

Antitumor Effects of Quercetin and Luteolin in MeWo Melanoma Cells: Potential Association with GPER Signaling

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Abstract

Introduction with high mortality rate, cutaneous melanoma (CM) is considered the most dangerous form of skin cancer, with steadily increasing incidence worldwide. Hormone-related therapies, especially those involving estrogen (E2), have emerged as promising strategies for treating CM. The phytoestrogens quercetin and luteolin have gained attention due to their antitumor effects across various cancers, including melanoma. However, the precise mechanisms by which they exert their effects and their association with G-protein-coupled estrogen receptor (GPER) signaling in melanoma remain unclear.

Methods we investigated the inhibitory effects of quercetin and luteolin in the MeWo melanoma cell line and their modulation of GPER-associated signaling. The effects of the flavonoids on cell viability, apoptosis and cell cycle were examined by MTT assay and flow cytometry. Cell migration was evaluated by transwell assay. GPER expression was verified by immunofluorescence staining, and the impact of the flavonoids on key signaling proteins was confirmed by Western blot. **Results** we showed that quercetin and luteolin exerted anti-proliferative and anti-migratory effects in melanoma cells. The two flavonoids also induced apoptosis and arrested the cell cycle at S and G2/M phases. Immunofluorescence and immunoblotting confirmed the presence of GPER in this cell line, and treatment with quercetin and luteolin, particularly at 100 μ M, upregulated its expression. Quercetin and luteolin, as well as the GPER agonist G-1, enhanced the expression of P-ERK and c-Myc but reduced the expression of P-Akt. These responses were attenuated or reversed by the specific GPER antagonist G15, supporting a potential association between these effects and GPER signaling.

Conclusion quercetin and luteolin have antitumor effects on the MeWo melanoma cell line in association with GPER signaling, suggesting that GPER-associated pathways are associated with their anti-melanoma effects and warrant further investigation.

1. Introduction

Cutaneous melanoma (CM) is considered the deadliest form of skin cancer, and its incidence has risen steadily worldwide in recent decades [1]. Globally, the age-standardized incidence and mortality rates of melanoma are 3.4 and 0.56 per 100,000 population, respectively. In the absence of substantial changes in current trends, the global burden is expected to increase significantly, reaching approximately 510,000 new cases and 96,000 deaths by 2040 [2].

CM originates from epidermal melanocytes and progresses through two main developmental stages: the radial growth phase, characterized by horizontal growth within the epidermis (*in situ*), and the vertical growth phase, during which tumor cells invade deeper layers of the dermis, increasing the likelihood of metastasis [3]. Several risk factors are associated with CM, including exposure to ultraviolet radiation, aging, immunosuppression, the number of nevi, genetic mutations, and family history [4]. Furthermore, sex may influence the risk of developing melanoma. Studies have indicated that melanoma incidence is higher in women at younger ages; however, this pattern shifts with increasing age, with men becoming more predominantly affected. Overall, both the incidence and mortality rates of melanoma are

consistently higher in men than in women [5]. Although the reasons underlying this sex disparity are not fully understood, these differences raise the possibility of a link between sex hormones, particularly 17 β -estradiol (E2), and melanoma development [6].

Surgery is the primary treatment for patients with primary CM lesions and those with resectable early-stage melanoma. Several additional treatment options are available, including chemotherapy, immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1), immunotherapy for advanced disease, BRAF/MEK-targeted therapy for unresectable or metastatic melanoma, and radiotherapy [7, 8]. However, these treatments have several limitations, including disease recurrence, toxicity, lack of response, and high medical costs [9]. Consequently, considerable interest has emerged in identifying novel and effective therapeutic approaches.

The relationship between CM and estrogen hormones has attracted increasing attention in recent years. Although CM is generally considered a non-hormone-dependent malignancy, growing evidence suggests a potential association between estrogen levels and disease outcomes [10]. Estradiol (E2) is a key hormone involved in regulating numerous physiological processes, including reproduction, immune function, and skin homeostasis [11]. The physiological effects of E2 are mediated primarily through estrogen receptors (ERs), ER α and ER β , which belong to the nuclear receptor superfamily [12]. Previous studies have reported that E2 exerts antiproliferative effects in CM, mediated in part by ER β , which is the predominant estrogen receptor expressed in this malignancy [10]. However, evidence regarding the influence of estrogen on CM progression remains controversial [13].

In addition to its genomic effects, E2 mediates rapid, nongenomic responses through binding to the G protein-coupled estrogen receptor (GPER). Identified in 1996, GPER is expressed in various tissues, where it mediates physiological and pathological processes independently of classical nuclear ER signaling, including metabolic regulation, immune responses, skin pigmentation, heart rate regulation, and cancer development [14, 15]. E2-induced GPER activation triggers several rapid downstream signaling events, including cyclic adenosine monophosphate (cAMP) production, Ca²⁺ mobilization, and activation of the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathways, primarily through transactivation of the epidermal growth factor receptor (EGFR) [16]. The role of GPER in cancer development remains under investigation, as it has been reported to exert either pro-tumorigenic or antitumorigenic effects, depending on the tumor type [17]. In melanoma, inhibitory effects of GPER activation have been documented in mouse K1735-M2 melanoma cells [18] and in uveal melanoma [19].

In addition to E2, several other natural and synthetic ligands have been reported to interact with GPER, including steroidal compounds such as estrone (E1), estriol (E3), 2-methoxyestradiol (2-MOE2), 2-hydroxyestradiol (2-OHE2), and aldosterone. These compounds may act as agonists or antagonists, depending on the ligand and experimental context [20]. However, investigating GPER-specific effects has been challenging because E2 and other steroidal ligands can also interact with classical ER α and ER β . The development of synthetic ligands with selectivity for GPER over classical ERs was therefore

important for distinguishing GPER-mediated effects from those mediated by ER α and ER β [21]. The first selective GPER agonist, G-1, was synthesized in 2006, followed by the selective antagonists G15 and G36 in 2009 and 2011, respectively [16].

Phytoestrogens are naturally occurring, plant-derived compounds found in a wide variety of foods and have demonstrated potential anticancer properties. They can be broadly classified into flavonoids and non-flavonoids [22]. Because of their structural similarities to E2, phytoestrogens can exert estrogen-like effects through interactions with classical ERs and GPER [23]. Daidzein, apigenin, genistein, quercetin, and resveratrol are phytoestrogenic compounds reported to interact with GPER and may act as agonists or antagonists, depending on the experimental context [20].

Quercetin (3,3',4',5,7-pentahydroxyflavone) is an important dietary flavonoid characterized by five hydroxyl groups on a C6–C3–C6 backbone, including a hydroxyl group at position 3 on the pyran ring [24]. Its antitumor effects have been extensively investigated in several cancer types, with proposed mechanisms including the induction of apoptosis and cell-cycle arrest, modulation of MAPK and Akt signaling pathways, and inhibition of cell proliferation [25]. In CM, the inhibitory effects of quercetin have also been documented. Soll et al. [26] demonstrated that quercetin inhibited proliferation and induced apoptosis in murine B16 melanoma cells in vitro. In addition, quercetin induced cytotoxicity, apoptosis, and oxidative stress in SK-MEL-28 cutaneous melanoma cells [23].

Luteolin (3',4',5,7-tetrahydroxyflavone) is another important flavonoid found primarily in vegetables and fruits. Its chemical structure is characterized by four hydroxyl groups and a double bond between C2 and C3. Luteolin has been investigated as an anticancer agent because of its reported ability to induce apoptosis, modulate angiogenesis, promote autophagy, and inhibit cell invasion [27]. Studies have demonstrated that luteolin inhibits tumor progression in murine B16F10 melanoma cells [28] and induces apoptosis and cell-cycle arrest in human choroidal melanoma cells [29]. Molecular docking studies have also suggested that luteolin can interact with ER α , ER β , and GPER [30].

In a previous study, we demonstrated that quercetin and luteolin suppress melanoma cell growth and migration in the A375 cutaneous melanoma cell line, which harbors a *BRAF* mutation, through GPER activation [31]. To determine whether these effects extend to other melanoma cellular contexts, the present study investigated the effects of these phytoestrogens in the MeWo cutaneous melanoma cell line, which exhibits distinct genetic and phenotypic characteristics and harbors wild-type *BRAF* [32]. Specifically, we investigated the potential GPER agonistic properties of quercetin and luteolin by examining their ability to activate GPER in MeWo cells and assessing their effects on the key Ras/Raf/ERK and PI3K/Akt signaling pathways. Our findings demonstrated that quercetin and luteolin increased phosphorylated ERK (p-ERK) and c-Myc expression while decreasing phosphorylated Akt (p-Akt) levels. These effects were partially attenuated by the selective GPER antagonist G15, supporting the involvement of GPER in the cellular responses to these flavonoids and suggesting their potential as compounds for further investigation in melanoma therapy.

2. Materials and Methods

2.1. Cell line and culture conditions

The MeWo melanoma cell line (cat no. 300285) was obtained from Cell Line Service (CLS GmbH, Eppellheim, Germany). MeWo cells were selected for this study based on their expression of the G protein-coupled estrogen receptor (GPER), the primary protein of interest [33], as well as their distinct genetic characteristics as a metastatic model harboring a wild-type BRAF mutation. Cells were cultured in minimum essential medium (MEM, EuroClone, Milan, Italy) supplemented with 10% fetal bovine serum (FBS), 1% antibiotic/antimycotic solution (100×; Gibco, Waltham, MA, USA), 1% glutaMAX (100×; Gibco, Waltham, MA USA), and 1% MEM non-essential amino acids (100×; EuroClone, Milan, Italy). The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂. Routine mycoplasma testing was conducted every 2–4 weeks to ensure the integrity of the cell cultures.

2.2. Chemicals and preparation

High-purity quercetin (> 98%) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA), while luteolin (> 98%) was sourced from Biosynth (Irvine, CA, USA). The GPER-selective agonist G1 [34] and the antagonist G15 [35] were purchased from Tocris Bioscience (Oxford, UK), and used according to protocols described in recent literature [36]. Stock solutions of 50 mM were prepared by dissolving each compound in dimethyl sulfoxide (DMSO; Fisher Scientific, Waltham, MA, USA) and stored at – 20°C. Prior to each experiment, working solutions were freshly prepared by diluting the stock solutions in culture medium, with the final DMSO concentration maintained at 0.2% for all treatments.

2.3. Cell viability assay

Cell viability and proliferation following treatment with different agents were assessed using a modified MTT assay [37]. Briefly, MeWo cells were seeded at 1×10^4 cells per well in 96-well plates with 100 μ L culture medium and incubated for 24 hrs. to allow cell attachment. The cells were subsequently treated with increasing concentrations (1, 3, 10, 30, and 100 μ M) of quercetin or luteolin, or 1 μ M of G1 (as a positive control). Vehicle controls received 0.2% DMSO. Following 24 hrs. of treatment, the medium was aspirated and the cells were washed with phosphate buffered saline (PBS) to protect against residual flavonoids. Fresh medium containing 10 μ L MTT reagent (Promega, Madison, WI, USA) was then added and the cells were incubated for an additional 3 hrs. at 37°C. The resulting formazan crystals were solubilized overnight in 100 μ L of Promega's solubilization solution in the dark. In parallel, cells pretreated with 3 μ M G15 for 24 hrs. were subsequently exposed to selected concentrations of flavonoids or G1 for 48 hrs. before performing the MTT assay. Absorbance was measured at 570 nm using a Biotek Cytation 5 microplate reader (Agilent, Santa Clara, CA, USA). Measurements were also taken at 48 and 72 hrs.

