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Exploring the antioxidant, anti-inflammatory and antiallergic potential of Brazilian propolis in monocytes

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ABSTRACT

Background: Propolis is a bee product used since ancient times due to its diverse biological applications. We aimed to investigate the antioxidant and anti-inflammatory action of Brazilian propolis in THP-1 cells stimulated with 1-fluoro-2,4-dinitrobenzene (DNFB) or lipopolysaccharide (LPS).
Methods: Cell viability was assessed by resazurin assay, antioxidant activity was evaluated by DPPH assay, superoxide dismutase (SOD) 1 and 2 activity was evaluated by colorimetric assay. CD86 expression was determined by flow cytometry. IL-1β and HMOX-1 were analysed by Western blot.
Results: Propolis did not affect cell viability and exhibited a potent antioxidant activity. Propolis alone induced SOD 1 activity. LPS stimulated SOD2, concomitantly or not with propolis. In the presence of LPS, propolis induced a higher HMOX-1 expression. Propolis inhibited CD86 expression stimulated by DNFB. LPS induced pro-IL-1β expression and propolis did not affect its action.
Conclusion: Propolis exhibited an antioxidant action in a cell-free system and in a cell based-model. Propolis exerted an anti-inflammatory/antiallergic action, probably due to its antioxidant activity.

1. Introduction

Propolis is made by bees from different plant sources, including the bark and buds of trees, containing a mixture of components like flavonoids, phenylpropanoids, terpenes, stilbenes, lignans, coumarins, and their prenylated derivatives. Its chemical composition varies according to the geographical location and botanical origin (Burdock, 1998; Huang et al., 2014; Popova et al., 2021).

Propolis is classified according to their geographic localization and botanic origin. Green propolis is extensively found in Brazil and its main vegetal source is *Baccharis dracunculifolia* DC. Brown propolis may be found in many regions of Brazil and Cuba, presenting a variable botanic source, such as *Luehea* sp. (Malvaceae), *Piptadenia falcate* Benth (Fabaceae), *Tabebuia* spp. (Bignoniaceae), *Tabebuia caraiba* (Mart.) Bureau (Bignoniaceae), *Vernonia* spp. (Asteraceae), and *Cecropia pachystachya* Trecul (Urticaceae). Red propolis is especially attributed to *Dalbergia ecastophyllum* (L.) Taub. (Fabaceae) and is found in Brazil, Cuba, Mexico, China and Venezuela (Santos et al., 2020).

Our sample is classified as green propolis and was previously analyzed, presenting the following constituents or chemical groups: benzoic acid, dihydrocinnamic acid, 3,5-diprenyl-4-hydroxycinnamic acid (artepillin C), *p*-coumaric acid, prenyl-*p*-coumaric acid, caffeic acid, 1,3- and 4,5-dicaffeoylquinic acids, 3,4,5-tricaffeoylquinic acid, flavones (6,8 di-*C*-hexosyl apigenin, 6-*C*-pentosyl-8-*C*-hexosyl apigenin and 6-*C*-hexosyl-8-*C*-pentosyl apigenin), trihydroxymethoxy flavanone, tetrahydroxy flavanone and triterpenes (Búfalo et al., 2013; Conti et al., 2015).

Propolis popular use comes from around 300 BCE, used by ancient civilizations for different purposes, such as wound treatment, as a mouth disinfectant, for mummification of corpses, anti-eczema, anti-myalgia, and anti-rheumatism agent, among other applications (Silva-Carvalho et al., 2015). Research on propolis action has intensified over the last decades, demonstrating remarkable immunomodulatory, antimicrobial

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Abbreviations

BCA	bicinchoninic acid
CD	cluster of differentiation
DNFB	1-fluoro-2,4-dinitrobenzene
DPPH	2,2-diphenyl-1-picrylhydrazyl
FBS	fetal bovine serum
FITC	fluorescein isothiocyanate conjugate
GC–MS	gas chromatography–mass spectrometry
HCl	*hydrochloric acid*
HEPES	2-[4-(2-*hydroxyethyl*)−1-*piperazinyl*]ethanesulfonic acid
HMOX-1	heme oxygenase-1
HPLC–PDA–ESI/MS^n	high performance liquid chromatography coupled to photodiode-array detector and interfaced with a electrospray ionization mass spectrometer
IC50	half-maximal inhibitory concentration
ICAM-1	intercellular adhesion molecule-1
IL	interleukin
IFN-γ	*interferon gamma*
LPS	lipopolysaccharide
NaCl	*sodium chloride*
Nrf2	*nuclear factor-erythroid-2–related factor 2*
PBS	phosphate buffered saline
PVDF	polyvinylidene fluoride
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
SDS-PAGE	*sodium dodecyl sulfate–polyacrylamide gel electrophoresis*
SOD	superoxide dismutase
TNF-α	tumor necrosis factor alpha
WST-1	[2-(4-Iodophenyl)- 3-(4-nitrophenyl)−5-(2,4-disulfophenyl)−2Htetrazolium.

and antitumor properties (Sforcin, 2016; Cardoso et al., 2022; Conte et al., 2021). Due to its diverse biological activities, propolis may be used in food supplementation, cosmetics and in the pharmaceutical industry (Silva-Carvalho et al., 2015; Berretta et al., 2020).

Oxidative stress is characterized by the production of reactive oxygen species (ROS) and the inability of the natural antioxidants to control their generation. When an imbalance occurs between the oxidant and antioxidant systems, cellular damage and tissue injury can be observed, which contributes to the development of the inflammatory process (Pisoschi and Pop, 2015). Since oxidative stress and inflammation are involved in several diseases, it is important to search for alternative treatments to minimize these effects and to promote human health. In recent years, research in natural products has grown due to their beneficial properties (Hussain et al., 2016) and less side effects compared to synthetic medication (Toreti et al., 2013).

