






Original Research

Cannabigerol Reduces Lipid Peroxidation Influencing Oxidative Stress and Inflammation Signaling Pathways in Melanocytes Exposed to UVA Radiation

Iwona Jarocka-Karpowicz¹, Agnieszka Gęgotek¹, Neven Žarković²,
Audrius Maruška³, Elżbieta Skrzydlewska^{1,*}¹Department of Analytical Chemistry, Medical University of Białystok, 15-222 Białystok, Poland²Ruder Bošković Institute, Laboratory for Oxidative Stress (LabOS), HR-10000 Zagreb, Croatia³Instrumental Analysis Open Access Centre, Faculty of Natural Sciences, Vytautas Magnus University, LT-44404 Kaunas, Lithuania*Correspondence: elzbieta.skrzydlewska@umb.edu.pl (Elżbieta Skrzydlewska)

Academic Editor: Alexandros G. Georgakilas

Submitted: 29 October 2025 Revised: 28 January 2026 Accepted: 11 February 2026 Published: 20 March 2026

Abstract

Background: Ultraviolet A (UVA) radiation is a major environmental factor contributing to melanoma development. Melanocytes synthesize melanin, which provides partial protection against UVA-induced oxidative damage; however, these cells remain highly susceptible to oxidative and pro-inflammatory effects of UVA exposure. **Methods:** In melanocytes, the following parameters were assessed: total antioxidant status (TAS-photometrically), reactive oxygen species (ROS-ESR), lipid peroxidation (4-HNE-GC-MS/MS), 4-HNE-protein adducts, and the expression/localization of key signaling proteins including phosphorylated nuclear factor erythroid 2-related factor 2 (pNrf2) and nuclear factor kappa B (NFκB) subunits [ELISA/fluorescence microscopy]. **Results:** Cannabigerol (CBG) is a cytoprotective phytocannabinoid. *In vitro* studies showed that CBG attenuated UVA-induced oxidative stress in human melanocytes exposed to UVA radiation and significantly reduces lipid peroxidation, as measured by the levels of 4-hydroxynonenal (4-HNE) and its protein adducts. The biosynthesis of antioxidants was also regulated by CBG, even when administered post-irradiation. CBG attenuated the effects of UVA radiation by downregulating Nrf2, Kelch-like ECH-associated protein 1 (Keap1), BTB domain and CNC homolog 1 (Bach1), potent cyclin-dependent kinase inhibitor (p21), KRAB-associated protein 1 (KAP1), and multifunctional adaptor protein (p62). CBG also partially inhibited the pro-inflammatory NFκB signaling pathway by reducing the level of the activator (pIκB) and increasing the levels of the inhibitors (IKKα/β). **Conclusion:** These results suggest that CBG may protect melanocytes from UVA-induced oxidative changes and lipid peroxidation by activating the Nrf2-dependent antioxidant system and inhibiting NFκB-based pro-inflammatory signaling. CBG can therefore create favorable conditions for the physiological functioning of melanocytes after UVA exposure, ultimately reducing the risk of inflammatory skin responses and neoplastic transformation.

Keywords: cannabigerol (CBG); melanocytes; UVA; inflammation; protein modification; 4-hydroxynonenal (4-HNE)

1. Introduction

Due to its protective role over the entire body, human skin is constantly exposed to physical and chemical environmental factors. Physical factors that affect the integrity and proper functioning of the skin include ionizing radiation, such as cosmic and environmental radiation (e.g., radionuclides), as well as non-ionizing radiation, such as solar radiation [1]. Ionizing radiation is capable of directly damaging DNA [2]. Non-ionizing radiation mainly affects the skin by inducing oxidative stress, which leads to photoaging, disorders of the epidermal barrier, and consequently photocarcinogenesis [3]. The skin is constantly exposed to radiation, and its cumulative effects constitute a major risk factor for metabolic modifications of skin cells [1]. Moreover, the skin has a number of functions, such as receiving various stimuli, maintaining water balance, thermoregulation, and, to some extent, vitamin D biosynthesis [4]. To meet these challenges under harsh environmental condi-

tions, skin cells, including melanin-producing melanocytes, protect other skin cells from the harmful effects of UV radiation from the sun [5] and from artificial sources [6].

While UVB radiation is absorbed primarily by epidermal keratinocytes, up to 50% of UVA radiation penetrates the basal layer of the epidermis, directly affecting melanocytes and stimulating melanin synthesis [7]. However, UVA exposure also promotes the oxidation of endogenous photosensitizers, leading to excessive production of reactive oxygen species (ROS) in melanocytes, often exceeding the levels observed in keratinocytes and fibroblasts [8]. This overproduction of pro-oxidant factors induces oxidative stress, leading to DNA damage as well as activation of proinflammatory signaling pathways [9,10]. Oxidative stress further modifies lipid metabolism, with enhanced non-enzymatic ROS-dependent peroxidation increasing the formation of reactive aldehydes such as 4-hydroxynonenal (4-HNE) [11,12]. In addition, enhanced enzymatic lipid



metabolism leading to cyclization products formation, including 15-deoxy- Δ 12,14-prostaglandin J2 [13], that can modulate nuclear factor erythroid 2-related factor 2 (Nrf2)- and nuclear factor kappa B (NF κ B)-dependent signaling [14,15]. This leads to metabolic dysregulation, including inflammation and increased melanocyte proliferation, contributing significantly to the development of melanoma [16]. It has been estimated that approximately 65% of melanoma cases are associated with excessive exposure to UV radiation [6,17].

There is currently great interest in bioactive substances, especially those of natural origin, that do not have adverse effects and could prevent the undesirable effects of UV radiation. These include antioxidants that reduce the level of oxidative damage and protect the associated metabolic pathways [18]. Among such compounds are non-psychoactive phytocannabinoids derived from *Cannabis sativa* L. These exhibit a range of therapeutic benefits for various conditions, including those affecting the skin [19]. The most widely studied phytocannabinoid is cannabidiol (CBD). CBD can protect melanocytes from the effects of UVB radiation, even increasing their survival, due to its antioxidant, anti-inflammatory, and cytoprotective properties [20]. The most recent *in vitro* data and pilot clinical studies have reported a reduction in oxidative stress-induced DNA damage and inflammation following topical application of CBD [21,22]. Moreover, CBD indirectly induces melanogenesis via MAPK-based stimulation of MITF gene expression, which plays a key role in the development of melanocytes [23]. Another increasingly studied phytocannabinoid is cannabigerol (CBG). Similar to CBD, CBG easily penetrates cell membranes and also exhibits antioxidant and anti-inflammatory properties. This is evidenced by its direct bioactive properties, and indirectly through interactions with G protein-coupled receptors (GPCRs) [24,25]. Compared to CBD, CBG acts more selectively on pathways related to inflammation, lipid metabolism, and mitochondrial function [26]. When applied topically to the skin, CBG enhanced the expression of key genes, including collagen (type I, III, and IV), elastin, fibronectin, hyaluronan synthase 2, and IL-10. In addition, CBG inhibited pro-inflammatory cytokines (e.g., IL-1 β , IL-6, IL-8, TNF α) more effectively than CBD, suggesting stronger anti-inflammatory potential in skin cells [27]. Emerging preclinical evidence has demonstrated the safety of long-term topical CBG use [28]. So far, the protective and metabolic regulating properties of CBG for skin cells have been studied in relation to psoriasis and atopic dermatitis [29,30]. CBG has also been shown to modulate lipid metabolism associated with UVA-induced pro-tumorigenic changes, and to reduce the secretion of immunosuppressive cytokines in melanoma models, which may slow tumor progression *in vivo* [31,32]. Hence, it can be concluded that CBG exhibits different cytoprotective effects compared to CBD, especially in UV-induced metabolic stress pathways,

justifying further investigation of its mechanistic role in skin photoprotection.

Despite the documented bioactive properties of CBG, its modulation of UVA-induced oxidative stress-inflammation interactions in human melanocytes has not been investigated. Therefore, in the present study, we examined the effect of CBG on UVA-induced metabolic changes in human melanocytes, with a particular emphasis on antioxidant and proinflammatory signaling pathways mediated by the Nrf2 and NF κ B transcription factors.

2. Materials and Methods

2.1 Cell Culture and Treatment

Standard human melanocytes isolated from the fore-skin of a newborn and purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA; Cat. PCS-200-012) were used in this study. The cell line was validated by STR profiling and tested negative for mycoplasma. In accordance with ATCC guidelines, cells were grown in specific melanocyte medium (Dermal Cell Basal Medium [DCBM], ATCC) supplemented with Melanocyte Growth Kit (ATCC). Penicillin (50 U/mL) and streptomycin (50 μ g/mL) were added to the medium to avoid bacterial contamination. During incubation, melanocyte cell cultures were kept at 37 °C in a humidified atmosphere containing 5% CO₂.

Once the melanocytes (passage 3) reached 90% confluence, the cells were placed in plastic dishes surrounded by ice and 15 cm from UV 5 lamps, each with 8 W power. They were then exposed for 70 min to UVA radiation at 365 nm (Bio-Link Crosslinker BLX /365; Vilber Lourmat, Germany) and an intensity of 4.08 mW/cm². The radiation dose was selected to achieve approximately 75% cell viability, as measured by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Merck Millipore, Burlington, MA, USA) [33]. In parallel, melanocytes not exposed to UVA radiation (control group) were kept under the same conditions but in the dark and protected from any light, including UVA radiation.

To assess the effect of CBG (Sigma-Aldrich, St. Louis, MO, USA) on melanocytes following exposure to UVA radiation, the cells were incubated for 24 h in medium supplemented with a final concentration of 1 μ M CBG, obtained after dilution of a 32 mM stock solution dissolved in ethanol. Because the CBG solution was prepared in ethanol, the respective control cultures were also treated with the same final concentration of ethanol (0.3%). The specific concentration of CBG was used because it does not alter cell morphology, proliferation, and viability as assessed by the MTT assay and as describe previously [34]. IC₅₀ (inhibitory concentration at 50%) of CBG was >30 μ M in tested melanocytes.

The melanocyte cultures were divided into four experimental groups (each consisting of 5 independent replicates) according to the treatment applied:

I. Control: melanocytes cultured in standard medium only;

II. CBG: melanocytes cultured in medium containing CBG (1 μ M) for 24 h;

III. UVA: melanocytes exposed to UVA radiation (18 J/cm²) and cultured in standard growth medium for 24 h;

IV. UVA+CBG: melanocytes exposed to UVA radiation (18 J/cm²) and cultured in medium containing CBG (1 μ M) for 24 h.

At the end of the respective treatments, cells were washed with PBS, sonicated on ice using a Sonic Ruptor 400 (Omni International, Kennesaw, GA, USA), and centrifuged for 15 min at 12,000 g to obtain lysates for quantitative determination. All parameters were adjusted according to the protein content, as determined by Bradford assay [35].

