

The citrus flavonoid hesperidin induces p53 and inhibits NF- κ B activation in order to trigger apoptosis in NALM-6 cells: involvement of PPAR γ -dependent mechanism

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Abstract

Background Hesperidin, a flavanone present in citrus fruits, has been identified as a potent anticancer agent because of its proapoptotic and antiproliferative characteristics in some tumor cells. However, the precise mechanisms of action are not entirely understood.

Aim The main purpose of this study is to investigate the involvement of peroxisome proliferator-activated receptor-gamma (PPAR γ) in hesperidin's anticancer actions in human pre-B NALM-6 cells, which expresses wild-type p53.

Methods The effects of hesperidin on cell-cycle distribution, proliferation, and caspase-mediated apoptosis were examined in NALM-6 cells in the presence or absence of GW9662. The expression of peroxisome proliferator-activated receptor-gamma (PPAR γ), p53, phospho-I κ B, Bcl-2, Bax, and XIAP proteins were focused on using the immunoblotting assay. The transcriptional activities of PPAR γ and nuclear factor-kappaB (NF- κ B) were analyzed by the transcription factor assay kits. The expression of PPAR γ and p53 was analyzed using the RT-PCR method.

Results Hesperidin induced the expression and transcriptional activity of PPAR γ and promoted p53 accumulation and downregulated constitutive NF- κ B activity in a PPAR γ -dependent and PPAR γ -independent manner. The

growth-inhibitory effect of hesperidin was partially reduced when the cells preincubated with PPAR γ antagonist prior to the exposure to hesperidin.

Conclusions The findings of this study clearly demonstrate that hesperidin-mediated proapoptotic and antiproliferative actions are regulated via both PPAR γ -dependent and PPAR γ -independent pathways in NALM-6 cells. These data provide the first evidence that hesperidin could be developed as an agent against hematopoietic malignancies.

Keywords Hesperidin · PPAR-gamma · p53 · Ikappa B · NF-kappaB

Introduction

Hesperidin, a flavanone glycoside found abundantly in citrus fruits, possesses a wide range of pharmacological properties including potential anti-inflammatory and anticancer effects [1, 2]. Hesperidin induces cell growth arrest and apoptosis in a large variety of cells including colon and pancreatic cancer cells [3, 4]. However, the molecular mechanisms involved in the growth-inhibitory effects of hesperidin remain to be elucidated. Some pieces of evidence show that hesperidin recruits peroxisome proliferator-activated receptor-gamma (PPAR γ) to exert biological actions [5]. PPAR γ is a member of the ligand-dependent nuclear receptor superfamily, which integrates the control of energy, lipid, and glucose homeostasis [6, 7]. PPAR γ is known for regulating cellular proliferation and differentiation inducing apoptosis in a wide spectrum of human tumor cell lines [6, 8].

Nuclear factor-kappaB (NF- κ B) and p53 tumor suppressor are important transcription factors involved in the regulation of cell proliferation and apoptosis. In contrast to

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p53 activation, which is associated with apoptosis induction, NF- κ B stimulation has been shown to promote resistance to apoptosis [9–12]. Therefore, sensitivity of tumor cells to apoptotic agents depends on the balance between these two transcription factors.

In the present study, a new insight is provided into the molecular mechanisms by which hesperidin induces the growth arrest and apoptosis in NALM-6 cells (wild-type p53-positive human pre-B cell line). The results of the present study showed that the apoptotic effects of hesperidin were triggered, at least in part, by the activation of PPAR γ . For this purpose, the specific PPAR γ antagonist, 2-chloro-5-nitrobenzanilide (GW9662), was applied in this study. GW9662 is a selective antagonist of PPAR γ (IC₅₀ 7.6 nM) that acts via the irreversible covalent modification of a cysteine residue (Cys285) in PPAR ligand-binding domain [13]. Moreover, hesperidin suppresses constitutive NF- κ B activation and accumulates p53 in a PPAR γ -dependent manner. The findings obtained in this paper provide evidence for a cross-talk between PPAR γ , p53, and NF- κ B in hesperidin-induced apoptosis in NALM-6 cells.

Materials and methods

Reagents and antibodies

Hesperidin, 2-chloro-5-nitrobenzanilide (GW9662), propidium iodide (PI), and 3-(4,5-methylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma (Sigma, St. Louis, USA). RNase A was obtained from Fermentase (Fermentas Life Sciences, Lithuania). Western blot analysis was performed using the primary antibodies including anti-p53 (DO1), anti-pIkappa B-Ser³² (B-9), anti-PPAR γ (E8), p21, Bax, Bcl2, anti-actin (C-2), and caspase-3 (all from Santa Cruz Biotechnology, CA, USA). Also, XIAP and caspase-9 were obtained from Cell Signaling (Cell Signaling Technology, MA, USA).

