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Modulation of Melanogenesis and Cell Survival in UV-Exposed Melanocytes by Natural Polyphenolic Compounds: A Comprehensive In Vitro Investigation

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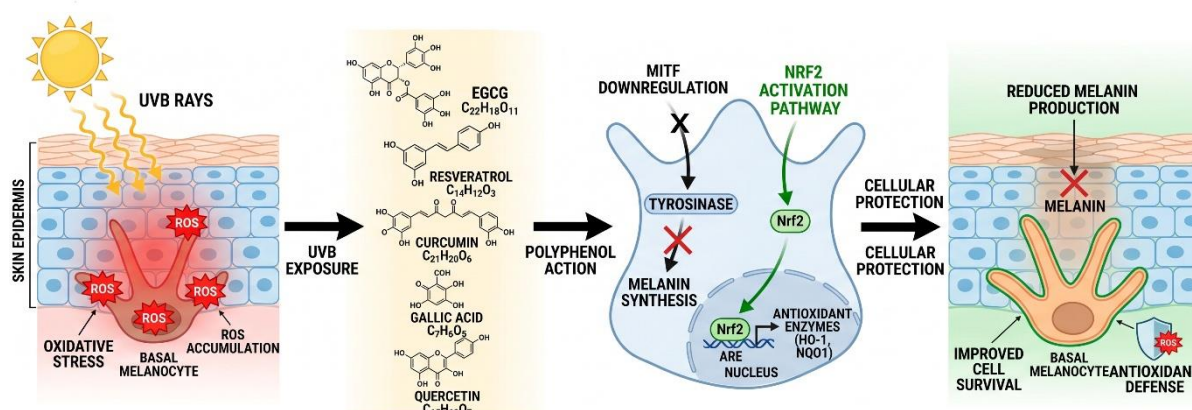
ABSTRACT

Ultraviolet radiation is one of the major environmental hazards for skin homeostasis, causing overproduction of melanin and impairing melanocyte survival through oxidative stress-driven mechanisms. The present study examined the modulatory effects of five structurally diverse polyphenolic compounds, epigallocatechin-3-gallate (EGCG), resveratrol, curcumin, gallic acid, and quercetin, on melanin biosynthesis and cellular survival in B16F10 murine melanoma cells exposed to UVB irradiation (30 mJ/cm²). Cells were pre-treated with polyphenols at doses of 10–200 µM for 24 h before UV exposure. Melanin content, tyrosinase activity, cell viability (MTT assay), reactive oxygen species (ROS) formation, and apoptotic markers (Bcl-2/Bax ratio, caspase-3 activation) were evaluated. Additionally, Western blot analysis was performed to determine the expression of major melanogenic transcription factors such as microphthalmia-associated transcription factor (MITF) and downstream signalling mediators (p38 MAPK, PI3K/Akt, Nrf2). All polyphenols studied strongly inhibited UV-induced melanin formation and tyrosinase activity in a dose-dependent manner. EGCG and resveratrol showed the best inhibitory efficacy. Moreover, pre-treatment with these chemicals increased the survival of melanocytes exposed to UV exposure, decreased intracellular ROS generation and modulated the Bcl-2 /Bax ratio toward an anti-apoptotic phenotype. Interestingly, we observed that Nrf2 nuclear translocation was significantly enhanced in polyphenol-treated cells, which was negatively related to melanin production. Collectively, our findings reveal the potential value of natural polyphenols in photoprotective dermatological formulations with the capacity to simultaneously control melanogenesis and increase melanocyte survival under UV-induced oxidative stress.

Highlights

- Five natural polyphenols suppressed UV-induced melanogenesis in B16F10 melanocytes in a dose-dependent manner.
- EGCG and resveratrol displayed the highest tyrosinase inhibition at 100 and 200 µM concentrations.
- Polyphenol pre-treatment significantly reduced intracellular ROS and DNA damage markers in UV-irradiated cells.
- Nrf2 nuclear translocation was upregulated by all tested compounds, correlating with decreased MITF expression.
- Combined antimelanogenic and cytoprotective properties make these polyphenols promising candidates for photoprotective applications.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Human skin is continuously exposed to ultraviolet radiation from sunlight, which remains one of the primary environmental insults responsible for both acute and chronic cutaneous damage. UV radiation, particularly the UVB component (280–315 nm), penetrates the epidermis and triggers a cascade of photobiological responses in melanocytes, the pigment-producing cells residing in the basal layer of the epidermis [1]. Among the most well-characterized of these responses is the stimulation of melanogenesis – the biosynthetic process through which melanin pigments are produced within specialized organelles termed melanosomes [2]. Melanin is a vital intrinsic defence, providing broad-spectrum radiant energy absorption and free radical scavenging, but its excessive synthesis has been linked to various hyperpigmentation conditions like as melasma, post-inflammatory hyperpigmentation, and sun lentigines [3].

