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1,2-Dihydroxy-*9H*-Xanthen-9-One, a Multifunctional Nature-Inspired Active Ingredient

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Abstract: Incorporating antioxidants into cosmetics is the mainstay for developing new products to mitigate skin aging. However, identifying novel multifunctional antioxidant ingredients with additional relevant properties that block the skin hallmarks of aging is a very striking strategy. Many natural compounds, including xanthones, have demonstrated biologically notable properties. In particular, 1,2-dihydroxy-9*H*-xanthen-9-one (1,2-DHX) has inhibitory activity against skin enzymes, and metal-chelating and radical-scavenging activities. Therefore, 1,2-DHX is an attractive molecule for cosmetic purposes. With this goal in mind, the anti-inflammatory, antioxidant, and anti-allergic potentials of 1,2-DHX were investigated. 1,2-DHX demonstrated anti-inflammatory properties by inhibiting the synthesis of specific pro-inflammatory mediators, including interleukin-6 (IL-6) and prostaglandin E2 (PGE2), in human macrophages. This xanthone did not elicit sensitization reactions and did inhibit allergic reactions triggered by a strong skin allergen, suggesting its potential as an anti-allergic compound. 1,2-DHX also revealed mitochondrial antioxidant activity by mitigating rotenone-induced oxidative stress in macrophages by up to 40%. Overall, 1,2-DHX displayed a safety profile and noteworthy biological activities, highlighting its multifunctional profile as an active cosmetic ingredient with anti-inflammatory, antioxidant, and anti-allergic properties.

Keywords: nature-inspired xanthone; anti-inflammatory; anti-allergic; antioxidant; multifunctional ingredient

1. Introduction

The appearance of deep and coarse wrinkles, compromised skin integrity, and age spots are clinical signs of skin aging [1]. These events mainly originate from external

factors, including solar radiation, contact with allergens, pollutants, and tobacco smoke [2]. These elements collectively lead to an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3]. They are both involved in oxidative damage to skin cells and in the pathophysiology of inflammation, resulting in the activation of the immune system [4,5]. Among the events evoked by oxidative stress are the degradation of several skin extracellular matrix proteins; the oxidation of lipids, carbohydrates, and nucleic acids [6]; and the production of pro-inflammatory mediators, triggering inflammatory cascade reactions [7]. Contact with allergens could also accelerate skin aging. The release of danger signals evoked after contact with skin allergens drives intracellular signaling pathways in antigen-presenting cells, enabling them to mature and prime naïve T lymphocytes [4]. Upon activation, these T cells transform into allergen-specific effector T cells, which circulate throughout the body, triggering a severe inflammatory response in following exposure. Incorporating antioxidants into cosmetic formulations is a key strategy for developing anti-aging products, since it helps to delay and prevent skin aging. This makes the discovery of innovative multifunctional antioxidant compounds highly relevant for enhancing skin protection. Natural products, especially xanthones, have been reported for their numerous biological activities [8]. Only two xanthones (α-mangostin and mangiferin) have been identified with potential to be used in cosmetic products, due to their antioxidant, anti-inflammatory, and anti-aging properties (Figure 1). Both xanthones are the main compounds identified in the respective natural extracts (α-mangostin: *Garcinia mangostana* extract; mangiferin: *Mangifera indica* extract) present in cosmetic products [9–11]. The presence of hydroxyl groups in this scaffold is responsible for their powerful antioxidant activity. It has already been reported that 1,2-dihydroxy-*9H*-xanthen-9-one (1,2-DHX) presents inhibitory activity against enzymes involved in the degradation of collagen (collagenase) and elastin (elastase) fibers and hyaluronic acid (hyaluronidase) [12]. Additionally, 1,2-DHX demonstrates metal-chelating effects [Fe(III) and Cu(II)] and DPPH radical-scavenging activity. Furthermore, the stability demonstrated at skin pH, together with the absence of phototoxicity in irradiated keratinocyte cells [12], reinforces its potential as a cosmetic active ingredient. Considering all the studies previously reported highlighting 1,2-DHX as a hit compound for cosmetic applications, the anti-inflammatory, anti-allergic, and mitochondrial antioxidant activities and skin sensitization potential were investigated, aiming to disclose the in vitro effectiveness and safety profile of 1,2-DHX (Figure 1).