Cell viability percentages were calculated relative to the control group using the following formula:

$$\text{Cell viability(\%)} = \frac{(\text{Optical density of treated sample})}{(\text{Optical density of control})} \times 100$$

IC₅₀ values were derived using GraphPad Prism 8 software.

2.4. Cell morphology

For morphological evaluation, MeWo cells were seeded at 2.5×10^5 cells per well in 6-well plates and allowed to adhere for 24 hrs. Cells were then incubated with quercetin or luteolin (1-100 μM), G1 (1 μM), or vehicle (0.2% DMSO) for 48 hrs. Morphological changes were documented using an EVOS XL Core inverted microscope (Invitrogen, Waltham, MA, USA).

2.5. Flow cytometry

2.5.1. Apoptosis assay

MeWo cells were seeded at a density of 2.5×10^5 cells per well in 6-well plates and incubated for 48 hrs. with quercetin or luteolin (1-100 μM), the GPER agonist G1 (1 μM), or 0.2% DMSO as a vehicle control. Cells were then trypsinized, washed with PBS, and stained using the Annexin V-FITC apoptosis detection kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Briefly, cells were incubated with Annexin V-FITC and propidium iodide (PI) in the binding buffer for 15 min. at room temperature in the dark. To investigate the involvement of GPER, MeWo cells were pretreated with the GPER antagonist G15 (3 μM) for 24 hrs. prior to exposure to the most effective concentrations of quercetin, luteolin (10, 30, and 100 μM), or G1 (1 μM) for 48 hrs. Cells were then processed and stained similarly. Samples were analyzed using a BD FACS Canto II flow cytometer, recording 10,000 events per sample. Apoptotic and necrotic populations were quantified using FlowJo software version 10, categorizing cells as viable (Annexin V-/PI-), early apoptotic (Annexin V+/PI-), late apoptotic (Annexin V+/PI+), or necrotic (Annexin V-/PI+) [38].

2.5.2. Cell cycle assay

Cells were treated with quercetin or luteolin (1-100 μM), the GPER agonist G1 (1 μM), or 0.2% DMSO as a vehicle control, harvested, and fixed overnight in 70% ethanol at -20°C . The fixed cells were then washed and incubated with PI/RNase staining solution (Tali Cell Cycle Kit, Invitrogen, Waltham, MA, USA) for 40 min. at 37°C . To investigate the involvement of GPER, cells were pretreated with the GPER antagonist G15 for 24 hrs., followed by treatment with 10, 30, and 100 μM of quercetin or luteolin, or 1 μM of the GPER agonist G-1 for 48 hrs. Subsequently, the cells were analyzed by flow cytometry to measure DNA content and determine their distribution across the G₁, S, and G₂/M phases of the cell cycle. Data analysis and histogram generation were performed using FlowJo software [31].

2.6. Transwell migration assay

Cell migration was evaluated using transwell inserts with 8 µm pore membranes (SPL Life Sciences, Pocheon-si, Korea). MeWo cells (5×10^5 per well) were cultured in 6-well plates and treated with 10, 30, or 100 µM quercetin or luteolin, 1 µM G1, or 0.2% DMSO for 48 hrs. For GPER involvement experiment, cells were pretreated with 3 µM G15 for 24 hrs. before incubation with quercetin or luteolin. Subsequently, 1×10^5 cells in serum-free MEM were seeded in the upper chambers, while the lower chambers contained MEM supplemented with 10% FBS as chemoattractant. After 48 hrs. at 37°C, non-migrated cells were removed from the upper membrane surface. Migrated cells adhering to the underside were fixed with 4% paraformaldehyde and stained with 0.3% crystal violet. Migration was quantified by counting stained cells in five random fields per insert under a 10× objective using an EVOS XL Core microscope [38].

2.7. Immunofluorescence staining

GPER expression was assessed by immunofluorescence as previously described [31]. Briefly, MeWo cells were seeded on sterile coverslips in 12-well plates at 1×10^5 cells per well and treated with 10, 30, or 100 µM quercetin or luteolin, 1 µM G1 or vehicle control (0.2% DMSO) for 48 hrs. Following PBS washes, cells were fixed with 4% paraformaldehyde for 15 min. and blocked for 1 h in a blocking solution. Cells were then incubated overnight at 4°C with anti-GPER rabbit primary antibody (1:1500; ELK Biotechnology, Denver, CO, USA). After washing, Alexa Fluor 488-conjugated goat anti-rabbit IgG secondary antibody (1:500; Invitrogen, Waltham, MA, USA) was applied for 2 hrs. at room temperature. Nuclear counterstaining was performed using DAPI (1:1500 dilution). Coverslips were mounted with anti-fade medium (Abcam, Cambridge, UK) and images were captured on a Carl Zeiss fluorescence microscope (Carl Zeiss, Jena, Germany). Mean fluorescence intensity (MFI) was quantified using Cell Profiler software, version 3 [39].

2.8. Western blot analysis

To evaluate the effects of flavonoids on GPER expression and downstream signaling (Ras/Raf/Erk and PI3K/AKT pathways), MeWo cells were grown in T25 flasks to 85–90% confluence. Cells were treated with 10, 30, or 100 µM quercetin or luteolin, 1 µM G1, or 0.2% DMSO for 24 hrs. For GPER inhibition experiment, cells were pretreated with 3 µM G15 for 24 hrs. before incubation with quercetin or luteolin. Total protein was extracted using RIPA buffer supplemented with phosphatase inhibitors. Protein concentration was quantified using a bicinchoninic acid assay (SMART BCA, INtRON Biotechnology, Seoul, Korea). Equal amounts (20 µg) of protein were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked overnight at room temperature with 3% BSA in tris-buffered saline (TBS), then incubated overnight at 4°C with primary antibodies (Table 1). After washing, membranes were incubated for 1 h with HRP-conjugated goat anti-rabbit or anti-mouse secondary antibodies (Abcam, Cambridge, UK; 1:1000). Protein bands were visualized using 4-chloro-1-naphthol substrate and imaged on a ChemiDoc™ XRS+ system (Bio-Rad, Hercules, CA, USA). Densitometric analysis was conducted using Image Lab 6 software (Bio-Rad) [40].

Table 1
Primary antibodies used in Western blots.

Antibodies	Host species	Cat. No.	Supplier	Dilution (WB)	Band size (kDa)
GP-ER	Rabbit	ES11471	ELK biotechnology	1:500	41
ERK 1/2	Rabbit	EA331	ELK biotechnology	1:500	42–44
P-ERK	Mouse	Sc-136521	Santa Cruz	1:500	42–44
Akt 1/2/3	Mouse	Sc-56878	Santa Cruz	1:500	62
P-Akt	Rabbit	Ab38449	Abcam	1:500	56
FAK	Mouse	NBP2-45923	Novus	1:500	119
P-FAK	Rabbit	ES1492	ELK biotechnology	1:500	119
c-Myc	Rabbit	EA053	ELK biotechnology	1:1000	57–65
B-actin	Rabbit	GW0061R	GenoChem World	1:1000	42

2.9. Statistical analysis

Statistical analyses were performed using GraphPad Prism software, version 8. Data are presented as means $\pm$ SD from three experiments. Apoptosis and cell cycle data were statistically analyzed using two-way ANOVA followed by Dunnett's multiple comparisons test. Data obtained from the MTT assay, immunofluorescence, transwell migration, and Western blot analyses were evaluated using one-way ANOVA followed by Dunnett's multiple comparisons test. Statistical significance was considered when p was < 0.05 .

3. Results

3.1. Cell viability assay

Quercetin and luteolin reduced MeWo cell viability in a dose-dependent manner compared to the DMSO control. The maximal inhibitory effects were observed after 48 hrs. of treatment, with no additional reduction in viability upon extending the incubation to 72 hrs. Therefore, a 48 hrs. incubation period was selected for all subsequent experiments.

As shown in Fig. 1 (A and B), the IC_{50} values were $46.9 \mu\text{M} \pm$ for quercetin and $20.9 \mu\text{M} \pm$ for luteolin. Treatment with $1 \mu\text{M}$ of G-1 (a GPER agonist, used as a positive control) significantly reduced cell viability to $30.2 \pm 1.3\%$ compared to the control. Pretreatment with the GPER-specific antagonist G15 markedly increased MeWo cell viability and partially abolished the inhibitory effects of both quercetin and luteolin, as shown in Fig. 1 (C and D). Similarly, the effect of G-1 was also reversed by G15 pretreatment.

3.2. Morphological alterations in MeWo cells

Under control conditions (DMSO treatment), MeWo cells exhibited a typical fibroblast-like morphology characterized by an elongated shape and firm adherence to the culture surface (Fig. 2). In contrast, treatment with the GPER agonist G-1 resulted in marked morphological alterations, including cellular shrinkage, reduced cell density, and the appearance of non-adherent, floating cells.

Incubation with low concentrations of quercetin or luteolin did not induce significant morphological changes; cells largely retained their normal morphology. However, at higher concentrations (10, 30, and 100 μ M), both compounds induced a concentration-dependent decline in cell confluence, accompanied by visible signs of cellular stress and detachment.

3.3. Flowcytometry

3.3.1. Quantification of apoptosis and necrosis

The effects of quercetin and luteolin on apoptosis and necrosis in MeWo melanoma cells were evaluated using Annexin V-FITC/PI dual staining followed by flow cytometry analysis. In DMSO-treated control cells, the percentages of apoptotic (early and late) and necrotic cells were 3.1% and 2.5%, respectively (Fig. 3).

Both quercetin and luteolin did not induce a significant increase in necrosis, even at high concentrations (30 and 100 μ M). However, both flavonoids elicited a significant, dose-dependent increase in apoptosis. Specifically, quercetin treatment increased the apoptotic population to 10.4% and 23.3% at 30 μ M and 100 μ M, respectively (representative blots and quantification are found in Supplementary Fig.S1), whereas luteolin increased apoptosis to 9.3% and 16.5% at the same concentrations.

Treatment with the GPER agonist G-1 (1 μ M) also significantly elevated apoptosis to 11.9%, without a corresponding increase in necrosis. Notably, pretreatment with the selective GPER antagonist G15 partially attenuated the pro-apoptotic effects of G-1 and quercetin (100 μ M), but caused no significant effect on luteolin-induced apoptosis (Fig. 3A and **B**, left panel).

3.3.2. Cell cycle analysis

Flow cytometry analysis showed that quercetin treatment led to significant alterations in cell cycle progression in MeWo cells. Specifically, exposure to 30 and 100 μ M of quercetin resulted in a marked accumulation of cells in the S phase (25.8% and 25.0%, respectively), accompanied by an increase in the G2/M population to 31.3% and 34.7% (blots and quantification are found in supplementary Fig. S2), indicating cell cycle arrest at both phases. Luteolin induced a distinct pattern of cell cycle disruption. While 30 μ M treatment increased the proportion of cells in the S phase to 13.0%, a substantial rise to 46.9% was observed at 100 μ M. In contrast, G2/M phase accumulation peaked at 23.2% with 30 μ M but declined to 17.3% following 100 μ M treatment, suggesting that higher concentrations of luteolin

preferentially induced arrest in the S phase (Fig. S2). Similarly, G-1 (1 μ M), a selective GPER agonist, led to an increase in cells arrested in the S (24.1%) and G2/M (28.9%) phases. Notably, pretreatment with the GPER antagonist G15 partially abrogated the effects of quercetin and luteolin, as well as G-1, on cell cycle distribution. (Fig. 4A and B).

