

Cannabidiol has a central role in the NF-κB inhibition of a *Cannabis sativa* L. extract in human keratinocytes



Fumagalli, M¹; Paladino, G²; Piazza, S¹; Rossini, N²; Ciriello, U²; Magnavacca, A¹; Martinelli, G¹; Pozzoli, C¹; Dell'Agli, M¹; Sangiovanni, E¹.

¹Department of Pharmacological and Biomolecular Sciences (DiSFeB), Università degli Studi di Milano, Milan, Italy.

²Linnea SA, Riazzino, Switzerland.

BACKGROUND

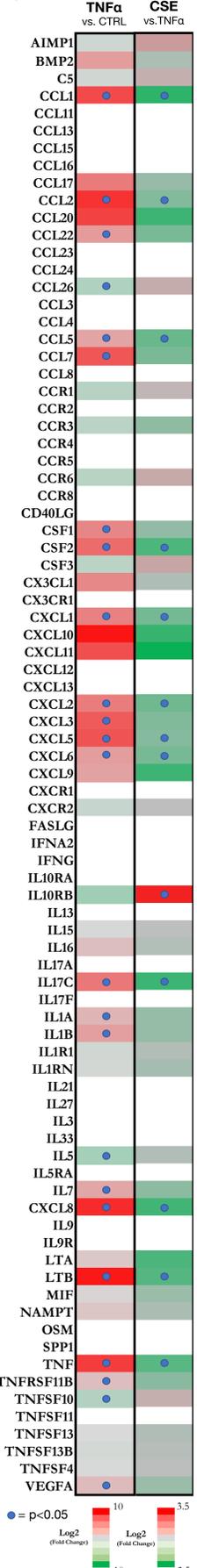
Cannabis sativa L. contains high concentrations of cannabinoids including cannabidiol (CBD), the second major cannabinoid and devoid of psychotropic activity, and Δ-9-tetrahydrocannabinol (THC). In our previous study, a *Cannabis sativa* L. ethanolic extract (CSE), characterized by medium chain triglycerides (MCT) as a carrier and standardized in 5% CBD and low concentration of THC, exerted anti-inflammatory effects in keratinocytes, including the downregulation of genes involved in inflammation*. CSE and CBD inhibited the nuclear transcription factor κB (NF-κB), but only CSE showed reduction in interleukin-8 (IL-8) secretion.

*Cannabis sativa L. extract and cannabidiol inhibit in vitro mediators of skin inflammation and wound injury. Sangiovanni, Enrico; Fumagalli, Marco; Pacchetti, Barbara; Piazza, Stefano; Magnavacca, Andrea; Khalilpour, Saba; Melzi, Gloria; Martinelli, Giulia; Dell'Agli, Mario
Phytotherapy research. , 2019, Vol.33(8), p.2083-2093.ISSN: 0951-418X, 1099-1573; DOI: 10.1002/ptr.6400; PMID: 31250491

Tab. 1 Composition of CSE subfractions obtained by subsequent extractions (1-4) from same plant material by solvents with increasing polarity.

Polarity increase	Percentage (%)	Cannabinoids											Terpens	Cannflavins		
		Compounds	CBD	CBDA	CBDV	CBDVA	CBDC4	THCV	CBG	CBGA	CBC	THC		THCA	Total	Cannflavin A
low	Fraction 1		28.0	0	0.3	0.1	0	0	0.7	0.1	1.0	1.0	0.1	1.8	0.1	0
	Fraction 2		13.0	0	0.3	0.2	0	0	1.0	0.1	0.6	0	0	0.2	0.4	0.3
high	Fraction 3		0	0	0	0	0	0	0	0	0	0	0	0.1	0	0
	Fraction 4		0	0	0	0	0	0	0	0	0	0	0	0.2	0	0

Fig. 2 Effect CSE on mRNA expression in keratinocytes challenged by TNFα



RESULTS

CSE was not toxic to keratinocytes till the maximum concentration tested of 50 μg/mL (Fig. 1A) and inhibited NF-κB driven transcription (Fig. 1B) and IL-8 secretion (Fig. 1C) induced by TNFα in a concentration dependent manner. CSE significantly downregulated 12 genes induced by TNFα treatment in HaCaT cells (Fig. 2).