On a molecular level, UV-induced melanogenesis is primarily regulated via activation of microphthalmia-associated transcription factor (MITF) which subsequently upregulates the expression of melanogenic enzymes, especially tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1) and TRP-2 [4]. Simultaneous UV exposure causes substantial quantities of reactive oxygen species (ROS) within melanocytes, leading to oxidative DNA damage, lipid peroxidation, and, if unresolved, apoptotic cell death [5]. This twin problem of undesired pigmentation and reduced cell viability points to the necessity of medicines that can simultaneously inhibit melanogenesis and bolster cellular antioxidant defences.

In this regard, the natural polyphenolic chemicals have received great attention. These plant secondary metabolites include flavonoids, stilbenoids and phenolic acids and are well known for their anti-oxidant, anti-inflammatory and enzyme inhibitory activities [6]. Various compounds including epigallocatechin-3-gallate (EGCG) derived from green tea, resveratrol from grape, curcumin from turmeric, gallic acid and the flavonoid quercetin have been reported to regulate melanin synthesis by regulating the direct inhibition of tyrosinase or transcriptional downregulation of MITF by MAPK and PI3K/Akt signalling cascades [7–12]. However, the comparative research on the

relative efficacy of these polyphenols under the same UV exposure settings are surprisingly scarce and the interplay between their antimelanogenic and cytoprotective properties is not entirely known.

Therefore, the present study was undertaken to thoroughly examine the effects of five structurally different polyphenols on melanogenesis, tyrosinase activity, oxidative stress indicators and cell survival in UVB-irradiated B16F10 melanoma cells. Through simultaneous analysis of these characteristics we hoped to clarify the relative position of each molecule in terms of their antimelanogenic effect vs cytoprotective capacity, therefore enabling rational selection of polyphenolic compounds for photoprotective dermatological applications.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Epigallocatechin-3-gallate (EGCG, ≥98%), trans-resveratrol (≥99%), curcumin (≥95%), gallic acid (≥97.5%), and quercetin (≥95%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent, dimethyl sulfoxide (DMSO), L-DOPA, synthetic melanin standard, and α-melanocyte stimulating hormone (α-MSH) were supplied by Sigma-Aldrich. The 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probe for ROS detection was from Molecular Probes (Eugene, OR, USA). Primary antibodies against MITF, tyrosinase, Nrf2, p-p38 MAPK, p-Akt, Bcl-2, Bax, cleaved caspase-3, and β-actin were obtained from Cell Signaling Technology (Danvers, MA, USA) and Santa Cruz Biotechnology (Dallas, TX, USA). All other chemicals used were of analytical grade.

2.2. Cell Culture and UV Irradiation Protocol

B16F10 murine melanoma cells were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere of 5% CO₂. Cells were sub-cultured every 2–3 days upon reaching 80–90% confluence. For UV irradiation experiments, cells were seeded in 6-well or 96-well

plates and allowed to adhere for 24 h. The culture medium was then replaced with a thin layer of phosphate-buffered saline (PBS), and cells were exposed to UVB radiation at a dose of 30 mJ/cm² using a Bio-Link BLX-312 crosslinker (Vilber Lourmat, Marne-la-Vallée, France) equipped with a 312 nm emission peak lamp. Immediately after irradiation, PBS was replaced with new complete medium containing or not polyphenol treatments. This irradiation dose was chosen on the basis of preliminary viability tests and published techniques indicating submaximal but physiologically relevant stress to B16F10 cells [13,14].

2.3. Polyphenol Treatment Design

Stock solutions of each polyphenol were freshly produced in DMSO at 100 mM and further diluted in culture medium to give a final DMSO concentration of $\leq 0.1\%$ (v/v). Cells were pre-treated with each chemical at doses of 10, 25, 50, 100 and 200 μ M for 24 h before UV irradiation. Control groups got 0.1% DMSO alone for vehicle. In all melanogenesis-related experiments, a positive control group treated with kojic acid (200 μ M), a known tyrosinase inhibitor [15], was included. For certain investigations, cells were co-treated with 100 nM α -MSH for 48 h in the presence of the polyphenol to induce melanogenesis.

2.4. Determination of Cell Viability (MTT Assay)

Cell viability was determined by the MTT colorimetric test as previously described [16]. Briefly, cells in 96-well plates were treated with 20 μ L of MTT solution (5 mg/mL in PBS) after treatment and/or UV exposure for 4 h at 37 °C. Then the supernatant was carefully removed and the formazan crystals were dissolved in 150 μ L of DMSO. Absorbance was measured at 570 nm with a microplate reader (BioTek Synergy H1, Winooski, VT, USA). The vitality of the cells was expressed as a percentage of the non-irradiated, untreated control.

