

HIGH CONCENTRATION OF BAICALINE AS A KEY AOX FOR LONG UVA PROTECTION

ABSTRACT

Scutellaria baicalensis root extract is a polyphenol extracted from the genus Scutellaria used as a Traditional Chinese Medicine for liver detoxifying properties. Among the UV wavelengths reaching the Earth surface, longwave UVA (UVA1, 340-400nm) represents up to 80% of total UV and penetrates skin deeply, reaching the deep dermis. Increasing body of literature indicates that UVA1 can have a significant contribution to the clinical consequences of solar UV. We have evaluated in tubo and in vitro the oxidative stress mitigating properties of Scutellaria baicalensis root extract alone or in combination with vitamin E and vitamin C. These studies showed Scutellaria baicalensis root extract has very good $\cdot O_2^-$ and good $\cdot OH$ and $\cdot ROO$ quenching properties that account for the inhibition of ROS (-12%) and lipid peroxidation (-66%) after UVA-exposure in normal human fibroblasts treated with Scutellaria baicalensis root extract. In a reconstructed skin model exposed to UVA1, closer to the real life situation, the Scutellaria baicalensis root extract containing cocktail allowed to mitigate fibroblasts apoptosis, MMP1 and pro-inflammatory mediator (IL-1ra, IL-8, GM-CSF) release. These findings led us to test in the clinic on a Caucasian and a Chinese panel exposed to long UVA and daily UV respectively. The efficiency of the cocktail is visible on the quality of the pigmentation, and highlighted by a pairwise comparison test. A naive panel of 60 volunteers is able to perceive the pictures illustrating the anti-oxidant cocktail formula as being significantly brighter, less red and less dull than pictures illustrating the vehicle formula.

INTRODUCTION

Human skin is daily exposed to solar UV exposure. Among the UV wavelengths reaching the Earth surface, longwave UVA (UVA1, 340-400nm) represents up to 80% of total UV and show high penetration properties, reaching the deep dermis. Increasing body of literature indicates that UVA1 can have a significant contribution to the clinical consequences of solar UV. Scutellaria baicalensis root extract is a flavone widely used in the Traditional Chinese Medicine (TCM) as a liver - detoxifying agent. A growing set of studies conducted in vitro or on animal models, allowed to confer Scutellaria baicalensis root extract strong anti-oxidant properties, alone or in mixture with other anti-oxidants [1-7]. In vivo on mice, Scutellaria baicalensis root extract was shown mitigating UVB induced skin Photo-aging [9]. Such effect more likely results from the anti-oxidant activity of Scutellaria baicalensis root extract since, although slightly absorbing in the UVB range (peaking at ≈ 318 nm), it does not convey a significant photo-protective value in the UVB domain (SPF). However, it has to be acknowledged that data dealing with its possible contribution to human skin protection, in vivo, are scarce. To our knowledge, only one work [5] depicts in vivo the interest of topical Scutellaria baicalensis root extract, associated to two other anti-oxidants (Resveratrol, Ascorbyl glucoside), in alleviating the signs of a mild to moderate photo-aged human skin during a 12 weeks application period. Such a paucity of data led us to explore, in vitro and in vivo the contribution of Scutellaria baicalensis root extract, to fight UVA1 induced oxidative stress in the skin. Evaluated alone and in an anti-oxidant cocktail, Scutellaria baicalensis root extract allowed protecting from the UVA1 induced damages in vitro and in vivo with providing a significant visible improvement of skin color.

MATERIALS AND METHODS

Scutellaria baicalensis root extract (MMP Inc, Plainfield, USA), Tocopherol (DSM Nutritional Products, Base, Switzerland), Ascorbyl glucoside (Hayashibara, Okoyama, Japan). An anti-oxidant mixture (named here as MIX) comprised Baicalin 0,2% (from Scutellaria baicalensis root extract), Tocopherol 1% and Ascorbyl glucoside 0,2% (all expressed as w/w) that were solubilized in a vehicle of the following composition (w/w): Di PropyleneGlycol (16%), Ethanol (62%) and Water (22%), all of analytical grade.