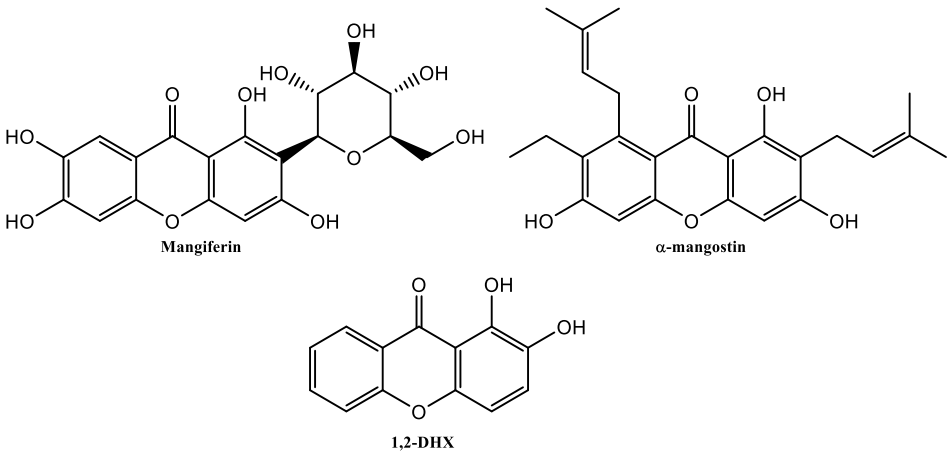

Figure 1. Chemical structure of natural xanthones found in cosmetic products and 1,2-DHX.

2. Materials and Methods

2.1. Materials

The compound 1,2-dihydroxy-*9H*-xanthen-9-one (1,2-DHX) was synthetized according to previously described procedures [13,14]. Briefly, the synthesis of 1,2-DHX started with the reaction between the two starting materials, methyl 2-bromobenzoate and 3,4-dimethoxyphenol, which gave methyl 2-(3,4-dimethoxyphenoxy)benzoate, which was

subsequently hydrolyzed to 2-(3,4-dimethoxyphenoxy)benzoic acid. Then, this intermediate was cyclized into 1,2-dimethoxy-*9H*-xanthen-9-one. Finally, the methoxy groups were deprotected, which gave 1,2-dihydroxy-*9H*-xanthen-9-one. Figure 2 illustrates the steps followed to synthesize 1,2-DHX.

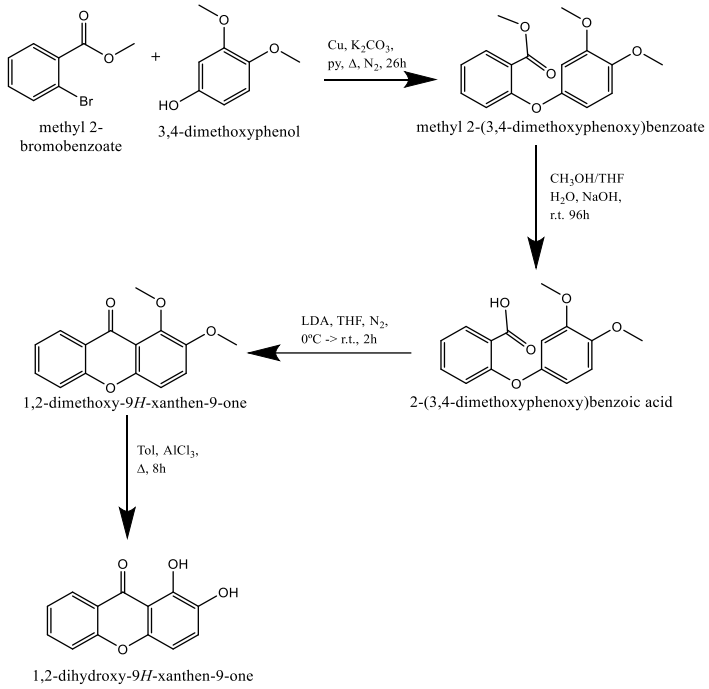

Figure 2. Synthesis of 1,2-DHX. Py: pyridine; Δ: heating; r.t.: room temperature; LDA: lithium diisopropylamide; THF: tetrahydrofuran; Tol: toluene.