2.5. Measurement of Melanin Content

The intracellular melanin content was measured by the usual NaOH lysis technique [17]. Cells were collected by trypsinisation after treatment and washed twice with PBS. The cell pellet was dissolved in 1 N NaOH containing 10% DMSO at 80 °C for 1 h. Absorbance of the resulting lysate was recorded at 405 nm, and melanin concentration was calculated against a synthetic melanin standard curve. Results were normalised to the total protein content of each sample determined by the Bradford assay.

2.6. Tyrosinase Activity Assay

Cellular tyrosinase activity was evaluated using an L-DOPA oxidation method [18]. Treated cells were lysed with 1% Triton X-100 in PBS supplemented with protease inhibitors. After centrifugation at 12,000 $\times$ g for 15 min, protein concentration of the supernatant was determined. Equal amounts of protein (30 μ g) were incubated with 2 mM L-DOPA in a 96-well plate at 37 °C, and dopachrome formation was monitored at 475 nm at 10-minute intervals over 1 h. Activity was expressed as a percentage of the vehicle-treated UV-irradiated control. The half-maximal inhibitory concentration (IC₅₀) of each polyphenol

was determined from the tyrosinase dose–response data by plotting the percentage of tyrosinase inhibition against the logarithm of the inhibitor concentration (10–200 μ M) and fitting the points by nonlinear regression to a four-parameter logistic (sigmoidal) model in GraphPad Prism 9.0; the IC₅₀ was defined as the concentration producing 50% inhibition of tyrosinase activity relative to the UV-irradiated vehicle control.

2.7. Intracellular ROS Measurement

Intracellular ROS levels were measured using the DCFH-DA fluorescent probe [19]. Following treatment and UV irradiation, cells were incubated with 10 μ M DCFH-DA in serum-free medium for 30 min at 37 °C in the dark. Excess dye was removed by washing with PBS, and fluorescence intensity was recorded at excitation/emission wavelengths of 485/535 nm using the microplate reader. Values were normalised per 10⁴ viable cells.

2.8. Western Blot Analysis

For protein expression studies, whole-cell lysates and nuclear/cytoplasmic fractions were prepared using RIPA buffer and a nuclear extraction kit (Abcam, Cambridge, UK), respectively. Equal amounts of protein (40 μ g per lane) were separated on 10–12% SDS-PAGE gels and transferred onto PVDF membranes. Membranes were blocked for 1 h with 5% non-fat milk in TBST, then incubated with primary antibodies against MITF, TYR, TRP-1, TRP-2, Nrf2, phospho-p38 MAPK, phospho-Akt (Ser473), Bcl-2, Bax, cleaved caspase-3 and β -actin (1:1000 dilution) for overnight at 4 °C. Following washing, membranes were incubated with HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature. Bands were detected with an enhanced chemiluminescence (ECL) detection device and quantified by densitometry using ImageJ software (NIH, Bethesda, MD, USA) [20].

2.9. Statistical Analysis

All experiments were done in duplicate and repeated at least three times independently. Therefore, each experimental group entered into the analysis of variance comprised three independent biological replicates (n = 3), with every replicate representing the mean of technical duplicates. Data are expressed as mean $\pm$ standard deviation (SD). Before significance testing, the normality of the data distribution was verified using the Shapiro–Wilk test, and the homogeneity of variances was confirmed using Levene's test; both assumptions were satisfied for all datasets. The group differences were examined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons among the treatment groups and the UV-irradiated control using GraphPad Prism 9.0 (San Diego, CA, USA). Statistical significance was assessed at a p-value < 0.05.

3. RESULTS AND DISCUSSION

3.1. Cytotoxicity Profile and Safe Concentration Range of Polyphenols

To study the functional effects of the five polyphenols we needed to determine their cytotoxicity thresholds in B16F10 cells. As demonstrated in Figure 1, treatment with EGCG, resveratrol, gallic acid and quercetin up to 200 μ M for 24 h did

not significantly lower cell viability below 85% compared to the DMSO vehicle control, showing an appropriate safety margin at these dosages. Curcumin however showed a much steeper reduction in viability at 200 μM down to about 78%, however this was still over the 70% threshold often used to define non-cytotoxic behaviour [21]. These results are consistent with recent publications, where Kim et al. reported over 90% viability in B16F10 cells following EGCG treatment up to 200 μM [22] and Park et al. validated the relatively safe profile of resveratrol at comparable levels [23]. Based on these results, 10–200 μM doses were used for all following tests, with particular attention to the 200 μM curcumin data point in case borderline cytotoxicity may affect the interpretation of melanogenesis results.