A set of 4 in tubo tests was performed to evaluate the activity of Scutellaria baicalensis root extract towards different forms of ROS: $\cdot ROO$ [26], $\cdot O_2$ [27], $\cdot OH$ [28], O_2^- [29]. Full thickness reconstructed skin model prepared by EpiskinTM laboratories (Lyon, France) were used. Formulas were topically applied for 48 hours prior and 72 hours after exposure to 40J/cm² long UVA with an Oriol UV solar simulator (Newport, USA) equipped with a WG 360 cut off filter (Schott, Clichy, France). The incident UV spectrum was analyzed with a spectro-radiometer (SR9910 Irradian, Tranent, Scotland, UK) as shown on Figure 1.

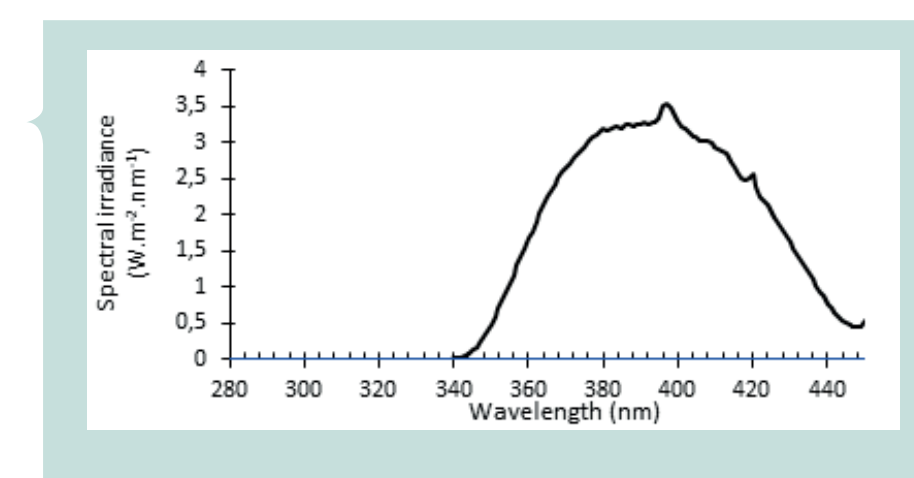


Figure 1: Spectrum of the UVA range delivered by the solar simulator equipped with the WG360 long UVA filter.

72 h after the UV exposure, the tissues were fixed and stained for HES analysis and the culture media was collected for matrix degrading and pro-inflammatory protein analysis. For each condition, the number of fibroblasts was counted and MMP1, IL1-ra, IL6, IL8 and GM-CSF release were measured through ELISA or Multiplex analysis (Bio-Rad, Marnes-la-Coquette, France).

Statistical analysis of the data was performed with a mixed model taking into account a heterogeneous variance by treatment. The model takes into account the interaction between the treatment and the batch of reconstructed skin on which the data were acquired. The statistical comparisons were adjusted biological marker by biological marker with the Benjamini-Hochberg method in multiple comparisons for evaluating the risk of false positive

K Lucet Levannier¹, E Planel², MJ Pygmalion², C. Tricaud¹

¹L'Oréal R&I, Chevilly Larue, France; ²L'Oréal R&I, Aulnay-sous-Bois, France;

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1/ Scutellaria baicalensis root extract containing anti-oxidant MIX effect against long UVA exposure: in vitro study

In previous experiments, the anti-oxidant potential of Scutellaria baicalensis root extract was determined according to its ability to quench different forms of ROS ($\cdot ROO$, $\cdot HO$, $\cdot O_2$ and O_2^-). These tests showed that Scutellaria baicalensis root extract displays a rather large anti-oxidant activity, against three of the ROS studied ($\cdot O_2^-$, $\cdot OH$, O_2^-). These results are detailed in another poster depicting the anti-pollution benefits of Scutellaria baicalensis root extract. The biological effect of the Scutellaria baicalensis root extract contained in MIX was evaluated on full thickness reconstructed skin model exposed to a moderate dose of long UVA wavelength previously known [18] for inducing measurable damages.

