

Development and validation of an RP-HPLC method to compare the apoptotic activity of quercetin found in marketed dietary supplements

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ABSTRACT: Quercetin is a ubiquitous bioactive flavonoid found in broad selection of dietary supplements. It has potential beneficial health effects and induces therapeutic activity in many diseases including different types of cancer. Quercetin exerts cytotoxic activity in cancer and induces apoptotic cell death. Albeit, each dietary supplement consists of impurities, which in turn diminish the biological activity of the quercetin. Herein, we extracted quercetin from 5 different marketed dietary supplements in tablet formulation and detected their quercetin content by developing and validating an RP-HPLC method. We further investigated the cytotoxic activity of quercetin and its role in apoptosis through mitochondrial pathway in MCF-7 and 4T1 breast cancer cell lines. Our findings demonstrated that the quercetin content in all formulations was less than the amount stated in the labeling information, nevertheless they exerted significant cytotoxic activities in breast cancer cells. We revealed that formulations with high quercetin content induced apoptosis through restoring the tumor suppression activity of p53 and activating downstream caspases, whereas the formulation with low quercetin content potentially induced non-apoptotic cell death. Our work illustrates the involvement of quercetin in apoptosis through the mitochondrial pathway in accordance with the quercetin content detected by the RP-HPLC.

KEYWORDS: RP-HPLC ; Quercetin ; supplement ; method validation ; flavonoids ; apoptosis ; breast cancer

1. INTRODUCTION

Quercetin (3,3',4',5,7'-pentahydroxyflavone) belongs to a family of flavonoids and is known to have significant biological activities. It is readily found in some fruits and vegetables such as berries, apples, onions, broccoli, pepper, citrus fruits, tea, and red wine [1]. In addition, quercetin is available in a broad selection of marketed dietary supplements.

As a naturally occurring phytochemical, quercetin and its derivatives have wide range of bioactive properties and their activities have been investigated in many diseases [2]. Quercetin exerts potential beneficial health effects and triggers therapeutic activity in various diseases and conditions [3]. Previous studies have revealed the anti-inflammatory, antioxidant, antitumor, neuroprotective, antiatherogenic, antidiabetic, anti-obesity, antiviral and antibacterial roles of quercetin [4]. There is a growing body of evidence suggesting that quercetin may have antiviral activity against various pathogens [5] including influenza [6], herpes simplex virus [7], and respiratory syncytial virus [8]. In addition, quercetin has been shown to stimulate immune function and modulate immune responses against SARS-CoV-2 infection [9].

The cytotoxic activities of quercetin have been shown in different cancer types. Through up-regulating the expression of the tumor-suppressive miRNAs, quercetin prevented human cervical cancer [10]. Quercetin was reported to suppress glycolysis and inhibit cell proliferation of oral squamous cell carcinoma [11]. Upon administration of quercetin-loaded thermosensitive hydrogels, improved apoptosis was observed in ovarian cancer models [12]. Similarly, quercetin-loaded hydrogel nanocomposite application caused cytotoxicity against breast cancer cells [13]. In some cases, prolonged quercetin intake was shown to be effective in cancer prophylaxis [14]. Interestingly, quercetin was reported to trigger a synergistic pharmacological effect, boost the anticancer activity of the chemotherapeutic drugs and reduce their adverse effects [4,12,15,16].

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Owing to global burden of cancer, quercetin has gained particular interest as a supplement to help mitigate the severity of the disease, in addition to its many health benefits. Regular consumption of dietary supplements containing quercetin may exert significant health effects and help preventing numerous types of cancers. However, the quercetin content of the marketed dietary supplements varies greatly since each supplier provide substance with different impurities. Besides, the dietary supplements are subjected to different sets of regulations compared to the drugs, which are controlled by the internal quality management systems of the companies. These factors, in turn, affect the quality of the supplement, the biological activity of the quercetin and its therapeutic role in cancer. Therefore, it is crucial to determine the quercetin content, compare them with the labeling information and detect the apoptotic activity accordingly. In this study, we aimed to determine the quantity of quercetin found in 5 different marketed dietary supplements in tablet formulation using high-performance liquid chromatography (HPLC) and demonstrate their apoptotic activities against breast cancer to allow providing the end-users with the proper dosage of this potentially beneficial dietary supplement.

2. RESULTS

2.1. RP-HPLC analysis to detect quercetin content in the dietary supplements

The quercetin content in the marketed dietary supplements varies greatly. Most products consist of impurities and the amount of quercetin rarely matches with the labeling information. Therefore, we initially analyzed the amount of quercetin in the marketed dietary supplements and compared the findings with the labeling information. To do this, we performed RP-HPLC analysis using 5 different dietary supplements containing quercetin in tablet formulation and encoded them as F1-F5. Quercetin dihydrate was used as the standard. Interestingly, the quercetin content in all formulations was less than the amount stated in the labeling information. We detected that F1-F5 formulations had $90.96\pm0.46\%$, $87.43\pm2.37\%$, $90.01\pm2.42\%$, $46.92\pm1.76\%$ and $78.4\pm0.93\%$ quercetin content compared to product labeling. The country of production and origin of the active pharmaceutical ingredients (API) declared on the label of the products and the comparison of the amounts experimentally determined in them with the label values are given in Table 1. The experimental quercetin content of F4 was dramatically low, whereas the quercetin content of F1, F2 and F3 formulations were comparable to the product labeling (Figure 1A).

Table 1. Quercetin contents in formulations

Formulation code, Product origin and API origin	Declared amount per one dosage form, mg	Found by validated method, per dosage form, mg \pm SD	Percentage of coverage	Standard Error
F1 (Türkiye, API from Italy)	100	90.96 \pm 0.79	90.96	0.46
F2 (Türkiye, API from India)	500	437.13 \pm 4.11	87.43	2.37
F3 (USA, API from USA)	500	450.06 \pm 4.19	90.01	2.42
F4 (Türkiye, API from India)	500	234.60 \pm 3.05	46.92	1.76
F5 (Türkiye, API from USA)	100	78.40 \pm 1.60	78.40	0.92

2.2. Quercetin supplements prevent viability and proliferation of breast cancer cells

We further investigated the cytotoxic activity of dietary supplements containing quercetin against breast cancer cell lines. To do this, MTT assay was carried out using 300 μ M or 500 μ M quercetin. The effect of quercetin was tested on hormone-responsive breast cancer cell line MCF7, metastatic stage IV human breast cancer cell line 4T1 and Human Embryonic Kidney cell line HEK293. We initially evaluated the dose dependent cell viability and proliferative activity of the different quercetin formulations. The cytotoxicity of 500 μ M quercetin extracts was more efficient than that of 300 μ M quercetin (data not shown). The MCF7 cell viabilities after quercetin treatment were within 55% and 82% (Figure 1B), whereas the viabilities of 4T1 cells were within 32% and 52% (Figure 1C). The HEK293 cells were used as control and the survival rates following quercetin application were above 70% (Figure 1D). F3, which had 90.01% quercetin content compared to product labeling, yielded the greatest cytotoxic activity in both breast cancer cell lines. Surprisingly, the survival rates of F4 formulation (46.92% drug content compared to product labeling) were similar to that of the standard. We further performed RP-HPLC analysis to detect cellular uptake of quercetin. Negligible amount of quercetin was detected in cell culture media suggesting that the cellular

uptake of quercetin was efficient. These findings revealed that the quercetin content of the dietary supplements was in accordance with their cytotoxic activities except F4, which was shown to be quite effective against breast cancer contrary to its low drug content.

