Characterization and anti-inflammatory activity of *Vitis vinifera* L. leaves in human keratinocytes

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The skin constitutes the first barrier between our organism and the environment. The epidermis consists mainly by keratinocytes that play an essential role in epidermal renewal and skin's defence, actively participating in the cutaneous immune responses through the expression of a variety of cytokines/chemokines. Despite their protective role, keratinocytes are also relevant to the pathogenesis of chronic inflammatory skin diseases such as psoriasis and atopic dermatitis. TNFα plays a key role in human psoriatic skin inflammation acting primarily on the NF-κB pathway (Wullaert et al., 2011). Once activated, NF-κB translocates into the nucleus, where it regulates the transcription of several cytokine and growth factor genes, including TNFα, IL-6 and IL-8. A genome-wide screening approach revealed a correlation between psoriasis and genes regulators of the NF-κB pathway (Nair et al., 2008).
Vitis vinifera L. (*V. vinifera*) is a plant native from Mediterranean region whose leaves showed various biological activities including hepatoprotective, spasmolytic, hypoglycemic and vasorelaxant effects (Fernandes et al., 2013). Extracts from *V. vinifera* leaves are used for the treatment of varicose veins (Rabe et al., 2011), both ingested or topically applied, but their anti-inflammatory effect on the skin was not previously investigated.
On these basis, the aim of the study was to investigate *in vitro* the anti-inflammatory effect of an aqueous extracts from *V. vinifera* leaves on the NF-κB pathway in human keratinocytes.
Experiments were carried out on the spontaneously immortalized keratinocyte cell line HaCaT. The extract was prepared according to the ESCOP suggestions: *V. vinifera* L. leaves extract (VE) was obtained after 4 hours in cold water (10 g of drug in 100 mL of distilled water). The NF-κB driven transcription was evaluated with a plasmid containing the luciferase gene under the control of three NF-κB responsive elements. TNFα, IL-1β (both at 10 ng/mL) and LPS (5 μg/mL) were used as pro-inflammatory stimuli. The NF-κB nuclear translocation was measured after 1 hour treatment with the previously reported stimuli by ELISA assay. IL-8 release was evaluated after 6 hours treatment by ELISA assay. Characterization of flavonoids occurring in VE was performed by HPLC-DAD. VE displayed a concentration-dependent inhibitory effect on the NF-κB driven transcription, ranging from 2.5 to 100 μg/mL. At the maximal concentration (100 μg/mL), VE inhibited the NF-κB driven transcription induced by TNFα (-80%), IL-1β (-56%), and LPS (-100%). To further investigate the effect of VE on the NF-κB pathway, the nuclear translocation was evaluated as well. VE (50 μg/mL) was able to decrease the NF-κB nuclear translocation induced by TNFα (-55%) and LPS (-100%); the effect was not found when IL-1β was used as stimulus. Since it has been demonstrated that the expression of IL-8 is NF-κB dependent, the following experiments were devoted to evaluate the effect of VE on the IL-8 secretion induced by TNFα, IL-1β, and LPS in human keratinocytes. VE reduced in a concentration-dependent manner IL-8 release induced by TNFα and LPS (complete inhibition at 100 μg/mL and 25 μg/mL respectively), but showed lower effect when cells were stimulated with IL-1β (-70% at 200 μg/mL). Characterization of VE showed that quercetin-3-glucoside, quercetin-3-glucuronide and caftaric acid were the most abundant flavonoids occurring in the extract.
These data show for the first time that VE is able to inhibit keratinocytes inflammation by acting on the NF-κB pathway. Thus, these results provide new insights on the use of extracts from the leaves of *V. vinifera* to ameliorate skin inflammatory diseases.

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