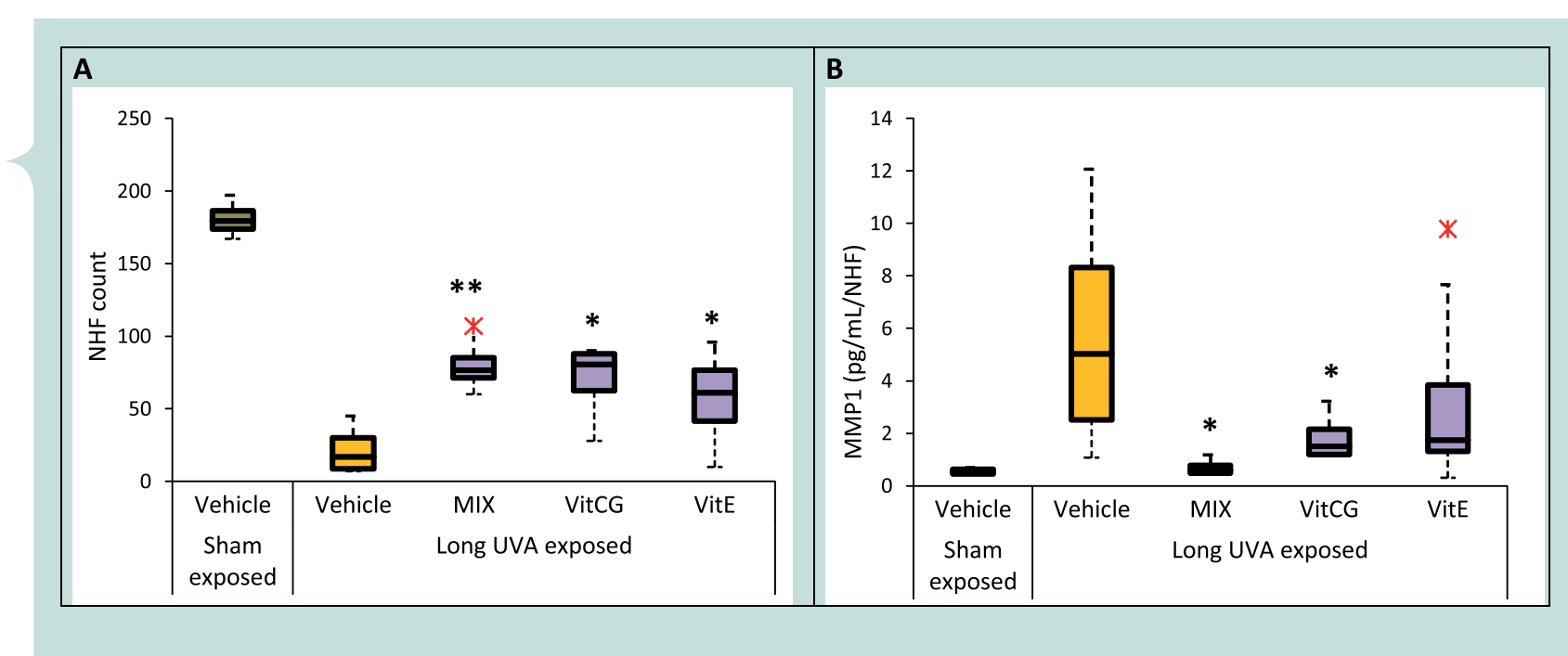


Figure 2A illustrates the impact of long-UVA exposure on the number of fibroblasts per section and its variable modulation by the three anti-oxidants. It shows that long-UVA has a strong impact upon fibroblast. Treatment by MIX or Ascorbyl glucoside partially prevented this negative impact. Tocopherol provides less protection with a lower median fibroblast count and higher dispersion of the data. With regards to MMP1 release (Fig. 2B), long-UVA exposure induces a significant release of MMP-1 into the culture medium. The application of the Scutellaria baicalensis root extract containing anti-oxidant MIX allows to maintain the basal level of MMP-1, whereas Ascorbyl glucoside and, to a lesser extent Tocopherol only partially prevents the release of MMP1. Finally, we have evaluated the release of IL1ra, IL6, IL8 and GM-CSF and found that exposition to long UVA induces a significant release of the soluble mediators from reconstructed skin. With regard the tested anti-oxidants, the Scutellaria