EFFECT OF CENTELLA ASIATICA EXTRACT (TITRATED IN ASIATICOSIDE, ASIATIC AND MADECASSIC ACIDS) COMBINED WITH C-XYLOSIDE AND HYDROLYZED RICE PROTEIN ON THE EXPRESSION OF HSP90, BY NORMAL HUMAN DERMAL FIBROBLASTS.

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BACKGROUND AND OBJECTIVE

Every living organism answer to environmental stresses by synthesis of protective biomolecules, particularly proteins of stress called « Heat-Shock Proteins » (HSPs). Heat Shock Proteins (HSPs) have a central role in the cells protection against environmental stresses they play an important role in reparation of damages induced by environmental stresses and in skin protection. They are able to eliminate aggressive free radicals and take part in the synthesis of functional proteins. By this way, they are able to maintain cells metabolic functions. Among all of them, HSP90 is a chaperone protein that assists other proteins to fold properly, stabilizes proteins against heat stress, and aids in protein degradation.
The study aim was to evaluate the effects of C-Xyloside, Centella asiatica extract and hydrolyzed rice protein tested alone or in association on the expression of the Heat Shot Protein HSP90 by human dermal fibroblasts.

MATERIAL & METHOD

1 The modulations of the HSP90 expression was evaluated in human dermal fibroplasts cultures. Different samples were compared:

1. Control skin,
2. Positive control: [vitamin C (20 µg/ml) + TGF-ß (10 ng/ml)]
3. Treated with C-Xyloside respectively at 1,2 x 10^{-2} % and 6 x 10^{-2} %
4. Treated with Centella asiatica extract at 4 x 10^{-4} % and 2 x 10^{-3} %
5. Treated with hydrolyzed rice protein at 2 x 10^{-3} % and 10^{-2} %
6. Treated with the association C-Xyloside + Centella asiatica extract + hydrolyzed rice protein at respectively at 1,2 x 10^{-2} %, 4 x 10^{-4} %, 2 x 10^{-3} % and 6 x 10^{-2} %, 2 x 10^{-3} %, 10^{-2} %.

2 Sample were analyzed by using quantification and picture analysis after in-situ immunolabelling.
At the end of 12-hour incubation, the cellular monolayers were isolated and rinsed with PBS and then fixed for 20 minutes with a solution of 4% PFA.
After multi PBS washes and at the end of the saturation step, the monolayers are incubated for 1 hour with antibody Anti-Hsp90 a/b (Enzo ALX-804-808-C100) then washed with 0,05% PBS-Tween 20 solution and incubated with antibodies.
In parallel, the cell nuclei are labeled with a cell-permeant nuclear counterstain. The cellular monolayers are observed by a technologic plateform for automac cellular imaging and analysis and quantification of markers is performed using a specific software.

RESULTS

1 RELATIVE QUANTIFICATION AFTER IN-SITU IMMUNOLABELLING AND IMAGE ANALYSIS

Positive control: Vitamin C (20 µg/ml) + TGF-ß (10 ng/ml) lead to an increase in the HSP90 expression.

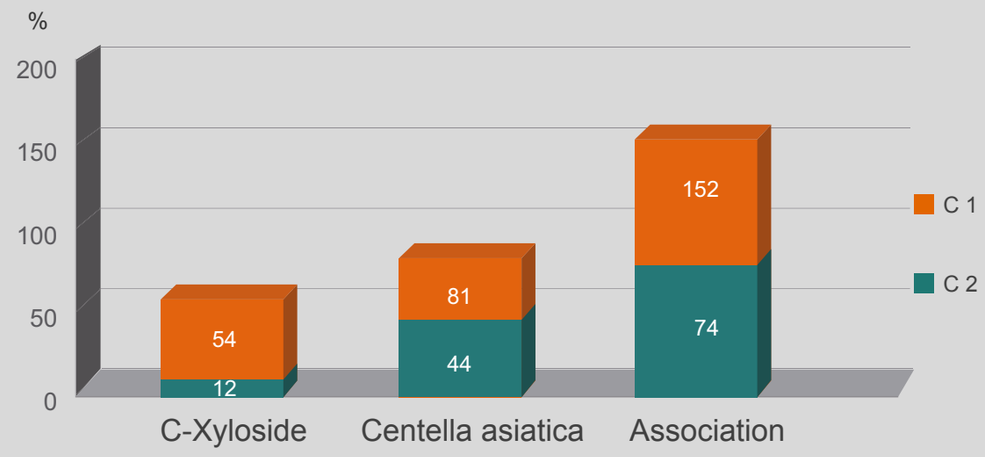
C-Xyloside significantly stimulates the expression of HSP90 in fibroblasts, with a dose dependent effect: +12% at 1.2 x 10^{-2} % and +54% at 6 x 10^{-2} % versus control. A similar effect is observed with **Centella asiatica**: +44% at 4 x 10^{-4}% and +81% at 2 x 10^{-3}%.