Data from literature has shown the beneficial action of propolis in inflammatory diseases such as neuronal degenerative disease, diabetes, chronic kidney disease, skin disorders, among others (Silva-Carvalho et al., 2015). However, the mechanisms by which propolis performs these activities are not yet fully understood and are somewhat controversial.

The main mechanisms involved in propolis anti-inflammatory action include the inhibition of cyclooxygenase and prostaglandin biosynthesis, free radical scavenging, inhibition of nitric oxide synthesis, and reduced inflammatory cytokines secretion. Propolis has also been described for its skin healing action and skin protection, stimulating skin tissue growth and regeneration, as well as improving collagen production and cell viability after exposed to oxidative stress (Braakhuis, 2019).

Our group has been investigating the action of propolis on human monocytes, which are phagocytic cells quickly recruited to sites of injury or infection, mediating the initial defense against pathogens (Geissmann et al., 2008) and playing an important role in many inflammatory diseases. THP-1 cell is a human monocytic cell lineage with relatively similar response patterns to primary monocytes. This cell line is widely used in vitro, avoiding genetic variability between individuals (Chanput et al., 2014) and providing a better comprehension of propolis mechanisms of action.

In this work, we aimed at investigating the antioxidant potential of propolis and its ability to prevent the activation of inflammatory/oxidative pathways in THP-1 monocytes stimulated with the skin allergen 1-fluoro-2,4-dinitrobenzene (DNFB), responsible for a skin inflammatory condition, or the Toll-like receptor (TLR)−4 agonist lipopolysaccharide (LPS) responsible for a systemic pro-inflammatory response.

2. Material and methods

2.1. Propolis sample

Propolis was produced in the Beekeeping Sector, UNESP, Botucatu. The plants visited by bees to produce propolis were identified and their vouchers specimens were stored at the Herbarium BOTU of the Institute of Biosciences, UNESP, obtaining the following registration numbers: *Araucaria angustifolia* (Bert.) O. Kuntze - BOTU 09866 - 18.03.98, *Baccharis dracunculifolia* DC - BOTU 09867 - 18.03.98 and *Eucalyptus citriodora* Hook - BOTU 04502 - 22.09.98. Plant names were taxonomically validated and may be checked at http://www.theplantlist.org.

2.1.1. Propolis extraction

Propolis was ground and ethanolic extracts were prepared (30 g of propolis/100 mL ethanol 70%), at room temperature, in the absence of light and under moderate shaking for one week, according to Sforcin et al. (2005). Then, the extracts were filtered and the dry weight was determined after complete evaporation of propolis solvent in an aliquot (1 mL), obtaining 140 mg of propolis per mL. Specific dilutions were prepared for each assay in RPMI 1640 culture medium (Sigma-Aldrich Chemical, USA) containing 18 mM sodium bicarbonate, 25 mM glucose, 10 mM 2-[4-(2-*hydroxyethyl*)−1-*piperazinyl*]ethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% (v/v) heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich Chemical, USA). The extract was filtered using a Millipore membrane filter 0.22 μm pore size, 30 mm diameter (Kasvi, Brazil). Propolis concentrations (1, 5, 10, 20 or 50 μg/mL) were previously standardized by our group in cell-based assays (Búfalo et al., 2014; Conti et al., 2016).

2.2. THP-1 cell culture

THP-1 human monocytic cell line (American Type Culture Collection TIB-202; InvivoGen, France) was cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin and incubated at 37 °C and 5% CO_2. Cells were maintained at 0.5×10^6 to 1×10^6 cells/mL and were sub-cultured every 2–3 days. All experiments were performed in duplicate with three independent assays.

2.3. Cell viability

In order to disclose the safe concentrations of propolis to be further exploitated in the subsequent biological assays, a dose-response curve was performed using the colorimetric Alamar Blue (Resazurin) assay, as previously described by O'Brien et al. (2000).

THP-1 cells were cultured at 0.2×10^6 cell/well in a final volume of 200 μL. Cells were treated with propolis (1, 5, 10, 20 or 50 μg/mL) or 70% ethanol with the same proportion found in 50 μg/mL. After 20 h of incubation at 37 °C and 5% CO_2, resazurin (20 μL – final concentrarion 50 μM) was added and incubated in the same conditions for 4 h. Absorbance was determined at 570 and 600 nm using the spectrophotometer Synergy HT Multi Detection Microplate Reader (Biotek Instruments, USA).

2.4. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Since much inflammatory pathology is fueled by oxidative stress, we further investigated whether propolis displays an antioxidant activity, using a cell free approach. The antioxidant capacity of propolis was determined by its ability to capture the free radical DPPH, according to Blois (1958). Thus, 10 μL of propolis samples (5 – 50 μg/mL) were assessed by their reactivity with 50 μL of DPPH methanolic solution 500 μM (Sigma-Aldrich Chemical, USA), with 100 μL of acetate buffer 0.2 M (pH = 6) and 140 μL of methanol (Merck, USA). The plate was incubated for 30 min at room temperature in dark conditions. The absorbances were read at 517 nm using the Multiskan FC spectrophotometer (Thermo Scientific, USA). Propolis inhibitory concentration necessary to decrease the absorbance of DPPH by 50% (IC50) was calculated from the calibration curve determined by linear regression.

2.5. Superoxide dismutase (SOD) 1 and 2 activity

SODs are present in eukaryotic cells as the main antioxidant defense systems against oxidative stress in the organism. Therefore, we investigated propolis antioxidant action on a cell based-model, focusing on SOD activity in THP-1 cells. SOD total activity was determined by the colorimetric assay, using the SOD Determination Kit (Sigma-Aldrich Chemical, USA), according to the manufacturer's instructions.