Detecting cell viability using MTT colorimetric assay

The hesperidin-induced cell death was determined by MTT assay [14]. Briefly, NALM-6 cells (obtained from National Cell bank of Iran, Pasteur Institute, Iran) were seeded at 5,000/well in flat-bottom 96-well culture plates. The cells were incubated with hesperidin in the presence or absence of GW9662 for 24 and 48 h. After medium removal, the cells were incubated with MTT solution (5 mg/mL in PBS) for 4 h and the resulting formazan was solubilized with DMSO (200 μ L). The absorption was measured at 570 nm (620 nm as a reference) in an enzyme-linked immunosorbent assay (ELISA) reader. The cell viability was expressed as the optical density at 570 nm.

Flow cytometric analysis of cell cycle and apoptosis

During apoptosis, activation of some nucleases results in DNA degradation. The sub-G1 method relies on the fact that after DNA fragmentation, there are small fragments of DNA capable of being eluted following the wash in either PBS or a specific phosphate-citrate buffer. This means that after staining with a quantitative DNA-binding dye, cells that have lost DNA will take up less stain and will appear to the left of the G1 peak. In brief, the NALM-6 cells were treated with hesperidin (10–50 μ M) in the presence or absence of hesperidin (10 μ M) for 48 h. Then, they were harvested, washed, and resuspended in 1 mL of PBS. One milliliter of 80% ethanol was added, and then the cells were fixed overnight at 4 °C. The fixed cells were centrifuged and resuspended in 0.5 mL of 500 μ g/mL RNase A and incubated for 45 min at 37 °C. They were centrifuged and resuspended again in 1 mL of 69 μ M PI in 38 mM sodium citrate and incubated at room temperature for a minimum of 30 min. The cells were then analyzed for DNA content using flow cytometry (Becton–Dickinson, Franklin Lakes, NJ). By analyzing the DNA histograms, the percentage of the cells in different cell cycle phases was evaluated. Finally, cells with a sub-G0/G1 DNA (sub-G1) were calculated as apoptotic cells.

cDNA synthesis and RT-PCR assay

Total RNA was extracted by RNA extraction kit according to the supplier's protocol (Cinagene, Karaj, Iran). First, strand complementary DNA (cDNA) was synthesized by cDNA synthesis kit using 1 μ g of the extracted RNA (Fermentas, Lithuania). The polymerase chain reaction (PCR) for PPAR γ , p53, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (as positive controls) was performed by the Master PCR Kit (Cinagene, Karaj, Iran) according to the manufacturer's instructions. The RT-PCR products were separated by gel electrophoresis on 1% agarose gel and stained with ethidium bromide. The applied primers were as follows: PPAR γ forward, 5'-TCTCTCCGTAATGGAAGACC-3', reverse, 5'-GCATTATGAGACATCCCCAC-3'; p53 forward, 5'-ACAGCCAAGTC TGTGACTTG-3', reverse, 5'-GTGATGATGGTGAGGATGG-3'; GAPDH, forward, 5'-TTGCCATCAATGACCCC TTCA-3', reverse, 5'-CGCCCCACTTGATTTTGG-3'.

PPAR γ transactivation assay

The analysis was carried out by a PPAR γ transcription factor ELISA Kit (Active Motif, CA, USA) in which an oligonucleotide containing the PPAR γ response element was immobilized onto a 96-well plate. The PPARs contained in nuclear extracts bound specifically to this

oligonucleotide and were detected through an antibody directed against PPAR γ . In short, NALM-6 cells were treated with hesperidin (50 μ M) in the presence or absence of GW9662 (10 μ M) for 24 h. Then, they were collected and the nuclear extracts were prepared by a Nuclear Extract Kit (Active Motif). The nuclear extracts of the same protein concentration obtained from individual treatments were subjected to the PPAR γ transcription factor assay according to the manufacturer's instructions.

NF- κ B transactivation assay

The NALM-6 cells were treated with hesperidin (10–50 μ M) in the presence or absence of GW9662 (10 μ M) for 24 h. The nuclear extract was prepared using the Nuclear Extract Kit (Active Motif). The NF- κ B activity assay was conducted using the NF- κ B assay Kit (Active Motif) according to the manufacturer's instructions.