Plant material was fractionated into 4 sub-fractions and analyzed for the content of cannabinoids, terpenes, and cannflavins (Tab. 1). Fraction 2 resulted cytotoxic at 25 μg/mL (Fig. 3A), but obtained the highest inhibition of NF-κB, considering also the lower concentration tested (Fig. 3B). None of the fractions significantly inhibited IL-8 secretion (Fig. 3C), but a higher inhibition trend was observed for fraction 1.

CBD was the most abundant compound of CSE (Tab. 2). None of purified cannabinoids were toxic to HaCaT cells till 5 μM (Fig. 4A) and, considering the inhibitory activity of CBD (Fig. 4B), this compound seems the main responsible for CSE inhibition of NF-κB.

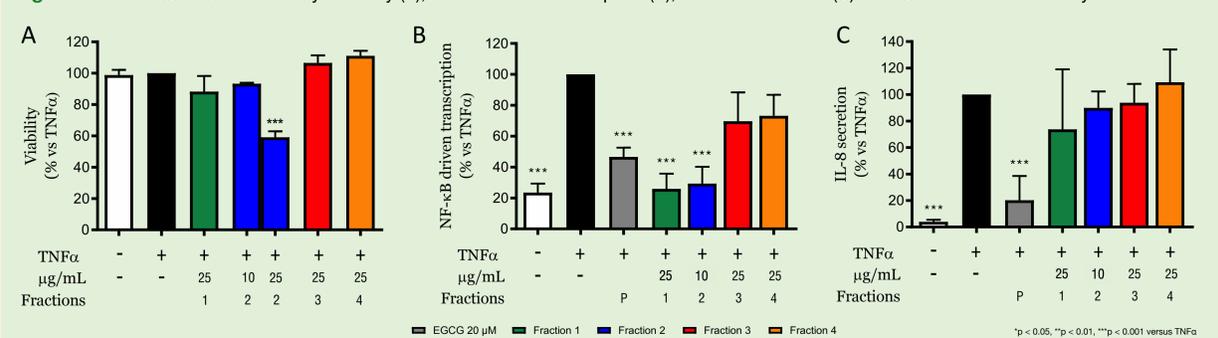
The evaluation of a reconstituted mixture of purified cannabinoids and terpenes (compounds proportional to 25 μg/mL of CSE, except for THC Fig. 5A), when combined inhibited NF-κB comparably to CSE (Fig. 5B). The mix of terpenes did not show significant inhibition alone or in addition to CBD (4 μM), not influencing the effect of the single cannabinoid (Fig. 5C).

AIM

The aim of the present study was to investigate the contribution of the main constituents of CSE to the anti-inflammatory activities in human keratinocytes (HaCaT) challenged by TNFα.



Fig. 3 Effects of CSE fractions on cytotoxicity (A), NF-κB driven transcription (B), and IL-8 secretion (C) in HaCaT cells stimulated by TNFα for 6h.



Tab. 2 Percentage of cannabinoids and terpenes in CSE. CBD is the most abundant compound in the extract.

Cannabinoids (%)	CBD	CBDA	CBDV	CBN	CBDC4	CBG	CBC	THC
CSE	4.836	0.020	0.019	0.001	0.020	0.138	0.235	0.222
Terpens (%)	γ-terpineol	β-caryophyllene	α-humulene	Farnesene isomer 1	Farnesene isomer 2	Farnesene isomer 3	Caryophyllene oxide	α-bisabolol
CSE	0.031	0.050	0.012	0.010	0.007	0.015	0.015	0.011

CONCLUSIONS

These results suggest that CBD plays a central role in the inhibition of pro-inflammatory mediators in human keratinocytes, acting on the NF-κB pathway. However, other cannabinoids can potentially participate in the inhibition of the IL-8 release, albeit quantitatively lower than CBD, for example THC.

Fig. 4 Effects of purified cannabinoids, canniprene, and cannflavin-A on cytotoxicity (A) and NF-κB driven transcription (B) in HaCaT cells stimulated by TNFα for 6h.