THP-1 cells (2×10^6 cells/well, final volume 2 mL) were incubated with propolis (50 μg/mL) or its solvent for 30 min before LPS (from *Escherichia coli* O26:B6, Sigma-Aldrich Chemical, USA, purity ≥ 95%; 1 μg/mL) stimulation. Cells were incubated at 37 °C and 5% CO_2 for 24 h.

After, cells were centrifuged at 300 *g* for 5 min at 4 °C and washed with cold PBS. Cells were lysed using 100 μL of RIPA buffer (50 mM Tris–HCl, pH 8.0, 1% Nonidet P-40, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate and 2 mM ethylenediaminetetraacetic acid) for 30 min on ice. After centrifugation at 12,000 *g* for 10 min at 4 °C, the supernatant containing the extracts was collected and protein concentration was determined by the bicinchoninic acid (BCA) method.

Briefly, 20 μL of each sample was mixed with 200 μL of the working solution containing WST-1 [2-(4-iodophenyl)- 3-(4-nitrophenyl)−5-(2,4-disulfophenyl)−2Htetrazolium, monosodium salt] and 20 μL of the working solution containing the enzyme. To evaluate SOD 2 activity, 2 μL of the SOD 1 inhibitor – potassium cyanide (final concentration 2 mM) were added. The reaction was incubated for 20 min at 37 °C and the absorbance was performed at 470 nm using the Synergy HT Multi Detection Microplate Reader (Biotek Instruments, USA). SOD 1 was determined by the formula: SOD 1 = total SOD – SOD 2.

2.6. CD86 expression by flow cytometry

Since several pro-inflammatory events are evoked by oxidative stress we hypothesize that, besides its antioxidant properties, propolis could also mitigate the production of pro-inflammatory molecules in monocytes. Therefore, we evaluated the effect of propolis on THP-1 cells stimulated with two different pro-inflammatory stimuli, the skin allergen DNFB and the TLR-4 agonist LPS. DNFB causes allergic contact dermatitis, a Type IV [delayed-type] hypersensitivity response, characterized by excessive ROS production and inflammation and manifests as a local skin rash, itchiness, redness, swelling, and lesions. THP-1 cells are frequently used to detect skin allergens since they evoke the upregulation of cell surface co-stimulatory molecules expression, for instance CD86. Since this assay could be used to screen molecules with anti-allergic potential, we investigated whether propolis could mitigate the increase on CD86 expression triggered by the strong skin allergen DNFB by flow cytometry (Luís et al., 2014).

THP-1 cells (0.8×10^6 cells/well, final volume 1.5 mL) were incubated in the presence of propolis (50 μg/mL) or its solvent (ethanol 70%) for 30 min at 37 °C and 5% CO_2. Then, 1.5 μL of the allergen DNFB (8 mM – Sigma-Aldrich Chemical, USA, purity ≥ 99%) was added to the culture and the cells were incubated for 24 h.

The cells were washed twice at 300 *g* / 5 min and resuspended in 100 μL of phosphate buffered saline (PBS) supplemented with 1% FBS. Cells were incubated with 3 μL of monoclonal antibody anti-CD86-Alexa488 (Clone IT2.2, BioLegend) for 30 min at 4 °C. For each sample, 10.000 events were analyzed using a BD AccuriTM C6 flow cytometer (BD, USA). The data represents a percentage (%) of cells expressing the surface markers.

2.7. Western blot analysis

THP-1 cells (2.4×10^6 cells/well, final volume 3 mL) were incubated in the presence of propolis (50 μg/mL) or 70% ethanol for 30 min before LPS (1 μg/mL) stimulation. Cells were incubated at 37 °C and 5% CO_2 for 24 h and western blot analysis was performed, adapted from Silva et al. (2020).

Cell lysates were obtained with 100 μL of RIPA lysis buffer supplemented with 1 mM dithiothreitol, protease and phosphatase inhibitor cocktails (Roche, Germany) for 30 min on ice. Cells were centrifuged at 12,000 *g* for 10 min at 4 °C to remove cell debris. Protein concentration was determined by the BCA method. Subsequently, the lysates were denatured at 95 °C for 5 min in a buffer containing 0.125 mM Tris pH 6.8, 2% (w/v) sodium dodecyl sulfate, 100 mM dithiothreitol, 10% glycerol and bromophenol blue.

Proteins were separated by 4–10% (v/v) SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Amersham Biosciences, Sweden). The membranes were incubated with the primary antibody for IL-1β (1:200; Santa Cruz Biotechnology, USA), heme oxygenase-1 (HMOX-1) (1:1000; Thermo Scientific, USA) and the control β-tubulin (1:20,000; Sigma-Aldrich Chemical, USA) overnight at 4 °C. The following day, the membranes were washed and incubated with secondary antibodies (1:20,000; GE Healthcare, UK) for 1 h at room temperature. The immunoreactive bands were visualized using the ECF substrate and the imaging system ThyphoonTM FLA 9000 (GE Healthcare, UK). The bands densitometry was quantified using the TotalLab TL120 software (Molecular Dynamics, Amersham Biosciences).

2.8. Statistical analysis

Data was analyzed by SAS for Windows (version 9.2) software. A normality analysis was performed using Shapiro Wilk test. Differences were determined using Gamma distribution followed by Wald's multiple comparison test. Results are presented as means ± standard deviation (SD) of 3 different assays, in duplicate. The differences were considered significant at $p < 0.05$. For DPPH assay, the IC50 was calculated from the calibration curve determined by linear regression.