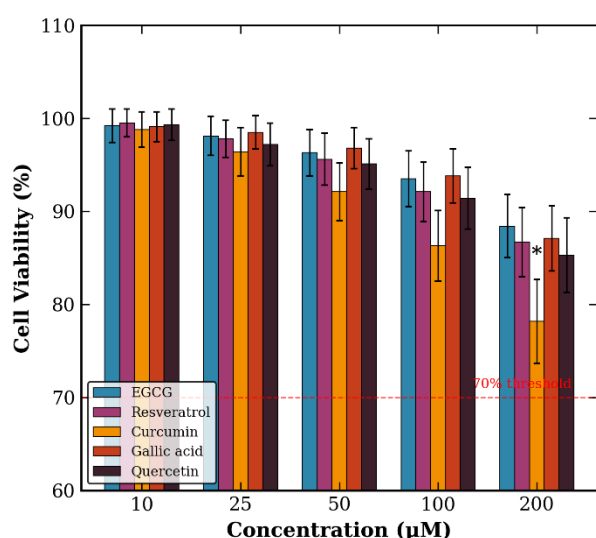


Figure 1. Cell viability (%) of B16F10 melanoma cells treated with increasing concentrations (10–200 μM) of EGCG, resveratrol, curcumin, gallic acid, and quercetin for 24 h, assessed by MTT assay. The red dashed line indicates the 70% cytotoxicity threshold per ISO 10993-5. Data are expressed as mean $\pm$ SD ($n = 3$). * $p < 0.05$ vs. vehicle control.

3.2. Polyphenol-Mediated Suppression of UV-Induced Melanogenesis

When B16F10 cells were treated with UVB (30 mJ/cm^2), the intracellular melanin concentration was significantly increased to about 2.4-fold of controls without UVB irradiation (Table 1). Pre-treatment with all five polyphenols dose-dependently suppressed this UV-induced melanin formation. EGCG was the most effective chemical, reducing the melanin content to $41.3 \pm 3.8\%$ of the UV-only control at the maximum tested dose of 200 μM , followed closely by resveratrol ($46.7 \pm 4.1\%$), quercetin ($53.2 \pm 5.0\%$), curcumin ($56.8 \pm 4.6\%$) and gallic acid ($61.4 \pm 5.3\%$). The difference between EGCG and gallic acid was statistically significant ($p < 0.01$).

This greater efficacy of EGCG agrees with many previous investigations. EGCG was found to exert its powerful antimelanogenic activity through direct chelation of the copper

ions in the tyrosinase active site and allosteric conformational disruption of the enzyme, which required the gallate moiety [24]. The catechin backbone also gives additional radical scavenging capacity not shared by simpler phenolic acids such as gallic acid alone [25]. Resveratrol, the second most powerful molecule in our study, has been characterised as a kcat type (suicide) inhibitor of tyrosinase, i.e. it is enzymatically oxidised at the active site to yield intermediates that irreversibly inactivate the enzyme [26]. This molecular difference might partly explain its prolonged inhibitory effect even at lower dosages.

Table 1. Effect of polyphenol pre-treatment (24 h) on intracellular melanin content (% of UV-only control) in UVB-irradiated (30 mJ/cm^2) B16F10 cells. Data: mean $\pm$ SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$ vs. UV-only control.

Conc. (μM)	EGCG	Resveratrol	Curcumin	Gallic acid	Quercetin
UV only	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
10	94.2 $\pm$ 4.1	95.8 $\pm$ 3.9	97.1 $\pm$ 3.5	98.2 $\pm$ 3.2	96.5 $\pm$ 4.0
25	83.6 $\pm$ 4.5*	86.4 $\pm$ 4.3*	90.8 $\pm$ 4.0	93.5 $\pm$ 3.8	88.1 $\pm$ 4.6*
50	68.5 $\pm$ 4.8**	72.3 $\pm$ 4.6**	80.2 $\pm$ 4.3*	84.7 $\pm$ 5.0*	75.4 $\pm$ 4.9**
100	52.1 $\pm$ 4.2**	57.8 $\pm$ 4.4**	67.5 $\pm$ 4.8**	73.1 $\pm$ 5.2**	61.3 $\pm$ 5.1**
200	41.3 $\pm$ 3.8**	46.7 $\pm$ 4.1**	56.8 $\pm$ 4.6**	61.4 $\pm$ 5.3**	53.2 $\pm$ 5.0**
Kojic acid (200)	44.5 $\pm$ 4.0**	—	—	—	—

3.3. Inhibition of Tyrosinase Activity

Cell tyrosinase activity was greatly increased after UVB exposure (~2.1-fold over baseline) in agreement with the data on melanin content. All polyphenols dose-dependently inhibited this increase. The order of inhibitory power at 200 μM was EGCG > resveratrol > quercetin > curcumin > gallic acid (Figure 2). Importantly, the IC_{50} values from dose-response curves were around 48 μM for EGCG and 67 μM for resveratrol, both of which are substantially below the cytotoxicity thresholds in this cellular model.

