

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 by-product 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 anti-carcinogenic**, among others.

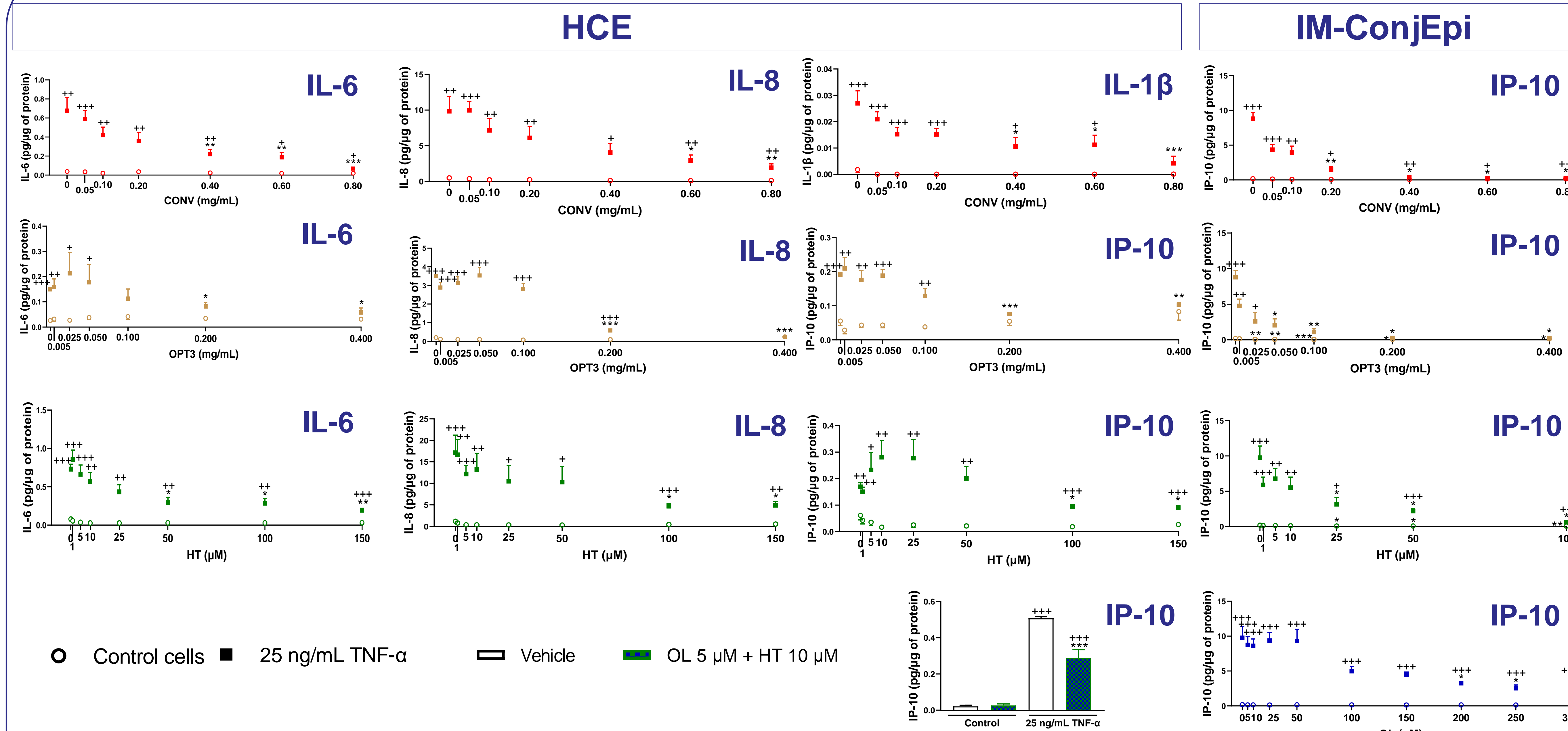
The aim of this study was to determine the **anti-inflammatory and antioxidant** activity of crude extracts derived from OP and their two major phenolic compounds (OL and HT), in human corneal (HCE) and conjunctival (IM-ConjEpi) epithelial cells.

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.
- HCE (Araki-Sasaki, IOVS 2005) and IM-ConjEpi (Innoprost) 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 μM OL + 10 μM HT), 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 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.