Western blot analysis

The cells were lysed in an RIPA buffer (50 mM Tris [pH 7.5], 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5 mM EDTA, 10 mM NaF, 5 mM *b*-glycerophosphate, 0.1 mM Na₃VO₄, 0.2 mM phenylmethylsulfonyl fluoride [PMSF], 10 mg/mL leupeptin, and 0.5% aprotinin), and the protein concentrations were determined using the Bradford method (Bio-Rad, CA, USA). Equal amounts of proteins were separated by a 10% SDS-PAGE and subsequently transferred onto a nitrocellulose membrane (Amersham Pharmacia Biotech, USA) using a semi-dry transfer cell (Bio-Rad). The proteins were detected using appropriate primary antibodies and the enhanced chemi-luminescence detection system (ECL Plus, Amersham Pharmacia Biotech) according to the manufacturer's protocol.

Statistical analyses

Statistical Package for the Social Sciences (SPSS) version 12.00 (SPSS Inc., Chicago, IL) was used to perform statistical analyses. The significance of differences between the experimental variables was determined using two-tailed Student's *t* test. A probability level of $p < 0.05$ was considered as statistically significant.

Results

PPAR γ antagonist partially abolishes hesperidin-induced inhibition of cell proliferation and apoptosis

In order to elucidate the potential role of PPAR γ in decreasing the cell viability and proliferation induced by

hesperidin in the NALM-6 cells, the effect of hesperidin on NALM-6 cells was studied using MTT assay with or without the antagonist of PPAR γ (GW9662, 10 μ M) for 24 and 48 h. As shown in Fig. 1a, treatment with hesperidin (10–100 μ M) for 24 h had a minimal effect on NALM-6 cells at these doses. There was a more pronounced decrease in the cell viability ($p < 0.05$) at the 48 h treatment. Pre-incubation of NALM-6 cells with GW9662 significantly prevented the antiproliferative effects of low doses of hesperidin (10 μ M), indicating a partial PPAR γ mediation. These results suggested that, at higher concentrations, hesperidin could recruit other molecular targets in order to exert its cytotoxic effects on NALM-6 cells.

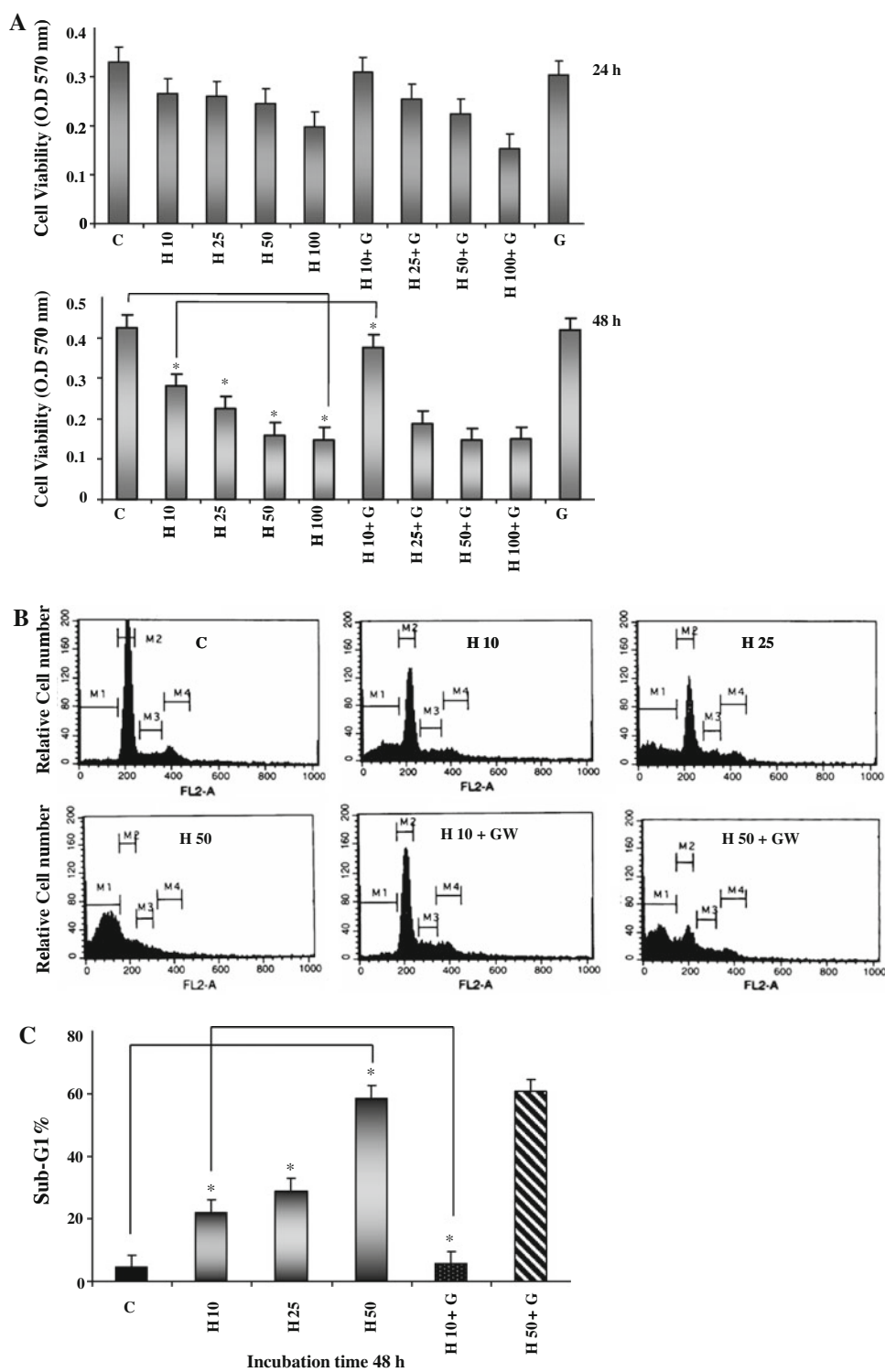
The apoptosis following treatment with hesperidin (10–50 μ M) for 48 h was measured by PI staining and flow cytometry, aiming to detect the sub-G1 peak that results from DNA fragmentation. As shown in Fig. 1b and c, treatment with hesperidin increased the percentage of cells in the sub-G1 phase ($p < 0.05$). The preincubation of NALM-6 cells with GW9662 (10 μ M) significantly decreased the sub-G1 cell population following the treatment of the cells with low concentration of hesperidin (10 μ M).