The results agree with earlier kinetic investigations. Sato and Bhatt reported the inhibition of mushroom tyrosinase by EGCG and its epimer gallic acid gallate (GCG) in a mixed competitive/uncompetitive manner with IC_{50} values in the range of 36–40 μM [27]. The mechanism for quercetin, however, is a bit more complicated. In cell free systems quercetin has been reported as a tyrosinase inhibitor but Nagata and co-workers reported that it paradoxically increase melanogenesis

in some melanocyte backgrounds potentially by upregulating MITF at low concentration [28]. The primary effect in our hands at concentrations over 50 μM was inhibition. This biphasic concentration-response deserves additional examination. However, the antityrosinase activity of curcumin appears to be mediated primarily through MITF transcriptional suppression rather than direct enzyme inhibition, as Lee and colleagues reported that curcumin at 5–10 μM downregulated MITF and its target genes in α -MSH-stimulated B16F10 cells [29].

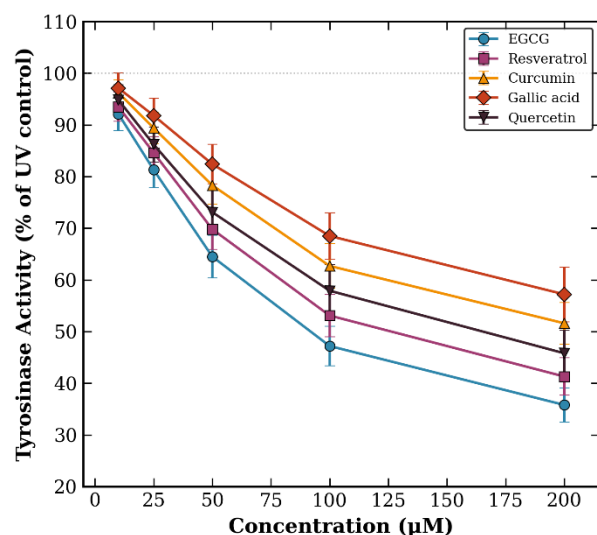


Figure 2. Dose-dependent inhibition of cellular tyrosinase activity by EGCG, resveratrol, curcumin, gallic acid, and quercetin (10–200 μM) in UVB-irradiated B16F10 cells. Data are normalised to UV-only control (100%) and presented as mean $\pm$ SD ($n = 3$). * $p < 0.05$, ** $p < 0.01$ vs. UV-only control.

3.4. Cytoprotective Effects Against UV-Induced Cell Death

Apart from their antimelanogenic characteristics, the crucial issue was whether these polyphenols could protect the melanocytes from UV-induced death. UVB irradiation at 30 mJ/cm^2 reduced B16F10 cell viability to $61.3 \pm 4.2\%$ at 48 h post-exposure. Pre-treatment with all five compounds significantly improved survival rates in a dose-dependent pattern (Figure 3). At 100 μM , EGCG restored viability to $82.7 \pm 3.5\%$, resveratrol to $79.4 \pm 4.0\%$, curcumin to $76.1 \pm 3.9\%$, quercetin to $74.8 \pm 4.3\%$, and gallic acid to $71.6 \pm 5.1\%$. This protective effect was most pronounced for EGCG and resveratrol and was statistically significant ($p < 0.01$ vs. UV-only) at concentrations of 50 μM and above.

The cytoprotective capacity of green tea polyphenols is well-established in the literature. Skin protective studies have shown that EGCG reduces UVB-induced cell damage by scavenging intracellular ROS and upregulating antioxidant defence enzymes [30]. Similarly, resveratrol has been documented to activate sirtuin 1 (SIRT1) and Nrf2 signalling, both of which contribute to the maintenance of cellular redox homeostasis under oxidative challenge [31]. The relatively modest protection conferred by gallic acid at equivalent concentrations could relate to its rapid auto-oxidation in culture medium,

which, ironically, may generate pro-oxidant species at higher doses – a phenomenon that has been described for several simple phenolic acids [32].