3.4. Migration of melanoma cells

G-1 (1 μ M) treatment significantly reduced the number of migrated cells to 47 cells/field, compared to 248 cells/field in DMSO-treated control cultures. Quercetin exerted a pronounced inhibitory effect on cell migration, with the number of migrated cells reduced to 57, 49, and 37 cells/field at 10, 30, and 100 μ M, respectively (Fig. 5A). Similarly, luteolin treatment caused a concentration-dependent reduction in migration, with 100, 43, and 10 cells/field were observed at 10, 30, and 100 μ M, respectively (Fig. 5B). Pretreatment with the selective GPER antagonist G15 partially attenuated the anti-migratory effects induced by quercetin, luteolin, and G-1.

3.5. Immunofluorescence staining of GPER

As illustrated in Fig. 6, GPER expression was evident in control cells treated with DMSO, exhibiting a mean fluorescence intensity (MFI) of 0.079. Quercetin treatment caused a modest change in GPER expression at lower concentrations. However, a significant elevation in MFI to 0.096 was detected following exposure to 100 μ M quercetin. In contrast, luteolin at 10 μ M significantly downregulated GPER expression (MFI = 0.069), while a concentration of 100 μ M led to a significant upregulation (MFI = 0.087). Exposure to the selective GPER agonist G-1 (1 μ M) produced a marked increase in GPER expression, as indicated by a significantly elevated MFI of 0.12.

3.6. Western blot

Quercetin treatment at 30 and 100 μ M significantly downregulated GPER protein expression, whereas G-1 (1 μ M) markedly increased its levels. Pretreatment with G15 (3 μ M) partially reversed the effects of both quercetin and G-1 on GPER expression.

Quercetin at 10 and 30 μ M led to a significant reduction in total Akt levels, although this effect was not observed at 100 μ M. Phosphorylated Akt (P-Akt) levels were significantly reduced across all quercetin concentrations. In contrast, G-1 increased total Akt expression while reducing P-Akt levels; these changes were abolished by G15.

Quercetin also significantly decreased total ERK levels at all tested concentrations, whereas G-1 induced a robust increase. Phosphorylated ERK (P-ERK) was significantly upregulated by 100 μ M quercetin and G-1, with both effects partially reversed by G15 pretreatment.

Quercetin also significantly downregulated focal adhesion kinase (FAK) at all concentrations, while exerting no notable effect on phosphorylated FAK (P-FAK). G-1 reduced both total FAK and P-FAK levels,

and these effects were reversed by G15. In contrast, c-Myc expression was upregulated following treatment with quercetin and G-1, and this effect was attenuated by G15 (Fig. 7A).

Luteolin treatment at 10 and 100 μ M significantly increased GPER expression, mimicking the effects of G-1; both responses were partially reversed by G15. Luteolin decreased total Akt levels, with more pronounced reduction in P-Akt observed at the same concentrations. Similar to the effects of quercetin, G-1 increased total Akt and decreased P-Akt, with these effects reversed by G15. Total ERK expression was significantly reduced by all concentrations of luteolin, while P-ERK was upregulated only at 100 μ M luteolin and in G-1-treated cells; both responses were partially suppressed by G15.

Luteolin at 10 and 100 μ M, as well as G-1, downregulated total FAK expression, whereas 30 μ M luteolin induced an increase. P-FAK levels were elevated only by 100 μ M luteolin and decreased by G-1; these changes were mitigated by G15. Finally, c-Myc expression was elevated in response to both luteolin and G-1, with G15 pretreatment significantly reducing this upregulation (Fig. 7B).

4. Discussion

Melanoma, an aggressive form of skin cancer, poses a major public health challenge because of its high mortality rate and increased global incidence [41]. Although advances in treatment have improved the survival rate in metastatic melanoma, issues such as toxicity, drug resistance, and rapid relapse continue to limit long-term success [42]. These challenges underscore the urgent need to identify novel therapeutic strategies. While melanoma has historically been classified as a non-hormone-related malignancy, growing evidence suggests that sex hormones, particularly estradiol (E2), may play a role in its development and progression. This has drawn attention to estrogen-related signaling pathways, especially those mediated by the G-protein coupled estrogen receptor, which has been increasingly studied in the context of various cancers, including CM [43].

In the present study, we examined the potential of two flavonoids, quercetin and luteolin, to exert anti-melanoma effects in the human MeWo CM cell line, a metastatic model harboring a wild-type BRAF mutation. We also investigated whether these effects are associated with GPER signaling. A previous study demonstrated that these flavonoids exert anti-melanoma effects via GPER activation in A375 cells, which represent primary melanoma with the BRAF V600E mutation [31]. To further explore this mechanism, we compared their effects to those of G-1, a selective GPER agonist known to induce physiological responses at nanomolar concentrations ($EC_{50} = 2$ nM) [44]. In addition, we tested whether their inhibitory activity could be reversed by G15, a selective GPER antagonist. We found that both quercetin and luteolin significantly reduced MeWo cell proliferation, with effects comparable to those of G-1. Notably, G15 markedly reversed the antiproliferative effects of these flavonoids, supporting the involvement of GPER-associated signaling in these effects. These findings suggest that quercetin and luteolin may exert their anti-melanoma effects partly through mechanisms involving GPER signaling, with potential relevance to the development of therapeutic strategies for metastatic melanoma, even in the context of BRAF wild-type status.

Our MTT assay results revealed that quercetin, luteolin, and G-1 significantly inhibited the proliferation of MeWo cells (Fig. 1). Quercetin demonstrated an IC_{50} of 42 μ M, while luteolin was more potent with an IC_{50} of 20.9 μ M. These findings are consistent with our earlier study using another cell line, A375, with IC_{50} values of 38.6 μ M and 19.5 μ M for quercetin and luteolin, respectively [31]. Furthermore, the consistent activity of luteolin across various cancer cell lines supports its potential as an anticancer agent [45].

Importantly, we found that blocking GPER with G15 partially attenuated the inhibitory effects of quercetin, luteolin, and G-1 on MeWo cell viability (Fig. 1). This indicates that GPER activation may contribute to the antiproliferative effects of these flavonoids. Additionally, higher concentrations of quercetin, luteolin, and G-1 significantly suppressed MeWo cell growth and altered cellular morphology consistent with apoptotic cell death (Fig. 2), providing additional support for the potential of these flavonoids as therapeutic agents for CM treatment.

The cell cycle is a cascade of events that governs cell growth and division. Apoptosis, on the other hand, is a natural mechanism that allows removal of damaged or abnormal cells [46]. Together, these processes are essential for maintaining normal cell growth and tissue homeostasis and are tightly controlled under normal circumstances. In cancer, these processes are frequently dysregulated, leading to uncontrolled proliferation and resistance to cell death [47]. By targeting these two critical processes of cancer, quercetin and luteolin may represent a promising strategy for cancer therapy that halts tumor progression and removes malignant cells [46]. This is because cell-cycle arrest not only prevents cells from completing division, thereby limiting tumor cell proliferation but may trigger apoptosis in some cases, thus eliminating tumor cells [48]. Therefore, molecules that induce cell-cycle arrest have been proposed as potential therapeutic agents for cancer [49].

At concentrations of 30 μ M and 100 μ M, both quercetin and luteolin significantly induced apoptosis without causing necrosis, consistent with findings from other melanoma models [50] as well as from other cancer models [51, 52]. Notably, treatment with the GPER antagonist G15 partially reduced the apoptosis induced by G-1, quercetin, and luteolin in MeWo cells (Fig. 3), supporting a potential contribution of GPER-associated signaling to these effects.

In MeWo cells, quercetin and luteolin caused cell-cycle arrest predominantly at the S and G_2 /M phases (Fig. 4), aligning with our earlier observations in A375 melanoma cells [31]. Supporting evidence from murine melanoma models demonstrated that quercetin similarly halted the cell cycle in B16-BL6 cells at the S and G_2 /M phases [53], whereas luteolin induced arrest at the G_2 /M phase in B16-F10 cells [54]. These findings collectively support the activity of these flavonoids across different melanoma models. The observation that GPER antagonist G15 partially attenuated the effects of both flavonoids and G-1 (Fig. 4) supports an association of GPER signaling and the cell-cycle effects of quercetin and luteolin in MeWo cells.

Cell migration is a crucial step in cancer metastasis, which accounts for a large proportion of cancer-related deaths [55]. It allows cells to spread to distant organs and to develop resistance to therapies

through multiple mechanisms, including epithelial-mesenchymal transition (EMT), activation of survival pathways, and evasion of apoptosis [56], in addition to the upregulation of drug-resistance genes [57]. Furthermore, metastatic cells can activate detoxifying enzymes, overexpress efflux pumps, and engage alternative signaling pathways like PI3K/AKT and MAPK, all of which contribute to therapy resistance [58, 59, 60, 61].

In this work, both flavonoids and G-1 significantly inhibited the migratory capacity of MeWo cells (Fig. 5). This inhibition was more pronounced in MeWo cells than in other melanoma models. For instance, quercetin has been shown to suppress migration in SK-MEL-28, G-361, and A375 cell lines [62], while luteolin reduced migration in SK-Mel2, SK-Mel28, WM3211, and A375 cells [31, 63]. Mechanistically, luteolin has been reported to inhibit matrix metalloproteinases MMP-2 and MMP-9 via the PI3K/AKT pathway in A375 cells, thereby reducing migration [64]. Similarly, quercetin suppressed migration by disrupting the HGF/c-Met signaling pathway [65]. These effects may lead to impaired extracellular matrix degradation and reduced cell motility, indicating that both compounds interfere with key molecular mechanisms required for melanoma metastasis. Importantly, the anti-migratory effects of both flavonoids and G-1 were partially reversed upon GPER blockade with G15, supporting a possible contribution of GPER-associated signaling to these effects in MeWo melanoma cells.

To further investigate the potential involvement of GPER, we performed immunostaining to evaluate GPER expression in MeWo cells. Consistent with the findings of Wróblewska et al. [33], who first demonstrated GPER protein expression in MeWo cells, we observed strong GPER expression (Fig. 6). Treatment with the selective GPER agonist G-1 significantly increased GPER expression. These results are consistent with our previous observations in A375 melanoma cells, as well as with findings by Almeida et al. [66] who reported elevated GPER levels in glioblastoma cells following 48-hour treatment with estradiol (E2) or G-1. Notably, quercetin and luteolin, particularly at 100 μ M, also increased GPER immunofluorescence, albeit to a lesser extent than G-1. This suggests that these flavonoids may exert GPER-mediated effects, potentially through partial agonistic activity in a manner similar to G-1 in this melanoma model.

Building on our observations, we investigated the mechanisms underlying the antiproliferative effects of quercetin and luteolin, focusing on their impact on GPER-associated pathways. Western blot analysis showed that G-1 (1 μ M) and luteolin (10 and 100 μ M) increased GPER protein expression, while quercetin (30 and 100 μ M) reduced it. These differences from the immunofluorescence findings likely reflect differences in methodology and incubation time (24 hrs. for Western blot versus 48 hrs. for immunofluorescence).

ERK, a key component of the MAPK signaling pathway, is dysregulated in about 40% of human malignancies. Although ERK signaling typically promotes cell survival, prolonged or excessive activation, often induced by stress or DNA damage, can lead to apoptosis [67]. Previously, we showed that ERK activation in BRAF-mutant A375 melanoma cells inhibits their proliferation. Here, we explored whether ERK activation has similar inhibitory effects in BRAF wild-type MeWo cells. Treatment with high

concentrations of quercetin or luteolin (100 μ M) and G-1 increased phosphorylated ERK (p-ERK) levels in MeWo cells, indicating activation of the Ras/Raf/ERK pathway. The antioxidant properties of these flavonoids may alter the cellular redox state, creating conditions in which ERK activation shifts from a pro-survival to a pro-apoptotic response under flavonoid-induced oxidative stress. This was supported by the observation that the GPER antagonist G15 partially reversed this effect, supporting a possible role for GPER signaling in these responses (Fig. 7).