For the biological assays, the materials and reagents used were obtained from different companies: RPMI-1640 media and Quant-iT PicoGreen dsDNA Kit from Thermo Fisher Scientific (Waltham, MA, USA); fetal bovine serum (FBS) from Gibco (Waltham, MA, USA); AlamarBlue from Bio-Rad (Hercules, CA, USA); human interleukin-6 (IL-6) DuoSet Enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Minneapolis, MN, USA); prostaglandin E2 (PGE2) ELISA Kit from Abcam (Cambridge, UK); ascorbic acid (AA), dexamethasone (DEX), lipopolysaccharide (LPS; *Escherichia coli* O26:B6), rotenone (Rot), and 1-fluoro-2,4-dinitrobenzene (DNFB) from Sigma-Aldrich (Darmstadt, Germany); celecoxib (CEL) from GmbH (Darmstadt, Germany); anti-CD86 (clone IT2.2) and anti-CD54 (clone HA58) antibodies from Biolegend (San Diego, CA, USA); and MitoSOX™ Mitochondrial Superoxide Indicators (MitoSOX Red Kit) from Invitrogen (Waltham, MA, USA).

2.2. Methods

2.2.1. Cell Culture

Healthy adult volunteers' blood donation units were used to acquire human peripheral blood mononuclear cells (PBMCs) through a collaborative agreement with the Portuguese Institute of Blood and Transplantation (IPST; Instituto Português do Sangue e Transplantação, Portugal). The collection of peripheral blood from healthy volunteers at Hospital of Braga, Portugal, was approved on 14 December 2018 by the Ethics Subcommittee for Life and Health Sciences (SECVS) of University of Minho, Portugal (No. 014/015). The principles expressed in the Declaration of Helsinki were followed, and participants provided their signed informed consent. Monocytes were isolated from human PBMCs according to the previous procedure [15]. Briefly, Histopaque-1077 solution and blood were mixed in a ratio of 1:1 and centrifuged at 400× *g*, for 30 min. Then, the PBMC ring was carefully harvested and washed with PBS. CD14 microbeads were used to isolate the monocytes from PBMCs through positive magnetic separation, in accordance with the manufacturer's

instructions. Isolated monocytes were resuspended in RPMI medium (cRPMI; RPMI-1640 medium containing 2 mM glutamine, 10% human serum, 1% penicillin/streptomycin, and 1% HEPES) and seeded (1×10^6 cells/mL) in a multi-well plate (MW-24) for 7 days, exposed to 20 ng/mL of recombinant human GM-CSF at 37 °C in a 5% CO_2 saturated atmosphere. The cell culture medium was substituted every 3 days. The morphology of human primary monocyte-derived macrophages (hMDMs) was verified by visualization with an inverted microscope (Axiovert 40; Zeiss, Oberkochen, Germany).

The human monocytic cell line THP-1 (ATCC® TIB-202™; American Type Culture Collection, Manassas, VA, USA) was cultivated in RPMI medium containing 25 mM (*D*)-glucose, 10% inactivated FBS, 10 mM HEPES, 100 U/mL penicillin, 100 µg/mL streptomycin, and 1 mM sodium pyruvate and kept at a cell density of 0.4×10^6 cells/mL. According to ATCC® instructions, THP-1 cells were sub-cultured every 2 or 3 days and kept in culture for a maximum of three months.

The mouse macrophage cell line RAW 264.7 (ATCC TIB-71; Manassas, VA, USA) was cultivated in DMEM containing 10% inactivated FBS, 1% antibiotic solution, 1 mM sodium pyruvate, and 1.5 g/L sodium bicarbonate. The cell culture of macrophages was mechanically separated with a cell scraper, sub-cultured in a 1:10 proportion (1 mL of cellular suspension to 10 mL of final volume of DMEM), and used after reaching 80–90% confluence.

2.2.2. Anti-Inflammatory Activity

A 50 mM stock solution of 1,2-DHX was prepared in DMSO and filtered by using a 0.22 µm sterile filter. Serial dilutions were made with cRPMI medium to obtain the final concentrations of 5, 12.5, 25, 50, and 100 µM in the wells. The hMDMs were exposed to 100 ng/mL of LPS in fresh cRPMI medium for 2 h. Afterwards, 1,2-DHX solutions at different concentrations were added to the LPS-stimulated hMDMs and incubated for 24 h. After that, the culture medium was collected, homogenized, and stored at −80 °C until analysis. Then, the metabolic activity of the cells and DNA content were determined. Controls containing 0.2% DMSO were also tested. Non-stimulated and LPS-stimulated hMDMs were used as negative and positive controls of the production of pro-inflammatory mediators, respectively. Dexamethasone (10 µM) and celecoxib (10 µM) were dissolved in ethanol and used as positive controls of the inhibition of the production of the pro-inflammatory mediators.

