Effective inhibition of Th17/Th22 pathway in in vitro and ex vivo models of psoriasis by Celastrol enriched plant cell culture extract (CEE)

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INTRODUCTION

Psoriasis is an immune-mediated inflammatory disease in which Th17 pathway is mainly involved. Systemic interventions with biologics that specifically block the Th17 pathway are effective to treat severe psoriasis. However, for efficient topical treatment, small molecules are more suitable than antibodies to penetrate and target epidermal keratinocytes, the key players in psoriasis. Celastrol, a well-described triterpene, is present in low amount in Tripterygium wilfordii roots. By using plant cell culture, we were able to boost Celastrol production in bioreactors. Here, we evaluated immune modulator effect of Celastrol enriched extract (CEE) in Th17/Th22 psoriasis induced 2D & 3D models in view of its dermatological usage.

CEE and Celastrol inhibited Th17 induced gene markers in a stimulated NHEK model

These gene markers: AMPs (DEFB4A, PI3 & S100A7A), SLPI, chemokines (CXCL1, IL-8, CCL5, CCL20), and cytokines (IL-19, IL-23A and IL-36γ) were inhibited in a dose dependent manner. A statistically inhibition was observed for all markers at the highest dose of 90 ng/ml for CEE and 135 ng/ml for Celastrol. These inhibitory effects were as high (or even better) as 10 µM JAK I inhibitor control.

		MIX	JAK inhibitor 10µM		CEE 10ng/ml	30ng/ml		90ng/ml		Celastrol 15ng/ml	45ng/ml		135ng/ml	
Antimicrobial peptides, innate immunity	DEFB4A	35300	1700	**	30000	11600		1600	**	31800	11700		790	**
	PI3	62	72		60	34		14	*	51	27		8	**
	S100A7A	71	17		74	33		14		67	37		8	*
	SLPI	77	7	***	10	5	***	2	***	9	4	***	1	***
Cytokines Chemokines Cytokine signaling	CXCL1	115	8	***	86	39		13	**	76	29		4	***
	CCL20	24	65		18	10		4	***	15	6	**	2	***
	CCL5	25	4	***	17	5	**	1	***	15	4	**	1	***
	IL8	252	16	**	188	90		14	**	137	45		4	***
	IL19	41800	627	***	60600	56700		18800		50100	45400		7600	**
	IL23A	22	15		18	10		4	*	13	5		2	**
	IL36G	64	100		67	52		19	**	43	27		9	***

*Table: Effects of positive control, CEE & Celastrol treatments on modulation of a genes panel in NHEK model after a stimulation by a cytokines cocktail [IL17 + OSM + TNF-α] for 24 H. *p < 0.05, **p < 0.01 & ***p < 0.001*

CEE or Celastrol were able to downregulate Th17 specific AMPs, cytokines and chemokines induced in NHEK at a low concentration of 90 ng/ml of equivalent Celastrol

CEE significantly inhibited IL-8 chemokine in a stimulated RHE model

CEE significantly inhibited IL-8 chemokine in a stimulated RHE at 30 ng/ml with 73% of inhibition (p<0.05) where JAK inhibitor (10 µM; positive control) had a 94% inhibition(p<0.01). The results were compiled from 2 independent experiments (n=2 donors for each).

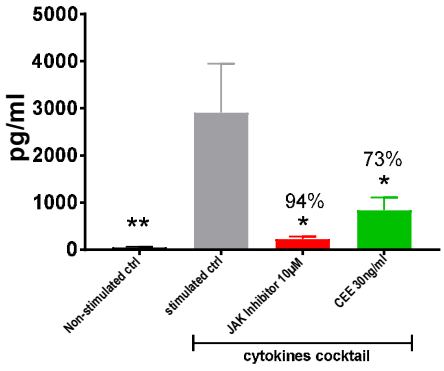

*Figure 1 : Histogram showing the effect of CEE & positive control treatments on IL-8 release by RHE model after a stimulation by a cytokines cocktail [IL17 + OSM + TNF-α] for 48 H. % of Inhibition were added. *p < 0.05, **p < 0.01*

CEE showed a high soothing effect in this model

CEE inhibited induced IL-17A, IL-22 and INF-γ in a CD4+ T cell model

In the presence of increasing concentrations of CEE, IL-17,IL-22 and IFN-γ were significantly reduced in a dose-dependent manner (p<0.01). The higher concentration of CEE used (30 ng/ml) had similar effect to Dexamethasone treatment reaching to 73%, 90% and 95% inhibition for IL-17A, INF-γ and IL-22 respectively. TNF-α, similarly to 2µM Dexamethasone control, was almost completely inhibited by the lowest dose of CEE.

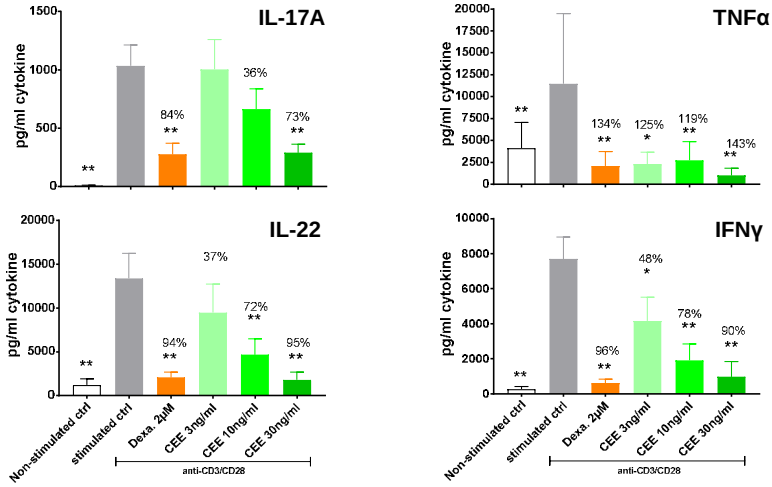

*Figure 2: Histogram showing the effect of CEE & positive control treatments on the release of a cytokines panel by Human CD4+ T cells after stimulation by anti-CD3 + anti-CD28 for 20 H. % of Inhibition were added.*p < 0.05, **p < 0.01*

CEE was able to suppress Th17 and Th22 cytokines release by activated CD4+ T cells

CONCLUSION

These findings showed clearly that CEE inhibited both in 2D and 3D human in vitro psoriasis models: Th17/Th22 cytokines, key inflammatory parameters and psoriasis associated biomarkers involved in the physiopathology of this cutaneous disease. Moreover, this high added value CEE was obtained by an ecofriendly bioprocess in contrast to extracts obtained from traditional roots. This is the first time, a well-defined immune-modulator CEE is proposed for adjuvant care in psoriasis prophylaxis.