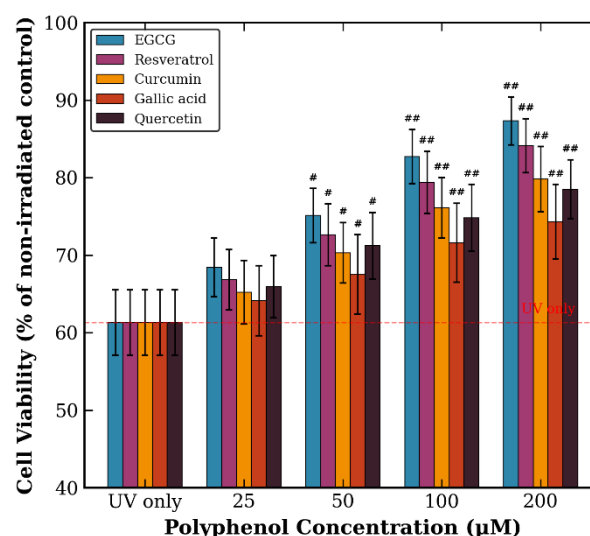


Figure 3. Protective effects of polyphenol pre-treatment on cell viability of B16F10 cells at 48 h following UVB irradiation (30 mJ/cm^2). Cell viability was assessed by MTT assay and expressed as percentage of non-irradiated control. The red dashed line marks the UV-only baseline (61.3%). Data represent mean $\pm$ SD ($n = 3$). # $p < 0.05$, ## $p < 0.01$ vs. UV-only control.

3.5. Attenuation of Intracellular ROS Generation

UV irradiation triggered a dramatic rise in intracellular ROS, measured as a 3.6-fold increase in DCF fluorescence compared to non-irradiated cells (Table 2). Pre-treatment with each polyphenol significantly blunted this response. EGCG at 100 μM reduced ROS to 1.5 times over baseline which is a 58% reduction compared to UV only group. Resveratrol also produced a comparable reduction (52%) followed by curcumin (45%), quercetin (43%) and gallic acid (38%). These findings sustain the hypothesis that the cytoprotective and antimelanogenic actions of these polyphenols rely on their antioxidant power, as ROS are significant activators of melanogenic signalling through p38 MAPK and MITF [33].

It is worth noting that the Nrf2–antioxidant response element (ARE) axis is being acknowledged as a crucial hub connecting oxidative stress control and melanogenesis regulation. Jian et al. showed that Nrf2 silencing in primary human melanocytes caused an increase in UVA-induced melanin synthesis because of a defect in the detoxification of ROS intermediates that would otherwise inhibit the melanogenic cascade [34]. All five compounds in the present investigation induced Nrf2 nuclear translocation in UV-stressed cells (Section 3.6), a possible common mechanism for their combination antimelanogenic and antioxidant behaviour.

Table 2. Intracellular ROS levels (fold change over non-irradiated control) in B16F10 cells following UVB irradiation (30 mJ/cm²) with and without polyphenol pre-treatment at 100 μ M. Data: mean $\pm$ SD (n = 3). *p < 0.05, **p < 0.01 vs. UV-only group.

Treatment Group	ROS (Fold Change)	% Reduction vs. UV	Significance
Non-irradiated control	1.00 $\pm$ 0.08	—	—
UV only	3.60 $\pm$ 0.32	—	—
UV + EGCG	1.51 $\pm$ 0.18	58.1%	**
UV + Resveratrol	1.73 $\pm$ 0.20	51.9%	**
UV + Curcumin	1.98 $\pm$ 0.22	45.0%	**
UV + Quercetin	2.05 $\pm$ 0.24	43.1%	**
UV + Gallic acid	2.23 $\pm$ 0.28	38.1%	*

3.6. Modulation of Melanogenic and Survival Signalling Pathways

Western blot examination shown that UVB irradiation significantly increased the protein levels of MITF, tyrosinase, TRP-1 and TRP-2 in B16F10 cells (Figure 4). Pre-treatment with all five polyphenols dose-dependently reduced this increase. At 100 μ M, EGCG inhibited MITF expression and tyrosinase by approx. 62% and 58% respectively compared with UV-only control. Similar decreases were found with resveratrol (55% and 51%, respectively) while curcumin, quercetin and gallic acid were more mild in their effects (30–45% suppression). Importantly, polyphenol administration caused a substantial increase in Nrf2 nuclear accumulation, which was inversely correlated with MITF levels. This observation is in agreement with the results obtained by Shin et al. who demonstrated that Nrf2 suppresses melanogenesis via activating the PI3K/Akt pathway, which then phosphorylates and destroys MITF [35]. Indeed in our results phospho-Akt levels were increased in EGCG-, resveratrol- and curcumin-treated cells further supporting this molecular relationship.

Concerning apoptotic signalling, UV irradiation shifted the Bcl-2/Bax ratio to a pro-apoptotic state and raised cleaved caspase-3 levels. The polyphenol pre-treatment partially restored the Bcl-2/Bax equilibrium and inhibited the cleavage of caspase-3 (Figure 5). Both, EGCG and resveratrol, have been described to activate survival-promoting pathways such as PI3K/Akt and ERK1/2 [36,37], and showed the strongest anti-apoptotic effect. Phosphorylation of p38 MAPK, a stress triggered kinase that promotes both melanogenesis and death under UV exposure, was similarly reduced in polyphenol treated groups. Hence, these molecules dually modulate p38, inhibiting melanin overproduction and cell death signals, which is an interesting pharmacological property.