- Metabolic activity

The metabolic activity of the non-stimulated or LPS-stimulated hMDMs was determined by using the alamarBlue assay, based on the manufacturer's instructions. After collecting the culture medium, the hMDM cells were gently washed with warm PBS. RPMI medium containing 10% AlamarBlue was added to each well and left to incubate at 37 °C for 3 h. At the end, the absorbance for each sample in triplicate was measured at 570 nm and 600 nm with a microplate reader (Synergy HT; BioTek, Winooski, VT, USA). The results were expressed in percentage in relation to the control.

- DNA quantification

DNA quantification was assessed by using the Quant-iT PicoGreen assay, according to the manufacturer's instructions. After the determination of metabolic activity, the hMDM cells were washed with DPBS. Then, 1 mL of ultrapure water was added to each condition, and the samples were frozen at −80 °C. For the analysis, samples were defrosted, collected into an eppendorf, and sonicated for 15 min. The samples or standards at concentrations between 0 and 2 µg/mL, in triplicate, were added to a 96-well plate, followed by PicoGreen solution and Tris-EDTA (TE) buffer. The plate was incubated for 10 min protected from light. Fluorescence was read with a microplate reader (excitation wavelength (EX) of 485 nm and emission wavelength (EM) of 528 nm). The DNA concentration in µg/mL was extrapolated by the standard curve plotted from the fluorescence intensity versus the DNA concentration. The results were expressed in relative DNA concentration of the control.

- Pro-inflammatory mediator quantification

The amounts of PGE2 and IL-6 were determined through ELISA kits, following the manufacturer's instructions. The PGE2 and IL-6 concentrations in pg/mL were obtained from a standard curve of the absorbance intensity versus the specific pro-inflammatory mediator. The values obtained were normalized to the respective DNA concentration. The results were presented in percentages relative to the positive control.

2.2.3. Sensitization Potential and Anti-Allergic Activity

The human monocyte THP-1 cell line was also employed as a dendritic cell (DC) surrogate for the skin sensitizing hazard, following OECD guidelines. By adjusting OECD test Guideline No. 442E [16], the THP-1 cell concentration was set to 0.5×10^6 cells/mL, and they were incubated overnight at 37 °C in a 5% CO_2 saturated atmosphere. On the following day, cells were plated in a 12-well plate (1.5 mL/well), and 1.2-DHX was applied at the final concentration of 100 µM. After pre-incubation (1 h at 37 °C) with the active compound, a solution of DNFB, prepared in DMSO, was added (final concentration of 8 µM), and cells were additionally incubated for another 24 h under the previously mentioned conditions. Afterwards, cells were transferred to eppendorfs and centrifuged at 300× *g* for 5 min at 4 °C. The supernatant was rejected, and the cell pellet was washed with PBS/1% FBS (2 × 1 mL) and centrifuged at 300× *g* for 5 min at 4 °C. Then, cells were further resuspended in 1 mL of PBS/1% FBS, and 200 µL was collected for two 1.5 mL eppendorfs equally divided for unstained (UNST) and stained (CD5486) conditions. Each stained condition contained 3 µL of each antibody, anti-CD86 and anti-CD54, and was further incubated in the dark for 30 min at 4 °C. Afterward, cells were washed with PBS/1% FBS and centrifuged at 300× *g* for 5 min at 4 °C. Then, the supernatant was discarded, and 100 µL of PBS/1% FBS solution was added to the cell pellet and gently homogenized for subsequent analysis through the flow cytometry technique (BD Accuri™ C6 cytometer (San Jose, CA, USA)). Flow cytometry was used to examine the levels of the cellular membrane markers CD86 and CD54 by using acquisition channels FL1 and FL4, respectively. For control cells and chemical-treated cells, the relative fluorescence intensity (RFI) of the membrane markers CD86 and CD54 was calculated by utilizing the geometric mean fluorescence intensity (MFI) and the following equation:

$$RFI = \frac{MFI\ of\ chemical\ treated\ stained\ cells - MFI\ of\ chemical\ treated\ unstained\ cells}{MFI\ of\ control\ stained\ cells - MFI\ of\ control\ unstained\ cells} \times 100$$

The findings from a minimum of three independent experiments were presented as percentages of the RFI values observed in relation to the control, which was set to 100%. Samples were categorized as skin sensitizers if the RFI values for CD54 and CD86 exceeded the thresholds outlined in the 442E OECD guideline, precisely 200% and 150%, respectively.