2.3. Quercetin supplements induce apoptosis through mitochondrial pathway

The cytotoxic activities of dietary supplements containing quercetin led to further investigation to understand whether quercetin induced breast cancer cell death was apoptotic. p53 has been known to regulate a wide range of cellular activities including apoptosis. Therefore, we inspected the expression of total p53 in MCF7, 4T1 and HEK293 cell lines after 500 μ M quercetin treatment. We observed increased p53 expression in breast cancer cell lines. The expression levels were higher in metastatic 4T1 cells. These findings indicated that the quercetin induced cell death might have occurred through the apoptosis pathway (Figures 2A-C). As expected, the levels of p53 expression after F3 treatment were greatest in MCF7 and 4T1 cell lines (Figures 2A-B), which was in agreement with its quercetin content. Strikingly, the cell density and thus p53 expression of F4 seemed to be less, which might have pointed out reduced apoptosis (Figures 2A-B).

Given that p53 mediated apoptosis has been known to occur through downstream activation of Caspase-9, we next explored its expression in breast cancer cell lines after administration of quercetin extracted from F1-F5 formulations. Almost no Caspase-9 was detected in HEK293 cells, whereas the breast cancer cells expressed significant levels of cleaved Caspase-9 following quercetin treatments (Figures 3A-C).

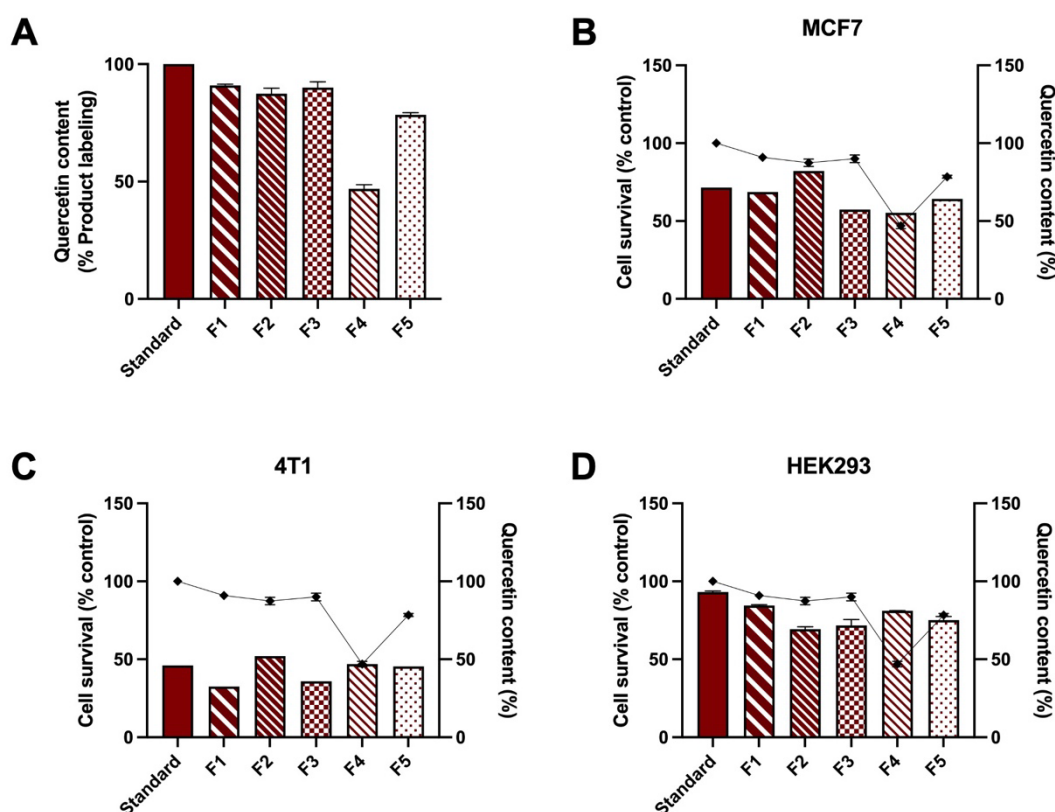


Figure 1. A) Quercetin content of 5 different dietary supplements detected by RP-HPLC. Products reduced the viability of B) MCF7 and C) 4T1 breast cancer cells, D) whereas the viability of HEK293 cells did not change.