Hesperidin-mediated apoptosis is associated with the cleavage of caspase-3, caspase-9, and the modulation of Bcl-2 family and XIAP

Since the findings in the present study showed that hesperidin caused strong apoptotic death of NALM-6 cells, first, an evaluation was made to see whether the hesperidin-induced apoptosis was caspase dependent. For this purpose, the NALM-6 cells were treated with the increasing concentrations of hesperidin (10–100 μ M) for 24 h. They were then harvested and subjected to the Western blot analysis using specific antibodies against the cleaved caspase-3 and caspase-9. As is shown by immunoblot analysis in Fig. 2a, the treatment of the cells with hesperidin resulted in a dose-dependent increase in the cleavage of caspase-3 and caspase-9.

Bax, Bcl-2, and XIAP proteins play an important role in apoptosis [15, 16]; therefore, the effect of hesperidin (10–100 μ M, 48 h) was studied on the constitutive protein levels of Bax and Bcl-2 in NALM-6 cells. The immunoblot analysis exhibited a significant increase in the protein expression of Bax by hesperidin (Fig. 2b). In contrast, the protein expression of Bcl-2 significantly decreased by the hesperidin treatment in a dose-dependent way (Fig. 2b). To explore the role of antiapoptotic XIAP in the apoptosis induced by hesperidin, the XIAP expression in the NALM-6 cells was analyzed after incubation by hesperidin. The results showed that the treatment with hesperidin for 48 h caused a significant inhibition of XIAP in the NALM-6 cells (Fig. 2b).

Fig. 1 Effects of PPAR γ inhibition on the decrease in cell viability induced by hesperidin in NALM-6 cells. **a** The NALM-6 cells were treated with hesperidin (10–100 μ M) for 24 and 48 h, and their cell viability was determined by the MTT assay. The data were expressed as absolute optical density values (570 nm OD) ($n = 3$; $*p < 0.05$ relative to the untreated cells). **b, c** The apoptotic effect of hesperidin was evaluated by the PI staining and flow cytometry analysis. The NALM-6 cells were pretreated with hesperidin (10–50 μ M, 48 h) in the presence or absence of GW9662. Then, they were harvested, fixed, stained with PI (50 μ g/mL), and analyzed by the flow cytometry. The percentage of cell population in sub-G0/G1 peak was assumed as apoptotic cells. Each value represents the mean of three independent experiments \pm SE ($n = 3$; $*p < 0.05$ relative to the untreated cells)