2.2.4. Mitochondrial Antioxidant Activity

Mitochondrial superoxide (O_2^-) generation was determined by using MitoSOX fluorescent probe according to the manufacturer's instructions. Briefly, 0.6×10^6 cells/mL were plated in a 12-well plate (1.2 mL/well) and incubated overnight at 37 °C. The next day, cells were exposed to 1,2-DHX (100 µM) and ascorbic acid (antioxidant control)) (500 µM) and pre-incubated for 1 h, using the conditions previously mentioned. Then, rotenone (final concentration of 20 µM) was added and incubated for 5 h at 37 °C. After the incubation period, the cells were carefully detached with a scrapper from each well, transferred to 2 mL eppendorfs, and centrifuged at 500× *g* for 3 min at room temperature. The cell supernatant was collected and rejected, and the cell pellet was washed with HBSS solution (2 × 1 mL) and centrifuged at 500× *g* for 3 min. Afterwards, the cell pellet was resuspended in 400 µL of PBS solution, and 300 µL was collected for two 1.5 mL eppendorfs: 150 µL for unstained and 150 µL for stained conditions. Stained cells were incubated with 100 µL of freshly prepared MitoSOX working solution (final concentration of 2.5 µM) at 37 °C for

15 min protected from light. Then, 400 µL of HBSS solution was added to the dilute stained condition and was further centrifuged. Then, the stained conditions were washed with HBSS solution (2 × 1 mL). Finally, the cell pellet was resuspended in 100 µL of PBS solution and analyzed by flow cytometry (BD Accuri™ C6 cytometer (San Jose, CA, USA)).

2.2.5. Statistical Analysis

GraphPad Prism8 for Windows (GraphPad Software, San Diego, CA, USA; www.graphpad.com; accessed on 5 December 2024) was utilized to conduct the statistical analysis. The results were presented as means ± standard deviations (SDs) or means ± standard errors of the means (SEMs), based on a minimum of three independent experiments, in triplicates. Statistical comparisons were carried out by using one-way ANOVA, followed by Sidak's multiple comparisons test (anti-inflammatory activity and levels of IL-6 and PGE2) and Tukey's multiple comparisons test (sensitization potential, anti-allergic activity, and mitochondrial antioxidant activity). The value of $p < 0.05$ was considered significant, and the statistical levels considered were * $p < 0.05$; ** $p < 0.01$, *** $p < 0.005$, and **** $p < 0.001$ relative to the respective control.

3. Results and Discussion

3.1. Anti-Inflammatory Activity

The metabolic activity and relative DNA content achieved for hMDMs that were stimulated by LPS in the absence or presence of 1,2-DHX are shown in Figure 3. 1,2-DHX did not affect hMDM metabolic activity (Figure 3A) or DNA content (Figure 3B) at the concentrations tested.

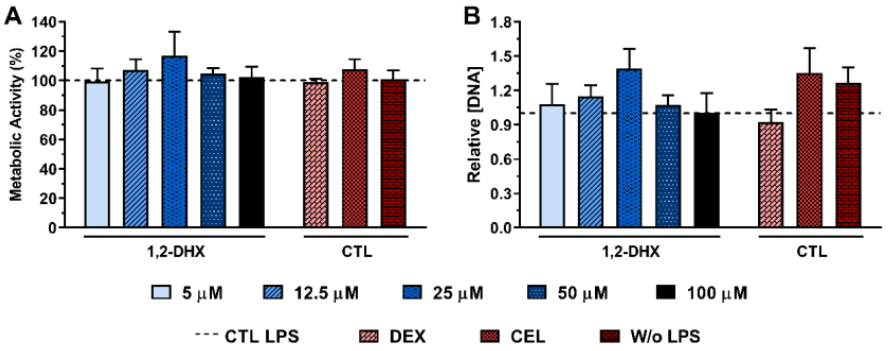

Figure 3. The metabolic activity (**A**) and relative DNA concentration (**B**) of non-stimulated (W/o LPS) and LPS-stimulated hMDMs cultured or not in the presence of different concentrations (5, 12.5, 25, 50, and 100 µM) of 1,2-DHX and clinically used anti-inflammatory compounds (dexamethasone (DEX) and celecoxib (CEL) at 10 µM). The dotted line represents the metabolic activity and the DNA concentration of the positive control (LPS-stimulated hMDMs without treatment (CTL LPS)). The results of three independent assays (n = 3) are expressed as means ± SDs (standard deviations).