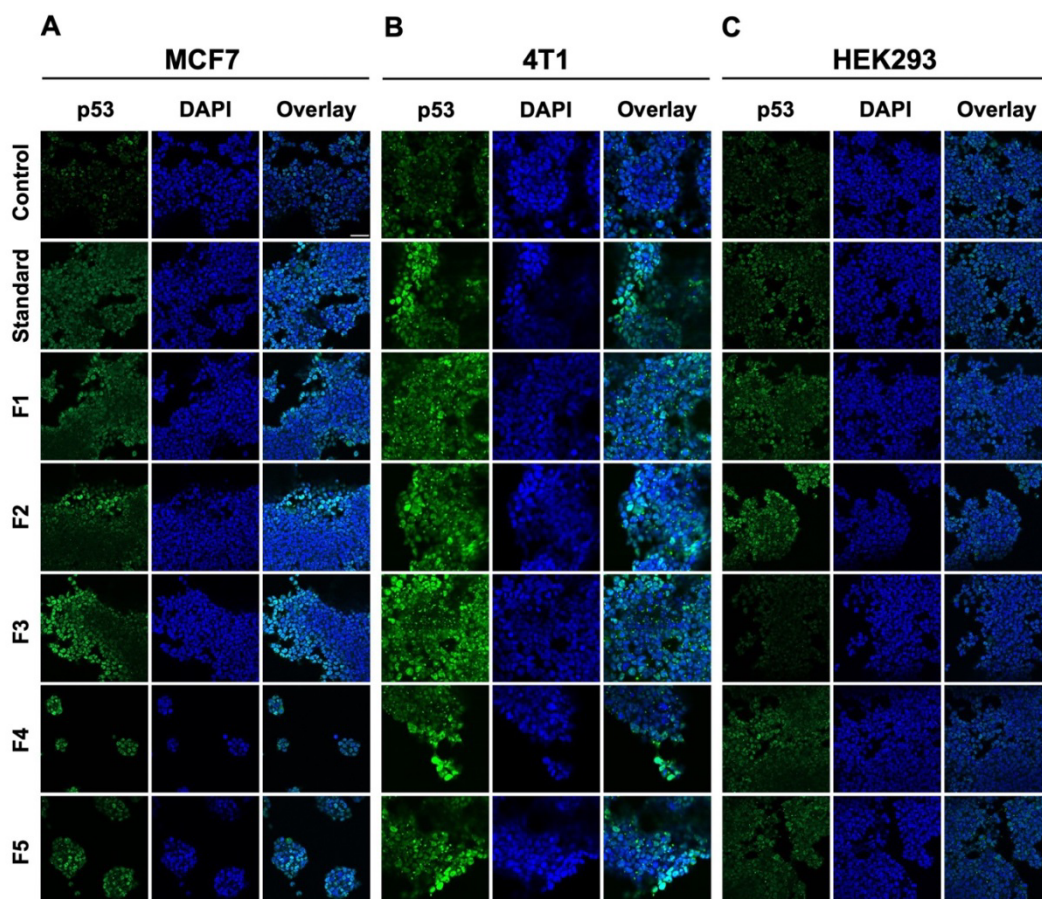


Figure 2. Quercetin extracted from 5 different marketed dietary supplements restored the tumor suppression activity of p53 in A) MCF7 and B) 4T1 cells in accordance with the content of the supplement. C) p53 expression in HEK293 cells after quercetin treatment remained low. Scale bar: 50 μm

Collectively, these results suggested that the dietary supplements containing quercetin, in parallel with the drug content compared to product labeling, mediated cell death through p53 induced apoptosis with downstream activation of Caspase-9.

To better understand the extend of apoptosis driven by quercetin treatment, we investigated the level of Caspase-3/7 activity in living cells. High Caspase-3/7 activity in MCF7 (Figures 4A, D) and 4T1 (Figures 4B, E) cells upon F1, F2, F3 and F5 application demonstrate that these formulations are effective in apoptosis mediated cell death, whereas F4 formulation yielded lower Caspase-3/7 activity in both breast cancer cell lines. Likewise, Caspase-3/7 activity in HEK293 cells were negligible (Figures 4C, F). Taken together, these findings suggest that quercetin formulations triggered apoptosis mediated cell death in breast cancer cells, except F4 formulation, which might have triggered non-apoptotic cell death.

3. DISCUSSION

Here, we identified the quantity of quercetin found in 5 different marketed dietary supplements in tablet formulation using HPLC and reported their role in apoptosis mediated cell death in breast cancer cell lines; MCF7 and 4T1. Specifically, formulations with high quercetin content (F1, F2, F3 and F5) had significant apoptotic activity against cancer cells.

Quercetin, a ubiquitous bioactive flavonoid, is found in various dietary supplements and widely used, albeit the majority of these products consist of impurities, which in turn alters the biological activity of the quercetin. A well-fitted method has been developed for quercetin extracts from 5 different marketed dietary

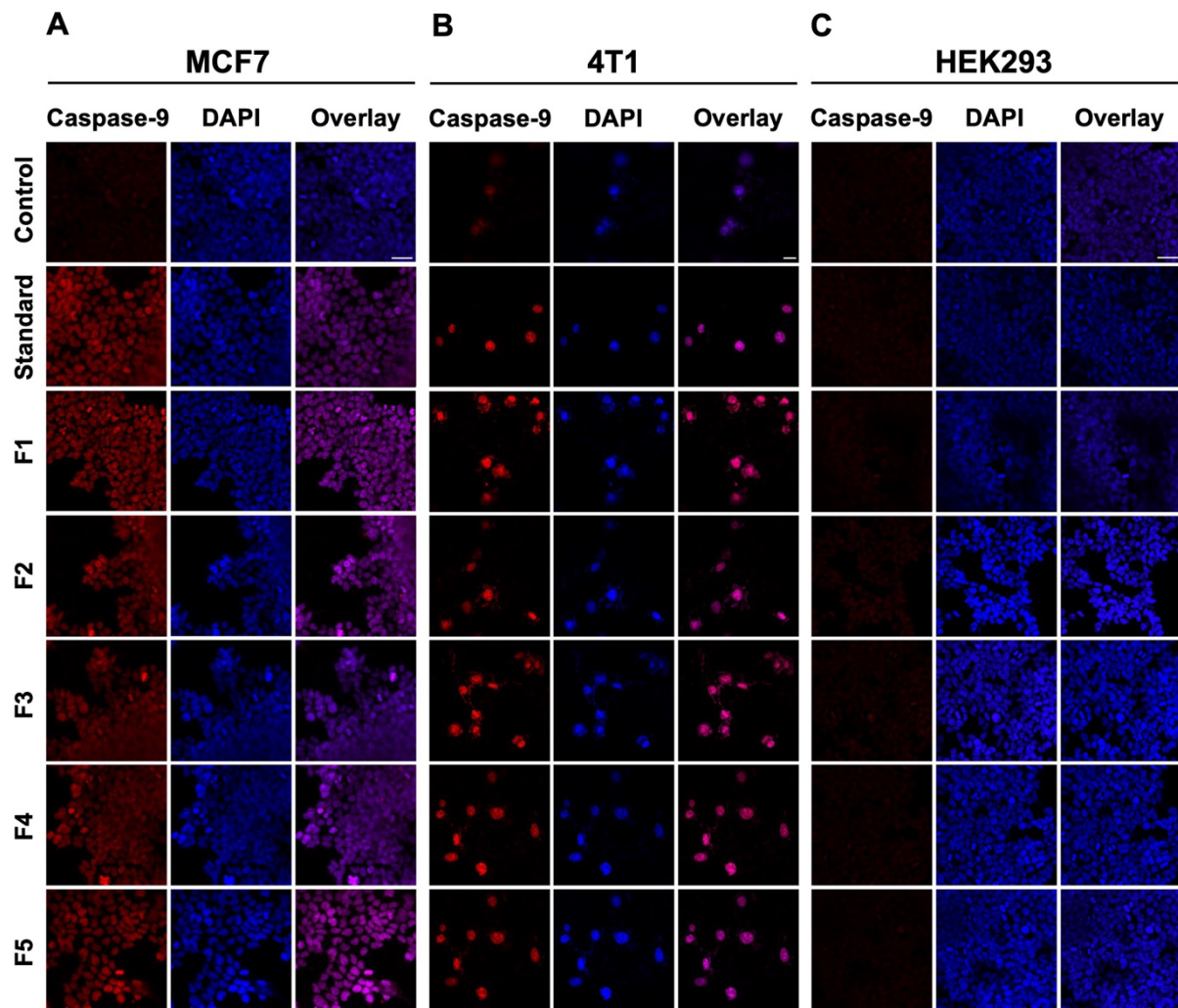


Figure 3. The activation of Caspase-9 increased in A) MCF7 cells (Scale bar: 50 μm) and B) 4T1 cells (Scale bar: 20 μm) upon application of quercetin extracted from 5 different marketed dietary supplements in accordance with the content of the supplement. C) No Caspase-9 expression was detected in HEK293 cells after quercetin application. Scale bar: 50 μm

supplements, which reduces solvent consumption as results are obtained in a shorter time, is cost effective and therefore more environmentally friendly. This developed and validated RP-HPLC method provides results with high accuracy, intermediate precision, and reproducibility. We provided this RP-HPLC method to detect the quercetin contents of 5 different marketed dietary supplements in tablet formulation and compared them with the product labeling.