Hesperidin induces PPAR γ expression and activity

Previous results of the present study showed that hesperidin exerted its action, at least in part, by PPAR γ activation. Therefore, hesperidin was proposed as an agonist for the

PPAR γ nuclear receptors. Enhanced PPAR γ expression is observed in many systems if the cells are first exposed to a PPAR γ agonist [17]. Hence, to investigate the role of hesperidin in the overexpression of PPAR γ , the NALM-6 cells were treated with 50 μ M hesperidin with or without

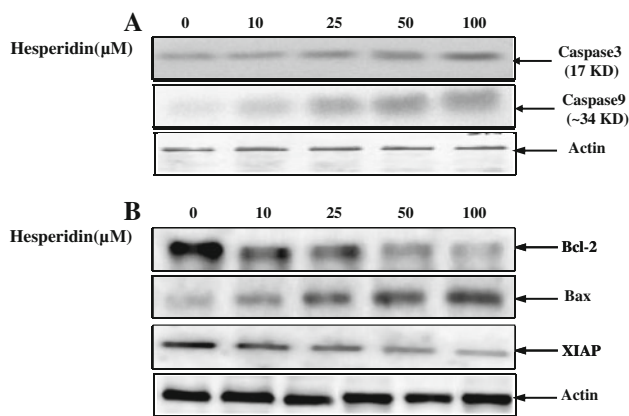


Fig. 2 a Hesperidin induces cleavage of procaspase-3 and procaspase-9 in NALM-6 cells. The NALM-6 cells were incubated with various amounts of hesperidin for 24 h and then harvested. Equal amounts of the cell lysates were subjected to the western blot analysis using specific antibodies against the cleaved caspase-3, caspase-9, and anti-actin antibody as a positive control. **b** Hesperidin enhances BAX and suppresses Bcl-2 and XIAP expression. The NALM-6 cells were incubated with hesperidin for 48 h. The cells were then harvested and subjected to the immunoblot analysis using specific antibodies against BAX, Bcl-2, and XIAP. As a loading control, β -actin levels were determined (the bottom panel). One of the three experiments is shown here as a representative

GW9662 (10 μ M) for 24 h and the level of PPAR γ mRNA was analyzed by RT-PCR. As shown in Fig. 3a, PPAR γ mRNA was undetectable in the untreated cells; however, the increased level of PPAR γ mRNA was seen in the NALM-6 cells treated with hesperidin after 24 h. In contrast, pretreatment of cells with GW9662 prior to the exposure to hesperidin markedly abrogated the induction of PPAR γ mRNA (Fig. 3a), suggesting that the expression of PPAR γ mRNA might be PPAR γ dependent.

The influence of hesperidin on PPAR γ protein expression assayed by the Western blotting revealed an increased expression by hesperidin in a concentration-dependent manner as compared with the untreated cells. The preincubation of NALM-6 cells by GW9662 (10 μ M) profoundly attenuated the levels of PPAR γ protein induced by hesperidin (Fig. 3b). These results suggested that PPAR γ expression was upregulated by hesperidin at both mRNA and protein levels.

To investigate whether hesperidin increased the transcriptional activity of PPAR γ , a modified electromobility shift assay was employed to show the PPAR γ DNA-binding activity. The results showed that hesperidin (25 and 50 μ M) partly induced the DNA binding of PPAR γ in a time-dependent manner (Fig. 3c). The inhibition of PPAR γ by a specific inhibitor GW9662 (10 μ M, 1 h) in NALM-6 cells abolished the hesperidin-induced PPAR γ activity. The attenuation of DNA-binding effect by GW9662 indicated that the major binding factor was PPAR γ .

Hesperidin induces p53 gene expression and activates p53-mediated transcription through PPAR γ

It has been shown that PPAR γ can upregulate the p53 gene expression [18]. Since hesperidin may recruit PPAR γ in order to inhibit the growth of NALM-6 cells, a RT-PCR analysis was performed to find out whether p53 mRNA level was enhanced in a PPAR γ -dependent manner. As depicted in Fig. 4a, hesperidin (50 μ M) induced p53 expression at the mRNA level 24 h after being exposed to hesperidin. Moreover, the GW9662 pretreatment blocked markedly the p53 induction of hesperidin (Fig. 4a). Also, the western blot analysis showed that the treatment of NALM-6 cells with various concentrations of hesperidin for 24 h induced p53 protein in a PPAR γ -dependent manner (Fig. 4b), confirming the involvement of PPAR γ in p53 induction.