3. Results

3.1. Cell viability

THP-1 cell viability was not affected after incubation with propolis concentrations (1 – 50 μg/mL) or its solvent 70% ethanol (Fig. 1), highlighting the safety profile of propolis to human cells.

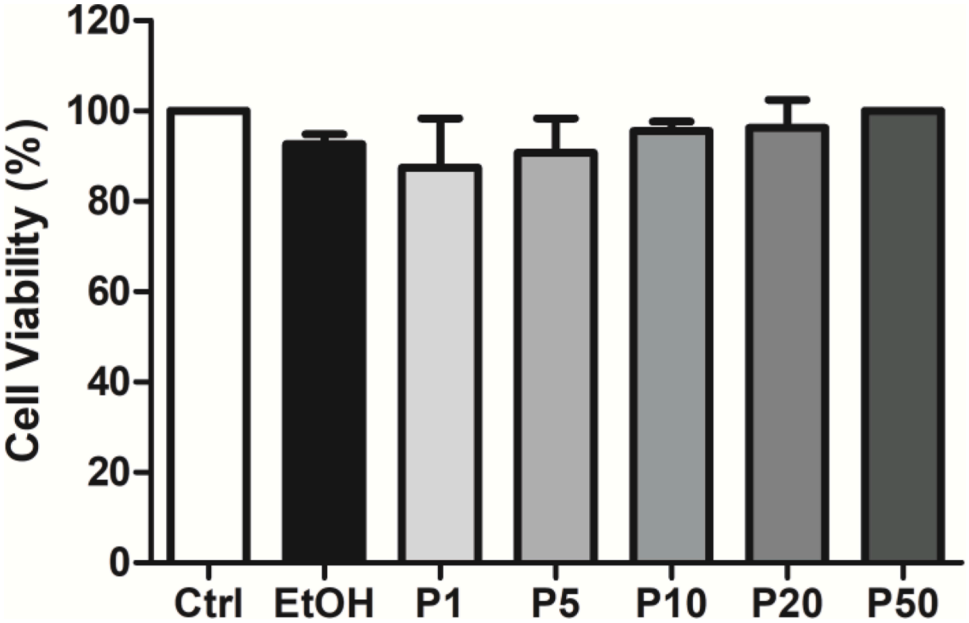

Fig. 1. Percentage (%) of THP-1 cells viability after incubation with culture medium (Ctrl), 70% ethanol (EtOH) or propolis (P – 1, 5, 10, 20 or 50 μg/mL) for 24 h by resazurin assay. Data represent mean ± SD of 3 independent assays performed in duplicate. Statistical analysis was performed using Gamma distribution followed by Wald's multiple comparison test ($p > 0.05$).

3.2. Propolis antioxidant activity

As shown in Fig. 2, propolis presented an antioxidant activity and the effective concentration for reducing 50% of DPPH was 18.52 μg/mL.

3.3. SOD 1 and SOD 2 activity and HMOX expression

Interestingly, propolis alone induced cytoplasmatic SOD 1 activity, while LPS and its association with propolis inhibited it. Regarding mitochondrial SOD 2, LPS stimulated it, concomitantly or not with propolis (Fig. 3).

Since HMOX-1 is upregulated during oxidative stress and it is critical in the response against oxidant-induced injury in many pathological conditions, we also investigated the effects of propolis on HMOX-1 expression in an attempt to further confirm its potential in the management of oxidative stress-related pathologies. A slight expression of HMOX-1 was observed in propolis or LPS-treated cells. Interestingly, in the presence of LPS, propolis significantly induced a higher expression of the antioxidant enzyme HMOX-1 (Fig. 4).

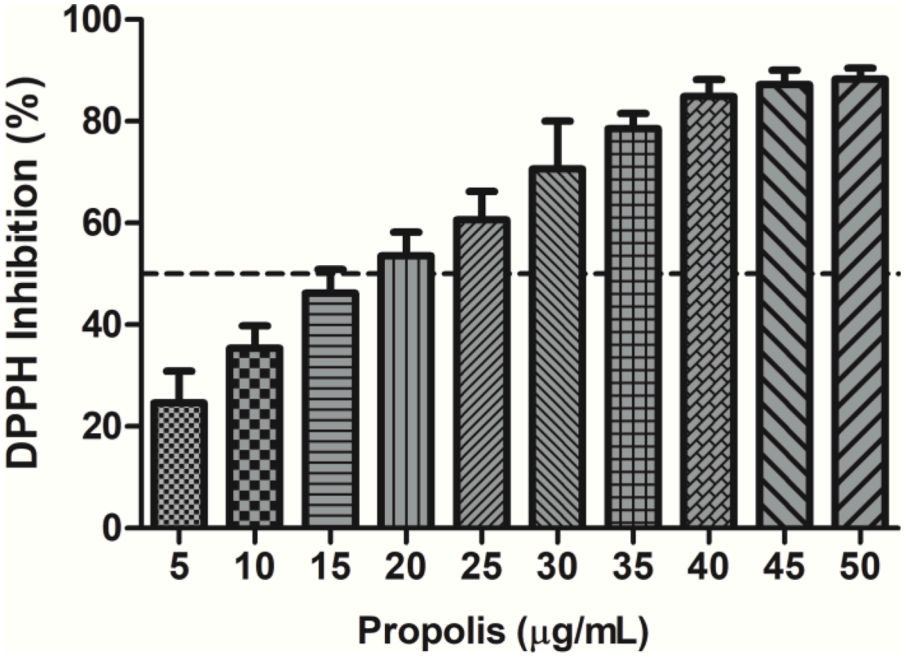

Fig. 2. DPPH inhibition (%) after incubation with different propolis concentrations (5–50 μg/mL) for 30 min. Data represent mean ± SD of 3 independent assays in duplicate. The dashed line indicates the concentration of propolis that inhibited 50% of DPPH. IC50 was calculated from the calibration curve determined by linear regression.