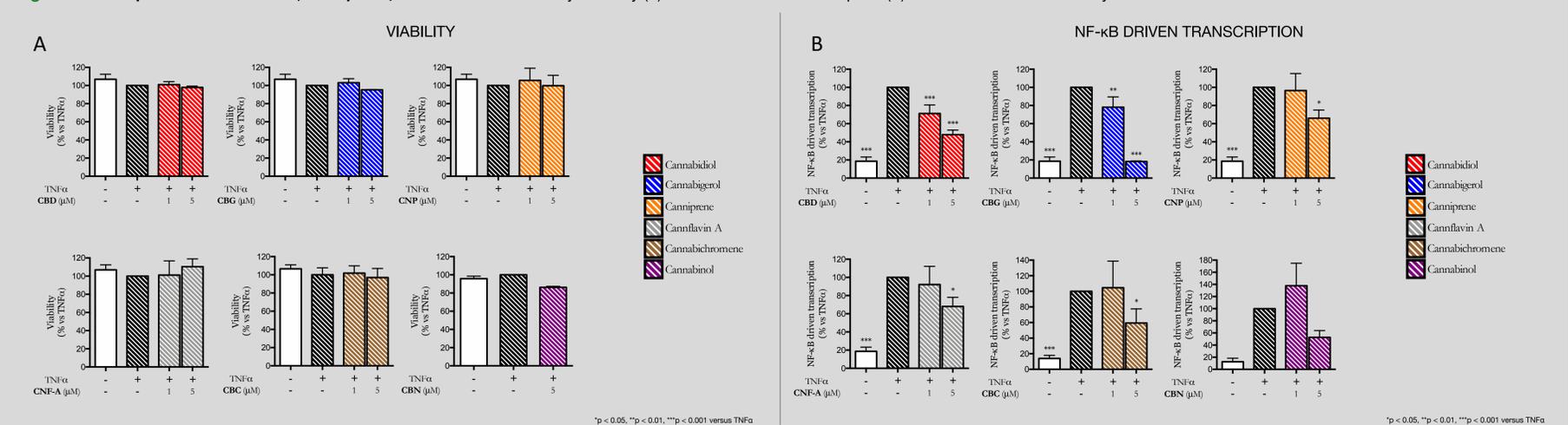
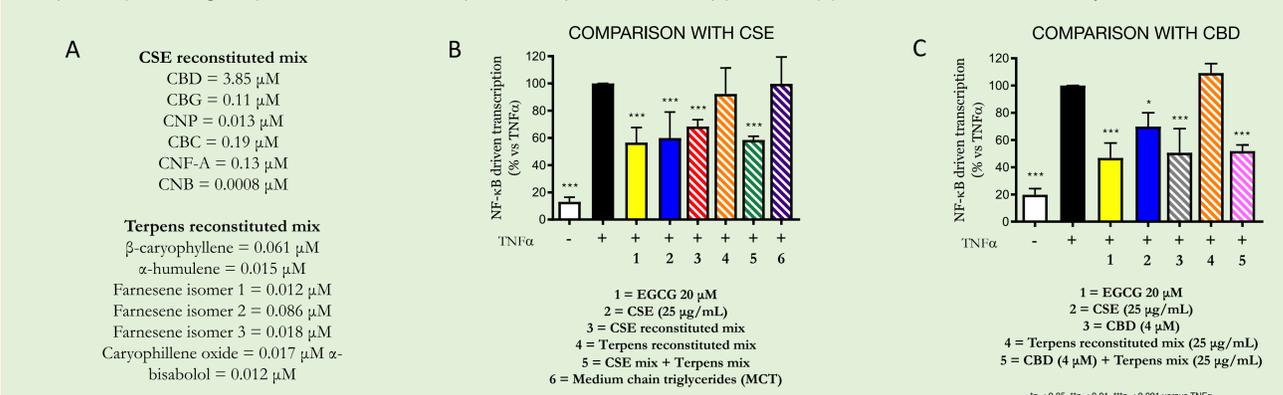


Fig. 5 Composition of the two reconstituted mixtures, considering compounds in 25 μg/mL of CSE (A). Effects of the reconstituted mixtures of compounds (resembling CSE) on NF-κB driven transcription in comparison with CSE (B) and CBD (C). HaCaT cells were stimulated by TNFα for 6h.



METHODS

EXTRACT AND COMPOUNDS: CSE, MCT and other purified constituents (cannabinoids, terpenes, and cannflavins) were provided by LINNEA SA (Riazzino, Switzerland) and assayed in human keratinocytes (HaCaT) challenged by TNFα (10 ng/mL) for 6 hours.
CELL VIABILITY: The cytotoxicity of CSE and compounds was evaluated, in HaCaT cells, by the 3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide method (MTT).
ASSAYS FOR ANTI-INFLAMMATORY ACTIVITY: IL-8 release was measured by an ELISA assay on cell culture media, while the NF-κB-driven transcription by transient transfection assay. The analysis of gene expression was performed using a 384-well polymerase chain reaction (PCR) array (QIAGEN Sciences, USA).