Hydrolyzed rice protein compound did not show significant modulation of HSP90 expression versus control.

The association of C-Xyloside, Centella asiatica extract and Hydrolyzed rice protein at respectively 1.2 x 10^{-2}%, 4 x 10^{-4} %, 2 x 10^{-3}% and 6 x 10^{-2}% , 2 x 10^{-3} %, 10^{-2} % **significantly stimulates the expression of HSP90, with a dependent dose effect: +74% and +152% versus control.**

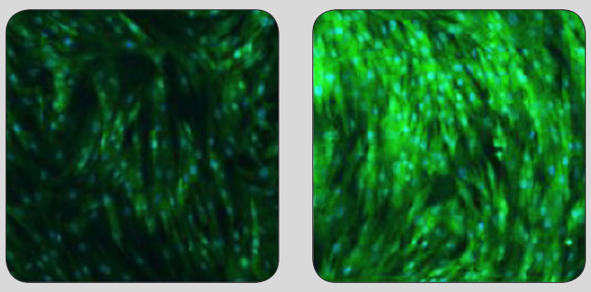
% of stimulation of the expression of HSP90

% of stimulation of the expression of HSP90 of C-Xyloside alone, Centella Asiatica alone and the combination C-Xyloside + Centella asiatica+ Hydrolyzed rice extract versus control.



2 VISUALIZATION BY IMMUNOLABELLING OF THE HSP 90 EXPRESSION

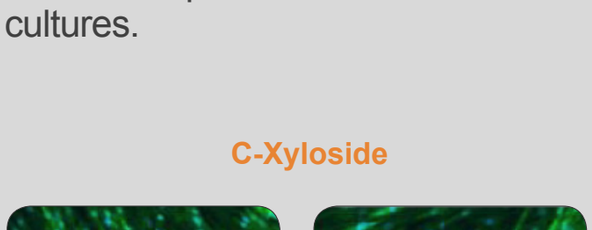
At the end of 72 hours of contact, the intensity of the fluorescence showed an increase of the expression of the HSP90 on positive control and treated cultures.



Control | Positive control

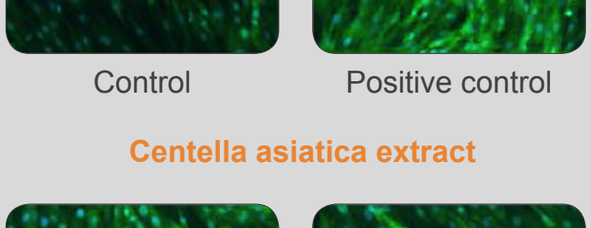
We also observed a superiority of the fluorescence intensity on cellular culture treated with the association compared those treated by alone actives.

C-Xyloside



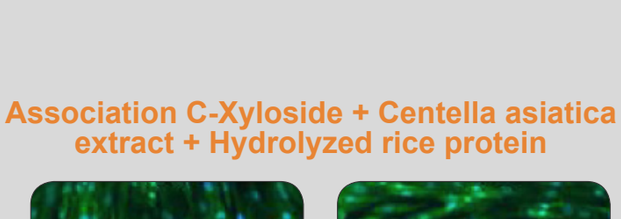
1.2 x 10^{-2} % | 6 x 10^{-2} %

Centella asiatica extract



4 x 10^{-4} % | 2 x 10^{-3} %

Association C-Xyloside + Centella asiatica extract + Hydrolyzed rice protein



1.2 x 10^{-2} % + 4 x 10^{-4} % + 2 x 10^{-3} % | 6 x 10^{-2} % + 2 x 10^{-3} % + 1 x 10^{-2} %

CONCLUSION & LIMITATIONS

This in vitro study showed the effects of the combination of C-Xyloside + Centella asiatica extract + hydrolyzed rice protein on the positive modulation of HSP 90 expression in dermal human fibroblasts.This effect was greater than those observed with the compounds C-Xyloside, Centella asiatica and Hydrolyzed rice protein alone, suggesting an additive effect between these three compounds. These data on cellular cultures could suggest the potential interest of an association containing C-Xyloside, Centella asiatica extract and Hydrolyzed rice protein in a daily skincare product.

The authors declare no conflict of interest

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