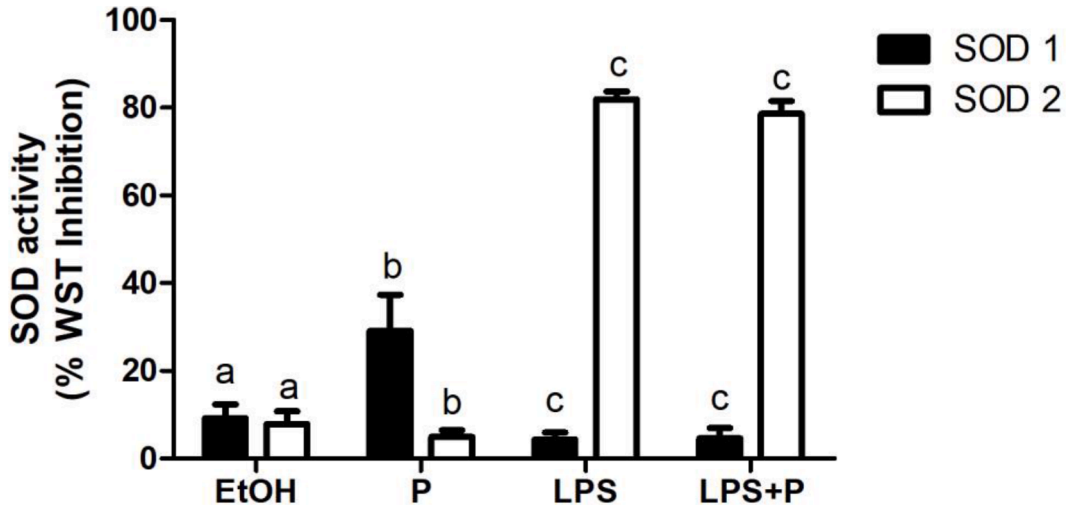

Fig. 3. SOD 1 and 2 activity (%WST inhibition) after THP-1 cells incubation with 70% ethanol (EtOH), propolis (P – 50 μg/mL), LPS (1 μg/mL) and LPS 1 μg/mL + propolis 50 μg/mL (LPS+*P*) for 24 h by a colorimetric test (SOD determination kit). Data represent mean ± SD of 3 independent assays performed in duplicate. Differences were determined using Gamma distribution followed by Wald's multiple comparison test. Means with the same letter do not differ at the 5% level of significance.

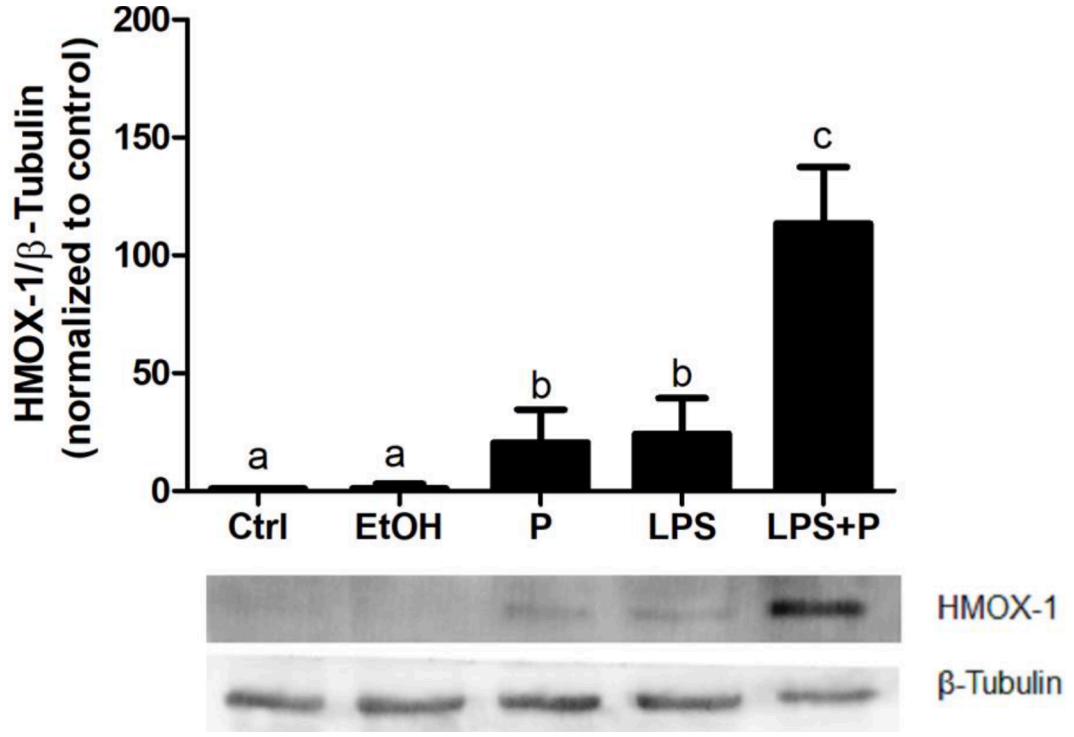

Fig. 4. HMOX-1 production by THP-1 cells after incubation with culture medium (Ctrl), ethanol (EtOH), propolis (P – 50 μg/mL), LPS (1 μg/mL) and LPS 1 μg/mL + propolis 50 μg/mL (LPS+*P*) for 24 h by Western blot. Data represent mean ± SD of 3 independent assays and a representative blot. HMOX-1 = 32 kDa; *β*-tubulin = 55 kDa. Differences were determined using Gamma distribution followed by Wald's multiple comparison test. Means with the same letter do not differ at the 5% level of significance.