The anti-inflammatory activity of 1,2-DHX was assessed by its ability to reduce the pro-inflammatory PGE2 and IL-6 mediators, produced by LPS-stimulated hMDMs (Figure 4). As expected, when the hMDMs were stimulated with LPS, we observed a significant increase in the levels of both PGE2 and IL-6 pro-inflammatory molecules. Celecoxib significantly decreased (29.4 ± 9.1%) PGE2 production, and dexamethasone effectively reduced IL-6 production (58.7 ± 5.1%). When LPS-stimulated hMDMs were treated with the 1,2-DHX, a decrease in the PGE2 level (34.2 ± 9.5% at 100 µM) in the culture medium was observed (Figure 4A). 1,2-DHX showed similar anti-inflammatory activity at all tested concentrations, leading to similar or inferior levels of PGE2 if compared with celecoxib, a clinically employed non-steroidal anti-inflammatory drug (NSAID). 1,2-DHX at 100 µM significantly decreased IL-6 production (34.7 ± 3.7%) by LPS-stimulated hMDMs (Figure 4B).

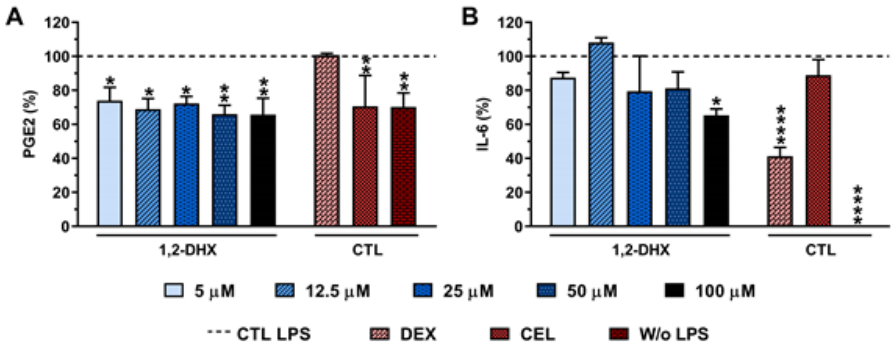

Figure 4. The PGE2 (**A**) and IL-6 (**B**) percentages of non-stimulated (W/o LPS) and LPS-stimulated hMDMs cultured in the presence or in the absence of different concentrations of 1,2-DHX and clinically used anti-inflammatory drugs (dexamethasone (DEX) and celecoxib (CEL) at 10 µM). The dashed line indicates the highest amount of generated pro-inflammatory mediators in the positive control (LPS-stimulated hMDMs without treatment (CTL LPS)). The results of three independent assays (n = 3) are expressed as means ± SDs, and statistically significant differences are indicated by * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$ in comparison with the respective positive control.

Cytocompatible concentrations severely reduced the PGE2 and IL-6 levels in LPS-stimulated hMDMs (Figure 4), being the highest concentration the most effective. This capacity is of utmost relevance, since PGE2 increases vasodilation, vascular leakage, hyperalgesia, and fever [17], and IL-6 promotes T-cell growth and activation, increases B-cell development, and modulates the chemokines that activate neutrophils [18]. Therefore, reducing the synthesis of these pro-inflammatory mediators ameliorates inflammation, specifically skin inflammation, avoiding skin premature aging. Moreover, 1,2-DHX demonstrated anti-inflammatory activity similar to or better than clinically used anti-inflammatory drugs. Considering the results obtained herein at the maximum concentration of 1,2-DHX used, the following biological activities explored for the compound were performed at 100 µM.

3.2. Sensitization Potential and Anti-Allergic Activity

The necessity for non-animal alternative studies has been recognized for the evaluation of the toxicity of chemicals to human health overall. It has become especially demanding for cosmetic active ingredients as a result of the European Union Commission's regulation banning the testing and marketing of products in which animal experiments have been used. Following an alternative validated test, OECD guideline 442E [16], the up-regulation of the co-stimulatory molecules CD54 and CD86 in THP-1 cells was used to evaluate the potential of the molecules to allay skin sensitization and xanthone's ability to mitigate skin allergy by using the strong allergen DNFB (Figure 5).