Quercetin has long been known for its anti-cancer activity in various cancer types. Previous studies revealed that cancer cells are readily available to the cytotoxic effects of quercetin [17–23]. These studies are in accordance with the findings of the current study, where we demonstrated that the breast cancer cell growth was inhibited after treating with quercetin extracts obtained from different dietary supplements. Restricted growth of cancer cells was earlier linked to suppression of survival signal proteins including AKT, ERK, protein kinase C (PKC- α) and activation of death signals including c-Jun N-terminal kinases (JNK) and PKC- δ [24–26].

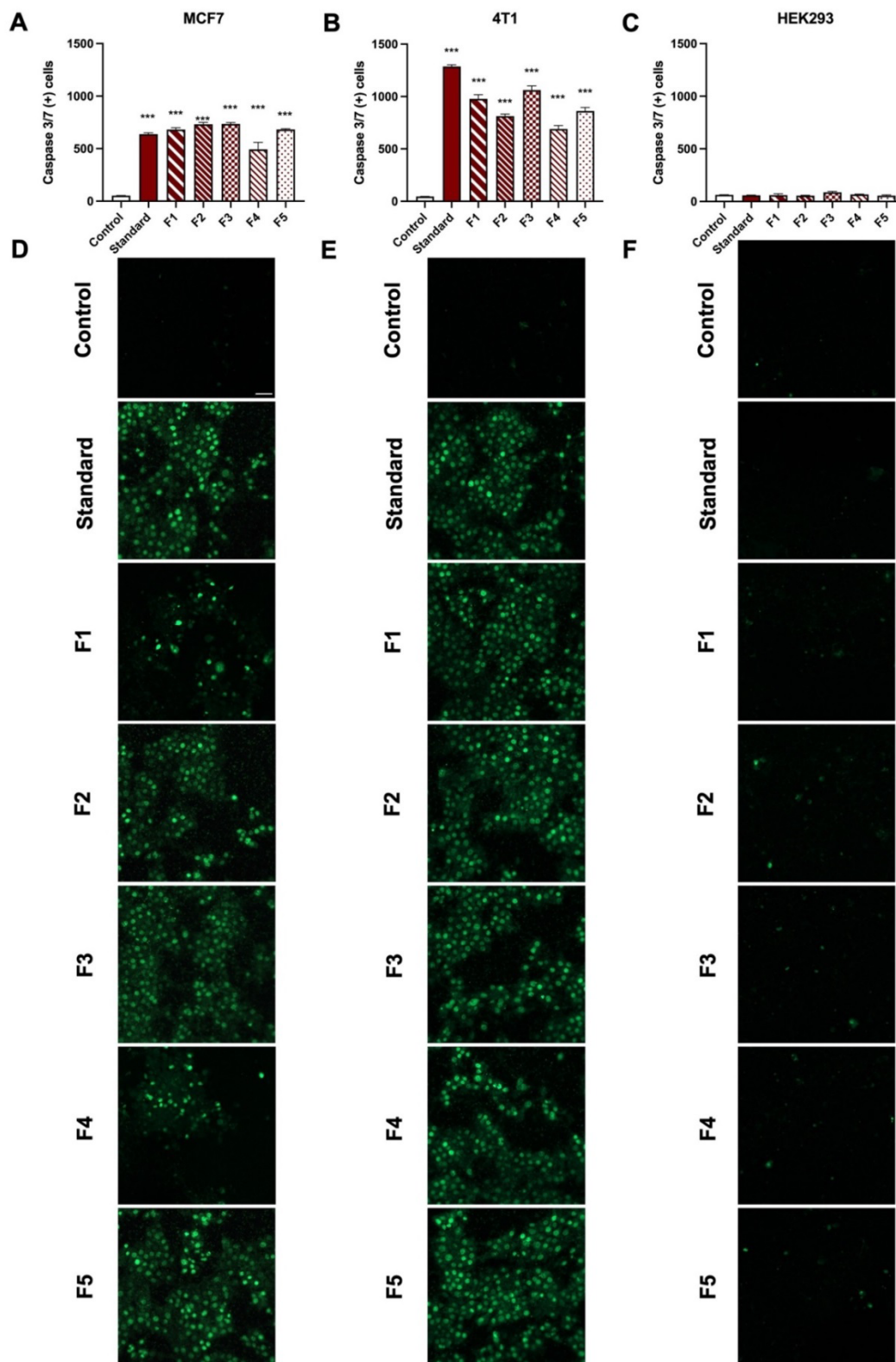


Figure 4. Live imaging demonstrated that quercetin extracted from 5 different marketed dietary supplements significantly increased Caspase-3/7 expression in A, D) MCF7 and B, E) 4T1 cells in accordance with the content of the supplement, C, F) whereas Caspase-3/7 levels in HEK293 were negligible. (***p* < 0.001). Scale bar: 50 μ m

The cytotoxic effect of quercetin is potentially modulated by the induction of the apoptotic pathways [17,27,28]. So far, disruption of mitochondria [29,30], generation of ROS [31], DNA intercalation [32] and cell cycle arrest [33,34] have been attributed to quercetin induced apoptosis. Quercetin was previously shown to

induce apoptosis through mitochondrial pathway [35]. In this pathway, apoptotic cell death is regulated by caspases, which belong to a family of cysteine proteases and allow cleavage of target proteins. The transcription factor p53 induces apoptosis and tumor suppression [36]. As a response to apoptosis inducing stress signals, mitochondrial translocation of p53 occurs, which in turn initiates release of cytochrome c release into cytosol and caspase activation [37]. After Caspase-9 is activated, Caspase-3 and Caspase-7, the downstream caspases, are activated as well, which altogether induces apoptosis [35,38]. Herein, our findings support the involvement of quercetin in apoptosis through the mitochondrial pathway (Figure 5). Our work indicates that quercetin extracted from F1, F2, F3 and F5 formulations restored the tumor suppression activity of p53 and induced downstream activation of Caspase-9 and Caspase-3/7. Treatment with F4 formulation did not promote p53 and downstream activation of caspases, nevertheless it reduced cancer cell viability, which may have led to non-apoptotic cell death.

