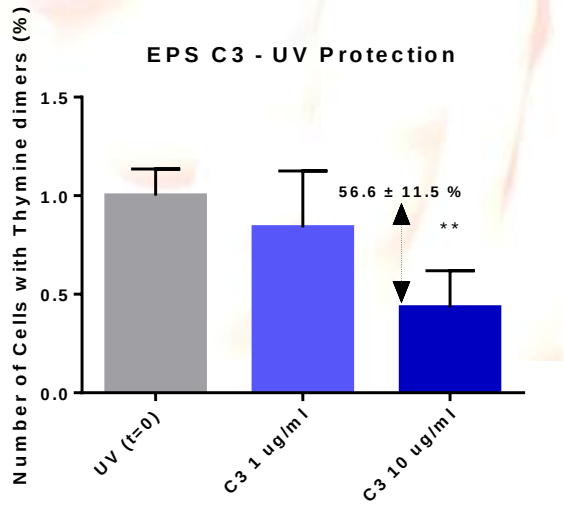

Figure 4: Graphical representation of the number of cells with thymine dimers, after treating cells with EPS C3 P.0236 at 1 µg/ml and 10 µg/ml and irradiating with UV-B light.

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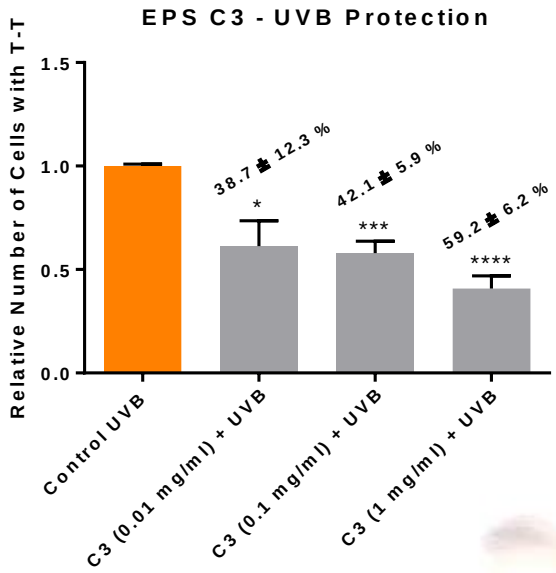

Figure 5: Graphical representation of the number of cells with thymine dimers, after treating medaka eleutheroembryos with EPS C3 P.0236 at 0,01 mg/ml, 0,1 mg/ml and 1 mg/ml irradiating with UV-B light.