2.2 Evaluation of Total Antioxidant Status (TAS)

Total antioxidant status (TAS) in melanocytes was determined using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS; Merck Millipore, Burlington, MA, USA) based on the method of Zhang *et al.* [36]. A microplate reader was used to measure absorbance at a wavelength of 734 nm. Results were calculated and presented as the percentage change in TAS in treated melanocytes compared to the control group.

2.3 Evaluation of Reactive Oxygen Species (ROS)

The generation of total ROS in melanocytes was determined using an electron spin resonance (ESR) spectrometer (Noxygen GmbH/Bruker Biospin GmbH, Germany). This measures the oxidation products of cyclic hydroxylamine spin trap (CMH) by ROS in cell lysates, as described in a previous study [37]. Results were calculated and presented as the percentage change of ROS in melanocytes compared to the control group.

2.4 Cellular Localization of pNrf2 and pNF κ B p52

Melanocytes were cultured in 96-well culture plates for 24 h, rinsed with PBS, and fixed with ice-cold methanol. Cell membranes were permeabilized with 0.1% Triton X-100, and the samples were incubated for 1 h with a 1:10,000 dilution of primary antibodies against phospho-Nrf2 (phospho-Ser40; Abcam, Cambridge, UK) or phospho-NF κ B (subunit p52, phospho-Ser222; Merck Millipore, Burlington, MA, USA). Goat anti-rabbit/mouse EnVision+ Dual Link/HRP solution (1:100; Agilent Technologies, Santa Clara, CA, USA) was used as the secondary antibody. The cells were subsequently visualized with a Nikon Eclipse Ti fluorescence microscope equipped with a DS-Qi2 camera (Nikon Instruments Inc., Tokyo, Japan) under 488 nm excitation and 525 nm emission wavelengths. The expression of pNrf2 and pNF κ B (p52) in the nucleus or cytosol was confirmed by MS-based proteomics as described in the **Supplementary material**.

2.5 Evaluation of Protein Expression Level

The expression levels of pNrf2 and NF κ B proteins, as well as those of their activators and inhibitors, were assessed by ELISA [38]. In brief, cell lysates were immobilized on ELISA plate wells (Nunc Immuno MaxiSorp, Thermo Scientific, Waltham, MA, USA), then blocked with a carbonate buffer containing 5% nonfat milk and subsequently incubated overnight with the respective primary antibodies targeting: phosphorylated nuclear factor erythroid 2-related factor 2 (pNrf2 (Ser40), Invitrogen, Waltham, MA, USA); heme oxygenase 1 (HO-1, Invitrogen, Waltham, MA, USA); KRAB-associated protein 1 (KAP1, Sigma, Shanghai, China); potent cyclin-dependent kinase inhibitor (p21); phosphorylated I κ B (pI κ B, Abcam, Cambridge, MA, USA); multifunctional adaptor protein (p62, Proteintech, Manchester, UK); phosphorylated extracellular signal-regulated kinase (pERK); phosphorylated mitogen-activated protein kinase (pMAPK); APC membrane recruitment protein 1 (AMER1; also known as WTX, Cell Signalling Technology, Danvers, MA, USA); BTB domain and CNC homolog 1 (Bach1); dipeptidyl peptidase 3 (DPP3); I κ B kinase beta (IKK β); Keap1; NF κ B (p52), NF κ B (p65); NLR family pyrin domain containing 3 (NLRP3); phosphoglycerate mutase family member 5 (PGAM5); tumor necrosis factor alpha (TNF α , Sigma-Aldrich, St. Louis, MO, USA) and I κ B kinase alpha (IKK α , Thermo Fisher, Waltham, MA, USA). After incubation with the primary antibodies (1:1000 dilution), the samples were treated with peroxidase blocking solution (3% H₂O₂, 3% nonfat dry milk in PBS) and then incubated with secondary antibody (anti-rabbit/mouse EnVision+ Dual Link/HRP; Agilent Technologies, Santa Clara, CA, USA). After removal of the secondary antibody, chromogenic substrate was added to the wells to initiate color development. The enzymatic reaction was subsequently terminated by sulfuric acid. Absorbance was measured at 450 nm within 10 min of reaction termination. Protein concentrations were determined using calibration curves generated from the corresponding standards of each protein: NF κ B p65, 0–5 μ g/mg total protein (TP); PGAM5, 0–20 μ g/mg TP; Bach1, 0–1000 pg/mg TP (OriGene, Rockville, MD, USA); NLRP3, 0–20 ng/mg TP; p21, 0–20 ng/mg TP; IKK α , 0–20 ng/mg TP; IKK β , 0–20 ng/mg TP; KAP1, 0–25 ng/mg TP; pI κ B, 0–20 ng/mg TP (Abcam, Cambridge, UK); TNF α , 0–5 ng/mg TP (Merck, Darmstadt, Germany); NF κ B p52, 0–20 ng/mg TP (LSBio, Seattle, WA, USA); HO-1, 0–5 ng/mg TP (Enzo Life Sciences, Farmingdale, NY, USA); p38, 0–25 ng/mg TP (Bio-technie, Minneapolis, MN, USA); Keap1, 0–20 ng/mg TP (Sino Biological Europe, Düsseldorf, Eschborn, Germany); phospho-Nrf2 (Ser40), 0–10 μ g/mg TP (Abcam, Cambridge, UK); p62, 0–25 ng/mg TP (Boster Biological Technology, Pleasanton, CA, USA); WTX, 0–20 ng/mg TP; pMAPK, 0–25 μ g/mg TP; pERK, 0–2000 pg/mg TP (Cell Signaling Technology,

Danvers, MA, USA); DPP3, 0–20 ng/mg TP (R&D Systems, Minneapolis, MN, USA).

2.6 Evaluation of Lipid Peroxidation Product (4-HNE)

The levels of 4-hydroxy-2-nonenal (4-HNE) were determined by the GC-MS/MS method [39]. 4-HNE was converted into its O-PFB-oxime-TMS derivative via derivatization with O-(2,3,4,5,6-pentafluorobenzyl) hydroxyamine hydrochloride (0.05 mol/L) (Sigma-Aldrich, St. Louis, MO, USA). Samples were then extracted with hexane and, after evaporation under argon, suspended in N,O-bis(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich, St. Louis, MO, USA). The samples were subsequently analyzed using a 7890A GC–7000 quadruple MS/MS (Agilent Technologies, Palo Alto, CA, USA) in a selected ion monitoring (SIM) mode: m/z ; 352.0, 271.2 (4-HNE-PFB-TMS) and m/z ; 307.0 (internal standard derivatives). Results were presented as percentage changes of 4-HNE in comparison to the melanocyte control group.

2.7 Evaluation of 4-HNE-Protein Adducts

The levels of 4-HNE-protein adducts in melanocytes were determined using an ELISA method [38]. Briefly, samples containing cell lysates were incubated overnight with a mouse monoclonal antibody against 4-HNE-His (Invitrogen, Burlington, ON, Canada). The primary antibody was then removed, and the samples were washed with PBS containing 0.1% Tween 20. After incubating with secondary antibody (goat anti-mouse antibody EnVision+ Dual Link/HRP; Agilent Technologies, Santa Clara, CA, USA), the samples were subsequently incubated with chromogen substrate solution (TMB; 0.1 mg/mL). Absorbance was then recorded at 450 nm, with 4-HNE-protein adduct levels expressed as a percentage relative to untreated control cells.

2.8 Statistical Analysis

Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test (GraphPad Prism 9, Dotmatics, Boston, MA, USA). Data are presented as the mean value with standard deviation (SD) for $N = 5$. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

Incubation of melanocytes with CBG for 24 h altered their endogenous antioxidant capacity. The total antioxidant potential (TAS) increased by approximately 30%, while ROS levels decreased (Fig. 1). Exposure of melanocytes to UVA radiation reduced TAS by approximately 50% compared to the control group. In contrast, treatment with CBG after UVA irradiation increased TAS, restoring it to the level of the control group.

The changes in melanocyte TAS may be related to the expression (Fig. 2) and cytosolic-nuclear localization

(Fig. 3 and **Supplementary material**) of the transcription factor Nrf2, which is responsible for the biosynthesis of antioxidant proteins. CBG was found to increase the level of pNrf2 and the expression of heme oxygenase (HO-1), which is an indicator of Nrf2 transcriptional efficiency (Fig. 2). Following cell exposure to UVA radiation, the levels of pNrf2 and HO-1 increased significantly by approximately 120% and 100%, respectively. Furthermore, microscopy results showed that exposure of melanocytes to UVA radiation induced strong increases in Nrf2 expression in both the nucleus and cytoplasm. However, treatment with CBG following UVA irradiation caused a significant decrease in pNrf2 levels, accompanied by an increase in HO-1 levels. CBG also modified the intracellular localization of Nrf2, partially preventing UVA-induced Nrf2 translocation to the nucleus (Fig. 3).

The levels of pNrf2 activators in melanocytes exposed to UVA and CBG are presented in Fig. 4. CBG was found to cause significant changes in the levels of activators (except p62), corresponding to the increased pNrf2 level (Fig. 2). Significant increases in activator levels were observed after UVA irradiation of cells, particularly for pMAPK (approximately 550%) and pERK (approximately 210%). However, addition of CBG to the medium after UVA exposure caused a decrease in the levels of KAP1 and pERK proteins, accompanied by a slight increase in p62 level.

CBG treatment also caused changes in the levels of pNrf2 inhibitors, including increases in Keap1 and PGAM5, and a decrease in the nuclear inhibitor Bach1 (Fig. 5 and **Supplementary material**). The response of these inhibitors to UVA radiation was also variable, with a decrease in Keap1 and an increase in Bach1 and PGAM5 levels. However, the addition of CBG to cells previously exposed to UVA radiation modified the response of Nrf2 inhibitors. Adding CBG to control cells increased Keap1 and PGAM5 levels and decreased Bach1 levels. Conversely, adding CBG to UVA-exposed melanocytes reversed the decrease in Keap1 level, and reversed the increase in Bach1 and PGAM5 levels. However, following CBG treatment, the levels of all Nrf2 inhibitors were different from those of the control group, with Keap1 and Bach1 being lower, and PGAM5 being higher.

Changes in pNrf2 levels are also influenced by the WTX and DDP3 proteins, which are activators of Keap1 (Fig. 6 and **Supplementary material**). CBG alone caused significant decreases in the levels of WTX and DPP3. In contrast, UVA radiation increased WTX by 60% and DPP3 by 270%. Moreover, CBG applied after UVA reversed the UVA-induced increase of both activators.