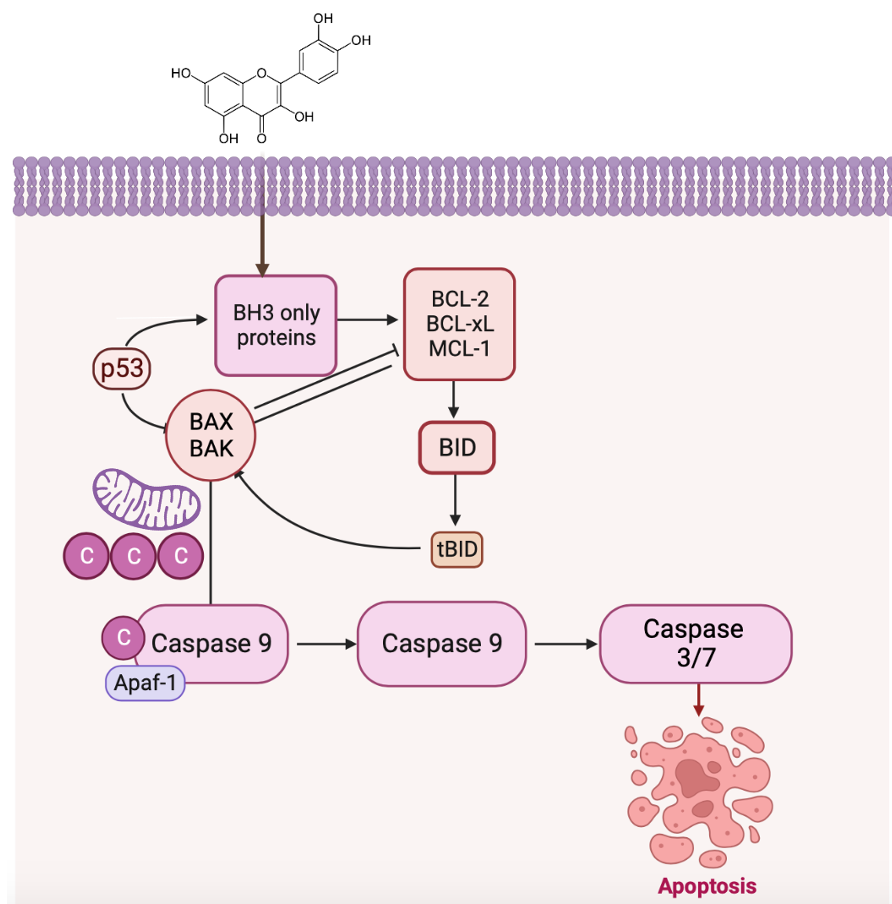


Figure 5. Schematic representation of the involvement of quercetin in apoptosis through the mitochondrial pathway (Created with Biorender.com)

4. CONCLUSION

We conclude that the quantity of quercetin extracted from 5 different marketed dietary supplements induce apoptosis through the mitochondrial pathway in accordance with the content described in the product labeling, which consequently provides an understanding of the proper dosing to maximize its therapeutic role in breast cancer.

5. MATERIALS AND METHODS

5.1. Materials

Quercetin dihydrate was used as the reference standard (Sigma-Aldrich, MO, USA). HPLC grade methanol (MeOH) and acetonitrile (ACN) were used to prepare the standard solutions and the mobile phase (Sigma-Aldrich). Formic acid was ACS reagent grade (Merck, Germany). Ultrapure water was obtained from Elga LabWater PURELAB flex 3 system.

5.2. Chromatographic conditions

An Agilent 1100 series HPLC system (USA) with an autosampler, column oven, gradient pump and UV detector was used during the analyses. An InertSustain C18 column with octadecyl silica particles (150x4.6mm, 5 μ m) was used for separation (GL Sciences, CA, USA). The column oven was thermostabilized at 30°C. A and B solvents mixed at different ratios and different time points were used during the separation that took place in binary gradient conditions. The ACN:Water:Formic acid combinations were 10:89:1 for mobile phase A and 89:10:1 for mobile phase B. The change of the mobile phase gradient over time started with 100% of A and decreased to 40% in 1.5 minutes, whereas the ratio of B increased to 60%. This ratio continued until the 3rd minute. The concentration of A increased to 55% at the 4th minute and to 100% 40 seconds later and continued at the same rate until the 7th minute. Retention time was 3.226 minutes. The method has been adapted from a previous study [39], modified and validated. Overlaid chromatograms of the standard quercetin and extracted quercetin are given in Figure 6.

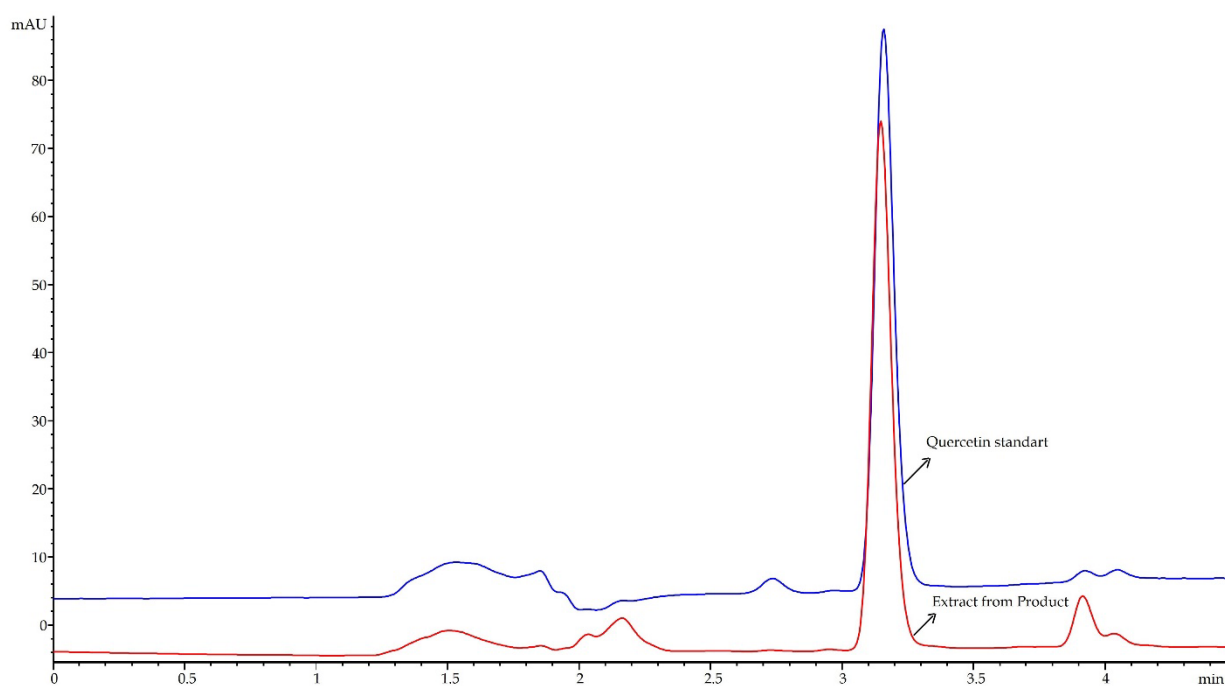


Figure 6. HPLC chromatogram of quercetin marketed products

5.3. Preparation of standard solution and extraction of marketed formulations

Quercetin dihydrate (11.2 mg) was weighed and dissolved in methanol assisted by ultrasound. 10 mL stock solution was prepared at a concentration of 1 mg/mL and it was used to obtain the calibration curve by diluting the standard solution in the range of 10-500 μ g/mL. Due to the stability of the quercetin solution and the volatility of methanol, the solution was prepared in an amber flask, covered with aluminum foil, and stored at +4°C. All solutions were filtered through 0.22 μ m membrane filters and analyzed in triplicate using the HPLC system.

