In vitro evaluation of olive pomace phenolic compounds as therapeutic agents for the dry eye disease

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INTRODUCTION

Inflammation is the major mechanism involved in the pathophysiology of the dry eye (DE). Natural compounds like polyphenols are increasingly getting attention due to their well-known antioxidant and anti-inflammatory properties.

Olive Pomace (OP) is the olive oil's industry main by-product and poses a great environmental concern due to its high organic load and phenolic content. At the same time, this phenolic content contains principally simple phenols (i.e. **Hydroxytyrosol – HT**) and secoiridoids (i.e. **Oleuropein – OL**) with numerous demonstrated biological activities, including **anti-inflammatory**.

The aim of this study was to valorize an environmentally hazardous agro-industrial by-product, the OP, as potential therapy for the DE, evaluating its **anti-inflammatory activity** on human corneal (HCE) and conjunctival (IM-ConjEpi) epithelial cells.

MATERIALS & METHODS

Phenolic extraction

Conventional extraction

0.5 g OP/mL 50% EtOH-H_2O, 70ºC, 1h, under stirring

VS

Pressurized Liquid Extraction (PLE)

Central composite design (24 experiments)
Three parameters:
- **Temperature (T):** 65.0-185.0ºC
- **EtOH% in H_2O:** 8.0-92.0%
- **Solid/Liquid ratio (S/L):** 0.2-0.8 g OP/mL solvent

Responses:
- Dry extract richness (DER) in OL & HT
- ORAC Antioxidant Activity (CAA)

Cell-based assays

HCE (Araki-Sasaki, IOVS 2005) and IM-ConjEpi (Innopoprost) cells were pre-treated for 2h with OP extracts dissolved in 0.4% EtOH (vehicle) and then stimulated with TNF-α (25 µg/ml) for 24h in the presence/absence of the treatments.
Cytokine production: Interleukin (IL)-1β, IL-6, IL-8 and interferon gamma-induced protein 10 (IP-10) secretion was analysed by an immune bead-based array (Milliplex, Merck) in a Luminex IS-100. Data were normalized to corresponding protein content.
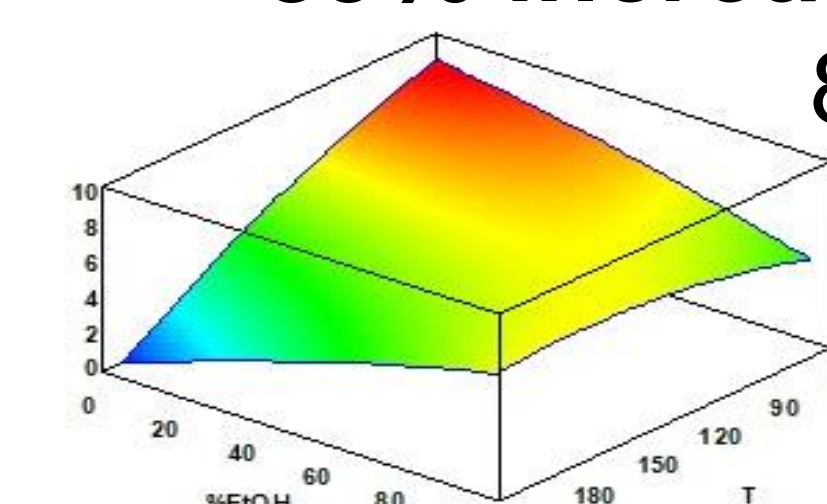
RESULTS