The shift in redox balance towards oxidation following UVA irradiation of melanocytes was reduced by the application of CBG (Fig. 1). This was reflected by the increase in ROS-dependent lipid peroxidation, which consequently manifested as increased levels of both 4-HNE and its protein adducts (approximately 117%) (Fig. 7). These

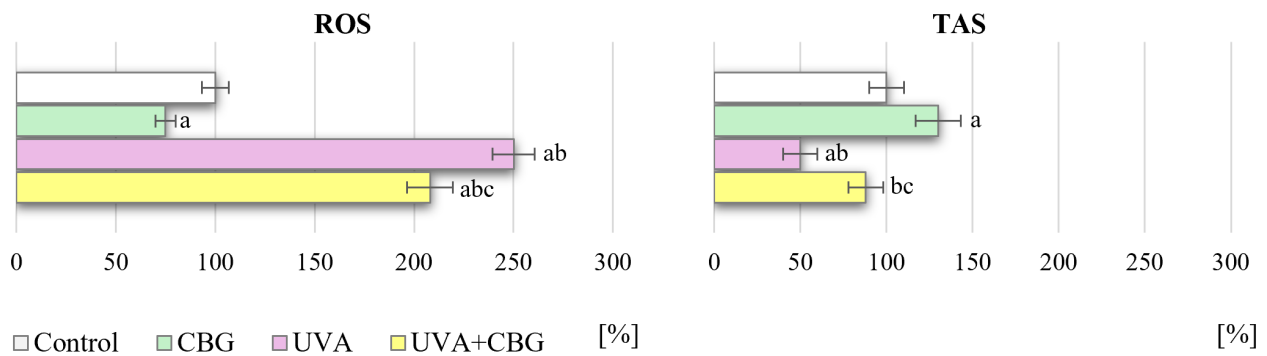


Fig. 1. Effect of CBG (1 μ M) on the level of reactive oxygen species (ROS) and total antioxidant status (TAS) in melanocytes (presented as a percentage of control cells). The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p \leq 0.05$). CBG, Cannabigerol.

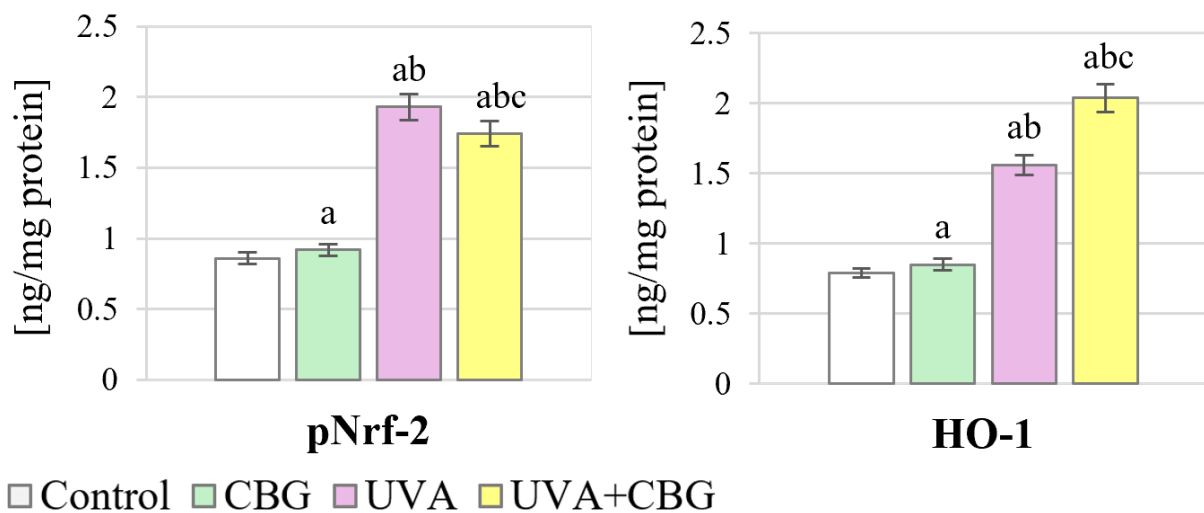


Fig. 2. Effect of CBG (1 μ M) on the level of phospho-Nrf2 (Ser40) (pNrf2) and heme oxygenase (HO-1) in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p \leq 0.05$). Nrf2, nuclear factor erythroid 2-related factor 2.

UVA effects were attenuated by treatment with CBG, which caused a significant reduction in lipid peroxidation. In melanocytes treated with CBG after UVA irradiation, the 4-HNE level decreased by approximately 40%, and 4-HNE-adducts with protein formation by approximately 24%.

The antioxidant transcription factor Nrf2 is known to interact with the proinflammatory transcription factor NF κ B. As a consequence, changes in the pNrf2 level resulted in modulation of NF κ B-dependent proinflammatory signaling (Fig. 8) and altered nuclear translocation of the NF κ B p52 subunit (Fig. 9 and **Supplementary material**).

Incubation of melanocytes with CBG was found to decrease the NF κ B p52 subunit level by approximately 20%, and the p65 subunit level by approximately 25%. However, this was accompanied by an approximately 30% increase in TNF α levels, possibly due to increased nuclear translocation of NF κ B p52. This was confirmed by microscopic assessment (Fig. 9) and by the reduced levels of pI κ B. pI κ B is an inhibitor of NF κ B p52, preventing its translocation to the nucleus and thus resulting in elevated levels of TNF α .

The exposure of melanocytes to UVA radiation led to increased expression of both NF κ B subunits and increased

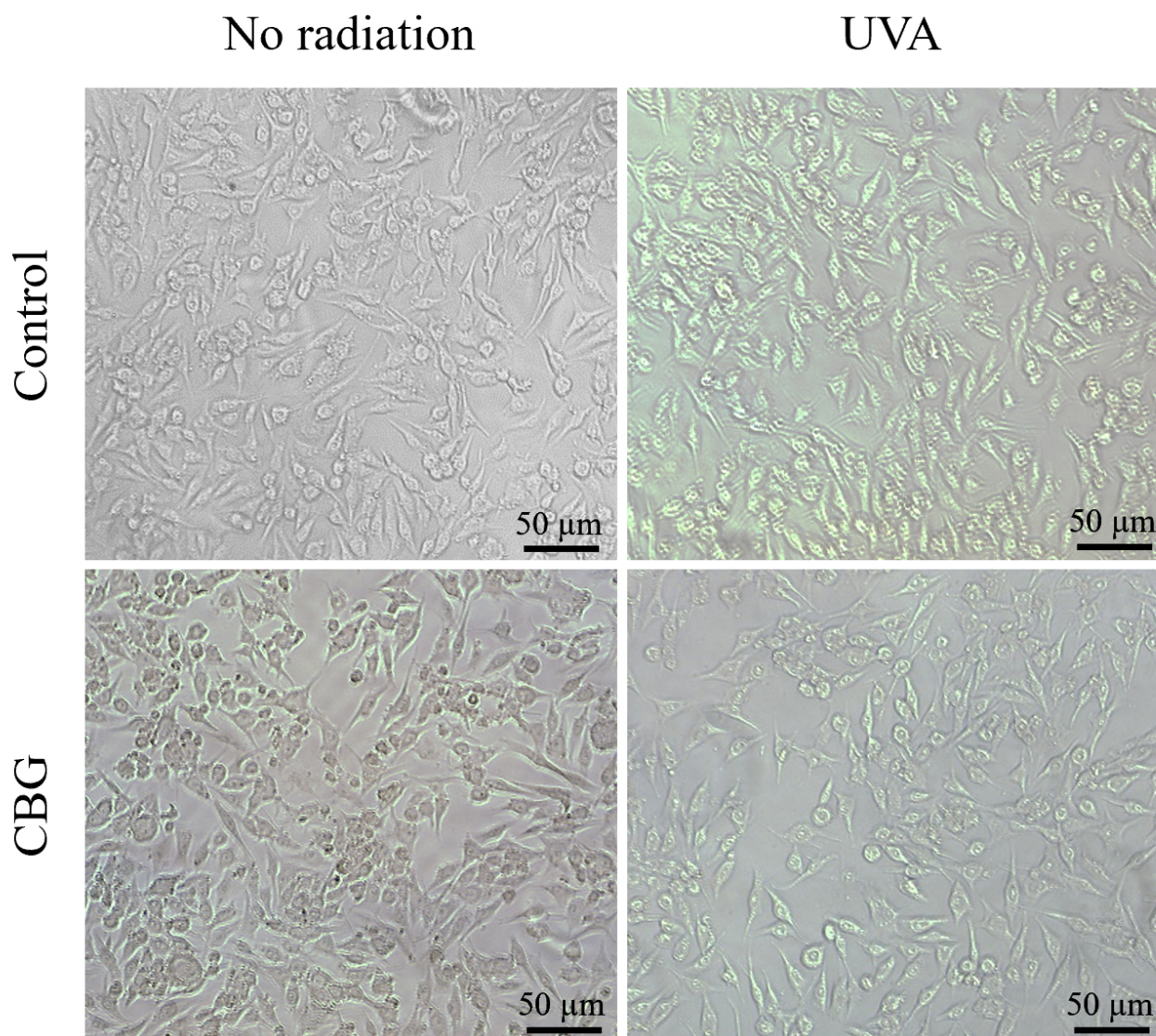


Fig. 3. Effect of CBG (1 μ M) on phospho-Nrf2 (Ser40) (pNrf2) intracellular distribution in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Localization of Nrf2 is shown in green. Scale bar = 50 μ m.

transcriptional activity, as assessed by increased TNF α levels (Fig. 8). This was also confirmed by microscopic assessment (Fig. 9). However, the addition of CBG to UVA-irradiated melanocytes decreased the NF κ B p52 subunit level (and consequently also the level of TNF α) compared to melanocytes exposed only to UVA. This effect was confirmed by the prevention of UVA-induced NF κ B p52 nuclear translocation (Fig. 9).

Changes in the levels of the NF κ B subunits p52 and p65 induced by UVA and/or CBG should also be assessed in the context of changes in the levels of NF κ B activators and inhibitors (Fig. 8B,C). Treatment with CBG reduced the levels of the IKK α activator and the pI κ B inhibitor in melanocytes. This was reflected in reduced expression of both NF κ B subunits p52 and p65. In turn, UVA

irradiation significantly increased the levels of NF κ B pathway components, and especially IKK β , which increased by approximately 190%. However, the addition of CBG to melanocytes previously exposed to UVA significantly attenuated the post-UVA increase in PGAM5, IKK α , and IKK β levels by approximately 20%, and the reduction in p62 by approximately 20% (Fig. 8C). Consequently, the different responses of components of the NF κ B-TNF α system to the action of UVA and/or CBG should be regarded as a cumulative factor regulating the inflammatory response of melanocytes.