Regarding the PI3K/Akt signaling pathway, treatment with quercetin, luteolin, or G-1 significantly decreased phosphorylated Akt (p-Akt) levels in MeWo cells. This suppression was partially attenuated by the GPER antagonist G15, indicating that the observed inhibition of cell-survival signaling may involve GPER-associated mechanisms. These findings contrast with our previous results in A375 melanoma cells, where the two flavonoids did not significantly alter p-Akt expression [31], and suggest that GPER-associated modulation of the PI3K/Akt pathway may be context-dependent [20], reflecting the complexity of GPER signaling in cutaneous melanoma. Cell line-dependent differences were also observed in the apoptotic and antimigratory responses to the flavonoids. In A375 cells, the flavonoids induced both apoptosis and necrosis, whereas the cytotoxic effects in MeWo cells were predominantly associated with apoptosis, with no detectable induction of necrosis. Similarly, the flavonoids exerted stronger antimigratory effects in MeWo cells than in A375 cells. The differential responses observed between A375 and MeWo cells may reflect not only the broader molecular heterogeneity of melanoma but also differences in GPER-associated signaling.

Furthermore, we evaluated the effects of the flavonoids on focal adhesion kinase (FAK), a non-receptor tyrosine kinase that plays an important role in regulating cell adhesion, motility, and survival. Quercetin and luteolin increased the ratio of phosphorylated FAK to total FAK (p-FAK/FAK) in MeWo cells, with G-1 showing a similar pattern (*Supplementary Fig. S3*). These effects were also partially reversed by G15. The increase in the p-FAK/FAK ratio may indicate increased FAK activation. This activation may appear paradoxical, as FAK is typically associated with pro-survival and pro-migratory signaling. However, FAK activation has been reported to occur under conditions of cellular stress such as those induced by cytotoxic agents like quercetin and luteolin. This activation may occur as an adaptive response that activates survival mechanisms such as enhanced cell proliferation, migration, and drug resistance [68]. Moreover, genetic or chemical stress may promote FAK nuclear localization, which may, in turn, promote degradation of the tumor suppressor p53, thereby enhancing cell survival [69]. In this context, the elevated p-FAK/FAK ratio may reflect a compensatory mechanism rather than a purely oncogenic signal, suggesting that MeWo cells may respond to flavonoid-induced cellular stress and GPER-associated signaling through an adaptive response involving FAK rather than through classical pro-survival pathways. Ultimately, these findings highlight the complexity of GPER-associated responses in melanoma and suggest that modulation of cytoskeletal and adhesion-related pathways may contribute to the effects of quercetin and luteolin beyond their pro-apoptotic actions.

We also observed elevated c-Myc expression following treatment with quercetin, luteolin, and G-1 in MeWo cells (Fig. 7). Although unexpected, this finding may reflect an apoptotic sensitization

mechanism. Similar c-Myc sensitization has been observed and attributed to limitations in the tricarboxylic acid cycle or due to an imbalance of metabolic/energetic supply and demand [70]. It has been proposed that c-Myc upregulation may represent a compensatory mechanism in response to DNA damage or toxicity by flavonoids. Alternatively, it may promote p53-dependent apoptotic signaling and reduce the expression of anti-apoptotic proteins such as Bcl-xL and Bcl-2 [71]. This increase was also partially reversed by G15, suggesting that GPER-associated signaling may contribute to the observed c-Myc response.

Finally, the structural features of flavonoids, particularly the number and position of hydroxyl groups and the presence of a double bond between carbons 2 and 3, can influence their estrogenic activity [72]. Quercetin and luteolin contain five and four hydroxyl groups, respectively, with the hydroxyl groups at position 7 of ring A and position 4' of ring B being particularly important, as they mimic the hydroxyl groups found at positions 3 and 17 β of estradiol (E2) [73]. Additionally, the double bond between C2 and C3 contributes to their high estrogenic potency. Based on our findings, 30 μ M appears to be an appropriate concentration for further mechanistic studies.

To our knowledge, this is the first study to demonstrate, in human MeWo cutaneous melanoma cells, that quercetin and luteolin exert anticancer effects associated with GPER signaling. Our results provide further insight into the effects of these flavonoids on the Ras/Raf/ERK and PI3K/Akt signaling pathways and suggest that GPER-associated signaling may contribute to their antiproliferative, pro-apoptotic, and anti-migratory effects. Together, these observations support a potential role for GPER-associated signaling in the cellular responses to quercetin and luteolin and support further investigation of this pathway in human cutaneous melanoma.

5. Conclusions

This study demonstrates that the phytoestrogens quercetin and luteolin exerted significant antitumor effects on the MeWo cutaneous melanoma cell line, with the G-protein-coupled estrogen receptor (GPER) potentially contributing to this effect. Both flavonoids effectively inhibited cell proliferation by inducing apoptosis and arresting cell cycle at S and G2/M phases. Furthermore, they significantly reduced the migratory capacity of melanoma cells. Mechanistically, the antitumor effects were associated with modulation of key signaling pathways, including increased expression of phosphorylated ERK (P-ERK) within the Ras/Raf/ERK cascade, and decreased levels of phosphorylated Akt (P-Akt) in the PI3K/Akt pathway. In addition, the regulation of the oncogenic transcription factor c-Myc may have contributed to the observed effects. Overall, this communication indicates that quercetin and luteolin have potential as anti-melanoma agents; their effects involve GPER signaling and associated modulation of critical cancer-related signaling pathways. Further studies are warranted to clarify the specific contribution of GPER signaling to these effects.

Declarations

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Author Contributions

Conceptualization, A.A. and S.A.; Methodology, S.A.H., N.T. and N.A.A; Software, N.T.; Validation, N.T., A.A. and S.A.; Formal analysis, S.A.H., N.T. and N.A. A; Investigation, S.A.H and N.T.; Resources, S.A.H., A.A. and S.A.; Data curation, S.A.H.; Writing—original draft, S.A.H. and N.T.; Writing—review and editing, N.T., A.A. and S.A.; Supervision, A.A. and S.A.; Project administration, S.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in this study are included in the article and in the *Supplementary Files*. Further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest in this work.

References

1. Guo W, Wang H, Li C. Signal pathways of melanoma and targeted therapy. *Signal Transduct Target Ther.* 2021; **6**: 424. doi: 10.1038/s41392-021-00827-6.
2. Roky AH, Islam MM, Ahasan AF, Mostaq S, Mahmud Z, Amin MN, Mahmu A, Overview of skin cancer types and prevalence rates across continents, *Cancer Pathog. Ther.* 3 (2025) 89–100, <https://doi.org/10.1016/j.cpt.2024.08.002>.
3. Naik PP. Cutaneous malignant melanoma: A review of early diagnosis and management. *World J. Oncol.* 2021; **12**: 7–19. doi: 10.14740/wjon1349.
4. Wunderlich K, Suppa M, Gandini S, Lipski J, White JM, Del Marmol V. Risk factors and innovations in risk assessment for melanoma, basal cell carcinoma, and squamous cell carcinoma. *Cancers.* 2024; **16**: 1–24. doi: 10.3390/cancers16051016.
5. Lopes J, Rodrigues CMP, Gaspar MM, Reis CP. Melanoma management: From epidemiology to treatment and latest advances. *Cancers.* 2022; **14**: 1–24. doi: 10.3390/cancers14194652.
6. Bhari N, Schwaertz R, Apalla Z, Salerni G, Akay BN, Patil A, Grabbe S, Goldust M. Effect of estrogen in malignant melanoma. *J Cosmet Dermatol.* 2022; **21**: 1905–1912. doi: 10.1111/jocd.14391.

7. Ernst M, Giubellino A. The current state of treatment and future directions in cutaneous malignant melanoma. *Biomedicines*. 2022; **10**: 1–30. doi: 10.3390/biomedicines10040822.
8. Champion PJ, Bluestein JR, Quinn AE, Bell SD, Kiley JH, Wakefield MR, Fang Y. A consolidated review of contemporary targeted and immunotherapeutic options for melanoma. *Biomedicines*. 2025; **13**:1–18. doi: 10.3390/biomedicines13061388.
9. Smetana K, Lacina L, Kodet O. Targeted therapies for melanoma. *Cancers*. 2020; **12**: 1–14. doi: 10.3390/cancers12092494.
10. Caerts D, Garmyn M, Güvenç C. A narrative review of the role of estrogen (receptors) in melanoma. *Int J Mol Sci*. 2024; **25**: 3456. doi: 10.3390/ijms25116251.
11. Waifalkar PP, Noh D, Derashri P, Barage S, Oh E. Role of estradiol hormone in human life and electrochemical aptasensing of 17-estradiol: A review. *Biosensors*. 2022; **12**: 1–23. doi: 10.3390/bios12121117.
12. Zhu J, Zhou Y, Jin B, Shu J. Role of estrogen in the regulation of central and peripheral energy homeostasis: From a menopausal perspective. *Ther Adv Endocrinol Metab*. 2023; **14**: 1–22. doi: 10.1177/20420188231199359.
13. Beiu C, Popescu MN, Dumitrascu MC, Petca A, Mehedințu C, Farcasanu PD, Pecta RC, Chibeleian C, Șandru F. The evaluation of estrogen receptor β as a potential prognosis factor in melanoma. *Rev Chim*. 2019; **70**: 4212–4216. doi: 10.37358/RC.19.12.7734.
14. Périan S, Vanacker JM. GPER as a receptor for endocrine-disrupting chemicals (EDCs). *Front Endocrinol*. 2020; **11**: 1–7. doi: 10.3389/fendo.2020.00545.
15. Prossnitz ER, Barton M. The G protein-coupled oestrogen receptor GPER in health and disease: An update. *Nat Rev Endocrinol*. 2023; **19**: 407–424. doi: 10.1038/s41574-023-00822-7.
16. Pepermans R, Sharma G, Prossnitz ER. G protein-coupled estrogen receptor in cancer and stromal cells: Functions and novel therapeutic perspectives. *Cells*. 2021; **10**: 1–16. doi: 10.3390/cells10030672.
17. Tirado-Garibay AC, Falcón-Ruiz EA, Ochoa-Zarzosa A, López-Meza JA. GPER: An estrogen receptor key in metastasis and tumoral microenvironments. *Int J Mol Sci*. 2023; **24**: 1–15. doi: 10.3390/ijms241914993.
18. Ribeiro MPC, Santos AE, Custódio JBA. The activation of the G protein-coupled estrogen receptor (GPER) inhibits the proliferation of mouse melanoma K1735-M2 cells. *Chem Biol Interact*. 2017; **277**: 176–184. doi: 10.1016/j.cbi.2017.09.017.
19. Ambrosini G, Natale CA, Musi E, Garyantes T, Schwartz GK. The GPER agonist LNS8801 induces mitotic arrest and apoptosis in uveal melanoma cells. *Cancer Res Commun*. 2023; **3**: 540–547. doi: 10.1158/2767-9764.crc-22-0399.
20. Xu F, Ma J, Wang X, Wang X, Fang W, Sun J, Li Z, Liu J. The role of G protein-coupled estrogen receptor (GPER) in vascular pathology and physiology. *Biomolecules*. 2023; **13**: 1–24. doi: 10.3390/biom13091410.

