Natural extract of *Cistus incanus* aerial parts blocks psychological stress signaling and reduces neurogenic inflammation and signs of skin aging.

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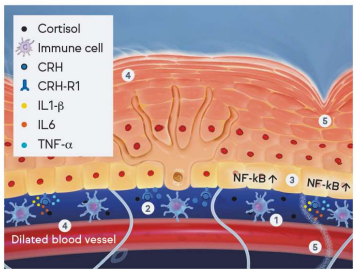
INTRODUCTION:

- Chronic psychological stress is recognized as a contributor to skin aging.
- The central stress signaling axis mediates states of stress, through production of corticotropin releasing hormone (CRH).



- Through its receptor CRH-R1, CRH activates NF-kB expression in keratinocytes, resulting in production of pro-inflammatory cytokines like IL1-β, IL6 and TNF-α.
- The inflammatory response increases blood flow, resulting in redness and edema.
- Inflammation also results in extracellular matrix degradation, leading to accelerated loss of skin elasticity and firmness, and the appearance of wrinkles.
- Here, we report on the use of an aqueous extract of *Cistus incanus*.
- *Cistus incanus* is a Mediterranean shrub used in traditional medicine as an anti-inflammatory agent and for treatment of various skin diseases.
- The extract contains Myricitrin, a flavonoid glycoside reported to block the activation of NF-kB and reduce the production of inflammatory markers.

MATERIALS & METHODS:

Extract: Aerial parts of *Cistus incanus* were ground and extracted in hot water. Solids were separated, and the resulting liquid was filtered, yielding a crude extract which was used as-is for *in-vitro* studies, and diluted in glycerin and preserved for use in clinical studies. The extract's myricitrin content was titrated by HPLC.

***In-vitro* CRH-R1 receptor blocking functional assay:** Recombinant CHO cells expressing the human CRH-R1 receptor were incubated in presence of *Cistus incanus* extract, and the antagonist effect of the extract was assessed via the evaluation of the response to a control agonist (30 nM Ovine CRH), through measurement of the cAMP signal by homogenous time-resolved fluorescence.

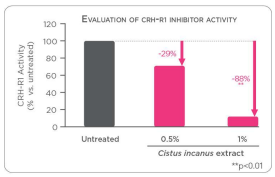
***In-vitro* inflammatory cytokine reduction, under CRH stimulation:** Primary human keratinocytes were incubated for 1 h in the absence (control) or presence of the extract at 0.05%; 0.1%; and 0.2% v/v. 100 nM CRH was added, and cells were incubated for a further 24 h. IL1-β, IL6, and TNF-alpha were quantified in culture medium by ELISA (R&D system Duoset).

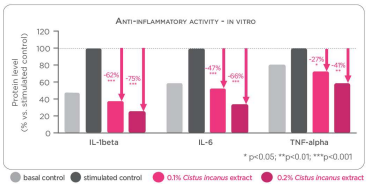
***Ex-vivo* inhibition of the NF-kB master inflammation regulator, under CRH induction:** Human skin explants were divided into the following groups: Untreated; Induction with 1 µM CRH for 72 h, otherwise untreated; Treatment with 0.125% and 0.25% extract for 24 h, followed by continued treatment for a further 48 h with simultaneous Induction with 1 µM CRH. Applications of CRH and extract were performed topically, in triplicate. NF-kB expression was analyzed by immunohistochemistry on fixed tissue samples using 3,3'-diaminobenzidine staining, and quantified by image analysis.

Clinical trial – signs of aging in a highly stressed population: 30 female panelists, aged 35-60 years old, with phototype (Fitzpatrick) II to IV, of all skin types, and presenting signs of aging (wrinkles or fine lines) were included in this double-blind trial. Panelists were selected for high stress levels, using a psychological questionnaire and cortisol levels measurement (in saliva). A formulation containing 1% preserved *Cistus incanus* extract was used opposite a matching placebo control, in a randomized split-face manner. Measurements were taken before application on D0 and after 14 and 28 days of twice-a-day application. Anti-inflammatory effects were evaluated by measurement of skin microcirculation (laser Doppler flowmetry) and skin redness (a*, Chromameter); red spots by image analysis (Visia-CA); and by evaluation of the skin's response to / recovery from chemical (SLS) insult (skin redness and microcirculation over 7 days). The anti-aging effect was evaluated by AEVA-3D image analysis.

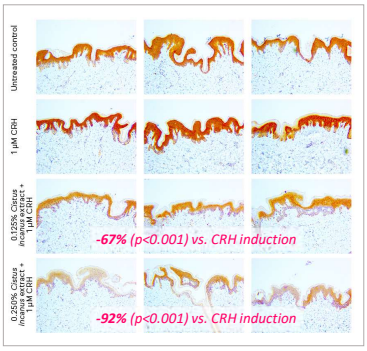
RESULTS AND DISCUSSION:

In-vitro results:



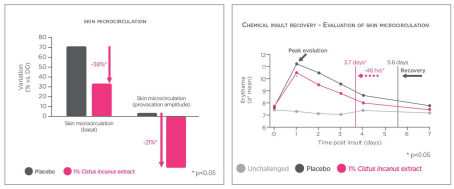


- **The *Cistus incanus* extract** effectively blocked the CRH-R1 receptor, indicating **a capacity to interrupt the neurogenic inflammation signaling chain.**
- In a keratinocyte culture under stimulation by CRH, **treatment with the extract decreased expression of IL1-β, IL6, and TNF-α, negating the increases induced by CRH**.
- In an *ex-vivo* model, **treatment with the extract blocked the effects of CRH stimulation and strongly decreased NF-kB levels in the explants**.



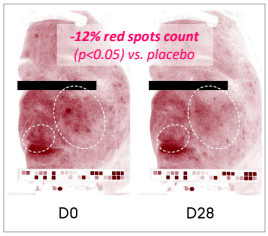
Clinical trial, highly stressed population (double-blind vs. placebo):

Anti-inflammatory effect:

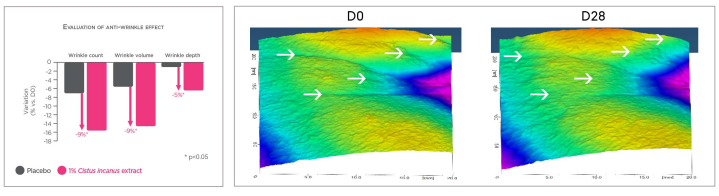


- **The extract showed significant anti-inflammatory effects (redness reduction, improved response to chemical insult).**

Reduction of skin redness:



Anti-aging effect:



- **The extract demonstrated significant anti-aging effects (wrinkle reduction).**

CONCLUSION:

The results presented above indicate that this *Cistus incanus* extract extract mitigates the effects of neurogenic stress on skin by interrupting the CRH stress signaling cascade (blocking the CRH-R1 receptor), and thus possesses significant potential as a skin care active ingredient.

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