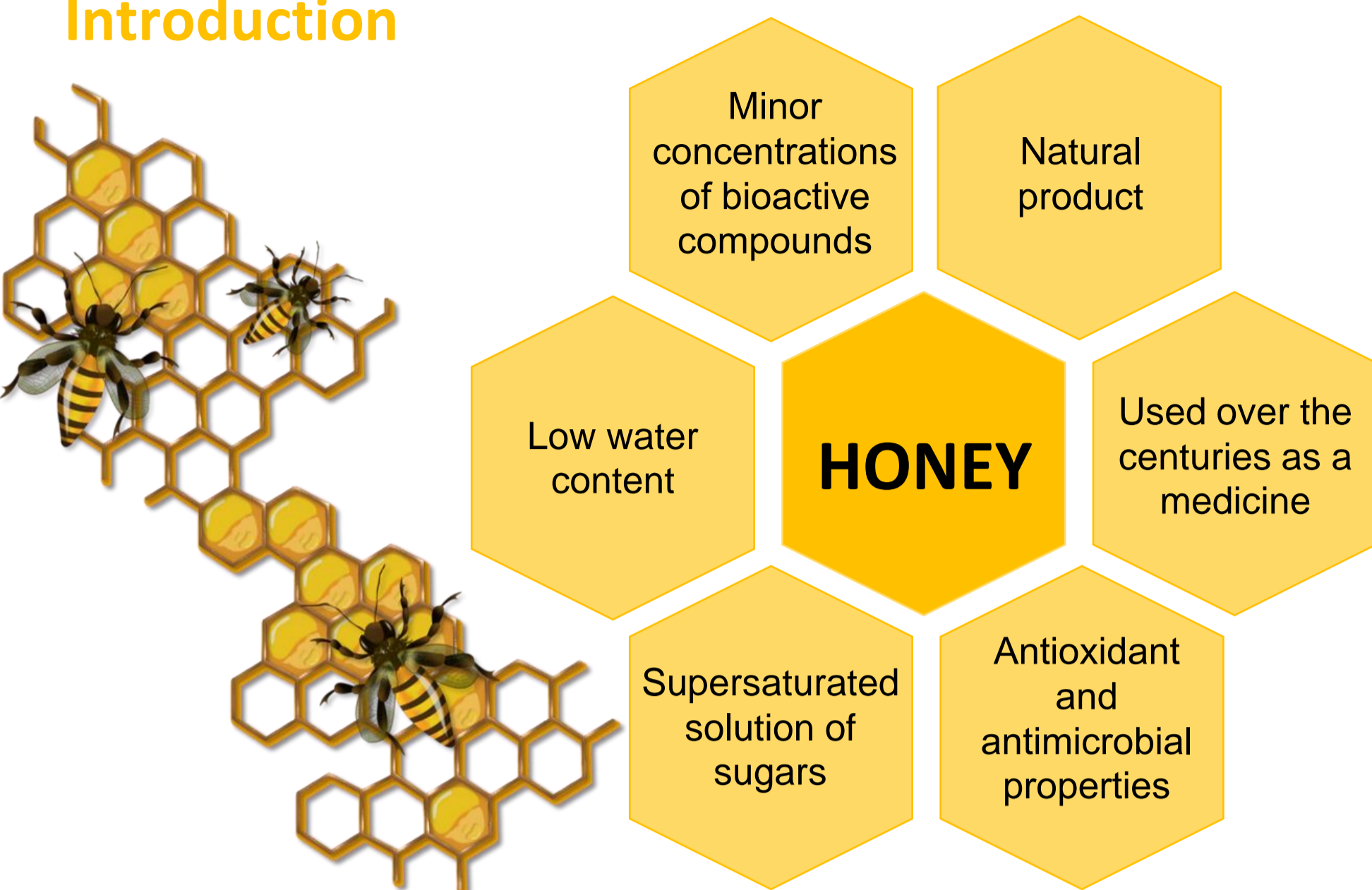


# Highly Effective Anti-inflammatory Potential of Centauri Cave Honey

Márcia Santos Filipe<sup>1,2</sup>, Przemysław Sitarek<sup>3</sup>, Tânia C. S. P. Pires<sup>4,5</sup>, Ricardo C. Calhella<sup>4,5</sup>, Ana María Diaz-Lanza<sup>1</sup>, Miguel Vilas-Boas<sup>4,5</sup>, Soraia Falcão<sup>4,5</sup>, Patrícia Rijo<sup>2,6\*</sup>

<sup>1</sup>Universidad de Alcalá de Henares. Facultad de Farmacia, Departamento de Ciencias Biomédicas, Madrid, España. <sup>2</sup>CBIOS – Universidade Lusófona's Research Center for Biosciences & Health Technologies, Portugal. <sup>3</sup>Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. <sup>4</sup>Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal. <sup>5</sup>Instituto de Investigação do Medicamento (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal. <sup>6</sup>Corresponding Author: [patricia.rijo@ulusofona.pt](mailto:patricia.rijo@ulusofona.pt)

## Introduction



Centauri Cave Nymph Honey is a Cave honey extracted from 2500 meters above sea level from a deep cave by professional speleologists in the Caucasus Mountains of Turkey.