21. Arterburn JB, Prossnitz ER. G protein–coupled estrogen receptor GPER: Molecular pharmacology and therapeutic applications. *Annu Rev Pharmacol Toxicol*. 2023; **63**: 295–320. doi: 10.1146/annurev-pharmtox-031122-121944.
22. Molina L, Bustamante FA, Bhoola KD, Figueroa CD, Ehrenfeld P. Possible role of phytoestrogens in breast cancer via GPER-1/GPR30 signaling. *Clin Sci*. 2018; **132**: 2583–2598. doi: 10.1042/cs20180885.
23. Roman A, Marcovici I, Chioibas R, Motoc A, Puenea G, Gyori Z, Crainiceanu Z, Bonte DC. Genistein and quercetin trigger cytotoxicity, apoptosis, and oxidative stress in SK MEL 28 cutaneous melanoma cells. *Farmacologia*. 2024; **72**: 622–630. doi: 10.31925/farmacologia.2024.3.16.
24. Kashyap D, Tuli H, Garg V, Bhatnagar S, Sharma A. Ursolic acid and quercetin: Promising anticancer phytochemicals with antimetastatic and antiangiogenic potential. *Tumor Microenv*. 2018; **1**: 9–15. doi: 10.4103/tme.tme_3_17.
25. Aghababaei F, Hadidi M. Recent advances in potential health benefits of quercetin. *Pharmaceuticals*. 2023; **16**: 1–31. doi: 10.3390/ph16071020.
26. Soll F, Ternent C, Berry IM, Kumari D, Moore TC. Quercetin inhibits proliferation and induces apoptosis of B16 melanoma cells in vitro. *Assay Drug Dev Technol*. 2020; **18**: 261–268. doi: 10.1089/adt.2020.993.
27. Almatroodi SA, Almatroudi A, Alharbi HOA, Khan AA, Rahmani AH. Effects and mechanisms of luteolin, a plant-based flavonoid, in the prevention of cancers via modulation of inflammation and cell signaling molecules. *Molecules* 2024; **29**: 1093. doi: 10.3390/molecules29051093.
28. Ruan JS, Liu YP, Zhang L, Yan LG, Fan FT, Shen CH, Wang, AY, Zheng SZ, Wang SM, Lu Y. Luteolin reduces the invasive potential of malignant melanoma cells by targeting $\beta 3$ integrin and the epithelial-mesenchymal transition. *Acta Pharmacol Sin*. 2012; **33**: 1325–1331. doi: 10.1038/aps.2012.93.
29. Shi ML, Chen YF, Liao HF. Effect of luteolin on apoptosis and vascular endothelial growth factor in human choroidal melanoma cells. *Int J Ophthalmol*. 2021; **14**: 186–193. doi: 10.18240/ijo.2021.02.02.
30. Arrigo DG, Gianquinto E, Rossetti G, Cruciani G, Lorenzetti S, Spyraakis F. Binding of androgen- and estrogen-like flavonoids to their cognate (non)nuclear receptors: A comparison by computational prediction. *Molecules*. 2021; **26**: 1–24. doi: 10.3390/molecules26061613.
31. Hussein SA, Ababneh NA, Tarawneh N, Ismail MA, Awidi A, Abdalla S. Antitumor effects of quercetin and luteolin in A375 cutaneous melanoma cell line are mediated by upregulation of p-ERK, c-Myc, and the upstream GPER. *Life*. 2025; **15**: 1–21. doi: 10.3390/life15030417.
32. Çolakoglu C, Hacıfendi A, Eryilmaz IE, Eskiler GG, Egeli U, Cecener G. The cytotoxic effect of usnic acid in malignant melanoma cells with different genomic profiles in the Braf aspect. *WCRJ*. 2022; **9**: 1–11. doi: 10.32113/wcrj_20223_2240.
33. Wróblewska M, Biały LP, Młynarczyk Biały I. Targeting G coupled estrogen receptor signaling in melanoma. *Rev Res Cancer Treat*. 2017; **3**: 44–53.

https://www.researchgate.net/publication/325321411_Targeting_G-coupled_estrogen_receptor_signaling_in_melanoma.

34. Zheng H, Triplett KD, Prossnitz ER, Hall PR, Daly SM. G protein-coupled estrogen receptor agonist G-1 decreases ADAM10 levels and NLRP3-inflammasome component activation in response to *Staphylococcus aureus* alpha hemolysin. *Microbiol Open*. 2024; **13**: e2345. doi: 10.1002/mbo3.1423.
35. Hall KA, Filardo EJ. The G protein-coupled estrogen receptor (GPER): A critical therapeutic target for cancer. *Cells*. 2023; **12**: 1–27. doi: 10.3390/cells12202460.
36. Clements L, Alexander A, Hamilton K, Irving A, Harvey J. G-protein coupled estrogen receptor (GPER1) activation promotes synaptic insertion of AMPA receptors and induction of chemical LTP at hippocampal temporoammonic-CA1 synapses. *Mol Brain*. 2023; **16**: 1–18. doi: 10.1186/s13041-023-01003-3.
37. Alwahsh M, Al-Doridee A, Jasim S, Awwad O, Hergenröder R, Hamadneh L. Cytotoxic and molecular differences of anticancer agents on 2D and 3D cell culture. *Mol Biol Rep*. 2024; **51**: 721. doi:10.1007/s11033-024-09669-1.
38. Tarawneh N, Hamadneh L, Alshaer W, Al Bawab AQ, Bustanji Y, Abdalla S. Downregulation of aquaporins and PI3K/AKT and upregulation of PTEN expression induced by the flavone scutellarein in human colon cancer cell lines. *Heliyon*. 2024; **10**: e39402. doi: 10.1016/j.heliyon.2024.e39402.
39. Liang Y, Fu W, Tang Y, Ye H, Wang Y, Sun C, Xiang Y, Xiong W, Cui M, Chen Y, Wang T, Deng Y. Selective activation of G protein-coupled estrogen receptor 1 (GPER1) reduces ER stress and pyroptosis via AMPK signaling pathway in experimental subarachnoid hemorrhage. *Mol Neurobiol*. 2025; **62**: 871–884. doi: 10.1007/s12035-024-04312-3.
40. Zhang J, Guo J, Qian Y, Yu L, Ma J, Gu B, Tang W, Li Y, Li H, Wu W. Quercetin induces apoptosis through downregulating P4HA2 and inhibiting the PI3K/Akt/mTOR axis in hepatocellular carcinoma cells: An in vitro study. *Cancer Rep*. 2025; **8**: e70220. doi: 10.1002/cnr2.70220.
41. Cosci I, Grande G, Nisio AD, Rocca MS, Fiore PD, Benna C, Mocellin S, Ferlin A. Cutaneous melanoma and hormones: Focus on sex differences and the testis. *Int J Mol Sci*. 2023; **24**: 1–14. doi: 10.3390/ijms24010599.
42. Boleti APDA, Jacobowski AC, Monteiro-Alfredo T, Pereira APR, Oliva MLV, Maria DA, Macedo MLR. Cutaneous melanoma: An overview of physiological and therapeutic aspects and biotechnological use of serine protease inhibitors. *Molecules*. 2024; **29**: 1–28. doi: 10.3390/molecules29163891.
43. Muhammad A, Forcados GE, Yusuf AP, Abubakar MB, Sadiq IZ, Elhussin I, Siddique AT, Aminu S, Suleiman RB, Abubakar YS, Katsayal BS, Yates CC, Mahavadi S. Comparative G-protein-coupled estrogen receptor (GPER) systems in diabetic and cancer conditions: A review. *Molecules*. 2022; **27**: 1–40. doi: 10.3390/molecules27248943.
44. Torres López L, Olivas Aguirre M, Dobrovinskaya O. The G protein coupled estrogen receptor GPER in the development and progression of cancer. *Receptors*. 2024; **3**: 220–254. doi: 10.3390/receptors3020012.

45. Fan JJ, Hsu WH, Lee KH, Chen KC, Lin CW, Lee YLA, Ko TP, Lee LT, Lee MT, Chang MS, Cheng CH. Dietary flavonoids luteolin and quercetin inhibit migration and invasion of squamous carcinoma through reduction of Src/Stat3/S100A7 signaling. *Antioxidants*. 2019; **8**: 1–16. doi: 10.3390/antiox8110557.
46. Abusaliya A, Jeong SH, Bhosale PB, Kim HH, Park MY, Kim E, Won CK, Park KI, Heo JD, Kim HW, Ahn M, Seong JK, Kim GS. Mechanistic action of cell cycle arrest and intrinsic apoptosis via inhibiting Akt/mTOR and activation of p38-MAPK signaling pathways in Hep3B liver cancer cells by prunetrin-a flavonoid with therapeutic potential. *Nutrients*. 2023; **15**: 1–16. doi: 10.3390/nu15153407.
47. Gousias K, Theocharous T, Simon M. Mechanisms of cell cycle arrest and apoptosis in glioblastoma. *Biomedicines*. 2022; **10**: 1–19. doi: 10.3390/biomedicines10030564.
48. Kluska M, Piastowska-Ciesielska AW, Tokarz P. Cell cycle status influences resistance to apoptosis induced by oxidative stress in human breast cancer cells, which is accompanied by modulation of autophagy. *Curr Issues Mol Biol*. 2023; **45**: 6325–6338. doi: 10.3390/cimb45080399.
49. Liu J, Peng Y, Wei W. Cell cycle on the crossroad of tumorigenesis and cancer therapy. *Trends Cell Biol*. 2022; **32**: 30–44. doi: 10.1016/j.tcb.2021.08.006.
50. Gębka N, Gębka Kępińska B, Adamczyk J, Mizgała Izvorska E. The use of flavonoids in skin cancer prevention and treatment. *J Preclin Clin Res*. 2022; **16**: 108–113. doi: 10.26444/jpccr/153023.
51. Pei SN, Lee KT, Rau KM, Lin TY, Tsai TH, Hsu YC. Luteolin (LUT) induces apoptosis and regulates mitochondrial membrane potential to inhibit cell growth in human cervical epidermoid carcinoma cells (Ca Ski). *Biomedicines*. 2024; **12**: 1–10. doi: 10.3390/biomedicines12102330.
52. Li N, Wang J. Quercetin induces cytotoxicity and apoptosis, reduces metastasis and drug resistance in oral cancer cells. *Turk J Biochem*. 2024; **49**: 148–156. doi: 10.1515/tjb-2023-0003.
53. Momtaz S, Niaz K, Maqbool F, Abdollahi M, Rastrelli L, Nabavi SM. STAT3 targeting by polyphenols: Novel therapeutic strategy for melanoma. *BioFactors*. 2017; **43**: 347–370. doi: 10.1002/biof.1345.
54. Sioud F, Maatouk M, Bzeouich IM, Ghedira LC, Kilani-Jaziri S. In vitro anti-melanoma effect of polyphenolic compounds. *Asian Pac J Trop Biomed*. 2022; **12**: 446–452. doi: 10.4103/2221-1691.357744.
55. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: A hallmark of cancer revisited. *Signal Transduct Target Ther*. 2020; **5**: 28. doi: 10.1038/s41392-020-0134-x.
56. Kim K, Choung J, Ko UH, Jung A, Choe W, Shin JH, Gweon B. Suppression of breast cancer cell migration and epithelial-mesenchymal transition by atmospheric pressure plasma. *Front Phys*. 2021; **9**: 1–8. doi: 10.3389/fphy.2021.694080.
57. Zhang H, Steed A, Co M, Chen X. Cancer stem cells, epithelial-mesenchymal transition, ATP and their roles in drug resistance in cancer. *Cancer Drug Resist*. 2021; **4**: 684–709. doi: 10.20517/cdr.2021.32.
58. Lie ZN, Tian Q, Teng QS, Worpel JND, Zeng L, Pan Y, Chen ZX. Understanding and targeting resistance mechanisms in cancer. *MedComm*. 2023; **4**: 1–38. doi: 10.1002/mco2.265.