3.4. Pro-inflammatory molecules: CD86 and pro-IL-1β expression

As expected, DNFB induced the expression of the co-stimulatory molecule CD86 and, interestingly, propolis inhibited CD86 expression stimulated by the allergen (Fig. 5), highlighting its antiallergic potential.

We further addressed the effect of propolis on THP-1 cells stimulated with LPS, specifically on the expression of the precursor form of IL-1*β* (pro-IL-1*β*). The results demonstrated that LPS strongly upregulated the pro-IL-1*β* expression, concomitantly or not with propolis (Fig. 6).

4. Discussion

In the last years, propolis has gained attention due to its potential application in the pharmaceutical industry, motivating a better understanding of its antioxidant and anti-inflammatory activity.

As expected, our propolis sample and its solvent did not affect cell viability, which was also observed in human monocytes using concentrations up to 100 μg/mL (Búfalo et al., 2014). As none of the concentrations used in our assay affected cell viability, the highest

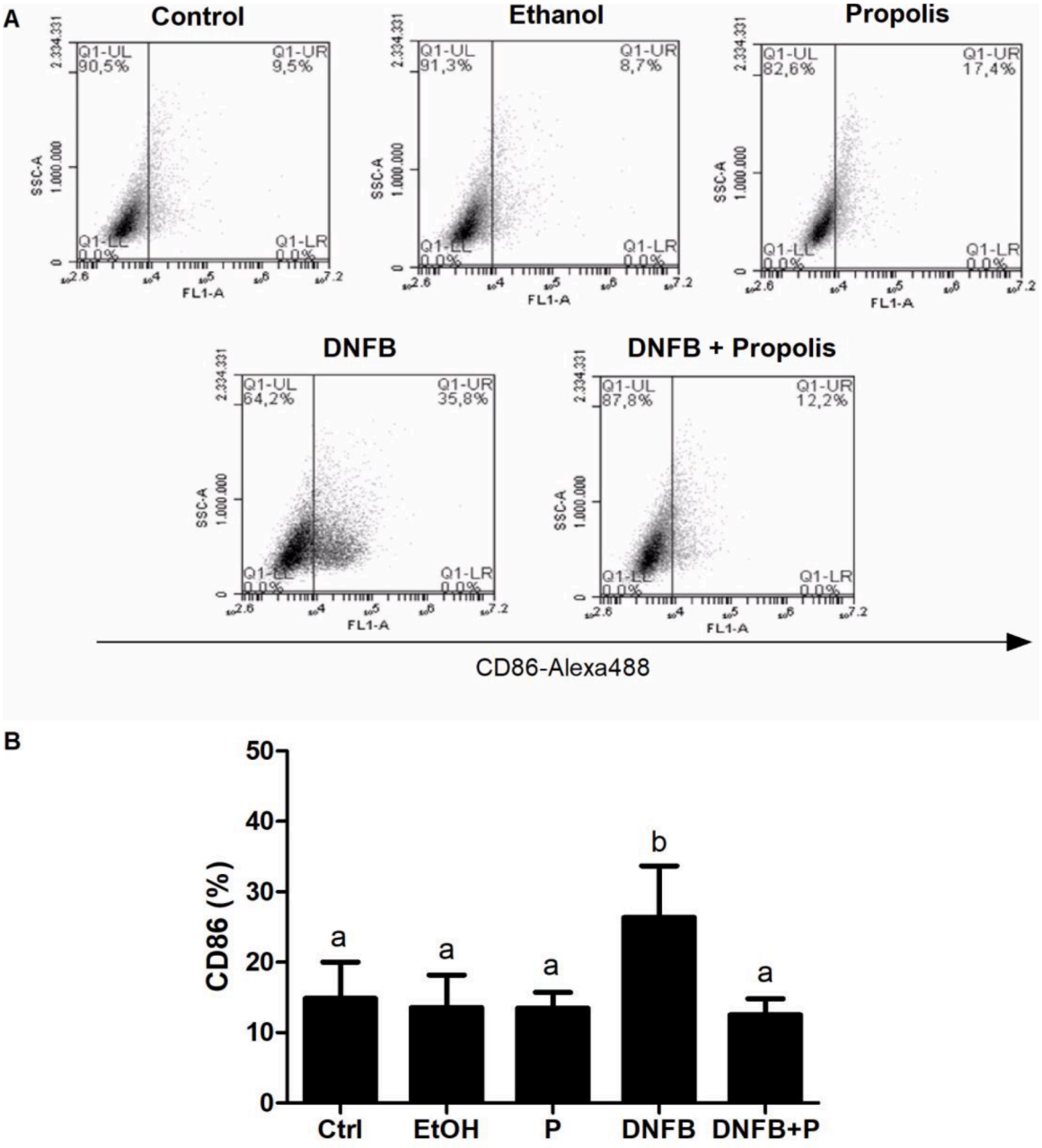

Fig. 5. CD86 expression (%) by THP-1 cells after incubation with culture medium (Ctrl), 70% ethanol (EtOH), propolis (P – 50 μg/mL), DNFB (8 μM) and DNFB 8 μM + propolis 50 μg/mL (DNFB + P) for 24 h by flow cytometry. a) Representative Dotplots. b) Data represent mean ± SD of 3 independent assays performed in duplicate. Differences were determined using Gamma distribution followed by Wald's multiple comparison test. Means with the same letter do not differ at the 5% level of significance.

concentration of propolis (50 μg/mL) was used in the subsequent assays.