The bee colony is located 50 kilometers from the nearest human residences away from other bee colonies.



## Aim

The aim of this work was to investigate the bioactivity of Centauri Cave Nymph Honey to validate the medicinal properties of this honey.

## Results and Discussion

### Quality and physicochemical parameters of honey

The quality and physicochemical parameters of the honey samples were assessed in previous studies [2] and herein we described the anti-inflammatory activity of Centauri honey.

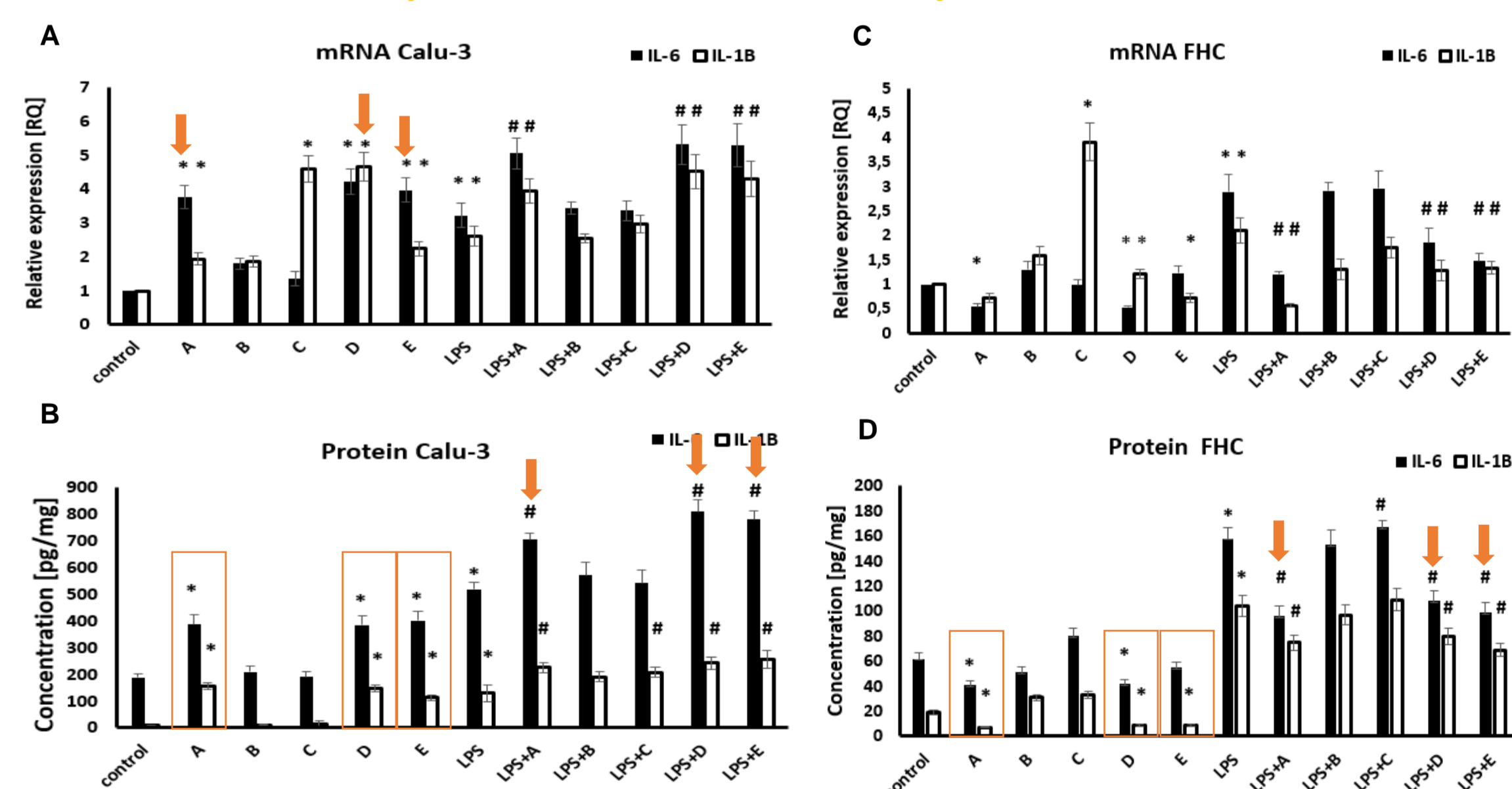
### Antioxidant and Anti-inflammatory Activity

**Table 1.** Antioxidant activity was tested through TBARS assay. The anti-inflammatory activity was tested using RAW 264.7 macrophages. Results are expressed as mean ± standard deviation.