Phenolic extraction

Conventional extract

CONV

DER in OL: 2.4 mg/g_DE
DER in HT: 1.8 mg/g_DE
CAA: 4.7 mmol_TE/g_DE

TE=Trolox Equivalents

VS

Optimal PLE extracts: comparison with CONV

OPT1
89% increase in CAA
8.9 mmol_TE/g_DE
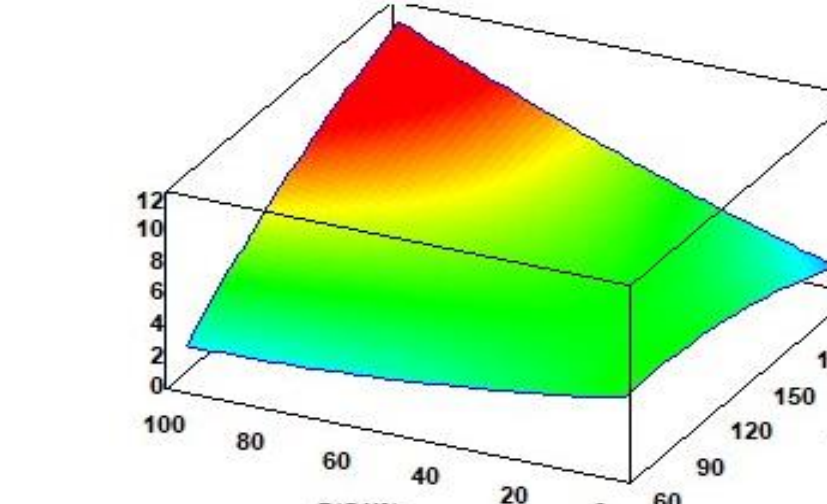T=66.0ºC, EtOH%=10.0%, S/L=0.8 g/mL

OPT2
475% increase in OL
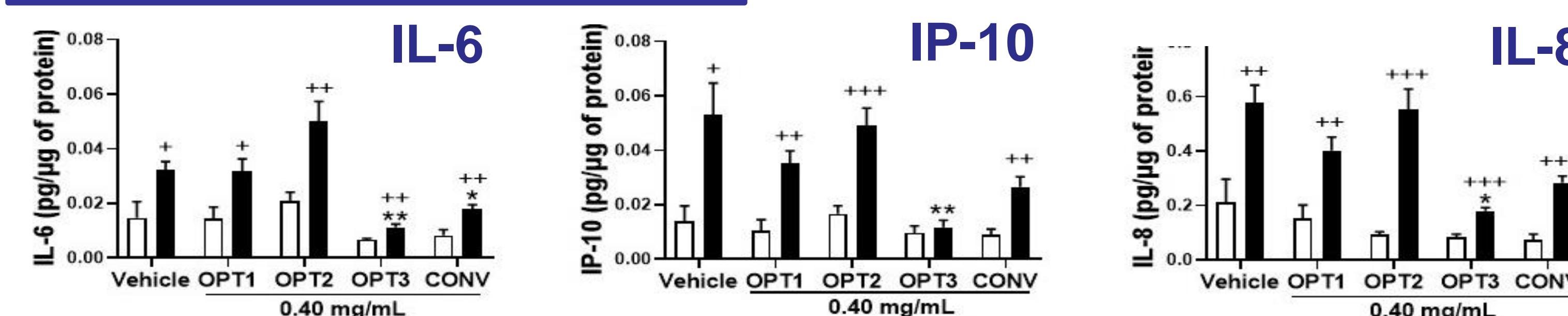13.8 mg_OL/g_DE
T=66.0ºC EtOH%=92.0% S/L=0.8 g/mL
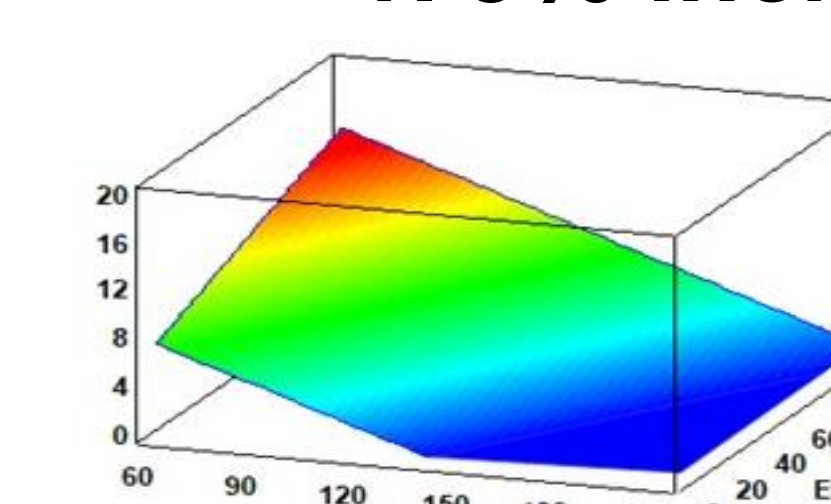
OPT3
428% increase in HT
9.5 mg_HT/g_DE
T=184.0ºC EtOH%=90.0% S/L=0.8 g/mL