4. Discussion

Epidemiological data indicate that UVA radiation induces oxidative stress in skin cells, including melanocytes,

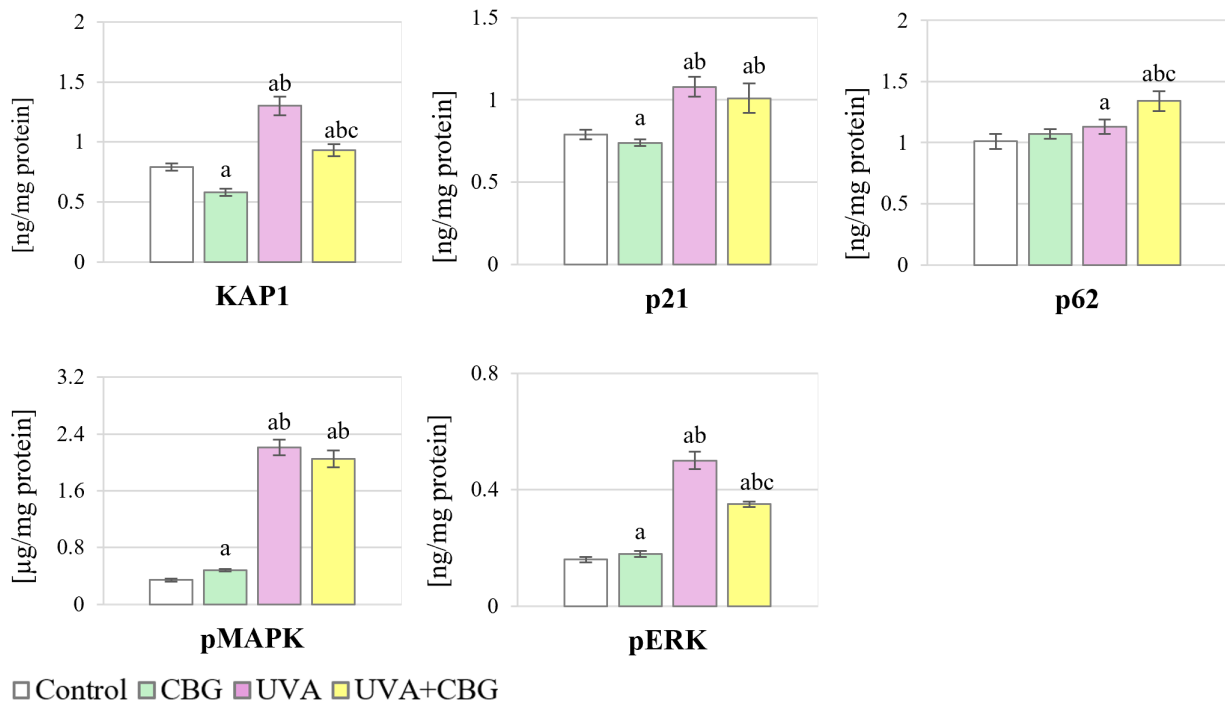


Fig. 4. Effect of CBG (1 μ M) on the level of Nrf2 activators in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p \leq 0.05$). KAP1, KRAB-associated protein 1; p21, potent cyclin-dependent kinase inhibitor; p62, multifunctional adaptor protein; pMAPK, phosphorylated mitogen-activated protein kinase; pERK, phosphorylated extracellular signal-regulated kinase.

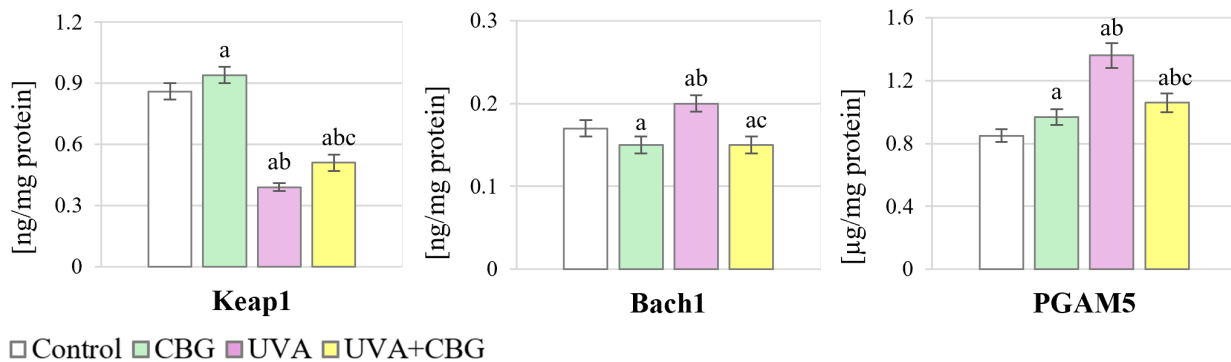


Fig. 5. Effect of CBG (1 μ M) on the level of Nrf2 inhibitors in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p \leq 0.05$). Keap1, kelch-like ECH-associated protein 1; Bach1, BTB domain and CNC homolog 1; PGAM5, phosphoglycerate mutase family member 5.

thereby altering the structure and function of macromolecules. This causes excessive cell proliferation and/or mutations [40] that subsequently exert cytotoxic, proinflammatory, and carcinogenic effects [41], leading to the

development of melanoma, a dangerous skin cancer type [42,43]. Hence, there is a need to protect skin from UVA radiation, preferably through the use of natural compounds such as phytocannabinoids that have a low probability of

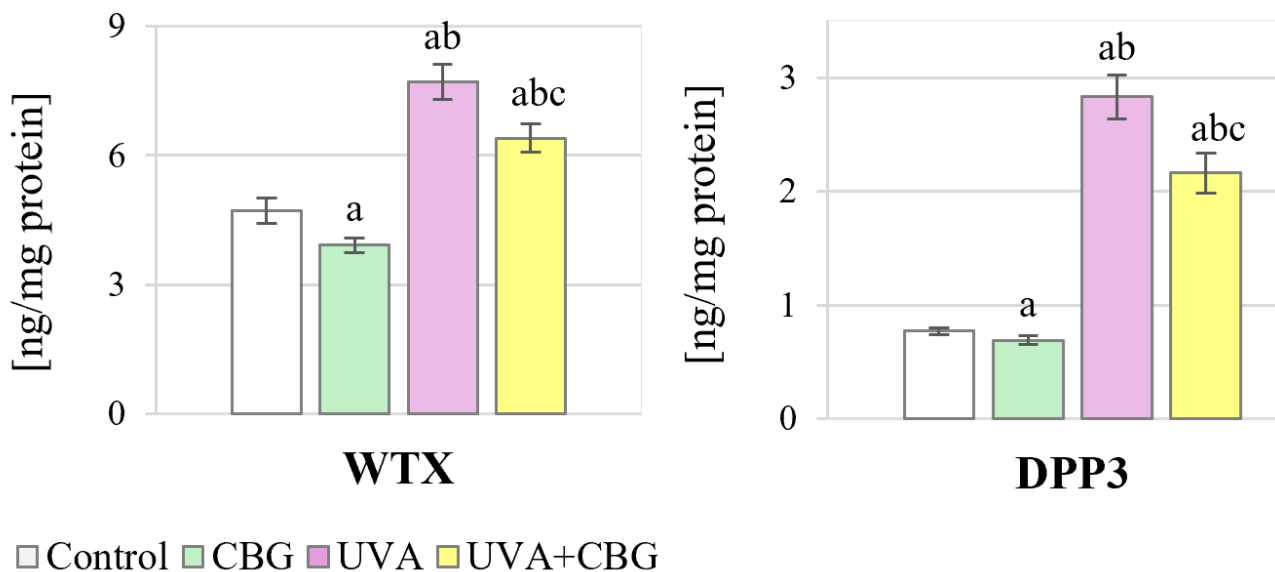


Fig. 6. Effect of CBG (1 μ M) on the level of Keap1 activators in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p \leq 0.05$). WTX, APC membrane recruitment protein 1 (AMER1); DPP3, dipeptidyl peptidase 3.

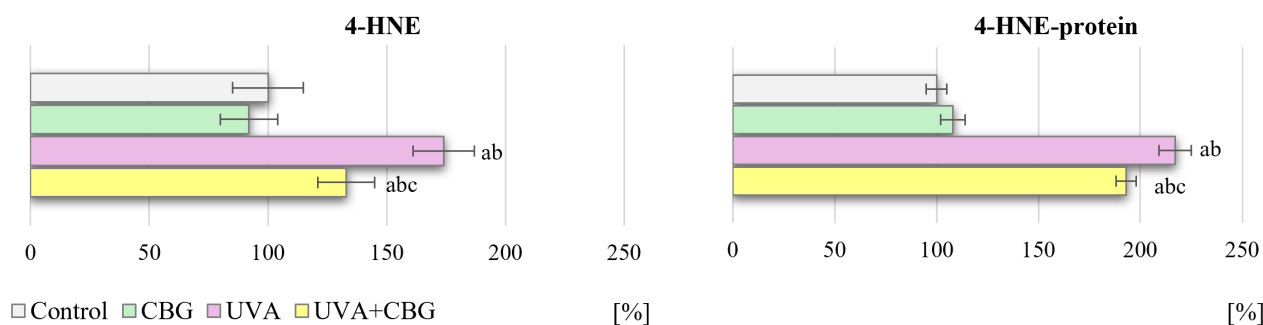


Fig. 7. Effect of CBG (1 μ M) on the level of 4-hydroxynonenal (4-HNE) and 4-HNE-protein adducts in melanocytes (presented as a percentage of control cells). The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p \leq 0.05$).

generating harmful effects. CBD and CBG have been shown to exhibit antioxidant and anti-inflammatory effects on keratinocytes and dermal fibroblasts [25,44]. Furthermore, CBG has greater metabolic efficiency than CBD [28]. The safety of long-term topical CBG use has already been demonstrated [28], suggesting it may be a promising bioactive ingredient in sunscreens and skin care products. Therefore, this study investigated the effect of CBG on melanocyte metabolism.