Five different best-selling dietary supplements, with quercetin being the API, were obtained from a pharmacy. All supplements contained quercetin only and were tablet formulations. The supplements were

coded as F1-F5 throughout the manuscript (F1 (Türkiye, API from Italy), F2 (Türkiye, API from India), F3 (USA, API from USA), F4 (Türkiye, API from India), F5 (Türkiye, API from USA) as manufacturer declared). Quercetin in the supplements was extracted with methanol for 3 times. The solvent was evaporated and dissolved with methanol to make the final volume 10 mL. The procedure to extract quercetin from the preparations was carried out in accordance with the monographs in the European pharmacopoeia (EUP). The extracts were freshly prepared, filtered through a 0.22 µm membrane filter before injection and injected into the HPLC device after 1:100 dilution.

5.4. Validation of HPLC Method

The linearity of the developed method was analyzed in the range of 10-500 µg/mL quercetin standard solutions. Effective parameters such as system suitability, precision, accuracy, specificity, selectivity, stability, theoretical plate number, resolution and tailing factor were carried out and the shown to be within the limits which is proposed by ICH Q2(R1) (International conference of harmonization) guidelines. In addition, linearity and system suitability data are given in Table 2.

Table 2. Linearity and system suitability parameters for the developed method (n=3)

Linear range (µg/mL)	10-500
Equation	$y=23.154x - 41.07$
Slope	23.154±0.0801
Intercept	-410.6967±2.6297
Correlation coefficient (R ²)	0.9995
Theoretical plate number (N)	7760
Resolution	8.35
Tailing factor	1.1
Intra-assay precision, Recovery, RSD	98.13%, 0.14%
Intermediate precision, (inter-day) Recovery, RSD	97.9 %, 0.65%
Intermediate precision (Intra-day) precision, Recovery, RSD	98%, 1.15%
Recovery range	98.87-104.26%
Short-term stability (96 hours), difference, RSD	1.5 %, 0.2%
LOD (3*S/N) (µg/mL)	0.0700
LOQ (10*S/N) (µg/mL)	0.2400

5.5. Cell lines and in vitro studies

All cell lines were obtained from American Type Culture Collection (ATCC®, Manassas, VA, USA). Hormone-responsive breast cancer cell line MCF7 (ATCC®: HTB-22) and triple-negative breast cancer cell line 4T1 (ATCC®: CRL-2539) were maintained in RPMI-1640 Medium (R0833; Sigma-Aldrich) supplemented with 10% Fetal Bovine Serum, 1% GlutaMAX™ (35050061; Gibco, Thermo Fisher Scientific, Waltham, MA) and 1% Antibiotic Antimycotic Solution (A5955; Sigma-Aldrich) at 37°C and 5% CO₂. Human Embryonic Kidney cell line HEK293 (ATCC®: CRL-1573) was maintained in Dulbecco's Modified Eagle Medium (DMEM-HPXA; Capricorn Scientific, Germany) supplemented with 10% Fetal Bovine Serum, 1% GlutaMAX™ (35050061; Gibco, Thermo Fisher Scientific, Waltham, MA) and 1% Antibiotic Antimycotic Solution (A5955; Sigma-Aldrich) at 37°C and 5% CO₂. Cells were seeded at a density of 5x10³ cells/well and incubated for 24 hours.

5.6. Cell viability and apoptosis

300 µM or 500 µM quercetin extracted from different marketed products (F1-F5) were added to each well. Quercetin dihydrate was used as positive control. The control wells were treated with DMSO or left untreated. The cells were incubated for 48 hours at 37°C and 5% CO₂.

After incubation, MTT assay was performed to assess the viability, proliferation, and cytotoxicity of cells by measuring their metabolic activity since it employs a colorimetric technique that relies on the conversion of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) into purple formazan crystals in metabolically active cells [40]. 10 μ L of the MTT labeling reagent (final concentration of 0.5 mg/mL) was added to each well, and the plate was incubated for 3 hours at 37°C and 5% CO₂. The medium was removed and 100 μ L of DMSO was added to each well. The plate was covered with aluminum foil, shaken for 20 minutes, and measured at 570 nm using a BioTEK Synergy HTX multimode reader. The findings were normalized to control value using the equation below.

$$\text{Cell Viability (\%)} = (\text{Sample Absorbance} - \text{DMSO absorbance}) / (\text{Control absorbance}) \times 100$$

All experiments were carried out in quadruplicate.

To detect apoptosis in living cells, CellEvent™ Caspase-3/7 Detection Reagent (C10423; Invitrogen, Thermo Fisher Scientific) was added to the cells by the end of 48-hour incubation in 1:100 concentration. The cells were incubated for 30 minutes and visualized using a confocal laser scanning microscope (LSM 800, Carl Zeiss, Jena Germany). To detect apoptosis, Caspase-3/7 expressing cells located at 5 adjacent regions of interest (ROI) were counted.

5.7. Immunocytochemistry (ICC)

For immunocytochemistry, cells were treated with 500 μ M quercetin extracts and quercetin dihydrate and incubated for 48 hours. Then, the cells were fixed with 4% paraformaldehyde (PFA) (158127; Sigma-Aldrich) for 15 minutes and washed with 1xPBS for 3 times. The cells were incubated with blocking solution containing 3:100 (v/v) bovine serum albumin, 3:1000 (v/v) sodium azide, 1:100 (v/v) fetal goat serum and 1:1000 (v/v) Triton X-100 (X100; Sigma-Aldrich) in 1xPBS for 30 minutes at room temperature. Cells were probed with anti-p53 (1:100; 2524; Cell Signaling, Beverly, MA) or anti-cleaved caspase-9 (Asp353) (1:100; 9509; Cell Signaling, Beverly, MA) in primary antibody solution containing 3:100 (v/v) bovine serum albumin, 3:1000 (v/v) sodium azide, 1:100 (v/v) fetal goat serum and 1:1000 (v/v) Tween-20 in 1xPBS and incubated overnight. Next day, the cells were washed with 1xPBS for 3 times and treated with secondary antibodies (1:1000; goat anti-mouse IgG (H+L) Alexa Flour 488 and 1:1000; goat anti-rabbit IgG (H+L) Alexa Flour 568; Invitrogen). After 3-hour incubation, cells were washed with 1xPBS for 3 times, counterstained with 4'-diamino-2-phenylindole (DAPI) for 5 minutes, washed again and evaluated using a confocal laser scanning microscope (LSM 800, Carl Zeiss).

