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Anti-inflammatory effects of *Cannabis sativa* L. in human keratinocytes

Enrico Sangiovanni[1], Marco Fumagalli[1], Barbara Pacchetti[2], Stefano Piazza[1], Mario Dell'Agli[1]

[1]Dept. of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy
[2]Linnea SA, Riazzino, Switzerland

Society for Medicinal Plant and Natural Product Research

INTRODUCTION

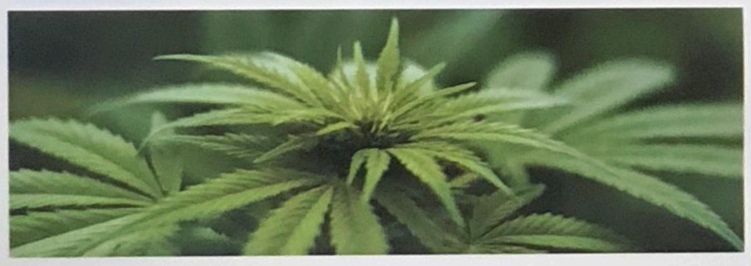
Dermatitis and psoriasis are inflammatory diseases in which keratinocytes, the most abundant cells in the epidermis, play a key role in the release of numerous pro-inflammatory factors (e.g. IL-8, MMP9, and VEGF). Chronic inflammation results from dysregulation and abnormal expression of inflammatory mediators or their receptors in keratinocytes. IL-8 is involved in neutrophil recruitment and VEGF regulates the angiogenesis process, while MMP9 contributes to the degradation of extracellular matrix. These pro-inflammatory mediators are regulated by different transcription factors, including NF-κB. The downregulation of keratinocytes inflammatory markers and the inhibition of their interaction with immune cells may be an effective target in the treatment of inflammatory skin diseases.

Cannabis sativa L. (*C. sat*… …ceous plant belonging to the family of Cannabaceae. The flow… …in the highest concentration of cannabinoids like delta… …HC), cannabidiol (CBD) and its carboxylated form… …*C. sativa* and its anti-CBD is the secon… …however, no studies on inflammatory act… human keratinocy…

The aim of thi… …rity of two *C. sativa* extracts, stan… …9-THC < 0.1%), in human kerati…

RESULTS

- Both the extracts were not cytotoxic till the highest concentration tested of 50 μg/mL.
- Both the extracts inhibited IL-8, MMP9 and VEGF release and NF-κB driven transcription induced by TNFα, with IC_{50}s below 50 μg/mL, while CBD was active only on NF-κB driven transcription (IC_{50}: 2.85 μM), suggesting that other compounds are involved in the biological activity.
- The extract containing 5% CBD+CBDA (25 μg/mL) and the corresponding concentration of cannabidiol (4 μM) were tested on the expression of 84 genes related to inflammation. The extract decreased the mRNA levels of several pro-inflammatory genes and for some of them CBD was responsible, at least in part, for the activity.



CONCLUSIONS

These results suggest that *C. sativa* extracts may counteract the cutaneous inflammatory processes by interfering with NF-κB pathway.

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… transcription by a luciferase reporter

Supported by Linnea

Shanghai, China, August 2018

Enrico Sangiovanni
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Cannabis sativa L. (*C. sativa*) is an annual herbaceous plant belonging to the family of Cannabaceae. The flowered tops of this plant contain the highest concentration of cannabinoids like delta-9-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD) and its carboxylated form (cannabidiolic acid, CBDA).

CBD is the second major cannabinoid occurring in *C. sativa* and its anti-inflammatory activity on skin has been demonstrated in mice; however, no studies on human keratinocytes inflammation have been reported so far.

AIM OF THE WORK

The aim of this work was to evaluate the anti-inflammatory activity of two *C. sativa* extracts, standardized in CBD and cannabidiolic acid (CBDA) (Δ9-THC < 0.1%), in human keratinocytes.

