

## Olive pomace phenolic compounds can inhibit inflammatory and oxidative – related diseases of human ocular surface epithelium

ROS production

HCE

**Travel Grant Recipient** 

**IM-ConjEpi** 

**OL** 5 μM + HT 10 μM

ROS production was significantly inhibited in

both cell lines by CONV, OPT3, OL and HT

dose-dependently and by 5 µM OL+10 µM HT.

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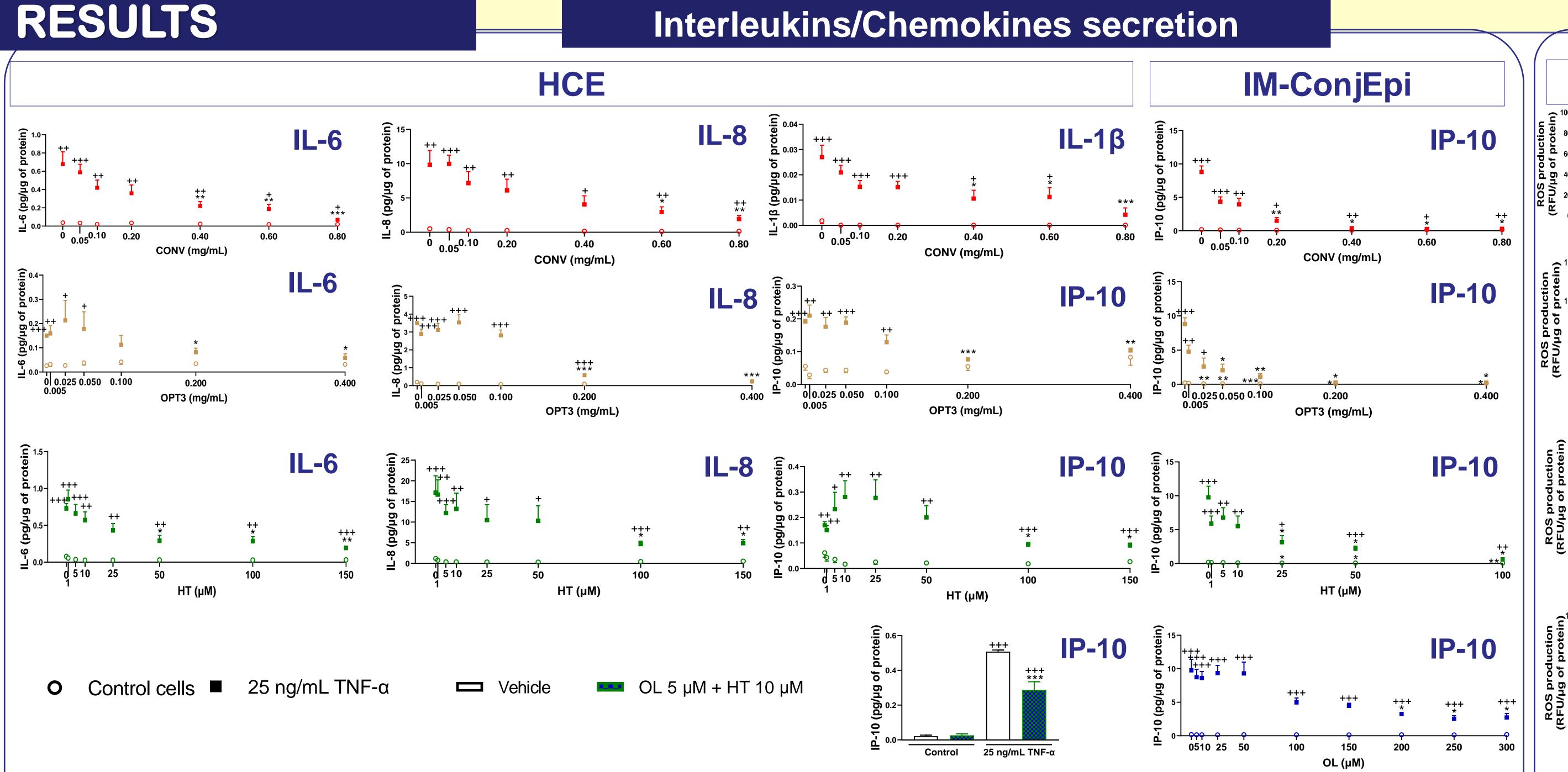
## INTRODUCTION