baicalensis root extract containing MIX brings better efficacy in most cases, to the exception of IL6 for which all 3 tested solutions provide weak protection

2/ Scutellaria baicalensis root extract containing anti-oxidant MIX effect against UV exposure : Clinical study

1/ UVA-1 induced erythema

The clinical test performed on the Caucasian panel exposed to monodose of long-UVA showed significant (t test student) efficacy of emulsion containing anti-oxidant Mix as compared to vehicle using Chromametry measurement.

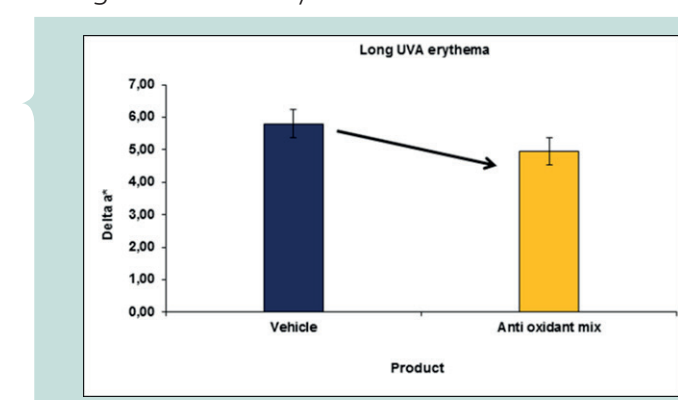
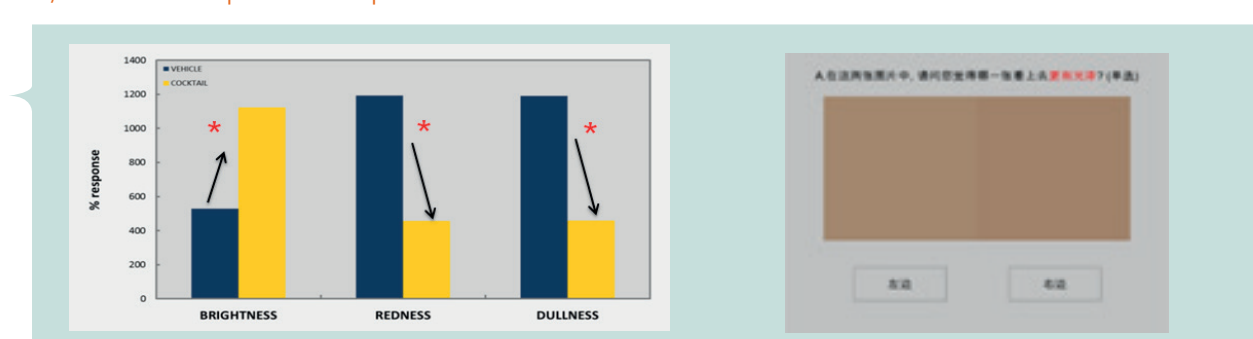


Figure 3: Effect of UVA1(340-440nm) mono dose, on Caucasian subjects receiving standardized application of 4mg/cm² of product on the small square on the back, 1h30 before exposure. Skin Color evolution has been assessed, 2h after UV exposure, using Minolta CR 400 Chromameter.* significant differences (p<0.005). To be closer to real life conditions, a clinical test using sub-erythematous daily UV exposures (UVB+UVA2+UVA1) was carried out.