RESULTS

- Both the extracts were not cytotoxic till the highest concentration tested of 50 μg/mL.
- Both the extracts inhibited IL-8, MMP9 and VEGF release and NF-κB driven transcription induced by TNFα, with IC_{50}s below 50 μg/mL, while CBD was active only on NF-κB driven transcription (IC_{50}: 2.85 μM), suggesting that other compounds are involved in the biological activity.
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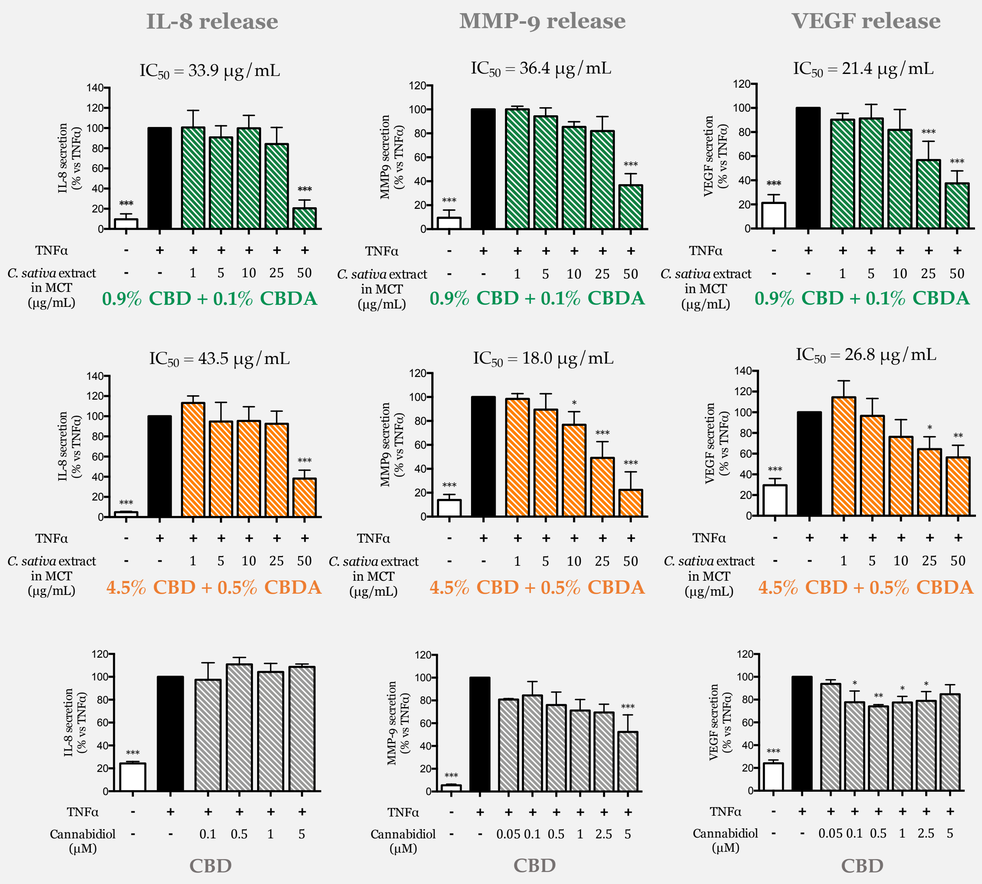
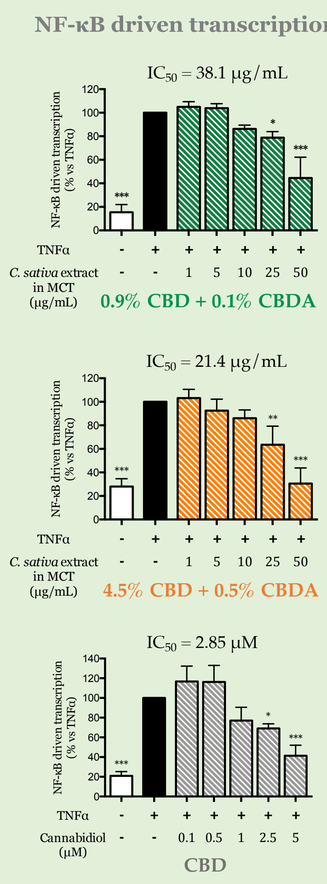
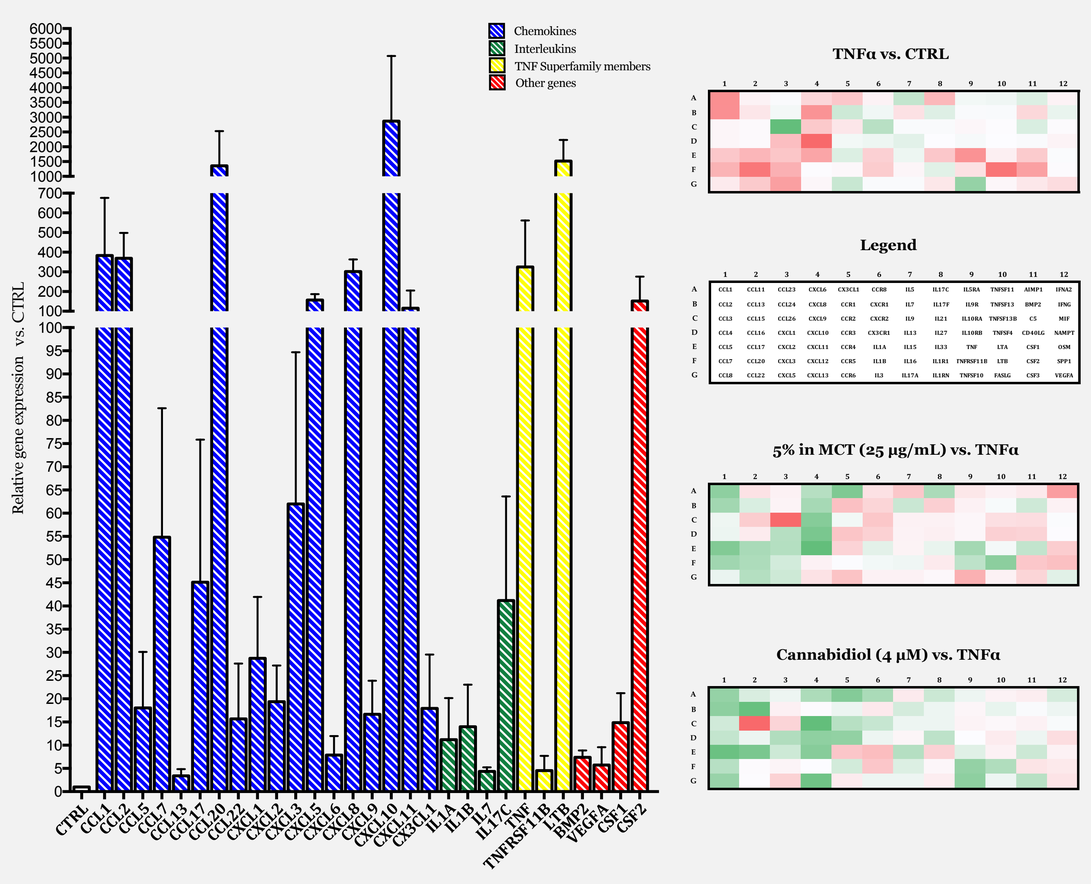
Effect of *C. sativa* extracts or CBD on pro-inflammatory mediators