Inflammation and oxidative stress are involved in several ocular surface diseases such as dry eye (DE) and acute and chronic ocular allergy. Olive Pomace (OP) is the main byproduct of the olive oil industry and poses a great environmental threat due to its high organic load and phenolic content. At the same time, it can be considered as a source of valuable bioactive compounds -> high content in simple phenols (i.e. Hydroxytyrosol - HT) and secoiridoids (i.e. Oleuropein - OL) with numerous biological activities reported, such as anti-inflammatory, antioxidant and anticarcinogenic, among others.

The aim of this study was to determine the antiinflammatory and antioxidant activity of crude extracts derived from OP and their two major phenolic compounds (OL and HT), in human corneal (HCE) and conjunctival (IM-ConjEpi) epithelial cells.

## MATERIALS & METHODS

- Two OP extracts were produced: CONV by a conventional solid-liquid extraction (conditions: 0.5 g OP/mL EtOH:H2O 50:50, 70°C, 1h), and OPT3 (conditions: 0.8 g OP/mL EtOH:H2O 90:10, 184°C, 20min), by a Design of Experiments Pressurized Liquid Extraction (PLE) optimization. HT and OL were obtained from Extrasynthese.
- (Araki-Sasaki, IOVS 2005) and IM-ConjEpi (Innopoprost) cell lines were used. Cells were pre-treated for 2h with CONV (0.05-0.80 mg/mL), OPT3 (0.005-0.400 mg/mL), OL (5–300 μM), HT (1–100 μM), and their mixture  $(5 \mu M OL + 10 \mu M HT)$ , and then stimulated with TNF- $\alpha$  (25) µg/ml) for 24h in the presence/absence of the treatments. Cytokine production (interleukin (IL)-1β, IL-6, IL-8 and interference comments in the first realiments. interferon gamma-induced protein 10 (IP-10) secretion was analysed by an immune bead-based array in a Luminex IS-100.
- Intracellular Reactive Oxygen Species (ROS) production was determined by H2DCF-DA dye assay in ultraviolet (UV)-B radiation-exposed cells in the presence/absence of same treatments as above for 1h (with 1h pre-treatment).
- Data were normalized to corresponding protein content.



On HCE cells, CONV inhibited the TNF-α stimulated secretion of IL-6 and IL-1β dose-dependently, and of IL-8 at 0.6 and 0.8 mg/mL. CONV also decreased IP-10 production by IM-ConjEpi cells in a dose-dependent manner. OPT3 reduced IL-6 and IL-8 secretion by HCE cells at 0.2 and 0.4 mg/mL, and IP-10 production by both cell lines dose-dependently. On HCE cells, HT decreased IL-6 levels in a concentration-dependent way and IL-8 levels at 100 μM. Also, HT significantly inhibited IP-10 production by both cell lines, dose-dependently. IP-10 secretion was also decreased in HCE cells by 5  $\mu$ M OL + 10  $\mu$ M HT, and in IM-ConjEpi by OL concentration-dependently.

3 independent experiments ± SEM, \*P<0.05, \*\* P<0.01,\*\*\*P<0.001, compared to vehicle – treated cells, +P<0.05, ++ P<0.01, +++P<0.001, compared to control cells

- CONV, OPT3, HT and OL demonstrate anti-inflammatory and antioxidant effects on both HCE and IM-ConjEpi cells. The effect of OL and HT can be higher when compounds are combined.
- An environmentally hazardous agro-industrial by-product can be transformed to a potential therapy for inflammatory and oxidative-related ocular surface diseases.
- These *in vitro* data consist an essential baseline for the treatment of these diseases in the future, while are paramount for the sustainable growth of related industries.

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