3/ Paired Comparison of pictures



2/ Daily UV induced pigmentation

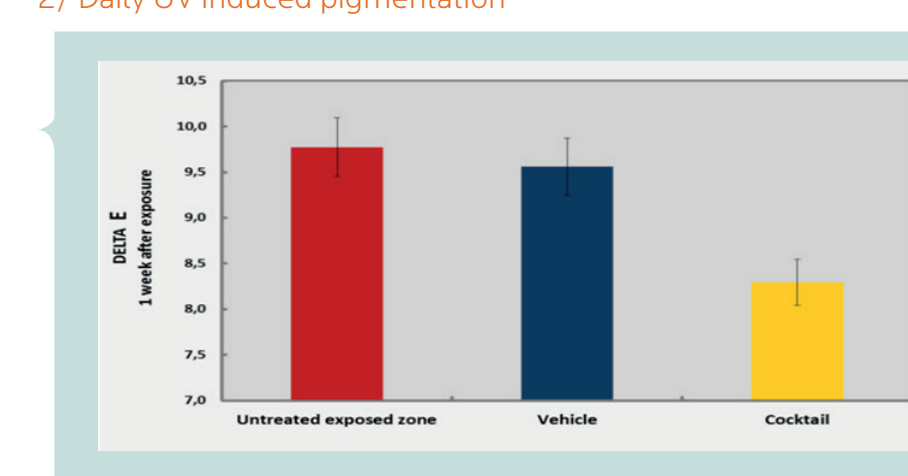


Figure 4: Effects of 4 days, 0.75MED of daily UV (290nm to 440nm), per day, on 25 volunteers receiving standardized application of 4mg/cm² of product, 1h30 before exposure. Measurement of pigmentation compared to the vehicle has been assessed using Chromameter CR 400 Minolta . * significant differences (p<0.005).

Under these conditions, the anti-oxidant mixture showed significantly efficient versus vehicle in preventing UV induced pigmentation through chromametric measurements in CIE L*a*b* space (DE (global pigmentation and Clinical assessments of pigmentation and erythema on 14 levels scales developed. To challenge the pigmentation results, reconstructed pictures from colorimetric measurements were proposed to naive panel (vehicle vs Mix).

Figure 5: Responses of 60 Chinese consumers comparing 28 pairs of pictures, (vehicle vs Mix). *significantly higher at 95% confidence level; The data is based on the count instead of percentage

Figure 5A: Example of paired comparison test between anti-oxidant Mix product and placebo, from 2 pigmentation squares.

The pictures illustrating the formula with the Scutellaria baicalensis root extract antioxidant MIX are significantly perceived brighter as well as less red and less dull than pictures illustrating the vehicle formula. The pictures illustrating the formula with the Baicalin antioxidant MIX are significantly perceived brighter as well as less red and less dull than pictures illustrating the vehicle formula. Thus Baicalin antioxidant MIX contributes of the improvement of skin color quality exposed to daily UV.

RESULTS

Figure 2: Effects of the 3 anti-oxidant solutions (and vehicle as control) on the maintenance of living fibroblasts, the release of MMP1 and of cytokines post exposure to long-UVA. Data are expressed as number of fibroblasts per skin section or quantity of soluble marker per milliliter per fibroblasts. **: Highly significant differences (p<0.005), *: significant differences (p<0.05) Benjamini-Hochberg test.

CONCLUSION

These studies tend to demonstrate the efficiency of Scutellaria baicalensis root extract containing anti-oxidant MIX, to fight UVA1 induced oxidative stress in the skin from in vitro models to clinical studies. This protection against long UVA induced oxidative stress was to be objectivized through visible clinical markers from measurements to consumer perception.

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