At the non-cytotoxic concentration of 100 µM, 1,2-DHX revealed a promising safety profile with RFI (%) values of 179.05 ± 29.45% and 90.85 ± 37.86%, with both values being lower than the thresholds for CD54 of 200% and CD86 of 150%, respectively, already established in the guidelines [16]; thus, a non-sensitizer label could be assigned to 1,2-DHX. As expected, the treatment with DNFB increased the levels of CD54 (RFI (%) = 621.34 ± 31.81%) and CD86 (RFI (%) = 367.00 ± 35.92%). 1,2-DHX showed inhibitory activity towards DNFB-induced cell maturation with RFI (%) values of 294.82 ± 34.00% for CD54 and 238.08 ± 35.92% for CD86. For the CD54 membrane cell marker, 1,2-DHX (294.82 ± 34.00%) demonstrated a stronger inhibitory effect (around 50%) towards the DNFB-induced cell maturation (621.34 ± 31.81%) than for the CD86 cell marker (238.08 ± 35.92%). The anti-allergic action of 1,2-DHX allows us to hypothesize that this compound could eliminate and prevent the harmful effects of other allergens that come into contact with the skin.

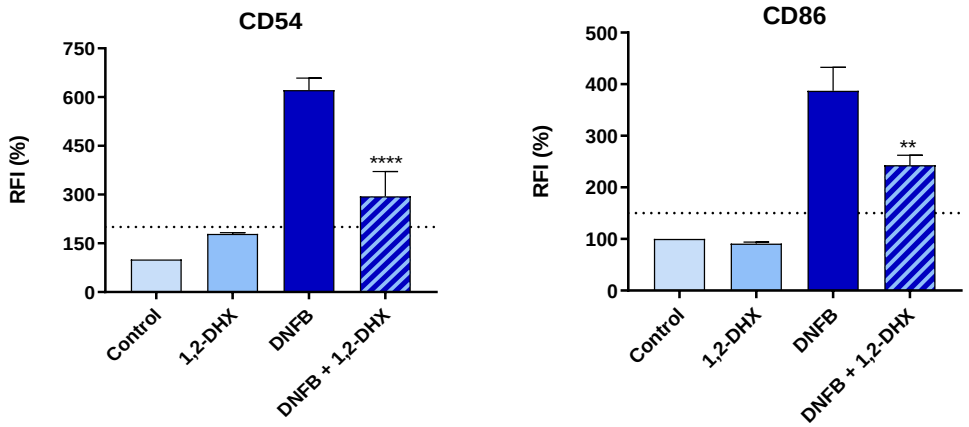

Figure 5. The non-sensitization potential (compared with control) and anti-allergic activity of 1,2-DHX towards the maturation of THP-1 cells induced by the strong allergen DNFB (compared with DNFB). The relative fluorescence intensity (RFI) of CD54 and CD86 expression was determined. The results of seven independent assays (n = 7) are expressed as means ± SEMs (standard errors of the means), and statistically significant differences observed are indicated by ** $p < 0.01$ and **** $p < 0.0001$ compared with the positive control (DNFB).

3.3. Mitochondrial Antioxidant Activity

Mitochondria are cellular organelles rich in oxidant species where the cellular oxidative stress process can be initiated more easily, triggered by an external stimulus capable to provoke an increase in oxidant species [19]. Oxidative stress activates other molecular events, including inflammatory and allergic reactions. Considering that mitochondria have been identified as a major source of ROS, mostly of superoxide anion ($O_2^{\bullet -}$), the mitochondrial antioxidant activity of 1,2-DHX was assessed in macrophage cells stimulated by rotenone (Figure 6).