Samples	TBARS (EC <sub>50</sub> , mg/mL)	NO Production Inhibition (IC <sub>50</sub> , µg/mL)
A	2.0 ± 0.1	62.75 ± 6.03
B	>166.7	17.86 ± 1.45
C	>166.7	32.74 ± 2.34
D	>166.7	63.76 ± 3.30
E	4.8 ± 0.2	>400
Positive control	0.0058 ± 0.0006	6.3 ± 0.4

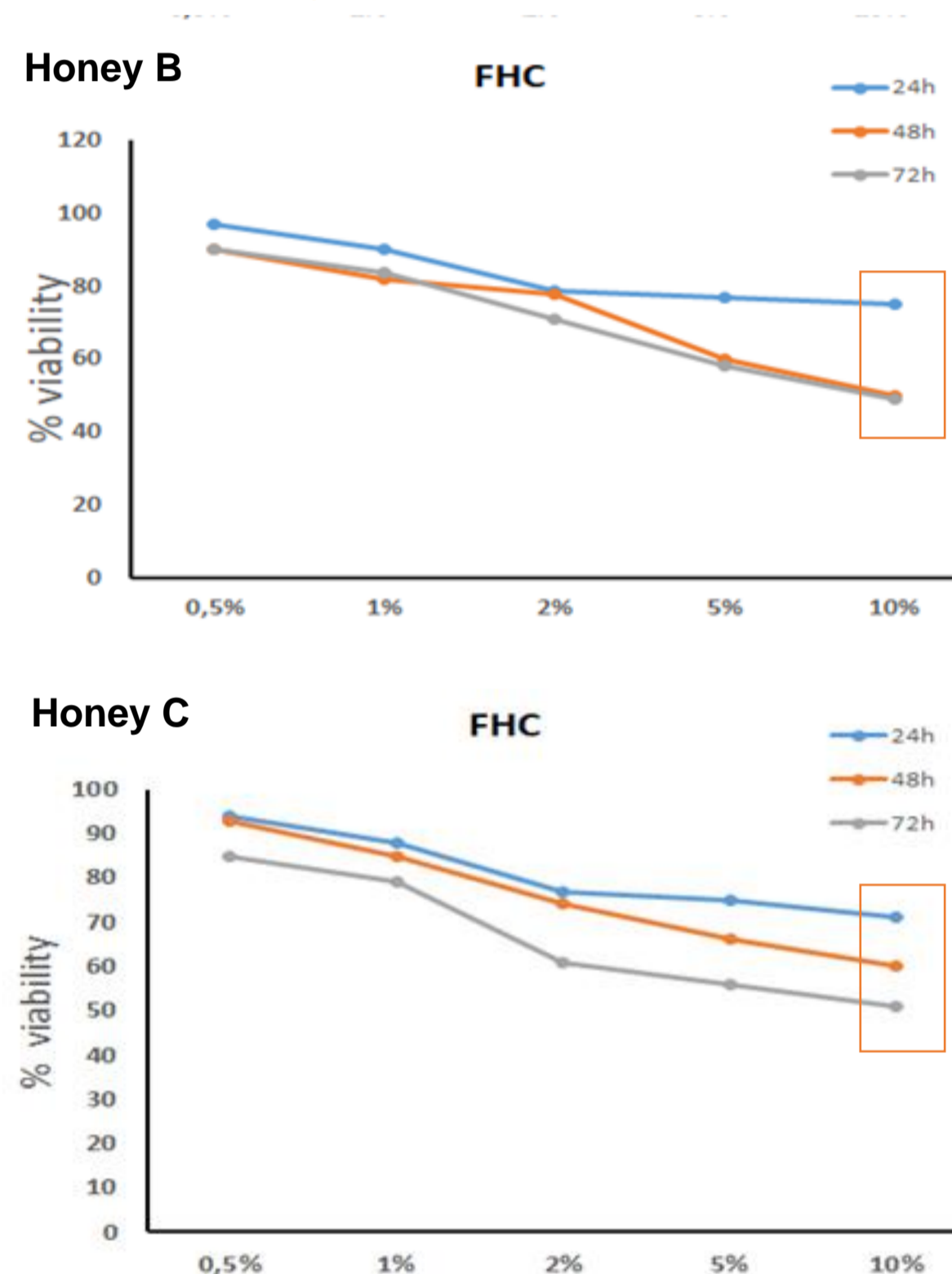
Positive controls: TBARS- Trolox. Anti-inflammatory- Dexamethasone.