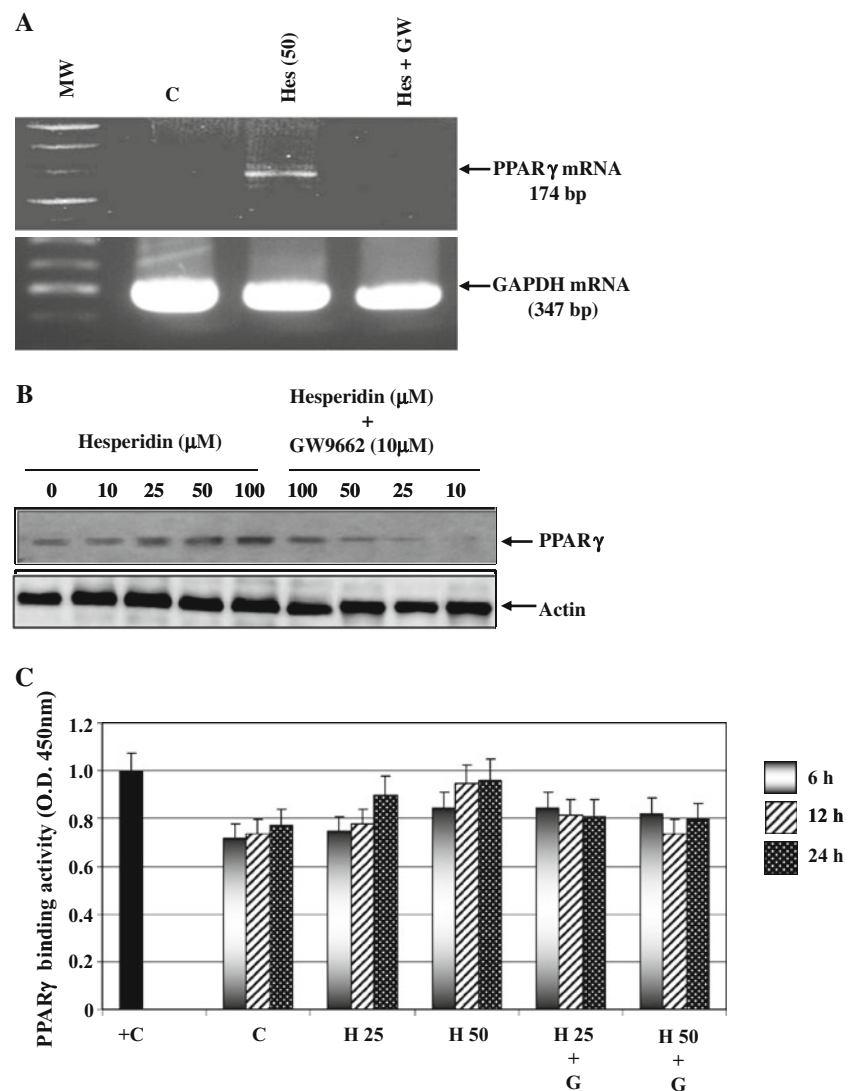
Since p53 protein is a mediator of cell cycle and apoptosis following the exposure to hesperidin, the cell-cycle-related protein, p21, was screened. As is shown in Fig. 4b, consistent with the accumulation of p53, p21^{Cip-1} protein was accumulated in the hesperidin-treated NALM-6 cells.

Hesperidin inhibits phosphorylation of I κ B and subsequent activation of NF- κ B through, at least in part, PPAR γ

In the absence of stimulatory signals, NF- κ B resides in the cytoplasm in the form of the heterodimeric complex with its inhibitory proteins, I κ B α . Stimulating the cells with various stimuli activates the I κ B kinases (IKKs) complex. Once activated, IKK phosphorylates the I κ B subunits of NF- κ B/I κ B complexes, triggering their ubiquitin-dependent degradation and activation of NF- κ B. [10, 19]. It was observed that the hesperidin treatment for 24 h resulted in a decrease in the constitutive phosphorylation of the I κ B α in a dose-dependent manner (Fig. 5a). As is shown in Fig. 5a, the inhibition of PPAR γ activation in NALM-6 cells attenuated the inhibitory effects of hesperidin on the I κ B phosphorylation.