Melanocytes are located in the basal layer of the epidermis and produce endogenous chromophores under the influence of UVA radiation, in particular the cytoprotective

agent melanin [17,42]. Melanin synthesis is regulated by numerous modulators, including cytokines such as TNF α (following the action of NF κ B), α -melanocyte-stimulating hormone (α -MSH), endothelin-1, and growth factors, including epidermal growth factor (EGF) [45,46]. Both melanin and its polymers are known to exhibit photo- and cytoprotective properties, acting as antioxidants by scavenging ROS [47]. However, in response to UVA radiation, melanocytes can overproduce melanin. By stimulating endogenous cytosolic chromophores (e.g., flavins, NADPH, urocanic acid) and mitochondrial chromophores, this can increase ROS levels [48]. The results of the current

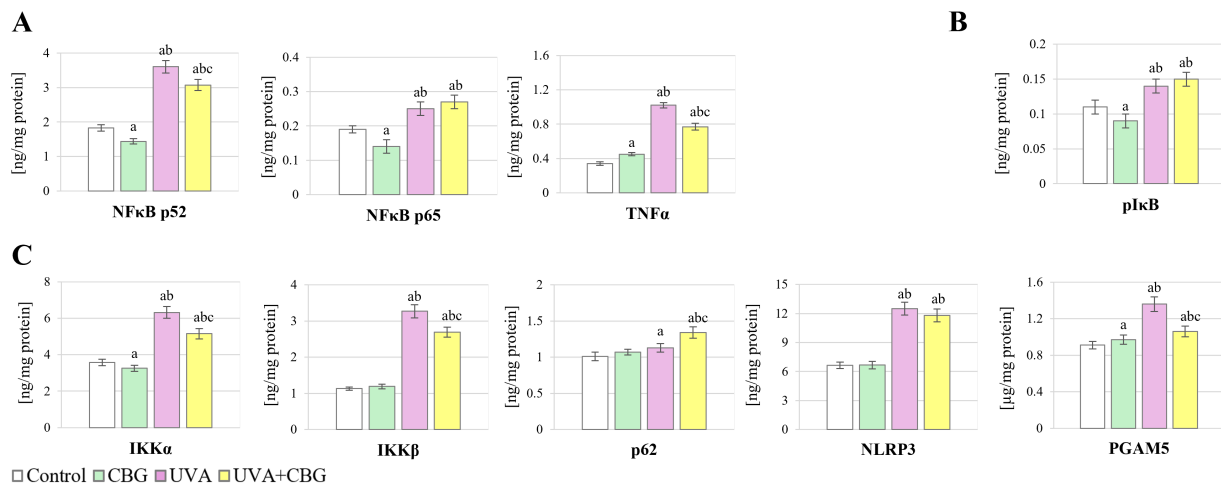


Fig. 8. Effect of CBG (1 μ M) on the level of (A) NF κ B subunits (p52 and p65) and TNF α as well as (B) NF κ B inhibitor (pI κ B) and (C) NF κ B activators in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm²) followed by culture for 24 h; and exposure to UVA (18 J/cm²) followed by treatment with CBG for 24 h. Values represent the mean \pm SD (N = 5). Statistical comparisons: *a*, difference from control; *b*, difference from CBG-treated cells; *c*, difference from UVA-treated cells ($p < 0.05$). NF κ B, nuclear factor kappa B; TNF α , tumor necrosis factor alpha; IKK α , I κ B kinase alpha; IKK β , I κ B kinase beta; NLRP3, NLR family pyrin domain containing 3.

study confirmed that a single dose of UVA radiation acting directly on melanocytes causes ROS overproduction and a decrease in total antioxidant status (TAS). This contributes to the generation of oxidative stress, promoting lipid peroxidation and an increase in the level of 4-HNE.

Previous studies have shown that sunlight (containing a UVA component) not only increases melanogenesis, but due to its reaction with dopaquinone, a pigment precursor [49], also decreases the antioxidant capacity [50,51]. It does this by reducing the levels of cysteine and consequently glutathione (GSH), which may influence the effectiveness of other cellular antioxidants. In the current study, CBG demonstrated cytoprotective effects by reducing ROS levels in both control and UVA-irradiated melanocytes, while simultaneously increasing the total antioxidant status (TAS), which is known to promote cell viability [34]. The metabolic bioactivity of CBG is believed to result from its terpene structure. CBG is estimated to have approximately twice the ROS-reducing capacity of CBD, making it a common ingredient in skin preparations [52].

The synthesis of a wide range of antioxidant proteins is regulated by the activity of the transcription factor Nrf2. The level and efficiency of Nrf2 is in turn regulated by cytoplasmic and nuclear activators and inhibitors [25]. The results of the current study showed relatively low levels of pNrf2 in the cytoplasm of control melanocytes. This indicates that it may form adducts with the cytosolic inhibitor Keap1, causing Nrf2 to be directed towards proteasomal degradation [53]. Similar to CBD, CBG can also modify Cys15 of Keap1, which is involved in the formation of the

Cul3–Keap1 complex necessary for interaction of this inhibitor with Nrf2 [54]. However, under the oxidative stress conditions of UVA-exposed melanocytes, Nrf2 dissociates from Keap1 and translocates to the cell nucleus, where it heterodimerizes with one of the small Maf proteins. Heterodimers recognize ARE sequences present in the regulatory regions of Nrf2 target genes, which are essential for the recruitment of key factors [55]. Nuclear Nrf2 induces the expression of a broad spectrum of genes involved in cellular antioxidant defense, detoxification, and redox homeostasis. In addition to the canonical redox-dependent mechanism, Nrf2 can also be activated via non-canonical pathways. In non-canonical pathways, proteins such as p62, p21, DPP3, and WTX interact directly with Keap1, impair Keap1-mediated ubiquitination of Nrf2, and promote its stabilization, nuclear accumulation, and transcriptional activity by disrupting the Nrf2–Keap1 complex [56].

A previous finding relevant to the present results is that UVA irradiation of melanocytes may increase pNrf2 levels and induce HO-1 expression, thereby inhibiting Bach1 degradation [57]. Furthermore, Bach1-mediated repression of Maf-regulated genes has been shown to dominate over Nrf2-mediated activation of these genes [58]. These results suggest that Nrf2 activation is able to offset the increase in Bach1 levels and activate the transcription of antioxidant genes, even in the presence of high Bach1 levels. Under reduced Keap1 levels following UVA exposure, Bach1 protein stabilization might be induced by Nrf2-dependent upregulation of HO-1, promoting a decrease in free heme levels.

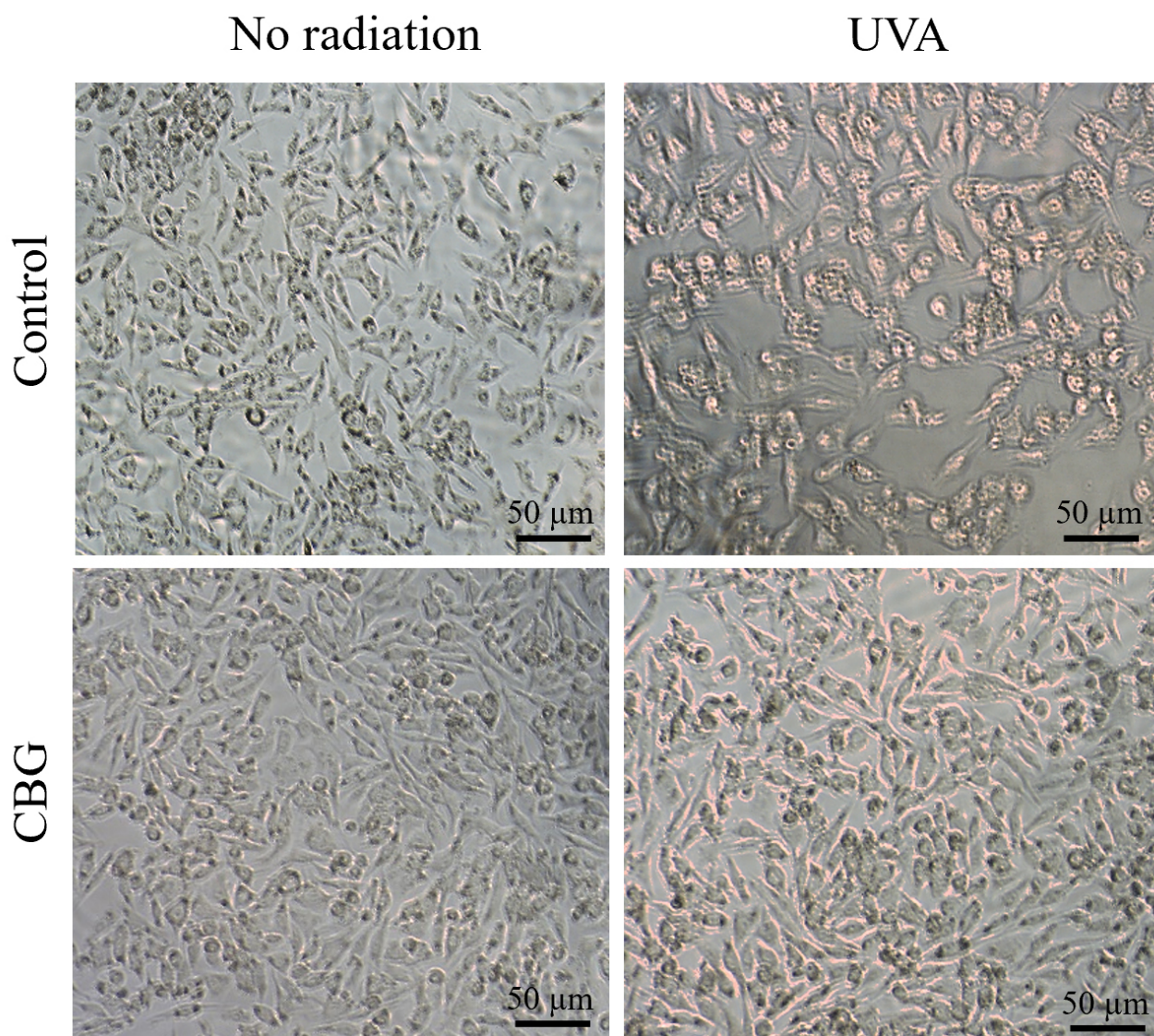


Fig. 9. Effect of CBG (1 μM) on phosphorylated form of subunit p52 $\text{NF}\kappa\text{B}$ (phospho-Ser222) ($\text{NF}\kappa\text{B}$ p52) intracellular distribution in melanocytes. The four experimental groups were: controls; treatment with CBG for 24 h; exposure to UVA (18 J/cm^2) followed by culture for 24 h; and exposure to UVA (18 J/cm^2) followed by treatment with CBG for 24 h. Localization of $\text{NF}\kappa\text{B}$ p52 is shown in red. Scale bar = 50 μm .

CBG increased the level of free Keap1 in control and UVA-irradiated melanocytes. This may indicate less involvement of the cytosolic Nrf2 inhibitor in the formation of Nrf2–Keap1 interactions, as the biological activity of Keap1 depends on the availability of functional cysteine thiol groups. However, these are highly susceptible to modification by ROS and electrophilic aldehydes, including 4-HNE generated during lipid peroxidation [59]. Increased levels of 4-HNE and its protein adducts in the cytosol were observed after UVA irradiation of melanocytes, but were reduced after CBG treatment, thereby maintaining the normal phospholipid structure of the cell membrane [34]. However, even after treatment with CBG, the level of 4-HNE-protein adducts did not reach the control values. This may

indicate the persistence of oxidative stress after CBG treatment, which is also supported by the increased level of ROS and 4-HNE. Modification of Keap1 by 4-HNE is known to increase the level of free Nrf2, thus enhancing its translocation to the cell nucleus, as confirmed by the microscopy results of our study. However, the levels of PGAM5, another cytosolic Nrf2 inhibitor [60], and Bach1, a nuclear Nrf2 inhibitor [61,62], were also observed to increase after UVA irradiation of melanocytes. In control melanocyte cultures and those treated with CBG, Bach1 was present primarily in the cytoplasm. In contrast, Bach1 shifted to the nucleus following UVA irradiation, where it is more effective at inhibiting Nrf2 activity. Furthermore, CBG treatment alone increased the expression of PGAM5 phosphatase, which

can interact with both Keap1 and Nrf2 [63], thereby confirming the regulatory role of this phytocannabinoid. Due to the reduced expression and likely structural modifications of Keap1 in UVA-irradiated and CBG-treated melanocytes, Nrf2 can more easily relocate to the nucleus compared to control cells. Thus, by increasing ROS generation, UVA irradiation simultaneously increases the expression of Nrf2 in the cytosol, inducing the transcription of cytoprotective proteins essential for maintaining redox balance under conditions of elevated ROS [59]. The level of the primary target of this transcriptional activity, HO-1 [64], was shown to increase after UVA exposure, and even more after CBG treatment.

