IMMUNOMODULATORY ACTIVITY OF BRAZILIAN RED PROPOLIS CONSTITUENTS IN J774A.1 MURINE MACROPHAGES *IN VITRO*

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ABSTRACT

Background: Brazilian red propolis (BRP) is a complex natural resin produced by honeybees that is rich in bioactive constituents that exhibit several pharmacological activities, including anti-inflammatory properties. Macrophages play crucial roles in the inflammatory process and repairing damaged tissues, which makes them a target for the development of new immunomodulatory agents. **Objectives:** The aim of this study was to investigate the immunomodulatory activity of the BRP constituents (biochanin A, 2-coumaric acid, catechin hydrate, p-coumaric acid, pinocembrin and chrysin) in macrophages *in vitro*. **Methods:** Initially, the compounds were screened for their cytotoxicity in J774A.1 murine monocyte/macrophage cell lineage by using MTT assay. For this purpose, cells were treated with the six compounds at concentrations of 25 and 50 µM for 48 h. To assess their immunomodulatory activity, J774A.1 cells were stimulated with LPS (200 ng/mL) and treated with 50 µM compounds for 48 h. The expression of F4/80 and the costimulatory molecule CD86 was then analyzed by flow cytometry, and the quantification of pro- and anti-inflammatory cytokines in the cell culture supernatant was assessed via cytometric bead array (CBA). The phosphorylation of the ERK1/2, p38 and JNK proteins was assessed via intracellular flow cytometry to evaluate MAPK (mitogen-activated protein kinase) activation. **Results and discussion:** Neither of the compounds affected cell viability at the concentrations and conditions tested. Only chrysin treatment significantly reduced the percentage of double-positive cells (F4/80+ CD86+) and the median fluorescence intensity (MFI) of both markers. LPS stimulation induced a significant increase in IL-6 and TNF-α levels, and chrysin treatment significantly reduced the levels of IL-6 but not TNF-α after 48 h. Furthermore, after CHRY treatment, there was a decrease in ERK1/2 phosphorylation and an increase in JNK phosphorylation. In contrast, p38 phosphorylation was not altered under the conditions tested. **Conclusion:** These results suggest that chrysin, as a compound with promising immunomodulatory activity, has the ability to modulate the activation status of macrophages. Further tests are needed to elucidate the biological mechanisms involved in the immunomodulatory effects of chrysin contributing for the development of new anti-inflammatory therapies.

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