It has been shown that NF- κ B activation blocks the apoptosis and promotes cell proliferation [20]. Since hesperidin treatment inhibits phosphorylation of I κ B, a test was conducted to investigate whether hesperidin inhibited a constitutive NF- κ B activation. Using the ELISA-based NF- κ B activity assay kit, the hesperidin treatment of the NALM-6 cells for 24 h resulted in a significant decrease in DNA-binding activity of NF- κ B in a relatively dose-dependent manner. However, the pretreatment of the NALM-6 cells with GW9662 attenuated, at least in part, the inhibitory effects of the low concentration of hesperidin (10 μ M) on the NF- κ B activity (Fig. 5b).

Fig. 3 Hesperidin induces PPAR γ expression and activity. **a** The NALM-6 cells were treated with hesperidin (50 μ M) in the presence or absence of GW9662 for 24 h. Total RNA was extracted, and the RT-PCR analysis for PPAR γ was performed. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression was examined for normalization purposes. **b** The NALM-6 cells were treated with hesperidin (10–100 μ M) in the presence or absence of GW9662 for 24 h, and PPAR γ protein levels were detected by the western blot analysis. **c** The NALM-6 cells were exposed to hesperidin (25 and 50 μ M, 24 h) with or without GW9662. Nuclear proteins then extracted aliquots containing equal amounts of proteins subjected to the PPAR γ activity assay. The COS-7 nuclear extract (PPAR γ -transfected) was provided as a positive control (+C) for PPAR γ activation. The values are represented as mean \pm SE



Discussion

Flavonoids are polyphenolic compounds present in plants with several potentially beneficial effects on human health [21]. The accumulated pieces of evidence show that these compounds have multiple modes of anti-cancer activities [2, 22]. Several mechanisms have been postulated for the tumor growth-inhibitory effects of flavonoids, including, but not limited to, the inhibition of MAPKs [23], NF- κ B signaling pathway [24, 25], cyclin expression [26], and AKT signaling pathway [27]. Hesperidin is the most common flavonoid found in citrus fruits. It has been shown that hesperidin possesses cancer chemopreventive and apoptotic effects in some tumor cells [1]; however, the mechanisms leading to its proapoptotic actions are largely unknown. Unraveling the mechanism behind the apoptotic effects of hesperidin could deliver insights into the hesperidin mode of action in cancer prevention.

Our findings in the present study show that hesperidin has a potent growth-inhibitory effect on human NALM-6 pre-B cell lines. The results also show that the incubation of NALM-6 cells with hesperidin induces the expression and the transcriptional activity of PPAR γ in NALM-6 cells. To establish the role of PPAR γ in the hesperidin apoptotic effects, PPAR γ -specific antagonist, GW9662, was employed in order to inhibit PPAR γ signaling. The obtained results demonstrate the partial prevention of hesperidin-induced apoptosis by inhibiting PPAR γ .

It has been shown that the activation of PPAR γ promotes p53 expression by a direct binding to the promoter region of the p53 gene in human MCF7 breast cancer cells [18, 28]. This study is a pioneer in demonstrating that p53 may be a mediator of hesperidin-induced apoptosis and revealing that hesperidin upregulates p53 expression via PPAR γ . These results, consistent with the findings of other

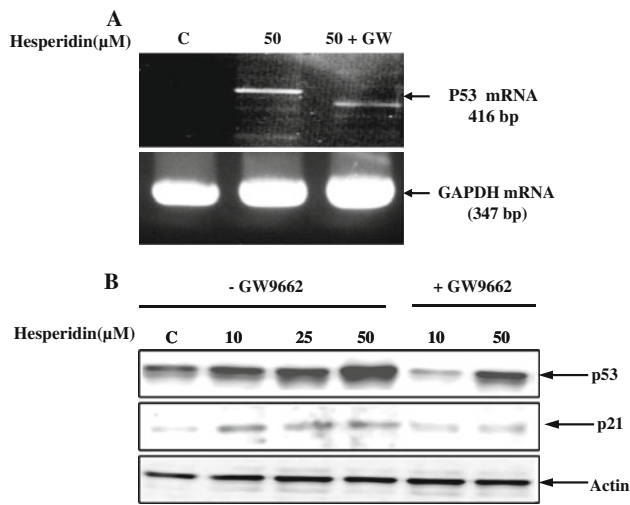


Fig. 4 Hesperidin induces p53 accumulation through PPAR γ . **a** The NALM-6 cells were treated with hesperidin (50 μ M) in the presence or absence of GW9662 for 24 h. Total RNA was extracted, and the RT-PCR analysis was performed to detect P53 mRNA. Each batch of reactions included positive controls for GAPDH mRNA. **b** The NALM-6 cells were exposed to various concentrations of hesperidin with or without GW9662 for 24 h. The cells were then harvested and subjected to the immunoblot analysis using specific antibodies against p53 and p21 proteins. An anti-actin antibody was used to ensure equal loading and quality of the protein extracts. These are the representative data of more than two experiments

studies, suggest that p53 may be involved in the PPAR γ -induced apoptosis [29].

