

MECHANISTIC STUDIES OF CYTOTOXICITY INDUCED BY A PORTUGUESE PROPOLIS EXTRACT, USING *SACCHAROMYCES CEREVISIAE* AS EUKARYOTIC CELL MODEL

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Propolis is a natural complex mixture produced by honey bees (particularly *Apis mellifera* L.) by collecting exudates from various plant sources. Characterized by a plethora of chemicals, propolis is generally rich in flavonoids, phenolic acids and terpene derivatives, bioactive compounds associated to its antimicrobial, anti-inflammatory, antimutagenic and antioxidant activities¹. Previous work had shown that the ethanol extract of a sample from the Portuguese region of Beira Alta exhibited unique dual genotoxic and antigenotoxic effects using the yeast *S. cerevisiae* eukaryotic model². In this work we prepared two ethanol extracts (EE) of propolis samples from Pereiro (P) - Beira Alta - collected in 2010 (P10.EE) and 2017 (P17.EE) to investigate the mechanisms of cytotoxicity and genotoxicity using specific *S. cerevisiae* mutants. While P17.EE didn't show any toxic effect, yeast cells exposed to P10.EE showed a considerable decreased viability along time, assessed by colony-forming units. Interestingly, the oxidative stress response-defective mutant *yap1* was more resistant than the wild type, suggesting that this cytotoxic effect was not mediated by oxidative stress. P.EE's genotoxicity was also analysed by the nucleus-cytosolic translocation of NHP6A protein, considered a marker of necrosis. P10.EE induced NHP6A protein translocation to the cytoplasm, observed by fluorescence microscopy, suggesting that cytotoxicity of this extract was indeed mediated by necrosis. Although P17.EE didn't seem to induce necrotic cell death, both extracts induced plasma membrane integrity loss, assessed by flow cytometry, using propidium iodide as marker. As recently observed for erythroleukemic cells with Brazilian propolis³, here we present the first evidence that also Portuguese propolis have necrotic-mediated cytotoxicity in yeast cells.

Acknowledgments:

This work is supported by: European Investment Funds by FEDER/COMPETE/POCI-Operational Competitiveness and Internationalization Programme, under Project POCI-01-0145-FEDER-006958 and National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UID/AGR/04033/2013.

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