5.8. Statistical Analysis

Data were expressed as mean \pm SEM. The differences between the two groups were tested with unpaired Student's t tests. Corrected p values, Pearson r and p values were calculated using Prism 9.0 (GraphPad Software Inc., Boston, MA, USA). A p value <0.05 was statistically significant.

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Author contributions: Ü.C.E conceived the study, performed the experiments, and prepared the manuscript. N.A designed and performed the experiments.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

- [1] Andres S, Pevny S, Ziegenhagen R, Bakhiya N, Schäfer B, Hirsch-Ernst KI, Lampen A. Safety Aspects of the use of quercetin as a dietary supplement. *Mol Nutr Food Res*. 2018;62(1). <https://doi.org/10.1002/mnfr.201700447>
- [2] Boots AW, Haenen GRMM, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol*. 2008;585(2-3): 325-337. <https://doi.org/10.1016/j.ejphar.2008.03.008>
- [3] Fideles SOM, de Cássia Ortiz A, Buchaim DV, de Souza Bastos Mazuqueli Pereira E, Parreira MJBM, de Oliveira Rossi J, da Cunha MR, de Souza AT, Soares WC, Buchaim RL. Influence of the neuroprotective properties of quercetin on regeneration and functional recovery of the nervous system. *Antioxidants* (Basel, Switzerland).

- 2023;12(1). <https://doi.org/10.3390/antiox12010149>
- [4] Azeem M, Hanif M, Mahmood K, Ameer N, Chughtai FRS, Abid U. An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: a review. *Polym Bull (Berl)*. 2023;80(1): 241–262. <https://doi.org/10.1007/s00289-022-04091-8>
- [5] Rahman MA, Shorobi FM, Uddin MN, Saha S, Hossain MA. Quercetin attenuates viral infections by interacting with target proteins and linked genes in chemico-biological models. *In Silico Pharmacol*. 2022;10(1):17. <https://doi.org/10.1007/s40203-022-00132-2>
- [6] Morimoto R, Hanada A, Matsubara C, Horio Y, Sumitani H, Ogata T, Isegawa Y. Anti-influenza A virus activity of flavonoids in vitro: a structure-activity relationship. *J Nat Med*. 2023;77(1): 219–227. <https://doi.org/10.1007/s11418-022-01660-z>
- [7] Lee S, Lee HH, Shin YS, Kang H, Cho H. The anti-HSV-1 effect of quercetin is dependent on the suppression of TLR-3 in Raw 264.7 cells. *Arch Pharm Res*. 2017;40(5): 623–630. <https://doi.org/10.1007/s12272-017-0898-x>
- [8] Vargas JE, Puga R, de Faria Poloni J, Saraiva Macedo Timmers LF, Porto BN, Norberto de Souza O, Bonatto D, Condessa Pitrez PM, Tetelbom Stein R. A network flow approach to predict protein targets and flavonoid backbones to treat respiratory syncytial virus infection. *Biomed Res Int*. 2015;2015:301635. <https://doi.org/10.1155/2015/301635>
- [9] Raghav A, Giri R, Agarwal S, Kala S, Jeong G-B. Protective role of engineered extracellular vesicles loaded quercetin nanoparticles as anti-viral therapy against SARS-CoV-2 infection: A prospective review. *Front Immunol*. 2022;13:1040027. <https://doi.org/10.3389/fimmu.2022.1040027>
- [10] Murata M, Komatsu S, Miyamoto E, Oka C, Lin I, Kumazoe M, Yamashita S, Fujimura Y, Tachibana H. Quercetin up-regulates the expression of tumor-suppressive microRNAs in human cervical cancer. *Biosci Microbiota Food Heal*. 2023;42(1): 87–93. <https://doi.org/10.12938/bmfh.2022-056>
- [11] Hu M, Song H-Y, Chen L. Quercetin acts via the G3BP1/YWHAZ axis to inhibit glycolysis and proliferation in oral squamous cell carcinoma. *Toxicol Mech Methods*. 2023;33(2): 141–150. <https://doi.org/10.1080/15376516.2022.2103480>
- [12] Xu G, Li B, Wang T, Wan J, Zhang Y, Huang J, Shen Y. Enhancing the anti-ovarian cancer activity of quercetin using a self-assembling micelle and thermosensitive hydrogel drug delivery system. *RSC Adv*. 2018;8(38): 21229–21242. <https://doi.org/10.1039/C8RA03274B>
- [13] Sabzini M, Pourmadadi M, Yazdian F, Khadiv-Parsi P, Rashedi H. Development of chitosan/halloysite/graphitic-carbon nitride nanovehicle for targeted delivery of quercetin to enhance its limitation in cancer therapy: An in vitro cytotoxicity against MCF-7 cells. *Int J Biol Macromol*. 2023;226: 159–171. <https://doi.org/10.1016/j.ijbiomac.2022.11.189>
- [14] Araújo JR, Gonçalves P, Martel F. Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. *Nutr Res*. 2011;31(2): 77–87. <https://doi.org/10.1016/j.nutres.2011.01.006>
- [15] Liu H, Lee JI, Ahn T-G. Effect of quercetin on the anti-tumor activity of cisplatin in EMT6 breast tumor-bearing mice. *Obstet Gynecol Sci*. 2019;62(4): 242–248. <https://doi.org/10.5468/ogs.2019.62.4.242>
- [16] Shen Y, TanTai J. Co-Delivery anticancer drug nanoparticles for synergistic therapy against lung cancer cells. *Drug Des Devel Ther*. 2020;14: 4503–4510. <https://doi.org/10.2147/DDDT.S275123>
- [17] Hashemzaei M, Delarami Far A, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, Sadegh SE, Tsarouhas K, Kouretas D, Tzanakakis G, Nikitovic D, Anisimov NY, Spandidos DA, Tsatsakis AM, Rezaee R. Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. *Oncol Rep*. 2017;38(2): 819–828. <https://doi.org/10.3892/or.2017.5766>
- [18] Kim H, Seo E-M, Sharma AR, Ganbold B, Park J, Sharma G, Kang Y-H, Song D-K, Lee S-S, Nam J-S. Regulation of Wnt signaling activity for growth suppression induced by quercetin in 4T1 murine mammary cancer cells. *Int J Oncol*. 2013;43(4): 1319–1325. <https://doi.org/10.3892/ijo.2013.2036>
- [19] Asgharian P, Tazekand AP, Hosseini K, Forouhandeh H, Ghasemnejad T, Ranjbar M, Hasan M, Kumar M, Beirami SM, Tarhriz V, Soofiyan SR, Kozhamzharova L, Sharifi-Rad J, Calina D, Cho WC. Potential mechanisms of quercetin in cancer prevention: focus on cellular and molecular targets. *Cancer Cell Int*. 2022;22(1): 257. <https://doi.org/10.1186/s12935-022-02677-w>
- [20] Ren M-X, Deng X-H, Ai F, Yuan G-Y, Song H-Y. Effect of quercetin on the proliferation of the human ovarian cancer cell line SKOV-3 in vitro. *Exp Ther Med*. 2015;10(2): 579–583. <https://doi.org/10.3892/etm.2015.2536>
- [21] Deng X-H, Song H-Y, Zhou Y-F, Yuan G-Y, Zheng F-J. Effects of quercetin on the proliferation of breast cancer cells and expression of survivin in vitro. *Exp Ther Med*. 2013;6(5): 1155–1158. <https://doi.org/10.3892/etm.2013.1285>
- [22] Ren K-W, Li Y-H, Wu G, Ren J-Z, Lu H-B, Li Z-M, Han X-W. Quercetin nanoparticles display antitumor activity via proliferation inhibition and apoptosis induction in liver cancer cells. *Int J Oncol*. 2017;50(4): 1299–1311. <https://doi.org/10.3892/ijo.2017.3886>
- [23] Busch C, Burkard M, Leischner C, Lauer UM, Frank J, Venturelli S. Epigenetic activities of flavonoids in the prevention and treatment of cancer. *Clin Epigenetics*. 2015;7(1): 64. <https://doi.org/10.1186/s13148-015-0095-z>
- [24] Sahpazidou D, Geromichalos GD, Stagos D, Apostolou A, Haroutounian SA, Tsatsakis AM, Tzanakakis GN, Hayes AW, Kouretas D. Anticarcinogenic activity of polyphenolic extracts from grape stems against breast, colon, renal and thyroid cancer cells. *Toxicol Lett*. 2014;230(2): 218–224. <https://doi.org/10.1016/j.toxiclet.2014.01.042>
- [25] Granado-Serrano AB, Angeles Martín M, Bravo L, Goya L, Ramos S. Time-course regulation of quercetin on cell survival/proliferation pathways in human hepatoma cells. *Mol Nutr Food Res*. 2008;52(4): 457–464. <https://doi.org/https://doi.org/10.1002/mnfr.200700203>