59. Tao L, Zhou Y, Luo Y, Qiu J, Xiao Y, Zou J, Zhang Y, Liu X, Yang X, Gou K, Xu J, Guan X, Cen X, Zhao Y. Epigenetic regulation in cancer therapy: From mechanisms to clinical advances. *MedComm Oncol*. 2024; **3**: 1–53. doi: 10.1002/mog2.59.
60. Liu HQ, Wu XD, Fang XW, An YS, Xia M, Luo XH, Li JZ, Wang GH, Liu T. Tumor targeted magnetic micelles for magnetic resonance imaging, drug delivery, and overcoming multidrug resistance. *ACS Omega*. 2024; **9**: 49566–49579. doi: 10.1021/acsomega.4c07132.
61. Tufail M, Hu JJ, Liang J, He CY, Wan WD, Huang YQ, Jiang CH, Wu H, Li N. Hallmarks of cancer resistance. *iScience*. 2024; **27**: 109979. doi: 10.1016/j.isci.2024.109979.
62. Seo SY, Ju WS, Kim K, Kim J, Yu JO, Ryu JS, Kim JS, Lee HA, Koo DB, Choo YK. Quercetin induces mitochondrial apoptosis and downregulates ganglioside GD3 expression in melanoma cells. *Int J Mol Sci*. 2024; **25**: 1–19. <https://www.mdpi.com/1422-0067/25/10/5146>.
63. Schomberg J, Wang Z, Farhat A, Guo KL, Xie J, Zhou Z, Liu J, Kovacs B, Smith FL. Luteolin inhibits melanoma growth in vitro and in vivo via regulating ECM and oncogenic pathways but not ROS. *Biochem Pharmacol*. 2020; **177**: 113956. doi: 10.1016/j.bcp.2020.113956.
64. Yao X, Jiang W, Yu D, Yan Z. Luteolin inhibits proliferation and induces apoptosis of human melanoma cells in vivo and in vitro by suppressing MMP-2 and MMP-9 through the PI3K/AKT pathway. *Food Funct*. 2019; **10**: 703–712. doi: 10.1039/c8fo02013b.
65. Cao HH, Cheng CY, Su T, Fu XQ, Guo H, Li T, Tse AKW, Kwan HY, Yu H, Yu ZL. Quercetin inhibits HGF/c-Met signaling and HGF-stimulated melanoma cell migration and invasion. *Mol Cancer*. 2015; **14**: 103. doi: 10.1186/s12943-015-0367-4.
66. Almeida CEG, Santerre A, Moreno LCL, Garcia IGA, Arellano RC, Jimenez SHD, Jimenez JMD. Proliferation and apoptosis regulation by G protein-coupled estrogen receptor in glioblastoma C6 cells. *Oncol Lett*. 2022; **24**: 1–9. doi: 10.3892/ol.2022.13338.
67. Cagnol S, Chambard JC. ERK and cell death: Mechanisms of ERK-induced cell death-apoptosis, autophagy and senescence. *FEBS J*. 2010; **277**: 2–21. doi:10.1111/j.1742-4658.2009.07366.x.
68. Schlaepfer DD, Ojalill M, Stupack DG. Focal adhesion kinase signaling-tumor vulnerabilities and clinical opportunities. *J Cell Sci*. 2024; **137**: jcs259522. doi:10.1242/jcs.261723.
69. Kleinschmidt EG, Schlaepfer DD. Focal adhesion kinase signaling in unexpected places. *Curr Opin Cell Biol*. 2017; **45**: 24–30. doi:10.1016/j.ceb.2017.01.003.
70. Edwards-Hicks J, Su H, Mangolini M, Yoneten KK, Wills J, Rodriguez-Blanco G, Young C, Cho K, Barker H, Muir M, et al. MYC sensitizes cells to apoptosis by driving energetic demand. *Nat Commun*. 2022; **13**: 4674. doi: 10.1038/s41467-022-32368-z.
71. Ahmadi SE, Rahimi S, Zarandi B, Chegeni R, Safa M. MYC: A multipurpose oncogene with prognostic and therapeutic implications in blood malignancies. *J Hematol Oncol*. 2021; **14**: 121. doi: 10.1186/s13045-021-01111-4.
72. Lavier MCC, Pelissero CB. Phytoestrogens and health effects. *Nutrients*. 2024; **15**: 317. Doi: 10.3390/nu150203177

Figures

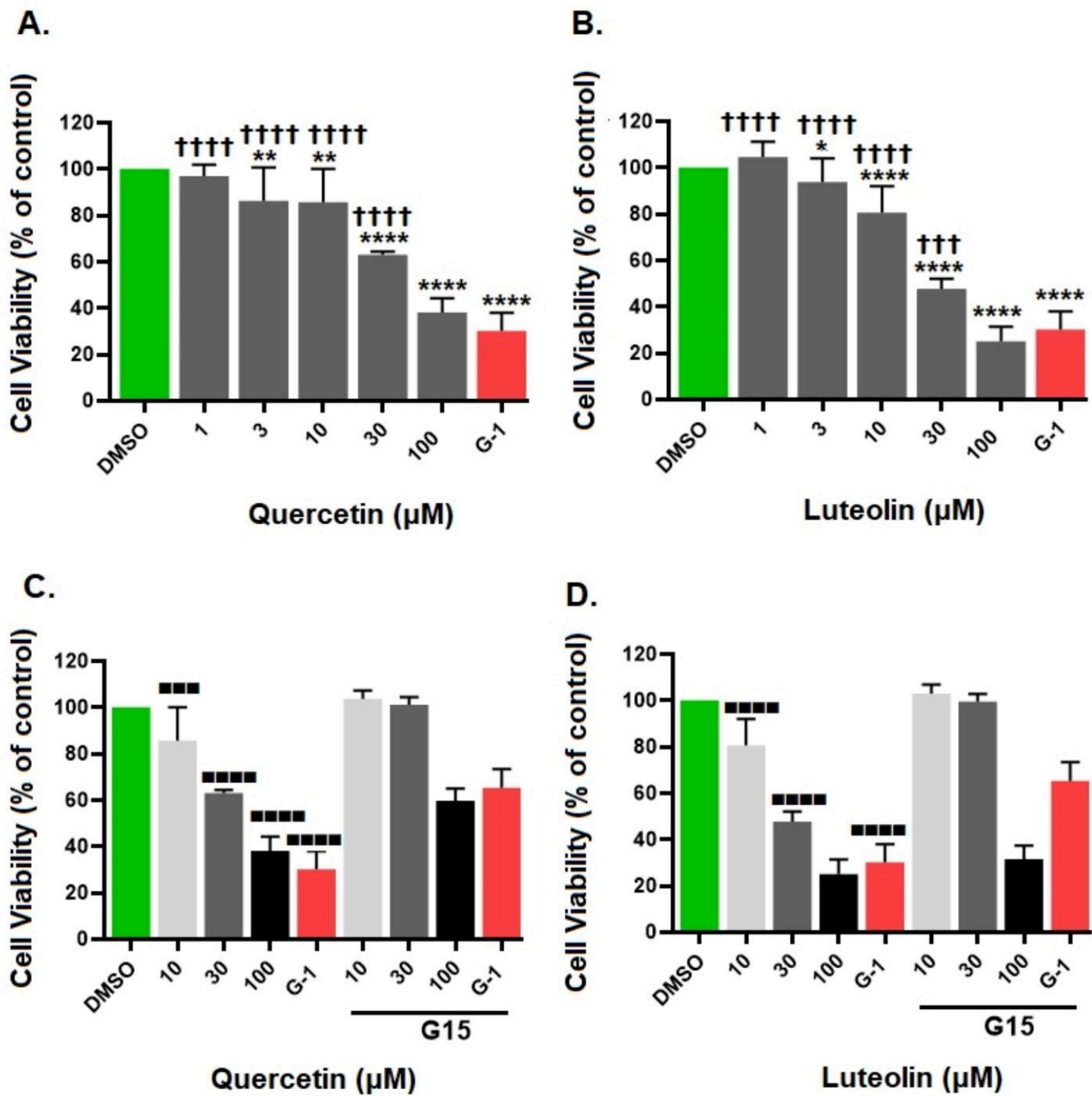


Figure 1

Effects of quercetin and luteolin on proliferation of MeWo cells in the absence (A and B) or presence (C and D) of G15. Cells were treated for 48 hrs. with 1, 3, 10, 30, and 100 μ M of quercetin or luteolin; G-1 (1 μ M) served as a positive control and DMSO (0.2%) as a negative control. Data are presented as the means $\pm$ SD of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **P < 0.01, ****p < 0.0001 compared to DMSO; ††† p <0.001, †††† p <0.0001 compared to G-1; ■■■P < 0.001, ■■■■P < 0.0001 compared to the similar concentrations of quercetin, luteolin, or G-1 in the presence of G15.

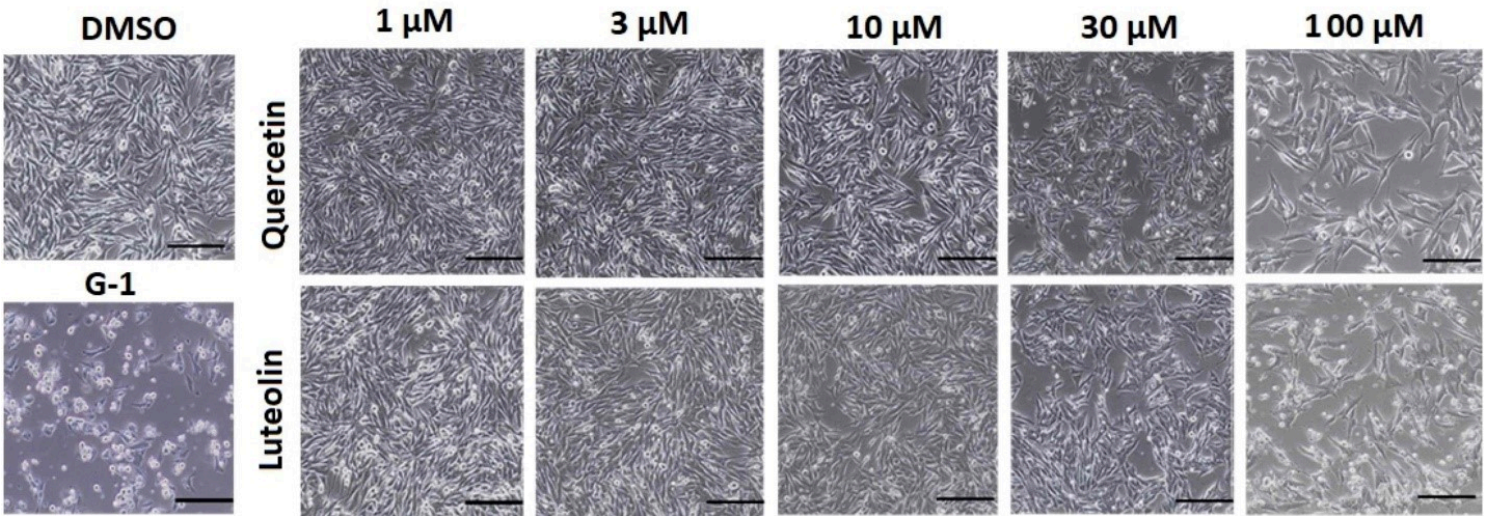


Figure 2

Effects of quercetin and luteolin on growth and morphology of MeWo cells. Cells were treated with DMSO (0.2%) as a negative control and G-1 as a positive control. The indicated concentrations of quercetin (*upper panel*) or luteolin (*lower panel*) were applied and images were captured. Scale bar_ 100 μ m.