It was also observed that hesperidin inhibited the constitutive phosphorylation of I κ B and the activation of NF- κ B through, at least in part, the PPAR γ activation. Different reports indicate the constitutive activation of NF- κ B in the lymphoid and myeloid malignancies, which underscores its implication in malignant transformation. The activity of NF- κ B transcription factors can prevent apoptosis in the normal and malignant hematologic cells [30, 31]. There are some pieces of evidence indicating that the flavonoids inhibit phosphorylation and activation of IKKs, subsequent phosphorylation of I κ B, and inhibition of NF- κ B activation [32]. It was also observed that hesperidin inhibited markedly constitutive activation of NF- κ B; however, pretreatment of the cells with PPAR γ antagonist lowered this inhibition, suggesting that PPAR γ is involved in the hesperidin-induced NF- κ B inhibition. It is known that activation of peroxisome proliferator-activated receptors (PPARs) can inhibit NF- κ B in several cell types such as smooth muscle cells [33], endothelial cells [34], and macrophages [35]. In a recent work conducted by the authors of this paper, it has been shown that hesperidin inhibits NF- κ B in Ramos cells in a PPAR γ -independent manner (data not shown). Accordingly, it was suggested that hesperidin may recruit several mechanisms in order to

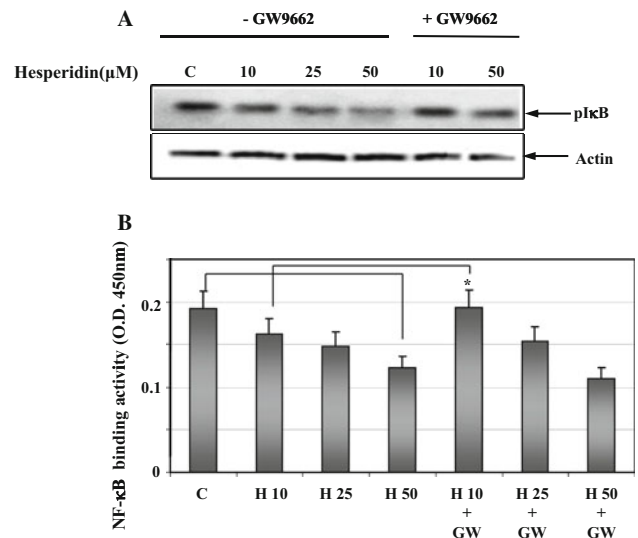


Fig. 5 Hesperidin suppresses I κ B phosphorylation and the subsequent NF- κ B activation. **a** The NALM-6 cells were preincubated with GW9662 (10 μ M) 1 h prior to being exposed to hesperidin which lasted for 24 h. The cells were then harvested and subjected to the western blot analysis using phospho-specific antibody against I κ B phosphorylated at Ser³², and anti-actin antibody was used as a positive control. **b** The NALM-6 cells were treated with hesperidin (10–50 μ M) in the presence or absence of GW9662. Then, they were harvested and the nuclear fraction was separated using the Nuclear Extraction Kit. The NF- κ B activity was quantified by enzyme-linked immunosorbent assay using the Trans-AM NF- κ B p65 Transcription Factor Assay Kit, according to the manufacturer’s instructions. The Jurkat (TPA + CI) nuclear extract was provided as a positive control (+C) for p65/NF- κ B activation. Values are represented as mean \pm SE ($n = 3$; * $p < 0.05$ relative to the untreated cells)

inhibit the constitutive NF- κ B activation. This is confirmed by the findings in the present study that the inhibition of PPAR γ partially suppressed the NF- κ B activation and the hesperidin-induced apoptosis.

In conclusion, this study provides evidence for the involvement of PPAR γ -dependent and PPAR γ -independent mechanisms in the proapoptotic and antiproliferative abilities of hesperidin. This appears to be triggered, at least in part, by the accumulation of p53 and modulation of NF- κ B, respectively. Therefore, the main importance of this study is the integration of several mechanisms in the apoptosis induced by the hesperidin in a hematopoietic cell line.

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