Regardless of the Nrf2-Keap1 interaction, metabolic links are known to exist between the Nrf2-activating protein p62 and the Keap-1 inhibitor. These links connect the Nrf2 and autophagy signaling pathways [65], which are essential for maintaining cellular antioxidant homeostasis [66] and increasing the biosynthesis of cytoprotective proteins [67]. Autophagy also acts as a cell survival mechanism under various physicochemical conditions, allowing p62 to form a positive feedback loop with Nrf2 [68], thus inhibiting p62 accumulation. UVA irradiation of melanocytes and their exposure to CBG significantly increased the level of p62, which may be important because autophagy plays a role in regulating melanin production and skin pigmentation [69]. Autophagy is also involved in melanin degradation via the PI3K/AKT/mTOR pathway [70]. Moreover, increased autophagy in melanocytes provides more effective protection against the accumulation of oxidatively modified macromolecules. Autophagy disorders can therefore increase the sensitivity of melanocytes to oxidative stress [71].

It is well established that overproduction of ROS leads to oxidative stress, which plays a major role in inflammatory processes [16]. At the same time, the NF κ B transcription factor family is known to play a significant role in the cellular environment. These transcription factors control the expression of important regulatory genes for inflammation, cell death, and proliferation. The NF κ B protein is located in the cytoplasm and can be activated by various cellular stimuli. On the other hand, UVA-induced oxidative stress in melanocytes may lead to overactivation of the AKT pathway through increased expression of Sestrin 2. As a consequence, increased expression of the NF κ B p65 subunit inhibits melanocyte apoptosis, including cells with altered pro-neoplastic metabolism [72], while promoting the proliferation of pre-neoplastic melanocytes [41,73,74]. UVA-induced oxidative stress and inflammation are thought to be associated with overexpression of TNF α , which in turn causes overexpression of Nrf2 and may also protect cells from overproduction of ROS and proinflammatory cytokines [25]. Since the Nrf2 and NF κ B cell signaling pathways interact, both the activation and inhibition of individual pathways may reflect interactions between the two pathways [75]. For example, the Keap1 pro-

tein participates not only in regulating the Nrf2 pathway, but also that of the NF κ B pathway [76]. Therefore, in light of the ability of CBG to modulate the Nrf2-dependent antioxidant system and to inhibit NF κ B-mediated inflammatory responses, it may also serve as an active ingredient in sunscreen formulations or post-sun exposure skincare products by enhancing cellular resistance to UVA-induced oxidative damage.

CBD is structurally similar to CBG and can also modify the structure of Keap1 [77]. It can therefore be assumed that CBG influences the regulation of both Nrf2 and NF κ B by modifying the structure of Keap1. Our study found that Nrf2 activation contributes to inhibition of the NF κ B signaling pathway in melanocytes exposed to both UVA and CBG. However, one possible NF κ B complex is NF κ B-4-HNE [78]. In line with the general increase in 4-HNE protein adducts, the level of this complex increased after UVA irradiation of melanocytes, but was partially reduced by CBG. Furthermore, CBG also reduced the level of I κ B-activated kinase (IKK), which phosphorylates NF κ B. The level of IKK increased after UVA irradiation, promoting the transcription of pro-inflammatory mediators such as TNF α , as well as IL-6, iNOS, and IL-1 [79]. The Nrf2 inhibitor complex, Keap1-Cul3, can target the IKK β subunit for ubiquitination and degradation [76]. Moreover, increased Keap1 levels following UVA and CBG treatment can result in Nrf2-Keap1 complexes. This prevents the continuous activation of Nrf2, which may otherwise promote the progression of melanocytes towards melanoma [59].

5. Limitations

CBG confers beneficial metabolic effects on melanocytes, especially those exposed to UVA radiation. However, it is important to note the limitations of this study. The experiments were conducted exclusively *in vitro* and using only monocultures of melanocytes, without considering possible interactions with other skin cells, such as fibroblasts. Furthermore, only a single dose of CBG and UVA radiation was used in the experiments, which especially in the case of UVA, does not reflect real-world conditions. Nevertheless, our study represents the first attempt to assess the protective effects of CBG on melanocytes, which may constitute a barrier to the neoplastic transformation of these cells. Further studies are planned to analyze the metabolic consequences of increased doses of CBG and the length of exposure to melanocytes. Furthermore, studies using co-cultures of fibroblasts and melanocytes are planned. Although there is no clear evidence of any harmful effects of CBG, an *in vivo* analysis of its effect on the metabolism of skin cells, including melanocytes, in experimental animals should also be conducted.

Furthermore, the results of this study only describe the mechanism of action of CBG, without comparing its effects with well-described and commonly used cytoprotec-

tive compounds such as ascorbic acid or CBD. These are characterized by both similar and diverse physicochemical properties, and consequently diverse biological effects that could significantly increase the interpretability of our results. Further analysis of the metabolic potential of CBG should include comparative studies of this phytocannabinoid with other protective compounds that have similar and diverse physicochemical and biological properties. Such research could then lead to the formulation of more rational conclusions.

6. Conclusion

CBG has regulatory effects on the metabolism of melanocytes, especially those exposed to UVA radiation. This phytocannabinoid generates favorable conditions for the physiological or near-physiological functioning of melanocytes, even after exposure to harmful environmental conditions such as UVA radiation. By increasing the antioxidant capacity of melanocytes and reducing inflammatory processes, CBG may reduce the risk of neoplastic transformation of these cells.

Skin exposure to solar radiation is not only a risk factor for premature skin aging, but also a trigger for metabolic changes leading to the development of skin cancer. Our results suggest that CBG protects melanocytes against UVA-induced oxidative changes by activating the Nrf2-dependent antioxidant system, and/or inhibiting pro-inflammatory signaling based on NF κ B. Consequently, CBG creates favorable conditions for the physiological functioning of melanocytes following UVA exposure, ultimately reducing the risk of inflammatory skin reactions or neoplastic transformation. These findings could help in the development of improved skin care/protection products.

Availability of Data and Materials

The datasets obtained during this study are available from the corresponding author upon reasonable request.

Author Contributions

IJ-K and ES designed the study. NZ and AM conducted the formal analysis. IJ-K and AG developed the methodology, conducted the research, and were responsible for data collection. ES managed the project administration and provided funding and supervision for the study. IJ-K, AG and AM prepared the visualizations and wrote the original manuscript, while NZ and ES analyzed and revised the final version. All authors contributed to editorial changes to the manuscript after peer review. All authors read and approved the final version of the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

The article has been supported by the Polish National Agency for Academic Exchange under the “Strategic Partnerships” programme (project no. BPI/PST/2024/1/00008).

Conflict of Interest

The authors declare no conflict of interest. Given his role as the Guest Editor and Editorial Board member, Neven Žarković had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Alexandros G. Georgakilas.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL47793>.

References

- [1] Parousis-Paraskevas O, Gkikoudi A, Al-Qaaod A, Vasilopoulos SN, Manda G, Beinke C, *et al.* Combined Radiations: Biological Effects of Mixed Exposures Across the Radiation Spectrum. *Biomolecules*. 2025; 15: 1282. <https://doi.org/10.3390/biom15091282>.
- [2] Talapko J, Talapko D, Katalinić D, Kotris I, Erić I, Belić D, *et al.* Health Effects of Ionizing Radiation on the Human Body. *Medicina* (Kaunas, Lithuania). 2024; 60: 653. <https://doi.org/10.3390/medicina60040653>.
- [3] Wei J, Balupillai A, Annamalai A, Selvababu AP, David E. Nobiletin enhances peroxiredoxin-1, preventing UVA radiation-induced oxidative stress and photoaging in human skin epidermal keratinocytes. *Journal of Functional Foods*. 2024; 114: 106066. <https://doi.org/10.1016/j.jff.2024.106066>.
- [4] McGrath JA, Uitto J. Structure and Function of the Skin. *Rook's Textbook of Dermatology* (pp. 1–50). John Wiley & Sons, Ltd.: Hoboken, Wiley. 2024.
- [5] Chambers ES, Vukmanovic-Stejić M. Skin barrier immunity and ageing. *Immunology*. 2020; 160: 116–125. <https://doi.org/10.1111/imm.13152>.
- [6] Tang X, Yang T, Yu D, Xiong H, Zhang S. Current insights and future perspectives of ultraviolet radiation (UV) exposure: Friends and foes to the skin and beyond the skin. *Environment International*. 2024; 185: 108535. <https://doi.org/10.1016/j.envint.2024.108535>.
- [7] Garbe C, Forsea AM, Amaral T, Arenberger P, Autier P, Berwick M, *et al.* Skin cancers are the most frequent cancers in fair-skinned populations, but we can prevent them. *European Journal of Cancer* (Oxford, England: 1990). 2024; 204: 114074. <https://doi.org/10.1016/j.ejca.2024.114074>.
- [8] Zhang X, Li H, Liu C, Yuan X. Role of ROS-mediated autophagy in melanoma (Review). *Molecular Medicine Reports*. 2022; 26: 303. <https://doi.org/10.3892/mmr.2022.12819>.
- [9] Cordero C, Mehta KPM, Weaver TM, Ling JA, Freudenthal BD, Cortez D, *et al.* Contributing factors to the oxidation-induced mutational landscape in human cells. *Nature Communications*. 2024; 15: 10722. <https://doi.org/10.1038/s41467-024-55497-z>.