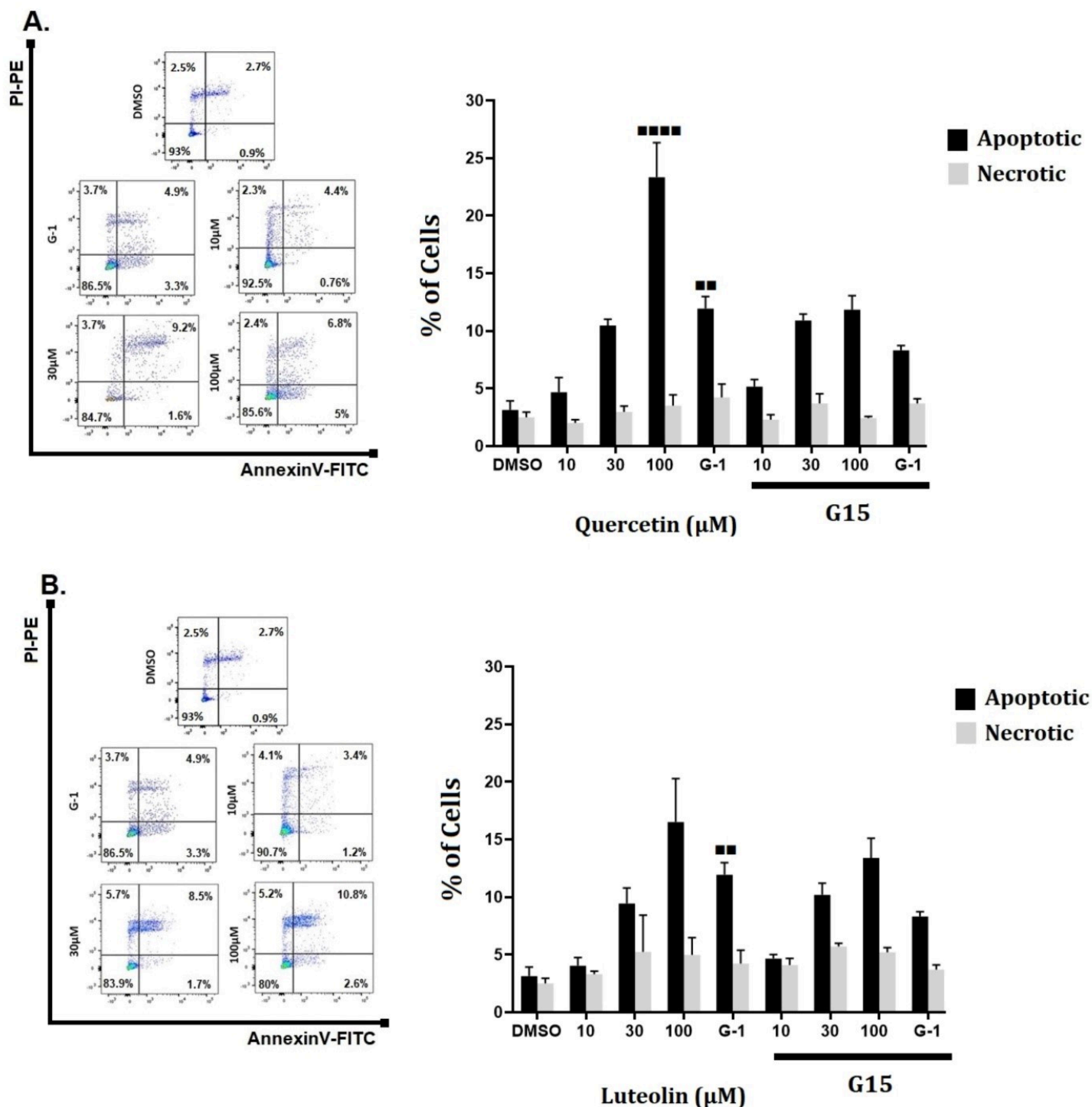


Figure 3

Effects of the GPER antagonist, G15, on apoptosis of MeWo cells. Cells were pretreated with 3μM of G15 for 24 hrs. before being treated with quercetin (**A**), or luteolin (**B**), at 10, 30 and 100 μM, DMSO (0.2%) as a negative control, and G-1 (1 μM) as a positive control. After 48 hrs., treated cells were stained with Annexin V/PI, and cell apoptosis was determined using flow cytometry. Representative flow cytometry plots in the presence of G15 are shown in the *left panels*). Data were quantified both in the absence and the presence of G15 using FlowJo software (*right panels*). Results are presented as the

means $\pm$ SD of three independent experiments. Statistical significance was determined using 2-way ANOVA followed by Dunnett's multiple comparisons test ■■ $p < 0.01$, ■■■■ $p < 0.0001$ compared to the similar concentrations of quercetin, luteolin, or G-1 in the presence of G15. Data of apoptosis and necrosis assay in the absence of G15 are shown in *supplementary Fig.S1*.

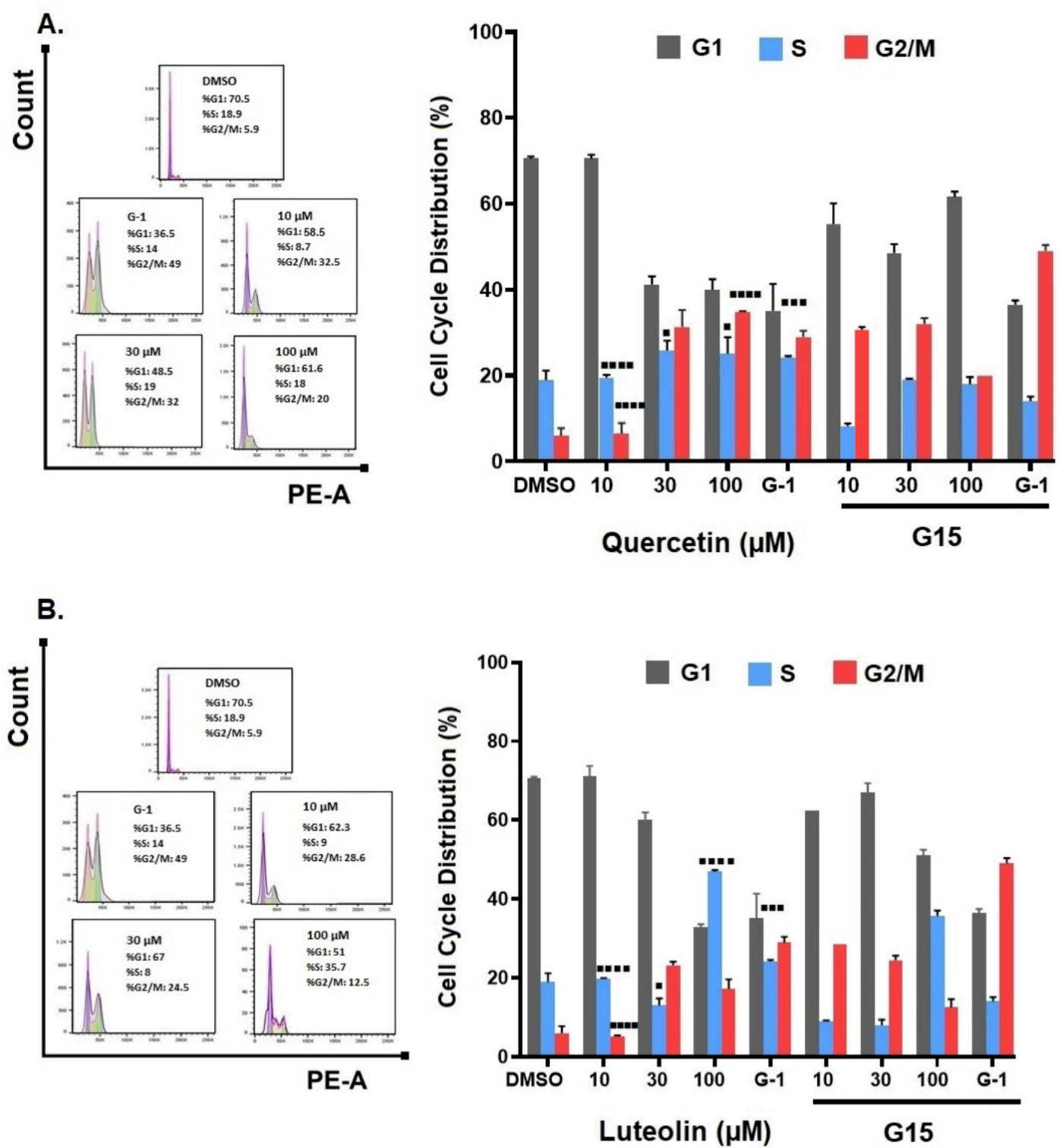


Figure 4

Effects of quercetin, luteolin, and G1 in the presence of GPER antagonist G15 on cell cycle distribution of MeWo cells. Cells were pretreated with the 3 μ M of G15 and then treated with 10, 30, and 100 μ M of quercetin (A), or luteolin (B); 1 μ M of G-1 as a positive control, and 0.2% DMSO as a negative control. Left panels are representative blots of cell cycle assay where the cell have been incubated with the GPER antagonist G15 for 24 hrs then incubated with quercetin or luteolin or the othe controls, then analyzed by flow cytometry (*left panels*). The percentage of cells in G1, S, and G2/M phases for each treatment was calculated and analyzed both in the absence and the presence of G15 (*right panels*). Data are presented as the means $\pm$ SD of three independent experiments. Statistical significance was determined using 2-way ANOVA followed by Dunnett’s multiple comparisons test. ■ $p < 0.05$, ■■■ $p < 0.001$, ■■■■ $p < 0.0001$ compared to the similar concentrations of quercetin, luteolin, or G-1 in the presence of G15. Data of cell cycle assay in the absence of G15 are shown in *supplementary Fig.S2*.