Propolis exerted a potent antioxidant action evidenced by the ability to reduce DPPH in low concentrations (IC50 = 18.52 μg/mL); moreover, our findings were exactly the same as reported by Búfalo et al. (2013), who used the same propolis sample and obtained an IC50 = 18.51 μg/mL, confirming its antioxidant action. Data from literature revealed that other propolis samples may have significantly higher IC50, varying from 0.0700 to 0.9320 mg/mL (Duca et al., 2019). These findings confirm the importance of working with chemically characterized propolis samples, since different chemical compositions may be responsible for divergence in results (Sforcin, 2016).

Because of the remarkable antioxidant activity observed in this work using the DPPH test, a cell-free assay, we aimed to confirm the antioxidant effect of propolis in THP-1 cells, investigating different cell pathways. Pro-inflammatory stimuli, like LPS, can induce SOD 2 activity in an attempt to contain the generated oxidative stress (Ishihara et al., 2015), as observed in our results. Although there are several works correlating propolis to total SOD activity, little is known about its action in SOD 1 and 2 pathways. Interestingly, our results demonstrated that propolis increased SOD 1 activity relatively to the vehicle, but not in the presence of LPS. Propolis may exert both pro- and antioxidant activity depending on the experimental conditions, raising the doubt whether propolis could be favouring a pro-oxidant activity and consequently inducing the expression of the gene encoding SOD1. In addition, propolis exerted an antioxidant activity by reducing DPPH (IC50 = 18.52 μg/mL) although a higher concentration (50 μg/mL) was used in all in vitro assays, as it did not affect cell viability. These results are interesting, showing a delicate balance between the generation and

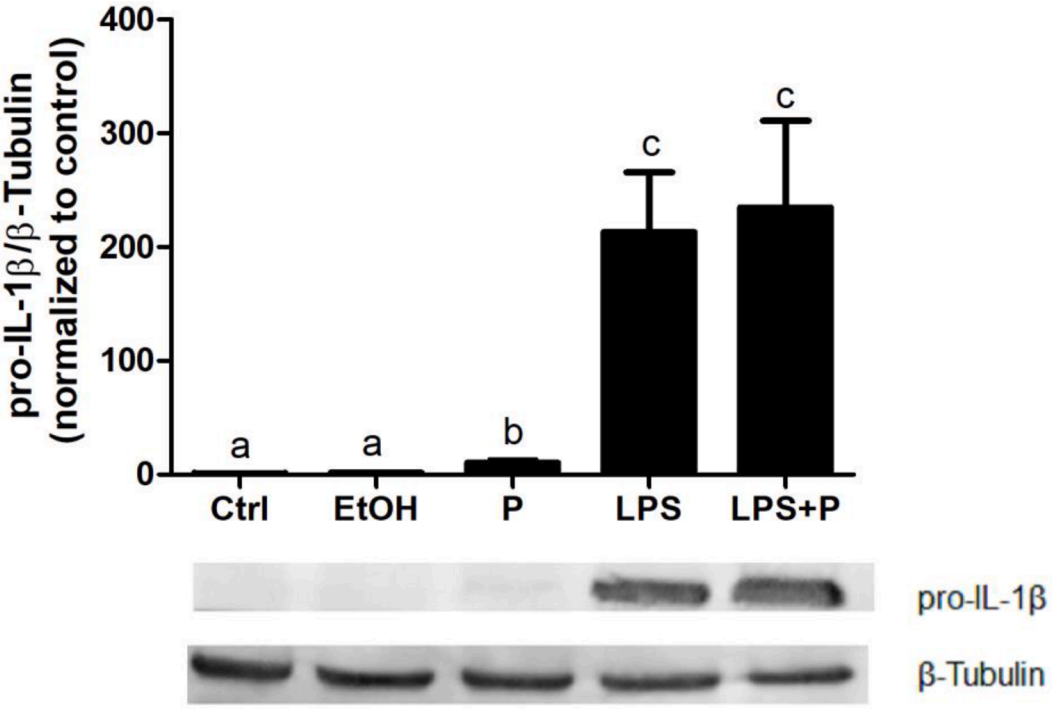

Fig. 6. Pro-IL-1β production by THP-1 cells after incubation with culture medium (Ctrl), ethanol (EtOH), propolis (P – 50 μg/mL), LPS (1 μg/mL) and LPS 1 μg/mL + propolis 50 μg/mL (LPS+*P*) for 24 h by Western blot. Data represent mean ± SD of 3 independent assays and a representative blot. Pro-IL-1β = 31 kDa; β-tubulin = 55 kDa. Differences were determined using Gamma distribution followed by Wald's multiple comparison test. Means with the same letter do not differ at the 5% level of significance.

destruction of oxidant agents, which may be beneficial or deleterious to the organism. Curti et al. (2019) observed increased SOD 1 in mice treated with brown propolis (250 mg/kg) – a sample rich in polyphenols, especially galangin, chrysin and pinocembrin – what was not observed using the concentrations 100 and 500 mg/kg. Similarly, an increased SOD 1 activity was seen in glioma cells incubated with a propolis sample from Turkey (250 and 500 μg/mL), but not with 100 μg/mL (Coskun et al., 2020). Thus, the potential of propolis to modulate the activity of SOD 1 and 2 enzymes may differ according to the models adopted in vitro and in vivo and to propolis source and concentrations.