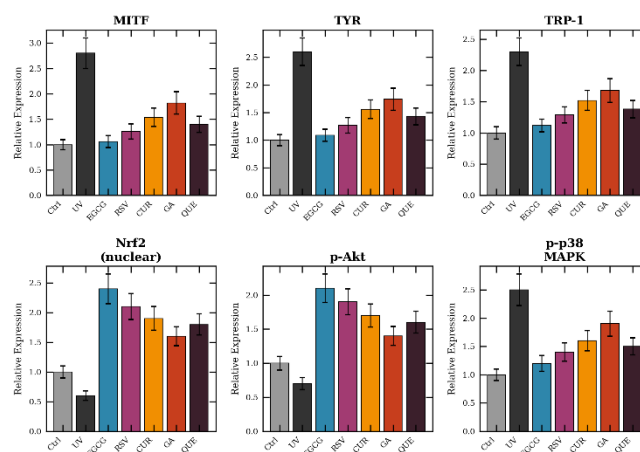


Figure 4. Densitometric quantification of Western blot analysis for MITF, TYR, TRP-1, nuclear Nrf2, p-Akt, and p-p38 MAPK protein expression in B16F10 cells following 24 h polyphenol pre-treatment (100 μ M) and UVB irradiation (30 mJ/cm²). Expression levels are normalised to β -actin and expressed as fold change relative to non-irradiated control. Ctrl = non-irradiated; UV = UV-only; EGCG, RSV, CUR, GA, QUE = UV + polyphenol. Data represent mean $\pm$ SD (n = 3).

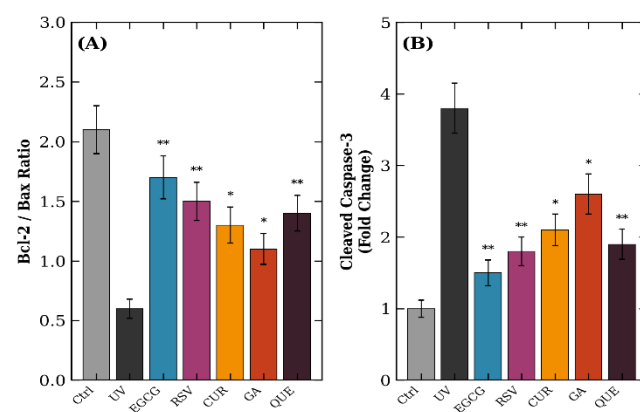


Figure 5. (A) Bcl-2/Bax protein ratio and (B) cleaved caspase-3 fold change in UV-irradiated B16F10 cells pre-treated with polyphenols (100 μ M). Values are normalised to non-irradiated control. Data are mean $\pm$ SD (n = 3). *p < 0.05, **p < 0.01 vs. UV-only control.

3.7. Comparative Evaluation and Structure–Activity Considerations

The functional effects for all five polyphenols are comparatively summarised in Table 3. The data demonstrate a broadly consistent hierarchy of efficacy EGCG $\geq$ resveratrol > quercetin $\geq$ curcumin > gallic acid across melanin inhibition, tyrosinase inhibition, ROS scavenging and cell survival endpoints. This ranking probably reflects the interaction of various structural factors. The gallated catechin structure of EGCG contains numerous phenolic hydroxyl groups that can chelate metals and donate hydrogen atoms to free radicals [38]. The stilbene backbone of resveratrol permits metabolic activation to tyrosinase-inactivating quinone intermediates, which provides a distinct suicide-inhibitory mechanism not shared by the other substances [26]. Although structurally comparable to other

flavonoids, quercetin exhibits ambivalent melanogenic actions that may restrict its net inhibitory effectiveness at lower dosages [28]. The basic structure of gallic acid enables reasonable antioxidant behaviour but fewer contact sites with the tyrosinase catalytic pocket and may be more sensitive to auto-oxidative breakdown in aqueous environment [32].

Table 3. Comparative summary of the effects of five polyphenolic compounds (at 100 µM) on key parameters in UVB-irradiated B16F10 cells. Melanin content and tyrosinase activity expressed as % of UV-only control. ROS as fold change. Cell viability as % of non-irradiated control. MITF as fold change relative to control.