Cell-based assays

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

First screening on HCE

IL-6 — IP-10 — IL-8

☐ Control cells   ■ 25 ng/mL TNF-α

At 0.40 mg/mL
OPT3 reduced significantly all measured ILs. CONV reduced significantly IL-6 secretion.

Final selection of:
CONV and OPT3
for dose-dependent studies on both HCE and IM-ConjEpi

Dose-dependent studies

On HCE, CONV inhibited IL-6 & IL-1β secretion dose-dependently, and IL-8 production at 0.6 & 0.8 mg/mL. CONV also decreased IP-10 levels on IM-ConjEpi dose-dependently.
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.

HCE — IL-6, IL-8, IL-1β
IM-ConjEpi — IP-10

○ Control cells   ■ 25 ng/mL TNF-α
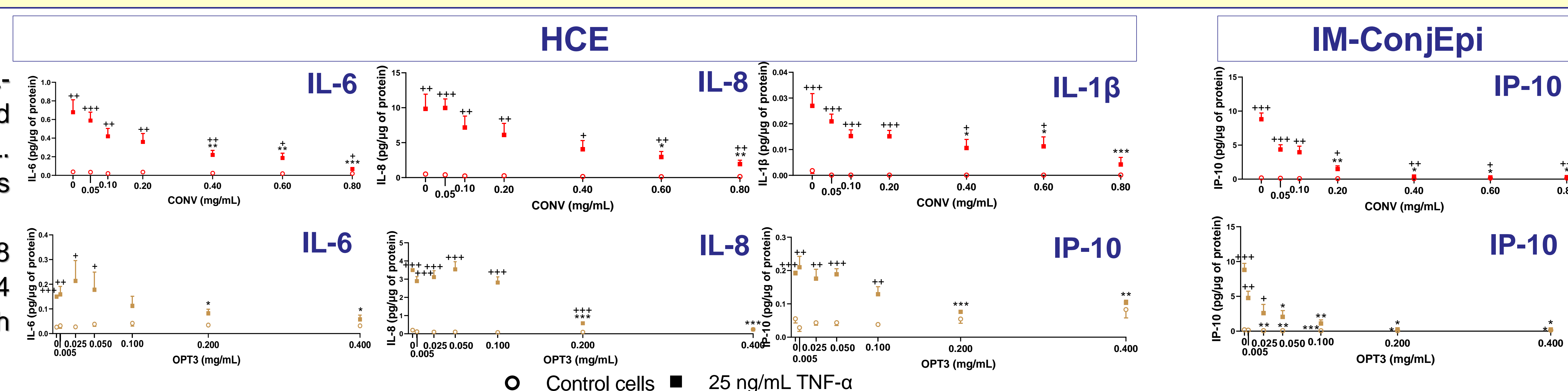
CONCLUSIONS

✓ Extracts with demonstrated anti-inflammatory activity on both HCE and IM-ConjEpi cells were produced from an environmentally hazardous agro-industrial by-product.

✓ The results of this study illustrate how sustainable and intensified extraction techniques are proved to remarkably increase selectivity towards biomarkers related to DE, as compared to conventional process.

✓ These *in vitro* data consist an essential baseline for the treatment of these diseases in the future.

✓ The use of these type of OP extracts is of chief importance for the green development of olive oil industries.

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