### Anti-inflammatory effect of LPS-induced honeys in an *in vitro* model



**Graph 1.** The effect of the tested honeys on the expression of pro-inflammatory cytokines in Calu-3 cells. Expression at the mRNA (A) and protein (B) levels. | Evaluation of the effect of selected honeys on mRNA (C) and protein expression (D) of IL-1β and IL-6 in FHC cells. Data presented as mean; \*p<0.05 comparing to control, #p<0.05 comparing to LPS.

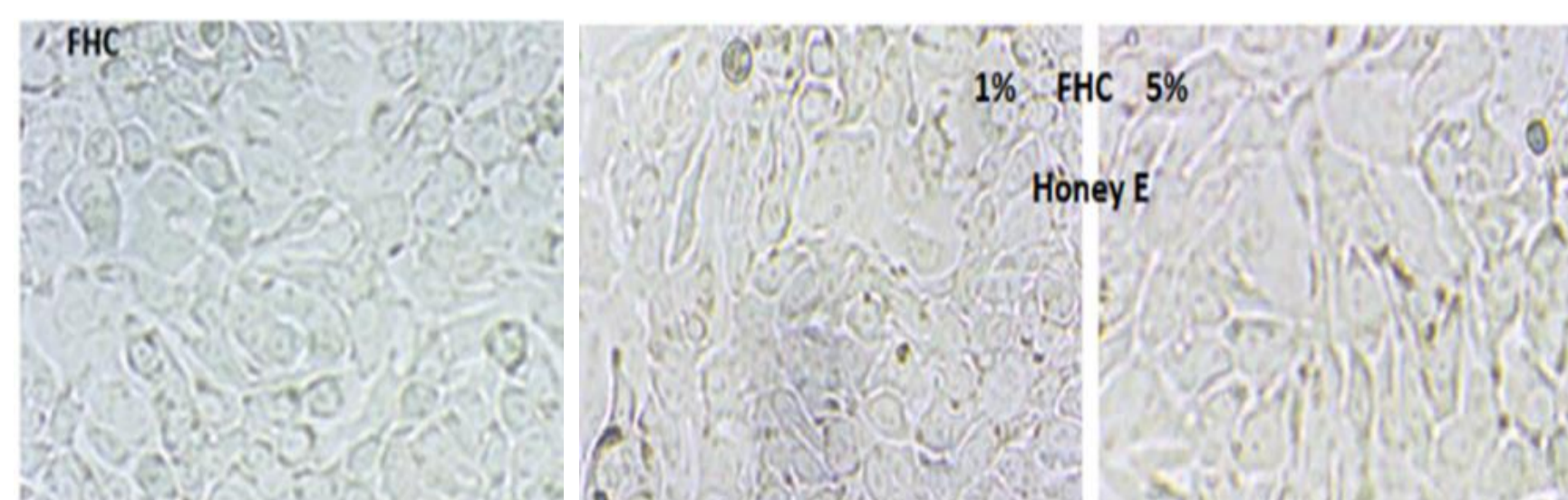
### Cell viability



No cytotoxic effect on FHC cell line (normal colon cell line) normal (after 24 h after exposure) but honey B and C were the least active at all times

**Graph 2.** Cell viability determined by MTT assay after stimulation for 24h, 48h and 72h: with honey B and C in FHC cells.

### Morphological changes after honey treatment in Calu-3 and FHC cells



**Figure 1.** Morphological changes after honey treatment in FHC (normal colon cell line) were not observed at 1 and 5% concentration (Cell morphology before and after honey stimulation: Magnification x40).

## Discussion

Remarkably, the strongest effect was observed for A, D and E honeys. In turn, in the normal FHC model, a decrease in the level of the same LPS-induced cytokines in cells was demonstrated. The best effect was observed for A, D and E honeys. These compelling findings suggest that Centauri honey possesses the potential to enhance immune responses.

Further studies are ongoing to scientifically validate the medicinal properties of Centauri Honey due to its exceptional chemical composition and thus to become an innovative Ingredient.

**Acknowledgments:** This work was financially supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) under projects UIDB/04567/2020 and UIDP/04567/2020 attributed to CBIOS. The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2020); and to the national funding by FCT, P.I., through the institutional scientific employment program-contract for R. C. Calhella's contract.

**References:** [1] Maddison Hunter *et al.*, Current Research in Food Science. 2021; 532–542. DOI: 10.1016/j.crf.2021.08.002. [2] GA-Congress, Dublin, Ireland, July 2023.