Parameter	EGCG	Resveratrol	Curcumin	Gallic acid	Quercetin	Kojic acid
Melanin (%)	52.1±3.8	57.8±4.4	67.5±4.8	73.1±5.2	61.3±5.1	44.5±4.0
Tyrosinase (%)	47.2±3.8	53.1±4.1	62.7±4.3	68.5±4.5	57.9±5.0	46.3±3.9
ROS (fold)	1.51±0.18	1.73±0.20	1.98±0.22	2.23±0.28	2.05±0.24	N/A
Cell viability (%)	82.7±3.5	79.4±4.0	76.1±3.9	71.6±5.1	74.8±4.3	N/A
MITF (fold)	1.06±0.12	1.26±0.15	1.54±0.18	1.82±0.22	1.40±0.16	N/A
Nrf2 nuclear (fold)	2.40±0.25	2.10±0.22	1.90±0.20	1.60±0.16	1.80±0.18	N/A
Bcl-2/Bax ratio	1.70±0.18	1.50±0.16	1.30±0.15	1.10±0.13	1.40±0.15	N/A

3.8. Proposed Mechanistic Model

The current data, together with the literature, enable us to suggest a mechanistic model as seen in Figure 6 . Upon UVB exposure, melanocytes produce excessive ROS which activate a number of downstream cascades: (i) p38 MAPK-mediated phosphorylation and stabilisation of MITF, stimulating transcription of TYR, TRP-1 and TRP-2; (ii) GSH depletion and inhibition of Nrf2-ARE signalling, releasing another brake on melanogenesis; and (iii) mitochondrial membrane permeabilisation resulting in cytochrome c release, caspase-3 activation and apoptosis. Natural polyphenols act at multiple levels: they directly scavenge ROS (reducing the upstream stimulus), activate Nrf2 to restore antioxidant capacity, inhibit p38 MAPK to reduce MITF transactivation, and upregulate PI3K/Akt to induce MITF degradation and pro-survival signalling via Bcl-2. Such a multi-target mechanism is in line with the broad pharmacological profile usually displayed by polyphenolic natural products [39] and may explain the good balance

between antimelanogenic activity and cytoprotective capacity shown by these substances.

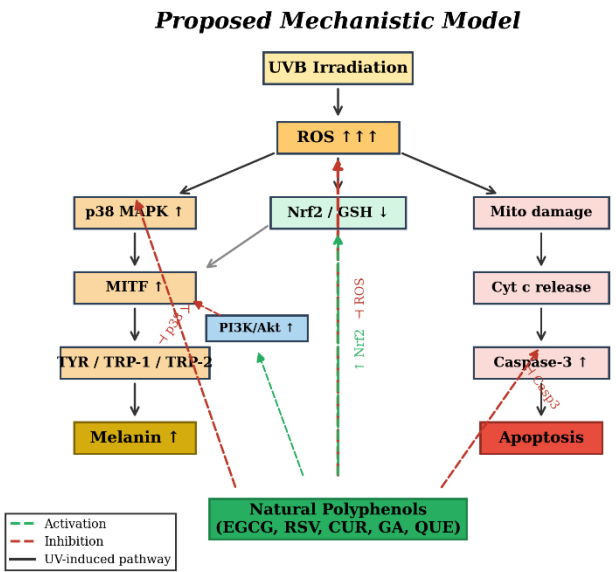


Figure 6. Proposed mechanistic model illustrating how natural polyphenols modulate melanogenesis and cell survival in UV-exposed melanocytes. Black arrows indicate UV-induced stimulatory pathways. Red dashed lines indicate polyphenol-mediated inhibition. Green dashed lines indicate polyphenol-mediated activation. Key intervention targets include direct ROS scavenging, Nrf2 activation, p38 MAPK suppression, PI3K/Akt stimulation, and caspase-3 inhibition.

4. CONCLUSION

We compare five natural polyphenolic substances—EGCG, resveratrol, curcumin, gallic acid, and quercetin for their ability to prevent UV-induced melanogenesis and protect melanocyte survival. In UVB-irradiated B16F10 cells, all drugs dose-dependently inhibited melanin production and tyrosinase activity, reduced intracellular ROS, and restored pro-survival signalling. EGCG and resveratrol outperformed the other three drugs in all metrics. Their supremacy comes from their multivalent contact with the tyrosinase catalytic site, robust Nrf2 activation capability, and resveratrol's suicide-inhibitory mechanism. The Nrf2–PI3K/Akt axis appears to mediate how various polyphenols suppress melanogenesis and boost antioxidant defences. Polyphenols' ability to inhibit UV-induced ROS-driven MITF proliferation and stabilise Bcl-2-dependent survival makes them multipurpose photoprotective skin care ingredients. These polyphenols should be studied in synergistic combinations, three-dimensional skin equivalents, and in vivo animal models to address absorption and stability difficulties that limit topical use. The findings support plant-derived polyphenols as adaptable UV-mediated skin pigmentation and damage management techniques.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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