We also analyzed the expression of HMOX-1 – a cytoprotective enzyme with a powerful antioxidant and anti-inflammatory action (Exner et al., 2004). Under normal conditions, HMOX-1 is expressed at low levels in most tissues; however, its activity is highly induced in response to oxidative stress, reducing ROS generation and controling the inflammatory response (Wu et al., 2011). Since propolis induced a high HMOX-1 expression in the presence of the inflammatory stimulus LPS, this may be one of the mechanisms related to propolis antioxidant activity in the presence of an inflammatory stimulus. Our data agrees with those in the literature, supporting propolis modulatory activity in the HMOX-1 pathway. An extract of Brazilian propolis induced the expression of HMOX-1 in human skin fibroblast cell line submitted to oxidative stress by ultraviolet A irradiation, which was associated with accelerated nuclear factor erythroid 2-related factor 2 (Nrf2) translocation (Saito et al., 2015). Yuan et al. (2019) also observed that Brazilian green propolis induced the expression of HMOX-1 and Nrf2 in human umbilical vein endothelial cells exposed to vascular endothelial injury. Thus, under oxidative stress, treatment with propolis can be beneficial by overexpressing the HMOX-1 pathway.

Subsequently, we investigated whether the antioxidant action exerted by propolis could be beneficial in the presence of the allergen DNFB. Indeed, skin allergens induce ROS production, which accounts for the overexpression of co-stimulatory proteins, for instance CD86, involved in the development of allergic contact dermatitis, a skin inflammatory condition. Propolis was able to protect THP-1 cells from DNFB action by inhibiting CD86. As far as we know, this is the first work to report propolis action in DNFB-induced cell activation. Phenolic compounds have been recognized for their ability to prevent or reduce the progression of various skin disorders (Dzialo et al., 2016). The extract from the leaves of *Sapium sebiferum* (L.) Roxb., rich in phenolic compounds, reduced symptoms of DNFB dermatitis, which was related to the antioxidant capacity of this extract (Fu et al., 2015). Also our propolis sample is rich in phenolic compounds, which may be related to the anti-allergic activity of the extract. The propolis sample used in our work contains several hydroxycinnamic acids and derivatives (Búfalo et al., 2013), widely believed to have an important antioxidant and anti-inflammatory potential, predominantly on the skin (Taofiq et al., 2017). Isolated compounds found in our propolis sample have been described for their ability to moderate DNFB-induced inflammation, such as caffeic acid (Jeon et al., 2015) and *p*-coumaric acid (Moon et al., 2021). Since natural compounds have been considered promising in reducing skin disorders and our propolis inhibited one of the cell activation mechanisms induced by DNFB, our results open perspectives for research on its potential antiallergic action.

As expected, LPS upregulated pro-IL-1β expression in our assay, associated or not with propolis. After pro-IL-1β processing, mature IL-1β is rapidly secreted by immune cells and plays a crucial role in the inflammatory response (Lopez-Castejon et al., 2011). Although its production is harmful in some circumstances, this cytokine is essential for the control and resolution of infections (Gabay et al., 2010). Propolis was able to modulate IL-1β production under different conditions. Brazilian green propolis at the same concentrarion (50 μg/mL) and incubation time (24 h) inhibited IL-1β, TNF-α and IL-6 production (measured by ELISA) by MG6 microglia submitted to hypoxia, demonstrating that propolis inhibits the hypoxia-induced activation of inflammatory pathway in microglia. (Wu et al., 2013). A sample of Brazilian green propolis - containing kaempferide (+ derivatives) and hesperitin as the major flavonoids; p-coumaric acid and prenylated cinnamic acid

derivatives (artepillin C, baccharin and drupanin) as the main phenolic acids – tested at 25 and 50 μg/mL for 24 h inhibited IL-1β production by J774A.1 macrophages stimulated with LPS + IFN-γ (Szliszka et al., 2013). Brazilian green propolis (30, 100 and 300 μg/mL) presenting caffeic acid, p-coumaric acid, trans-cinnamic acid, aromadendrin and artepillin C reduced IL-1β secretion by murine bone marrow-derived macrophages stimulated with LPS + nigericin or *Legionella pneumophila* (Hori et al., 2013). Although in general propolis may exhibit an inhibitory effect on IL-1β production, this was not reported in our assay conditions. Although all this studies utilized Brazilian green propolis, the samples are from different regions, with different extract preparations and used under different assay conditions, demonstrating the complexity of propolis and the need for standardization.

On these bases, our data indicated that propolis has a potent antioxidant activity and may be beneficial in preventing skin allergy via CD86 inhibition, probably by inducing the activation of antioxidant pathways, such as SOD and HMOX-1. In addition, our data are attributed to the constituents of our propolis sample, which may vary according to the phytogeographic conditions. Its chemical composition was previously analyzed, revealing presence of benzoic acid, dihydrocinnamic acid, artepillin C, *p*-coumaric acid, prenyl-*p*-coumaric acid, caffeic acid, 1,3- and 4,5-dicaffeoylquinic acids, 3,4,5-tricaffeoylquinic acid, flavones (6,8 di-C-hexosyl apigenin, 6-C-pentosyl-8-C-hexosyl apigenin, 6-C-hexosyl-8-C-pentosyl apigenin), trihydroxymethoxy flavanone, tetrahydroxy flavanone and triterpenes (Búfalo et al., 2013; Conti et al., 2015).

In conclusion, propolis exerted an antioxidant activity, potentially involved in its anti-inflammatory/antiallergic action. The findings based in a cell culture-model stimulated with pro-inflammatory agents open perspectives for the inclusion of propolis in the treatment of diseases and conditions that generate oxidative stress/inflammation.

CRediT author statement/author contribution

Conceptualization: Fernanda Lopes Conte, José Maurício Sforcin, Maria Teresa Batista and Maria Teresa Cruz. Methodology and laboratory experiments: Fernanda Lopes Conte. Writing - original draft preparation: Fernanda Lopes Conte, Ana Catarina Pereira, Gonçalo Brites, Isabel Ferreira, Ana Cristina Silva, Ana Isabel Sebastião, Patrícia Matos, Cláudia Pereira, Maria Teresa Batista, José Maurício Sforcin, Maria Teresa Cruz.

Declaration of Competing Interest

There is no conflict of interest.

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