- [10] Zhao J, Dan Y, Liu Z, Wang Q, Jiang M, Zhang C, *et al.* Solamargine Alleviated UVB-Induced Inflammation and Melanogenesis in Human Keratinocytes and Melanocytes via the p38 MAPK Signaling Pathway, a Promising Agent for Post-inflammatory Hyperpigmentation. *Frontiers in Medicine*. 2022; 9: 812653. <https://doi.org/10.3389/fmed.2022.812653>.
- [11] Wang B, Wang Y, Zhang J, Hu C, Jiang J, Li Y, *et al.* ROS-induced lipid peroxidation modulates cell death outcome: mechanisms behind apoptosis, autophagy, and ferroptosis. *Archives of Toxicology*. 2023; 97: 1439–1451. <https://doi.org/10.1007/s00204-023-03476-6>.
- [12] Jaganjac M, Zarkovic N. Lipid Peroxidation Linking Diabetes and Cancer: The Importance of 4-Hydroxynonenal. *Antioxidants & Redox Signaling*. 2022; 37: 1222–1233. <https://doi.org/10.1089/ars.2022.0146>.
- [13] Biernacki M, Skrzydlewska E. Metabolic pathways of eicosanoids-derivatives of arachidonic acid and their significance in skin. *Cellular & Molecular Biology Letters*. 2025; 30: 7. <https://doi.org/10.1186/s11658-025-00685-y>.
- [14] Takabe P, Kärnä R, Rauhala L, Tammi M, Tammi R, Pasonen-Seppänen S. Melanocyte Hyaluronan Coat Fragmentation Enhances the UVB-Induced TLR-4 Receptor Signaling and Expression of Proinflammatory Mediators IL6, IL8, CXCL1, and CXCL10 via NF-κB Activation. *The Journal of Investigative Dermatology*. 2019; 139: 1993–2003.e4. <https://doi.org/10.1016/j.jid.2019.03.1135>.
- [15] Atalay Ekiner S, Gegotek A, Skrzydlewska E. Inflammasome activity regulation by PUFA metabolites. *Frontiers in Immunology*. 2024; 15: 1452749. <https://doi.org/10.3389/fimmu.2024.1452749>.
- [16] Meierjohann S. Oxidative stress in melanocyte senescence and melanoma transformation. *European Journal of Cell Biology*. 2014; 93: 36–41. <https://doi.org/10.1016/j.ejcb.2013.11.005>.
- [17] Sarkar S, Gaddameedhi S. Solar ultraviolet-induced DNA damage response: Melanocytes story in transformation to environmental melanomagenesis. *Environmental and Molecular Mutagenesis*. 2020; 61: 736–751. <https://doi.org/10.1002/em.22370>.
- [18] Verma A, Zanoletti A, Kareem KY, Adelodun B, Kumar P, Ajibade FO, *et al.* Skin protection from solar ultraviolet radiation using natural compounds: a review. *Environmental Chemistry Letters*. 2024; 22: 273–295. <https://doi.org/10.1007/s10311-023-01649-4>.
- [19] Liu Y, Liu H-Y, Li S-H, Ma W, Wu DT, Li HB, *et al.* Cannabis sativa bioactive compounds and their extraction, separation, purification, and identification technologies: An updated review. *TrAC Trends in Analytical Chemistry*. 2022; 149: 116554. <https://doi.org/10.1016/j.trac.2022.116554>.
- [20] Gohad P, McCoy J, Wambier C, Kovacevic M, Situm M, Stanimirovic A, *et al.* Novel cannabidiol sunscreen protects keratinocytes and melanocytes against ultraviolet B radiation. *Journal of Cosmetic Dermatology*. 2020. <https://doi.org/10.1111/jocd.13693>. (Online ahead of print)
- [21] McCormick E, Han H, Abdel Azim S, Whiting C, Bhamidipati N, Kiss A, *et al.* Topical nanoencapsulated cannabidiol cream as an innovative strategy combating UV-A-induced nuclear and mitochondrial DNA injury: A pilot randomized clinical study. *Journal of the American Academy of Dermatology*. 2024; 91: 855–862. <https://doi.org/10.1016/j.jaad.2024.06.088>.
- [22] Li Y, Hao D, Wei D, Xiao Y, Liu L, Li X, *et al.* Photoprotective Effects of Cannabidiol against Ultraviolet-B-Induced DNA Damage and Autophagy in Human Keratinocyte Cells and Mouse Skin Tissue. *Molecules (Basel, Switzerland)*. 2022; 27: 6740. <https://doi.org/10.3390/molecules27196740>.
- [23] Hwang YS, Kim YJ, Kim MO, Kang M, Oh SW, Nho YH, *et al.* Cannabidiol upregulates melanogenesis through CB1 dependent pathway by activating p38 MAPK and p42/44 MAPK. *Chemico-biological Interactions*. 2017; 273: 107–114. <https://doi.org/10.1016/j.cbi.2017.06.005>.
- [24] Perez Almeria CV, Setiawan IM, Siderius M, Smit MJ. G protein-coupled receptors as promising targets in cancer. *Current Opinion in Endocrine and Metabolic Research*. 2021; 16: 119–127. <https://doi.org/10.1016/j.coemr.2020.10.005>.
- [25] Jarocka-Karpowicz I, Stasiewicz A, Olchowik-Grabarek E, Sękowski S, Kacprowska A, Skrzydlewska E. Antioxidant and membrane-protective effects of the 3-O-ethyl ascorbic acid-cannabigerol system on UVB-irradiated human keratinocytes. *Free Radical Biology & Medicine*. 2025; 228: 251–266. <https://doi.org/10.1016/j.freeradbiomed.2025.01.008>.
- [26] Li S, Li W, Malhi NK, Huang J, Li Q, Zhou Z, *et al.* Cannabigerol (CBG): A Comprehensive Review of Its Molecular Mechanisms and Therapeutic Potential. *Molecules (Basel, Switzerland)*. 2024; 29: 5471. <https://doi.org/10.3390/molecules29225471>.
- [27] Perez E, Fernandez JR, Fitzgerald C, Rouzard K, Tamura M, Savile C. In Vitro and Clinical Evaluation of Cannabigerol (CBG) Produced via Yeast Biosynthesis: A Cannabinoid with a Broad Range of Anti-Inflammatory and Skin Health-Boosting Properties. *Molecules (Basel, Switzerland)*. 2022; 27: 491. <https://doi.org/10.3390/molecules27020491>.
- [28] Luz-Veiga M, Mendes A, Tavares-Valente D, Amorim M, Conde A, Pintado ME, *et al.* Exploring Cannabidiol (CBD) and Cannabigerol (CBG) Safety Profile and Skincare Potential. *International Journal of Molecular Sciences*. 2024; 25: 12224. <https://doi.org/10.3390/ijms252212224>.
- [29] Pagano C, Ciaglia E, Coppola L, Lopardo V, Raimondo A, Giuseppe M, *et al.* Cannabidiol exerts multitarget immunomodulatory effects on PBMCs from individuals with psoriasis vulgaris. *Frontiers in Immunology*. 2024; 15: 1373435. <https://doi.org/10.3389/fimmu.2024.1373435>.
- [30] Jeong GH, Kim KC, Lee JH. Anti-Inflammatory Effects of Cannabigerol In Vitro and In Vivo Are Mediated Through the JAK/STAT/NFκB Signaling Pathway. *Cells*. 2025; 14: 83. <https://doi.org/10.3390/cells14020083>.
- [31] Łuczaj W, Dobrzyńska I, Skrzydlewska E. Differences in the phospholipid profile of melanocytes and melanoma cells irradiated with UVA and treated with cannabigerol and cannabidiol. *Scientific Reports*. 2023; 13: 16121. <https://doi.org/10.1038/s41598-023-43363-9>.
- [32] Wyrobnik I, Steinberg M, Gelfand A, Rosenblum R, Eid Mutlak Y, Sulimani L, *et al.* Decreased melanoma CSF-1 secretion by Cannabigerol treatment reprograms regulatory myeloid cells and reduces tumor progression. *Oncoimmunology*. 2023; 12: 2219164. <https://doi.org/10.1080/2162402X.2023.2219164>.
- [33] Fotakis G, Timbrell JA. In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters*. 2006; 160: 171–177. <https://doi.org/10.1016/j.toxlet.2005.07.001>.
- [34] Gegotek A, Mucha M, Skrzydlewska E. Skin cells protection against UVA radiation - The comparison of various antioxidants and viability tests. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2024; 181: 117736. <https://doi.org/10.1016/j.biopha.2024.117736>.
- [35] Nouroozi RV, Nouroozi MV, Ahmadzadeh M. Determination of Protein Concentration Using Bradford Microplate Protein Quantification Assay. *International Electronic Journal of Medicine*. 2015; 4: 11–17.
- [36] Zhang Z, Zhang Q, Xue Y, Chen G, Wu Z, Fang H. Serum levels of total antioxidant status, nitric oxide and nitric oxide synthase in minor recurrent aphthous stomatitis patients. *Medicine*. 2019; 98: e14039. <https://doi.org/10.1097/MD.00000000000014039>.
- [37] Dikalov S, Griendling KK, Harrison DG. Measurement of re-