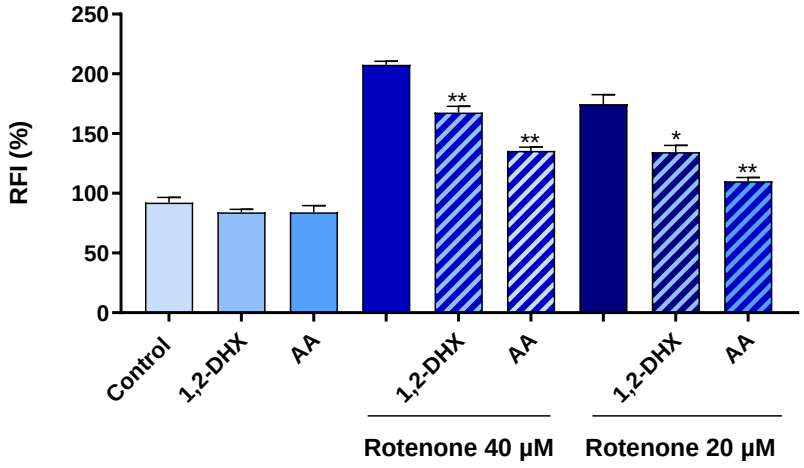

Figure 6. The pro-oxidant potential (compared with control; 100%) and mitochondrial antioxidant activity of 1,2-DHX (100 µM) under rotenone-induced oxidative stress in RAW264.7 cells (compared with rotenone). The antioxidant control ascorbic acid (AA) was also tested. The relative fluorescence intensity (RFI) of the fluorescent probe was measured. The results of eight independent assays (n = 8) are expressed as means ± SEMs, and statistically significant differences observed are indicated by * $p < 0.05$ and ** $p < 0.01$ compared with the positive control (Rot) at the tested concentrations.

Two control molecules, AA and rotenone, with antioxidant activity and potential to induce oxidative stress, respectively, were used. 1,2-DHX (84.14 ± 5.07%) and AA (83.97 ± 4.88%) did not demonstrate the ability to induce oxidative stress in macrophages, with RFI (%) values inferior to the control (100%) (Figure 6). The antioxidant response of 1,2-DHX was further investigated by using rotenone as a positive control. Rotenone triggered

mitochondrial oxidative stress, increasing mitochondrial $O_2^{\bullet -}$ production [20], reflected by an increase in fluorescence. 1,2-DHX showed inhibitory effect on rotenone-induced mitochondrial $O_2^{\bullet -}$ production with RFI (%) values of 167.59 ± 5.41% and 134.57 ± 4.92% compared with rotenone at 40 µM (207.55 ± 4.25%) and rotenone at 20 µM (110.18 ± 7.05%), respectively (Figure 6). Mitochondrial $O^{2\bullet -}$ is one of the most reactive species that contribute to the oxidation of biomolecules, the acceleration of skin aging, the regulation of apoptosis, and senescent events [21,22]. Thus, a reduction in $O^{2\bullet -}$ production can help to avoid cellular oxidative processes, preventing the acceleration of the aging of the skin. Other antioxidant molecules are being used in cosmetic products with the purpose of neutralizing ROS [23]; however, there is not specific information about their antioxidant activity directed to the mitochondria. Ascorbic acid is one of the antioxidants used in cosmetic products that has been already reported for its antioxidant activity, including as an untargeted and mitochondria-targeted antioxidant [24]. With our results, it is also possible to observe the effective inhibitory response of AA towards rotenone-induced mitochondrial $O_2^{\bullet -}$ production at different concentrations of rotenone used as a stimulus (40 µM: 135.42 ± 3.08%; 20 µM: 110.18 ± 6.37%), corroborating the results already reported in the literature [24]. The existence of a few mitochondria-targeted antioxidants in cosmetic products, aligned with the results obtained for 1,2-DHX, highlights it as a potential active cosmetic ingredient.

4. Conclusions

Skin is constantly exposed to external factors that contribute to the aging process, so its protection and equilibrium maintenance are essential. Sources of new active ingredients with multifunctional action are must-haves in the development of new cosmetic products, specifically anti-aging formulations. Nature has been used as a valuable source in the discovery of new active compounds with diverse daily cosmetic applications. 1,2-DHX is a nature-inspired xanthone with promising anti-aging and antioxidant activities and a physicochemical profile suitable for topical application. The evaluation of skin sensitization potential and anti-inflammatory, anti-allergic, and mitochondrial antioxidant activities is unveiled herein for the first time. Non-cytotoxic concentrations of 1,2-DHX demonstrated the ability to reduce PGE2 and IL-6 pro-inflammatory mediator release. 1,2-DHX did not elicit skin sensitization and inhibited DNFB-triggered allergic reactions, highlighting its preventive potential for use after skin contact with allergens. Furthermore, 1,2-DHX significantly reduced mitochondrial superoxide anion production. Based on these results, the application of this ingredient could be diversified in cosmetic products that help to fight skin inflammation, such as those activated by exposure to solar radiation and, in this particular case, aftersun products. 1,2-DHX could also help to minimize the effects of allergic contact dermatitis, due to its ability to mitigate allergen-induced skin sensitization. By acting as an untargeted and mitochondria-targeted antioxidant ingredient, 1,2-DHX could prevent premature skin aging by impeding the deregulation of the biological and metabolic processes of the skin that depend on the equilibrium state of mitochondria. Overall, 1,2-DHX can block relevant hallmarks of skin aging, namely, inflammaging and oxidative stress, and is thus a promising anti-aging ingredient.

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