RESULTS

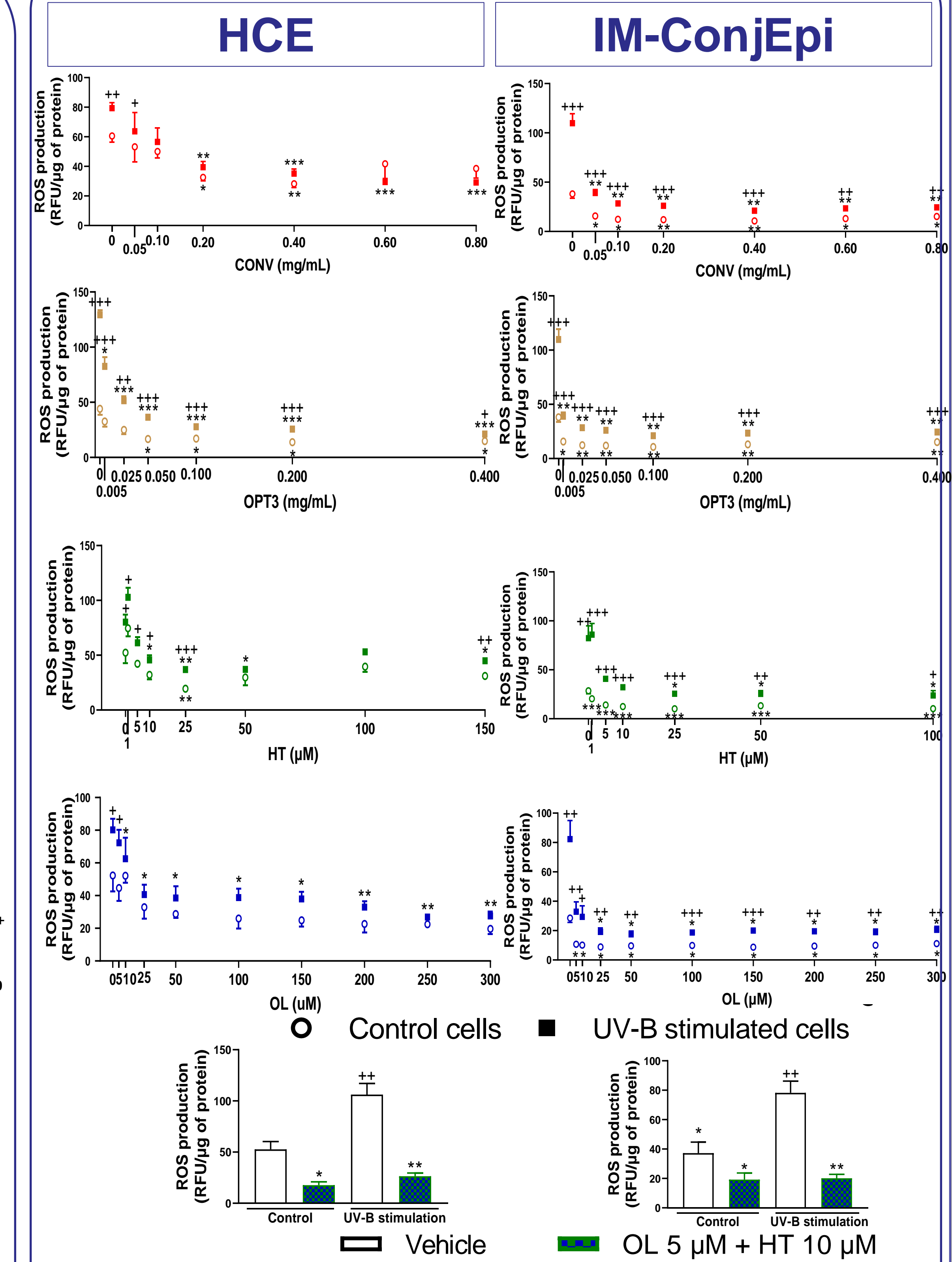
Interleukins/Chemokines secretion



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 μM **OL** + 10 μM **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

ROS production



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**.

CONCLUSIONS

- ✓ **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.**