- [26] Youn H, Jeong J-C, Jeong YS, Kim E-J, Um S-J. Quercetin potentiates apoptosis by inhibiting nuclear factor-kappaB signaling in H460 lung cancer cells. *Biol Pharm Bull.* 2013;36(6): 944–951. <https://doi.org/10.1248/bpb.b12-01004>
- [27] Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. *J Nutr Biochem.* 2007;18(7): 427–442. <https://doi.org/10.1016/j.jnutbio.2006.11.004>
- [28] Jakubowicz-Gil J, Paduch R, Piersiak T, Głowniak K, Gawron A, Kandefer-Szerszeń M. The effect of quercetin on pro-apoptotic activity of cisplatin in HeLa cells. *Biochem Pharmacol.* 2005;69(9): 1343–1350. <https://doi.org/10.1016/j.bcp.2005.01.022>
- [29] Ferraresi R, Troiano L, Roat E, Lugli E, Nemes E, Nasi M, Pinti M, Fernandez MIG, Cooper EL, Cossarizza A. Essential requirement of reduced glutathione (GSH) for the anti-oxidant effect of the flavonoid quercetin. *Free Radic Res.* 2005;39(11): 1249–1258. <https://doi.org/10.1080/10715760500306935>
- [30] Kim H, Moon JY, Ahn KS, Cho SK. Quercetin induces mitochondrial mediated apoptosis and protective autophagy in human glioblastoma U373MG cells. *Oxid Med Cell Longev.* 2013;2013:596496. <https://doi.org/10.1155/2013/596496>
- [31] Kim GT, Lee SH, Kim J Il, Kim YM. Quercetin regulates the sestrin 2-AMPK-p38 MAPK signaling pathway and induces apoptosis by increasing the generation of intracellular ROS in a p53-independent manner. *Int J Mol Med.* 2014;33(4): 863–869. <https://doi.org/10.3892/ijmm.2014.1658>
- [32] Srivastava S, Somasagara RR, Hegde M, Nishana M, Tadi SK, Srivastava M, Choudhary B, Raghavan SC. Quercetin, a natural flavonoid interacts with DNA, arrests cell cycle and causes tumor regression by activating mitochondrial pathway of apoptosis. *Sci Rep.* 2016;6:24049. <https://doi.org/10.1038/srep24049>
- [33] Jeong J-H, An JY, Kwon YT, Rhee JG, Lee YJ. Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. *J Cell Biochem.* 2009;106(1): 73–82. <https://doi.org/10.1002/jcb.21977>
- [34] Mu C, Jia P, Yan Z, Liu X, Li X, Liu H. Quercetin induces cell cycle G1 arrest through elevating Cdk inhibitors p21 and p27 in human hepatoma cell line (HepG2). *Methods Find Exp Clin Pharmacol.* 2007;29(3): 179–183. <https://doi.org/10.1358/mf.2007.29.3.1092095>
- [35] Zhang J, Yi T, Liu J, Zhao Z, Chen H. Quercetin Induces Apoptosis via the Mitochondrial Pathway in KB and KBv200 Cells. *J Agric Food Chem.* 2013;61(9): 2188–2195. <https://doi.org/10.1021/jf305263r>
- [36] Tokino T, Nakamura Y. The role of p53-target genes in human cancer. *Crit Rev Oncol Hematol.* 2000;33(1): 1–6. [https://doi.org/10.1016/s1040-8428\(99\)00051-7](https://doi.org/10.1016/s1040-8428(99)00051-7)
- [37] Vaseva A V, Moll UM. The mitochondrial p53 pathway. *Biochim Biophys Acta.* 2009;1787(5): 414–420. <https://doi.org/10.1016/j.bbabi.2008.10.005>
- [38] Frank AK, Pietsch EC, Dumont P, Tao J, Murphy ME. Wild-type and mutant p53 proteins interact with mitochondrial caspase-3. *Cancer Biol Ther.* 2011;11(8): 740–745. <https://doi.org/10.4161/cbt.11.8.14906>
- [39] Gulsoy Toplan G, Kurkcuoglu M, Goger F, İscan G, Ağalar HG, Mat A, Baser KHC, Koyuncu M, Sariyar G. Composition and biological activities of *Salvia veneris* Hedge growing in Cyprus. *Ind Crops Prod.* 2017;97: 41–48. <https://doi.org/10.1016/j.indcrop.2016.11.055>
- [40] van Meerloo J, Kaspers GJL, Cloos J. Cell sensitivity assays: the MTT assay. *Methods Mol Biol.* 2011;731: 237–245. https://doi.org/10.1007/978-1-61779-080-5_20

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