- active oxygen species in cardiovascular studies. *Hypertension* (Dallas, Tex.: 1979). 2007; 49: 717–727. <https://doi.org/10.1161/01.HYP.0000258594.87211.6b>.
- [38] Hnasko R, Lin A, McGarvey JA, Stanker LH. A rapid method to improve protein detection by indirect ELISA. *Biochemical and Biophysical Research Communications*. 2011; 410: 726–731. <https://doi.org/10.1016/j.bbrc.2011.06.005>.
- [39] Tsikas D, Rothmann S, Schneider JY, Gutzki FM, Beckmann B, Fröllich JC. Simultaneous GC-MS/MS measurement of malondialdehyde and 4-hydroxy-2-nonenal in human plasma: Effects of long-term L-arginine administration. *Analytical Biochemistry*. 2017; 524: 31–44. <https://doi.org/10.1016/j.ab.2016.08.009>.
- [40] Zhang X, Li E, Kuang Y, Gai Y, Feng Y, Huang Y, *et al.* MTCH2 regulates NRF2-mediated RRM1 expression to promote melanoma proliferation and dacarbazine insensitivity. *Cell Death & Disease*. 2025; 16: 268. <https://doi.org/10.1038/s41419-025-07618-9>.
- [41] Speeckaert R, Belpaire A, Speeckaert M, van Geel N. The delicate relation between melanocytes and skin immunity: A game of hide and seek. *Pigment Cell & Melanoma Research*. 2022; 35: 392–407. <https://doi.org/10.1111/pcmr.13037>.
- [42] Fadadu RP, Wei ML. Ultraviolet A radiation exposure and melanoma: a review. *Melanoma Research*. 2022; 32: 405–410. <https://doi.org/10.1097/CMR.0000000000000857>.
- [43] Remigante A, Spinelli S, Marino A, Pusch M, Morabito R, Dossena S. Oxidative Stress and Immune Response in Melanoma: Ion Channels as Targets of Therapy. *International Journal of Molecular Sciences*. 2023; 24: 887. <https://doi.org/10.3390/ijms24010887>.
- [44] Wroński A, Dobrzyńska I, Sękowski S, Łuczaj W, Olchowik-Grabarek E, Skrzydlewska E. Cannabidiol and Cannabigerol Modify the Composition and Physicochemical Properties of Keratinocyte Membranes Exposed to UVA. *International Journal of Molecular Sciences*. 2023; 24: 12424. <https://doi.org/10.3390/ijms241512424>.
- [45] Pillaiyar T, Manickam M, Jung SH. Downregulation of melanogenesis: drug discovery and therapeutic options. *Drug Discovery Today*. 2017; 22: 282–298. <https://doi.org/10.1016/j.drudis.2016.09.016>.
- [46] Yun WJ, Bang SH, Min KH, Kim SW, Lee MW, Chang SE. Epidermal growth factor and epidermal growth factor signaling attenuate laser-induced melanogenesis. *Dermatologic Surgery: Official Publication for American Society for Dermatologic Surgery [et Al.]*. 2013; 39: 1903–1911. <https://doi.org/10.1111/dsu.12348>.
- [47] Snyman M, Walsdorf RE, Wix SN, Gill JG. The metabolism of melanin synthesis-From melanocytes to melanoma. *Pigment Cell & Melanoma Research*. 2024; 37: 438–452. <https://doi.org/10.1111/pcmr.13165>.
- [48] Dumbuya H, Hafez SY, Oancea E. Cross talk between calcium and ROS regulate the UVA-induced melanin response in human melanocytes. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2020; 34: 11605–11623. <https://doi.org/10.1096/fj.201903024R>.
- [49] Meyskens FL, Jr, Farmer P, Fruehauf JP. Redox regulation in human melanocytes and melanoma. *Pigment Cell Research*. 2001; 14: 148–154. <https://doi.org/10.1034/j.1600-0749.2001.140303.x>.
- [50] Upadhyay PR, Ho T, Abdel-Malek ZA. Participation of keratinocyte- and fibroblast-derived factors in melanocyte homeostasis, the response to UV, and pigmentary disorders. *Pigment Cell & Melanoma Research*. 2021; 34: 762–776. <https://doi.org/10.1111/pcmr.12985>.
- [51] Kohandel Z, Farkhondeh T, Aschner M, Samarghandian S. Nrf2 a molecular therapeutic target for Astaxanthin. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2021; 137: 111374. <https://doi.org/10.1016/j.biopha.2021.111374>.
- [52] Dawidowicz AL, Olszowy-Tomeczyk M, Typek R. CBG, CBD, Δ9-THC, CBN, CBGA, CBDA and Δ9-THCA as antioxidant agents and their intervention abilities in antioxidant action. *Fitoterapia*. 2021; 152: 104915. <https://doi.org/10.1016/j.fitote.2021.104915>.
- [53] Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the Hallmarks of Cancer. *Cancer Cell*. 2018; 34: 21–43. <https://doi.org/10.1016/j.ccell.2018.03.022>.
- [54] Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2-Keap1 signaling in oxidative and reductive stress. *Biochimica et Biophysica Acta. Molecular Cell Research*. 2018; 1865: 721–733. <https://doi.org/10.1016/j.bbamcr.2018.02.010>.
- [55] Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 System: a Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis. *Physiological Reviews*. 2018; 98: 1169–1203. <https://doi.org/10.1152/physrev.00023.2017>.
- [56] Silva-Islas CA, Maldonado PD. Canonical and non-canonical mechanisms of Nrf2 activation. *Pharmacological Research*. 2018; 134: 92–99. <https://doi.org/10.1016/j.phrs.2018.06.013>.
- [57] Lignitto L, LeBoeuf SE, Homer H, Jiang S, Askenazi M, Karakousi TR, *et al.* Nrf2 Activation Promotes Lung Cancer Metastasis by Inhibiting the Degradation of Bach1. *Cell*. 2019; 178: 316–329.e18. <https://doi.org/10.1016/j.cell.2019.06.003>.
- [58] Reichard JF, Motz GT, Puga A. Heme oxygenase-1 induction by NRF2 requires inactivation of the transcriptional repressor BACH1. *Nucleic Acids Research*. 2007; 35: 7074–7086. <https://doi.org/10.1093/nar/gkm638>.
- [59] An Y, Zhang Q, Zhao J, Zheng N. Tangeretin regulates oxidative stress in cutaneous melanoma cells via the Nrf2 signaling pathway. *Archives of Dermatological Research*. 2025; 317: 550. <https://doi.org/10.1007/s00403-025-03958-w>.
- [60] O'Mealey GB, Plafker KS, Berry WL, Janknecht R, Chan JY, Plafker SM. A PGAM5-KEAP1-Nrf2 complex is required for stress-induced mitochondrial retrograde trafficking. *Journal of Cell Science*. 2017; 130: 3467–3480. <https://doi.org/10.1242/jcs.203216>.
- [61] Zhu H, Itoh K, Yamamoto M, Zweier JL, Li Y. Role of Nrf2 signaling in regulation of antioxidants and phase 2 enzymes in cardiac fibroblasts: protection against reactive oxygen and nitrogen species-induced cell injury. *FEBS Letters*. 2005; 579: 3029–3036. <https://doi.org/10.1016/j.febslet.2005.04.058>.
- [62] Gęgotek A, Skrzydlewska E. The role of transcription factor Nrf2 in skin cells metabolism. *Archives of Dermatological Research*. 2015; 307: 385–396. <https://doi.org/10.1007/s00403-015-1554-2>.
- [63] Mammanna S, Cavalli E, Gugliandolo A, Silvestro S, Pollastro F, Bramanti P, *et al.* Could the Combination of Two Non-Psychotropic Cannabinoids Counteract Neuroinflammation? Effectiveness of Cannabidiol Associated with Cannabigerol. *Medicina (Kaunas, Lithuania)*. 2019; 55: 747. <https://doi.org/10.3390/medicina55110747>.
- [64] Chairprasongsuk A, Panich U. Role of Phytochemicals in Skin Photoprotection via Regulation of Nrf2. *Frontiers in Pharmacology*. 2022; 13: 823881. <https://doi.org/10.3389/fphar.2022.823881>.
- [65] Hseu YC, Vudhya Gowrisankar Y, Wang LW, Zhang YZ, Chen XZ, Huang PJ, *et al.* The in vitro and in vivo depigmenting activity of pterostilbene through induction of autophagy in melanocytes and inhibition of UVA-irradiated α-MSH in keratinocytes via Nrf2-mediated antioxidant pathways. *Redox Biology*. 2021; 44: 102007. <https://doi.org/10.1016/j.redox.2021.102007>.
- [66] Peng L, Lu Y, Zhong J, Ke Y, Li Y, Liang B, *et al.* Lycium barbarum polysaccharide promotes proliferation of human melanocytes via activating the Nrf2/p62 signaling pathway

- by inducing autophagy in vitro. *Journal of Food Biochemistry*. 2022; 46: e14301. <https://doi.org/10.1111/jfbc.14301>.
- [67] Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, *et al*. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Molecular Cell*. 2013; 51: 618–631. <https://doi.org/10.1016/j.molcel.2013.08.003>.
- [68] Wu X, Sun R, Wang H, Yang B, Wang F, Xu H, *et al*. Enhanced p62-NRF2 Feedback Loop due to Impaired Autophagic Flux Contributes to Arsenic-Induced Malignant Transformation of Human Keratinocytes. *Oxidative Medicine and Cellular Longevity*. 2019; 2019: 1038932. <https://doi.org/10.1155/2019/1038932>.
- [69] Pangilinan C, Klionsky DJ, Liang C. Emerging dimensions of autophagy in melanoma. *Autophagy*. 2024; 20: 1700–1711. <https://doi.org/10.1080/15548627.2024.2330261>.
- [70] Huang P, Yang Z, Wang H, Wang C, Luo M, Zhou R, *et al*. Imperatorin promotes melanin degradation in keratinocytes through facilitating autophagy via the PI3K/Akt signaling pathway. *Archives of Dermatological Research*. 2024; 317: 70. <https://doi.org/10.1007/s00403-024-03559-z>.
- [71] He H, Xiong L, Jian L, Li L, Wu Y, Qiao S. Role of mitochondria on UV-induced skin damage and molecular mechanisms of active chemical compounds targeting mitochondria. *Journal of Photochemistry and Photobiology. B, Biology*. 2022; 232: 112464. <https://doi.org/10.1016/j.jphotobiol.2022.112464>.
- [72] Zhou J, Ling J, Song J, Wang Y, Feng B, Ping F. Interleukin 10 protects primary melanocyte by activation of Stat-3 and PI3K/Akt/NF- κ B signaling pathways. *Cytokine*. 2016; 83: 275–281. <https://doi.org/10.1016/j.cyto.2016.05.013>.
- [73] Bagati A, Moparthy S, Fink EE, Bianchi-Smiraglia A, Yun DH, Kolesnikova M, *et al*. KLF9-dependent ROS regulate melanoma progression in stage-specific manner. *Oncogene*. 2019; 38: 3585–3597. <https://doi.org/10.1038/s41388-019-0689-6>.
- [74] Malakoutikhah Z, Mohajeri Z, Dana N, Haghjooy Javanmard S. The dual role of Nrf2 in melanoma: a systematic review. *BMC Molecular and Cell Biology*. 2023; 24: 5. <https://doi.org/10.1186/s12860-023-00466-5>.
- [75] Gao S, Guo K, Chen Y, Zhao J, Jing R, Wang L, *et al*. Keratinocyte Growth Factor 2 Ameliorates UVB-Induced Skin Damage via Activating the AhR/Nrf2 Signaling Pathway. *Frontiers in Pharmacology*. 2021; 12: 655281. <https://doi.org/10.3389/fphar.2021.655281>.
- [76] Chen H, Nguyen NH, Magtoto CM, Cobbold SA, Bidgood GM, Meza Guzman LG, *et al*. Design and characterization of a heterobifunctional degrader of KEAP1. *Redox Biology*. 2023; 59: 102552. <https://doi.org/10.1016/j.redox.2022.102552>.
- [77] Talebi M, Sadoughi MM, Ayatollahi SA, Ainy E, Kiani R, Zali A, *et al*. Therapeutic potentials of cannabidiol: Focus on the Nrf2 signaling pathway. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2023; 168: 115805. <https://doi.org/10.1016/j.biopha.2023.115805>.
- [78] Ioannidis M, Tjepkema J, Uitbeijerse MRP, van den Bogaart G. Immunomodulatory effects of 4-hydroxynonenal. *Redox Biology*. 2025; 85: 103719. <https://doi.org/10.1016/j.redox.2025.103719>.
- [79] Gugliandolo A, Pollastro F, Grassi G, Bramanti P, Mazzon E. In Vitro Model of Neuroinflammation: Efficacy of Cannabigerol, a Non-Psychoactive Cannabinoid. *International Journal of Molecular Sciences*. 2018; 19: 1992. <https://doi.org/10.3390/ijms19071992>.