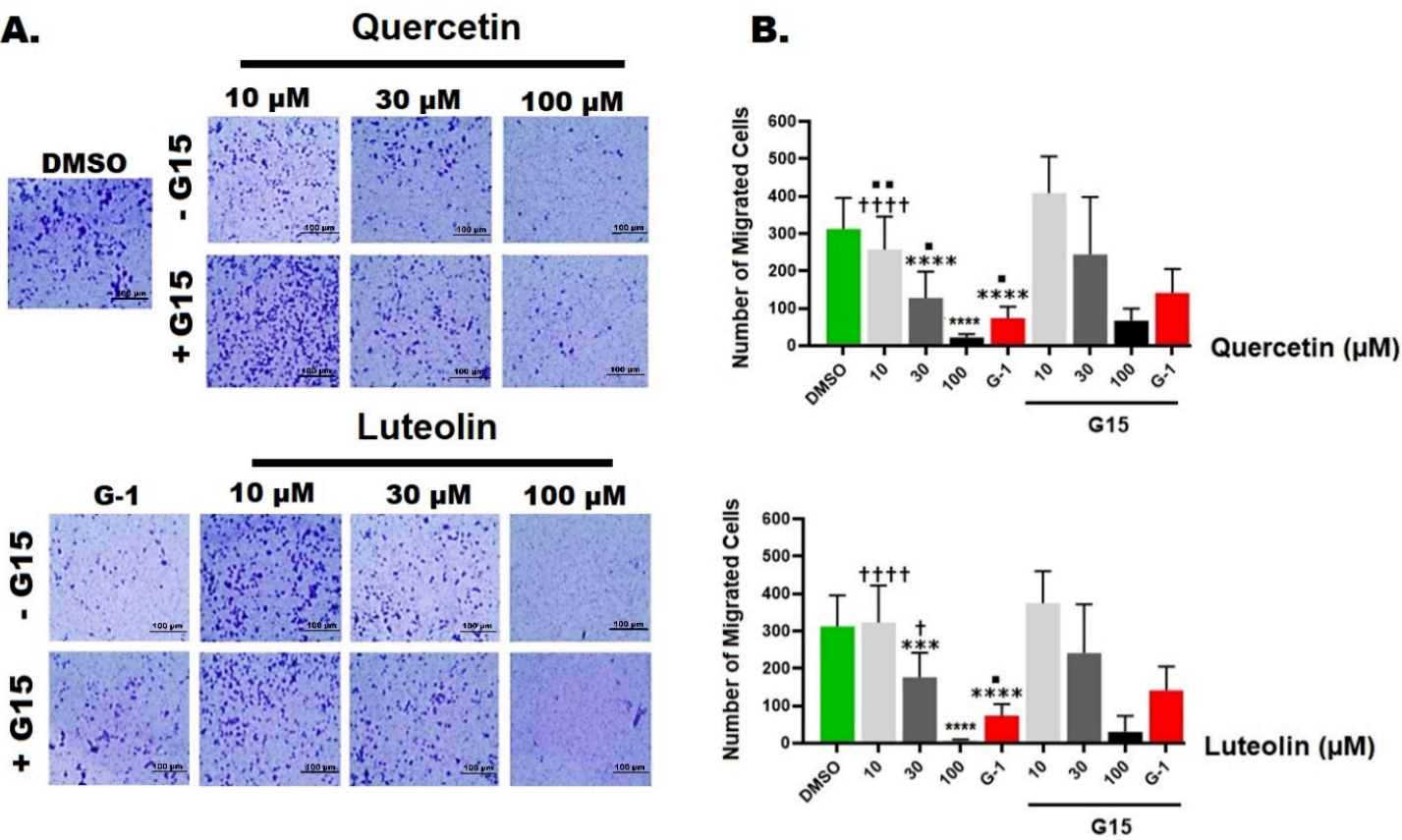


Figure 5

Migration of MeWo cells under different treatments. Cells were exposed for 48 hrs. to the indicated concentrations of quercetin (A) or luteolin (B), G-1 (positive control), and 0.2% DMSO (negative control), in the absence or the presence of 3 μ M of G15. Transwell migration was allowed for 48 hrs. and the absolute number of migrating cells is shown in the *right panels*. Images for DMSO (the negative control) and for G-1 (the positive control) are the same for the two flavonoids. Values are means $\pm$ SD of 10 fields/treatment. Statistical significance was determined using one-way ANOVA followed by Dunnett’s multiple comparisons test. **** $p < 0.0001$ compared to DMSO; † $p < 0.05$, ††† $p < 0.001$ compared to G-1.

In the absence of 3μM of the antagonist G15: ■ p < 0.05, ■■P < 0.01 compared to the same concentration and ligand in its presence.

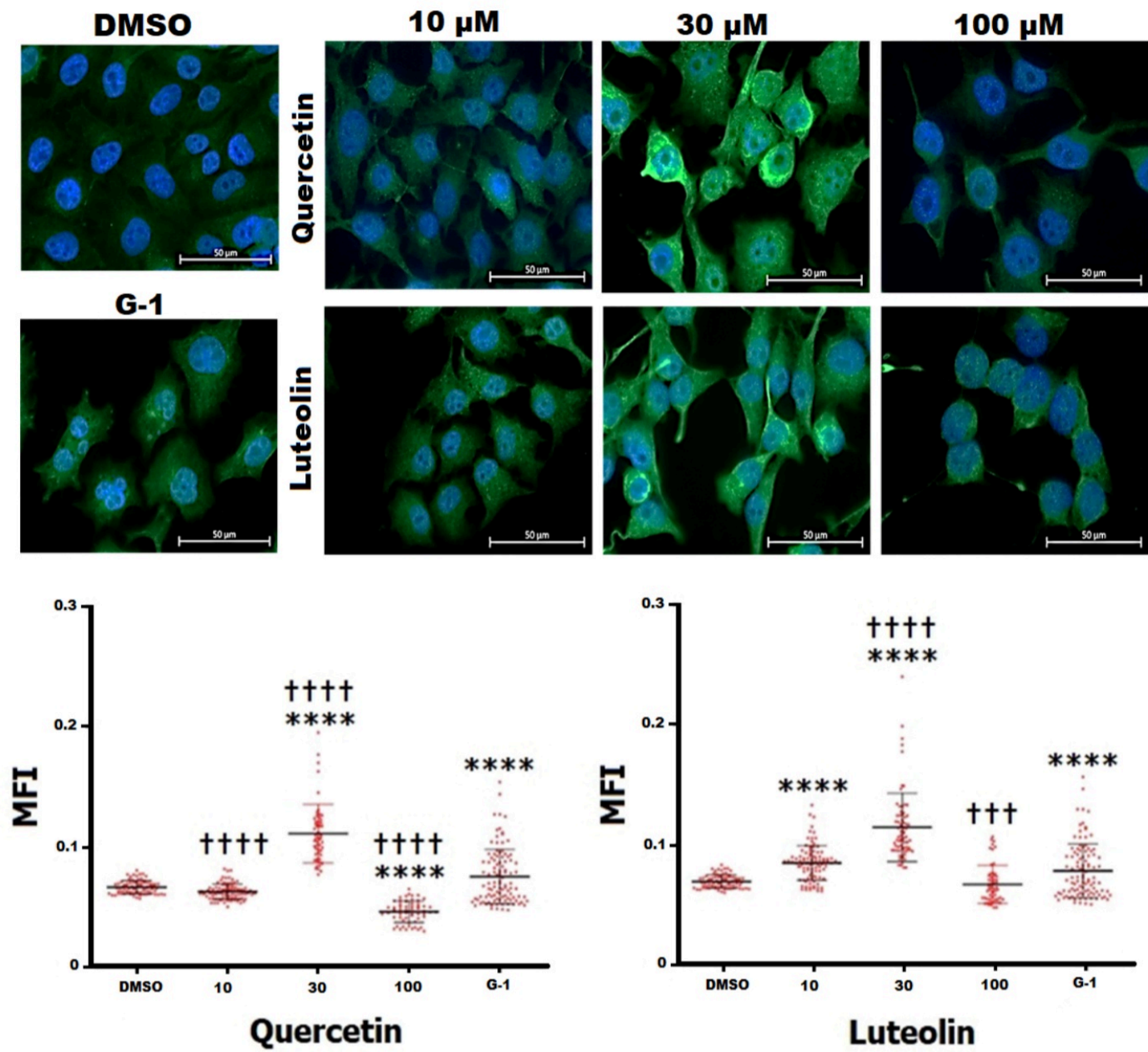


Figure 6

Detection of GPER expression in MeWo cells by immunofluorescence staining. Cells were treated for 48 hrs. with 10, 30 and 100 μM of quercetin, luteolin, or 1μM G-1 as a positive control. DMSO (0.2%) was used as a negative control. Representative immunofluorescence images captured by time-lapse fluorescence microscope are shown (*upper panels*). Green signal indicates positive staining for GPER, while the blue signal (DAPI) represents the nuclei. Scatter dot plots show the mean fluorescence intensity (MFI) for GPER determined by cell profiler software (*lower panel*). Statistical significance was

determined using one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **P < 0.01, ***p < 0.001, ****p < 0.0001 compared to DMSO; ††† p< 0.001, ††††p <0.0001 compared to G-1. Scale bar =50µm.

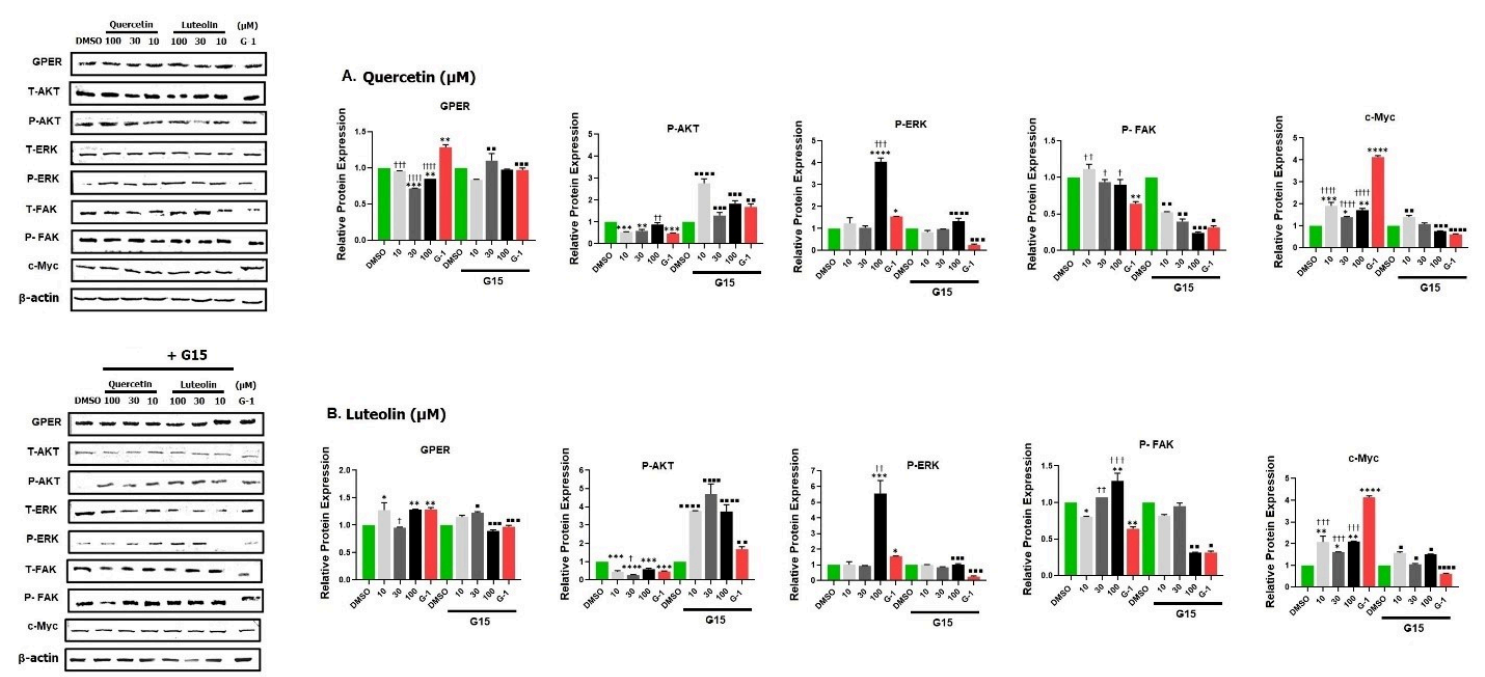


Figure 7

Effects of quercetin and luteolin on protein expression of MAPK/ERK and PI3K/AKT pathways. MeWo melanoma cell line were treated with quercetin (A), luteolin (B), G-1 (positive control), and 0.2% DMSO (negative control) in the absence or the presence of 3µM of G15. Representative Western blots images for the expression of GPER, P-Akt, P-ERK, P-FAK, and c-Myc are shown (*left panels*). Densitometric analysis was performed and the relative protein expression was normalized to β-actin (*right panels*). Statistical significance was determined using one-way ANOVA followed by Dunnett's multiple comparisons test. *p < 0.05, **P < 0.01, ***p < 0.001, ****p < 0.0001 compared to DMSO; †p < 0.05, †† p< 0.01, ††† p < 0.001, †††† p < 0.0001 compared to G-1. In the absence of the antagonist G15, the relative protein expressions were significantly reversed: ■P < 0.05, ■■P < 0.01, ■■■P < 0.001, ■■■■P < 0.0001 when compared to the same concentration and ligand in its presence. Densitometry analysis for total Akt, p-Akt/total Akt, total ERK, p-ERK/total ERK, total FAK, and p-FAK/total FAK for treatment with quercetin and luteolin is shown in the *Supplementary Figure (Figure S3)*.

Supplementary Files

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- [SupplementaryMaterialQuercetin.docx](#)