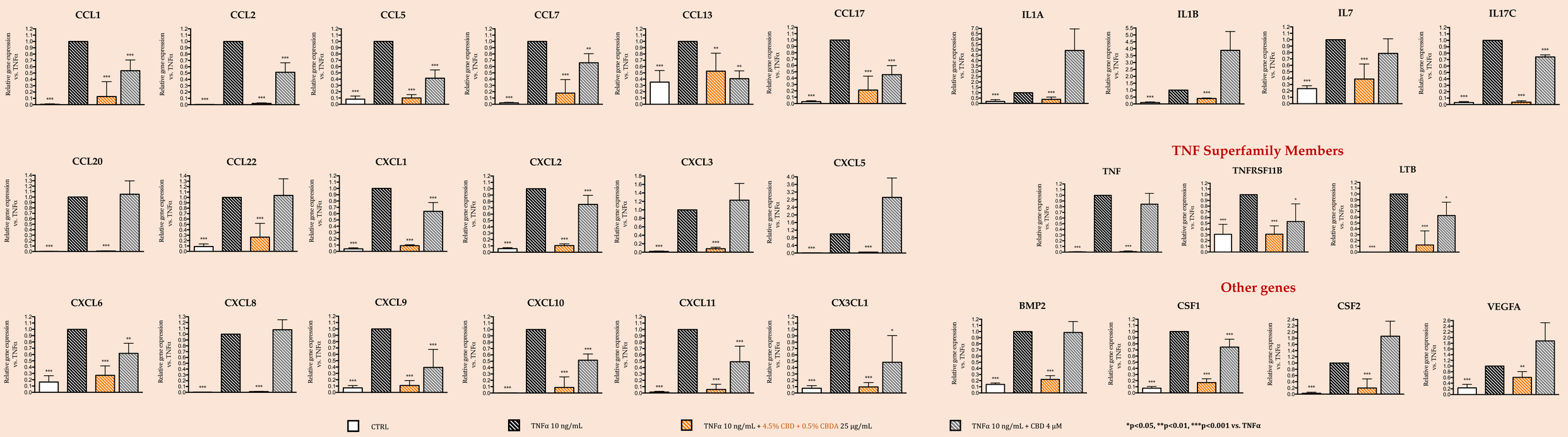
Effect on NF-κB pathway



Genes overexpressed by TNFα treatment in HaCaT cells



Genes modulated by *C. sativa* extract (4.5% CBD + 0.5% CBDA) or CBD



MATERIALS AND METHODS

- The extracts, containing 1% or 5% CBD+CBDA, were prepared by LINNEA SA (Riazzino, Switzerland).
- HaCaT cells were grown in 24-well plates (6× 10^5 cells/well) for 48 h; cells were treated with TNF-α at 10 ng/mL and extracts/compound under study. IL-8 release, NF-κBluc and gene expression were evaluated at 6 h, while MMP-9 and VEGF release at 24 h.
- The cytotoxicity of the extracts and CBD was evaluated by MTT test at 6 and 24 h.
- IL-8, MMP-9 and VEGF release were analysed by ELISA assays, NF-κB driven transcription by a luciferase reporter plasmid, while gene expression by real-time PCR.

CONCLUSIONS

These results suggest that *C. sativa* extracts may counteract the cutaneous inflammatory processes by interfering with NF-κB pathway.

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